Phytoplankton dynamics and bio-optical variables associated with Harmful Algal Blooms in Aquaculture Zones

Julia A. Busch
Phytoplankton dynamics and bio-optical variables associated with Harmful Algal Blooms in aquaculture zones

Dissertation

Zur Erlangung des Akademischen Grades eines Doktors der Naturwissenschaften

-Dr. rer. nat. -

im Fachbereich 2 (Biologie/Chemie)

der Universität Bremen

vorgelegt von

Julia A. Busch

Bremen, Oktober 2013

1. Gutachter: Prof. Dr. Allan D. Cembella
Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research
University Bremen

2. Gutachter: Prof. Dr. Oliver Zielinski
Institute for Chemistry and Biology of the Marine Environment (ICBM)
Carl von Ossietzky University Oldenburg
1. Gutachter: Prof. Dr. Allan D. Cembella
2. Gutachter: Prof. Dr. Oliver Zielinski
3. Prüfer: Dr. Marcel Wernand
4. Prüfer: Prof. Dr. Kai Bischof
Tag des Promotionskolloquiums: 09.12.2013
ABSTRACT

The surveillance of Harmful Algal Blooms (HABs) in aquaculture zones is a crucial component in monitoring and mitigation of adverse effects caused by accumulation of high biomass of algal cells and/or associated toxins. The high diversity among HAB species, their harmful impact and spatio-temporal distributional patterns necessitates regionally adopted observational approaches that cover a broad spectrum of temporal and spatial scales. General objectives of this thesis were firstly, to outline how bio-optical techniques support the coverage of adequate spatio-temporal dimensions for HAB surveillance, in particular in the aquaculture zones of the Ebro Delta embayments of Alfacs and Fangar, and secondly, to gain insights into distribution, adaptive strategies and habitat preferences of harmful taxa that would eventually lead to prediction of algal growth and proliferation patterns. In addition to data generated continuously from a hyperspectral radiometric sensor system, extensive field samples on inherent optical properties, environmental variables, phytoplankton taxa and associated phycotoxins completed the picture on bio-optical characteristics and phytoplankton dynamics in the area during seasonal study periods over two years.

The findings of this study established a sound basis for the operational enhancement of spatio-temporal scales for the surveillance of selected HAB taxa by means of a bio-optical sensor system. In particular, the installation of an environmental observatory in the aquaculture area, and the capability of a radiometric sensor system as key component are highly motivated by study results. One crucial factor is the high variability of phytoplankton, and in particular of HAB dynamics during the study period, which profoundly changed within weeks and formed small sized patches in the water column. In the temporal aspect, phytoplankton dynamics thereby clearly exceeded the limits of conventional weekly sampling, whereas the temporal resolution of bio-optical measurements was sufficiently resolved to detect and locate changes in proliferation patterns, such as the initiation of algal blooms.

The second convincing result of this study is the establishment of regionally adapted techniques that allow a rapid automated processing of long-term remote sensing data-sets. These include the correction or elimination of remote sensing spectra which were compromised by boat traffic and specular sun reflection, and the subsequent retrieval of the algal biomass proxy chlorophyll $a$ (Chl $a$) by a parameterised algorithm. In the Ebro Delta, these techniques permit the operational detection of algal biomass anomalies of a defined group of HAB taxa. Their applicability was demonstrated for the study period, but may require adjustment due to expected seasonal changes in bio-optical characteristics. Enhancement of areal scales was not effectively achieved by the single bio-optical test unit, but would be solved by additional components of an environmental observatory at key locations of the embayments.
ABSTRACT

In the Ebro Delta embayments the biomass proxy Chl $a$ is applicable to detect harmful blooms of several taxa that often dominate algal biomass at critical cell abundances, such as that of the ichthyotoxic dinoflagellate *Karlodinium*. Yet it was clearly shown that for the interpretation of bio-optical data, detailed knowledge on bloom characteristics is crucial. For instance, based on findings of this study Chl $a$ is not likely to be a regional indicator for toxic blooms of the diatom *Pseudo-nitzschia* spp., as high abundances of this genus were not coinciding with the presence of the associated toxin domoic acid, and were therefore not classified as HAB. Hence the general limitation of bio-optical approaches to determine whether or not an observed bloom is harmful, or to delineate taxa, implies the use of complementary methods. One exception outlined in this study is the case for high abundances of *K. veneficum* and *K. armiger*, as their rare pigment gyroxanthin-diester is likely to be a regional indicator for this genus as outlined in this study.

Bloom dynamics observed during the study period were typical for the season. Notable is the first observed development of high *Karlodinium* spp. abundances in Fangar Bay, which could be identified as *K. veneficum* in this study. Furthermore, a potentially new toxigenic species of the genus *Azadinium* was identified by means of light microscopy. Comparative findings of this thesis work indicate that *Karlodinium* spp. and *Dinophysis* spp. constitute alternative species types with different preferable habitats during the study period in spring/summer. Such understanding of bloom dynamics may be transferable to other embayments in a comparative approach and eventually lead to the delineation of habitat preferences of harmful taxa.

Integrated findings of this thesis strongly stress the significance of synoptic bio-optical and conventional measures for efficient surveillance of HABs and environmental triggers. By the effective coverage of bloom dynamics, combined with insights on environmental scenarios that promote the proliferation of certain taxa, public and private responses can be optimised. By following such regional approaches, mitigation actions may be anticipated by preventive measures for certain HAB taxa in the future.
ZUSAMMENFASSUNG


ZUSAMMENFASSUNG


Die integrierte Betrachtung dieser Arbeit unterstreicht die Notwendigkeit des komplementären Einsatzes von bio-optischen und konventionellen Methoden für die
RESUMEN

La detección de floraciones de algas nocivas (FAN) en zonas de acuiculturas es uno de los objetivos primordiales en el contexto del seguimiento y mitigación de los efectos desfavorables provocados por una gran acumulación de biomasa debido al incremento células y/o toxinas asociadas. La gran diversidad de especies FAN, sus efectos desfavorables, y su distribución sobre múltiples escala espacio-temporales exige la aplicación de métodos adecuados de observación, que cubran todas estas escalas.

Los objetivos generales de esta tesis fueron en primer lugar, describir cómo las técnicas bio-ópticas permiten la adecuada cobertura de las escalas espacio-temporales requeridas para el seguimiento de FAN; en particular en las actividades de acuicultura de las zonas de las bahías Alfacs y Fangar, en el delta del Ebro. En segundo lugar, obtener conocimientos sobre la distribución, estrategias de adaptación y las preferencias de hábitat de los taxones nocivos que eventualmente llevarían a la predicción del crecimiento de las algas y los patrones de proliferación. Los datos generados por sistemas de sensores radiométricos hiperespectrales se complementaron con los datos generados por muestreos convencionales, tales como extensivas observaciones de campo de propiedades ópticas inherentes, variables ambientales, así como muestras de taxones de fitoplancton y las ficotoxinas asociadas. Los datos complementarios permitieron obtener una visión más completa de la dinámica del fitopláncton y las propiedades bio-ópticas del área a lo largo del periodo de dos años de estudio.

Los resultados de este estudio establecen una base sólida para la mejora operativa del seguimiento de taxones de FAN, detectables por medio de sensores bio-ópticos, a las escalas espacio-temporales requeridas. En particular, el establecimiento de un observatorio medio ambiental en el área de acuicultura que incorpore un sistema de sensores radiométricos como componente clave están altamente motivados por los resultados del presente estudio. Un factor crucial es la alta variabilidad de fitoplancton, particularmente en la dinámica de FAN observada durante el periodo de estudio, detectándose cambios importantes en la comunidad en cuestión de semanas así como la formación de pequeños parches en la columna de agua. En el aspecto temporal, la dinámica del fitoplancton excedió claramente los límites de muestreo semanal convencional, mientras que la resolución temporal de las medidas bio-ópticas fueron los suficientemente resolutivas para detectar y localizar cambios en los patrones de proliferación, tales como la iniciación de la proliferación de algas. El segundo resultado relevante de este estudio es el establecimiento de técnicas de adaptación regionales que permiten un procesamiento rápido y automático de largas series de observaciones ópticas remotas. Se ha propuesto un algoritmo parametrizado que permite la eliminación o corrección
de espectros afectados por el tráfico fluvial o reflexión especular del sol durante el proceso de estimación de la concentración de Chl $a$, como proxy de la biomasa algal.

En el delta del Ebro, estas técnicas permiten la detección funcional de anomalías de biomasa de algas de un grupo definido de taxones FAN. Su aplicabilidad fue demostrada durante el periodo de estudio, pero puede requerir de ajustes futuros debido a los esperables cambios estacionales de las características bio-ópticas. No se lograron mejoras de las escalas espaciales a través de una única unidad de observación bio-óptica, pero podría resolverse incluyendo observaciones adicionales en locaciones clave de la bahía.

En las bahías del delta Ebro la estimación de la Chl $a$, como proxy de biomasa, es un parámetro indicador para la detección de proliferaciones de varios taxones que a menudo dominan la biomasa algal en condiciones de abundancia celular críticas, como el caso del dinoflagelado ictiotóxico Karlodinium. Sin embargo, está claramente demostrado que es crucial un conocimiento detallado acerca de las características de proliferación para la interpretación de los datos bio-ópticos. Por ejemplo, sobre la base de los resultados de este estudio, no es probable que la concentración de Chl $a$ se sea un indicador regional para proliferaciones tóxicas de la diatomea Pseudo-nitzschia spp., ya que los altos niveles de abundancia de este género no coinciden con la presencia de la toxina asociada, ácido domoico y por eso no se clasificaron como FAN.

Por lo tanto, la limitación general en los enfoques bio-ópticos para determinar si una proliferación observada es perjudicial, así como para discriminar taxones, implica el uso de métodos complementarios. Una excepción indicada en el presente estudio es el caso de gran abundancia de K. veneficum y K. armiger, ya que su rara pigmentación, diester-giroxantina, sea probablemente un indicador regional para este género como se ha indicado en este estudio.

Las dinámicas de proliferación observadas durante este estudio fueron las típicas asociadas a esa estación. Es de destacar la primera observación de un incremento de abundancia de Karlodinium spp. en la bahía Fangar, el cual se identificó como K. veneficum en este estudio. Por otra parte, una nueva especie toxigénica potencial del género Azadinium fue identificada a través de microscopía óptica. Resultados comparativos de este trabajo de tesis indican que Karlodinium spp. y Dinophysis spp. constituyen tipos de especies alternativos con diferentes preferencias de hábitats durante el periodo de estudio primavera-verano. Esta comprensión de dinámica de proliferación puede ser transferida a otras bahías en un enfoque comparativo y eventualmente conducir a la demarcación de las preferencias de hábitats de taxones nocivos.
Resultados integrados de esta tesis destacan encarecidamente la importancia de muestreos bio-ópticos sinópticos con medias convencionales para un seguimiento efectivo de FAN y sus factores ambientales desencadenantes. Mediante una cobertura efectiva de las dinámicas de proliferación, en combinación con las perspectivas sobre escenarios medio ambientales que puedan promover la proliferación de ciertos taxones, se pueden optimizar las respuestas de los sectores públicos y privados. Utilizando estas aproximaciones aplicables a escala local, se pueden anticipar acciones de mitigación a través de medidas preventivas para ciertos taxones de FAN en el futuro.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Abbreviations, acronyms &amp; mathematical notations</td>
<td>xii</td>
</tr>
<tr>
<td>ii</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>iii</td>
<td>Conceptual framework</td>
<td>11</td>
</tr>
<tr>
<td>iv</td>
<td>Scope of the thesis</td>
<td>16</td>
</tr>
<tr>
<td>v</td>
<td>List of original articles and author’s contribution</td>
<td>21</td>
</tr>
<tr>
<td>vi</td>
<td>Introduction to the research fields</td>
<td>24</td>
</tr>
</tbody>
</table>

**THEME CHAPTER I Advantages and limits of bio-optical tools for HAB surveillance** | 27 |

**Publication I: Detecting marine hazardous substances and organisms: sensors for pollutants, toxins, and pathogens** | 28 |
| 1.1 | Introduction | 29 |
| 1.2 | Marine health hazards | 31 |
| 1.3 | Detection of health hazards: Status and developments | 33 |
| 1.3.1 | Status of sensor techniques with decreasing spatial coverage | 34 |
| 1.3.1.1 | Detection on a large scale: remote sensing | 34 |
| 1.3.1.2 | Detection on intermediate scales: *in situ* platforms | 36 |
| 1.3.1.3 | Detection on a small scale: *in situ* – point measurement | 40 |
| 1.3.2 | Coverage and gaps | 42 |
| 1.4 | Future demands and emerging technologies | 43 |
| 1.5 | Conclusions and Outlook | 48 |

**Publication II: Optical assessment of Harmful Algal Blooms** | 49 |
| 2.1 | Addressing the diversity of harmful algal blooms | 50 |
| 2.2 | Algal features for bio-optical assessment | 55 |
| 2.3 | Scale and resolution in surveillance of algal blooms | 60 |
| 2.3.1 | Remote sensing | 60 |
| 2.3.2 | *In situ* ocean sensing | 68 |
| 2.4 | Emerging advancement in bio-optical sensor technologies | 72 |
| 2.5 | Transfer to operational oceanography | 74 |

**THEME CHAPTER II The large scale: Bio-optical assessment of phytoplankton in the Ebro Delta** | 78 |

**Publication III: Correction of hyperspectral reflectance measurements for surface objects and direct sun reflection on surface waters** | 79 |
| 3.1 | Introduction | 80 |
| 3.2 | Methods and data | 83 |
| 3.2.1 | Sensor system set-up | 83 |
| 3.2.1.1 | Remote sensing reflectance from an above water fixed platform | 83 |
| 3.2.1.2 | Digital photos of sea state and sky conditions | 83 |
| 3.2.2 | Automated algorithm development | 84 |
| Publication IV: Bio-optical variability and phytoplankton biomass retrieval with hyperspectral reflectance measurements in the optically complex aquaculture zone of the Ebro Delta, NW Mediterranean | 97 |
| 4.1 Material and Methods | 103 |
| 4.1.1 Characteristics of the study sites | 103 |
| 4.1.2 Discrete determination of bio-optical and biogeochemical properties | 103 |
| Measurement, calculation and correction of the Remote Sensing Reflectance ($R_{rs}$) | 104 |
| Determination of backscattering properties | 106 |
| Quantitative determination of Chl $a$ and TSM | 107 |
| Determination of variability of absorption properties | 108 |
| 4.2 Results | 110 |
| 4.2.1 Variability of phytoplankton biomass, TSM and CDOM | 110 |
| 4.2.2 Contribution of optically active components to absorption and backscattering | 113 |
| 4.2.3 Variability of remote sensing spectra and their relation to IOPs | 117 |
| 4.2.4 Selection of algorithms for estimation of phytoplankton biomass | 119 |
| Ocean Chlorophyll (OC) algorithms | 119 |
| Three wavelength ratio in the red | 120 |
| Fluorescence line height | 120 |
| 4.2.5 Application of OC algorithm for estimation of phytoplankton biomass | 121 |
| 4.3 Results | 125 |
| 4.3.1 Variability of optically active substances | 125 |
| 4.3.2 Phytoplankton biomass retrieval by remote sensing | 125 |
| 4.3.3 Spatio-temporal scales for operational algal bloom surveillance by means of reflectance measurements | 128 |
| 4.4 Discussion | 129 |
| 4.4.1 Variability of optically active substances | 125 |
| 4.4.2 Phytoplankton biomass retrieval by remote sensing | 125 |
| 4.4.3 Spatio-temporal scales for operational algal bloom surveillance by means of reflectance measurements | 128 |
| 4.5 Conclusion | 129 |

| Publication V: Comparative studies on phytoplankton dynamics and bio-optics for HAB monitoring in the Ebro Delta, NW Mediterranean | 131 |
| 5.1 Introduction | 132 |
| 5.2 Field sites and sampling strategy | 133 |
| 5.3 Continuous observation of phytoplankton biomass | 133 |
| 5.4 Phytoplankton bloom dynamics | 135 |
TABLE OF CONTENTS

5.5 Conclusions .................................................................................................................. 137

Theme Chapter III The small scale: HAB diversity and patchiness in the Ebro Delta .......... 138

Publication VI: An integrated approach for the assessment of HAB dynamics in two NW Mediterranean bays ........................................................................................................................................... 139
6.1 Introduction .................................................................................................................... 140
6.2 Materials and methods .............................................................................................. 141
6.3 Results and discussion .............................................................................................. 141
6.4 Summary & conclusions .......................................................................................... 144

Publication VII: Vertical distribution of toxigenic algae and associated phycotoxins during freshwater runoff in two coastal embayments in the Ebro Delta (NW Mediterranean) .... 145
7.1 Introduction .................................................................................................................. 147
7.2 Material and methods ................................................................................................ 150
  7.2.1 Location and field sampling of the study sites .................................................... 150
  7.2.2 Determination of physical properties of the water ............................................. 150
  7.2.3 Inorganic nutrients ............................................................................................. 151
  7.2.4 Characterisation of the harmful phytoplankton community .............................. 151
  7.2.4.1 Enumeration and identification of HAB taxa/genera by inverted light microscopy .............................................................................................................. 151
  7.2.4.2 Molecular diversity and phylogeny .............................................................. 152
  7.2.5 RDA of phytoplankton abundances with respect to environmental data ......... 155
  7.2.6 Identification and quantification of phycotoxins ................................................ 156
7.3 Results .......................................................................................................................... 157
  7.3.1 Freshwater contribution and phytoplankton biomass distribution .................... 157
  7.3.2 Occurrence and proliferation patterns of HAB taxa and phycotoxins ............... 159
  7.3.2.1 Presence of harmful species ........................................................................ 159
  7.3.2.2 Distributional and proliferation patterns of harmful taxa .............................. 161
  7.3.2.3 Distribution of phycotoxins and assignment of toxins to taxa ...................... 167
7.4 Discussion and conclusions ........................................................................................ 168
  7.4.1 Contribution of freshwater to defining stratification in the two regimes .......... 168
  7.4.2 Dynamics of the usual suspects and phycotoxins under the two regimes ......... 169
  7.4.2.1 Dynamics of HAB taxa and phycotoxins under regime I ................................ 169
  7.4.2.2 Dynamics of HAB taxa and phycotoxins under regime II ............................. 171
  7.4.2.3 Dynamics of HAB taxa and toxins during both regimes ............................. 172
7.5 Final remarks on HAB dynamics and environmental scenarios .............................. 175

Synopsis and conclusions .................................................................................................. 178

Future perspectives ........................................................................................................ 184

Acknowledgments ........................................................................................................ 188

References ...................................................................................................................... 190

Annex .............................................................................................................................. 209
# Abbreviations, Acronyms & Mathematical Notations

## List of Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>Amnesic Shellfish Poisoning</td>
</tr>
<tr>
<td>AOP</td>
<td>Apparent Optical Property</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>AMG</td>
<td>Autonomous Microbial Genosensor</td>
</tr>
<tr>
<td>AUV</td>
<td>Autonomous Underwater Vehicle</td>
</tr>
<tr>
<td>AZP</td>
<td>Azaspiracid Shellfish Poisoning</td>
</tr>
<tr>
<td>AVHRR</td>
<td>Advanced Very High Resolution Radiometer</td>
</tr>
<tr>
<td>AVIRIS</td>
<td>Airborne Visible/Infrared Imaging Spectrometer</td>
</tr>
<tr>
<td>CDOM</td>
<td>Coloured Dissolved Organic Matter</td>
</tr>
<tr>
<td>CWA</td>
<td>US Clear Water Act</td>
</tr>
<tr>
<td>CFP</td>
<td>Ciguatera Fish Poisoning</td>
</tr>
<tr>
<td>CHEMTAX</td>
<td>Chemical Taxonomy</td>
</tr>
<tr>
<td>CZCS</td>
<td>Coastal-Zone Color Scanner</td>
</tr>
<tr>
<td>IFCB</td>
<td>Imaging FlowCytobot</td>
</tr>
<tr>
<td>DSP</td>
<td>Diarrhetic Shellfish Poisoning</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>ERTS-1</td>
<td>Earth Resources Technology Satellite</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ESP</td>
<td>Environmental Sample Processor</td>
</tr>
<tr>
<td>ENVISAT</td>
<td>Environmental Satellite</td>
</tr>
<tr>
<td>EnMAP</td>
<td>Environmental Mapping and Analysis Program</td>
</tr>
<tr>
<td>FLH</td>
<td>Fluorescence Line Height</td>
</tr>
<tr>
<td>FOV</td>
<td>Field Of View</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography coupled with Mass Spectrometry</td>
</tr>
<tr>
<td>GESAMP</td>
<td>Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection</td>
</tr>
<tr>
<td>GEOHAB</td>
<td>Global Ecology and Oceanography of Harmful Algal Blooms</td>
</tr>
<tr>
<td>GLI</td>
<td>Global Imager</td>
</tr>
<tr>
<td>GSM</td>
<td>Garver-Siegel-Maritorena, semi-analytical algorithm</td>
</tr>
<tr>
<td>GOOS</td>
<td>Global Ocean Observing System</td>
</tr>
<tr>
<td>HIS</td>
<td>Hyperspectral Imager</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HELCOM</td>
<td>Helsinki Commission (Baltic Marine Environment Protection Commission)</td>
</tr>
<tr>
<td>HAB</td>
<td>Harmful Algal Bloom</td>
</tr>
<tr>
<td>HAB-OFS</td>
<td>HAB Operational Forecast System</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer</td>
</tr>
<tr>
<td>LCA</td>
<td>Last Common Ancestor</td>
</tr>
<tr>
<td>LC-FD</td>
<td>Liquid Chromatography coupled with Fluorescence</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography coupled with Mass Spectrometry</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid Chromatography coupled with Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>LOKI</td>
<td>Lightframe On-sight Keyspecies Investigation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>IOP</td>
<td>Inherent Optical Property</td>
</tr>
<tr>
<td>LSU</td>
<td>28S Large Subunit rRNA</td>
</tr>
<tr>
<td>LWCC</td>
<td>Liquid Waveguide Capillary Cell</td>
</tr>
<tr>
<td>IOC-UNESCO</td>
<td>Intergovernmental Oceanographic Commission - United Nations Educational, Scientific and Cultural Organization</td>
</tr>
<tr>
<td>MEMS</td>
<td>Micro Electro Mechanical Systems</td>
</tr>
<tr>
<td>MSFD</td>
<td>EU Marine Strategy Framework Directive</td>
</tr>
<tr>
<td>MAA</td>
<td>Mycosporine-like Amino Acids</td>
</tr>
<tr>
<td>MERIS</td>
<td>Medium Resolution Imaging Spectrometer</td>
</tr>
<tr>
<td>MODIS</td>
<td>Moderate Resolution Imaging Spectroradiometer</td>
</tr>
<tr>
<td>NOAA</td>
<td>National Oceanic and Atmospheric Administration</td>
</tr>
<tr>
<td>NSP</td>
<td>Neurotoxic Shellfish Poisoning</td>
</tr>
<tr>
<td>OC</td>
<td>Ocean Chlorophyll (and related algorithms, e.g. OC4)</td>
</tr>
<tr>
<td>OCM</td>
<td>Ocean Colour Monitor</td>
</tr>
<tr>
<td>OCTS</td>
<td>Ocean Color Temperature Scanner</td>
</tr>
<tr>
<td>OLCI</td>
<td>Ocean Land Colour Instrument</td>
</tr>
<tr>
<td>OPD</td>
<td>Optical Plankton Discriminator (‘Brevebuster’)</td>
</tr>
<tr>
<td>OSPAR</td>
<td>Oslo Paris (Oslo and Paris conventions for the protection of the marine environment of the North-East Atlantic)</td>
</tr>
<tr>
<td>PSICAM</td>
<td>Point Source Integrating Cavity Absorption Meter</td>
</tr>
<tr>
<td>RDA</td>
<td>Redundancy Analysis</td>
</tr>
<tr>
<td>RGB</td>
<td>Red – Green – Blue</td>
</tr>
<tr>
<td>RMSE</td>
<td>Root Mean Square Error</td>
</tr>
<tr>
<td>ROV</td>
<td>Remotely Operated Vehicle</td>
</tr>
<tr>
<td>PSP</td>
<td>Paralytic Shellfish Poisoning</td>
</tr>
<tr>
<td>POP</td>
<td>Persistent Organic Pollutants</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal Ribonucleic Acid</td>
</tr>
<tr>
<td>SeaDAS</td>
<td>SeaWiFS Data Analysis System</td>
</tr>
<tr>
<td>SeaWiFS</td>
<td>Sea-viewing Wide Field-of-view Sensor</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface Enhanced Raman Spectroscopy</td>
</tr>
<tr>
<td>SIPPER</td>
<td>Shadowed Image Particle Profiling Evaluation Recorder</td>
</tr>
<tr>
<td>SRM</td>
<td>Selected Reaction Monitoring (SRM)</td>
</tr>
<tr>
<td>SST</td>
<td>Sea Surface Temperature</td>
</tr>
<tr>
<td>SWaP</td>
<td>Size, Weight and Power consumption</td>
</tr>
<tr>
<td>TACCS</td>
<td>Satlantic Tethered Attenuation Coefficient Chain Sensor</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Program</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>QAA</td>
<td>Quasi-Analytical Algorithm</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative Polymerase Chain Reaction</td>
</tr>
<tr>
<td>WFD</td>
<td>EU Water Framework Directive</td>
</tr>
</tbody>
</table>
# List of Frequently Used Mathematical Notations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Units</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>nm</td>
<td>Wavelength</td>
</tr>
<tr>
<td>$A$</td>
<td></td>
<td>Absorbance</td>
</tr>
<tr>
<td>$a$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient</td>
</tr>
<tr>
<td>$a_t$</td>
<td>m$^{-1}$</td>
<td>Total absorption coefficient</td>
</tr>
<tr>
<td>$a_p$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient of pigments</td>
</tr>
<tr>
<td>$a_p$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient of particles</td>
</tr>
<tr>
<td>$a_{NAP}$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient of non-algal particles</td>
</tr>
<tr>
<td>$a_{CDOM}$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient of CDOM</td>
</tr>
<tr>
<td>$a_w$</td>
<td>m$^{-1}$</td>
<td>Absorption coefficient of pure water</td>
</tr>
<tr>
<td>$l$</td>
<td>m</td>
<td>Optical path length</td>
</tr>
<tr>
<td>$\beta$</td>
<td>m$^{-1}$ sr$^{-1}$</td>
<td>Volume scattering function</td>
</tr>
<tr>
<td>$\beta_p$</td>
<td>m$^{-1}$ sr$^{-1}$</td>
<td>Volume scattering function of particles</td>
</tr>
<tr>
<td>$\beta_w$</td>
<td>m$^{-1}$ sr$^{-1}$</td>
<td>Volume scattering function of water</td>
</tr>
<tr>
<td>$b_b(\lambda)$</td>
<td>m$^{-1}$</td>
<td>Total backscattering coefficient</td>
</tr>
<tr>
<td>$b_p(\lambda)$</td>
<td>m$^{-1}$</td>
<td>Particulate backscattering coefficient</td>
</tr>
<tr>
<td>$b_{pw}$</td>
<td>m$^{-1}$</td>
<td>Backscattering coefficient of pure water</td>
</tr>
<tr>
<td>$E_s$</td>
<td>Wm$^{-2}$</td>
<td>Downwelling planar irradiance above surface</td>
</tr>
<tr>
<td>$E_d$</td>
<td>Wm$^{-2}$</td>
<td>Downwelling planar irradiance below surface</td>
</tr>
<tr>
<td>$L_w$</td>
<td>Wm$^{-2}$ sr$^{-1}$</td>
<td>Water leaving spectral radiance</td>
</tr>
<tr>
<td>$L_{sky}$</td>
<td>Wm$^{-2}$ sr$^{-1}$</td>
<td>Sky radiance</td>
</tr>
<tr>
<td>$L_{sfs}$</td>
<td>Wm$^{-2}$ sr$^{-1}$</td>
<td>Radiance above sea surface</td>
</tr>
<tr>
<td>$L_u$</td>
<td>Wm$^{-2}$ sr$^{-1}$</td>
<td>Upwelling radiance</td>
</tr>
<tr>
<td>$\phi$</td>
<td>°</td>
<td>Relative azimuth angle sun azimuth angle</td>
</tr>
<tr>
<td>$\rho$</td>
<td></td>
<td>Fresnel reflectance factor</td>
</tr>
<tr>
<td>$R$</td>
<td></td>
<td>Reflectance</td>
</tr>
<tr>
<td>$R$</td>
<td></td>
<td>Pixel corrected reflectance</td>
</tr>
<tr>
<td>$R_{rs}$</td>
<td>sr$^{-1}$</td>
<td>Remote sensing reflectance</td>
</tr>
<tr>
<td>Min$_{NIR}$</td>
<td></td>
<td>Ambient NIR level of reflectance</td>
</tr>
<tr>
<td>$D$</td>
<td></td>
<td>Oxygen absorption depth of reflectance</td>
</tr>
<tr>
<td>$K_d$</td>
<td>m$^{-1}$</td>
<td>Diffuse attenuation coefficient for downwelling irradiance</td>
</tr>
<tr>
<td>SD</td>
<td>m</td>
<td>Secchi disk depth</td>
</tr>
</tbody>
</table>
II INTRODUCTION

HARMFUL ALGAL BLOOMS: EFFECTS AND THEIR ASSESSMENT

When in 1963 Alfred Hitchcock released his famous horror film *The Birds*, in which unexplained violent bird attacks devastate Bodega Bay, California, he may have had a real event in mind. Only two years before, in August 1961, seabirds were observed to commit inexplicable kamikaze-like suicides in Monterey Bay, California. Now, 50 years later, the presence of toxic species of the diatom genus *Pseudo-nitzschia* in corresponding zooplankton gut samples was confirmed. This underlines an argument that Hitchcock’s scenario was inspired not only by Daphne du Maurier’s novelette on bird attacks in Cornwall, England, but also by witnessing the effects of a Harmful Algal Bloom (HAB). In this scenario, the production of the neurotoxin domoic acid (DA) by the bloom organism led to the disorientation of birds in the area (Bargu et al., 2012) (Fig. ii.1). A strong coherence of the toxigenic diatom *Pseudo-nitzschia* cell abundances with the abnormal behaviour and death of seabirds in the same geographical region was found in 1991, when DA and the culprit species were found in the gut content of the seabird’s prey (Fritz et al., 1992). This illustrative anecdote gives a first glance upon one of the manifold effects that harmful algae can pose to human health, the ecosystem and socio-economic interests.

Fig. ii.1. The local newspaper Santa Cruz Sentinel reported on the seabird invasion in Monterey Bay, California on the front page from August 18, 1961. The event possibly inspired Alfred Hitchcock to shoot his famous horror film *The Birds*, in which unexplained violent bird attacks devastate Bodega Bay, California (Source: Bargu et al., 2012, Supplementary Fig. 1). For original figure see Annex Fig. 1).

1 Use of figure from Bargu et al., 2012 granted by License Number: 3226670398399
Throughout the world, the formation of algal blooms represents a seasonal natural phenomenon in aquatic ecosystems with a great contribution to the food web and the global carbon cycle (Assmy & Smetacek, 2009). The differentiation of a HAB from such a typical seasonal bloom is simply through negative consequences or devastating effects from the human perspective. Therefore the term HAB is not of scientific, but rather of societal origin. Often the effects of HABs result from the formation and degradation of high biomass that leads to fish kills or deaths of other marine fauna through anoxia or to damage of the underlying vegetation by shading. A HAB is, however, not necessarily associated with such high biomass. Moderate algal cell abundances can also have devastating effects due to the production of a wide spectrum of potent phycotoxins (toxins of algal origin). The two primary transvectors of human illnesses caused by toxic algae are the exposure to toxins in water or aerosols, and the consumption of phycotoxin-contaminated fish and shellfish. Therefore the occurrence of HABs in aquaculture zones or commercial fishery areas can lead to tremendous economic losses due to temporal harvesting closures and associated costs for monitoring and medical treatments in cases of human poisoning (Hoagland et al., 2002). In addition, the consumer’s negative perception of possibly contaminated and precarious seafood reduces the market demand and value even when no phycotoxins are present. This consumer’s reaction seems reasonable when regarding the wide range of adverse effects that different phycotoxins can cause in humans, ranging from mild symptoms, such as nausea, fever, or eye irritation, to more severe cases associated with short term memory losses, paralysis, neurological disturbances and cardiorespiratory failure, depending on toxin type, dose and exposure time (for references on harmful effects see Wright, 1995, Codd et al., 2005, Campás et al., 2007, Campás et al., 2008).

More than 100 taxa among various taxonomic groups are listed in the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae (Moestrup et al., 2009 onwards). Among the eukaryotic lineages, dinoflagellates are most strongly represented in the marine environment. Additionally, the prokaryotic Cyanophyta, often referred to as blue-green algae, include many toxic species and are present in fresh-, brackish-, and marine waters (Carmichael, 2001, Codd et al., 2005, O’Neil et al., 2012). By trend, the number of HAB species is increasing, not only due to taxonomic diversification and revisions, but also through the discovery of novel toxic species, such as the description of the dinoflagellate *Azadinium spinosum* that was successfully attributed as the proximal source of azaspiracid toxins (Tillmann et al., 2009).
The large diversity in HAB species and their respective ecological niches also reflects a large set of spatio-temporal patterns that range from centimetres to kilometres and from days to several months (see Fig. ii.2). Especially in aquaculture areas, where the safety of food products and the perception of consumers to seafood quality are at stake, appropriate regional monitoring frameworks are crucial. Key monitoring components comprise the record on presence, magnitude, movement and taxon-composition of HABs (Stumpf et al., 2003). The comprehensive determination of HAB magnitude and extent over the required spatio-temporal scales is highly challenging, but of great importance to initiate adequate public and authority responses to prevent harm with respect to health issues, ecological damage and economic losses. This underlines a strong need for regional adaptive solutions for HAB assessment and monitoring, with the inclusion of real-time observational sensor systems.

Fig. ii.2. Spatio-temporal scales for algal blooms and other ocean processes and phenomena with relevance in biogeochemical cycling (figure from Dickey, 2001; reprinted with permission from TOS). Thin-layer algal blooms can also concentrate in vertical ranges of centimetres.
**INTRODUCTION**

**BIO-OPTICS FOR ALGAL BLOOM SURVEILLANCE**

The rationale for an adoption of bio-optical techniques in HAB surveillance is their synoptic coverage of large spatio-temporal dimensions, in a non-intrusive, near real-time and operational manner—i.e. *in situ* and independent of man-power. Measurement principles of such sensors for the assessment of phytoplankton are based on the optical characteristics of algal cells that absorb, scatter, fluoresce and sometimes also phosphoresce light, according to their cell size, pigment suites and other intrinsic cell components, such as gas vacuoles. Spectral intensity and shape of bio-optical measures such as remote sensing reflectance and absorption spectra are thus determined by algal presence and group composition.

Remote sensing operations have gained prominence for the detection of algal proliferations, bloom magnitude and movement, by retrieval of biomass anomalies over large areas in near real-time (Stumpf *et al.*, 2003, Hu *et al.*, 2005). Algal biomass is conveniently measured in concentrations of chlorophyll *a* (Chl *a*), that generally represents the major pigment in algae. As an exception, Prochlorophytes contain divinyl-Chl *a* instead. Especially remote sensing measurements of clear open ocean water are processed with standard Ocean Chlorophyll (OC) algorithms to derive algal biomass. The measurement principle that underlays OC algorithms is based on the ratio of an increased absorption due to increasing algal abundance in the blue spectral region that is set against maximum reflectance in the green region (O’Reilly *et al.*, 2000). In costal zones, however, the ocean colour signal is often strongly influenced by other optically active constituents in the water, such as suspended sediment or coloured dissolved organic matter (CDOM), transported by riverine or agricultural runoff or generated *in situ*. Satellite borne standard measurements and general open ocean algorithms therefore often fail in near-shore areas and regionally adjusted approaches need to be implemented. Yet, especially for aquaculture operations, such near-shore areas are of high importance, as many aquaculture and fishing activities are located in open coastal zones and embayments.

In this ‘missing kilometre’ along the coastline, vicarious investigations of local bio-optical characteristics, and the development of local algorithms are crucial for the retrieval of algal biomass. *In situ* remote sensing applications are of twofold profit for this task: 1) continuous *in situ* applications yield a high temporal resolution while space borne measurements depend on overflight frequencies that may exceed a week, and 2) measurements that cover a concise spot allow a comparison with spatially matching laboratory samples,
whereas applications from space require ground truthing that covers the averaged signal over pixel sizes of kilometres. Finally, radiometric *in situ* measurements are essential for calibrations of satellite borne data, e.g. for atmospheric corrections (Zibordi *et al.*, 2002, McClain, 2009).

In addition to radiometers, a large number of alternative bio-optical instruments, e.g. fluorometers, transmissometers, or scatterometers, are commercially available for operational deployment on variable platforms (Moore *et al.*, 2009). The rapid progress in technical development with respect to the bio-optical assessment of HABs on a large variety of platforms is also reflected in numerous publications (see volume of Babin *et al.*, 2008). The combination of such platforms covers the spatial dimension and temporal resolution that are necessary for the surveillance of HABs. Besides satellite and ground-based above-surface constructions, a variety of sub-surface fixed and mobile platforms, such as remotely or autonomously operated underwater vehicles (ROVs or AUVs) can be assorted for the retrieval of algal proliferations in vertical and horizontal patches (Fig. ii.3). Such environmental observatories can be implemented to determine the presence, magnitude and movement of algal blooms in near real-time, thereby covering a key component for HAB surveillance.

![Fig. ii.3. Hypothetical coastal observing network for HAB monitoring in fjords and coastal embayments with sensors on a variety of platforms (adapted from Cembella *et al.*, 2005). Highlighted instrumentations are surface fixed arrays of radiometers providing data to calculate the diffuse attenuation coefficient \(K_d(\lambda)\) (5); surface buoys with upwelling radiance and downwelling irradiance sensors to measure remote sensing reflectance \(R_{rs}(\lambda)\) (6); and a bottom-fixed array of fluorometers (4). Reprinted with permission from TOS.](image-url)
Recently, the spectral resolution of such measurements has increased with the introduction of hyperspectral sensors that allow a precise measurement of the water constituent characteristics in a fine-scaled spectral resolution; typically better than 2nm. Still, regarding taxon-specificity, such measurements show apparent limitations and the question if a detected bloom is harmful or not is not always resolvable with bio-optical methods. Consequently, taxon-composition needs to be addressed by additional methods, e.g. light microscopy or molecular diversity techniques for algal species, and the presence of toxins can only be confirmed by analysis of chemical composition. Yet, there are a few notable exceptions for the successful performance of bio-optical tools for species discrimination in a HAB context. The Optical Plankton Discriminator (OPD) targets unique absorption features of the toxic dinoflagellate *Karenia brevis*. These unique features arise due to the presence of the rare pigment gyroxanthin diester that is exclusive to this taxon in Florida coastal waters. Regionally, the OPD is therefore operating as species-specific bio-optical *in situ* system (Kirkpatrick *et al.*, 2000). Another prominent example for species delineation is the Imaging FlowCytoBot (IFCB), an imaging system that bases on the distinction of morphological characteristics of algal cells on images (Olson & Sosik, 2007b). The IFCB allowed the detection of the toxic dinoflagellate genus *Dinophysis* spp. from bloom emergence until decline by automated classification of images over several months in 2008 in the Gulf of Mexico (Campbell *et al.*, 2010).

In summary, bio-optical methods cover the large spatio-temporal dimensions that allow an appropriate surveillance of HABs, yet only a couple of instruments allow the retrieval of detailed information on the *harmfulness* of a bloom. Instruments with a high informational value or laboratory measures in turn show limitations in sampling frequency over space and time. With a set of complementary methods, however, regionally adapted instrumentation can close these gaps between HAB surveillance measures and thereby contribute to now-cast of bloom presence, size and movement as well as to forecasting models.
**HABs in Coastal Embayments**

Why is it advantageous to study HABs in coastal embayments? The answer to this question is within a key challenge for HAB research: to determine factors that control the population dynamics of diverse HAB taxa which occur in multi-faceted aquatic environments. This may be physical processes on meso- and sub-mesoscale such as advection by oceanic currents, on stratification and turbulence scales, or biological interactions, such as aggregation behaviour, grazing or allelopathic interactions on a cellular level (GEOHAB, 2013). Due to multifarious physical, chemical, and biological processes that determine phytoplankton dynamics, it is difficult to address this question with a laboratory approach. In natural systems, however, an experimental setup is hindered by influences from a vast amount of parameters that cannot be controlled. An alternative to determine the complex processes and mechanisms that underlay HAB formation is the comparison of algal dynamics in different ecosystems. The extent to which similar processes influence HAB formation in heterogenetic environments may be similar and a comparative approach may enable the quantification of effects on HAB formation. Likewise, the comparison of similar ecosystems with distinct HAB patterns is applicable and may reveal common habitat characteristics and processes that initiate blooms of certain species. Eventually, the determination and quantification of responses of HABs to environmental processes may lead to enhanced predictive capabilities (GEOHAB, 2001), which is a key component in HAB monitoring and management.

Embayments are well suited for comparison, as they form semi-enclosed research environments with mesocosm-like properties, due to the more or less confined system boundaries. While experiments based on mesocosms are often criticised to miss the significance of scale and to operate with a reduced biological and physical complexity, a critical setup and application of such systems may aid in understanding scaling relationships in nature (Petersen & Hastings, 2001). The same holds especially true for embayments as natural systems and consequently, defined boundaries and small spatial scales allow detailed studies with respect to HAB presence, population dynamics, and relations to triggers and forcing functions. Such systems are often characterised by an inflow of seawater from the surrounding ocean and freshwater from rivers, irrigation channels or ground-water. Additional hydrodynamic forcing functions are determined by bottom characteristics and shapes of the bays and weather properties, such as rain, wind and wave action. In this aspect, coastal embayments do not differ from other coastal systems. In isolated systems with limited material and water fluxes, however, effects of these hydrodynamic forcing functions are amplified. Freshwater inflow has a stronger impact on a small than on a large area, and enclosed systems have longer retention times of water as compared to open coastal systems. These longer
The embayments Alfacs and Fangar in the Ebro Delta system are the major aquaculture sites in Catalonia. Due to the presence of harmful phytoplankton taxa and phycotoxins, both bays are subject to frequent harvesting closures. Retention times of water may lead to a ‘batch culture’ effect and promote algal bloom development from a starter inoculum of a HAB species. This ‘seed population’ may have been present in the water in low cell concentrations, encysted in sediment or introduced by oceanic waters or anthropogenic sources such as aquaculture spat or ballast water from shipping industry, leading to increased persistence of blooms in enclosed systems. The point source introduction of freshwater, nutrients or both is creating vertical layers or distinct high nutrient zones and therefore promotes patchiness of phytoplankton proliferations that are in favour of the resulting micro-habitats (Cembella et al., 2005). Consequently, HAB surveillance in embayments needs to adequately address the patchiness of HABs in an appropriate spatio-temporal resolution.

**HABs in the Coastal Embayments of the Ebro Delta**

The two embayments Alfacs and Fangar in the Ebro Delta are located on the Catalan coast in the NW Mediterranean and are semi-enclosed from the sea by two sandbar arms (Fig. ii.4). The Delta provides important ecosystem goods and services to the region: the flat terrestrial basin is used for agricultural activities, predominantly for rice cultivation, and numerous water sheds are inhabited by a rich flora and fauna. The region was declared as a Natural Park in the 1980s and attracts tourists in summer, due to distinct nature characteristics, wide beaches and seafood varieties. The fisheries and aquaculture sector provides employment and income for approximately 2000 persons and contributes about 15% to the annual production of Catalonia (in EUCC, 2013). Alfacs and Fangar Bay are Catalonia’s major aquaculture sites, with an annual mussel production of about 3000 t (Ramón et al., 2005a). The primary aquaculture species is the Mediterranean mussel *Mytilus galloprovincialis*, which is grown in suspension culture on ropes that are attached to fixed wooden raft constructions (Ramón et al., 2005a) (Fig. ii.4).

Due to the presence of HAB taxa and phycotoxins, both bays are subject to regular shellfish harvesting closures. Major HAB events are usually related to paralytic- (PSP) and diarrhetic shellfish poisoning (DSP) toxins that accumulate in the suspension feeding mussels. Shellfish tissues are tested regularly for PSP toxicity with the mouse bioassay method (AOAC, 1995) for PSP toxicity and for DSP toxicity in lipophilic fractions by an alternative mouse bioassay (Yasumoto et al., 1978). Since 2001, also amnesic shellfish poisoning (ASP) was included to routine sampling efforts by HPLC-UV. Events that lead to closures are mainly attributed to the dinoflagellates *Dinophysis sacculus, D. caudata, Protoceratium reticulatum* (Table ii.1) and *Alexandrium minutum* (Diogene et al., 2008, Fernández-Tejedor et al., 2008).
Proliferations of these HAB taxa are considered critical at low abundances such as 500 cells L\(^{-1}\) in the case of *Dinophysis* spp. and at 1000 cells L\(^{-1}\) for *A. minutum* and *A. catenella*.

Likewise, HAB taxa that usually occur in high abundances are present in both embayments. The ichthyotoxic dinoflagellate genus *Karodinium* forms high biomass blooms which could be attributed to fish kills in Alfacs Bay (Fernández-Tejedor *et al.*, 2008). Two taxa, *Karodinium veneficum* and *K. armiger* (in this region previously referred to as *Gyrodinium corsicum* (Garcés *et al.*, 2006)) were responsible for these events and both have been regularly observed in Alfacs Bay since 1994 (Delgado *et al.*, 1995). Furthermore, the diatom *Pseudo-nitzschia* forms high abundance blooms in the embayments, which were frequently exceeding critical cell numbers of 200,000 cells L\(^{-1}\) (Fernández-Tejedor 2008). Up to now, DA was not detected in phytoplankton samples and could not be related to a certain *Pseudo-nitzschia* sp., but recently, investigations of shellfish samples from production sites along the Catalan coast from the years 2008 – 2011 revealed the presence of DA in harvested mollusc samples (Giménez Papiol *et al.*, 2012). Based on a long term dataset over 30 years in this region, an increase of *Pseudo-nitzschia* abundances has been observed over time (Fernández-Tejedor *et al.*, 2010). This poses a possible future threat to food safety in the aquaculture area, and stresses the importance to identify the culprit for DA production.

**Fig. ii.4.** a) Satellite image\(^2\) of the Ebro Delta area with river mouth, rice field cultivation in the flat data basin, and the two semi-enclosed embayments Alfacs (southern) and Fangar Bay (northern); b) rice field with seedlings in the Ebro Delta area. During spring time, rice fields are submerged by river water; c) the Mediterranean mussel *Mytilus galloprovincialis* is grown in suspension culture on ropes; d) ropes for mussel cultivation are fixed on anchored wooden constructions.

Table ii.1. Harvesting closures for aquaculture in Alfacs and Fangar Bay between 2003 and the first quarter of 2007 with start- and end days, duration and assigned species. Cases where no potential species was detected in sufficient numbers are indicated as ND (not detected). All events were attributed to DSP toxicity (Modified from Fernández-Tejedor et al., 2008).

<table>
<thead>
<tr>
<th>Year</th>
<th>Start- → end date</th>
<th>Duration of closure [days]</th>
<th>Assigned taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfacs Bay</td>
<td>2003</td>
<td>02-05-03 → 04-06-03</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>31-12-04 → 27-01-05</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>09-02-05 → 03-03-05</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14-07-05 → 22-08-05</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-12-05 → 23-12-05</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>27-04-06 → 04-05-06</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-07-06 → 01-08-06</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>09-11-06 → 18-12-06</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>21-12-06 → 29-01-07</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-01-07 → 21-03-07</td>
<td>49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Start- → end date</th>
<th>Duration of closure [days]</th>
<th>Assigned taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fangar Bay</td>
<td>2003</td>
<td>06-08-03 → 14-08-03</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>06-10-03 → 10-10-03</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>13-08-04 → 23-08-04</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>02-09-04 → 13-09-04</td>
<td>11</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>11-11-04 → 22-11-04</td>
<td>11</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>21-07-06 → 28-07-06</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>05-01-07 → 15-01-07</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>16-02-07 → 05-03-07</td>
<td>17</td>
<td>P. reticulatum</td>
</tr>
<tr>
<td></td>
<td>06-03-07 → 21-03-07</td>
<td>13</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>29-03-07 → 12-04-07</td>
<td>2</td>
<td>ND</td>
</tr>
</tbody>
</table>

With average depths of 2 m in Fangar Bay and 4 m in Alfacs Bay (Camp & Delgado, 1987), the bays allow a detailed assessment of HAB patchiness in thin layers through the entire water column. The horizontal and vertical dimensions of the bays allow addressing HAB patchiness at adequate spatio-temporal scales, provided that operational monitoring systems are mounted at strategic locations. As yet, there is no such system installed to support weekly bloom assessment in the bays. Spatio-temporally enhanced assessment of bloom dynamics would, however, provide valuable insights into bloom dynamics and support monitoring and management efforts by the assessment of bloom presence, magnitude and movement in a complementary way. Furthermore, the comparative study of HABs in the two coastal embayments Alfacs and Fangar provides the opportunity to address bloom dynamics with enhanced spatio-temporal dimensions, from fine-scale vertical distributions of algal taxa to large scale observations over time.
The conceptual framework of this thesis is clustered into dynamics of phytoplankton and bloom development in time and space, and the coverage of these spatio-temporal dimensions by operational bio-optical tools in near real-time. This allows for an adequate and regionally adapted surveillance of HABs (for schematic view of conceptual framework see Fig. iii.1).

Phytoplankton and HAB dynamics are influenced by biotic and abiotic forcing functions that determine the presence of HAB taxa and toxin production in an area. The distribution of...
phytoplankton taxa within the water column is commonly interpreted as a result of the species’ survival strategies and habitat preferences based upon biotic and abiotic forcing functions. These strategies cross taxonomic boundaries, and especially dinoflagellates show diverse bloom patterns from oligotrophic waters in the Gulf of Mexico (Stumpf et al., 2003) to turbulent upwelling systems (Trainer et al., 2010). Bloom forming marine dinoflagellates could, however, be assigned to nine type species based on three adaptive strategies – C (good competitors), S (stress-tolerant), and R (disturbance-tolerant), as identified originally for freshwater species (Reynolds, 1987), and the three habitat variables mixing, irradiance, and accessibility of nutrients (Smayda & Reynolds, 2001) (Fig. iii.2). The assignment of phytoplankton groups within a matrix of nutrient availability and mixing (Margalef, 1978, Margalef et al., 1979), well known as Margalef’s Mandala\(^3\) is included in the figure. This grouping based on adaptive strategies and habitat preferences is a step towards a functional grouping of phytoplankton that can simplify models of HAB proliferation. Besides physical (mixing, shear stress, irradiation) and chemical (nutrients) forcing, biological interactions (grazing, species communication, allelopathic interactions, behaviour) may lead to a favoured proliferation of one algal species over another.

In open natural systems, horizontal displacement of algal cells due to alongshore advection can be unfavourable for proliferations of phytoplankton. In case of dinoflagellates, the current velocities are often stronger than their motile capacity, and the cells are not able to maintain in their respective life-form habitat (Smayda, 2002). Coastal systems with confined- or semi-confined boundaries, however, are generally characterised by lower current velocities and the transport of algal cells out of the system is restricted. Such systems with increased retention times of water can act as ‘bloom incubators’ and thus provide favourable conditions for the study of biological, chemical and physical influences on algal proliferations. Furthermore, environmental effects, such as freshwater inflow or nutrient introduction have stronger effects on a small confined water body with respect to the open ocean. A comparative approach among embayments enables the identification of key species or functional groups of species, and this may eventually allow discrimination of habitat preferences and an improvement in understanding key elements that drive bloom dynamics in time and space. Such detailed information is crucial for the inclusion into models that allow forecasting of HAB development.

\(^3\) Margalef’s Mandala (Margalef 1978) allocates diatoms to nutrient rich mixed waters, and dinoflagellates to stratified and oligotrophic conditions, thereby explaining the typical yearly bloom cycles in temperate regions. Persistent accumulations of autotrophic dinoflagellates were assigned to nutrient-rich, low-turbulence conditions by a red tide trajectory in this concept (Margalef et al., 1979). Nevertheless, there are numerous exceptions, such as the occurrence of the dinoflagellate *Karenia brevis* in the oligotrophic seas in the Gulf of Mexico (Stumpf et al., 2003), to a number of toxic dinoflagellate species in upwelling systems (Trainer et al., 2010).
III CONCEPTUAL FRAMEWORK

Fig. iii.2. Schematic view of nine species types or functional groups of bloom forming dinoflagellates (in roman numerals) and the different pelagic marine habitats in which they bloom (black boxes) along an onshore-offshore gradient separating deep-mixed and well-stratified, but nutrient-deficient systems. I* refers to irradiance level received by cells within water column; H_m represents depth of mixed-layer. Overlap of types within the habitat template schema does not always imply their contiguity. The diagonal approximates the main successional sequence depicted in Margalef et al. (1979). Dinoflagellate life form types associated with the turbulence-nutrient matrix are Type I = gymnodinioids; Type II = peridinioids and prorocentroids; Type III = ceratians; Type IV = frontal zone species; Type V = upwelling relaxation taxa; Type VI = coastal current entrained taxa; Type VII = dinophysoids; Type VIII = tropical oceanic flora; Type IV = tropical shade flora (modified from Smayda & Reynolds, 2001. For original figures see Annex Fig. 2).

Owing to the large diversity of algal species and environmental conditions that allow bloom development, spatio-temporal dimensions of a bloom are highly variable and distributions are patchy. Blooms can extend over kilometres in surface waters, or they can align within thin layers within the water column, and persist from days to months. Surveillance programmes for HABs are determined by these regional spatio-temporal bloom patterns and organised on three basic levels (see Fig. iii.1): 1) detection of algal bloom presence, dimension and movement; 2) assessment of the composition of the algal assemblage and presence of HAB taxa; and 3) determination of the presence of phycotoxins.

The proxy for the first level is Chl a, as algal biomass is conveniently measured in concentrations of this ubiquitous pigment. Furthermore, major groups of HAB species are typically primarily phototrophic and therefore contain this pigment for photosynthesis.

---

4 Use of figures from Smayda et al., 2001 granted by License Number: 3226670398399
Whereas satellite-borne bio-optical sensor systems cover vast spatial areas, such systems provide only a moderate spatial resolution due to large pixel sizes, and often struggle with the ‘missing kilometre’ along the shoreline. Here, in situ radiometric measurements are highly valuable for calibration of measurements from satellites, as well as for independent functions at a high temporal and resolution. Radiometric measurements are commonly embraced as ocean colour measurements, by capturing the light that leaves the water after interactions with the water components, the water leaving radiance $L_w$. Typical ocean colour variables are $L_w$ or remote sensing reflectance $R_{rs}$, which is defined as the ratio of $L_w$ to the solar plane irradiance $E_s$ within the visible spectral range (VIS; 300 to 700 nm) (Mobley, 1999). Information on the water constituents of different water types is derived in the visible spectral range (VIS) via the magnitude and spectral form of the signal over the wavelengths ($\lambda$) (Fig. iii.3).

Radiometric measurements are assigned to apparent optical properties (AOP), as they are not solely dependent on the water constituents, but also on the distribution of the ambient light field influenced by the solar angle, sky and sea state. Direct interactions of water components that are only a function of the constituents and wavelengths are referred to as inherent optical properties (IOPs). Fundamental IOPs are absorption ($a$) and the volume scattering function ($\beta$). With respect to reflectance, absorption and backscattering are antagonistic processes, as
absorbed downward traveling photons cannot be scattered backwards. Remote sensing reflectance is in relation to IOPs via the particulate backscattering coefficient $b_b$ derived from $\beta$ and the total absorption $a_t$, and a dimensionless proportionality factor $f/Q$, which accounts e.g., for properties of the radiance field (compare Tzortziou et al., 2007).

$$R_{rs}(\lambda) \sim \frac{f/Q \cdot b_b(\lambda)}{(a_t(\lambda) + b_b(\lambda))} \quad (\text{iii.1})$$

The algal biomass proxy Chl $a$ is derived by remote sensing reflectance measurements with standard empirical algorithms, such as the OC algorithms. Most of these are based on the ratio of increased Chl $a$ absorption in the blue and high reflectance in the green spectral region caused by high phytoplankton abundances (where most absorbing components have a minimum, but indirectly lead to a high reflectance due to the high backscatter) (see O’Reilly et al., 1998, O’Reilly et al., 2000).

IOPs are additive, and groups of optically active substances have typical spectral signatures that can be split into partial components. Total absorption is therefore the sum of phytoplankton absorption ($a_\phi$), absorption of non-algal particles ($a_{\text{NAP}}$), absorption of CDOM ($a_{\text{CDOM}}$) and pure water itself ($a_w$). Typical shapes of the absorption spectra for phytoplankton particles include a maximum peak at 433 nm and a local maximum at 674 nm due to strong Chl $a$ absorption, whereas CDOM and total suspended matter (TSM) are strong absorbers at short wavelengths (around 400 nm), decreasing towards longer wavelengths. Especially in coastal waters with high sediment input and CDOM concentrations, phytoplankton absorption is masked by contributions of both in the blue spectral range. To tackle the challenge of distinguishing relevant signals the variability of all optically active substances needs to be accessed in a regional approach.

The composition of an algal assemblage is determined by assessing the morphology of algal cells, genetic diversity, and, for certain algal groupings, by measuring a characteristic suite of accessory pigments for light harvesting or protection besides Chl $a$. The bio-optical determination of an algal group composition is predominantly based on characteristic pigment compositions, and thus the spectral shape of the phytoplankton absorption spectrum, but can also include other parameters such as a changed refraction index due to gas vacuoles in certain cyanobacteria (Westberry et al., 2005) or a predominantly red colour in reflectance spectra for certain dinoflagellate blooms (Ahn & Shanmugam, 2006).

To answer the questions of whether or not a detected bloom is harmful or not or if phycotoxins are present is beyond the capability of bio-optical systems and molecular diversity of taxa and the identification of chemical components need to be addressed by complementary methodologies.
IV Scope of the Thesis

This doctoral thesis was prompted by the need to nowcast and to predict HABs in aquaculture zones, with the purpose to warn, mitigate, and prevent adverse effect that arise due to the production of high biomasses and/or toxins of harmful taxa. The surveillance of HABs is impaired by the large diversity among algal taxa, their harmful effects, spatio-temporal distributional patterns and assemblage behaviour that demand for locally adjusted surveillance measures.

The focus of this research is primarily on a contribution to HAB surveillance on all relevant monitoring levels as outlined by Stumpf et al. (2003), with a regional emphasis on the aquaculture zone in the Ebro Delta embayments, NW Mediterranean. This implies the coverage of appropriate spatio-temporal scales for now-casting of blooms on an operational level, and knowledge on adaptive strategies and habitat preferences of HAB taxa as a first step to predictive models for algal growth and accumulation patterns. The overall aim is to apply adequate methods and to bridge the gap between a large spatio-temporal coverage and appropriate taxonomic resolution in HAB surveillance. In particular, the integration of automated bio-optical measurements into HAB assessment on all relevant monitoring levels is focal area of this work.

Under the common theme of ‘HAB surveillance’, the following general objectives are addressed:

- Review advantages and limits of bio-optical techniques to address the diversity of HABs
- Evaluate and test the potential for a spatio-temporally enhanced automated retrieval of algal blooms by means of radiometric hyperspectral measurements
- Address diversity and patchiness of HAB taxa and phycotoxins in a comparative approach in the two coastal embayments of the Ebro Delta
THEME CHAPTER I: ADVANTAGES AND LIMITS OF BIO-OPTICAL TOOLS FOR HAB SURVEILLANCE

The diverse distributional patterns of HAB taxa imply a demand for the coverage of adequate spatio temporal dimensions. These may vary regionally, but always require the coverage of vertical, horizontal and temporal scales that allow the detection of algal patches and the unambiguous specification of HABs and/or their toxins. Bio-optical tools are highly valuable to enhance the surveillance-range of over space and time, as traditional methods with subsequent handling in the laboratory are in most cases restricted to point sampling and, in addition, do not deliver critical information in real time. In turn, only few operational bio-optical measures reach the required species specificity. To comply with demands for HAB surveillance, advantages and limitations of bio-optical applications need to be weighed to allow an adequate adoption of such tools for HAB monitoring, in particular with respect to regional settings. General objectives approached in Theme Chapter I are the determination of advantages and limits of bio-optical state-of-the-art technology for the surveillance of HABs and phycotoxins. Focus is set on the operational - i.e. in situ and independent of man-power – long-term deployment of sensors and sensor systems from satellite-based platforms, and from in situ above- or underwater devices. In detail, the following specific objectives are addressed in two comprehensive reviews:

- Determine general advantages and limits of operational bio-optical measurements for HAB surveillance
- Outline optically distinguishable features and indicators for HABs
- Review existing sensors and sensor systems for continuous operational HAB assessment and their suitability for HAB species detection
- Assess future perspectives for research on HAB monitoring with optical sensors
THEME CHAPTER II: THE LARGE SCALE. BIO-OPTICAL ASSESSMENT OF PHYTOPLANKTON IN THE EBSO DELTA

Within Theme Chapter II, the adoption of a radiometric hyperspectral sensor system for HAB surveillance in the Ebro Delta aquaculture zone is in focus. The essential objective addressed here is to follow algal biomass dynamics over enhanced spatio-temporal dimensions. Anomalies in the observed patterns may indicate potentially harmful proliferations. With respect to the regional small scale conditions - the Ebro Delta embayments are highly vibrant and phytoplankton dynamics may vary below sub-meso-scales (< 1 km) – *in situ* measures are well suited as they enable data acquisition at high temporal frequency, are independent of cloud cover and can be integrated in an operational setup that covers large spatial dimensions. In addition, radiometric *in situ* data are important for the validation of satellite sensor signals in coastal zones, especially in a system with such apparent changes over a year’s cycle, e.g., due to agricultural freshwater influences, such as found in the Ebro Delta bays. Continuous long-term applications, such as the deployed *in situ* radiometric system, deliver large data-sets. Therefore, automated data processing needs to be assured and tailored to regional conditions. Furthermore, the regional parameterisation of algorithms for algal biomass calculations is, especially in near-shore areas, crucial to comply with the introduction of terrestrial material. Accordingly, the assessment of interferences of the phytoplankton biomass signal with additional optically active components in turbid coastal zones is highly beneficial for bio-optical measurements. Consequently, specific objectives addressed in Theme Chapter II are to:

- **Develop a quality procedure that allows the elimination of external sources of disturbances from remote sensing spectra in an automated way**

- **Bio-optically characterise the Ebro Delta embayments and assess sources of variability for optically active water constituents**

- **Select appropriate spectral regions for algal biomass retrieval within remote sensing reflectance spectra, based on seasonal local conditions**

- **Generate a regionally parameterised local algorithm for Chl a retrieval by means of *in situ* hyperspectral reflectance measurements as an indicator for phytoplankton biomass that allows an automated surveillance of algal bloom development**
THREE CHAPTER III: ASSESSMENT ON A SMALL SCALE: HAB DIVERSITY AND PATCHINESS IN THE EBBRO DELTA

Theme Chapter III is dedicated to taxonomic diversity and small-scale patchiness of HAB taxa and phycotoxins in the two coastal embayments of the Ebro Delta, addressed in a comparative approach. Focus is set on the identification of HAB taxa, their proliferation patterns and habitat preferences. The clear assignment and quantification of harmful taxa is mandatory for HAB surveillance and management responses, yet beyond the capacity of most bio-optical sensors and sensor systems. Therefore, alternative approaches to comply with demands of HAB assessment are integrated. This includes traditional measures, such as microscopic identification and enumeration of taxa, but also their molecular diversity. These measures need to address regionally known culprit HAB species, as well as yet undetected potentially harmful taxa. Furthermore, the determination of factors which regulate population dynamics and lead to proliferations of harmful taxa is necessary to enable forecasting activities. Eventually, knowledge on triggering forcing functions that determine the growth of certain HAB taxa would lead to predictive measures. In the case of the Ebro Delta embayments, it remains an open question, whether or not the seasonal freshwater introduction from agricultural activities in the delta basin, and resulting stratification of the water column, forms microhabitats for certain HAB taxa in the Ebro Delta embayments. Accordingly, specific objectives addressed in this part of the thesis are to:

- Identify and quantify HAB taxa and phycotoxins, and assign toxins to taxa, with emphasis on taxa known to produce toxins in the area, as well as potentially new species
- Determine spatio-temporal patchiness of HAB taxa and phycotoxins by comparing abundance patterns in two geographically close coastal embayments
- Assess the distribution of HAB taxa and phycotoxins within thin- and mixed-layers, in an effort to determine the extent to which freshwater inflow contributes to stratification of the water mass and creates an ecological niche for HAB species

The thesis closes with a short synopsis and conclusions, final remarks and future perspectives on the inclusion of bio-optics in HAB surveillance, with emphasis on the Ebro Delta aquaculture region.
The publications that form the basis of this doctoral thesis treat the above given objectives in a multidisciplinary approach. The publications (I-VII) are allocated to the following research fields:

**HAB Surveillance**
I, II, IV, V, VI, VII

**Operational Oceanography**
I, II, IV, V, VI

**Ocean Optics**
I, II, III, IV

**Phytoplankton Dynamics**
V, VI, VII

**Aquaculture**
II, V, VII
V LIST OF ORIGINAL ARTICLES AND AUTHOR’S CONTRIBUTION

The cumulative thesis is based upon the following publications:

**THEME CHAPTER I:**

**OVERVIEW ON THE ASSESSMENT OF HABs WITH OPTICS**


**THEME CHAPTER II:**

**THE LARGE SCALE: BIO-OPTICAL ASSESSMENT OF PHYTOPLANKTON IN THE Ebro Delta**


THEME CHAPTER III:

THE SMALL SCALE: HAB DYNAMICS IN THE EBRO DELTA


***

PUBLICATION I. This article is based on the session ‘Health Hazards from the Ocean’ of the OceanSensors08 workshop held 31 March–4 April 2008 in Warnemünde, Germany. I was responsible for overall structure of the paper, and input on all three presented scales of detection, especially the sections on harmful algal blooms. Further contributors to this section were Daly and Cembella. I created all figures and tables of this publication. The article is not printed in full length, but comprises all parts that are relevant for HABs and bio-optics.

PUBLICATION II. This book chapter is based on a presentation at the EOS topical meeting on ‘Blue Photonics’ - Optics in the Sea - held from 18-19 August 2009 in Aberdeen, Scotland, UK. As first author, I constructed the first draft of the chapter. After significant written contributions from Cembella, minor revisions were conducted by all authors.

PUBLICATION III. Field work and data analysis were planned and carried out by me. Furthermore, I generated the first draft version and all figures. The article was further developed by substantial contributions to structure and content by Hedley, and revised by all authors.

PUBLICATION IV. Field- and laboratory work as well as data analysis were planned and carried out by me. The first version of the publication and all figures and tables were developed by me. Revisions by all authors followed.
Publication V. Field- and laboratory work, data analysis and structure of the article were planned and conducted by me. Principal discriminant analysis was carried out by Jan Schulz and Andrea Mentges. All authors revised the publication with minor revisions.

Publication VI. Field- and laboratory, data analysis and structure of article were planned by me, except the identification and enumeration of Karlodinium spp. and associated environmental variables that origin from the IRTA long-term monitoring trial which were carried out by Fernández-Tejedor. Statistical analysis was strongly supported by Schulz; minor revisions were conducted by all authors. The original publication was extended by material from the poster presented at the 14th International Conference on Harmful Algae, held from 1-5 November 2010 in Crete, Greece.

Publication VII. Field work and experiments were planned and carried out by me. Toxin analysis by LC-MS-MS was conducted by Krock with support from A. Müller and W. Drebing, for karlotoxins also with support from B. Noriega. The 454 sequencing and related analyses were done by G. Reintjes and John, the qPCR by Andree, and the redundancy analysis by Schulz; all with respective contributions to the methods part of the manuscript. Tillmann provided identification of Azadinium spp. in microscopic samples. Multiple written input to the entire manuscript was provided by Cembella, revisions of the article were conducted by all authors.
VI INTRODUCTION TO THE RESEARCH FIELDS

In the following, the research fields to which this study is allocated are briefly introduced and key aspects tackled in the publications (by roman numerals) are highlighted.

**HAB SURVEILLANCE:** HAB development has raised increasing societal concerns due to the worldwide occurrence of harmful events with augmenting intensity and awareness. Besides the detection of new potentially harmful species, known taxa are subject to geographical extension due to anthropogenic activities, such as ballast water tanks disposal in the natural environment or dispersal of aquaculture spat from distinct regions, as manifested in the Global Spreading Hypothesis (Hallegraeff, 1993). The high diversity of HAB distribution and harmful effects requires not only the coverage of large spatio-temporal scales, but also species specificity to ensure food safety, environmental health, and also to prevent economic losses due to the detraction of environmental goods and services (closure of aquaculture sites or touristic value).

**OPERATIONAL OCEANOGRAPHY:** “Operational Oceanography can be defined as the activity of systematic and long-term routine measurements of the seas and oceans and atmosphere, and their rapid interpretation and dissemination” (EuroGOOS). Sensors for the near real-time and long-term retrieval of oceanographic parameters have been mounted on a large variety of platforms, reaching from sea-borne, such as research vessels, to stationary buoys, piles in the water up to air-borne platforms such as aircrafts, unmanned vehicles to satellites. The complementary picture of sensors of different preciseness in information depth and with different spatio-temporal resolutions leads to a synoptic view on the ocean bio-geochemical processes. Operational oceanography has gained high relevance in the context of HAB monitoring, as large spatio-temporal dimensions are involved. Also, ocean observatories with multiple sensors are by now gaining prominence for the assessment of HAB dynamics.

---

5 http://www.eurogoos.org/content/content.asp?menu=0090000_000000_000000
**OCEAN OPTICS:** The colour of the sea bears information on its multiple bio-geochemical constituents. The retrieval of single water constituents, such as phytoplankton, out of this ocean colour has been approached by means of various sensors and sensor systems. In the field of ocean optics, by now a large variety of optical sensors with different measurement principles and different ranges of accuracy can be mounted on various platforms for short- or long-term applications (see operational oceanography). Optical attributes that are measured with such sensors can be divided in two basic principles: the measurement of IOPs that are directly linked to water constituents, such as absorption and backscattering, and the measurement of AOPs. AOPs are dependent on geometric characteristics of the natural light-field (solar angle, light intensity etc.), and include radiometric quantities, such as upwelling radiance. Disturbing factors for the measurement of water leaving radiance or remote sensing reflectance include surface reflections of sun and sky, as well as surface objects, such as buoys and boats. Both, AOPs and IOPs hold valuable information on water constituents. The inversion of AOPs to IOPs, has been approached by various algorithms. Generally, a regional sensor system setting requires a regional approach for the correction of data and the subsequent calculation of water constituents from optical measurements.

**PHYTOPLANKTON DYNAMICS:** Phytoplankton is a highly diverse group of organisms and algal blooms have been found in marine and freshwater (Assmy & Smetacek, 2009), and in harsh environments, such as under arctic sea ice (Arrigo et al., 2012) or in hot springs (Krienitz et al., 2003). Factors that trigger the growth of certain species over others are commonly attributed to physical, chemical and biological factors, such as temperature, salinity, shear stress by water mixing, nutrients, as well as grazing or allelopathic interactions (GEOHAB, 2013). Comparative studies of algal proliferations in different ecosystems enable the organization of HAB species into ‘functional groups’ in relation to environmental drivers to provide a basis for simplification to improve forecasting abilities relative to environmental components of the system.
AQUACULTURE: The culture of marine organisms has contributed to human nutrition for many centuries and in various facets from subsistence to industrial scales. Since the worldwide exploitation of natural abundances of marine organisms cannot meet the increasing demand of seafood, the importance of aquaculture is further enhanced. For marine species, most of the production is conducted in natural environments and regular controls for food safety due to hazardous substances are mandatory to secure the health of consumers. Hazardous substances include chemical components of anthropogenic origin as well as bacteria and viruses and harmful algae and the disastrous consequences of their occurrence that may result in large economic losses or even jeopardise the basis of livelihood for subsistence farmers. In the Ebro Delta in Catalonia, NW Mediterranean, Catalonia’s largest aquaculture sites are located in the two semi-enclosed bays Alfacs and Fangar. Monitoring to ensure food safety is conducted by the IRTA on a regular basis, and the inclusion of an ocean observatory for HAB monitoring is a future perspective for enhanced spatio-temporal coverage of HAB monitoring.
THEME CHAPTER I:
ADVANTAGES AND LIMITS OF BIO-OPTICAL TOOLS FOR
HAB SURVEILLANCE

PUBLICATION I: Detecting marine hazardous substances and organisms: Sensors for pollutants, toxins, and pathogens

PUBLICATION II: Bio-optical approaches for the operational assessment of harmful algal blooms

Image acquired by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the NASA Aqua satellite on February 23, 2004. Credit to NASA/GSFC, Rapid Response
Abstract

Marine environments are influenced by a wide diversity of anthropogenic and natural substances and organisms that may have adverse effects on human health and ecosystems. Real-time measurements of pollutants, toxins, and pathogens across a range of spatial scales are required to adequately monitor these hazards, manage the consequences, and to understand the processes governing their magnitude and distribution. Significant technological advancements have been made in recent years for the detection and analysis of such marine hazards. In particular, sensors deployed on a variety of mobile and fixed-point observing platforms provide a valuable means to assess hazards. In this review, we present state-of-the-art of sensor technology for the detection of harmful substances and organisms in the ocean. Sensors are classified by their adaptability to various platforms, addressing large, intermediate, or small areal scales. Current gaps and future demands are identified with an indication of the urgent need for new sensors to detect marine hazards at all scales in autonomous real-time mode. Progress in sensor technology is expected to depend on the development of small-scale sensor technologies with a high sensitivity and specificity towards target analytes or organisms. However, deployable systems must comply with platform requirements as these interconnect the three areal scales. Future developments will include the integration of existing methods into complex and operational sensing systems for a comprehensive strategy for long-term monitoring. The combination of sensor techniques on all scales will remain crucial for the demand of large spatial and temporal coverage.
1.1 Introduction
The quality of marine environments is influenced by a range of anthropogenic and natural hazards, which may adversely affect human health, living resources and the general ecosystem. The focus of this review is on biological marine hazards, including those produced by organisms or the organisms themselves, and on chemically mediated deleterious effects, rather than on physical hazards (rogue waves, tsunamis, storm surge, meteorological effects, etc.). Major components of such bio-hazards are typically endogenous to marine systems, but may also be contributed from freshwater aquatic and terrestrial habitats via run-off and coastal erosion. Identification of types of hazards and their temporal and spatial scale are crucial for an analysis of the associated risks. In this review, we address the assessments of ecological status and the protection and restoration of ecological potential of habitats. These issues are regulated by law under global, regional or national statutes, such as the EU Water Framework Directive (WFD) (2000/60/EC), the EU Marine Strategy Framework Directive (MSFD) (2008/56/EC), and the US Federal Water Pollution Control Act (Clean Water Act) of 1948 and its amendments (33 U.S.C. 1251-1376).

One goal of the marine science community has been to detect hazardous substances and organisms and to monitor related parameters in the ocean to improve understanding of critical processes and to prevent and mitigate adverse effects. Significant advances in the detection and analysis of hazards have been achieved in recent years, in particular in expanding the temporal and spatial scales of observational technologies and in improving resolution. These monitoring techniques are, in most cases, complementary to methods applied to discrete point-source samples. A close cooperation between remote- and in situ disciplines has also emerged, if somewhat belatedly. During the last decade, a range of global and regional monitoring programs have been developed to protect human- and environmental health and prevent economic losses caused by marine hazardous substances in an integrated manner. Amongst these programs are the following: 1) Global Ocean Observing System (GOOS, 2008), 2) Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB, 2008), 3) Harmful Algal Bloom Forecasting System (NOAA, 2008c), 4) Coast Watch (NOAA, 2008b), and 5) United Nations Environment Program (UNEP) on Global Monitoring for Persistent Organic Pollutants (POPs) (2004), and monitoring in accordance with regional sea conventions such as OSPAR (2009), and HELCOM (2009).

To tackle extant and emerging environmental problems, flexible approaches and methodologies must be linked with decision-making strategies of managers. Ecological risk assessment is currently undergoing a shift from the evaluation of particular health impacts, often on a small scale in a specific environment, towards more complex assessments of whole populations and communities across ecologically meaningful landscapes on larger scales.
(Landis, 2003, Hope, 2006). This conceptual approach was designed primarily with terrestrial ‘landscapes’ in mind, but it is no less valid for consideration of ‘seascapes’, albeit that the fluxes, dynamics, and community structures are somewhat different in the sea. Increasingly, remote observations will be performed on an operational basis from a variety of in situ platforms and enabling technologies, including profiling moorings and floats, autonomous underwater vehicles (AUVs), gliders, drifters, ships-of-opportunity, and nodes attached to cable networks. Since successful remote ocean operations for marine hazards fundamentally depend on the sensing techniques, we have to examine the state-of-technology and derive demands for upcoming methodologies, sensors, and sensor systems.

Sensors may be generally characterised as devices that capture and transduce a signal related to the presence and/or concentration of a compound or organism, including related physical properties, which can then be stored or transmitted to a receiver at a different location. The captured signal can then be related to biological, chemical, or physical processes affected by or affecting the compounds or organisms detected. Smart sensors additionally comprise the ability of the sensor to process and evaluate the captured signal to yield information upon which the receiver or the sensor platform can directly respond.

A variety of platforms are needed to support sensing systems in the ocean, including multiplex and integrated observational technologies. Fixed-point profiling moorings are essential to resolve a wide range of temporal variability (short-lived episodic events, subtle changes over decades, etc.) of physical, chemical, and biological processes that occur between the sea surface and the sea floor. Mobile platforms (floats, gliders, AUVs) with appropriate sensors provide measurements of spatial variability to complement the fixed sites. Satellites can yield broad spatial synoptic measurements of the surface ocean, but are of limited use in the vertical dimension.

A vast number of articles have been published on the detection of hazardous substances and organisms. A recently published comprehensive volume on observational technologies for coastal ecosystems, with a focus on Harmful Algal Blooms (HABs) (Babin et al., 2008) is illustrative of the rapid advancements in such fields. The coverage of all hazards and upcoming technologies in this active field of development would have to be accomplished separately. Rather than providing a detailed review of all groups of hazardous substances and organisms, including all possible sensors, here we restrict our purview to advanced techniques for detection of marine pollution, toxins, and pathogens in the ocean, with a focus on sensors applicable for remote deployment.
1.2 Marine health hazards:
Hazardous substances and organisms in marine waters may derive from anthropogenic or natural sources. In this review we distinguish between anthropogenic marine pollution, natural marine toxins, and pathogenic agents (Fig. 1.1). Unfortunately these categories are not clear cut – formation of many marine pollutants is facilitated by the combination and transformation of anthropogenic components with naturally occurring substances. Furthermore, hazardous ‘natural’ occurrences of toxic organisms (e.g., HABs) or bacterial and viral pathogens may be stimulated by human activities, such as sewage inflow and eutrophication or long distance human-mediated transport, as in ship ballast water. Finally, natural pathogenic organisms can be enhanced in diversity and biogeographical extent through human interventions such as agricultural run-off and improper sewage treatment. It is, therefore, unwise to treat these phenomena as unrelated events for observational and management purposes.

![Fig. 1.1. Classification of marine hazards of anthropogenic and natural origin, as structured within this review and schematic of marine and terrestrial systems imperilled by these harmful substances and organisms. Marine systems are sensitive to bioaccumulation in food webs.](image)

By consensus MP is considered to be derived exclusively from human activities. The term pollution is defined by GESAMP (1983) as:

".. the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance of marine activities, including fishing, impairment of quality of use of seawater, and reduction of amenities.”
According to the EU WFD and the US CWA, priority substances that represent a significant risk to or via the aquatic environment range from toxic metals to organic contaminants, such as persistent hydrocarbons, organochlorine compounds and pesticides, as well as organometallic compounds. […]

Health hazards with a natural origin comprise both MT and PA, although recent observations indicate an increased prevalence and distribution due, at least partially, to anthropogenic influences on the marine environment (Anderson et al., 2002). A large number of marine animals from many different phyla, including certain snails, jellyfish, sea anemones, sea urchins, sponges, and fish, etc. produce highly bioactive substances, including potent toxins and venoms for prey capture or defence. These substances can also be hazardous to human health. Because the effects are extremely localized and the toxins themselves cannot usually be monitored with in situ or remote sensors, they are not dealt with in detail in this review. […]

Our focus here on marine toxins comprises biotoxins synthesized by living organisms, with emphasis on toxins produced by microorganisms, such as microalgae, fungi, and bacteria, including cyanobacteria. These MTs are widely associated with contamination of seafood. The most widespread classification of these microorganism-derived toxins linked to seafood poisoning is based on associated toxin syndromes (Campás et al., 2007), e.g., okadaic acid and dinophysistoxin analogues causing diarrheic shellfish poisoning (DSP); saxitoxin and related derivatives causing paralytic shellfish poisoning (PSP); DA associated with amnesic shellfish poisoning (ASP); brevetoxins causing neurologic shellfish poisoning (NSP); azaspiracids causing azaspiracid shellfish poisoning (AZP); ciguatoxin and maitotoxin analogues linked to ciguatera fish poisoning (CFP); and tetrodotoxin causing pufferfish (fugu) poisoning (Geistdoerfer & Goyffon, 2004, Campás et al., 2007).

Human illness caused by marine toxins can also be divided into their primary transvectors - those associated with seafood consumption, and those due to exposure to water (or aerosols) containing toxins. These human health effects are highly diverse, ranging from mild to acute (even fatal) especially when neurotoxins are involved, and can include nausea, neurological disturbances, paralysis, short term memory loss, fevers, ear and eye irritation, and pulmonary consolidation. Longer term exposure may be associated with kidney- and liver damage, even resulting in carcinogenesis and/or tumour promotion (for references see Codd et al., 2005).

Most toxins associated with marine microorganisms are naturally produced by microalgae, including the prokaryotic cyanobacteria (informally known as ‘blue-green algae’). There are also occasional reports of mycotoxins in the marine environment, such as those of the toxic fungus Aspergillus fumigatus, which can accumulate in mussels (Grovel et al., 2003).
Toxins of algal origin (phycotoxins) may be transferred through the food chain via the consumption of toxic microalgae and then can accumulate in higher trophic levels (fish, marine mammals, seabirds) with devastating consequences. As well, these toxins in seafood pose a health risk for human consumers. Phycotoxins in the marine environment regularly lead to restrictions on commercial and recreational shellfish harvesting and negatively impact tourism and public health resulting in high economic losses each year. Cyanobacterial toxins in fresh and brackish water are another critical and emerging problem, with evidence of effects on adjacent linked marine ecosystems related both to toxicity and high biomass production.

The term Harmful Algal Bloom (HAB) is often applied operationally to algal occurrences that cause harm through the production of toxins and/or by excessive accumulation of biomass – but not all HABs meet both criteria (Anderson et al., 2002, Masó & Garcés, 2006). Blooms are generally characterised by development, maintenance, and decline phases. The detection of such events occurs mainly during later development and maintenance stages when significant biomass and/or toxic effects are present; early warnings of impending events are thus rare and bloom prediction and modelling remains a major challenge that is being addressed in only a few key areas (e.g., the Gulf of Maine, reviewed by Anderson, 2005). High biomass accumulation alone may lead to environmental damage, such as hypoxia, anoxia, and harmful shading of underlying vegetation, such as seagrass beds and corals. Furthermore, certain toxins, including some from cyanobacteria, can persist in the water phase after extracellular release (Lawton et al., 1994), thus the absence of the bloom does not necessarily indicate absence of toxins. […].

1.3 Detection of health hazards: Status and developments

The health hazards described above can be classified based on source of origin: a) point sources, such as discharges from urban waste waters, oil spill, or aquaculture; b) diffuse sources, like losses from agriculture or leakages; and c) spread sources, such as the atmospheric deposition on water bodies. This areal dependence on the origin of the potential hazards introduces a spatial dimension that can also be transferred to the corresponding sensing technologies. However, there is a reciprocal relationship in the spatial coverage of a research area of interest and the information depth with the majority of sensor methodologies associated to the level of areal coverage (Fig. 1.2). The coverage of a large area is of importance as well as precise measurements of a smaller pixel. Subsequently, we review the state-of-technology for the detection of marine hazards following this area-approach, from large to small scales.
Fig. 1.2. The reciprocal dependency between sensor sampling resolution and sampling area influencing the extent to which information on hazardous substances and organisms in marine environments can be obtained on large-, intermediate-, and small-scales. Illustrative examples of technologies are provided. In situ point measurements include liquid chromatography coupled with detection by fluorescence (LC-FD) and mass spectrometry (LC-MS).

1.3.1 Status of sensor techniques with decreasing spatial coverage

1.3.1.1 Detection on a large scale: remote sensing

Remote sensing techniques are required to obtain broad spatial synoptic coverage of the ocean surface. In general remote sensing is the detection and identification of phenomena at a distance from the object of interest using human capabilities or special sensors. Modern remote sensing instruments are normally based on optical, electronic or, less frequently, chemical techniques. During the last few decades, many improvements have been achieved in the development of new sensors and in the improvement of existing sensors and their application (Bonn Bonn Agreement, 2007).

Remote sensing of the ocean on a larger scale is commonly, though not exclusively, applied from above the water surface via satellite or aircraft. Most wavelengths for optical or radio-sensing techniques are strongly attenuated in seawater, which prohibits a deep penetration of the water column, and, thus, are limited to sensing the surface layer of the ocean. Satellites can detect marine surface films, for example those generated by oil-spills; however, any sub-surface blooms, such as those of harmful algae, remain undetected, if low water transparency prohibits upwelling radiation from being detected at the relevant depths.
Other limitations of remote sensing are its dependency on the radiative transfer within the atmosphere, which is especially important for optical sensors. This critical feature also highlights that calibration and validation exercises are imperative. Another limitation is the restricted availability of remote optical sensing data due to cloud cover and orbital path and temporal coverage in the case of satellite-borne systems. Similar constraints may also affect the availability of airborne remote sensing data, which depends on the range, technical status, and obligations of the carrier platforms (Zielinski et al., 2001).

Remote ocean sensors, in general, require a change in the absorption, scattering, and/or reflection of water for a given wavelength, using either natural (denoted as passive sensing) or artificial (active sensing) illumination sources. Airborne sensors basically draw on the same techniques developed for satellite observations, reducing the atmospheric influences by operating at lower altitudes, but concurrently reducing their aerial coverage. The increased flexibility and mobility of airborne sensors makes them a prominent choice for surveillance tasks and supporting actions, e.g., to complement shipboard observations. Here we examine satellite and airborne sensors as remote sensing systems and discuss existing approaches being used to address marine toxins and mass occurrence of toxigenic organisms.

[...].

The presence of toxins in the water column or within marine organisms is not detectable by remote sensing since their concentrations and optical properties do not provide significant changes neither in ocean colour nor in other electromagnetic features. However, remote sensing provides for detection of mass aggregations (blooms or swarms) of biotoxin-bearing organisms. For example, the location and mass characteristics of large aggregations of the jellyfishes, e.g. *Rhizostoma octopus*, *Cyanea capillata*, and *Chrysaora hysoscella*, have been identified via aerial surveys (Houghton et al., 2006). Such successful applications of remote detection methods provide a means of monitoring potential primary transvectors of toxins.

Whereas toxins do not change the optical properties of seawater, high biomass algal blooms certainly do so and can be detected by passive remote sensing, that takes advantage of the distinct absorption characteristics of chlorophyll *a* (Chl *a*) in microalgae and the corresponding influence on ocean colour. Both airborne and satellite-based optical remote sensing systems have been widely applied for monitoring the magnitude and distribution of algal blooms, both benign and harmful. In HAB research and monitoring, remote sensing offers the possibility to track mass-surface aggregations based upon pigment spectral signatures, although not toxins or events with low cell concentration. In cases where the species identification and toxic or otherwise harmful potential has been established by independent means, such as *in situ* sampling or access to historical data on bloom
characteristics, remote sensing is a valuable method of conducting broad scale synoptic surveys. For example, remotely sensed chlorophyll data have been used as a proxy for abundance of the Florida red-tide dinoflagellate, *Karenia brevis*, from which the cell abundance estimates can serve as a proxy for the brevetoxins produced during blooms (Tester *et al.*, 2008). Further successful development of other remote sensing techniques to detect and track *K. brevis* blooms on the west Florida shelf are now being implemented (Carder & Steward, 1985, Hu *et al.*, 2005, Hu *et al.*, 2008). Recently, a novel classification approach combining high chlorophyll-low backscatter measurements allowed improved satellite detection of *K. brevis* (Cannizzaro *et al.*, 2008a).

We emphasize that it is not possible to discriminate toxic species or populations from non-toxic ones by large-scale remote sensing. Such methods are also not applicable for the detection of putatively toxic or harmful blooms when the organisms are present only in low biomass. It is, however, possible to identify anomalies and typical situations with high probabilities for HAB events that can be used as triggers to enable countermeasures for aquafarming or tourism (Stumpf, 2001, Stumpf *et al.*, 2003, Reinart & Kutser, 2006). Aircraft observations can be automated with optical equipment, such as still- and motion-cameras, mounted on light-weight platforms such as Unmanned Aircraft Systems (Patterson & Brescia, 2008). In regions where jellyfish swarms or HABs constitute a common interference with marine enterprises and activities, such as tourism, aquaculture, navigation, etc., protocols could be developed for aerial observatory operations or satellite-based systems to detect, enumerate, and predict the development and distributional pattern of such events.

1.3.1.2 Detection on intermediate scales: in situ platforms

Assessing processes on intermediate temporal and spatial scales, including transient events, requires even higher resolution of measurements than for large-scale remote sensing. Long time-series stations and ocean observatories need robust, reliable instruments for long duration deployments (Dickey, 2001, Daly *et al.*, 2004), as well as an appropriate and consistent accuracy, sensitivity and selectivity that is required for use in monitoring programmes. Sensors must have sampling rates high enough to detect the development of transient events and operate over time scales at least comparable to those of physical processes (and physical sensors for conductivity, temperature, and pressure). To give an illustration: the application of an optical nutrient sensor on a winch onboard a ship produces a nearly one-dimensional data set (a depth profile) without any sample preparation on board. As part of an undulating towfish or a glider, the same sensor can even yield quasi two-dimensional information. The point of this example is that the dimensions of the area that can be probed depend on the capabilities of the mobile platform in combination with sensor characteristics (e.g., sampling rate).
course the integrated sampling area will be smaller than the vast areas covered by satellites or aircraft remote-sensing, but larger than that covered by discrete shipboard water sampling from fixed depths, which often require sophisticated (non-real-time) laboratory analysis to generate results.

With respect to the recent development of mobile platforms such as floats, gliders, or AUVs, the intermediate scale is also the most relevant scale for sensor applications and development. We therefore review the portable *in situ* sensor technologies for toxin- and taxon specific detection and the detection of high biomass HABs.

**Toxin- and taxon-specific detection**

The identification of marine biotoxins, either phycotoxins or those produced by marine macrofauna (e.g., jellyfish, fish, sea snakes, cone snails), at the intermediate scale from deployable systems is (with a couple of notable exceptions) not yet realizable. One of these exceptions is the detection of the phycotoxin domoic acid (DA) produced by several species of toxigenic pennate diatoms, *Pseudo-nitzschia* spp., based upon a specific antibody method for the toxin (Doucette *et al.*, 2009) and integrated into the moored Environmental Sample Processor (ESP) developed at the Monterey Bay Aquarium, Monterey, California (see detailed description in Scholin *et al.*, 2008, Scholin *et al.*, 2009). The ESP system was originally designed for *in situ* near real-time detection of harmful algal taxa based upon their unique ribosomal DNA signatures. The molecular probes can be multiplexed for simultaneous detection of many putatively harmful species and can be hierarchically designed to reflect the closeness of target affiliations (class, order, genus, species, geographical population, etc.). Hybridization of compatible rRNA from *in situ* cells extracted on-line in the ‘sandwich hybridization assay’ can be detected optically by either fluorescence or photometric sensing, which also provide a semi-quantitation of total hybridisable rRNA as a proxy for target cell number. This ESP system is now past the advanced prototype stage, and in the latest configuration has been deployed over several months on moorings in Monterey Bay, California and the Gulf of Maine, USA. Commercial production is expected to follow within the near future.

Since most marine toxins are non-volatile compounds they are not readily amenable to certain chemical analytical techniques, such as gas chromatography coupled with mass spectrometry (GC-MS), and appropriate derivatisation methods for detection are not commonly available. Current applications of liquid chromatography with mass spectrometry (LC-MS) to marine biotoxin analysis are limited to laboratory extracted and serially injected discrete samples (Quilliam, 2003) and do not include in-water profiling or moorage deployment. The successful deployment of an advanced coupled tandem mass spectrometer (LC-MS/MS) with linear ion-trap (ABI SCIEX 4000 with Q-trap) for shipboard measurements
of marine phycotoxins harvested from the water column particulate fraction in the North Sea (Krock et al., 2008) is an example of transitional analytical technology for intermediate temporal and spatial scales. In precursor ion scan mode, a wide array of putative phycotoxins belonging to different structural grouping can be assessed qualitatively and quantitatively from a single injection in <1 hour run time, providing quasi-synoptic spatial coverage in near-real time for these toxins while underway (Krock et al., 2009). This on board laboratory technique provided the chemical signal for the identification of the organismal source of azaspiracid toxins (Tillmann et al., 2009) – previously a mystery and major issue for shellfish toxins monitoring programs.

It would of course be significantly advantageous if LC-MS systems were available for in situ applications (Marr et al., 1992) and recent developments towards miniaturization of both LC and MS technology (Taylor et al., 2001) indicate that in situ toxin analysis directly from seawater may be feasible in the not too distant future. Underwater mass spectrometers are available commercially (e.g., Applied Microsystems In-Spectr), although they are limited to analysis of very small molecules such as methane. Through the use of MEMS (Micro Electro Mechanical Systems) based mass spectrometers (Taylor et al., 2001) the size and power demand of these systems could probably be reduced even further. On-chip or capillary LC with microfluidics would reduce the consumption of the mobile phase and the need for the vacuum pumps to remove large amounts of vapour from the interface, as well as improve sensitivity. The relatively low sample throughput (minutes to hours per sample) as well as power and space requirements of such a sensor system would likely make it best suited for larger/stationary platforms, short targeted deployments, or for ground-truthing of other sensors.

Detection of high biomass HABs

Profiler or mooring-based systems for detection of HABs are almost all based upon inherent- or apparent optical properties of the bloom and are hence generally both less sensitive and less specific than the techniques described for taxon- and toxin-specific sensors. Bloom detection with the former instrumentation, therefore, typically requires high biomass (or high concentration of a proxy parameter such as Chl a or phycobilin-pigments), while yielding only very low taxonomic resolution (Cullen et al., 1997b). Such systems also perform best when the species composition is relatively well defined and where the bloom tends to be monospecific. For the continuous detection of microalgal blooms or particle concentrations on vertical and horizontal scales, a range of commercial in situ bio-optical instruments, such as fluorometers, transmissometers, or turbidometers, are commonly available (Moore et al., 2009). The discrimination of valid information on microalgae or bulk material in the water is mainly solved by the relatively large amount of information obtained over temporal and
spatial scales. Commercial in situ bio-optical instruments use inherent optical properties (IOPs) of substances contained in seawater, such as the specific adsorption, attenuation, scattering, and backscattering, at an increasing number of wavelengths (Babin et al., 2005). The underwater IOPs range from bulk hyperspectral to miniature multispectral instruments, and are being deployed on all types of fixed and mobile in situ platforms, e.g., buoys, ROVs, AUVs (Mitchell et al., 2000, Bishop et al., 2002, Zielinski et al., 2006).

Fluorometers with internal light sources are used as indicators for Chl a concentration, a proxy for phytoplankton abundance and humic/coloured dissolved organic matter (CDOM). A second group of optical instruments employ passive sensors, which measure the distribution of light in the water column (measurement of apparent optical properties - AOPs). Values of reflectance and diffuse attenuation can be derived, e.g., from the vertical gradient in irradiance, and inversion techniques can be used to derive IOPs and water constituents (Moore et al., 2009). Passive measurements are dependent on external light sources, such as daylight and are subject to potential sources of environmental variation and uncertainty. Recently, an increasing number of hyperspectral AOP sensors are being deployed enabling sophisticated spectral fitting algorithms that can be used to derive substance concentrations in complex water bodies, e.g., in coastal areas. However, discriminating harmful from non-harmful algae species is still an open challenge for optical sensors, except if the hazard is due to relatively high algal concentrations (Kirkpatrick et al., 2000).

The most advanced development of an optical plankton discriminator (OPD, also called the ‘Brevebuster’) has been successfully deployed to monitor and track blooms of the Florida red-tide organisms *K. brevis* (Kirkpatrick et al., 2000). Blooms of this red-tide species in Florida present a typically ideal suite of characteristics – high surface concentrations, high dominance and monospecific tendencies, plus an unusual pigment signature - that lends itself to optical detection systems. The ‘Brevebuster’ uses a liquid waveguide capillary cell for the in vivo optical detection of the rare pigment, gyroxanthin-diester, which occurs in *K. brevis* in the eastern Gulf of Mexico and is in constant proportion to cellular Chl a (Millie et al., 1997). Comparing light absorption by particles in ambient water to the light absorption fingerprint characteristic of the unusual pigment signature provides a species-specific in situ detection system. The comparison yields a similarity index which is related to the fraction of phytoplankton community biomass contributed by *K. brevis*. Such OPDs are routinely deployed on underwater gliders to map subsurface *K. brevis* blooms on the west Florida shelf (Robbins et al., 2006). Further characterisation of *K. brevis* multi-wavelength spectral properties should allow more sensitive detection with underwater spectrophotometers (Spear et al., 2009).

[…].
1.3.1.3 Detection on a small scale: in situ – point measurement

The application of highly accurate and precise methods is necessary to quantify specific harmful substances and associated organisms and to provide unambiguous identification of the toxic components and their affiliations with particular taxa. Most conventional approaches are constrained by a time delay in delivery of results, high implementation costs, the need for highly trained personnel, and the requirement for technologically advanced equipment and laboratories. For some toxic substances, the objectives of low cost and ease of use procedures can be partially attained by access to biochemical and biomarker assays (Wells et al., 2001, Cembella et al., 2003), which can often be run in parallel for additional time saving in high-throughput screening systems. Such assays can serve for toxicity testing from a variety of sample matrices including organisms and seawater, and can be configured to be highly specific for the analytes of interest. For most environmental monitoring, structural or functional assays, frequently supplemented with chemical analytical techniques for confirmatory analysis, have largely replaced testing with whole live mammals. The one major exception for marine hazards testing is the retention of the intraperitoneal mouse bioassay (Fernández et al., 2003) for potentially phycotoxin-contaminated seafood by many regulatory agencies around the world. In addition to the well calibrated AOAC mouse bioassay for acute toxicity (AOAC, 1995), many mammalian subjects are also sacrificed for long-term toxicity trials of marine hazardous substances for which alternative dose-response model systems are not available. Nevertheless, increasing concerns for animal rights, as well as the confounding disadvantages of mammalian test organisms, such as effect of age, gender, acclimation history, and natural variation, and which can affect the reliability of bioassays, strongly underscore the necessity of developing alternative detection methods for marine hazardous substances.

In recent years, there has been a tremendous expansion in the use of liquid chromatography with mass spectrometry (LC-MS), especially since the advent of atmospheric pressure ionization systems in the late 1980s (Quilliam, 2003). In spite of the major breakthroughs in monitoring hazardous compounds by instrumental methods (LC with fluorescence or diode-array detection; LC-MS, etc.) or in vitro assays (immunological, biomarker, biochemical, etc.) most of these approaches remain confined to the laboratory. A few advances towards near real-time techniques suitable for field deployment have been made in attempts to transduce the signal from assays via sensors, thereby facilitating the transition from single-shot probing to continuous measurements. In the following section we focus on these sensor technologies, including biosensors and electrochemical, optical, and mass-sensitive sensors.

In addition, most of the sensor technologies for small scale detection still require validation with advanced analytical equipment and laborious laboratory analysis. The new
methods, therefore, can be considered as an alternative or complementary to conventional laboratory methods, such as chromatography coupled with mass spectrometry, standard culturing and microscopic examination methods, immunoassays, etc, and not necessarily as complete replacements.

[...].

Marine toxins and mass occurrence of toxigenic organisms
As outlined before, the detection of many marine toxins via whole mouse assays, e.g., the AOAC intraperitoneal mouse bioassay for PSP toxins, remains a method in widespread use and is internationally accredited. For example, the mouse bioassay is still an EU reference method for detection of certain phycotoxins in shellfish (Aune et al., 2007). For replacement in the current EU legislation, alternative methods need to be validated according to an internationally recognised protocol. Such methodologies focus by now on laboratory methods, such as LC-MS (Alexander et al., 2008).

Several alternative in vitro assays, including in receptor binding assays, biochemical assays, immunoassays and electrochemical immunosensors have been developed and are increasingly applied in seafood toxin monitoring programmes (Cembella et al., 2003, Fernández et al., 2003). Chemical analytical methods such as chromatographic or electrophoretic techniques and mass spectrometry are now widely employed for the detection of marine toxins (Quilliam, 2003). The large variety of functional and structural assays for phycotoxin monitoring are unfortunately mainly targeted to a specific toxin or selected group of toxins and, therefore, do not provide a broad spectrum screening (Rossini, 2005). Furthermore, interference by nonspecific matrix effects or limited availability of standard reference materials may also impair the application of these techniques for general routine measurement (Campbell et al., 2007).

There is no ideal method for toxin determination and, therefore, methods that reliably detect toxic substances in a rapid, low-cost and easy-to-use way are still required. Rapid developments are occurring in the leap from whole animal and tissue culture assays to biosensors, and from simple immunoassays (e.g., colorimetric or fluorometric ELISA) to sophisticated immunosensors (Campás et al., 2007). Biosensors also have potential as a partial alternative and/or complementary tool to long established technologies. For example, Campás et al. (2007) developed an amperometric immunosensor assay which was compared with the protein phosphatase inhibition assay and conventional HPLC analysis of cyanotoxins. The immunosensor proved its applicability as a screening tool for fast and reliable cyanotoxin detection. Given the success in detecting low level chemical contaminants in food, optical
biosensors based on surface plasmon resonance technology also have the potential to be an alternative strategy for monitoring PSP toxins in seafood (Campbell et al., 2007).

Detection of high biomass HABs

The detection of HABs on the toxin- or species level is crucial for HAB monitoring, as the harmful effects are often attributable to single or at least dominant species. In particular, sensors are needed to detect HABs at low background concentrations to allow early warning of bloom development and possible mitigation strategies. Traditional observation techniques for algal species on a small spatial scale include light microscopy and laboratory analysis, which are labour-intensive methods that do not deliver real-time results or broad coverage (LaGier et al., 2007). Emerging techniques for near real-time monitoring of phytoplankton include the benchtop FlowCAM®, combining microscopy and flow cytometry in measuring light-scattering and fluorescence from chlorophyll and phycoerythrin on individual particles larger than 5 µm coupled with image capture capabilities allowing for the recognition of species (Sieracki et al., 1998). The FlowCytobot is an automated submersible flow cytometer that has been used to analyze pico- and nanoplanckton (Olson et al., 2003). Techniques depending on single cell analysis, however, may be inappropriate for the colonies or coiled filaments of cyanobacteria (Codd et al., 2005). Another autonomous in situ flow cytometer, the CytoBuoy, has been employed to quantify marine plankton, including the difficult HAB organism Phaeocystis spp., which tends to form amorphous gelatinous colonies (Rutten et al., 2005). The CytoBuoy allows phytoplankton analysis in the size range 1 - ~50 µm (for more details see, e.g., Thyssen et al., 2008).

Molecular techniques have already been developed for in situ detection of HAB organisms even at low biomass concentrations. One deployable molecular-based detection system, the moored Environmental Sample Processor (ESP) uses a rRNA hybridization approach (Scholin et al., 2008). An alternative system, the Autonomous Microbial Genosensor (AMG) (Paul et al., 2007) can also collect and process plankton samples in the ocean. The AMG operates by nucleic acid sequence-based amplification, with an initial configuration designed to detect K. brevis. This instrument is designed to be deployed on moorings and transmit data to shore in near real-time. Development of ‘phylochips’ and DNA microarrays for selected taxa including harmful algal species are underway (Metfies & Medlin, 2008) but are not yet configured for in situ deployment.

[...].

1.3.2 Coverage and gaps

Review of the state-of-technology reveals well established and proven, as well as not yet mature sensing approaches for marine health hazards on different spatial and temporal scales.
Efforts to detect, monitor, track, and predict harmful substances and organisms by remote sensing techniques, *in situ* measurements with sensors and sensor systems, as well as fine-scale laboratory analysis, reveals that each methodology has advantages as well as limits to its range of implementation.

Remote sensing on a large scale is not a complete solution, but is useful for synoptically monitoring harmful substances or proxies. At the other end of the spectrum, dedicated laboratory measurements provide accurate and extensive measurements of a single water sample, but owing to the labour and time-intensive methods, they cannot yield higher spatial or temporal resolution within affordable budgets and resources. A combination of these applications, specifically an integration of large-scale quasi-synoptic data with high resolution surveys and laboratory in-depth analysis, can partly overcome the constraints of a single approach. This combination of scales will provide additional insight and decision making information. A gap remains for new sensing technologies, especially on the intermediate scale, where remote sensing and laboratory measurement intersect. The application of *in situ* sensors and sensor systems on moorings, ships of opportunity, etc. hold the possibility of combining some advantages of precise laboratory methods and remote sensing to address the demand for high resolution long-term data sets with broad spatial coverage.

Many sensors described herein still require research and development. Especially for detection of heavy metals, POPs, and pathogenic agents full commercialization has often not been achieved, whereas other devices (e.g., for detection of chlorophyll and nitrate) are already available often from multiple manufacturers (Table 1.1).

### 1.4 Future demands and emerging technologies

Some of the categories of health hazards are already addressed by a variety of commercialized sensor techniques. Whereas the areas where few sensors are available might be interpreted as an indication of critical immediate future needs, not all approaches are technically feasible or even recommended for the end-user community. For example, the demand for species identification via remote sensing from satellites or aircraft is simply not feasible as the required specifications typically exceed the laws of physics. In many cases, the analysis of marine hazard parameters has been approached by measuring what is easy (e.g., Chl *a* by fluorosensors or particle spectra and fluorescence of picoplankton by flow cytometry) merely because the technology is available, but not because the results are always relevant. Hope is, however, justified in the proposed adoption of the underlying principles of laboratory analytical measurements to be applied to the field. With respect to *in situ* technologies, there are grounds for optimism that many demands will eventually be satisfied, in spite of the technical and financial constraints in transferring laboratory prototypes to deployable sensor systems. The need for detection of certain substances may not yet be strong
enough on all scales to catalyse the required efforts for technical development. Furthermore, the replacement of statutory laboratory methodologies by sensor technology is only possible if comparable (or better) sensitivity and selectivity towards the target analyte is accomplished and can be proven. Proof may take the form of various quality assurance procedures, which include visual inspection and performance monitoring of the sensor, pre- and post-deployment calibration, and inter-comparison of measurements with established analytical methodologies (Waldmann et al., 2009). Combining technology gaps with social demands will drive the needs and priorities for future development. For the monitoring programmes robust and reliable instruments for long duration deployment are needed. Here, development should focus on consistent accuracy, sensitivity, and selectivity of the sensors during deployment. The effect of fouling on the quality of data is an issue and sensor performance needs to be underpinned by quality assurance data using reference methods. For this purpose deployment of autosamplers alongside sensors could enable collection of reference samples.

 [...] .

For the assessment of the chemical status of marine ecosystems, as is for example required for the EU WFD, a variety of parameters need to be tracked over large temporal- and spatial scales in a rather precise resolution. Substances include chemical polluting elements as well as physico-chemical elements, such as nutrients. The WFD also requires the assessment of ecological status. Phytoplankton is included within the biological elements considered in the WFD. Established indicators in this respect are phytoplankton biomass, taxonomic composition and abundance, as well as the frequency of blooms (OJEC, 2000). The accurate and timely identification of harmful algal species and measurement of their toxins is fundamentally important to both HAB research and management. Mitigation could also be facilitated by early detection of toxic blooms. Cell counts of putatively toxic microalgae are often used as a proxy for inferring the presence of phycotoxins (Steidinger et al., 1999, Kirkpatrick et al., 2000), but these quantitative estimates are not very reliable because of large differences in cell toxin content among members of the same morphospecies and the ephemeral nature of the associated blooms.
Table 1.1. Compilation of commercially available *in situ* sensors for long-term applications in marine environments.

<table>
<thead>
<tr>
<th>Target analyte</th>
<th>Sensor principle</th>
<th>Sensor</th>
<th>Company, location</th>
<th>Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollutant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trace metals Cu(II), Pb(II), Cd(II) Zn(II) (ppt level); Mn(II), Fe(II) ppb level</td>
<td>Electro-chemical/ Voltammetric</td>
<td>Voltammetric <em>in situ</em> profiling system (VIP)</td>
<td>Idronaut, Italy</td>
<td><a href="http://www.idronaut.it">www.idronaut.it</a></td>
</tr>
<tr>
<td>Hydrocarbons, PAH</td>
<td>Optical</td>
<td>EnviroFlu HC</td>
<td>TriOS, Germany</td>
<td><a href="http://www.trios.de">www.trios.de</a></td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>Optical</td>
<td>Hydrocarbon Fluorometer</td>
<td>Sea &amp; Sun, Germany</td>
<td><a href="http://www.sea-sun-tech.com">www.sea-sun-tech.com</a></td>
</tr>
<tr>
<td>Humic acids, amino acids, BTXE, PAH</td>
<td>Optical</td>
<td>HydroC™/PAH</td>
<td>Contros, Germany</td>
<td><a href="http://www.contros.eu">www.contros.eu</a></td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>Optical</td>
<td>UViLux and UV AQUAtracka</td>
<td>Chelsea Technologies Group, UK</td>
<td><a href="http://www.chelsea.co.uk">www.chelsea.co.uk</a></td>
</tr>
<tr>
<td>Crude oil</td>
<td>Optical</td>
<td>Cyclops-7 Submersible Sensors</td>
<td>Turner Designs, USA</td>
<td><a href="http://www.turnerdesigns.com">www.turnerdesigns.com</a></td>
</tr>
<tr>
<td>Nitrate, Nitrite</td>
<td>Optical</td>
<td>ProPs UV</td>
<td>TriOS, Germany</td>
<td><a href="http://www.trios.de">www.trios.de</a></td>
</tr>
<tr>
<td>Nitrate, Nitrite</td>
<td>Optical</td>
<td>ISUS V3</td>
<td>Satlantic, Canada</td>
<td><a href="http://www.satlantic.com">www.satlantic.com</a></td>
</tr>
<tr>
<td>Nitrate</td>
<td>Optical</td>
<td>SUNA (Submersible UV Nitrate Analyzer)</td>
<td>Satlantic, Canada</td>
<td><a href="http://www.satlantic.com">www.satlantic.com</a></td>
</tr>
<tr>
<td>Nitrate</td>
<td>Ca Reduction/ Diazonisation</td>
<td>9600 Nitrate Monitor</td>
<td>YSI, USA</td>
<td>wwwysi.com</td>
</tr>
<tr>
<td>Sulphide</td>
<td>Amperometric</td>
<td>Submersible Sulphide/H2S Probe</td>
<td>Sea &amp; Sun, Germany</td>
<td><a href="http://www.sea-sun-tech.com">www.sea-sun-tech.com</a></td>
</tr>
<tr>
<td>Ammonia</td>
<td>Chemical/ Optical</td>
<td>MARCHEM</td>
<td>SubChem Systems, Inc., USA</td>
<td><a href="http://www.subchem.com">www.subchem.com</a></td>
</tr>
<tr>
<td>Microalgae species composition</td>
<td>Optical</td>
<td>Multi-Exciter <em>in vivo</em> multi-wavelength excitation fluorescence</td>
<td>JFE ALEC Co. Ltd., Japan</td>
<td><a href="http://www.jfe-alec.co.jp">www.jfe-alec.co.jp</a></td>
</tr>
<tr>
<td>Microalgae class composition/ Total Chl analysis</td>
<td>Optical</td>
<td>bbe FluoroProbe</td>
<td>bbe moldaenke GmbH, Germany</td>
<td><a href="http://www.bbe-moldaenke.de">www.bbe-moldaenke.de</a></td>
</tr>
<tr>
<td>Chl</td>
<td>Optical</td>
<td>MicroFlu chl</td>
<td>TriOS, Germany</td>
<td><a href="http://www.trios.de">www.trios.de</a></td>
</tr>
<tr>
<td>Phytocyanin</td>
<td>Optical</td>
<td>MicroFlu blue</td>
<td>TriOS, Germany</td>
<td><a href="http://www.trios.de">www.trios.de</a></td>
</tr>
<tr>
<td>Chl</td>
<td>Optical</td>
<td>ECO BB2F</td>
<td>Wetlabs, USA</td>
<td><a href="http://www.wetlabs.com">www.wetlabs.com</a></td>
</tr>
<tr>
<td>Chl, rhodamine, fluorescein, phycocyanin, phycoerythrin, nephelometer</td>
<td>Optical</td>
<td>AQUAtracka III and UniLux/TriLux series</td>
<td>Chelsea Technologies Group, UK</td>
<td><a href="http://www.chelsea.co.uk">www.chelsea.co.uk</a></td>
</tr>
<tr>
<td>Chl <em>in vivo</em>, phycocyanin, phycoerythrin, cyanobacteria</td>
<td>Optical</td>
<td>Cyclops-7 Submersible Sensors</td>
<td>Turner Designs, USA</td>
<td><a href="http://www.turnerdesigns.com">www.turnerdesigns.com</a></td>
</tr>
<tr>
<td>HABs and Toxins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A Florida State task force (Steidinger et al., 1999) identified six priority areas of study regarding HABs and their toxins, but this list is also reflective of global requirements: 1) determine the distribution of toxic and non-toxic strains, 2) develop epidemiological studies to determine public health risks, 3) develop economic impact studies to evaluate losses by location or industry, 4) determine the roles of nutrient enrichment and managed freshwater flow in blooms, 5) determine fate and effects of toxins in the food web, and 6) investigate control and mitigation methods, including hand-held and autonomous biosensors. The development and application of sensor methodologies would support the Member States of the EU in the WFD objective to reach a good surface water status by the year 2015.

Biosensors are a clear priority for detection of harmful algae and their respective toxins. Approaches such as membrane-ion channel biosensors, surface plasmon resonance-based biosensors (see Campbell et al., 2007), and molecular and biochemical diagnostic procedures (e.g., immunoassays) must be further advanced to comply with the sensitivity requirements to replace the AOAC mouse bioassay.

For marine biotoxins, a single procedure covering multiple classes of toxins would provide the best standard for consumer protection (Rossini, 2005). Unfortunately such a method does not exist – the application of LC-MS/MS to toxin analysis comes closest, but has the major drawback of not directly measuring toxicity and cannot effectively screen for new classes of toxins without prior knowledge of chemical structure and evidence of toxicity. There remains a residual requirement for development of functional assays to determine toxin potency to at least partially replace whole animal bioassays. The range of biosensors for seafood toxicity screening allows detection of phycotoxins at adequate sensitivities, but their limited availability, primarily as research tools, hinders their broader utilization in monitoring programmes. Commercial exploitation could be enhanced by combining existing knowledge in interdisciplinary areas, such as nanoelectronics, bioelectronics, micromachining, and microfluidics (Campás et al., 2007). This would also contribute to the implementation of these devices on deployable measurement platforms.

[…].

The development of ecogenomic sensors is a future domain of investigation. Within this field, besides the definition of target compounds, methods for detection and signal transduction need to be established (Scholin, 2010). In addition to biosensor and ecogenomic sensor-based applications, Raman and imaging-based techniques are promising tools to reach a higher sensitivity towards the target analytes and organisms. Although there has been some success in the application of Raman spectroscopy in the detection of health hazards (Brewer et al., 2004, Kronfeldt et al., 2004), this technique may be also regarded as an emerging
technique for field deployment, due to the high potential for measuring inorganic and organic compounds even under extreme conditions. In addition to conventional Raman scattering, sophisticated techniques such as SERS or resonance Raman can be employed to increase the sensitivity for specific compounds in a complex mixture, e.g., carotenoids and chlorophyll pigments in algae.

A different approach towards the aim of detecting hazardous organisms is the use of image forming devices. Systematic efforts in underwater imaging have been carried out since the 1970s (see Wiebe & Benfield, 2003, and references therein). Today digital technology allows sensing of object size classes below 100 µm and on spatial scales in the decimetre range. However, the required high magnification results in small volumes scanned per frame. Thus, particles with low abundances have a higher probability to remaining undetected until their number increases (Davis et al., 1992, Benfield et al., 1996). Another imaging system, the SIPPER, utilizes a high-speed linescan camera to continuously image all particles passing through a relatively larger volume of water (Remsen et al., 2004) and an image analysis software to measure and identify plankton (Luo et al., 2004). Recent research also is being conducted towards automatic species identification based on research platforms, such as the Lightframe On-sight Keyspecies Investigation method (LOKI) (Schulz et al., 2008). The LOKI acquires images of objects in a defined volume and assigns them to environmental parameters. The challenge is to ensure the reliability of the post-processing with autonomous and correct identification of particles. In addition to standard parameters, like Hu-moments, Fourier-descriptors or texture analysis, the classification algorithms includes new form based feature extractions (Latecki & Lakämper, 2000, ISO/IEC TR 15938-8, 2002), increasing classification success.

Considering the increased computational and network capacities onboard modern in situ observation platforms, it is possible to realize their autonomous, adaptive response. For example, modeling can be applied to help cast projections of biological, chemical and physical properties. By directing small fleets of mobile platforms or altering the operation of a fixed array of sensors and samplers within that domain, a distributed network could be variably tuned to remotely detect specific phenomena.

Further progress in sensor technology is expected to depend largely on the development of small-scale laboratory sensor technologies with a high sensitivity and specificity towards the target analyte or organism. Deployable systems, however, must comply with platform requirements, as the latter connect the small- to the large scale. In any case, the combination of sensor techniques applicable to all scales will remain crucial for the coverage of all spatial and temporal dimensions.
1.5 Conclusions and Outlook

In the past several decades, a large variety of measurement devices and sensing systems have been designed. This interdisciplinary field is characterised by a rapid technical development in disciplines such as science, systems engineering and field operation systems. We used the reciprocal relationship between the area coverage and the information depth obtained by the available sensors for these different spatial dimensions to organize our review. From this status quo, a large window of opportunity is evident for the advancement of sensors in marine hazard detection on all scales. Ancillary requirements for monitoring and operational oceanography are improvements in the SWaP-factor (size, weight, and power consumption), biofouling prevention, handling, reagent free operation, real-time data availability, as well as simplified deployment and maintenance. Additional issues of stability and reliability and the testing of techniques, e.g., in ring trials to reach comparable results of multiple users, must also be addressed. Current ocean-observation efforts are limited in scope and as yet do not have clear mechanisms for translating large-scale, international ocean experiments into long-term, operational observation efforts, or for transitioning emerging new ocean-observation technologies to operational use (NOAA, 2008a). This is particularly true with respect to monitoring of (non-physical) marine hazards. The focus here should be on the operational oceanography aspects of in situ sensors with more precise measurements and integration with data via space- and air-borne systems, especially on the intermediate scale.

The future of ecological risk assessment will, according to Hope (2006), focus increasingly on larger spatial scales and the need for scientific, defendable, and implementable assessment tools beyond single organisms to large ecosystems. This will require a continued application and development of sensors to cover (spatially and temporally) an assessment of multiple stressors, including meta-data storage and analysis capacities.

Furthermore, improved communication amongst all decision makers, stakeholders, and lay audiences is required. This is beyond the scope of a sensor review paper. It is, however, important for the creation of data protocols, analysis tools, and for clear, effective management strategies and for the consideration of the socioeconomic consequences of marine hazards. The protection and restoration of habitats via improved detection and monitoring of hazardous substances, organisms, and linkages with associated critical processes, through sensors and sensor systems will contribute to the prevention and mitigation of adverse effects.
**Abstract:**

The surveillance of Harmful Algal Blooms (HABs) in aquatic environments is a crucial component in monitoring and mitigation of adverse effects caused by accumulation of high biomass of algal cells and/or associated toxins. The high diversity among HABs necessitates observational approaches that cover a broad spectrum of temporal and spatial scales. Current approaches range from remote sensing to in situ discrete and profiling observations. The challenge is to develop new systems and approaches driven by the need for sensitive and discrete detection of HAB species and associated bio-optical properties. Herein we review state-of-the art technology and address the diversity of HABs with an appropriate set of approaches for operational long-term monitoring.
2.1 Addressing the diversity of harmful algal blooms

Proliferations of phytoplankton in aquatic ecosystems are a natural phenomenon and are observed worldwide. As pasture of the sea, phytoplankton constitutes the nutritional basis of aquatic food webs, and as photosynthesizing organisms they are also a primary contributor to the global carbon cycle. Yet among the phytoplankton and benthic microalgae there are many species that form high biomass and/or toxic aggregations of cells characterised as HABs (Smayda, 1997a). The occurrence of such HABs is documented worldwide and they can have adverse and often devastating effects on aquatic ecosystems, human and aquatic faunal health and socio-economic interests. The term ‘HAB’ is not a scientific, but rather a societal one, referring informally to algal events that have a detrimental effect from the human perspective. The manifestation of these effects is highly complex but often, although not necessarily, results from the generation of high algal biomass and/or from the production and propagation of potent marine toxins. Many HABs are caused by rapid cell growth and aggregation of particular taxa, and can even tend towards monospecificity in the phytoplankton assemblage. High biomass accumulation of algal cells can result in local oxygen depletion and shading of the underlying vegetation, which may then lead to a shift of ecosystem states, e.g. from coral-to algal-dominated, and weaken ecologically important seagrass beds. Algal-derived scums and foams aggregate on the water surface or on adjacent beaches and reduce their touristic and recreational value. Many algal species can also produce toxins, frequently leading to large economic losses, such as temporary closures of commercial fisheries and aquaculture and associated costs of toxin monitoring and medical intervention in cases of human poisoning (Hoagland et al., 2002). These phycotoxins can be harmful even in low concentrations and can accumulate in seafood species following exposure to toxic cells or released toxin components. Human exposure to phycotoxins can be via direct contact with water or aerosols, but mainly through consumption of contaminated seafood. The mode of action and potency of the phycotoxins is very diverse and in humans the acute effects can range from mild to fatal. Symptoms of poisoning may include nausea, fever and eye irritation, as well as short term memory loss, paralysis, neurological disturbances and cardiorespiratory failure, depending upon the type of toxin, dosage and exposure regime. Long-term and chronic effects of phycotoxins may include damage to kidneys and liver, and in certain cases promotion of tumour development (for references see Wright, 1995, Codd et al., 2005, Campás et al., 2008).

The microalgal taxa associated with HABs are numerous and belong to diverse taxonomic and phylogenetic lineages, but among these groups, marine flagellates, particularly dinoflagellates, are dominant. The IOC-UNESCO Taxonomic Reference List of Harmful Microalgae comprises more than 100 species within five algal classes: Bacillariophyceae (diatoms), Prymnesiophyceae (haptophytes), Dinophyceae (dinoflagellates), Raphidophyceae (raphidophytes), and Dictyochophyceae (associated to silico-flagellates) (Moestrup et al.,
2009 onwards) (Fig. 2.1). A few members of the Pelagophyceae, known informally as ‘brown tide algae’, are also harmful, due to negative effects on vegetation and shellfish, even though no direct human health issues arise (Gobler et al., 2005). In addition, an estimated 40 genera of the Cyanophyceae, known as ‘blue-green algae’ but more properly as cyanobacteria, contribute to HABs. Cyanobacterial HABs occur primarily in freshwater, but also in brackish- and marine systems, where they include toxic species of *Trichodesmium*, *Planktothrix* and *Nodularia*, among other genera (Carmichael, 2001, Codd et al., 2005, O’Neil et al., 2012). The list of HAB species is constantly being amended, but is generally increasing in length because of taxonomic revisions, shifts in biogeographical distribution and the description of new taxa associated with known toxin syndromes, e.g. the discovery of the novel dinoflagellate *Azadinium spinosum* as the causative organism of azaspiracid toxicity (Tillmann et al., 2009).

The geographical extent and distribution of HAB species has also undergone an apparent increase over the past several decades, both as a result of increased scientific awareness of formerly cryptic species and their toxins, as well as the consequences of true range extension of taxa (Hallegraeff, 1993). The Global Spreading Hypothesis attributes the general increase in the spatial distribution of harmful species to shifts in ocean currents and environmental conditions, and to anthropogenic factors such as coastal eutrophication, physical alteration of shoreline regimes, and transport of invasive species in ballast water tanks of vessels or shellfish transplantation (Smayda, 2007, Anderson et al., 2012).

The spatio-temporal distribution of HABs ranges from sub-metre scales to hundreds of kilometres and such blooms may persist from days to months (Fig. 2.2). With notable exceptions, most HABs occur within coastal or shelf seas, including in adjacent estuaries, narrow fjords and coastal embayments, rather than in the open ocean. Some species, such as the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*) often accumulate in dense surface aggregations in daylight (Tester et al., 2008), whereas others tend to form thin layers within the water column, such as species of the diatom *Pseudo-nitzschia* (Velo Suárez et al., 2008).

Comprehensive assessment of HABs on relevant spatio-temporal scales and with appropriate resolution is crucial for effective management, to adequately address and respond to the event. Monitoring strategies comprise warning, prevention and mitigation of negative effects, but also require a thorough understanding of the underlying processes of HAB formation, magnitude and distribution for early warning measures (see Stumpf et al., 2003). HAB forecasting efforts must integrate long-term observational data from field surveys based on remote and *in situ* sensing technology with interpretative and numerical modelling (Table 2.1).
Figure 2.1. Examples of HAB taxa associated with blooms potentially detectable by bio-optical approaches. Clockwise from top left quadrant: the dinoflagellate *Dinophysis acuminata*, the pennate diatom *Pseudo-nitzschia* sp., and the dinoflagellates *Karlodinium* sp. and *Karenia* sp. Normarski interference light microscopy of field samples (Ebro Delta, NW Mediterranean) fixed in Lugol’s iodine solution.

Figure 2.2. Left: Algal bloom identified as that of the dinoflagellate *Akashiwo sanguinea* in early April along the Pacific coast off Peru (see Kahru *et al*., 2004), stretching from Lima to the important industrial and artisanal fishery landing sites San Andrés, El Chaco and Lagunillas around the Paracas Peninsula and Laguna Grande. Image acquired by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the NASA Aqua satellite on February 23, 2004. Credit to NASA/GSFC, Rapid Response. Right: Aerial photograph (19 June 2011) of a phytoplankton bloom attributed to the potentially ichthyotoxic haptophyte *Chattonella* sp. (identified by IRTA) near Ampolla, Tarragona, in the Ebro Delta, NW Mediterranean, off l’Arenal beach in the vicinity of aquaculture sites. Credit to the Agència Catalana de l’Aigua. Visualisation of bloom water masses is increased by enhanced colours and separation by drawn line.
Despite a few promising forecasting efforts in pilot areas (e.g., the Gulf of Maine, see Anderson et al., 2005a, McGillicuddy et al., 2008), the prediction of blooms and successful early warning of impending events are still considered a major challenge. The large range of detrimental effects caused by different HAB taxa and their biological complexity emphasizes the importance of species identification for detection and mitigation efforts. Furthermore, the high importance of spatial and temporal bloom dynamics in determining the magnitude and extent of HAB events underlines the need for real-time observational systems equipped with sensors capable of discriminating among key taxa while simultaneously providing quantitative information on a continuous basis throughout the water column. The detection of these extremely diverse phenomena has been approached by a variety of techniques, of which optical sensors present a highly suitable and non-intrusive means. A large variety of optical sensors have been applied for HAB detection on various spatial scales, and numerous approaches for phytoplankton detection exist, including those based on apparent optical properties (AOP) and inherent optical properties (IOP) (see Cunningham & McKee, 2013, Zielinski, 2013). Large-scale ocean and coastal zones can be surveyed synoptically and over large spatial scales (e.g., > 10 km$^2$) with sensors on satellites, whereas meso-scale HABs can be more effectively studied via airborne sensors. Localized blooms and small-scale HABs, particularly sub-surface aggregations, can be addressed with in situ bio-optical sensors providing information via profiling or from discrete depths on moored or mobile platforms. HAB-relevant technical development in the bio-optics sector is proceeding rapidly; a comprehensive volume on observational technologies for coastal ecosystems with a focus on HABs (Babin et al., 2005) is illustrative of the rapid advancements in this field. The question is how far the resolution of these sensors allows the detection of harmful algal species or toxins in natural waters, and how they otherwise contribute to HAB monitoring components.

The objective of this chapter is to address the diversity of HABs with appropriate optical approaches: (1) to outline optically distinguishable features of HABs; (2) to review existing sensors and sensor systems for continuous operational HAB assessment on various spatial scales (from satellites, airborne and above water or in situ underwater sensors) and their suitability for HAB species detection); and (3) to assess future perspectives for research on HAB monitoring with optical sensors.
Table 2.1. HAB monitoring components as listed in Stumpf et al. (2003) combined with but not directly corresponding to key issues and their indicators for HAB monitoring and forecasting (a, b, c, d). Routine field surveys with remote and *in situ* sensing are necessary for Type 1 and 2 components, whereas Types 3 and 4 include interpretative and numerical modelling.

<table>
<thead>
<tr>
<th>HAB monitoring component</th>
<th>Key issue</th>
<th>Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1: monitoring movement of previously identified HAB</td>
<td>a) Bloom location and dimension</td>
<td>a) Phytoplankton biomass increase/anomaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell/toxin concentration (in time and space)</td>
</tr>
<tr>
<td>Type 2: detecting new blooms as HAB or non-HAB</td>
<td>b) Species identification and composition</td>
<td>b) Morphology (cell size and shape)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pigment composition or (regional) marker pigment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unique or rare features (e.g., gas vacuoles)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Molecular targets (e.g., DNA/RNA)</td>
</tr>
<tr>
<td>Type 3: predicting the movement of an identified HAB</td>
<td>c) Toxin determination</td>
<td>c) Toxicity and toxin composition</td>
</tr>
<tr>
<td>Type 4: predicting conditions favourable for a HAB to occur where blooms have not yet been observed</td>
<td>d) Linked environmental parameters</td>
<td>d) Bloom transport and hydrodynamic vectors (winds and currents), temperature, salinity, CDOM, nutrients, etc.</td>
</tr>
</tbody>
</table>
2.2 Algal features for bio-optical assessment

Phytoplankton cells characteristically absorb, scatter, and fluoresce light, and thereby provide bio-optical information on algal cell size, shape and intracellular components. The success of bio-optical measurements in providing taxonomic information depends on the selection of an appropriate spectral resolution and/or appropriate wavelengths, as well as on interferences by external optically active substances, such as coloured dissolved organic matter (CDOM) (see Coble, 2013), and suspended non-algal particles. The extraction of discrete optical signatures from these bulk signals is still a major challenge, especially if the target species does not represent a large fraction of the overall phytoplankton biomass.

Most bio-optical approaches for algal discrimination are based on pigment signatures. Pigments are arranged in pigment-protein complexes in the thylakoid membranes of the chloroplast and function in light harvesting and photo-protection. Allocation within thylakoids hampers the determination of single pigments with bio-optics due to the packaging effect of pigments within the light harvesting complex. Pigment discrimination is, however, of high value for taxonomic, phylogenetic and physiological assessment of phytoplankton (Johnsen et al., 2011). The pigment chlorophyll $a$ (hereinafter denoted as Chl $a$) is a universal index for phytoplankton biomass (except for some prochlorophytes that contain divinyl-Chl $a$), and the Chl $a$ signature in the oceans has been derived from absorption and fluorescence properties for many decades (Kreps & Verjbinskaya, 1930, Lorenzen, 1966). As an indicator for algal bloom location and movement, Chl $a$ is often used in HAB detection even though this pigment is not species-specific.

Improvements in chemotaxonomic methods have led to the identification of more than 70 pigments that provide an array of chemotaxonomic markers for algal classes (Jeffrey et al., 2011). The presence of certain chlorophylls and accessory pigments in algal taxa allows the assignment to typical pigment suites (for HAB species see Table 2.2). Suites of algal group-specific ratios of extracted pigments have been incorporated into automated analysis systems to disentangle these predetermined groups from a mixed algal population. The software CHEMTAX (chemical taxonomy), is one of these automated computation entities that iteratively fits these suites to a measured natural pigment composition (Mackey et al., 1996, Lewitus et al., 2005). In oceanographic applications, this laboratory analysis of extracted phytoplankton pigments is widely used to discriminate between phytoplankton classes and has also been applied in a HAB context (Trice et al., 2004).
Table 2.2: Illustrative representative examples of pigment suites for HAB species given in the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae (Moestrup et al., 2009 onwards), plus certain members of the Pelagophyceae and Cyanophyceae. Pigment suites are adapted from data in Jeffrey et al. (2011), except for *Pseudo-nitzschia* spp. chlorophyll c grouping (Zapata et al., 2011). Chl a is present in all groups and is therefore not included. Dinoflagellate groups are separated into: 1) peridinin-containing; 2) pigments of haptophyte origin; 3) pigments of diatom origin; 4) pigments of cryptomonad origin; 5) pigments of prasinophyte origin (as an exception, represented by a non-HAB species*).

<table>
<thead>
<tr>
<th>Division</th>
<th>Class</th>
<th>Pigment group</th>
<th>Species</th>
<th>Chlorophylls</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterokontophyta</td>
<td>Bacillariophyceae</td>
<td>DIATOM 1</td>
<td><em>Pseudo-nitzschia australis</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DIATOM 2</td>
<td><em>Pseudo-nitzschia calliantha</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DIATOM 3</td>
<td><em>Pseudo-nitzschia fraudulenta</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td>Raphidophyceae</td>
<td>RAPIDO-1</td>
<td><em>Chlorastrum globosa</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td>Dictyochophyceae</td>
<td>DICTYO-1</td>
<td><em>Pseudo-chaetocystis farrinensis</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td>Pelagophyceae</td>
<td>PELAGO</td>
<td><em>Aureococcus anophagefferens</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td>Haptophyta</td>
<td>Prymnesiophyceae</td>
<td>HAPTO-4</td>
<td><em>Prymnesium parvum</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HAPTO-7</td>
<td><em>Chrysochromulina polyedra</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HAPTO-8</td>
<td><em>Phaeocystis poucheti</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td>Dinophyta</td>
<td>Dinophyceae</td>
<td>DINO-1</td>
<td><em>Amphidinium carterae</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DINO-2</td>
<td><em>Karenia brevis</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DINO-3</td>
<td><em>Kryptoperidinium foliaceum</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DINO-4</td>
<td><em>Dinophysis norvegica</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DINO-5</td>
<td><em>Gymnodinium chloroformum</em></td>
<td>× × × × ×</td>
<td>× × × × ×</td>
</tr>
<tr>
<td>Cyanophyta</td>
<td>Cyanophyceae</td>
<td>CYANO groups</td>
<td><em>Trichodesmium</em> spp.</td>
<td>× ×</td>
<td>× ×</td>
</tr>
</tbody>
</table>

** MG-2,4-divinyl pheophorpin a5 monomethyl ester; *** 7’, 8’dihydreonxanthin-20’-al-3’-β-lactoside
Certain accessory pigments are restricted to only few taxonomic classes or even individual species, and thus can serve as markers, e.g. peridinin (dinoflagellates, Type 1), alloxanthin (cryptophytes), and phycobilipigments (cyanobacteria, rhodophytes and cryptophytes). Pigment suites and markers often appear to cross taxonomic boundaries, due to symbiotic associations, phagotrophy upon other algae, or inheritance of chloroplasts and associated pigments from other algal classes over evolutionary time-scales. Dinoflagellates may thus contain pigments of haptophyte or cryptomonad origin; the presence of alloxanthin in the HAB dinoflagellate *Dinophysis norvegica* is an example of the latter acquisition (Meyer-Harms & Pollehne, 1998) (Table 2.2). Furthermore, the presence of a marker pigment may not always reflect the presence of the source organism in the water column, but rather their ingested cell relicts in mixotrophic species, including certain predatory dinoflagellates. The toxic species *Karenia brevis* belongs to a dinoflagellate group with pigments of haptophyte origin (Table 2.2). As a minor exception to most other dinoflagellates, species of the three related genera *Karenia*, *Karlodinium*, and *Takayama* lack peridinin, and instead contain fucoxanthin and derivatives as major accessory pigments. Most species of these genera also contain at least small amounts of the rare pigment gyroxanthin-diester or a similar pigment (hereinafter denoted as gyroxanthin); among the few exceptions are the non-toxic species *Karlodinium australae* (De Salas *et al.*, 2005) and *Takayama helix* (De Salas *et al.*, 2003). Gyroxanthin is highly suitable as a marker for *K. brevis*, as it is restricted to only a few algal species which are not typically co-dominant (Table 2.3). In *K. brevis* this pigment is consistently detectable and quantifiable through various physiological states (e.g. under different irradiance regimes), and has specific absorption characteristics that allow a definite determination (Millie *et al.*, 1995). Furthermore, the pigment ratio of gyroxanthin:Chl *a* corresponds to cell counts in cultures and natural waters and thus allows the determination of *K. brevis* abundance (Millie *et al.*, 1997, Berg *et al.*, 2004). By enhancement of spectral absorption characteristics of the algal cells by accessing the fourth derivative (Butler & Hopkins, 1970) and subsequent application of a similarity index with a *K. brevis* culture as a reference, this HAB species can be distinguished from other phytoplankton in mixed field samples (Millie *et al.*, 1997, Kirkpatrick *et al.*, 2000). The presence of gyroxanthin for the differentiation of *K. brevis* in phytoplankton assemblages has been successfully incorporated into CHEMTAX (Örnólfsdóttir *et al.*, 2003) and even though on a global basis the pigment is not unique to the species, in a defined region such as the Florida coast where *K. brevis* is the overwhelmingly dominant producer, this pigment can be used as a reliable species-specific marker.

Algal species display morphological features, such as cell size and shape, which serve in the identification and enumeration of cells in natural water samples with traditional light microscopy. Bio-optical characteristics of these features are exploited in imaging flow
cytometry, providing size distributions in natural samples through scattering and also images of single cells (Sieracki et al., 1998). This allows the detection and discrimination of harmful genera or even species, as shown for Dinophysis spp. (Campbell et al., 2010). Cell size distributions have also been derived by bulk optical signals from remote applications (Ciotti et al., 2002, Hirata et al., 2008, Fujiwara et al., 2011), and have led to the differentiation of HABs of *K. brevis* from non-HABs, due to weak backscattering of the large sized cells of this species on a regional basis (Cannizzaro et al., 2008b).

The intracellular organisation of algal cells provides further characteristics, such as the alignment of thylakoids, storage material, and shape of the nucleus or presence of gas vesicles. Many bloom-forming cyanobacteria change their position in the water column by means of gas vacuoles (see examples in O’Neil et al., 2012), and as these change the refraction index of light, backscattering signals are augmented across all wavelengths (Subramaniam et al., 1999). This has been incorporated into specific remote sensing algorithms for the cyanobacterial HAB genus *Trichodesmium* (Westberry et al., 2005).

Furthermore, considerable spectral information, e.g. for *K. brevis*, can be gained by dividing the cell into multiple compartments, including cell size, density (‘granularity’) and internal components, such as nucleic acids and proteins (Spear et al., 2009). This spectral approach to internal features on a cellular level may also prove useful for discrimination of other HAB taxa, e.g. gonyaulacoid dinoflagellates such as *Alexandrium* spp. contain a characteristically large horseshoe-shaped nucleus (Figueroa et al., 2006).

Many toxic phytoplankton species contain mycosporine-like amino acids (MAA) (Vernet & Whitehead, 1996, Callone et al., 2006). The function of these components is not yet fully understood (Singh et al., 2008), although they have often been attributed to photo-protection. MAAs have absorption maxima in the UV range (between 310 and 340 nm) and this characteristic has been exploited for HAB detection, e.g. for cells of the cyanobacterium *Trichodesmium* (Subramaniam & Carpenter, 1999). When excreted into the water, e.g. by the harmful dinoflagellate *Lingulodinium polyedrum*, MAAs also contribute to optically detectable CDOM and UV absorption (Vernet & Whitehead, 1996, Whitehead & Vernet, 2000). CDOM in turn may an indirect bio-optical indication for harmful species by marking a species-specific environmental setting that is associated with or leads to bloom formation, such as high CDOM concentrations linked to *Trichodesmium* blooms (Steinberg et al., 2004).

Certain toxins extracted from toxigenic microalgae, such as DA (Bouillon et al., 2008), karlotoxins (Bachvaroff et al., 2008), and brevetoxin B-type (Satake et al., 2005), absorb light in the UV range due to the presence of conjugated double-bonds. UV absorption has been applied for qualitative and quantitative detection of these toxins in cell-free algal extracts and
Table 2.3. Phytoplankton species that contain the rare gyroxanthin-diester (or gyroxanthin-diester-like) pigment. In cases of HAB species harmful effects are indicated; non-HAB taxa are indicated in bold.

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Reference for gyroxanthin (or like)</th>
<th>Harmful effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinophyceae</td>
<td><em>Karenia brevis</em></td>
<td>Bjørnland <em>et al.</em>, 2003</td>
<td>Marine animal mortalities, neurotoxic shellfish poisoning, respiratory irritation</td>
</tr>
<tr>
<td></td>
<td><em>Karenia mikimotoi</em></td>
<td>Hansen <em>et al.</em>, 2000, Johnsen &amp; Sakshaug, 1993</td>
<td>Fish and invertebrate mortality. A cytotoxic polyether, named gymnocin-A, has been isolated from a Japanese culture [1]</td>
</tr>
<tr>
<td></td>
<td><em>Karenia cristata</em></td>
<td>Botes <em>et al.</em>, 2003</td>
<td>Fish killing. Cultured seaweed (Porphyra tenera) also affected, resulting in irregular cell growth [1]</td>
</tr>
<tr>
<td></td>
<td><em>Karenia umbella</em></td>
<td>De Salas <em>et al.</em>, 2004</td>
<td>Implicated in fish kills in Tasmania - rainbow trout and salmon [1]</td>
</tr>
<tr>
<td></td>
<td><em>Karenia papilionacea</em></td>
<td>Laza-Martinez <em>et al.</em>, 2007</td>
<td>Produces suspected brevetoxin or brevetoxin-like compounds as indicated by immunosassay (ELISA), but has not been confirmed with LC-MS (L. Flewelling, Florida Fish and Wildlife Conservation Commission, pers. comm., in: Steidinger <em>et al.</em>, 2008)</td>
</tr>
<tr>
<td></td>
<td><em>Karlodinium decipiens</em></td>
<td>Laza-Martinez <em>et al.</em>, 2007</td>
<td>Toxic to a range of marine invertebrates and fish. Produces karlotoxins [1]</td>
</tr>
<tr>
<td></td>
<td><em>Karlodinium veneficum</em></td>
<td>Bjørnland &amp; Tangen, 1979, Bjørnland <em>et al.</em>, 2000, Johnsen &amp; Sakshaug, 1993</td>
<td>Ichthyotoxic [1]</td>
</tr>
<tr>
<td></td>
<td><em>Karlodinium armiger</em></td>
<td>Garcés <em>et al.</em>, 2006</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td></td>
<td><em>Takayama tasmanica</em></td>
<td>De Salas <em>et al.</em>, 2003</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td>Prymnesiophyceae</td>
<td><em>Emiliania huxleyi</em></td>
<td>Zapata <em>et al.</em>, 2004, in Zapata, 2005, Seoane <em>et al.</em>, 2009</td>
<td>P. globosa was reportedly toxic in China, but the short published abstract p only mentions non-specific hemolysin(s) [Qi <em>et al.</em>, 2002 in 2].</td>
</tr>
<tr>
<td></td>
<td><em>C. acanthi, C. pringsheimii</em></td>
<td>Seoane <em>et al.</em>, 2009</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td></td>
<td><em>C. simple C. cf cymbium, C. thronsdennii</em></td>
<td>Seoane <em>et al.</em>, 2009</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td></td>
<td><em>Isochrysis galbana</em></td>
<td>Seoane <em>et al.</em>, 2009</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td></td>
<td><em>Phaeocystis globosa</em></td>
<td>Seoane <em>et al.</em>, 2009</td>
<td>Ichthyotoxic (C. leadbeateri) [2]</td>
</tr>
<tr>
<td>Pelagophyceae</td>
<td><em>Pelagomonas calceolata</em></td>
<td>Bjørnland <em>et al.</em>, unpublished* in Bjørnland et al., 2003</td>
<td>Adversely impacts the growth, survival, and reproduction of grazers (e.g. shellfish) and underlying vegetation (Gobler &amp; Sunda, 2012)</td>
</tr>
<tr>
<td></td>
<td><em>Pelagococcus subviridis</em></td>
<td>Jeffrey &amp; Zapata (cited in Zapata, 2005)</td>
<td>Adversely impacts the growth, survival, and reproduction of grazers (e.g. shellfish) and underlying vegetation (Gobler &amp; Sunda, 2012)</td>
</tr>
<tr>
<td></td>
<td><em>Aureococcus anophagefferens</em></td>
<td>Jeffrey &amp; Zapata (cited in Zapata, 2005)</td>
<td>Adversely impacts the growth, survival, and reproduction of grazers (e.g. shellfish) and underlying vegetation (Gobler &amp; Sunda, 2012)</td>
</tr>
</tbody>
</table>

solvent matrices, but the method is not very sensitive (Quilliam, 2003). As a consequence of this low sensitivity, UV absorption properties for toxigenic cells based upon their toxin content have not been successfully exploited in vivo nor in situ in natural bloom populations. Such absorption approaches are completely ineffective with respect to other phycotoxins, such as spirilides, azaspiracids, paralytic shellfish poisoning toxins, yessotoxins and pectenotoxins, which do not contain such chromophores.

For dinoflagellates that form resting cysts, these may be an indicator for upcoming bloom events, as shown for the forecasting of *Alexandrium* blooms in the Gulf of Maine (Anderson *et al.*, 2005b). The optical assessment of cysts during their presence in the water column has been attempted in a few studies, e.g. through characteristic features in absorption spectra of field samples with a high amount of mixed algal cysts (Barocio-León *et al.*, 2008).

In summary, there are no optically detectable features that allow the taxonomic discrimination of algal cells in natural assemblages at the species level by means of unique AOPs and IOPs. Nevertheless, the combination of different characteristics, such as pigment signatures, inner- and outer-cell structure, combined with regional knowledge on favourable environments and historical HAB patterns, provide a basis to address all crucial levels of HAB detection.

### 2.3. Scale and resolution in surveillance of algal blooms

The high diversity of HABs in terms of assemblage behaviour, adaptive strategies and taxonomic variety necessitates multidimensional observational systems for nowcasting and early warning. In the following section, examples of HAB detection by their optically detectable features at different spatio-temporal scales and levels of taxonomic specificity are given for remote sensing and in situ applications.

#### 2.3.1 Remote sensing

The practical access of satellite-based systems for HAB detection began in the late 1970s. The tracking of a *K. brevis* bloom in Florida with MSS 5 imagery from the ERTS-1 satellite (Murphy *et al.*, 1975) was among the first of such applications. A turbidity patch outlined by a Coastal-Zone Color Scanner (CZCS) prototype in 1977 could be aligned to the same species (Mueller, 1979 in Steidinger & Haddad, 1981). In 1978, data from the CZCS on the Nimbus-7 satellite were used for the delineation of blooms of this dinoflagellate via high chlorophyll discoloured water patches in Florida (Steidinger & Haddad, 1981, Tester & Steidinger, 1997, for history on *K. brevis* see Steidinger, 2009). Back then, data acquisition and processing were assigned to a time delay, but the following satellite generation already met the near real-time demands of HAB monitoring. In 1999, Sea-viewing Wide Field-of-view Sensor (SeaWiFS) satellite imagery was routinely integrated into a HAB monitoring system within the NOAA
CoastWatch programme in the Gulf of Mexico. Data on *K. brevis* blooms were derived with a regional bio-optical algorithm and an anomaly flag that compared Chl *a* values with the mean value of two antecedent months with a time span of 2 weeks from the new dataset (Stumpf *et al.*, 2003).

Since these early studies, detection of algal blooms by satellite-based sensors has been approached via various optical properties of phytoplankton and by applying alternative analytical methods, such as defining empirical relationships, semi-analytical models, anomalies and indexation. The concentration of the pigment Chl *a*, as well as the normalized water leaving radiance (*L*<sub>wn</sub> (λ)) (Gordon & Clark, 1981), belong to the baseline satellite-based ocean colour sensor products (listed in Mueller *et al.*, 2003). Chl *a* concentration is widely derived by an empirical relationship of *L*<sub>wn</sub> (λ) or remote sensing reflectance (*R*_<sub>rs</sub> (λ)) and Chl *a* (O’Reilly *et al.*, 1998). Standard empirical algorithms for Chl *a* retrieval, such as the Ocean Chlorophyll (OC) algorithms, use a radiance ratio of two bands or a maximum band ratio (O’Reilly *et al.*, 1998). Improved versions of the OC maximum band ratio algorithms exist for the most commonly used sensors, such as SeaWiFS (OC4), CZCS (OC3C), moderate resolution imaging spectroradiometer (MODIS) (OC3M), ocean colour temperature scanner (OCTS) (OC4O), and medium resolution imaging spectrometer (MERIS) (OC4E) (O’Reilly *et al.*, 2000), as well as global imager (GLI) (Mitchell & Kahru, 2009).

In optically complex waters, the derived Chl *a* values are often biased, and calculated high concentrations may in fact be due to artefacts owing to the abundance of high CDOM or suspended matter concentrations (Tang *et al.*, 2003, Ahn *et al.*, 2006). To more accurately gather single constituents of highly complex water ingredients and thus increase the accuracy for Chl *a* estimations, semi-analytic (or semi-empirical) models have been developed based on spectral matching (see O’Reilly *et al.*, 1998, Lee *et al.*, 2002, Maritorena *et al.*, 2002) or neural networks (Tanaka *et al.*, 2004). These algorithms allow the inversion of single reflectance spectra to IOPs of seawater (backscatter and absorption coefficients) and thus to multiple optically active constituents. The capability of these models depends on the precise parameterisation of these IOPs. The GSM (Garver-Siegel-Maritorena) (Maritorena *et al.*, 2002) and the QAA (Quasi Analytical Algorithm) (Lee *et al.*, 2002) are the principal algorithms used for HAB detection and both are also included in the SeaWiFS Data Analysis System (SeaDAS) software. Whereas it is possible to derive more products with inversion algorithms, they are, however, quite complex and thus not always the first choice for robust derivation of Chl *a*. Another challenge in the application of semi-analytic algorithms is their sensitivity to atmospheric correction procedures, especially at lower wavelengths. To separate algal patches from other constituents, further statistical approaches can be applied. As an example, Ahn *et al.* (2006) used a supervised classification scheme (forward principal
component analysis and minimum spectral distance), widely employed for land applications, to distinguish patches of the fish-killing dinoflagellate *Cochlodinium polykrikoides* in Korean coastal waters from sediment dominated and mixed areas in coastal zones with high spatial resolution Landsat-7 ETM+ images.

Other approaches for the delineation of Chl *a* take advantage of the sun-induced natural fluorescence of the pigment. Among these, the fluorescence line height (FLH) algorithm is the most prominent, taking into account the fluorescence maximum of Chl *a* (approximately at 683 nm) and a baseline of two more wavelengths for backscatter correction, as described for MODIS with bands centred at 665.1, 676.7 and 746.3 nm (Letelier & Abbott, 1996). This algorithm has been used in combination with the OC3M to estimate Chl *a* for investigation of blooms of the toxic dinoflagellate *K. brevis* (Hu *et al.*, 2005) and, combined with enhanced true-colour images (red-green-blue [RGB]), revealed the movement of a *C. polykrikoides* bloom in the Persian Gulf (Moradi & Kabiri, 2012). As an alternative to chlorophyll absorption, backscattering can serve as a proxy for phytoplankton biomass (Dall’Olmo *et al.*, 2009) if phytoplankton is the dominant scattering component in the water. An advantage of light scattering properties is that they are less dependent on physiological forcing that might induce intracellular changes in pigment amount and packaging than Chl *a*.

By detection of Chl *a* or backscattering properties alone from these satellite-based measurements it is possible to detect plankton aggregations but not necessarily the presence of a HAB. There have been investigations towards the differentiation of HAB and non-HAB blooms, and also regional approaches for the retrieval of a few harmful algal species. Ahn & Shanmugam (2006) presented a red tide index for a regional study in Korean waters. Their index is based on the assumption that due to an increased cell abundance of the bloom species, light at lower green to blue wavelengths is more strongly absorbed whereas green wavelengths is are more strongly reflected. The combined ratio of three bands, water-leaving radiance at the wavelengths 510/555 normalized with the absolute values of water-leaving radiance at 443 nm, was used to quantify the ‘redness’ of a bloom, with the highest possible redness indexed with ‘1’.

Another approach to producing a red tide index is based on increased concentration of MAAs in dinoflagellate blooms and thus high absorption in the UV. This red tide UV index accesses the 380 nm band that was available on GLI, in the ratio of $L_{wn}$ 380/412 (Mitchell & Kahru, 2009), and was presented for the differentiation of the HAB dinoflagellate *Lingulodinium polyedrum* (formerly *Gonyaulax polyedra*) (Kahru & Mitchell, 1998). Inversion models have also been used to infer different phytoplankton groups. Phytoplankton absorption spectra can be derived empirically with the QAA directly from measured remote sensing reflectance-absorption spectra and can thus be used without prior knowledge of the
spectral shape of absorption spectra (Lee & Carder, 2004). Accessory pigments of phytoplankton can be derived from hyperspectral absorption spectra, and thus valuable information for distinguishing phytoplankton classes is obtained (Hoepffner & Sathyendranath, 1993). The GMS was extended and adapted for cyanobacteria, specifically for *Trichodesmium* spp., with a specific reflection model, taking into account some of the distinct optical properties of the cyanobacterium (Westberry *et al.*, 2005). *Trichodesmium* spp. were also tracked based upon the high reflectivity resulting from their gas vacuoles and phycoerythrin absorption from CZCS data along with ground-truthing in Atlantic, Indian and Pacific regions (Subramaniam & Carpenter, 1994). High reflection has also served for the detection of annual high biomass blooms of other cyanobacteria, such as *Nodularia spumigena* in the Baltic Sea, with a supervised classification method with the Advanced Very High Resolution Radiometer (AVHRR) (Kahru *et al.*, 1994, 2000, Kahru, 1997). In a study for the suitability of various sensors for the mapping of cyanobacterial blooms in the Baltic Sea both MERIS and MODIS fine resolution bands were capable of quantitative detection of cyanobacterial blooms, but only MERIS sensors have suitable bands for the specific detection of cyanobacteria by accessing the phycocyanin absorption feature near 630 nm and a peak near 650 nm (Reinart & Kutser, 2006). Algorithms for phycobilin retrieval as an indicator for cyanobacteria mainly target this phycocyanin absorption around 620 nm (Simis *et al.*, 2006) and have been applied to satellite-based sensors such as MERIS (here for inland waters) (Simis *et al.*, 2006) and Hyperion (Kutser, 2004). The Ocean Colour Monitor (OCM), now on Oceansat-1 has also been tested for phycocyanin retrieval (Dash *et al.*, 2011). OCM measurements are comparable to the by now decayed SeaWiFS, but have a high spatial resolution of 360 m for local area products.

In contrast to the high reflectance of cyanobacteria, *K. brevis* blooms backscatter weakly. The ratio of Chl *a* to the particulate backscattering coefficient (*b*<sub>bp 550</sub>) was successfully applied to SeaWiFS data to differentiate *K. brevis* blooms from diatom blooms with higher backscattering (Cannizzaro *et al.*, 2008b). This technique may be inversely applied for the detection of high biomass diatom blooms, such as those of *Pseudo-nitzschia* spp..

Among the major limitations of satellite data for surveillance of HABs are the relatively large pixel sizes, i.e. low spatial resolution. Most sensors are only appropriate for detecting blooms over larger spatial scales, but priority areas for HAB monitoring are typically coastal, and often relevant bloom patches are too small to be effectively resolved. Whereas a typical pixel size of ocean colour products derived by MODIS is 1 km 2, there are also medium resolution bands of 250 and 500 m available. Kahru *et al.* (2004) accessed these medium resolution bands for the detection of a coastal bloom in Paracas Bay, Peru, an important location for commercial and artisanal fisheries (Fig. 2.2). In this case study, dimension and
concentration of bloom of the HAB dinoflagellate *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum*) were retrieved with MODIS medium resolution bands. Bloom boundaries were made visible with an RGB image, while phytoplankton concentration was calculated with the turbidity index of particulate material, consisting mainly of phytoplankton.

The use of pigment signatures for HAB detection is limited where the causative species are not contributing significantly to the overall phytoplankton biomass. Many examples for such highly toxic but low biomass blooms are associated with members of the dinoflagellate genus *Alexandrium* (McGillicuddy *et al.*, 2008, Anderson *et al.*, 2012). Indirect links to biological and physical oceanographic patterns provide the possibility to track such blooms via advection of water masses. Luerssen *et al.* (2005) connected *A. fundyense* populations and toxicity along the western Gulf of Maine with AVHRR derived sea surface temperature (SST) patterns, and documented the utility of these data for monitoring and prediction of conditions that are linked to toxic events. The SST data can also be used to follow the water masses of blooms in real-time (e.g., Tester & Steidinger, 1997).

In areas with large seasonal differences in water masses, a seasonal splitting of methodologies may be appropriate for the detection of harmful taxa. The complexity of the water components may strongly differ between seasons and relevant dynamics, such as of nutrients, may be missed by remote sensing models. A chlorophyll anomaly method with SeaWiFS data correlated well during the *K. brevis* bloom season from August to April on the west coast of Florida, whereas in the spring and early summer period anomalies were falsely observed (Tomlinson *et al.*, 2004). As suggested by Anderson *et al.* (2011), a fall/winter model based on the satellite derived Chl *a* anomaly method for *Pseudo-nitzschia* spp., and a subsequent spring/summer model with focus on simulations of nutrient levels may be feasible to distinguish toxic *Pseudo-nitzschia* spp. from other phytoplankton in the Santa Barbara Channel.

HAB detection via satellites only targets algal populations at or near the surface, deeper or sub-surface fine-structured layers of phytoplankton remain undetected. Furthermore, the temporal resolution of data acquisition is dependent on overflight frequency over the desired area and the quality of the data with respect to cloud coverage. An example to increase the revisit intervals is given by RapidEye – a constellation of five satellites with identical sensors in the same orbital plane (DLR, 2013b). Since the first use of CZCS for satellite-based HAB detection, a newer generation of successors, such as MODIS and SeaWIFS, has enabled near real-time monitoring of HABs. The SeaWIFS mission ended in December 2010, and ENVISAT with MERIS stopped communication in spring 2012, but new sensors have followed and more are in development, some of which are already addressing the need for hyperspectral data acquisition for ocean colour. In 2014, the launch of OLCI, a MERIS
ENVISAT heritage, is planned with Sentinel-3; the wavelength centres are as for MERIS plus an additional six bands. The launch of EnMAP (Environmental Mapping and Analysis Program), with the HIS hyperspectral imager possessing 94 spectral bands between 420 and 1000 nm with a ground sampling distance of 30 m², has been scheduled for 2015 (DLR, 2013a).

Despite evident limitations in species retrieval, satellite-based observations meet two of the components for HAB management as listed in Stumpf et al. (2003), Type 1: monitoring the movement of an algal bloom that has previously been identified as a HAB; and Type 2: detecting new blooms as HAB or non-HAB. Detection and monitoring movement of HABs has been feasible for different algal species in a wide range of environments, and from various satellite platforms (Table 2.4). The identification of species via satellite is not directly possible, but some species with distinct features can be tracked in regional settings, where they are known to occur regularly. Examples include K. brevis blooms on the Florida coast or those of certain cyanobacterial species in the Baltic Sea. For other blooms, the prior identification of a harmful species by in situ characterisation is necessary.

Reports from airborne sensing systems on sea surface coloration have been available for many decades, including serial aerial surveys for algal blooms that have been recommended earlier and later implemented as a monitoring strategy for red tides in Florida (Ingle et al., 1959). Such aerial surveys are still being conducted, even though nowadays regular monitoring of large sea surface areas is broadly covered by satellite-based sensors. In many areas, airborne surveillance of coastal areas is undertaken only upon demand, e.g. during oil spills or where potentially harmful algal blooms have been observed beforehand.

Airborne sensors for ocean colour measurements are widely used to validate satellite data, but can also be employed to derive Chl a concentrations and thus serve a valuable function for detection and determination of spatial dimensions and movement of algal blooms. The appropriate algorithms are essentially similar to those described for satellite-based observations and rely on empirical and semi-empirical relationships. In a HAB context, the SeaWiFS OC4v4 algorithm was used for calculation of Chl a concentrations during an algal bloom in Monterey Bay, from data retrieved by an aircraft-mounted Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) in swaths of 11 km (Ryan et al., 2005). The extent and dynamics of a toxic Pseudo-nitzschia bloom was surveyed by aircraft equipped with multi-spectral radiance sensors; in combination with field measurements, pigment concentrations could be related to these spectral data with local empirical algorithms (Sathyendranath et al., 1997). There exists a large variety of commercially available sensors with high spectral resolution capable of generating data that can be inverted to hyperspectral absorption spectra of phytoplankton pigments (Lee & Carder, 2004). Hyperspectral absorption spectra of
phytoplankton pigments can be used to differentiate among major phytoplankton groups (Hoepffner & Sathyendranath, 1993). There are a handful of examples for the delineation of algal species in high biomass blooms based upon hyperspectral airborne radiometer data. For example, for the detection of *K. brevis*, absorption spectra were calculated with the QAA (Lee *et al.*, 2002), and these were compared to directly measured absorption spectra of *K. brevis* as a reference, to then apply an similarity index (Millie *et al.*, 1997) based on fourth derivative calculations for the rare pigment gyroxanthin. Even though high CDOM concentrations and low cell concentrations would impede the approach, the applicability of *K. brevis* detection based on hyperspectral remote sensing reflectance data is shown in a case study from Florida (Craig *et al.*, 2006). For high biomass blooms, Roesler & Boss (2004) generated an inverse ocean colour model with reflectance as a function of phytoplankton biomass, composition, and size distribution. The algal absorption spectrum was further divided into five algal group components: for diatoms, dinoflagellates, cryptophytes, and chlorophytes. Additionally, large cells of species of the toxic dinoflagellate genus *Dinophysis* that were present in high concentration could be separated. In this case, spectral reflectance was calculated with hyperspectral downward irradiance and upward radiance data (from a Satlantic Hyperspectral Tethered Spectroradiometer Buoy), with irradiance derived in air at the surface and upwelling spectral radiance within one meter below the sea surface. The five algal groups and one potentially toxic genus could thus be distinguished by simple radiometric measurements. Despite the fact that some basic model vectors were retrieved by microscopic and spectrophotometric laboratory analysis, these results display the sensitivity of hyperspectral ocean colour signals for determination of algal cell concentration, composition, and size distribution.

In contrast to satellite-based systems, an atmospheric correction factor is not essential for airborne measurements close to the sea surface, and a higher spatial resolution (metre-scale) allows the detection of smaller algal blooms. Aircraft-mounted systems are highly flexible in that they can be rapidly deployed to target regions, whereas sensor systems mounted on other platforms can provide continuous data. Hyperspectral sensors mounted on aircraft or other monitoring platforms, such as floating buoys or anchored poles, provide high spectral resolution and thus the possibility for resolving taxon-specific differences. Nevertheless, in attempts to identify spectral regions characteristic of algal pigments via absorption and fourth derivative calculations based upon airborne measurements, possible interferences must be taken into account, e.g. with Fraunhofer lines at 431 nm, thereby pointing out the necessity to critically review the interpretation of absorption bands from remote sensing data with respect to phytoplankton pigments (Szekielda *et al.*, 2009).
Table 2.4: Case studies on bio-optical HAB detection from biomass to species-level with instrumentation mounted on satellite-, aircraft- or *in situ* platforms.

<table>
<thead>
<tr>
<th>Sensor (platform)</th>
<th>Method</th>
<th>Feature detected</th>
<th>HAB taxon</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CZCS (Nimbus-7)¹</td>
<td>Reflectance</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;</td>
<td><em>Karenia brevis</em></td>
<td>Steidinger &amp; Haddad, 1981</td>
</tr>
<tr>
<td>CZCS (Nimbus-7)²</td>
<td><em>Trichodesmium</em>-protocol</td>
<td>Phycoerythrin, Chl and high reflectance due to gas vacuoles</td>
<td><em>Trichodesmium</em> spp.</td>
<td>Subramaniam &amp; Carpenter, 1994</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>Regional optical algorithm and anomaly flag; complementary physical data.</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;</td>
<td><em>Karenia brevis</em></td>
<td>Stumpf et al., 2003</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³ &amp; Landsat-7 ETM+</td>
<td>OC4v4 and supervised classification scheme (FPCA and MSD)</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;</td>
<td><em>Cochlodinium polykrikoides</em></td>
<td>Ahn et al., 2006</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>OC4 and normalized ratio</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt; and Red Tide Index ‘Redness’</td>
<td><em>Cochlodinium polykrikoides</em></td>
<td>Ahn &amp; Shannugam, 2006</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>Semi-analytical and adapted GSM</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;, <em>Trichodesmium</em>-specific</td>
<td><em>Trichodesmium</em> spp.</td>
<td>Westberry et al., 2005</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>Semi-analytic</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt; and particulate backscattering coefficient</td>
<td><em>Karenia brevis</em></td>
<td>Cannizzaro et al., 2008</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>Red tide index Chl algorithm</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt; and Red Tide Index ‘Redness’</td>
<td><em>Karenia brevis</em></td>
<td>Chu &amp; Kuo, 2010</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>Bio-optical algorithm</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt; and Sea surface temperature with AVHRR</td>
<td><em>Gymnodinium catenatum</em></td>
<td>Tang et al., 2003</td>
</tr>
<tr>
<td>MODIS (Aqua)⁴</td>
<td>OC3M and FLH</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;</td>
<td><em>Karenia brevis</em></td>
<td>Hu et al., 2005</td>
</tr>
<tr>
<td>MODIS (Aqua/Terra)⁴</td>
<td>True-colour images (red-green-blue) Reflectance</td>
<td>Distribution (colour signature) Turbidity (as proxy for cell concentration)</td>
<td><em>Akashiwo sanguinea</em></td>
<td>Kahru et al., 2004</td>
</tr>
<tr>
<td>MODIS (Aqua)⁴</td>
<td>OC3 Semi-analytic</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt; Particulate backscatter</td>
<td><em>Karenia brevis</em></td>
<td>Carvalho et al., 2011</td>
</tr>
<tr>
<td>MODIS (Aqua)⁴</td>
<td>Semi-empirical and linear HAB classifier</td>
<td>HAB likelihood map</td>
<td><em>Karenia mikimotoi</em></td>
<td>Davidson et al., 2009</td>
</tr>
<tr>
<td>SeaWiFS (OrbView-2)³</td>
<td>OC4 Semi-analytic (QAA) and band ratio/OC3</td>
<td>Chl*&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Cyanobacteria</td>
<td>Reinart &amp; Kutser, 2006</td>
</tr>
<tr>
<td>MERIS (ENVISAT)⁵</td>
<td>Algal 2 (semi-analytic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperion (EO-1)⁵</td>
<td>Semi-analytic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.2 In situ ocean sensing

Approaches for bulk properties

For continuous monitoring of phytoplankton in the water column, a large range of bio-optical instruments, equipped with multi- to hyper-spectral sensors, are commercially available (see review of Moore et al., 2009). Systems for deployment of subsea sensors include buoys, semi-permanent structures, such as moorings, wind mill masts and oil platforms, as well as autonomous underwater vehicles, drifting profilers and flow-through systems on research
vessels. Many more alternative underwater systems are available for the measurement of at least the basic parameters: phytoplankton biomass proxy, turbidity, salinity, and temperature (ICES, 2009). As for all measurements of bulk optical properties, the performance of these instruments depends on the concentration of algal cells and of other optically active water constituents, such as CDOM and suspended matter. As a passive optical approach, the diffuse attenuation coefficient ($K_d$), e.g., calculated with two or more radiometers at different depths, or a profiling radiometer may be used to derive information on phytoplankton assemblages (Cembella et al., 2005) (Fig. 2.3). The measurement of Chl $a$ by prompt fluorescence has been performed in situ for decades (Lorenzen, 1966) as a simple and economical means for rapid phytoplankton biomass estimation. Fluorescence techniques are based on the inner structure of the Photosystem II (PS II) that consists of a Chl $a$ containing core antenna and a peripheral antenna, which varies among algal groups and is often species-dependent. Chl $a$ measurements are generally derived from fluorescence emissions around 685 nm from PS II (Yentsch & Menzel, 1963). The peripheral antenna influences the excitation spectrum and the colour of algal cells.

Besides the applications for Chl $a$ detection as a proxy for algal biomass, there are some bio-optical systems that offer an increased taxonomic resolution, including underway sensing. Phycocyanin fluorescence measurements have been obtained with a flow-through system based on ships of opportunity to assess bloom-forming filamentous cyanobacteria in the Baltic

![Fig. 2.3. Deployment of a Satlantic tethered attenuation coefficient chain sensor (TACCS) at a shellfish aquaculture site in a coastal fjord at Ship Harbour, Nova Scotia. Such a system is capable of continuous autonomous monitoring of passive optical signatures (‘ocean colour’), yielding downwelling irradiance ($E_d$) at the surface and upwelling radiance ($L_u$) at different depths, from which the diffuse attenuation coefficient $K_d$ can be calculated. TACSS monitoring systems can be equipped multi- or hyperspectral sensors on the K-chain for continuous monitoring of HABs and seston depletion and advection. Photo courtesy of Diego Ibarra, Dept. of Oceanography, Dalhousie University, Halifax, Canada.](image)
Sea (Seppälä et al., 2007). For detection of other algal groups, commercially available instruments can exploit different fluorescence excitation wavelengths. Beutler et al. (2002) describe a submersible instrument with excitation wavelengths of 450, 525, 570, 590 and 610 nm, and emission measured at 680 nm for the decomposition of ‘spectral groups’ of microalgae (green group, Chlorophyta; blue, cyanobacteria; brown, Heterokontophyta, Haptophyta, Dinophyta; mixed, Cryptophyta) that are characterised by a specific excitation spectrum (Beutler et al., 2002). The instrument has been successfully deployed on ships for the differentiation of algal groups in natural communities (Richardson et al., 2010), and forms also part of FerryBox automated monitoring systems on ships of opportunity in the North Sea (Petersen et al., 2011). In combination with instruments that provide information on cell size, such as the laser in situ scattering and transmissometry (LISST) system (Anglès et al., 2008), ‘spectral groups’ can be further subdivided to possible taxonomic divisions.

In addition to algal group-specific systems, submersible instruments have been designed for the identification of a few algal species. The optical plankton discriminator (OPD) is one of the most advanced in situ instruments for the detection of HABs via a bulk optical signal. The instrument is also called ‘Brevebuster’, as it aims at the detection of K. brevis blooms by enhancing the spectral absorption signature of the rare pigment gyroxanthin by augmenting the pathway with a liquid waveguide capillary cell (LWCC) as a sampling cuvette, attached to a fibre-optic spectrometer. The instrument has been successfully applied to detect and to specifically monitor the presence and concentration of K. brevis cells in red tides in Florida (Kirkpatrick et al., 2000). The OPD has been integrated into AUVs and served to quantify short term movement of K. brevis blooms (Robbins et al., 2006). Deployed on such platforms, the instrument thus holds a large potential for local species-specific delineation of bloom dimension and movement and thus greatly improves monitoring and predictive efforts.

Cellular-based approaches

In situ techniques for the accurate examination of individual algal cells have already proven to be a valuable adjunct to the extraction of algal optical signatures from bulk properties of water samples, particularly with respect to HAB taxa. With flow cytometers, light scattering by algal cells provides information about particle size and internal structure (e.g., ‘granularity’), whereas multi-channel fluorosensors provide information on pigment composition and concentration. Fluorescence with 435–470 nm excitations and an emission range of 520–700 nm distinguishes Chl a - and phycobilin-containing phytoplankton from other particles. Imaging flow cytometers, such as the FlowCAM®, can also provide images of single cells thereby yielding detailed morphotaxonomic features. Thus, real-time cell counts, cell size measurements, cell images, and a dot-plot of particle size and fluorescence allow rapid characterisation of phytoplankton in natural samples (Sieracki et al., 1998). Such a system has
been transferred to an automated submersible flow cytometer, the Flow CytoBuoy that is capable of in situ high-frequency monitoring of up to one sample every 5 min (Dubelaar et al., 1999). This instrument has provided real-time imaging of dominant phytoplankton species based on pulse-shape analysis, including the HAB haptophyte *Phaeocystis* spp., even though species of this genus tend to form amorphous, gelatinous colonies (Rutten et al., 2005, for detailed presentation of CytoBuoy see Thyssen et al., 2008).

The FlowCytobot is a similar device, but it is not autonomous and must be connected to the shore by power and communication cables (Olson et al., 2003). The Imaging FlowCytobot (IFCB) is a more recent development based upon the FlowCytobot (Olson & Sosik, 2007a). Manual inspection of IFCB images revealed a bloom of the toxic dinoflagellate *Dinophysis* spp. that could be followed from emergence until decline by automated classification over several months in 2008, from a pier laboratory at the entrance to the Mission-Aransas estuary, Gulf of Mexico (Campbell et al., 2010). The continuous measurement at a high temporal resolution further revealed increased cell abundances during water flow into the estuary; this indicated an offshore origin of the bloom. Although there are difficulties with strong currents and biofouling, successful long-term deployment (over two months) of the instrument has already been proven at the Woods Hole Oceanographic Institution dock (Sosik & Olson, 2007). There remain, however, limitations of flow cytometric measurements due to chain formation of cells, e.g. filaments of cyanobacteria (Codd et al., 2005) or chain-forming pennate diatoms, such as *Pseudo-nitzschia* spp. (Olson & Sosik, 2007a).

Precise and accurate identification and quantitation of phytoplankton taxa, even at the specific and infra-specific level is now possible with ecogenomic sensors that employ wet chemistry coupled with molecular techniques to assess specific organisms, their genes, metabolites, or other biomarkers within an environmental context (Scholin et al., 2008). To enhance the species-specificity of submarine instruments, bio-optics may be combined with such cellular-based techniques. As an example, FlowCAM measurements have been combined with molecular markers for *K. brevis* for an improvement of automated species identification (e.g., Rhodes et al., 2004).

Taxon-specific molecular probes have been developed for a variety of HAB taxa, including key genera such as *Alexandrium* (John et al., 2003, 2005) and *Pseudo-nitzschia* (Miller & Scholin, 1998), originally for application to field samples and cultured isolates in the laboratory. The high sensitivity and specificity of molecular probes renders them suitable for taxon detection even in pre-bloom conditions, such as for the dinoflagellate *Cochlodinium polykrikoides* in Korean coastal waters (Mikulski et al., 2008). Furthermore, a portable semi-automated multi-target system for the simultaneous detection of up to 14 different algal
species with molecular markers has been developed (Diercks-Horn et al., 2011), although it is not yet configured for in situ autonomous deployment.

Development of species-specific gene probes for key HAB taxa, supplemented with novel screening methods for toxins (e.g., multiplex immunodiagnostic assays), has led to rapid incorporation into automated deployable systems for in situ detection of harmful species and their toxins. The second generation of the Environmental Sample Processor (ESP) is an automated system for the concentration of plankton and other particulates from subsurface water samples upon filters and subsequent molecular processing. On line extraction of the filtered particulate sample is followed by a sandwich hybridization assay based on ribosomal RNA. The method has been applied successfully for the detection and discrimination of a number of HAB taxa, including *Pseudo-nitzschia* spp. (*P. australis, P. multiseries, P. multiseries/pseudodelicatissima*), *Alexandrium catenella*, and *Heterosigma akashiwo*, at cell concentrations even below the limits for human health concerns regarding toxicity (Greenfield et al., 2008). The ESP system has been further equipped with an immunodiagnostic sensor for the detection of the toxin DA from *Pseudo-nitzschia* spp., and successful measurements have been achieved in situ (Doucette et al., 2009). In this configuration the ESP has already been deployed on three sites in Monterey Bay, California for HAB ecology research, and was equipped for assays for the above mentioned species and the toxin domoic acid (DA) (Ryan et al., 2011). The instruments were part of an ocean observing system in which relationships between environmental parameters and species measurements were revealed. The ESP is now commercially available and is now being deployed for HAB detection in other areas, such as for the toxic dinoflagellate *A. fundyense* in the Gulf of Maine (D. M. Anderson, personal communication; NCCOS, 2011).

The Autonomous Microbial Genosensor (AMG) (Fries et al., 2007, Paul et al., 2007) is a similar system based on molecular genetic detection. The principle of the AMG is based on RNA-amplification; the nucleic acid sequence-based amplification is then measured as an increase in fluorescence and holds potential to quantitatively measure *K. brevis* cell concentrations (Paul et al., 2007). Instruments such as the ESP and AMG provide platforms for the deployment of multiple techniques based on taxon- and toxin- specific molecular probes.

### 2.4 Emerging advancement in bio-optical sensor technologies

For HABs characterised by low cell concentration, target species detection and discrimination remains an open challenge for optical sensors. There are some promising examples for the in situ application of regional species-specific and highly sensitive instruments, such as the OPD for the toxic dinoflagellate *K. brevis* that enhances the absorption spectrum with a capillary cell, or in situ flow cytometric systems that couple optical parameters to retrieve algal groups
with rapid image processing. An alternative principle to enhance measurement sensitivity is that of the Point Source Integrating Cavity Absorption Meter (PSICAM) (Kirk, 1997, Röttgers et al., 2007). Such a system has been transferred to an in situ device and successfully deployed in a profiling mode (Dana & Maffione, 2006). The optical diagnostic value of rare pigments such as gyroxanthin has until now been applied only for *K. brevis* in Florida. Even though efforts to optically track the HAB dinoflagellate *Karlodinium* spp. in the Southern Ocean were impeded by the presence of other gyroxanthin containing species (as stated by Llewellyn et al., 2011), the fourth derivative approach may be adjusted to detect *Karlodinium* spp. in regions where high biomass blooms of this ichthyotoxic species occur regularly (or for other HAB species in Table 2.4). The thorough examination of HAB species pigment patterns may reveal further rare pigments with diagnostic value.

With respect to recent advances in chemotaxonomic methods for pigment differentiation, there is astonishingly little comprehensive published information on pigment signatures of HAB species (for a collection of some available material see Appendix 14A in Johnsen et al., 2011). In a recent chemotaxonomic approach, various species and strains of the potentially toxic diatom *Pseudo-nitzschia* could be divided in three chlorophyll c pigment pattern groups (Zapata et al., 2011). One of these groups contains *P. australis* and *P. multiseries*, two potentially causative species of amnesic shellfish poisoning. In monospecific bloom situations, such grouping may lead to the identification of regionally occurring *Pseudo-nitzschia* spp. Nevertheless, for the critical identification of *Pseudo-nitzschia* spp., transmission electron microscopy or genetic probes are often used because key morphological features are at the limit of resolution of classical light microscopy.

The in situ and or in vivo detection of phycotoxins in natural HABs remains a major challenge for optical techniques. To date the fluorescence derivatisation properties and absorbance characteristics of these toxins has proven amenable only to detection from extracted samples. Nevertheless, attempts to link the influence of toxins to the structure of the photosynthesis machinery have been proposed for a toxigenic cyanobacterium. Differences in fluorescence induction curves (‘fluorescence fingerprinting’) due to peaks of Photosystem I and II, and differences in their ratios, were used to distinguish in vivo between toxic and non-toxic algal cells of *Nodularia* sp. (Keränen et al., 2009).

The similarity in taxonomic distribution of phycotoxins and MAAs, as documented by Klitsch & Häder (2008), may also yield relevant bio-optical information. Further insights into the currently hypothetical relationships between algal toxins and MAAs and their release into the water may increase the value of MAAs for optical monitoring of toxic microalgae.
There remain many cellular components, such as nucleotide and protein composition, that could be further exploited to yield information on species identification and discrimination, including of HAB taxa. These increased classification parameters may also hold true for the assessment of algal resting cysts (cellulose wall and pigments), as an indicator of an upcoming bloom. Progress on the use of bio-optical approaches to provide highly detailed information of this kind arises in spectroscopic techniques that are aimed at the molecular and elemental composition of the target, e.g. Raman spectroscopy, surface plasmon resonance spectroscopy and laser induced breakdown spectroscopy.

Integrated multi-functional approaches linking highly targeted molecular-based methods (e.g., qPCR or DNA microarrays) and/or toxin detection (e.g., automated immunoassays) with bio-optical sensors for bulk properties of bloom taxa on field deployable underwater platforms are emerging rapidly. The transfer of such promising techniques to subsea operational applications and sophisticated data processing procedures (e.g. artificial neural networks), combined with regional knowledge of HAB properties, are anticipated to lead to further progress towards successful species discrimination.

2.5 Transfer to operational oceanography

Given the urgent requirements for operational systems for assessment of HABs over appropriate horizontal, vertical, and temporal dimensions it is rather surprising that few comprehensive systems are currently deployed. This is in spite of the fact that optical sensing technology has evolved rapidly in combination with both remote sensing and in situ platforms. The first two component types for HAB monitoring defined by Stumpf et al. (2003), namely, monitoring the movement of an algal bloom previously identified as a HAB (Type 1), and the detection of a new bloom as HAB or non-HAB (Type 2) are both feasible by means of proxies for algal biomass with remote sensing and in situ applications. Remote sensing applications from satellites and airborne surveillance sensors cover large surface areas, whereas stationary and mobile bio-optical in situ sensors systems may now be integrated into networks within three dimensional submarine areas (Cullen et al., 1997a, Cembella et al., 2005). Nevertheless, since differentiation of algal blooms into HAB vs. non-HAB types necessitates the discrimination of the target organism at the species level and/or via an independent monitoring of toxigenic properties, it appears that these issues can only be resolved through in situ sensors deployed within the water column (Table 2.1), provided that for long-term deployment, size, weight, and power (SWaP) factors, and antifouling measures (see e.g., Lehaitre et al., 2008), are adjusted.

Bulk optical measurements and interpretations are in a development stage, such that they provide useful adjuncts to address both types of HAB monitoring, but there are only a few examples of validated case studies for which the required high taxonomic resolution has been
achieved. With these few exceptions, precise identification at the species level and the enumeration of target cells remain critical limitations and challenges for field deployable instruments.

Ecogenomic sensors based on molecular targets, such as ESP and AMG, are highly sensitive for detection and quantitation of a few key HAB species, but in operational mode they are currently limited by the paucity of validated probes to address the high diversity of HAB species and their toxins. To date only a small selection of pre-defined species have been integrated for simultaneous processing. The detection of unexpected or new HAB species within an area is not possible with these highly specific probes; however, the technological challenges to the incorporation of novel probes within current sensor packages are not insurmountable. For example, recent developments in DNA microarrays for HAB species offer the possibility to screen simultaneously thousands of genes from a multitude of species. Incorporation of such sophisticated technology on gene expression patterns into operational systems has not yet been accomplished, and current in situ platforms for continuous deployment over extended times periods will require further refinement for operation, especially in areas that are not easily accessible for maintenance.

The techniques and systems for sensor deployment described satisfy some of the levels that are required for HAB monitoring, but also have limits in either spatio-temporal coverage, or target specificity. Space-based and airborne sensor systems provide measurements over large geographical areas, but suffer from restricted resolution of single water constituents and do not address events occurring within the water column (e.g., thin layer sub-surface concentrations). In an inverse relationship, laboratory based analysis of point-source and discrete samples provides high sensitivity and resolution of target organisms and/or their toxic properties, but does not deliver continuous real-time observations over appropriate spatial scales. Bio-optical sensors of each level thus form a valuable complementary element in a HAB monitoring chain, in which the combination of different techniques links spatial and temporal scales with enhanced resolution (Fig. 2.1). Due to the high diversity of HABs and their biogeographical distribution along horizontal axes and within the water column, there is no technological solution over all temporal and spatial scales. The appropriate temporal and spatial scale may also vary from region to region, with respect to the time–space development of HABs and the potential for advection and dispersion. Therefore, an observing system needs to be adjusted to comply with the local situation with respect to the required horizontal, vertical and temporal dimensions. The acquired optical information, in combination with local expertise and biological and physical oceanographic knowledge of the respective area, can provide advice for surveillance strategies.
There are few case examples of the successful integration of optical sensors in long-term ocean observatories, such as Coast Watch in Florida. This programme now provides contemporary access to multiple satellite ocean remote sensing data and products for a range of biological applications, including Chl \( a \) and Chl \( a \) anomaly retrieval for HAB monitoring in various regions. These data are then included in the NOAA HAB operational forecast system (HAB-OFS), and provided in a near-real-time bulletin. In addition to satellite imagery, field observations, models, public health reports, and buoy data are also made public by federal, state, and local authorities (NOAA, 2012). The analysis also includes data from the OPD and Flow Cytobot. Other ocean observing networks that employ optical sensor systems for an enhanced spatio-temporal coverage are ADEOS in the Gulf of Maine (Ramp et al., 2009) and Hong Kong with a four year frequency data set (Lee et al., 2005). Harmful algal events are also included in the Global Ocean Observing System (GOOS) coastal module, within the societal benefit area ecosystem health (Malone et al., 2010). Within GOOS, global data and information on coastal ecosystems will be provided and support interdisciplinary knowledge on environmental processes and observational methodologies for HABs. On a regional scale, more observatories for HAB monitoring arise worldwide, often combining continuous in situ measurements of environmental variables with surveillance of shifts in biodiversity. Some of these observatories include near real-time information complementary to traditional HAB monitoring programmes.

By integrating the relationship of oceanographic processes with the continuous long-term bio-optical detection of blooms, ocean observatories present a promising constellation of technologies to gain insight into drivers and constraints on HAB bloom development. These integrative approaches would then also tackle the forecasting required for HAB monitoring and mitigation that demand knowledge on HAB ecology and include interpretative and numerical modelling. The construction of these HAB scenarios would then address the final two categories proposed by Stumpf et al. (2003): predicting the movement of an identified HAB (Type 3), and predicting conditions favourable for a HAB to occur where blooms have not yet been observed (Type 4). Within this application of technological methods and conceptual approaches to operational oceanographic systems, bio-optical tools are an important element for integrated HAB monitoring and forecasting of bloom dynamics.

In addressing the diversity of HABs and their impacts with a combination of such observatories and complementary methods, the effectiveness of public and private responses to harmful events can be optimised, and diverse impacts reduced. As early as 1988, an early warning of a surveillance system in the North Sea (within the Seawatch project of EURMAR) was reported to having saved $30 million worth of aquaculture fish in southern Norway in 1988, due to early measures of the fish farmers (in this case farmers moved net cages to safe
sites) (Stel & Mannix, 1996). The prevention and mitigation of economic losses, as well as threats to human and environmental health caused by harmful blooms, need to be integrated into an appropriate and regional coastal zone management. Providing increased knowledge on HAB species composition, frequency, magnitude and biogeography, with critical observations of their impacts, justifies the critical necessity and long-term value of development and application of bio-optical tools in HAB monitoring and coastal zone management systems.

Fig. 2.4. Spatial coverage and resolution (information depth) of optically derived parameters in operational oceanography 'compartments' for a HAB assessment context. Apparent- (AOPs) and inherent- (IOPs) optical properties can be derived by remote surface to submarine in situ optical sensing, from discrete point sampling or by profiling in a sensor network. More accurate laboratory determinations of other properties of HAB species can be conducted from discrete samples by techniques such as liquid chromatography coupled with detection by fluorescence (LC-FD) or tandem mass spectrometry (LC-MS/MS), and by quantitative polymerase chain reaction (qPCR) of organismal DNA. (Source: Adapted from Zielinski et al., 2009).
THEME CHAPTER II

THE LARGE SCALE: BIO-OPTICAL ASSESSMENT OF PHYTOPLANKTON IN THE EBRO DELTA

PUBLICATION III: Correction of hyperspectral reflectance measurements for surface objects and direct sun reflection on surface waters

PUBLICATION IV: Bio-optical variability and phytoplankton biomass retrieval with hyperspectral reflectance measurements in the optically complex aquaculture zone of the Ebro Delta, NW Mediterranean

PUBLICATION V: Comparative studies on phytoplankton dynamics and bio-optics for HAB monitoring in the Ebro Delta, NW Mediterranean
CORRECTION OF HYPERSPECTRAL REFLECTANCE MEASUREMENTS FOR SURFACE OBJECTS AND DIRECT SUN REFLECTION ON SURFACE WATERS

Abstract:

Satellite, airborne, or platform-based remote sensing reflectance measurements of aquatic targets are frequently compromised by water-surface effects such as specular sun reflection (glint) or transient objects like buoys or boats. For temporal or spatial data series where sub-surface reflectance is of interest, the elimination of affected data may require time-consuming manual selection of spectra and substantial data loss. Here, we present a method for the automated elimination of data points containing surface objects or strong sun reflection, which is based on the spectral slope in the ultra-violet to blue (350 nm to 450 nm). To minimize data loss, an automated sun glint correction combining two previously published methods is also presented. The method operates by subtracting a glint spectrum by means of a regression curve characterised from low to medium glint data points and is further automated by selecting these low glint data on the basis of the oxygen absorption depth in the near infrared (NIR). The elimination and correction algorithms facilitate rapid automated processing of large bio-optical data sets for both spatial and temporally resolved remote-sensing reflectance data sets. Here we demonstrate their efficacy on a three-month data set of hourly light field measurements from a fixed platform in the northwest Mediterranean.
3.1 Introduction

Remote-sensing systems on fixed platforms are a valuable method for continuous real-time assessment of aquatic bio-geochemical parameters. Especially in near-shore regions, where remote sensing based on satellite platforms often struggles due to influences from land and high complexity of bio-optical substances, high-quality in situ radiometry is a highly beneficial tool. Space- and airborne products cover large spatial areas but have limited temporal flexibility, and therefore omit temporal continuity that is necessary for assessing certain environmental processes. Discrete near-surface radiometer systems only retrieve a single ‘pixel’ of 1–10 m², but when integrated into coastal time-series and observation systems, they allow a synoptic coverage of large spatio-temporal dimensions in aquatic environments (Reuter et al., 2009, Helmholtz-Zentrum-Geesthacht, 2013). Furthermore, in situ radiometric measurements in diverse ocean provinces are crucial for vicarious sensor data calibration and validation, and for atmospheric corrections of satellite data (Zibordi et al., 2002, McClain, 2009).

The application of near-surface autonomous systems offers certain advantages to sub-surface instrument moorings. They provide a high pointing stability to facilitate calculations of the geometric light-field, are less prone to bio-fouling, and can be more accessible for maintenance. Yet, fixed settings also entail some disadvantages, as they are inflexible with respect to solar angle and sea state, and are exposed to weather conditions (Zibordi et al., 2002). Also, direct surface sun reflection or ‘sun glint’ cannot always be avoided by fixed above-surface sensors. All above-surface data will to some extent be contaminated by sun and sky reflections as a function of solar angle and the deviations in the surface slope caused by wind and wave actions (Mobley, 1999). Furthermore, surface objects such as boats, buoys, and other constructions such as platforms for aquaculture contaminate the signal if they fall within the sensor field of view (FOV). Such data points need to be eliminated, which is time consuming if quality assurance is manually applied to large data sets. To maintain the quality of data in continuous measurement setups or for real-time applications, an automated data quality routine that eliminates the influence of surface objects and direct sun reflectance is crucial (Fig. 3.1).

Elimination of low quality data points can result in losses of large parts of the dataset, so wherever possible it is desirable to apply corrections to the data so that it can be usefully retained. Correction for specular sun-glint reflection from the air-water interface has been proposed by various methods (see review of Kay et al., 2009). Techniques for large pixel sizes of 100 m or more, as derived by ocean colour space-borne measurements such as MERIS, are typically based on models of surface reflectance parameterised on wind and the solar and view geometry. These approaches are not useful for high spatial resolution imagery or sensors.
Fig. 3.1 Remote-sensing reflectance measurements $R_{rs}(490)$ from a fixed platform, performed autonomously every quarter of an hour throughout approximately two months of measurement in a coastal environment, display two major sources of disturbances: direct sun reflection during noon time (grey), and traffic (boats below the sensor system), mainly at the favoured fishing time before noon.

where the field of view is smaller than the surface wave scale, because the glint reflection varies from pixel to pixel. In this context the removal of sun effects by subtraction of a glint spectral shape scaled on a pixel to pixel basis has been proposed (Hochberg et al., 2003, Kutser et al., 2009). This spectral glint shape, as well as its proportional influence in each pixel can be determined from light and dark glint pixels in an image, using the near-infrared (NIR) 710 – 910 nm spectral region as a ‘glint indicator’ (Hochberg et al., 2003). Another proposed method is to use the depth of the oxygen absorption region in the NIR (Kutser et al., 2009). These high spatial resolution methods are a promising route for correction of fixed platform measurements, where spatial variations are replaced by comparable temporal changes.

Therefore, appropriate glint correction methods require a consistent relationship between signals in the NIR and the surface reflectance. The influence of shallow water bottom reflectance in the NIR can often be neglected, since this is likely to be close to zero for all but the shallowest waters (< 1 m). Measurements taken when surface objects are present, however, affect the spectrum in the NIR and should therefore be removed manually from the selected pixels prior to the application of the glint correction. The Hochberg et al. (2003) method proposed utilising only few reference spectra from low or high NIR reflectance measurements. To mitigate the possibility that these might be unrepresentative outliers, and to translate the correction procedure to a simple routine application, Hedley et al. (2005) revised the Hochberg method by using linear regression to establish the glint spectrum and its relationship between NIR reflectance, $R_{NIR}$, minus the image-wide ambient NIR level, $Min_{NIR}$. To ensure
the glint characterisation is representative of the whole image, a subset of variable glint-level pixels are selected from different regions of the image; this is typically done manually by visual assessment (Hedley et al., 2005). The glint intensity for each visible wavelength is determined by the difference between the pixel $R_{\text{NIR}}$ value and the ambient NIR level, or ambient light field ($\text{Min}_{\text{NIR}}$) projected onto $b (\lambda)$, the regression slope for the respective wavelength. The subtraction of the glint shape scaled by this factor results in the pixel corrected reflectance $R^\prime$:

$$R^\prime (\lambda) = R (\lambda) - b (\lambda) (R_{\text{NIR}} - \text{Min}_{\text{NIR}})$$ (3.1)

The derivations of the regression slopes, $b (\lambda)$, are relatively insensitive to the influence of surface objects because other pixels in the regression will outweigh any outliers, assuming these outliers are comparatively rare. However, the surface object measurements themselves would not be valid data after the application of the correction, and to improve the procedure it is desirable that they be eliminated.

In common with spatial spectral data (images), temporal spectral measurements consist of wavelength-dependent data in a certain spectral resolution collected under different water regimes, variations in solar geometries and sea surface state. We therefore hypothesized that spatially developed approaches for detection and correction of above-surface reflectance anomalies may be applicable to temporal in situ radiometric data, with some modification. The approach for glint correction described in Hedley et al. (2005) requires visual selection of pixels that represent a range of glint severity to guide the calculation of glint shapes. For a procedure applicable to temporal data another selection procedure is required. In addition, the development of an automated procedure for the elimination or correction of surface objects and direct sun would be valuable for processing of both spatial and temporal remote sensing data.

The objectives of the work presented here were:

1. To establish a procedure for an automated elimination of measurements containing surface objects by means of appropriate spectral features.

2. To test the transferability of sun glint correction methods typically applied to spatial data (imagery) to a temporal set of spectra from a fixed platform.

3. To develop a procedure for automated selection of representative low glint spectra for establishing the NIR-glint relationship with the oxygen absorption depth, as presented by Kutser et al. (2009) and to compare this procedure with the original method (Hochberg et al., 2003, Hedley et al., 2005) for an automated process of glint shape subtraction.
3.2. Methods and data

3.2.1 Sensor system set-up

3.2.1.1 Remote sensing reflectance from an above water fixed platform:

Hyperspectral measurements were made in a coastal embayment of the NW Mediterranean (40.620083°N, 0.658167°E) from a fixed elevated pole approximately four meters from the water surface, every quarter of an hour from 08:00 to 20:00 local time from beginning of May to July 2010. As the sensor setup was pointing southwards, the optimum relative azimuth angle of $90^\circ \leq \varphi \leq 135^\circ$ to the sun (suggested by Mobley, 1999) was exceeded close to noon, resulting in strong sun reflections on the water surface (see Fig. 3.1). Remote-sensing reflectance $R_r$ was calculated as the ratio of downward plane irradiance $E_s$, (measured directly with a zenith directed RAMSES-ACC-VIS irradiance sensor, TriOS, Germany) to the water-leaving spectral radiance $L_w$. Radiance $L_w$ was estimated using a sky reflectance correction (described below) based on measurements from two TriOS RAMSES-ARC radiance sensors (7° FOV in air). The first instrument measured radiance ($L_{sfc}$) leaving the surface at an angle of 30° from zenith. From this measurement a portion, $\rho$, of the sky radiance ($L_{sky}$) was subtracted, as measured by the second instrument pointing to the sky in a reciprocal angle (i.e., the instrument was upward directed at 30° from zenith) (Fig. 3.2). For the setup angle of 30° and an average wind speed of 3 ms$^{-1}$, a $\rho$ value of 0.024 was assumed (based on Figure 8 in Mobley, 1999). Data with insufficient incoming light ($E_s$ at 480 nm less than 20 mWm$^{-2}$ nm$^{-1}$) or with the influence of red sky at dusk or dawn apparent on the spectral shape (as when $E_s$ at 680 nm larger than $E_s$ at 470 nm, as described in Wernand, 2002) were eliminated. Spectra below zero and data taken under rain conditions, as flagged by data from a nearby meteorological station, were also deleted. Negative spectra occurred occasionally, possibly caused by cleaning of the sensors or by a full reflection of the sky under calm water conditions. Such spectra also were omitted from further processing. The complete dataset consisted of 2396 valid spectra, recorded at the instrument resolution of 3.3 nm steps from 320 to 950 nm, but then interpolated linearly to 0.5 nm steps. Estimated uncertainties of $R_r$ ($\lambda$) for the RAMSES instrument vary approximately between 4% and 6% in the spectral range of interest. The considered sources of uncertainty include system calibration, stray light effects and non-cosine response of the $E_s$ sensor (see Zibordi et al., 2012, Table 7 for uncertainties).

3.2.1.2 Digital photos of sea state and sky conditions:

Images of the sea surface and the sky were automatically taken at the same time as radiometric measurements with a DualDome D12 (Mobotix, Germany) camera system, consisting of two cameras pointing to the sky and sea surface at an angle of 45°, the latter covering the footprint of the radiometric measurements.
3.2.2 Automated algorithm development

3.2.2.1 Selection of an appropriate spectral region for surface object determination

Digital photographs were used to identify a subset of 31 spectra taken with boats below the sensor system, in anchorage or passage. In this subset a strong ascending slope in the UV-blue spectral range was prominent in comparison to ‘clean’ spectra (Fig. 3.3). The slope between 350 and 450 nm, $\text{slope} = \frac{(y_2 - y_1)}{(x_2 - x_1)}$, where $x_2$ and $x_1$ are the wavelengths 450 and 350 nm, and $y$ represents the spectral intensity at these wavelengths, was then tested as an indicator of surface objects for the entire dataset. The resulting selected data points and appropriate ranges were validated by eye, utilizing the corresponding camera images. In contrast to the slope in the blue wavelengths, visual examination indicated that surface object reflectance in the NIR did not show sufficient characteristics to distinguish boats from sunglint spectra.
3.2.2.2 Selection of representative spectra for the sub-surface transmitted light field and transfer of sun glint correction methods to a temporal dataset

For the automated selection of spectra that are least effected by solar reflection and hence best represent the sub-surface transmitted light field, we tested the oxygen absorption depth $D$, calculated by $D = \left[ R_{rs}(739) + R_{rs}(860) \right] / 2 - R_{rs}(760.5)$. This formula, presented by Kutser et al. (2009), takes the difference between the oxygen absorption wavelength and a baseline in the NIR from wavelengths on either side. We adopted the baseline wavelengths of the original formula, but identified 760.5 nm as minimum in our data, instead of 760 nm as in the original formula. Following Kutser et al. (2009), $D$ was assumed to be proportional to the glint intensity in each spectrum and it was further assumed that there would be no spectral feature at 760.5 nm if it did not contain glint. This implicitly presumes that surface objects have been eliminated from the dataset, which was the objective of the previously described step. The rationale for the use of $D$ is the possibly lower influence of optically active substances in the water on this metric, rather than on the overall NIR level.

As in the original application of $D$ by Kutser et al. (2009), negative values of $D$, presumably due to noise, were changed to zero. As expected, most of the spectra with lowest $D$ were of low to moderate intensity (and thus low glint effects), as opposed to the spectra with
a high $D$ (strong glint effects) (Figure 4a, b). Examination of the camera images corresponding to the 20 lowest $D$ measurements further proved the general relation of $D$ to sun glint. Based on this test, the spectrum with the lowest $D$ was selected as representative of the lowest glint conditions, with the spectral value of this spectrum at 760.5 nm taken as reference for the glint correction procedure, $R_{rs,D_{\text{min}}}(760.5)$. As comparison to $D$, a second reference for the glint correction was selected by determining the lowest spectral value at 739 nm, $\text{NIR}_{\text{min}}(739)$, a wavelength which is in the NIR, but far from an atmospheric absorption band. Low and high values of the spectra at 739 nm also corresponded well to typical spectral effects of sun and sky reflection (Fig. 3.4c, d), indicating a general positive relationship of the reflectance at 739 nm to sun glint. temporally resolved radiometric dataset and compared for performance in glint removal. Two of the methods used as a glint indicator the absolute NIR, in the oxygen absorption wavelength.

Three variations of the regression method for sun glint correction were applied to the 760.5 nm and far from it at 739 nm, the third used the depth of the oxygen absorption band, i.e. $D$ as previously defined. A reduced dataset of about 450 spectra was used as a validation data set for the other spectra. The reduced set consisted of measurements taken with the sun in an optimal relative azimuth angle ($90^\circ \leq \phi \leq 135^\circ$, Mobley, 1999) and assumed that these measurements were least affected by sun reflection. These measurements were clustered in the morning and evening hours throughout the sampling time.

The first approach used the absolute NIR in the oxygen absorption band as a glint indicator, so a linear relationship $b(\lambda)$ was established between the oxygen absorption wavelength 760.5 nm and each wavelength, $\lambda$, in the measurements in the VIS region (Fig. 3.5 a). The resulting slopes were then used to subtract the effects of glint as described before, with the low glint spectrum selected with $R_{rs,D_{\text{min}}}(760.5)$ (Equation (3.1)):

$$R_{rs,corrOx}(\lambda) = R_{rs}(\lambda) - b(\lambda) [R_{rs}(760.5) - R_{rs,D_{\text{min}}}(760.5)]$$ (3.2)

As a second variation and for comparison with the original regression method without any influences of an atmospheric absorption band, a linear relationship $b(\lambda)$ between $R_{rs}(739)$ and each wavelength ($\lambda$) measurement in the VIS region (Fig. 3.5 b) was conducted. The resulting slopes were then used to subtract the effects of glint to the ambient light level NIR$_{\text{min}}(739)$ as described before (Equation 3.1):

$$R_{rs,corrNIR}(\lambda) = R_{rs}(\lambda) - b(\lambda) [R_{rs}(739) - \text{NIR}_{\text{min}}(739)]$$ (3.3)

Thirdly, by assuming that $D$ is fully proportional to the sun glint, a direct exchange of $R_{NIR}$ in the original Equation (3.1) by $D_{\text{min}}$ should be possible. As a relative value it may be insensitive to some sources of variation in NIR reflectance and lead to a more robust
Fig. 3.4. Comparison of methods to select remote-sensing ($R_{rs}$) spectra that are least and most affected by solar radiation. (a) subset of 100 minimum (least reflectance ($R_{rs}, D_{min}$)) and (b) maximum (highest reflectance ($R_{rs}, D_{max}$)) spectra selected by the relative value of the absorption depth ($D$); (c) 100 minimum (least reflectance ($R_{rs}, NIR_{min}$)) and (d) maximum values (highest reflectance ($R_{rs}, NIR_{max}$)) selected by the value at 739 nm. Objects and strongest sun glint situations were deleted before selection.

Fig. 3.5. Regression of all spectra was conducted to all VIS wavelengths, here shown for $R_{rs}$ (490) to a) 760.5 nm with $r^2 =0.951$; b) to 739 nm $r^2 =0.953$; and c) to $D$ $r^2 =0.464$. 
correction of data. Therefore, the regression procedure was also tested with the appropriately modified Equation (3.1):

\[ R_{rs,\text{corr}}(\lambda) = R_{rs}(\lambda) - b(\lambda)[D - D_{\text{min}}] \]  (3.4)

In the original method used for correction of imagery, Hedley et al. (2005) selected a subset of sun-affected pixels over deep water for the determination of glint effects on the regression line. Deep water is required to ensure that all variations in reflectance are due to sun glint and not effects from underlying seabed or vegetation. As the remote sensing data of this study were measured over time from a fixed platform at the same spot, the selection of a subset over similar water-bodies for the establishment of a regression slope is not applicable. Instead, we tested the inclusion of the whole dataset to establish the linear relationship \( b(\lambda) \), with the assumption that this would average out any effects due to variation in optically active substances (Fig. 3.5).

### 3.3 Results and Discussion

#### 3.3.1 Elimination of surface objects

For the detection of boats, a strongly ascending slope between 350 and 450 nm reliably indicated the presence of vessels in the sensor field of view. Of the 2396 total spectra, 31 were previously identified as influenced by passing or anchoring vessels or surface objects. All 31 spectra were captured by a threshold of slope greater than \( 15 \times 10^{-5} \text{ sr}^{-1} \text{ nm}^{-1} \). Validity of this identification was confirmed by visual inspection of the corresponding camera images for each spectrum. In total, 53 spectra were selected by this procedure, of which the first 34 contained 90% of all the images containing boats. Images for the first 22 spectra are shown in Fig. 6. The effectiveness of the UV-blue slope criteria for removal of surface objects might be explained by the reflectance due to protective coatings commonly used to prevent damage from UV on boat hulls (Stewart, 2011). However, in addition to sport or fishing boats with intact boat hulls weathered surfaces of floats for aquaculture maintenance were correctly identified by this method (Fig. 3.6). The UV-blue slope criteria may not be valid for dark surface objects or vegetation mats but the study did not yield data to test this. All the false positives where UV-blue slope was greater than \( 15 \times 10^{-5} \text{ sr}^{-1} \text{ nm}^{-1} \) but the corresponding images did not contain surface objects were influenced by strong solar reflection. In fact, for our dataset a second range of the slope, from \( 15 \times 10^{-5} \) to \( 8 \times 10^{-5} \text{ sr}^{-1} \text{ nm}^{-1} \) returned measurements that were strongly affected by direct sun reflection. The application of this second range was therefore used to exclude the strongest sun-glint events from the dataset (Fig. 6). This range also included two measurements that partly contained boats within the sensor FOV (with a slope of \( 11 \times 10^{-5} \text{ sr}^{-1} \text{ nm}^{-1} \)). The application of both ranges was also validated with a second dataset taken in 2011 under the same conditions as in 2010 (data not shown). The identification of sun glint by a slope in the UV-blue was unexpected and may be
Fig. 3.6. Graphs: Remote-sensing reflectance $R_{rs}$ of all data over wavelength (left panels) and at $R_{rs}(490)$ over time (right panels) with stepwise subtraction of spectra (in downward direction) that were identified as surface objects by range 1 and direct sun reflection by range 2 with the degree of ascending slope between 350 and 450 nm. Images: 53 images taken with the camera system, corresponding to range 1 (selected boat spectra from the maximum value to $15 \times 10^{-5}$ sr$^{-1}$ nm$^{-1}$) are shown sorted and descending. Outliers are all strongly affected by direct solar reflectance (framed red).

due to a strong reflection in the UV-blue range under glint conditions. However, only a trend was observed and not a strict proportionality of strong solar reflection and the slope in the UV-blue spectral range. To demonstrate a general applicability of this observation, further studies in other geographical regions would have to tackle its validity for a broad range of sky and sea state conditions.

3.3.2 Correction of remaining sun effects

Subsequent to the removal of data contaminated by surface objects and strong solar reflection, the remaining moderately sun glint effected data were corrected towards a zero glint condition by all three methods described in Section 3.2.2.1 (Fig. 3.7). Even though in this dataset $D$ was not found to be strictly proportional to the amount of glint, a robust selection of the lowest glint spectra was clearly demonstrated (Fig. 3.4) and the lowest value could be used as representative to a minimal glint condition. In our dataset some spectral variability was visible in the NIR range, hence the reflectance in wavelengths used to calculate the baseline for $D$ were a source of variation in $D$. These spectral variations may be a consequence of sun reflection, as they were most prominent in spectra that were strongly influenced by sun glint (compare the NIR region of spectra with high reflectance and low spectral intensity in Fig. 3.4). Yet, an alternative calculation of $D$ which attempted to average out these variations by
Fig. 3.7. a) $R_\alpha$ spectra of the original data set with considerable influence of sun reflection, b) after correction by means of the regression technique with regression to 760.5 nm, c) the subtracted glint shape of these spectra; d) spectra corrected by means of regression to 793 nm, e) glint shape of these spectra; f) by regression to $D$, g) corresponding glint shape.
using the average of wavelengths 739 to 750 nm and 860 to 875 nm as a baseline, instead of the values at 739 nm and 860 nm, did not yield better results (data not shown here). Since $D$ is only a small numerical difference between adjacent bands the effect of noise on this relative value is greater than the on absolute NIR value. In our dataset $D$ was not proportional to the glint. The next step in the procedure, the regression of $D$ to the reflectance in the VIS wavelength 490 nm, resulted in a poor fit, $r^2 = 0.464$, whereas the fit for the other two method variations with regressions against reflectance at 760.5 and 793 nm was quite good, $r^2 = 0.951$ and 0.953 (Fig. 3.5). As in Kutser et al. (2009) we set negative $D$ values to zero before the regression. Allowing $D < 0$ returned a worse fit, $r^2 = 0.285$. Due to the poor performance of $D$ to indicate the proportion of glint in the measurements, the use of $D$ for glint correction was not pursued further. The following only considers the methods based on absolute NIR reflectance.
The glint spectral shape subtraction with the two NIR method variations resulted in a correction of both the absolute level and shape of the reflectance spectra. Fig. 3.8 shows this for a clear sky day with few clouds, the dominant weather condition throughout the study.

There were no clear advantages of the method variation with regression against reflectance at 760.5 over regression at 739 nm. Both result in a similar spectral intensity and shape of corrected $R_n$ spectra and the subtracted glint (Fig. 3.7b – e). While the contribution of glint has often been assumed as a fixed and additive component over all wavelengths (Gould et al., 1998), more recent simulations concerned with top of atmosphere (TOA) measurements show an increase of glint contributions towards the NIR due to greater transmittance and less Rayleigh scattering in the NIR, see Fig. 5 of Doerffer et al. (2008). A similar argument follows for bottom of atmosphere (BOA) measurements: the direct solar illumination is relatively less blue than the downwelling irradiance due to Rayleigh scattering, which preferentially redirects blue wavelengths from the direct illumination to the diffuse. This can be readily verified by using a radiative transfer model such as libRadtran (Mayer & Kylling, 2005) to plot the direct BOA solar irradiance divided by the total irradiance, which results in a curve almost identical to Fig. 3.7c, e, g (Fig. 3.9). The need for a greater correction towards longer wavelengths is also supported by findings of Lee et al. (2010, compare Fig. 3), who investigated an increasing value of $\rho$ (percentage of $L_{sky}$ to be subtracted from $L_{sfc}$) over wavelengths. In our study we set $\rho$ as a constant along all wavelengths, thereby possibly underestimating the portion of sky radiance to be subtracted from the total water leaving radiance. In conclusion, the spectral shape of sun glint effects and corrected spectra derived by the described methods is consistent with expectations.

---

Fig. 3.9. Typical bottom of atmosphere (BOA) ratio of direct sun irradiance to total irradiance under clear sky conditions, aerosol-free atmosphere, and high sun position, as modelled by libRadtran. The spectral shape approximates to that of solar glint reflection, since Fresnel reflectance at the air–water interface is approximately spectrally flat.
The accuracy of the correction procedure was examined by comparisons between uncorrected data, corrected data and reference data taken with the sun in an optimal angle of $90^\circ \leq \phi \leq 135^\circ$. Comparisons of the two corrected datasets to the reference are visualized at $R_{rs}$ (490) in Fig. 3.10a. When compared to the uncorrected dataset, which retains the temporally variable effect of direct solar reflection, the corrected spectra are clearly disposed of these dominant variations (Fig. 3.7a – e). Furthermore, both the correction approaches: 1) the selection of minimal sun glint by the relative $D$ and regression at 760.5 nm, and 2) the determination of low glint by absolute NIR value and the regression at 739 nm; performed well in the reduction of glint effects over time and both complied well with the reference dataset (Fig. 3.10b). The exchangeability of wavelengths for regression, and also of the low-glint selection procedure, is an indication of robustness of the method.

The presented approach has limitations when the glint reflectance is large compared to the water leaving radiance. A stricter selection of strong sun-glint effects (e.g. by extending the second range of the UV-blue slope used for testing which spectra to exclude), may aid in cleaning the dataset prior to applying a correction procedure. Furthermore, the reflectance in the NIR spectral region used by all variations of the method may be influenced by the presence of suspended sediments. The potential of this spectral range to obtain information on suspended sediments in turbid waters has recently been emphasised (Doron et al., 2011). For the presented dataset, total suspended material was low to moderate at 2 – 11 mg L$^{-1}$ (Busch et al., submitted), therefore strong influences of sediments were not expected. By performing the regression on the entire dataset, instead of only a subset as originally described by Hedley et al. (2005) or Hochberg et al. (2003), errors in the glint correction due to optically active constituents are minimized.

The issue of possible negative reflectance values due to bottom reflectance or other factors, as well as further considerations regarding the application of the method have been discussed in detail in Hochberg et al. (2003) and Hedley et al. (2005) and are not repeated here.

Further limitations of the method can be seen when comparing the fit of corrected spectra to the reference, $R_{rs}$, along the full spectral range. Neither approach is valid for the correction of the NIR spectral range (Fig. 3.11), which is expected, as originally the regression method was established for benthic mapping, assumes zero NIR reflectance and limits the correction to visible wavelengths. For radiometric datasets that are established for the calculation of optically active water components such as phytoplankton, total suspended matter or coloured dissolved organic matter (CDOM), the method may have limitations for subsequent application of certain algorithms. This includes any methods that make use of the NIR spectral region, such as those for retrieval of suspended material (Doron et al., 2011), or retrieval of
Fig. 3.10. Time series of $R_s$ (490) over a three-month trial in the field. a) Before correction, the sun reflection dominates the variability of $R_s$ data (grey dots), even though boat spectra as well as strongest sun glint reflections are already deleted. After the correction by means of subtraction of the glint spectral shape derived by regression of all VIS spectra to the oxygen absorption band at 760.5 nm (black open diamonds) and to 793 nm (green open squares), the strong influences of the sun are eliminated. b) Comparison of both correction methods with a reduced data set that includes only spectra taken with an optimal sun azimuth angle ($90° \leq \phi \leq 135°$) (open red circles).

the algal pigment Chl $a$ as biomass indicator (Dall'Olmo & Gitelson, 2005, Gitelson et al., 2008). The derivation of inherent optical properties, such as phytoplankton absorption coefficients, may be affected due to uncertainties in certain spectral ranges. Alternative correction methods, such as a spectral optimisation approach (Lee et al., 2010), may be more appropriate. In comparison to the reference dataset $R_{s,r}$ for both method variations (the regression at 760.5 and at 739 nm) the best spectral fit occurred between 490 and 630 nm, with $r^2 > 0.85$ (Fig. 3.11). For calculations of water constituents by means of ratios, especially in this best fit range, the method is applicable. This conclusion is supported by the successful calculation of phytoplankton biomass over time from the presented dataset (Busch et al., submitted). It should be noted that $R_{s,r}$ is not a direct measure of ‘real’ $R_s$, but rather a selection of spectra by means of a solar angle that is regarded as optimal for remote sensing measurements. However, as $R_{s,r}$ is a subset of the same dataset that was used for the correction methods it is not subject to biases in measurement conditions, such as the arrangement of the sensor system, or influences of the geometric light-field (e.g. solar zenith angle), wind, wave, and cloud coverage. Thus, it is an appropriate dataset to confirm the overall performance of the correction method as applied to time-resolved radiometric measurements.
Fig. 3.11. All spectra of the two corrected data sets $R_{rs\_corrNIR}$ and $R_{rs\_corrOx}$ were compared wavelength-wise to the reduced data set $R_{rs\_r}$ by means of regression. The resulting mean fit $r^2$ over the whole data set is plotted here over the wavelengths, thereby revealing differences in fit over wavelengths. The dashed horizontal line marks an $r^2 > 0.85$, the dotted vertical lines frame the wavelengths with a better fit than $r^2 = 0.85$.

3.4. Conclusions

This study was aimed at the automated processing of large datasets of remote sensing reflectance, to eliminate outlying features and to reduce the loss of data due to sun reflections by means of a correction procedure. The key conclusions are:

- The slope between 350 and 450 nm can be used for the selection and elimination of spectra influenced by surface objects.

- Furthermore, the same slope is an indication of strongest sun reflectance and can be used to eliminate spectra that are strongly influenced by sun glint. The slope is, however, not strictly proportional to sun-glint intensity.

- The regression technique as applied by Hedley et al. (2005) can be applied not only to imagery, but also to a temporally resolved dataset, and is robust with respect to the selection of NIR wavelengths used (here 760.5 and 739 nm). A reduction of spectral intensity and correction of spectral shape is reached by both variations of the method, for the present dataset the correction works best in the spectral range between 490 and 630 nm.

- The oxygen absorption depth $D$ is basically proportional to sun reflectance and is valid for a selection of spectra with minimal influences of solar radiation. Yet, strong noise in the NIR reduces the proportionality and the ability of the relative value $D$ to determine the
proportion of a spectral shape to be subtracted is, at least for the presented dataset, not demonstrated.

The presented methods provide a rapid and effective means for the automated elimination of undesired spectra in terms of surface objects and strong sun reflections, as well as the correction of remaining spectra from partial sun glint effects. This has been shown for temporally resolved remote sensing measurements, but should be also applicable for spatially resolved images from space-borne measurements of the sea surface. The presented procedure does not require manual selection of spectra and can be applied in a fully automated way. The enhanced data quality will have positive impact on further processing steps and calculations to derive bio-geochemical parameters.
**BIO-OPTICAL VARIABILITY AND PHYTOPLANKTON BIOMASS RETRIEVAL WITH HYPERSPECTRAL REFLECTANCE MEASUREMENTS IN THE OPTICALLY COMPLEX AQUACULTURE ZONE OF THE EBBRO DELTA, NW MEDITERRANEAN**

**Abstract:**

Ocean colour measurements are highly suited for spatio-temporally resolved monitoring of anomalies in phytoplankton abundances that may indicate the presence, magnitude, and movement of an algal bloom. Such measurements are especially important in coastal zones, where the occurrence of harmful algal species with the ability to produce potent phycotoxins and/or high biomass may pose a threat to seafood production for human consumption and components of the marine environment. The overarching objective of this study was to evaluate the inclusion of hyperspectral light-field ocean colour measurements as a tool for continuous monitoring of phytoplankton biomass by the unambiguous pigment chlorophyll a (Chl a) as a proxy in the aquaculture zone of two bays in the Ebro Delta, NW Mediterranean. Radiometric measurements with a sensor system were conducted continuously in two field campaigns from May to July in 2010 and 2011, accompanied by weekly casts for backscattering retrieval by means of a multi-parameter profiling system and laboratory measurements of content and absorption of Chl a, suspended matter and coloured dissolved organic matter (CDOM). Chl a as proxy for phytoplankton biomass was generally below the yearly average of 3 µg L\(^{-1}\) in both bays, with an increase up to 9 µg L\(^{-1}\) at the end of the 2010 study period. Major hindrances for determining remotely-sensed phytoplankton biomass variations were external influences, such as boat traffic or sun and sky reflectance, and non-co-varying bio-optically active substances, particularly CDOM. A locally adjusted pre-processing allowed an automated and rapid selection of valid datasets – a reduced and a corrected version. Both were successfully correlated to laboratory measurements of the algal pigment Chl a by simple and robust locally adjusted OC algorithms with the ratios \([R_{rs_s}(555)-1 \times R_{rs_s}(490)]\) \((r^2 = 0.85/0.96, \text{RMSE} = 0.68/0.36, p < 0.05)\) and \([R_{rs_s}(555)-1 \times R_{rs_s}(510)]\) \((r^2 = 0.86/0.97, \text{RMSE} = 0.67/0.34, p < 0.05)\) in a second and third degree polynomial relationship.
Unexpectedly, additional approaches based on absorption or fluorescence signals in the red/NIR spectral range with less influences by CDOM did not succeed, possibly owing to the relatively low concentrations of phytoplankton with respect to original applications. In any case, for continuous monitoring of anomalies in phytoplankton biomass, the remote sensing system is highly appropriate to support local monitoring of high biomass algal blooms including harmful events in the Ebro Delta bays.
4.1 Introduction

The Ebro Delta system comprises a nature reserve situated on the Catalonian coast of the Mediterranean Sea and is the source of manifold ecosystem goods and services in this region. The wide terrestrial Delta basin is covered predominantly by rice fields that are irrigated with river water by a system of irrigation channels and dams. Numerous watersheds are home to a diverse flora and fauna, and wide beaches make the area a popular holiday destination for tourists during summer months. The coastal region is also well known for diverse seafood varieties, such as fish, shrimps, and bivalve shellfish. The main aquaculture sites for bivalve shellfish along the entire Catalonian coast are situated in the Ebro Delta, specifically within Alfacs and Fangar bays, semi-enclosed embayments partially isolated from the Mediterranean Sea by two sandbank arms (Ramón et al., 2005b) (Fig. 4.1).

Due to the presence of potent natural phycoxotoxins produced by certain phytoplankton species, both bays are subject to frequent periodic closures for shellfish harvesting. Suspension-feeding bivalve shellfish, such as the dominant aquaculture species, the Mediterranean mussel *Mytilus galloprovincialis*, are known to accumulate phycoxotoxins above regulatory limits via feeding upon such toxigenic plankton, particularly when these plankton are present in high cell concentrations in the form of harmful algal blooms (HABs). In addition to the accumulation of toxins in mussels, fish kills have been related to occurrences of harmful algae, such as species of the ichthyotoxic dinoflagellate *Karlodinium*.

To ensure seafood safety for human consumption, harmful algal taxa and associated phycoxotoxins are monitored weekly within a governmental programme in both bays. Laboratory measurements provide detailed information on algal species and the presence of phycoxotoxins in mussels, but are limited in spatial and temporal frequency. Harmful algal proliferations, however, are characterised by a large diversity in spatial patterns of bloom formation (including surface blooms, sub-surface thin-layers, small- versus large plankton patches) and duration. The implementation of observational systems for continuous automated *in situ* data retrieval is therefore highly desirable for bloom surveillance and the development of dynamic models for their occurrence and persistence. Bio-optical sensors, in particular, allow a spatio-temporal coverage for HAB assessment in continuous settings, and over spatial scales from meters to hundreds of kilometres, but are limited with respect to accurate quantitative assessment of species-specific biomass and species identification, with few exceptions (Busch et al., 2013b). Nevertheless, although it is often not possible to determine whether or not a bloom is harmful, bloom presence, magnitude and movement patterns can be
derived in near real-time and then used to initialise additional detailed laboratory measurements for model parameterisation.

Ocean colour measurements have often been included in HAB assessment by targeting the algal pigment chlorophyll \( a \) (Chl \( a \)) as a proxy, because this pigment is present unambiguously in photosynthetic algal cells throughout all species (except of Prochlorophytes, which contain divinyl Chl \( a \)) (Stumpf et al., 2003, Hu et al., 2005). Furthermore, all known major groups of toxigenic phytoplankton associated with HABs are obligate or facultative photoautotrophs and hence contain Chl \( a \) as the primary photosynthetic pigment. Especially in coastal regions, where space-borne systems often struggle to resolve the ‘missing kilometre’ and to address bio-optical complexity along the coastline, in situ radiometric measurements provide useful information for the validation of space-borne sensor data and for regional model parameterisation, leading to the development of algorithms for determining the respective contributions of water constituents. Such in situ systems can also provide independent measurements at high temporal resolution that are necessary for optically describing particular environmental processes, but which cannot be obtained by satellite-borne systems that depend on overflight frequencies, and are subject to interference by cloud cover.

Ocean colour is determined by the light that leaves the water after interactions with in-water components and is derived in radiometric units, such as the water-leaving spectral radiance \( L_w \) or as the ratio of \( L_w \) to incoming light (downwelling solar plane irradiance \( E_s \)), defined as remote sensing reflectance \( R_{rs} \) over wavelengths (\( \lambda \)) within the visible spectral range (VIS; 380 to 700 nm) (Mobley, 1999). Information on the water constituents is derived via the magnitude and spectral form of the signal over the wavelengths. The remote sensing reflectance is dependent on the solar angle, sky and sea-state and is thus referred to as an apparent optical property (AOP), whereas inherent optical properties (IOPs) are independent of the ambient light and are solely a function of the optically active water constituents and wavelength (\( \lambda \)). Absorption (\( a \)) and the volume scattering function (\( \beta \)) are fundamental IOPs that can be measured directly with a variety of commercially available instruments over wavelengths (in the case of \( \beta \) usually over limited angles and wavelengths) (Moore et al., 2009). The radiometric quantities of ocean colour are in relation to the IOPs via

\[
R_{rs}(\lambda) \sim \frac{f}{Q} b_h(\lambda) \times (a_t(\lambda) + b_b(\lambda))^{-1}
\]  

(4.1)

where \( b_h \) is the particulate backscattering coefficient derived from \( \beta \), \( a_t \) is the total absorption and the proportionality factor \( f/Q \) accounts for complex effects, such as those from the radiance field (compare Tzortziou et al., 2007). Due to this relationship, spectral absorption
maxima of algal pigments are also (inversely) reflected in the same spectral regions of ocean colour measurements. Standard empirical algorithms, such as the Ocean Chlorophyll (OC) algorithms for Chl $a$ retrieval, are based on the ratio of increased Chl $a$ absorption in the blue and high reflectance in the green spectral region caused by high phytoplankton abundances (where most absorbing components have a minimum, but phytoplankton amounts indirectly lead to high reflectance due to high backscatter) (see O’Reilly et al., 1998, O’Reilly et al., 2000).

The IOPs are additive and the contribution of phytoplankton pigments, non-algal particles (NAP) or coloured dissolved organic matter (CDOM) to the bulk signal of absorption or scattering over the spectral range can therefore be isolated to single components. The separation of phytoplankton signals for biomass retrieval from other optically active components in clear open ocean waters, commonly referred to as Case 1, is not necessarily addressed, because phytoplankton is usually the dominant contributor to optical signals. Furthermore, suspended particles and CDOM are expected to be mainly related to phytoplankton, thus co-varying, and therefore not disturbing the signal. Mediterranean waters, however, are characterised by low phytoplankton abundances, but tend to be ‘greener’ than they should be with respect to phytoplankton content (Claustre et al., 2002). The frequent overestimation of phytoplankton by remote sensing measurements has been attributed to high CDOM content in the Mediterranean (see Morel & Gentili, 2009 and references therein). A strong influence of CDOM on phytoplankton absorption in the Ebro Delta bays would need to be tackled by a regional algorithm, if the signal was co-varying. Yet, in coastal areas with manifold additional origins and sources of CDOM and NAP, the contributions of these components to the bulk optical signal are higher and generally not co-varying (i.e. they are independent) with phytoplankton abundance. The relevant spectral areas for phytoplankton biomass are therefore superimposed in optically complex ecosystems, such as the Ebro Delta. Many of the standard algorithms for Chl $a$ retrieval are thus ineffective for the quantitative and qualitative discrimination of phytoplankton in coastal zones. The contribution of CDOM and suspended particulates to absorption exponentially decreases towards longer wavelengths and is comparatively low in the red spectral region. For turbid highly productive areas, algorithms for the retrieval of algal biomass that target the local red absorption maximum of Chl $a$ near 674 nm have been developed.

One of these approaches was first described by Gitelson et al. (2003) for the retrieval of leaf Chl $a$ content in terrestrial vegetation and successfully applied in optically complex turbid productive waters (Dall'Olmo & Gitelson, 2005, Gitelson et al., 2008, Gitelson et al., 2011).
Additionally, sun-induced Chl a fluorescence in the red spectral region has been used for the retrieval of algal biomass (Hu et al., 2005, Wernand et al., 2006). These approaches are promising for the coastal optically complex Ebro Delta region, where large contributions of CDOM are expected. Most of these methods have been applied with phytoplankton biomasses up to 100-fold of the average algal biomasses that varied around 3 µg L⁻¹ Chl a for 2000 – 2004 (Llebot et al., 2011) and 1982 – 1986 (Camp & Delgado, 1987) in Alfacs and Fangar bays.

The success of the different approaches based on ocean colour depends on the presence and contribution of absorbing and scattering water components over the spectral range. Consequently, the variability of absorption and backscattering of algal and non-algal particles, as well as dissolved organic matter is of high importance for the selection of appropriate algorithms for Chl a retrieval from ocean colour. There is, however, no comprehensive background on the variability of optically active components in the Ebro Delta, or in other words for the type of water body and dependencies (co-variations) of other optically active substances and phytoplankton abundances from a bio-optical point of view. To our knowledge, no regional coastal algorithms for the optical retrieval of phytoplankton have been developed for the Ebro Delta bays. Neither have tests of performance of the standard OC algorithms been conducted. Accordingly, there is no observational bio-optical system for a spatio-temporally enhanced surveillance of phytoplankton installed in the Ebro Delta.

The early detection of biomass anomalies over time and space would support HAB surveillance in the Ebro Delta twofold: 1) in the monitoring aspects of bloom presence, magnitude and movement; and 2) in enhancing knowledge on environmental triggers of HAB development.

The present work is an evaluation of a sensor system based upon hyperspectral light-field measurements as a tool for continuous monitoring of phytoplankton biomass in two field campaigns from May to July in 2010 and 2011 in two bays of the Ebro Delta. The specific objectives were as follows: 1) to assess sources of variability in absorbing water constituents and remote sensing reflectance $R_{rs}$ for the study region; 2) to investigate appropriate spectral regions for algal biomass retrieval by means of ocean colour based on local conditions; and 3) to determine a local algorithm for algal biomass retrieval by means of *in situ* hyperspectral reflectance measurements, as an indicator for phytoplankton bloom detection in an automated way. Specifically, the standard OC ratios and calculations based on the red spectral region and fluorescence were considered for their applicability in the local environment.
4.2 Material and Methods

4.2.1 Characteristics of the study sites

Alfacs and Fangar bays are shallow coastal embayments, separated from the Mediterranean Sea by two sandbar arms (Fig. 4.1). With a surface area of 50 km² and a maximum water depth of 6 m, Alfacs Bay is larger than Fangar Bay, with an area of 12 km² and a maximum depth of only 4 m (Camp & Delgado, 1987). These embayments are not largely influenced by tidal action, but they are strongly affected by winds and anthropogenic factors, such as the operation of an irrigation system from river water for rice agriculture (Llebot et al., in press). The opening and closure of irrigation channels and dams regulates freshwater inflow into the bays, and also the stratification regime. Anchored wooden platforms in both bays are used for aquaculture activities; on a few of these platforms, shelter and room for storage is provided by a small hut. The area is frequented by small vessels with fishermen and tourists, and floats are maintained for aquaculture and harvesting activities.

Fig. 4.1. a) The Ebro Delta is situated on the NW Mediterranean coast of Catalonia, between Barcelona and Valencia. Two semi-enclosed embayments, Alfacs Bay (southern) and Fangar Bay (northern), comprise the largest aquaculture cultivation area for the Mediterranean mussel Mytilus galloprovincialis in Catalonia. A radiometric measurement system was installed on a fixed wooden aquaculture platform in Alfacs Bay (marked with a star); b) mussels are cultivated in suspension from fixed wooden platforms aligned parallel to the coast; image of platform system in Fangar Bay.

4.2.2 Discrete determination of bio-optical and biogeochemical properties

Field work was conducted from May to the beginning of July in two consecutive years, 2010 and 2011. Ocean colour measurements were conducted continuously throughout the study seasons, from a remote sensing system installed on an aquaculture platform in Alfacs Bay (raft station: 40.620083 °N, 0.658167 °E). Weekly casts with a multi-parameter profiling system were carried out at the raft station in Alfacs Bay and a station in Fangar Bay (40.778767 °N, 0.749233 °E) for comparison. Contemporary to the weekly casts with the multi-profiling
system, water samples were taken at three depths. Besides surface samples from both bays, integrated samples between 1.0 – 2.5 m and 2.5 – 4 m (in 0.5 m steps) were collected in Alfacs Bay, and at discrete depths at 2 and 4 m in Fangar Bay. Samples for all described laboratory measurements were collected with an on-board pumping system and immediately stored in one 10 L container (pre-rinsed with sampling seawater) per depth in cooling boxes on ice and kept in the dark until arrival in the laboratory (note modification for CDOM intersect samples in Alfacs Bay described below). All samples were processed immediately upon arrival at the laboratory, with a priority on measurements involving algal pigments. Descriptive statistics of the optically active substances in Alfacs Bay surface waters are displayed in Table 4.1.

**Measurement, calculation and correction of the Remote Sensing Reflectance (Rs)**

Radiometric measurements were conducted from a fixed station on an aquaculture raft in Alfacs Bay continuously from 08:00 to 20:00 h in intervals of 15 min between measurements. Remote-sensing reflectance Rs was calculated as the ratio of the water-leaving spectral radiance Lw to downwelling spectral plane irradiance Es. The Es was derived directly with an irradiance sensor pointing to the zenith (RAMSES-ACC-VIS, TriOS, Germany), whereas the water leaving radiance was derived by measurements of the surface upwelling radiance Lsfc, with a percentage ρ of Lsky subtracted to account for sky reflections on the water surface. Remote-sensing reflectance was calculated from the following equation:

\[
Rs(\lambda) = \frac{L_w}{E_s} = \frac{[L_{sfc} - 0.024 L_{sky}]}{E_s}
\]

The sensors for Lsfc and Lsky retrieval (RAMSES-ARC-VIS) were pointing to the water surface at an angle of 30° or to the sky, respectively in a reciprocal angle. With an average wind speed of 3 m s⁻¹ at a nearby weather station, a ρ value of 0.024 was assigned to the described setting (based on Fig. 8 in Mobley, 1999).

Power was supplied with a liquid fuel cell in 2010 and with two lead-acid batteries in 2011, positioned in a shaded hut on the aquaculture raft. Due to a fuel system breakdown at the end of the field phase in 2010, Rs data from 1 July 2010 onwards are not available. Radiometric data acquisition was automatically synchronized using a global triggering function and then recorded (Tribox2, TriOS, Germany). Spectral sampling resolution was 3.3 nm per pixel in a wavelength range of 320 – 950 nm (field of view 7° for ARC-VIS, cosine for ACC-VIS); total valid spectra numbered 2396 in 2010 and 2379 in 2011. The integration time of the sensors was adjusted automatically for each measurement and sensor. The stored data were extracted from the Tribox2 to Matlab® (2012a, The MathWorks, USA) and underwent a linear interpolation in 0.5 nm steps and a quality evaluation. The latter included the
elimination of the following spectra: 1) where data were obtained under insufficient incoming light, and strong influences of red colours in dusk and dawn, by the application of two data quality flags based on $E_s$ (Wernand, 2002); 2) where data were acquired during rain events, by means of an adjustment with precipitation data from the nearby weather station; and 3) where spectral values fell below zero. Specific local challenges for $R_s$ measurements in the area were boat traffic, boat anchorage, and direct sun reflection, as the sensor system had to be faced southwards on the platform to prevent shading, and at the same time profit from shelter for the power supply. Measurements interfering with surface objects below the sensor system were eliminated automatically by applying a newly established quality flag based on steep linear slopes in the UV-blue spectral region from 350 to 450 nm; direct sun reflections were identified and deleted by a second range of this slope (Busch et al., 2013a). A subset of data from the resulting dataset was selected at the sun optimum azimuth angle ($\phi$) of $90^\circ \leq \phi \leq 135^\circ$ according to Mobley (1999), in our article referred to as $R_{s, r}$. Spectra in this dataset were least affected by sun and sky reflections, but suffered a data loss >50%. These spectra largely exclude daytimes of increased sun reflection around midday. To prevent these losses due to residual sun reflections, a correction procedure was applied by the subtraction of a glint spectral shape, resulting in a fully automated reduction of glint effects on $R_s$ spectra (Busch et al., 2013a), in the following section referred to as $R_{s, corrOx}$. In brief, the glint spectral shape was calculated for each spectrum by regressing all VIS wavelengths to a NIR band that was selected to represent the ambient light field (Hedley et al., 2005), in a revision of the method of Hochberg et al. (2003). For the presented dataset, the atmospheric absorption band for oxygen 760.5 nm was used for regression, while representative spectra of the ambient light field were automatically selected with the depth of the oxygen absorption feature (Busch et al., 2013a), calculated as described by Kutser et al. (2009). For regression analyses, up to three valid spectra closest to the water sampling time (between 11:30 and 13:00) were averaged for all datasets. For algorithm development, additional spectra, referred to as $R_{s,s}$ that were closest to water reference sampling and least affected by sun influences were selected from the original dataset.

Complementary to the remote sensing measurements, images of the sea surface and the sky were automatically taken in adjusted sampling intervals with a DualDome D12 (Mobotix, Germany) camera system. In a waterproof housing, two cameras pointed to the sky and sea surface in an angle of 45° (field of view 90°). The images covered the footprint of the radiometric measurements (for complete system setup see Fig. 4.2). The bio-optical system was controlled weekly to ensure optimal technical functioning, or, in 2011, for a weekly battery change, and cleaned if necessary.
Determination of backscattering properties
Weekly casts with a free-falling multi-profiling system (HyperPro II, Satlantic, Canada) were conducted at the stations in Alfacs and Fangar bays from a small vessel (Fig. 4.2). The instruments on the platform were connected to a power source (12 V battery on board) and measurements were immediately controlled with data acquisition software SatView on a laptop. After a pressure tare for the profiling platform on board the instrument was operated in profiling mode, at a distance of approximately 10 - 15 m off the boat with five repetitive casts per station. Casts with an inclination of >5% of the instrument were immediately repeated. Total volume scattering at 117° [\(\beta (117^\circ)\)] was obtained at 470 and 700 nm with a scattering meter (ECO Puck™ BB2F, WET Labs, USA) mounted on the profiling platform. Data processing was conducted with scale factors from the manufacturer in the programme ProSoft (Version 7.7, for details see Satlantic, 2009): Volume scattering of particles \(\beta_p (117^\circ)\) was derived by subtracting the volume scattering of water \(\beta_w (117^\circ)\) (from Morel, 1974) from \(\beta\).

The particulate backscattering coefficient \(b_{bp}\) was then calculated as:

\[
b_{bp} = 2\pi \times \beta_p (117^\circ)
\]  

(4.3)

Fig. 4.2. a) The above-water sensor system was installed on a fixed wooden aquaculture platform in Alfacs Bay, NW Mediterranean (40.620083 °N, 0.658167 °E). Remote sensing reflectance was derived with a zenith pointing RAMSES-ACC-VIS irradiance sensor and two RAMSES-ARC-VIS radiance sensors (TriOS, Germany) adjusted in an angle of 30° to the sea surface and to the sky. Below the hyperspectral radiometer setup, two cameras were installed in a waterproof housing (DualDome D12, Mobotix, Germany) to take contemporaneous control images of the sea state and sky. b) Volume scattering measurements were conducted weekly at stations in Alfacs and Fangar bays with a scattering meter (ECO Puck™ BB2F, WET Labs, USA) on a free-falling multi-parameter profiling system (HyperPro II, Satlantic, USA).
with $X = 1.1$ resulting in an uncertainty of 4% (Boss & Pegau, 2001). The total backscattering coefficient was derived by adding the backscattering of pure water $b_{bw}$ to $b_{bp}$:

$$b_b(\lambda) = b_{bp}(\lambda) + b_{bw}(\lambda)$$  \hspace{1cm} (4.4)

For this study, $b_b$ and $b_{bp}$ were used for the analysis of water component properties with relevance for remote sensing reflectance measurements. Mean values of the five casts per station were calculated after prior examination for obvious outliers.

**Quantitative determination of Chl a and TSM**

Total suspended matter (TSM) determination included inorganic (e.g., suspended sediments) and organic particles (e.g., algal cells). The TSM (in mg L$^{-1}$) and extracted chlorophyll $a$ (Chl $a_{extr}$ in µg L$^{-1}$) were determined quantitatively for all depths in triplicate samples, with Chl $a_{extr}$ as quantitative reference for algal biomass in Alfacs and Fangar bays. Upon arrival in the laboratory, samples were immediately processed as described below.

**Extracted chlorophyll $a$**

Triplicates of 1 L seawater were filtered through a 47 mm glass-fibre filter (Whatman, GF/F; ~0.7 µm nominal pore size), at vacuum pressure not exceeding 20kPA and in subdued light to protect algal pigments. The filter with the suspended solids was immediately placed in a screw cap tube with 10 mL 90% acetone. The tube was cooled in crushed ice, and algal cells on the filter were probe-ultrasonicated in subdued light for 5 min, in pulses of three seconds sonication followed by one second pause. The pigments were extracted overnight in the dark at 4 °C. Whenever extraction was not possible on the same day, filters were stored in cryovials at −80 °C and processed within one week. Following extraction, the samples were shaken by hand to assure homogeneity, and then filters and cellular debris were centrifuged for 10 min (centrifuge MR 22i, Jouan, 2538 × g). A 1 mL aliquot of the supernatant was mixed with 40 mL 90% acetone in a cuvette and the Chl $a_{extr}$ concentrations were determined by fluorometry (Model 10-005R, Turner Designs, USA). To account for degraded pigments (e.g., phaeopigments) that may result from senescent or dead phytoplankton cells, fecal pellets of zooplankton, etc., fluorescence was measured before and after acidification with 200 µL hydrochloric acid. The concentration of Chl $a_{extr}$ and phaeopigments were calculated according to protocols and equations provided by Strickland and Parsons (1972).

**Total Suspended Matter**

The dry weight of total suspended matter (TSM) was determined gravimetrically, following Strickland and Parsons (1972). In detail, clean 47 mm GF/F filters, were combusted at 450 °C for 4 h, washed with 350 mL of MilliQ water and dried in an oven at 60 °C for approximately
12 h. Filters were weighted on an analytical balance (MX5, Mettler Toledo, USA) after adapting to room temperature for at least three hours and then stored in PetriSlides (Millipore, Eschborn, Germany) until further use. For the determination of TSM, filters were placed on the filtration unit and first soaked with deionized water to prevent salt residues at the edges, and then 1 L of the seawater sample was filtered, not exceeding a vacuum of 20kPa. To wash out the remaining salt 20 mL of ammonium formate was added and filtered after 5 min. Filters were then placed in plastic PetriSlides (Millipore, Eschborn, Germany) and dried at 60 °C for approximately 12 h. After adopting room temperature for at least three hours, the filters and filtered matter were re-weighed. The dry weight of total suspended solids (mg L⁻¹) was then calculated as the difference between final and initial weight of the filter, divided by the sample volume.

Determination of variability of absorption properties
The IOPs of optically active water constituents are additive, therefore the total absorption in aquatic environments per wavelength \( a_t (\lambda) \) can be split into phytoplankton absorption \( a_\phi \) and the absorption of non-algal particles \( a_{NAP} \), the absorption of dissolved organic matter (CDOM) \( a_{CDOM} \), and pure water itself \( a_w \):

\[
a_t (\lambda) = a_\phi (\lambda) + a_{NAP} (\lambda) + a_{CDOM} (\lambda) + a_w (\lambda)
\] (4.5)

The absorption of dissolved and particulate matter was determined spectrophotometrically at room temperature for all depths in both bays as follows. Total absorption \( a_t \) was calculated as the sum of the single measured components plus values for \( a_w \) (Pope & Fry, 1997).

Determination of CDOM absorption \( (a_{CDOM}) \)
For spectroscopic analysis of CDOM, triplicate samples were filtered through 0.2 µm pore-sized polycarbonate membrane filters (Millipore, Germany) on a glass filtration apparatus in subdued light. For all surface waters and the two deeper samples from Fangar Bay, 300 mL of seawater were filtered through one filter, whereas for the two intersect depths in Alfacs Bay, 300 ml of seawater that was pumped in 0.5 m steps were filtered through three separate filters, and each filtrate was thoroughly mixed. The filtrate was then transferred into transparent 50 mL glass bottles and immediately stored in the dark at 4 °C until analysis. All glass bottles and filtration apparatus were combusted and washed with a hydrochloric acid solution and MilliQ water prior to use. Absorbance \( (A) \) was determined at room temperature in a pre-cleaned quartz cuvette with a path-length \( (l) \) of 1 cm. In 2010, \( A \) was measured with a dual beam spectrophotometer (Shimadzu UV-2450, Japan) with absorbance scans from 200 to 700 nm at a resolution of 0.5 nm, and in 2011 with a simultaneous absorbance and fluorescence system.
with absorbance scans from 240 to 450 nm in 2 nm steps (Aqualog, Horiba Scientific, Germany) using MilliQ water as a reference. An additional baseline correction was applied in 2010 via subtraction of an average of values between 685 and 700 nm. In 2011 no additional baseline correction could be applied as data at wavelengths longer than 450 nm were not available. The specific absorption coefficient of CDOM ($a_{\text{CDOM}}$) was then calculated following Kirk (1994):

$$a_{\text{CDOM}}(\lambda) = 2.303 \frac{A(\lambda)}{l} \quad (4.6)$$

where $A$ is the corrected spectrophotometrically derived absorbance at wavelength $\lambda$ and $l$ is the optical path length (m). The $A$ in the range for baseline correction between 685 and 700 nm did not exceed values above 0.001 in 2010; yet, this corresponded to an $a_{\text{CDOM}}$ of 0.2303 m$^{-1}$, which is considered to be a conservative detection limit in the following. With respect to other absorbing components, and especially $a_p$, this value is quite high and a direct comparison of components at longer wavelengths is therefore questionable. At 440 nm, a good estimate for the determination of CDOM was possible. In 2010 calculated raw spectra were smoothed with a moving average.

**Particulate matter**

The absorption of particulate matter was determined by a modified quantitative filter technique (QFT) on 47 mm GF/F filters, by filtration of 100 – 250 mL of seawater, not exceeding 20 KPa vacuum in subdued light. Filters were then blotted on clean tissue, immediately placed in a Petri-Slide and stored at -80 °C until analysis. Samples were analysed in both years within three to five months after filtration with a dual-beam UV/VIS spectrophotometer (Cary 4000, Varian, USA, equipped with a 150 mm integrating sphere (external DRA-900, Varian and Labsphere, made from Spectralon™). Filters were positioned in the sphere’s centre with a centre-mount filter holder so that the sample light beam was perpendicular to the filter (as described in Taylor et al., 2011, Röttgers & Gehrke, 2012). Absorbance scans from 300 to 850 nm were carried out with a 2 nm slit width at a scan rate of 150 nm min$^{-1}$. Baseline corrections were conducted with a clean GF/F filter, soaked in Milli-Q water for more than 30 min, and a clean dry filter. Measurements of these wet and dry blank filters were conducted in between measurements of five to six sample filters. The absorption coefficient of particulate matter $a_p$ was calculated with a general path length amplification factor of $\beta_{\text{f}} = 4.5$, as recommended for $A_s - A_t < 0.1$ (Röttgers & Gehrke, 2012):

$$a_p (\text{m}^{-1}) = 2.303 \frac{(A_s - A_t) F / V \times 1}{\beta_{\text{f}}} \quad (4.7)$$
where $A_s$ is the $A$ (absorbance) of the sample filter, $A_r$ is $A$ of the reference filter ($A = -\log_{10}T$, with $T$ being the transmittance), $V$ is the filtered sample volume in m³ and $F$ is the filter clearance area (m²).

For the determination of the absorption coefficient of algal particles ($a_\phi$), pigments on filters were quickly bleached with sodium hypochlorite (see Tassan & Ferrari, 1995, Ferrari & Tassan, 1999) to derive the absorption coefficient of non-algal particles ($a_{NAP}$) and measured with the same procedure as described for $a_p$. This value was then subtracted from $a_p$ to derive the absorption of algal pigments $a_\phi$ according to Equation 4.8. As the sodium hypochlorite bleached all pigments on the filter, this value may include also phaeopigments that were associated with other algal particles or small zooplankton.

$$a_\phi(\lambda) = a_p(\lambda) - a_{NAP}(\lambda) \quad (4.8)$$

### 4.3 Results

#### 4.3.1 Variability of phytoplankton biomass, TSM and CDOM

The variability of absorbing components as reported here is focussed primarily on the surface water of Alfacs Bay, where the radiometric measurements were applied to derive phytoplankton biomass from $R_{rs}$, and on relevant spectral regions of phytoplankton absorption. Nevertheless, certain results of further measurements at lower depths and from Fangar Bay are included for comparative purposes and to optically characterise the respective water bodies, to evaluate the potential transferability of the sensor system.

Descriptive statistics for both study years indicate optical conditions that are typical for coastal waters, including from the Mediterranean Sea. Yet, there was only low to moderate phytoplankton production with a mean Chl a concentration 2.16 µg L⁻¹ in 2010 and 1.10 µg L⁻¹ in 2011. One exception to the low algal biomass in surface waters during the study was an increase in the last two weeks of the sampling period in 2010, when a maximum of 9.97 µg L⁻¹ was measured. The two maximum measurements of Chl $a_{\text{exr}}$ are responsible for the high coefficient of variance (CV) (>150%) in this year, as compared to 38% and a maximum of 1.10 µg L⁻¹ in 2011 (Table 4.1). Frequency distribution plots for all depths in both bays display the same trend; phytoplankton biomass in 2010 is skewed towards values <3 µg L⁻¹ Chl a (Fig. 4.3). In addition to the chlorophyll, TSM also reached higher concentrations in 2010 than in 2011, leading to an increased range of optically active particulate matter in this year. As expected, Chl a, TSM and CDOM did not strongly co-vary in both years when observed over all depths in both bays (Fig. 4.4).
Table 4.1 Values of optically active water parameters measured for Alfacs Bay surface waters in 2010 and 2011, including extracted chlorophyll $a$ (Chl$_{a \text{ extr}}$), total suspended matter (TSM), particulate absorption at 440 nm ($a_p$), comprising two components algal pigments ($a_p$) and non-algal particles ($a_{\text{NAP}}$), absorption by coloured dissolved organic matter ($a_{\text{CDOM}}$) and particulate backscattering coefficients ($b_{bp}$) at 470 and 700 nm.

<table>
<thead>
<tr>
<th>Study period 2010</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± STD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $a_{\text{extr}}$ [µg L$^{-1}$]</td>
<td>11</td>
<td>0.19</td>
<td>9.97</td>
<td>2.16 ± 3.35</td>
<td>155</td>
</tr>
<tr>
<td>TSM [mg L$^{-1}$]</td>
<td>11</td>
<td>2.30</td>
<td>11.33</td>
<td>5.41 ± 2.77</td>
<td>51</td>
</tr>
<tr>
<td>$a_p$ (440) [m$^{-1}$]</td>
<td>11</td>
<td>0.03</td>
<td>0.17</td>
<td>0.09 ± 0.04</td>
<td>46</td>
</tr>
<tr>
<td>$a_{\text{NAP}}$ (440) [m$^{-1}$]</td>
<td>11</td>
<td>0.05</td>
<td>0.14</td>
<td>0.09 ± 0.03</td>
<td>33</td>
</tr>
<tr>
<td>$a_{\text{CDOM}}$ (440) [m$^{-1}$]</td>
<td>11</td>
<td>~0.23</td>
<td>1.25</td>
<td>0.55 ± 0.43</td>
<td>78</td>
</tr>
<tr>
<td>$b_{bp}$ (470) [m$^{-1}$]</td>
<td>10</td>
<td>0.0150</td>
<td>0.0381</td>
<td>0.0220 ± 0.0067</td>
<td>31</td>
</tr>
<tr>
<td>$b_{bp}$ (700) [m$^{-1}$]</td>
<td>10</td>
<td>0.0124</td>
<td>0.0298</td>
<td>0.0169 ± 0.0052</td>
<td>31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study period 2011</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± STD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $a_{\text{extr}}$ [µg L$^{-1}$]</td>
<td>9</td>
<td>0.19</td>
<td>1.10</td>
<td>0.68 ± 0.23</td>
<td>38</td>
</tr>
<tr>
<td>TSM [mg L$^{-1}$]</td>
<td>9</td>
<td>2.57</td>
<td>4.33</td>
<td>3.39 ± 0.66</td>
<td>20</td>
</tr>
<tr>
<td>$a_p$ (440) [m$^{-1}$]</td>
<td>9</td>
<td>0.04</td>
<td>0.22</td>
<td>0.12 ± 0.05</td>
<td>39</td>
</tr>
<tr>
<td>$a_{\text{NAP}}$ (440) [m$^{-1}$]</td>
<td>9</td>
<td>0.07</td>
<td>0.23</td>
<td>0.18 ± 0.05</td>
<td>28</td>
</tr>
<tr>
<td>$a_{\text{CDOM}}$ (440) [m$^{-1}$]</td>
<td>9</td>
<td>0.02</td>
<td>0.22</td>
<td>0.08 ± 0.06</td>
<td>77</td>
</tr>
<tr>
<td>$b_{bp}$ (470) [m$^{-1}$]</td>
<td>9</td>
<td>0.0171</td>
<td>0.0311</td>
<td>0.0208 ± 0.0047</td>
<td>23</td>
</tr>
<tr>
<td>$b_{bp}$ (700) [m$^{-1}$]</td>
<td>9</td>
<td>0.0141</td>
<td>0.0211</td>
<td>0.0164 ± 0.0024</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 4.3. Variations in extracted chlorophyll $a$ (Chl $a_{\text{extr}}$) [µg L$^{-1}$] (left panels) and total suspended matter (TSM) [mg L$^{-1}$] (right panels) for all three depths in frequency diagrams for Alfacs Bay: a) in 2010 and b) in 2011, and Fangar Bay: c) in 2010 and d) in 2011.
Fig. 4.4. Scatterplots of relationships between optically active substances total suspended matter (TSM), extracted chlorophyll a (Chl $a_{\text{extr}}$), and coloured dissolved organic material ($a_{\text{CDOM}}(440)$) at three depths, derived from Alfacs Bay and Fangar Bay from May–July 2010 and 2011 reveal non-covariance of these parameters: a) TSM vs Chl $a_{\text{extr}}$ ($r^2 = 0.099$; RMSE = 2.380; n = 82); b) Chl $a_{\text{extr}}$ vs $a_{\text{CDOM}}(440)$ ($r^2 = 0.055$; RMSE = 2.445; n = 81); c) TSM vs $a_{\text{CDOM}}(440)$ ($r^2 = 0.0004$; RMSE = 2.095; n = 83).
4.3.2 Contribution of optically active components to absorption and backscattering

The absorption spectra of algal pigments, TSM, and CDOM over VIS wavelengths were characteristically shaped (compare Babin et al., 2003) and displayed similar magnitude throughout both years for both bays (Fig. 4.5). Pigment absorption spectra were characterised by a distinctive maximum around 430 nm, the absorption maximum of Chl \( a \). Besides the main peak, a local maximum in the red spectral range (at 674 nm), also mainly attributable to Chl \( a \), was visible in the pigment absorption spectra. Additionally, the shape of the absorption curves was affected by the presence of accessory pigments with their respective local maxima, mainly at shorter wavelengths. The CDOM and NAP contributions were high in the UV-blue spectral range, but exponentially descending towards longer wavelengths. In particular, large contributions of CDOM to the total absorption at shorter wavelengths mask the distinctive Chl \( a \) maximum absorption peak in the green region. A minimum for all algal and non-algal absorbing components was evident in the range between 550 and 650 nm for all spectra and at all depths (Fig. 4.5).

The large contribution of CDOM was also visible when considering the percentage contribution of the three parameters to total absorption, excluding that of pure water, at 440 nm – a wavelength at which all parameters contribute to the total absorption (Fig. 4.6a). Based on these spectra, the optical properties of the water bodies in Alfacs and Fangar bays can be classified as mainly determined by CDOM and to a minor extent by phytoplankton for both years. When considering the contribution at 674 nm, namely the Chl \( a \) red absorption maximum, \( a_\phi (674) \) clearly dominates over \( a_{NAP} (674) \) and \( a_{CDOM} (674) \) (Fig. 4.6b).

When considering the mean contribution of only algal and non-algal particles over the VIS spectral range, the algal signal always dominated at wavelengths longer than 450 nm (Fig. 4.5). This tendency remains when the percentage contribution of algal and non-algal particles is compared for each single sampling event at wavelengths relevant for phytoplankton absorption. Algal absorption dominated non-algal absorption at 443 nm and 490 nm in most cases (50 – 75% contribution), and strongly exceeded that of non-algal particles (65 – 100% contribution) at 674 nm (Fig. 4.5). In general, NAP contributed only moderately to the total absorption when compared to the other optically active components.

In summary, CDOM contribution to absorption was dominant in most relevant regions for Chl \( a \) absorption, excluding the local red absorption maximum of Chl \( a \). With respect to NAPs, algal particles dominated at spectral regions that are relevant for the retrieval of Chl \( a \) as phytoplankton biomass proxy.
Fig. 4.5. Contribution of optically active substances to absorption in average and over the study time for (a, b) Alfacs and (c, d) Fangar Bay from May to July 2010 and 2011. Spectral magnitude and shape of absorbing components is shown as averaged over the study time from 300 to 750 nm (left plots). Components are divided into particulate absorption $a_p$, split into pigment $a_{φ}$ and non-algal particulate absorption $a_{NAP}$, and the absorption of coloured dissolved organic matter ($a_{CDOM}$ only available until 450 nm in 2011) and water $a_w$. In the right plots, the percentage contribution of algal $a_{φ}$ and non-algal particles $a_{NAP}$ to total particulates for each sampling event is shown for each bay and year at relevant wavelengths at three wavelengths that are relevant for phytoplankton biomass retrieval, at 433, 490 and 674 nm. Each bar represents one sampling event, the horizontal line indicates the 50% mark, where $a_{φ}$ or $a_{NAP}$ dominate $a_p$. 
Fig. 4.6. Relative [%] contribution of pigments $a_{\phi}$ and non-algal particles $a_{\text{NAP}}$ and coloured dissolved organic matter $a_{\text{CDOM}}$ over the study time from May to July 2010 and 2011 in Alfacs and Fangar bays to absorption at a) 440 nm and b) 674 nm. For 2011 no CDOM absorption values >450 nm are available.

Fig. 4.7. Regression (solid line) showing the relation of absorption coefficient of phytoplankton pigments $a_{\phi}$ at 443 nm to Chl+Phaeo $a_{\text{extr}}$ for three depths in Alfacs and Fangar bays from May – July 2010 and 2011 ($n = 86$) ($r^2 = 0.49$, RMSE = 0.576, $p = 5.59e-14$). Surface measurements in Alfacs Bay 2010 and 2011 are marked with squares; regression line for this sub-sample selection is given by the dotted line ($n = 20$, $r^2 = 0.64$, RMSE = 3.72, $p = 2.6e-05$).
The absorption of algal particles at relevant wavelengths (443, 490, 510 and 674 nm) could be well related to fluorometrically derived algal pigments from the same water samples. As $a_{\phi}$ was derived by the QFT and may also contain degradation products or inactive Chl $a$, e.g. from relicts of the contents or faecal pellets of small grazers on the filter, the sum of Chl $a_{\text{extr}}$ and Phaeo $a_{\text{extr}}$ was used to establish the relationship. At all wavelengths and years, $a_{\phi}$ was positively correlated to Chl+Phaeo $a_{\text{extr}}$ in surface waters, with best values for 443 nm ($r^2 = 0.64$, $n = 20$) (Fig. 4.7). Over all depths and years, the positive relationship remained ($r^2$ of 0.49, root mean square error (RMSE) = 5.76, $n = 86$).

The contribution of algal and non-algal particles to particulate backscattering was examined in the blue (470 nm) and red (700 nm) spectral region for all depths over both years in Alfacs Bay (Fig. 4.8). By trend, absorption and backscattering are positively correlated in both spectral regions. At both wavelengths, variations in the backscattering signal were explained by the absorption of non-algal particles by approximately 30%, whereas algal pigment absorption explained about 40% of the backscattering signal at 700 nm and was equal.
to $a_{\text{NAP}}$ 30% at 470 nm. Consequently, there was no dominant particulate contributor to the particulate backscattering signal.

### 4.3.3 Variability of remote sensing spectra and their relation to IOPs

Remote sensing spectra were quite similar in magnitude and shape during the study time in both years, with few events of higher intensity notable in 2010 (Fig. 4.9b, c). The spectral shape can be attributed to a predominantly (blue-)green water colour in Alfas Bay throughout both study years. As a first approximation, when compared with the reverse spectrum of total absorption, the shape of $R_\text{n}$ also generally reflects the representation of optically active substances as described in the results. Dominant similarities include a maximum reflectance in the green region around 570 nm, coinciding with a minimum absorption in this spectral range. Furthermore, in the blue and red regions, the remote sensing spectrum tended to decrease from the maximum towards shorter and longer wavelengths. Correspondingly, the influence of absorption to the spectrum in these regions is shaped predominantly by the exponentially decreasing absorption of CDOM and the absorption of pure water (Fig. 4.9a), with a strong contribution of CDOM from blue to green, and of water from 555 nm onwards. Effects of phytoplankton at the relevant wavelengths for Chl $a$ are not clearly visible in either spectra, as the phytoplankton absorption is masked by the effect of other matter and water, but there are indicated troughs in the inverse total absorption spectrum and also in the $R_\text{n}$ spectrum at relevant wavelengths. A local maximum at 680 – 685 nm that should be assigned to Chl $a$ sun-induced fluorescence is, however, not clearly visible in the $R_\text{n}$ spectrum. The NIR region (>740 nm) of $R_\text{n}$ spectra is characterised by strong absorption of water and is therefore generally low, with a local minimum at 760.5 due to the oxygen absorption band depth, where this element absorbs most. Variability in the light intensity in the NIR spectral region is mainly assignable to remaining sun and sky reflections.

In coastal waters, the effect of backscattering on remote sensing spectra is assumed to be quite high, as $R_\text{n}$ can be related to IOPs following Equation 4.1. To test the sources of variability on $R_\text{n}$ by both, absorption and backscattering, the remote sensing spectra were examined by regression at 440 and 700 nm.

Whereas the general shape of the curve can be well attributed to absorption of the water components, the effects to the intensity of the $R_\text{n}$ spectrum at the selected wavelengths indeed are strongly attributable to backscattering, when considering the whole dataset for Alfas Bay surface waters (Table 4.2). For Alfas Bay surface waters, 40% of the variability in $R_{\text{n,r}}$ spectra at 440 nm, and 70% of the variability at 690 nm is explained by $b_\theta$. Absorption explains only 23% of the variability in $R_{\text{n,r}}$ spectra at 440 nm, but considerably more (43%) at
670 nm. Whereas the contribution is even stronger when examined separately for the 2010 dataset, no clear connection between absorption and backscattering with remote sensing spectra emerges in 2011.

Fig. 4.9. a) Reverse average total absorption (continuous black line) $a_\text{a}$, the sum of particulate matter absorption ($a_\text{p}$), and the absorption of coloured dissolved organic matter ($a_{\text{CDOM}}$) and water ($a_\text{w}$) for Alfacs surface waters from May to July 2010 (note the reverse scale on y-axis). Particulate matter absorption ($a_\text{p}$) is divided into phytoplankton pigment ($a_\phi$) and non-algal particle absorption ($a_{\text{NAP}}$). Remote sensing reflectance spectra taken at an optimal sun’s azimuth angle ($R_{\text{rs_r}}$, corrected for surface objects) for all measurements in b) 2010 and c) 2011 are shown over wavelengths from 350 – 800 nm. The main Chl $a$ absorption wavelengths 412, 433, 490, 510 and 674 nm are indicated by red dotted vertical lines.
### Table 4.2. Relationship between remote sensing reflectance ($R_{rs}$) at 440 and 670 nm vs. inherent optical properties total absorption ($a$) and particulate backscattering ($b_b$) in Alfacs Bay surface waters in 2010, 2011 and for both years combined. An $r^2$ above 0.5 is marked bold.

<table>
<thead>
<tr>
<th>Parameter (X)</th>
<th>Parameter (Y)</th>
<th>Combined years</th>
<th>Study period 2010</th>
<th>Study period 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{rs}(440)$</td>
<td>$b_b(470)$</td>
<td>9+9</td>
<td>0.407</td>
<td>0.005</td>
</tr>
<tr>
<td>$R_{rs}(670)$</td>
<td>$b_b(700)$</td>
<td>9+9</td>
<td>0.691</td>
<td>0.002</td>
</tr>
<tr>
<td>$R_{rs}(440)$</td>
<td>$a(440)$</td>
<td>9+9</td>
<td>0.233</td>
<td>0.337</td>
</tr>
<tr>
<td>$R_{rs}(670)$</td>
<td>$a(670)*$</td>
<td>10+9</td>
<td>0.425</td>
<td>0.017</td>
</tr>
<tr>
<td>$R_{rs}(440)$</td>
<td>$b_b(470) \times (a(440)+b_b(470))^{-1}$</td>
<td>8+9</td>
<td>0.069</td>
<td>0.114</td>
</tr>
<tr>
<td>$R_{rs}(670)$</td>
<td>$b_b(700) \times (a(670)^*+b_b(700))^{-1}$</td>
<td>9+9</td>
<td>0.660</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* $a(670)$ in 2011 was calculated without CDOM absorption $a_{CDOM}$, as data were only available until wavelength 450 nm. CDOM absorption at such wavelengths was, however, close to zero as observed with data from 2010, and also negligible for the relationship in 2011.

#### 4.3.4 Selection of algorithms for estimation of phytoplankton biomass

In the following, the performance of three empirical approaches for Chl $a$ retrieval were tested with respect to the above described regional variability of optically active components: 1) Standard Ocean Chlorophyll (OC) algorithms; 2) a three band approach in the NIR where lesser influences of CDOM contribute to the spectral signal; 3) the sun-induced Chl $a$ fluorescence signal, likewise in the red spectral region. For algorithm development, all $R_{rs}$ datasets were tested. As sun effects were strongest at midday, when the field reference samples for Chl $a$ were taken, the dataset that based on an optimal sun’s azimuth angle $R_{rs_s}$ was often 2-4 h before the water sampling, whereas the manually selected spectra with least sun influences $R_{rs_s}$ were closer to the sampling event. The spectra corrected for sun and sky reflection $R_{rs_corrOx}$ were fully aligned with sampling.

**Ocean Chlorophyll (OC) algorithms**

Standard empirical OC algorithms are built up of ratios between the blue spectral regions with high absorbance and inversely decreased reflectance due to increases in algal biomass, and the green spectral regions, characterised by low absorption and thus a high reflectance that is further increased by backscattering of algal cells. For the presented dataset, indeed the presence of algal cells could be positively related to the $a_\phi$ absorbance at 443, 490 and 510 nm. A defined relationship to $R_{rs}$ spectra was theoretically possible if the signal was not masked by the strong CDOM absorption. The CDOM signal is highly dominating in these spectral areas, but as it decreases exponentially with increasing wavelengths it cannot be assumed to be responsible for local minima and maxima. There are, however, visible troughs in both the
inverse total absorption and the $R_{rs}$ spectra. These troughs may be attributable to phytoplankton absorption. With respect to particulate matter, algal particles contributed on average to >50\% in spectral range >450 nm to the NIR in both study years. On this basis, the two ratios $[R_{rs} (555)^{-1} \times R_{rs} (490)]$ and $[R_{rs} (555)^{-1} \times R_{rs} (510)]$, as well as a maximum value algorithm $[R_{rs} (555)^{-1} \times (R_{rs} (510) > R_{rs} (490) > R_{rs} (433))]$ were tested for their performance in Alfacs Bay, with the divisor 555 nm being close to the minimum absorption and maximum reflectance observed in the spectra. The maximum value algorithm proved to equal the product $[R_{rs} (555)^{-1} \times R_{rs} (510)]$ on all occasions and was therefore not further considered. For the dataset obtained in 2010, a simple quadratic linear regression between $R_{rs}$ spectra and Chl $a_{extr}$ was demonstrated with all OC band ratios, with best fits for the corrected dataset in 2010 $[R_{rs,corrOx} (555)^{-1} \times R_{rs,corrOx} (490)]$ ($r^2 = 0.79$, RMSE = 1.16, $p < 0.05$). By inclusion of the 2011 data set for validation, the best fit for linear regression was achieved with $[R_{rs,corrOx} (555)^{-1} \times R_{rs,corrOx} (510)]$ ($r^2 = 0.43$, RMSE = 1.31, $p< 0.05$). The separate investigation of the validation dataset, however, resulted in poor fits.

Three wavelength ratio in the red

A second approach, the inclusion of the local Chl $a$ absorption maximum in the red spectral region, with the assumption that the influences of CDOM absorption would superimpose the impact of phytoplankton pigments in the green, did not comply with Chl $a_{extr}$ data. For the presented dataset, $\lambda_1$ (674), $\lambda_2$ (700), and $\lambda_3$ (755) were selected following the examination of our $R_{rs}$ and absorption spectra, as well as original values used by Dall’Olmo and Gitelson (2005): $\lambda_1$ (673), $\lambda_2$ (710), and $\lambda_3$ (740). For both selections, wavelength $\lambda_1$ has a high sensitivity for Chl $a$ absorption (close to the red local absorption maximum), and a lesser effect of TSM and CDOM. $\lambda_1$ is reduced by subtraction of $\lambda_2$ with a minimal sensitivity to Chl $a$, but a similar effect of TSM and CDOM as $\lambda_1$. Therewith, the effects of the two undesired non-algal absorbing components are subtracted and the effect of algal absorption at $\lambda_1$ remains. Contributions of backscattering are taken into account by dividing the difference by $\lambda_3$ with minimal contribution of absorption but strong influence of backscattering. The relationship to the Chl $a_{extr}$ was, however, poor for all datasets from both years.

Fluorescence line height

Algorithms based on the fluorescence line height (FLH) were also tested, to circumvent masking of the phytoplankton absorption signal in the green by CDOM. Among several two- and three-wavelength approaches (Hu et al., 2005, Wernand et al., 2006), a positive relationship was found by a two-band approach that targets the peak magnitude assigned to the sun induced Chl $a$ fluorescence at 686 nm to a minimum at 672 nm by simple subtraction $[R_{rs}$
Most of the obtained differences were, however, assigned to spectral values below zero.

### 4.3.5 Application of OC algorithm for estimation of phytoplankton biomass

Polynomial regressions were performed for OC band ratios as these were the most promising approaches based on the first estimations by linear regression. Best fits for second degree polynomials were achieved with the ratios \(\frac{R_{rs_s}(555)}{R_{rs_s}(490)} \times R_{rs_s}(490)\) \((r^2 = 0.85, \text{RMSE} = 0.683, p < 0.05)\) and \(\frac{R_{rs_s}(555)}{R_{rs_s}(510)} \times R_{rs_s}(510)\) \((r^2 = 0.858, \text{RMSE} = 0.671, p < 0.05)\) for the selected spectra of the original dataset; when considering only the data from 2010, the sun and sky reflectance corrected spectra \(\frac{R_{rs_CorrOx}(555)}{R_{rs_s}(510)} \times R_{rs_s}(490)\) \((r^2 = 0.992, \text{RMSE} = 0.247, p < 0.05)\) performed best. With third degree polynomials, the same ratios performed slightly better with \(\frac{R_{rs_s}(555)}{R_{rs_s}(490)} \times R_{rs_s}(510)\) \((r^2 = 0.963, \text{RMSE} = 0.355, p < 0.05)\) and \(\frac{R_{rs_s}(555)}{R_{rs_s}(510)} \times R_{rs_s}(510)\) \((r^2 = 0.965, \text{RMSE} = 0.343, p < 0.05)\), and likewise for the year 2010 with the corrected dataset \(\frac{R_{rs_CorrOx}(555)}{R_{rs_CorrOx}(490)} \times R_{rs_CorrOx}(490)\) \((r^2 = 0.995, \text{RMSE} = 0.213, p < 0.05)\) (Fig. 4.10, Table 4.3). Algal biomass variations over time were then retrieved by applying the algorithms obtained from the second and third degree polynomial regressions for both, the original dataset with spectra influenced by sun reflectance excluded, and the corrected dataset. To reduce remaining disturbances, e.g., due to remaining sky reflections, the retrieved algal biomass was filtered with a multiple moving average. When considered over the sampling time in 2010 and 2011, the continuous time-series of variations in phytoplankton biomass were in good agreement with the field sample reference Chl \(a_{ext}\) data for both polynomials and for both ratios (Fig. 4.11). As an independent measure, Chl \(a_{ext}\) values from weekly depth integrated measurements at a nearby station \((40.622133^\circ N, 0.658183^\circ E)\) were also included in the time series. Particularly for the first week of the study in 2010, when no surface data for Chl \(a_{ext}\) were available, the additional value was an indicator for the validity of the algorithm. In general, all values from 2010 yielded a good fit to the derived dataset, even though these data were not included in the regression and algorithm development. In 2011, the validation with an external dataset from the same location was not comparably successful. It should be noted that all external values were considerably higher than at the original sampling point at the aquaculture platform. Furthermore, strong salinity-dependent stratification of the water column in this year may have impeded the comparison of our surface samples with these depth integrated samples.
Fig. 4.10. a) Regression relationships (n = 19, p < 0.05) between Chl $a_{ex}$ and $R_{rs_s}$ in Alfacs and Fangar Bay with second degree (a, b) and third degree (c, d) polynomial regression for $R_{rs_s}(555)^{-1} \times R_{rs_s}(490)$ (b, d) and $R_{rs_s}(555)^{-1} \times R_{rs_s}(510)$ (a, d). Details on fits are given in Table 4.3.
Table 4.3. Linear regression relationships between Chl \textit{a}_{\text{ext}} and \textit{R}_{\text{ns}} in Alfacs Bay surface waters for tested algorithms. Results for both years are presented combined (\textit{n} = 18) and for each year separately (\textit{n} = 9). \textit{r}^2 values > 0.5 are indicated in bold, best fits are underlined.

<table>
<thead>
<tr>
<th>Parameter (X)</th>
<th>Combined years</th>
<th>Study period 2010</th>
<th>Study period 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{r}^2</td>
<td>RMSE</td>
<td>\textit{p}</td>
</tr>
<tr>
<td>\textit{OC Band ratios}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.403</td>
<td>1.332</td>
<td>0.005</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.374</td>
<td>1.364</td>
<td>0.007</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(490)}</td>
<td>0.422</td>
<td>1.312</td>
<td>0.004</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(510)}</td>
<td>0.421</td>
<td>1.312</td>
<td>0.004</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(510)}</td>
<td>0.391</td>
<td>1.347</td>
<td>0.006</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(434/490/510)}^{\max}</td>
<td>0.426</td>
<td>1.306</td>
<td>0.003</td>
</tr>
<tr>
<td>\textit{Three band red NIR}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns}}(673) - \textit{R}</em>{\text{ns}}(710)^{1} \times \textit{R}_{\text{ns}}(740)</td>
<td>0.025</td>
<td>1.703</td>
<td>0.531</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns}}(673) - \textit{R}</em>{\text{ns}}(710)^{1} \times \textit{R}_{\text{ns}}(740)</td>
<td>0.028</td>
<td>1.701</td>
<td>0.509</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(673)} - \textit{R}</em>{\text{ns,corr}(710)}^{1} \times \textit{R}_{\text{ns}}(740)</td>
<td>0.064</td>
<td>1.669</td>
<td>0.313</td>
</tr>
<tr>
<td>\textit{FLH}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns}}(686) - \textit{R}</em>{\text{ns}}(672)</td>
<td>0.285</td>
<td>1.459</td>
<td>0.023</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns}}(686) - \textit{R}</em>{\text{ns}}(672)</td>
<td>0.168</td>
<td>1.573</td>
<td>0.091</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(686)} \textit{R}</em>{\text{ns,corr}(672)}</td>
<td>0.139</td>
<td>1.600</td>
<td>0.127</td>
</tr>
<tr>
<td>\textit{OC ratios, second degree polynomial}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.718</td>
<td>0.945</td>
<td>7.44e-05</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.853</td>
<td>0.683</td>
<td>5.69e-07</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(490)}</td>
<td>0.834</td>
<td>0.726</td>
<td>1.43e-06</td>
</tr>
<tr>
<td>\textit{R}_{\text{ns,corr}(490)}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(510)}</td>
<td>0.707</td>
<td>0.965</td>
<td>0.0001</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(510)}</td>
<td>0.858</td>
<td>0.671</td>
<td>4.37e-07</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(510)}</td>
<td>0.783</td>
<td>0.830</td>
<td>1.05e-05</td>
</tr>
<tr>
<td>\textit{OC ratios, third degree polynomial}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.866</td>
<td>0.674</td>
<td>2.26e-06</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(490)}</td>
<td>0.963</td>
<td>0.355</td>
<td>3.02e-10</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(490)}</td>
<td>0.911</td>
<td>0.551</td>
<td>1.38e-07</td>
</tr>
<tr>
<td>\textit{R}_{\text{ns,corr}(490)}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(510)}</td>
<td>0.832</td>
<td>0.756</td>
<td>1.09e-05</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns},(555)}^{1} \times \textit{R}</em>{\text{ns},(510)}</td>
<td>0.965</td>
<td>0.343</td>
<td>1.86e-10</td>
</tr>
<tr>
<td>\textit{R}<em>{\text{ns,corr}(555)}^{1} \times \textit{R}</em>{\text{ns,corr}(510)}</td>
<td>0.882</td>
<td>0.632</td>
<td>9.29e-07</td>
</tr>
</tbody>
</table>
Fig. 4.11. Calculation of Chl $a$ concentrations from remote sensing reflectance measurements $[R_{rs_s}(555) - 1 \times R_{rs_s}(490)]$ throughout the study seasons in 2010 (a, c) and 2011 (b, d), derived by a second degree polynomial regression (a, b). As the original dataset contains strong sun and sky reflections, only data omitting spectra taken between 12 – 17 h were plotted. These data are still influenced by daily variations of sun and sky reflections (grey), and are filtered with a moving average (red filled diamonds). The corrected full dataset $[R_{rs_{corrOx}}(555) - 1 \times R_{rs_{corrOx}}(490)]$ is also plotted (red empty diamonds), generally well complying with the pattern. The same dataset is also shown as calculated with an algorithm derived by a third degree polynomial with measurements $[R_{rs_s}(555) \times R_{rs_s}(510)]$ and $[R_{rs_{corrOx}}(555) \times R_{rs_{corrOx}}(510)]$ (c, d). Corresponding Chl $a_{extr}$ values used for the algorithm development are also shown (green circles), as well as depth-integrated Chl $a_{extr}$ data taken during a weekly monitoring routine at a nearby station. These data were not included in algorithm development but are included for a validation of the applied algorithm.
4.4 Discussion

4.4.1 Variability of optically active substances

Throughout the study period from May to the beginning of July in 2010 and 2011, the bio-optically active components of the Ebro Delta bays were similar in magnitude over all spectral ranges between the bays and years. The Ebro Delta Bays can indeed be described as optically complex because, as expected, variations in phytoplankton, non-algal particles and CDOM are independent of each other. The study region is strongly CDOM dominated, with respect to absorption at 440 nm, with a minor influence of phytoplankton particles. This basic view is also reflected by the remote sensing signal. The spectral shape is comparable to remote sensing spectra of other water types with moderate sediment and CDOM with some phytoplankton (IOCCG, 2000). The water colour is indicated as predominantly green by the remote sensing (ocean colour) signal. While the overall shape of the remote sensing spectra are essentially determined by the exponentially decreasing absorption of CDOM from shorter to longer wavelengths, and by the increasing water absorption towards longer wavelengths, local minima and maxima at relevant spectral regions for phytoplankton biomass monitoring are visible and are attributable to phytoplankton abundances. As opposed to the spectral shape, there are indications that backscattering explains most of the changes in the remote sensing signal at blue-green (470 nm) and red (700 nm) wavelengths (Table 4.2) and is therefore probably strongly determining the spectral magnitude.

In the Ebro Delta bays, freshwater input from the management of rice fields is an important element that determines stratification of the water column in the bays. During the two studied seasons May to July (2010 and 2011), continental water from the rice fields was discharged into the embayments. The yearly freshwater inflow results in a stronger stratification of water with a freshwater layer on top and a salt wedge entering from the open ocean on the bottom (Camp & Delgado, 1987). During this study, a surface freshwater layer was well pronounced, especially in 2011, which explains the lower phytoplankton biomass in surface layers during the study period (Publication VII this thesis). As opposed to the presented situation, the water column tends to be better mixed and transparent in winter. This may imply a change of the variability of bio-optically active water components. The presented dataset therefore represents a seasonal excerpt of bio-optically active components in the Ebro Delta Bays.

4.4.2 Phytoplankton biomass retrieval by remote sensing

The main objective of the present work was to evaluate the inclusion of hyperspectral light-field ocean colour measurements as a tool for automated and continuous monitoring of
phytoplankton biomass in the Ebro Delta bays. Practically speaking, ocean colour was evaluated by automated continuous measures of remote sensing reflectance in order to estimate phytoplankton biomass from an above water platform and consequently to detect biomass anomalies and algal blooms. Surprisingly this estimate was best and most successfully represented by the application of a simple OC algorithm. Two OC ratios, \([R_n(555)^{1} \times R_n(490)]\) and \([R_n(555)^{1} \times R_n(510)]\), resulted in very good compliance with measured Chl \(a\). The algorithms performed well when calculated from the first dataset (2010) and with the combination of both datasets (2010 and 2011). When regarding the second dataset separately, however, none of tested methodologies led to successful representation. As there were no large differences in optical complexity, the main difference between the years - a considerably lower concentration of Chl \(a\) (with maximum values of 1.1 \(\mu g\) L\(^{-1}\) in 2011) - was possibly responsible.

The calculation of derived algal biomass from remote sensing spectra yielded similar amounts of Chl \(a\) over time, when comparing a dataset for which spectra around midday were deleted, with a dataset where these \(a\ priori\) removed spectra were included, but corrected for sun and sky reflectance. This allows two conclusions: first, the algorithm is generally robust, which is also indicated by the similar fits for two different OC ratios, and second, the correction of spectra was successful when compared to outcomes of the reduced dataset (Fig. 4.11). The derived algal abundances revealed variations that could not be covered by the weekly reference water samples throughout the study time. Additional data points of an external sampling set of Chl \(a\) \(_{ext}\) allowed an independent measure of these variations and generally did fit well into the algal biomass pattern derived by the local OC algorithm in 2010. These Chl \(a\) \(_{ext}\) data were derived by the same method and instrumentation as the data in this study and therefore can function as a validation dataset. Nevertheless, although the sampling location is geographically close (approximately 200 m), it may temporarily represent a different water-mass as it is located at the other side of the aquaculture raft systems. This may explain why the last data point of the independently derived dataset is below the Chl \(a\) \(_{ext}\) value and the derived algal biomass values by remote sensing at the end of the study period in 2010. Furthermore, samples from the external dataset were not restricted to the water surface as in our study, but represent depth integration through the whole water column. This compromises the use of the external dataset for validation in the year 2011. Chl \(a\) \(_{ext}\) concentrations of the external dataset were considerably higher throughout the study time in this year, which may be due to the depth-integrated sampling regime. In 2010, a pronounced freshwater layer developed during our study time, which was characterised by lower Chl \(a\) fluorescence than in deeper layers in this year, whereas such a layering of water was not apparent during most of
the study period in 2010. Therefore, the use of this dataset for validation is questionable even through some values comply with peaks in our remotely sensed algal biomass in 2011 (Fig. 4.11).

During the study period 2010, only one $R_{rs}$ spectrum for regression to increased Chl $a$ concentrations at the end of 2010 was available for inclusion in algorithm development, due to a breakdown of the liquid fuel cell. Therefore, the observed increase of algal biomass as indicated by the last laboratory value was not covered by remote sensing spectra and consequently the algorithm would profit from the inclusion of additional high biomass events.

The overall success of the application of this algorithm may be favoured by the homogeneity observed in averaged contribution of IOP characteristics (Fig. 4.5) and the resulting similarity of remote sensing spectra throughout the study. A change in these characteristics may negatively influence results of the local algorithm. CDOM provides the dominant disturbing optical signal at relevant spectral wavelengths (490, 510 and 555 nm). Whereas strong CDOM contributions at the respective OC algorithm wavelengths may have resulted in overestimations of phytoplankton abundances in previous studies in the Mediterranean Sea, as found by Morel and Gentili (2009), such effects were not observed during our study in Alfacs Bay, as phytoplankton abundances and CDOM were not correlated. In any case, a seasonal pattern of maxima in the winter and decreases in spring to a minimum in summer was found in the north-western basin of the Mediterranean (Morel & Gentili, 2009), where the Ebro Delta is situated. Such seasonal variability may be due to influences of photo-bleaching combined with the stratification of the water column in summer, and strong vertical mixing, also bringing CDOM from bottom to surface waters, in winter (Morel & Gentili, 2009). Such effects could also influence CDOM contributions in the Ebro Delta bays and thereby cause alterations in the contribution of optically active substances. Especially with respect to seasonal changes in water mass characteristics due to freshwater inflow from the opening and closure of dams for the irrigation of rice fields that lead to changes in stratification and mixing in the bays. Continued tests for the validity of the OC algorithm are necessary to confirm its long-term applicability. As opposed to the situation during the study time, in winter the water of the bays is usually transparent, and thus effects from bottom reflectance would have to be included in the quality procedure.

The reference of algal biomass, Chl $a$ concentration of field samples as retrieved fluorometrically, averaged 1.39 µg L$^{-1}$ over the two study periods and was therefore well below the seasonal mean of 4.2 µg L$^{-1}$ at central Alfacs Bay (integrated sample, May-July 2001 – 2013, M. Fernández-Tejedor et al., unpublished data). With respect to the surrounding
Mediterranean Sea, Alfacs and Fangar bays exhibit high primary production, thus enabling a viable aquaculture industry. As compared to other study regions for algal biomass retrieval in optically complex waters with Chl \(a\) concentrations reaching \(>100 \mu g \, L^{-1}\) (Dall'Olmo & Gitelson, 2005, Gitelson et al., 2008, Blondeau-Patissier et al., 2009) the average biomass in the Ebro Delta bays is low. This may be the reason for a failure of the otherwise promising \(R_{rs}\) algorithm approaches based on the red spectral range and thus omit the CDOM dominated short wavelength region. In particular, the three wavelength approach by other authors (Dall'Olmo & Gitelson, 2005, Gitelson et al., 2008) has been successful for cases where algal biomass spanned three orders of magnitude, with minimum Chl \(a\) concentrations of 4.4 \(\mu g \, L^{-1}\) (Dall'Olmo & Gitelson, 2005), 4.4, 1.7, and 1.2 \(\mu g \, L^{-1}\) (Gitelson et al., 2008), and 2.27, 3.97 \(\mu g \, L^{-1}\) (Gurlin et al., 2011). A general relationship of the local red absorption maximum to phytoplankton to algal biomass was observed for the two study seasons, but the relatively low contribution of algal biomass in relation to the strong signal of pure water in the red spectral region may have impeded the successful application of this approach. The same holds true for the application of algorithms to retrieve algal biomass by the FLH method, for which we retrieved negative values. Values below zero were also observed by Wernand et al. (2006) for a lower Chl \(a\) concentration and therefore a lower limit of 2.5 \(\mu g \, L^{-1}\) Chl \(a\) was proposed for this algorithm. Consequently, this approach is not suitable for the consistent monitoring of algal biomasses in the Ebro Delta region.

### 4.4.3 Spatio-temporal scales for operational algal bloom surveillance by means of reflectance measurements

A persuasive rationale for the application of an \textit{in situ} remote sensing system for algal bloom surveillance is the coverage of spatio-temporal dimensions from days to months and from centimetres to kilometres in an operational/automated and near-real-time approach. With respect to the automation of data-collection and processing, the presented system reliably retrieved the basic variables for the calculation of remote sensing reflectance. Complementary digital images by a camera system allowed the examination and tests for exceptional events, but would not necessarily need to be included into a long-term measurement or observational system (even though its presence would provide video surveillance against vandalism, theft and physical damage to the system). The data-logger (TriBox2) would allow the remote inspection and control of incoming spectra, as well as an automated processing of spectra for a subsequent quality control for such a long term application. A similar system is currently in operation in the North Sea (see COSYNA, Helmholtz-Zentrum-Geesthacht, 2013). In our study, a data processing scheme included an elimination of low quality spectra that was adjusted to the local conditions. This included spectra with insufficient incoming light, red
spectral influences of dusk and dawn (Wernand, 2002), sub-surface objects and strong sunglint spectra, as well as the subtraction of remaining sun reflection from spectra (Busch et al., 2013a). All of these are based on distinct characteristics of $E_s$ or $R_n$ spectra. Rain events for elimination of affected spectra were obtained from an external source, in this case a nearby weather station. The pre-located and locally adjusted processing permits automated retrieval of a long-term dataset for algal biomass estimation.

The sensor system on an aquaculture raft allowed a highly temporally resolved data retrieval in 15 minute intervals over daytime. Therefore the temporal resolution that is necessary for algal bloom surveillance was met. The footprint of the remote sensing reflectance was about 1 m² and thus small when compared to the bay. To extend the horizontal spatial scale, the installation of further sensor systems over a larger area is applicable (see COSYNA, Helmholtz-Zentrum-Geesthacht, 2013), also considering that data handling, power supply and cost of the sensors can be reduced by the selection of a multispectral instrument as opposed to the hyperspectral sensors for ratio measurements. Furthermore, remotely derived spectra from space-borne measurements or airborne instruments may be used to test for compliance with the local algal biomass algorithm. Given the similarity of optical variability in Alfacs and Fangar bays, a transferability of such a system or network of system to this other embayment is feasible. For the enhancement of algal biomass assessment over an extended vertical space, additional radiometers or fluorometers could be installed. At least during spring/summer months, measurements with submerged instruments are strongly impeded by biofouling and require at least weekly manual cleaning or an automated cleaning mechanism such as a wiper or brush. Geographical locations for the installation of such integrated sensor systems would be determined by the location of the aquaculture rafts to primarily cover the area of food production, but also with respect to environmental triggers of HABs and hydrodynamic models of the bays. From a strategic viewpoint, the northeast part of Alfacs Bay is an important location, as it has an increased retention time of the water mass in relation to other parts of the bay and therefore possibly acts as an incubator for outbreaks of the ichthyotoxic dinoflagellate *Karlodinium* spp. (Berdalet et al., in press). Furthermore, installation of a radiometric system at locations with incoming water masses from the Mediterranean would improve observations and aid in determining the origin of phytoplankton blooms and particularly HABs within the Bay or from the surrounding sea.

4.5 Conclusion
The overall objective of this study was to evaluate remote sensing measurements from a fixed platform for an operational retrieval of algal biomass in the Ebro Delta. The two semi-
enclosed Ebro Delta bays Alfacs and Fangar are characterised by non-covarying contributions of CDOM, algal- and non-algal particles and can thus be considered optically complex. Main interfering factors for algal biomass retrieval were external influences, such as boat traffic or sun- and sky reflectance, as well as the non-covarying nature of optically active substances, especially CDOM. External influences were successfully approached by the application of an easy-to-apply rapid pre-processing of data adjusted to the local conditions. Measurement of algal biomass out of the bulk signal including non-algal particles and CDOM was best approached by a simple OC algorithm with the ratios $[R_{rs,s}(555) - 1 \times R_{rs,s}(490)]$ and $[R_{rs,s}(555) - 1 \times R_{rs,s}(510)]$ in second and third degree polynomial regressions. Unexpectedly, algorithm approaches that base on absorption or sun induced fluorescence in the red / NIR spectral range with less influences by CDOM did not succeed, possibly owing to the relatively low concentrations of phytoplankton.

The application of remote sensing measurements allowed a continuous and automated near real-time retrieval of changes in phytoplankton biomass that cannot be covered by weekly sampling for laboratory analysis. Horizontal and vertical spatial scales or such an in situ above-surface system may be further enhanced by the integration of additional radiometer systems in a network or additional sub-surface sensors. Such a comprehensive multi-sensor system allows the detection of irregularities in biomass, or movement of algal patches and can result in adequate responses, such as additional surveys for high biomass HAB species and, in succession, the closure of aquaculture harvesting.

Inversion of remote sensing to phytoplankton absorption spectra in a hyperspectral resolution may even reveal more specific details on the composition of algal groups or even genera, thereby increasing the target specificity (for examples of phytoplankton pigment absorption derived from remote sensing spectra taken at Alfacs Bay 2010 see Annex Fig. 3). Continuous observations of algal biomass variations with bio-optical systems may, in alliance with environmental parameters, also enhance knowledge on forcing functions and triggers of HAB proliferations and their geographical origin. This knowledge is the key to predictive models of HAB development, a prospective goal in HAB assessment which is still challenging due to the high diversity in taxon-compositions and behaviours. With contributions to near-real time assessment of HABs as well as to research on possible causes, remote sensing systems are a valuable component in HAB surveillance and coastal zone management systems.
**COMPARATIVE STUDIES ON PHYTOPLANKTON DYNAMICS AND BIO-OPTICS FOR HAB MONITORING IN THE EBRO DELTA, NW MEDITERRANEAN**

**Abstract**

Alfacs and Fangar bays are semi-enclosed embayments in the Ebro Delta system, where sustainable shellfish aquaculture requires harmful algal bloom (HAB) surveillance at the appropriate spatio-temporal scale. We therefore deployed a hyperspectral light-field observational system in Alfacs Bay, in late spring through summer over two successive years. The vertical distribution and bloom dynamics of two HAB-genera, the toxigenic dinoflagellate *Karlodinium* and the diatom *Pseudo-nitzschia* were assessed by weekly laboratory analyses of phytoplankton and ancillary parameters in a comparative approach in both bays. Bio-optical measurements provided continuous time-series proxy data for changes in phytoplankton biomass adjacent to aquaculture sites. Small scale dynamics of *Karlodinium* spp. and *Pseudo-nitzschia* spp. displayed a patchiness that could be related to temperature and salinity patterns. While we do not consider these parameters as direct forcing functions, they are related to other relevant parameters, such as stratification and nutrient regime, and thus may act as proxy indicators for bloom initiation. Continued studies on a species level are necessary to fully assess bloom dynamics for this region, but future integration of both bio-optical and phytoplankton patterns at the sub-mesoscale will support regional oceanographic models for HAB forecasting and enhance surveillance.
5.1 Introduction
The two semi-enclosed embayments Alfacs and Fangar bays in the Ebro Delta system are the major aquaculture sites in Catalonia (NW Mediterranean). Bivalve shellfish, predominantly the Mediterranean mussel *Mytilus galloprovincialis*, are grown on fixed rafts in suspension culture. Due to the presence of harmful microalgal taxa and accumulation of their phycotoxins, both bays are subject to occasional harvesting closures.

Semi-confined environments, such as the Ebro Delta bays are characterised by an increased retention time of water and associated plankton, and are more influenced by point source input (e.g., of nutrients or freshwater) than open coastal systems exposed to long-shore advection. These conditions can lead to increased stratification and therefore increased vertical patchiness of phytoplankton proliferations. Consequently, the surveillance of HABs must adequately address bloom patchiness at an appropriate spatio-temporal resolution (Cembella et al., 2005).

Bio-optical tools have gained prominence in synoptic and long-term assessment of HABs, especially for documentation of the presence, dimensions and movement of blooms. Ocean colour analysis is widely applied to track chlorophyll *a* (Chl *a*) as an algal biomass proxy (Stumpf et al., 2003), although this approach provides poor taxonomic resolution and it is not often possible to distinguish harmful from benign blooms.

In the Ebro Delta, the ichthyotoxic dinoflagellate *Karlodinium*, represented by co-occurring species, *K. armiger* and *K. veneficum* (formerly *Gyrodinium corsicum*, Garcés et al., 2006), has caused marine faunal mortalities (Fernández-Tejedor et al., 2010). An increase in cell abundance of *Pseudo-nitzschia* spp. has also been observed in the region over the past 20 years (Fernández-Tejedor et al., 2010), including detection several species known to produce the neurotoxin domoic acid (DA). Even though the presence of DA has not led to shellfish harvesting closures, the increase in abundances poses a future threat to the regional shellfish industry.

The comparison of HAB dynamics in two semi-enclosed embayments is an approach to increase knowledge on bloom dynamics. The ultimate objective of the current study is to address the spatio-temporal patchiness of HABs and to determine which factors regulate the population dynamics of diverse HAB taxa in these multi-faceted marine environments. We applied a hyperspectral light-field observational system for continuous monitoring of phytoplankton in Alfacs Bay and assessed the dynamics of target genera in the two bays to address this objective.
5.2 Field sites and sampling strategy

Field work was conducted in the Ebro Delta bays from May to July in 2010 and 2011. Remote sensing reflectance ($R_n$) was continuously retrieved throughout both study seasons with a radiometric sensing system installed on an aquaculture raft in Alfacs Bay (40.620083 °N, 0.658167 °E) (Fig. 5.1). $R_n$ was calculated via $R_n(\lambda) = \frac{L_{sfc} - 0.024 \times L_{sky}}{E_d}$, where $L_{sfc}$ is surface upwelling radiance, $L_{sky}$ is sky radiance, and $E_d$ solar plane irradiance. Spectra influenced by surface objects and strong sun glint were deleted and remaining sun and sky reflections were corrected following Busch et al., (2013a).

![Fig. 5.1: a) Study site in the Ebro Delta on the NW Mediterranean coast within the two semi-enclosed embayments Alfacs (northern) and Fangar (southern); b) sensor system setup with radiometers for the near real-time assessment of anomalies in chlorophyll a as algal biomass proxy and a camera system to monitor surface and sky conditions.](image)

The analysis of biological and physical parameters was conducted weekly at a vertical resolution of 0.5 m in Alfacs and Fangar Bay (40.778767°N, 0.749233 °E). This included CTD (conductivity, temperature, depth) casts as well as water samples for laboratory analyses of nutrients with an auto-sampler, and the phytoplankton community by means of inverted microscopy. The extracted Chl $a$ (Chl $a_{ext}$) content of surface samples was determined fluorometrically as reference to bio-optical measurements.

5.3 Continuous observation of phytoplankton biomass

Retrieval of Chl $a$ as phytoplankton biomass proxy by means of $R_n$ measurements was approached by an algorithm employing the ratio $[R_n(555 \text{ nm}) \times R_n(490 \text{ nm})]$ (Fig. 5.2), despite interference of coloured dissolved organic matter (CDOM) at these spectral ranges. Algorithms targeting the absorption peak of Chl $a$ in the red region (~674 nm), to avoid masking of the signal by CDOM, as well as those targeting sun-induced natural Chl $a$ fluorescence from the $R_n$ signal, failed in explaining variations of reference Chl $a_{ext}$ (Busch et al., submitted). The radiometer system was operated at a 15 min sampling interval and
Fig. 5.2: Phytoplankton biomass proxy retrieved by reflectance measurements in Alfacs Bay over the study time in 2010 (upper panel) and 2011 (lower panel). Remaining influences of sun and sky reflections (grey), filtered by means of a moving average (red) complied well with laboratory measurements (Chl $a_{\text{extr}}$, dark green circles). Corresponding Chl $a_{\text{extr}}$ values at a nearby station were not included in algorithm development, but also complied well with the overall pattern (light green squares), only available for 2010.

therefore recorded variations in phytoplankton biomass with a high temporal resolution in a fully automated mode. Values complied well with fluorometrically derived Chl $a_{\text{extr}}$ from weekly sample extracts that were used for algorithm development. In addition, an external dataset from a nearby station, derived from a local weekly routine monitoring programme for food safety conducted by the IRTA, was used as validation dataset for the applied algorithm, and could be aligned to the bio-optical data (Fig. 5.2).

Highest Chl $a$ concentrations were measured at the end of the 2010 study season. Even though increased cell abundances of Karlodinium spp. were also identified by microscopic counts, this information could not be isolated by data from the bio-optical system.

The applied radiometer system measured spectra at hyperspectral resolution (3 nm steps). Such measurements allow retrieval of detailed spectral data on phytoplankton pigment absorption and based on this information more details on algal composition. As an example, high cell abundances of the toxic dinoflagellate Karenia brevis have been identified from bio-optical data by an inversion of $R_n$ to phytoplankton absorption spectra over the spectral range (Craig et al., 2006). The identification was based on distinct absorption characteristics of $K$. 
brevis due to the presence of the rare pigment gyroxanthin-diester. This technique may also be applicable for high biomass and ‘mono-generic’ blooms of Karlodinium in the Ebro Delta bays, as these dinoflagellates also contain this rare pigment.

5.4 Phytoplankton bloom dynamics

In Alfacs and Fangar bays, the stratification regime is mainly formed by freshwater inflow from rice-field irrigation channels. Due to irrigation patterns, an increased seasonal intensity was expected during the study time. A freshwater layer in the surface meter was apparent in both bays over the whole study period in 2011, whereas in 2010 it was restricted to Alfacs Bay in the beginning of May. While an assemblage of the two target genera due to this layer was not visible, an irregular vertical patchiness pattern was clearly shown (Fig. 5.3). In 2010, the highest cell counts of Karlodinium spp. were from samples from around 3 m depth in both bays, with highest abundance on 1 July 2010 in Fangar Bay. A general correspondence of high cell abundances within a window of temperature (20 – 27 °C), as apparent with data from January to August 2010 (Busch et al., 2012), was basically confirmed with this dataset.

Cell abundances of Pseudo-nitzschia spp. were patchy at various depths, but an exceptional bloom occurred in Fangar Bay in May 2011 (>7 \times 10^6 cells L^{-1}). This event could only be captured from surface sampling during harsh wind and wave conditions that presumably initiated the decline phase of the bloom. Pseudo-nitzschia cell abundances exceeded the alert level (2 \times 10^5 cells L^{-1}) from 9 to 30 May, by which time concentrations still remained rather high at 5.4 \times 10^4 cells L^{-1}).

A redundancy analysis was conducted to examine if the target genera Pseudo-nitzschia and Karlodinium indeed respond similarly to the increased freshwater inflow in both bays. For both bays, variances of Pseudo-nitzschia spp. occurrences could be best explained by salinity (freshwater patterns), whereas variances of Karlodinium spp. were associated more closely with temperature (Fig. 5.4). These factors are not considered key elements that trigger blooms of these genera, but are related to other factors, such as stratification or nutrient input, and may aid in outlining proxies for HAB dynamics.

All results presented here are shown on a genus level, as the discrimination of species within these genera by light microscopy is problematic. Further information on the species level is required to address questions regarding which biotic and abiotic factors control population dynamics, because even populations within a single species may express different adaptive strategies for growth and survival that determine bloom dynamics. Preliminary results on the species composition of Pseudo-nitzschia by means of 454 DNA sequencing showed the presence of a local species mixture with potentially toxic and non-toxic taxa (Publication VII, this thesis,). This complies well with prior findings for the area (Andree et al., 2011). Along the Catalan shellfish production areas, DA has been detected in shellfish, but
was below quantification level in the Ebro Delta Bays most of the time (Giménez Papiol et al., 2012). Whereas both *K. armiger* and *K. venificum* are prominently associated with HAB events in the Ebro Delta, it is not clear which *Pseudo-nitzschia* species are responsible for the production of DA in this area. This is one of the questions that will be addressed in the next step of this study.

**Fig. 5.3:** Abundances (cells L$^{-1}$) of the dinoflagellate *Karlodinium* spp. and the diatom *Pseudo-nitzschia* spp. in Alfacs (a, b, e, f) and Fangar (c, d, g, h) bays in 0.5 m vertical steps (black dots represent water samples) for the years 2010 and 2011.
Fig. 5.4. Redundancy analysis of *Pseudo-nitzschia* and *Karlodinium* spp. in Alfacs Bay (black) and Fangar Bay (green), for the variables salinity, temperature and inorganic phosphorus.

### 5.5 Conclusions

An automated long term retrieval of phytoplankton biomass by a radiometric sensor system was successfully applied in the coastal embayments of the Ebro Delta to record the presence of algal blooms. Inclusion of such a system into an environmental observatory provides a synoptic view of phytoplankton blooms over the appropriate spatio-temporal scales for aquaculture installations.

Strategic location for an observatory is especially critical for areas that have been identified as bloom incubators. For example, the NE area of Alfacs Bay may function as bloom incubator for *Karlodinium* spp. due to increased retention time of water (Bardalet et al., in press). Taxon specificity is as mandatory for HAB surveillance as a large spatio-temporal coverage, but the former cannot be determined by this regional bio-optical approach. The inclusion of additional environmental parameters and alternative techniques for determining species specificity (e.g., molecular genotyping) within a comparative approach will allow insights into determining factors of bloom dynamics. Finally, incorporation of bio-optical patterns and phytoplankton datasets into local and regional oceanographic models will lead to improved forecasting and surveillance for HAB events.
THEME CHAPTER III

THE SMALL SCALE: HAB DIVERSITY AND PATCHINESS IN THE EBRO DELTA

PUBLICATION VI: An integrated approach for the assessment of HAB dynamics in two NW Mediterranean bays

PUBLICATION VII: Vertical distribution of toxigenic algae and associated phycotoxins during freshwater runoff in two coastal embayments in the Ebro Delta (NW Mediterranean)
AN INTEGRATED APPROACH FOR THE ASSESSMENT OF HAB DYNAMICS IN TWO NW MEDITERRANEAN BAYS

Abstract

Alfacs and Fangar Bay in the Ebro Delta, NW Mediterranean are the major sites in Catalonia for shellfish cultivation. These bays are subject to occasional closures in shellfish harvesting due to the presence of phycotoxins. Fish kills have also been associated with harmful algal blooms. The comparison of phytoplankton dynamics in both bays offers the opportunity to reveal differences in bloom patterns of species known to be harmful for the ecosystem and aquaculture activities. Field research is underway under the GEOHAB framework within the Core Research Project on HABs in Fjords and Coastal Embayments. The overall objective of this study is to improve our understanding of HAB biogeographical patterns, and key elements driving bloom dynamics in time and space within these semi-constrained embayments. Via the comparative approach we aim to improve the prediction for monitoring purposes, with a focus on Karlodinium associated with massive kills of aquaculture species. This objective is addressed by incorporating long-term time series of phytoplankton identification and enumeration with the first results of recent field work in both bays. The latter includes the application of optical sensors, to yield a complementary view with enhanced spatial and temporal resolution of bloom phenomena.
6.1 Introduction
The two semi-enclosed embayments Alfacs and Fangar bays in the Ebro Delta system, NW Mediterranean, are the major aquaculture sites in Catalonia. Due to the presence of phycotoxins, both bays are subject to occasional harvesting closures. High mixed abundances of the ichthyotoxic species *Karlodinium veneficum* and *K. armiger* (in this area previously referred to as *Gyrodinium corsicum* (Garcés et al., 2006) have been found in Alfacs Bay since 1994. In 2010 the species were also detected in Fangar Bay. In spite of their proximity and similar climatic conditions, Alfacs and Fangar Bay profoundly differ in HAB dynamics. Circulation patterns and retention time of water in both bays are differently affected by winds, coastal currents, and freshwater inflow from agriculture. The comparison of environmental forcing functions and bloom characteristics in both bays therefore provides the opportunity to improve our understanding of the key elements that drive bloom dynamics in time and space.

![Fig. 6.1. Study area with Alfacs and Fangar bays in the Ebro Delta, Spain, NW Mediterranean.](image)

Via the comparative approach we aim to improve the prediction for monitoring purposes, with a focus on *Karlodinium* spp. associated with massive kills of aquaculture species (Delgado et al., 1995). This objective is addressed by incorporating a time series of phytoplankton identification and enumeration with the first results of recent field work in both bays. The latter includes the application of oceanographic and optical sensors, to yield a complementary view with enhanced spatial and temporal resolution of bloom phenomena. The objective of the presented work is the comparison of high *Karlodinium* spp. abundances in both embayments to outline environmental proxies for bloom development of this genus.
6.2 Materials and methods

Cell numbers of *Karlodinium* spp. were retrieved within the regular monitoring programme from depth-integrated samples at five stations in each of both bays between January and August 2010. Three abundance classes for the genus were generated, with a low abundance/presence group with <1000 cells L$^{-1}$, moderate abundance of <10,000 cells L$^{-1}$, and a high abundance class in excess of 10,000 cells L$^{-1}$. These abundance classes were plotted separately for Alfacs and Fangar bays, with respect to temperature-, salinity- and oxygen conditions. In addition, variations of environmental parameters over depth were retrieved by CTD casts between May and July 2010.

6.3 Results and discussion

Increased *Karlodinium* spp. abundances were contemporaneously detected in depth integrated samples of both embayments. While first sightings were concentrated in the port and near-coastal area in Alfacs Bay, the genus was first detected at the entrance of Fangar Bay (Fig. 6.2). Cell concentrations of the highest abundance class of *Karlodinium* spp. were only reached in Alfacs Bay, while in Fangar Bay only up to 10,000 cells L$^{-1}$ were counted from January to August 2010. Abundances differed between stations, and hence over horizontal scales. Highest abundances were detected in a sample from the inner part of Alfacs Bay (21 June), situated in between the fixed mussel rafts and the coast (Fig.6.2a). At this time, fine-scaled difference of about 1 °C in temperature and 1 in salinity were observed at this station.

![Image](image_url)

*Fig. 6.2. Karlodinium* spp. cell concentration (depth integrated) from May to July 2010 a) at five sampling stations in Alfacs Bay, and b) at Fangar Bay. Station of maximum abundance of the genus in Alfacs Bay (CIA), and or first appearance in Fangar Bay (EF) are marked with arrows.
from surface to bottom in both embayments (Fig. 6.3). In Fangar Bay, increased abundances were first detected at the station which is located at the entry of the bay (1 June) (6.2b). In the Fangar Bay transect, the influence of saline water entering from the Mediterranean is visible during this time (Fig. 6.3). In spring/summer months, dams are open for rice field irrigation in the Delta area. Consequently, flow of freshwater increases from the rice fields to the bays and stratification predominates in both bays. This is due to a lateral fresh water inflow through a series of channels of the main land, and sea water inflow from the Mediterranean as a salt wedge along the bottom (Camp & Delgado, 1987). A trend of *Karlodinium* spp. blooms in stratified waters was recognized in a 20 years’ time series of monitoring in the Ebro Delta Bays (Fernández-Tejedor et al., 2010).

From January to August 2010, *Karlodinium* spp. have been detected throughout a wide range of environmental conditions (Fig. 6.4). As Fangar Bay is smaller than Alfacs Bay, the influences of freshwater inflow, as well as from the southwesterly coastal currents, have a stronger effect on this bay. Indeed, when comparing the range of water samples in which all abundance classes of *Karlodinium* spp. were detected in both embayments, the range from below 20 to 40 is much wider in Fangar Bay than in Alfacs Bay with 30 -37. The two higher abundance classes of the genus were, however, restricted to a limited range of corresponding environmental conditions, in particular with salinity and temperature. The two highest abundance classes of the genus were, however, restricted to a limited range of corresponding environmental conditions, in particular with salinity and temperature. Notably, these ranges were similar in both embayments and therefore may reflect optima for these Mediterranean strains of *Karlodinium*. These were given with a salinity of 32 - 35, and temperature of 20 - 27 °C in in both embayments (Fig. 6.2). In Alfacs Bay, the ranges were even more defined with the presence of the highest abundance class. The combination of these small ranges in salinity and temperature may not be the key elements that trigger high *Karlodinium* spp. abundances, but provide an environmental setting of this year’s bloom patterns. This can be an indication of the presence of proxies for algal proliferations that can be used for the early detection of blooms, e.g. by means of optical sensors.
Fig. 6.3. CTD casts on a transect of sampling stations (vertical dotted lines) in a) Alfacs and b) Fangar bays reveal fine-scaled differences of temperature and salinity in both embayments during the time of increased *Karlodinium* spp. abundances. For Alfacs Bay, a distance of 0.5 km from the sampling point between aquaculture constructions and coast at 0 km, southwards towards the inner part of the bay is plotted for 23 June 2010. In Fangar Bay, the 4 km long section from the mouth of the bay to the central part is plotted for 1 July 2010.

Fig. 6.4. Pair plot of *Karlodinium* spp. detection in three abundance classes (<1000 cells L$^{-1}$=black dots; <10,000 cells L$^{-1}$=red squares; >10,000 cells L$^{-1}$=green triangles) from 4 Jan – 30 Aug 2010 in Alfacs (upper three scatter plots) and Fangar (lower three scatter plots) with respect to temperature, salinity and oxygen saturation. The distribution of total *Karlodinium* spp. abundances in environmental ranges of both bays is given in the bar charts.
6.4 Summary & conclusions

- Increased abundances of *Karlodinium* spp. were detected in Fangar Bay for the first time. This allowed the comparison of environmental settings of genus abundances in both embayments.
- Abundances started to increase at the same time in both embayments, and were heterogeneously distributed over horizontal space and time.
- Abundance classes of *Karlodiniums* spp. defined optimum temperature and salinity values for this genus in a comparative approach. These indicators may aid in the definition of habitat preferences for this genus.
- Such habitat preferences of their proxies can be used for the early detection of algal blooms, e.g., by means of optical sensors.
Abstract

Harmful Algal Bloom (HAB) surveillance is hampered by the high taxonomic diversity of species, their habitat preferences, assemblage behaviour and phycotoxins. Insights on population dynamics of harmful algae on a species level are therefore crucial for the effective mitigation of adverse effects on human health, the ecosystem or economy. Information on environmental scenarios which may eventually lead to HAB prediction is expected to arise by comparative studies of algal assemblages in similar ecosystems. Accordingly, the main objective addressed in the presented study was to identify HAB taxa, toxins, and proliferation dynamics in the shallow Ebro Delta embayments Alfacs and Fangar in a comparative approach, and to determine if agricultural freshwater inflow forms microniches which favour their development. Environmental variables, phytoplankton taxa and associated phycotoxins were addressed in weekly cruises during two study periods from May to July in 2010 and 2011. CTD casts on key stations and transects revealed two major stratification patterns, of which one was characterised by a freshwater influenced surface layer, separated by a well-defined pycnocline, and the other displayed less stratified conditions. These patterns were comparably stable along transects and similar over time in both embayments. At key stations, more than 23 HAB taxa were identified to species-, and four additional to genus-level by light microscopy and/or by their molecular diversity. HAB species were heterogeneously abundant over depth and time, with profound changes in time spans as small as weeks. Notably, alert abundances of dinoflagellate Dinophysis spp. in particular D. sacculus and D. acuminata and associated phycotoxins were reached within defined patches, which were restricted to the first regime in both embayments. Hence, this high stratification regime has presented a favourable habitat type for the present species, but findings do not permit the determination of triggering forcing functions. Prominent patterns during the second regime include increased abundance of the dinoflagellate Karlodinium veneficum, notably for the first time observed in Fangar Bay in 2010. The presence of the genus was associated to moderate salinity and increasing temperatures in both embayments and these two parameters may serve as main input parameters for a habitat model of these taxa. The diatom genus Pseudo-nitzschia was heavily represented in Fangar Bay during both regimes, with heterogenic distribution of potentially toxic taxa. Findings of this study indicate that the producer species is unlikely to be the main
contributor to total genus abundance as the associated toxin domoic acid was found in samples which did not coincide with highest cell abundances. It was neither possible to assign a Pseudo-nitzschia species to toxin production, yet it is highly probable, that additional taxa of this genus were present. Noteworthy is the identification of Azadinium sp. with as potential new HAB species in the region. Cells identified by means of light microscopy were characterised by a unique combination of morphological features when compared to the so far described members of this genus. Most taxa that were present during the study periods are typical for the season. Yet, a clear trend which was evident in both embayments in a comparative approach was the separation of the bloom season in two regimes of water stratification which apparently complied with habitat preferences of harmful taxa. In this scenario, Dinophysis spp. is assigned to stratified areas, and Karlodinium spp. to a lower degree of stratification. Hence, association to species types with their favoured habitat types is worth to be followed for HAB surveillance in the Ebro Delta with a long-term dataset.
7.1 Introduction
The surveillance of harmful algal blooms (HABs) in coastal ecosystems is hampered by the large diversity of harmful species, and the confounding effects of plankton behavioural responses to hydrodynamic factors such as advection, turbulence and sub-mesoscale processes. Further complications arise from biological factors, amongst them grazing, cell mortality and allelopathic interactions between taxa.

Harmful algal blooms are notorious for forming concentrated patches comprising high cell concentration of the dominant species, and can even tend towards monospecificity. Such heterogeneous algal patches vary widely in spatio-temporal dimensions, over both the horizontal and vertical axes. Algal patches may form on the water surface or in deeper zones of the water column, and also can occur in sub-meter (often centimetre-thin) horizontal layers (Martin, 2003). Such layering of algae is often attributed to stratified water systems, but is not necessarily a physical cause and effect situation, but rather may be due to formation of microhabitats, e.g. particular nutrient regimes, with better fitting suites of growth conditions for certain taxa (Smayda, 1997b). In particular, cell accumulations of dinoflagellate HAB taxa are frequently highest in sub-surface layers, as formed by water-column stratification (Gentien et al., 2005). As example, the dinoflagellate Dinophysis acuta was shown to aggregate in a salinity driven pycnocline in the Galician Rías Baixas over a 24 h study period (Pizarro et al., 2008), whereas thin layers of D. acuta were also found along a coastal jet in waters off the south coast of Ireland (Farrell et al., 2012). Also thin layers of putatively toxic pennate diatoms species are common in coastal ecosystems, including those of the diatom Pseudo-nitzschia along pycnoclines in the Galician Rías Baixas (Velo Suárez et al., 2008), and in fjords of the San Juan Islands (USA) (Rines et al., 2002).

Determination of the dimensions of HAB patches, as well as identification of harmful taxa and associated phycotoxins, are crucial for establishing a time-series record of their presence and distribution. This will eventually lead to implementation of adequate management strategies, and, furthermore, to enhanced knowledge on triggering functions for bloom development. Nevertheless, the diversity in algal cell distribution, species, and toxins pose severe challenges for operational HAB detection which cover the required spatio-temporal scales (Busch et al., 2013b). Such effective HAB monitoring of coastal areas is best approached by continuous near-real time surveillance, based upon a combination of remote and in situ instrumentation. Systems based upon the quantitative detection of pigments, such as the ubiquitous algal pigment Chlorophyll a (Chl a) and other accessory pigments can often yield a valuable algal biomass proxy, but are highly limited in taxonomic resolution of the target HAB taxa, particularly in complex coastal waters.
In the current study we integrated a number of alternative approaches for surveillance of HAB events within selected coastal embayments linked to intensive shellfish aquaculture activities in the Ebro Delta, Mediterranean Sea. Particular attention was paid to the scale of temporal and spatial resolution of HAB species occurrence even at sub-bloom magnitude. Alfacs and Fangar bays are two semi-enclosed embayments in the Ebro Delta System, NW Mediterranean (Fig. 7.1). Alfacs Bay, with a surface area of 50 km² and a water depth of 6 m in the central bay is larger than Fangar Bay, which covers 12 km² and has a depth of 4 m in the centre (Camp & Delgado, 1987). The Ebro river discharges directly into the open Mediterranean Sea, but in addition river water is diverted to rice fields in the delta basin by irrigation channels located about 25 km upstream of the mouth of the Ebro river (Xerta, Baix Ebre, Mañosa et al., 2001). Especially from March/April to October/November, freshwater enters the embayments through water discharge channels. These play an important role in supplying inorganic nutrients, which for Alfacs Bay are mainly introduced along the northern shore, close to the water inlet (Delgado & Camp, 1987). The contribution of organic nutrients by this system is only poorly understood. In any case, this northern shore is also an important area for the development of phytoplankton biomass in the bay (Delgado, 1987). The freshwater inflow leads to stratification by a freshwater-influenced layer from riverine origin, over a salt wedge entering on the bottom of the bay from the Mediterranean Sea. Ocean currents outside the bays are mainly determined by the south-westerly current of the Catalan Sea (Font et al., 1990) and the main water exchange between the bays and sea is via the entrance to the bays. As compared to the oligotrophic conditions in the Mediterranean Sea, the embayments are characterised by a relatively high phytoplankton biomass of approximately 3 µg L⁻¹ (proxy Chl α) (Camp & Delgado, 1987, Llebot et al., 2011). Consequently, the majority of aquaculture activities in Catalonia are conducted in these bays, predominantly consisting of cultures of the suspension feeding Mediterranean mussel Mytilus galloprovincialis. The aquaculture industry is, however, subject to episodic closures of shellfish harvesting in both bays, due to proliferation of harmful phytoplankton and/or to phycotoxin accumulation in shellfish to levels above regulatory limits (Fernández-Tejedor et al., 2008, Fernández-Tejedor et al., 2010). Within a long-term weekly monitoring program, most harvesting closures due to paralytic shellfish poisoning (PSP) toxins have been attributed to the dinoflagellate Alexandrium minutum, whereas diarrhetic shellfish poisoning (DSP) toxins in shellfish from this area typically coincide with occurrence of species of the dinoflagellate genus Dinophysis, such as D. sacculus or D. caudata (Fernández-Tejedor et al., 2008, Fernández-Tejedor et al., 2010). The appearance of yessotoxin in shellfish has been related to the dinoflagellate Protoceratium reticulatum (Diogene et al., 2008), but other dinoflagellates known to produce lipophilic phycotoxins, e.g., Lingulodinium polyedrum and Prorocentrum lima, are also present in both bays. Critical cell numbers of potentially toxigenic species of the diatom genus
The taxonomic distribution of *Pseudo-nitzschia* species by quantitative Polymerase Chain Reaction (qPCR). We assessed the overall molecular biodiversity of the phytoplankton community of selected samples by means of high throughput (Roche 454) DNA pyrosequencing. The latter sequencing approach was designed.
to confirm the presence of certain HAB taxa that were not clearly identified by light microscopy due to pure fixation capability (naked flagellates) low abundance or lacking of clear taxonomic features (cryptic diversity), and hence to reveal the presence of HAB taxa that are not routinely resolved by light microscopy or targeted molecular methods. Similarities and differences in spatio-temporal HAB patchiness were revealed by comparison of the two geographically adjacent Ebro Delta bays, and this comparison provided evidence for the establishment of microhabitats for HAB taxa, such as may be created by stratification, over a fine-scale resolution on the vertical axis.

7.2 Material and methods

7.2.1 Location and field sampling of the study sites

In two consecutive years, 2010 and 2011, weekly measurements were conducted from May to beginning of July at key stations in Alfacs Bay (40.620083 °N, 0.658167 °E) and Fangar Bay (40.778767 °N, 0.749233 °E) (Fig. 7.1). From a small vessel, a total of 30 weekly cruises were conducted. At both key stations, physical parameters were taken from the vessel using a CTD (Conductivity, Temperature, Depth) probe in profiling mode as outlined in section 7.2.2. Additional CTD casts were taken weekly along transects of 1 -2 km in Alfacs Bay, and of 5 km in Fangar Bay. Subsequent to CTD casts, water samples were collected at the two key stations from an on-board pumping system at discrete depths in 0.5 m steps from surface to bottom, which resulted in 11 samples in Alfacs Bay, and in 9 samples in Fangar Bay. The submersible system was operated from the deck of the small vessel and consisted of a weighted hose that pumped water from the desired depth to the deck of the ship. Due to small movements of the boat by wind and waves, and consequently vertical movement of the sampling hose, the entire water column was sampled. Subsamples for the determination of phytoplankton taxa, toxins and nutrients were taken at each depth and treated as explained in detail in section 7.2.3 and 7.2.4. All samples were immediately stored in the dark and on ice in isolation containers until further processing in the laboratory.

7.2.2 Determination of physical properties of the water

Physical parameters were derived during the weekly casts with a Sea-Bird SBE19plus SeaCAT Profiler CTD instrument package mounted on a frame. While lowering the package with approximately 0.5 m s⁻¹ from the research vessel, depth (m), temperature (ITS-90, °C), salinity, density (kg m⁻³) and Chl a fluorescence (Wetlabs Wetstar, corresponding to μg L⁻¹ Chl a) were taken with at least 10 measurements per meter. These were then binned to 0.25 m. In total, 25 CTD casts were conducted at the key stations in Alfacs and Fangar Bay; 145 casts when considering transects.
Fig. 7.1. Location of the study sites, Alfas Bay (A) and Fangar Bay (F), in the Ebro Delta on the Catalan coast (NW Mediterranean). Key sampling stations in both embayments are marked with a star, start- and endpoint of transects with a dot. Shellfish aquaculture of the Mediterranean mussel *Mytilus galloprovincialis* is conducted on fixed constructions in both bays (dashed lines).

### 7.2.3 Inorganic nutrients

Samples were taken each 1 m throughout the water column. For each depth, a plastic syringe was used to filter sampling water through a syringe filter (nominal pore size 0.45 µm Sartorius, Minisart, single use, non-sterile, hydrophilic). The first filtered 10 mL were discarded, and then $5 \times 10$ mL were filtered into five 12 mL sterile screw cap plastic tubes. The first two were used as duplicates for the analysis of silicate, the remaining three as triplicates for analysis of ammonium, nitrate, nitrite, and phosphate. Samples were kept upright in a rack, at cool and dark conditions. Upon arrival in the laboratory, all samples were stored in upright position, the two samples for silicate at 4 °C; the remaining three at –20 °C. All samples were analysed within three months after sampling with standard auto analyser techniques (AutoAnalyser 3, Bran+Luebbe, Germany), yielding concentrations of $\text{NH}_4$, $\text{NO}_3$, $\text{NO}_3+\text{NO}_2$, Si, $\text{PO}_4$. $\text{NO}_2$ was then calculated by $c(\text{NO}_2) = (c(\text{NO}_3) + c(\text{NO}_2)) - \text{NO}_3$.

### 7.2.4 Characterisation of the harmful phytoplankton community

#### 7.2.4.1 Enumeration and identification of HAB taxa/genera by inverted light microscopy

For enumeration and identification of phytoplankton by light microscopy, 100 mL of sampling water were collected directly from the on-board pumping system. The samples were immediately fixed with Lugol’s iodine neutral solution and stored in cool (4 – 15 °C) and dark conditions until analysis. Microscopic analysis of all collected samples was accomplished within nine months in 2010 (n = 156) and within one year in 2011 (n = 136). Analysis was conducted with a 50 mL subsample. Samples were adjusted to room temperature, re-
suspended and settled in a sedimentation tube attached to a circular counting chamber (both Hydro-Bios Apparatebau GmbH). After the proposed sedimentation time of 24 h (CEN, 2006), the sedimentation tube was slid from the settlement chamber to discard the supernatant. The counting chamber was protected with a coverslip and the sample was then investigated with an inverted microscope by means of the Utermöhl method, based on the European Standard (Utermöhl, 1931, CEN, 2006). Most samples were analysed with a ZEISS Axiovert 40, or 35, few samples with an inverted microscope by NIKON and LEITZ 4080. After confirmation that all cells were randomly distributed on the counting chamber bottom, the whole phytoplankton community was identified and enumerated up to species or genus level whenever possible with inverted microscopy. Except high species distribution, in which case higher magnification was chosen, all large cells (> 20 µm) were identified and counted in the whole chamber at a magnification of 100 ×. Subsequently, small cells (< 20 µm) were counted on transects through a grid in the ocular at a magnification of 200 ×. In case of high abundances of certain taxa, a magnification of 400 × or random fields at 200 × were selected. In the rare cases of high phytoplankton abundances, or if the identification was compromised by a high load of flocculating particles, the sample was diluted or a lower volume was settled. Identification of species was mainly based on following references: Tomas, 1997, Hoppenrath et al. 2009, and the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009 onwards).

7.2.4.2 Molecular diversity and phylogeny

Determination of HAB molecular diversity by means of 454 amplicon pyrosequencing (28S large subunit rRNA (LSU))

For an analysis by means of high throughput sequencing technology (Roche 454) and DNA based molecular markers, a 300 mL sub-sample was filtered through a polycarbonate filter (nominal pore size 0.2 µm, Millipore). The filter was then folded and stored in a 1.5 mL safe lock tube (Eppendorf, Hamburg, Germany) at -80 °C until further analysis. Three samples collected from the upper and lower water column in Fangar Bay were selected for analysis. For DNA extraction, the content of three selected sample filters was rinsed off with preheated (65 °C) AP1 buffer (DNeasy; Qiagen, Hamburg, Germany) by pipetting and mixed in a vortexer for 30 seconds. DNA was then extracted with a Qiagen mini shredder kit (Qiagen, Hamburg, Germany) as follows: To the cells in 400 µL AP1, three spatula tips (approx. 300 - 500 µL) of acid washed glass beads (80 – 200 µm) (Sigma-Aldrich, Munich, Germany) were added. The mixture and glass beads were incubated for 10 minutes at 65 °C and vortexed in intervals of 5 minutes. Subsequently, cell lysis was completed by two 20 second long incubations at 6.5 in a TissueLyserTM (Qiagen, Hamburg, Germany). Then, 4 µL RNase (100 mg mL⁻¹) was added and while vortexing in intervals of five minutes, the mixture was
incubated at 65 °C for 15 min. After adding 130 µL AP2 buffer, the tube was kept on ice for 5 min and then centrifuged at 12,000 × g for 5 min. The supernatant was then transferred to a QIAshredder® mini spin column and centrifuged at 12,000 × g for 2 min. The supernatant was transferred into a new 2 mL tube and 750 µL of AP3 was added with a pipette. 650 µL of this mixture were then added to the DNeasy® spin column and centrifuged at 5,900 × g for 1 min. After discarding the filtrate, the previous step was repeated once. The DNA was washed by adding 500 µL AW buffer to the column; this was left to incubate for 30 sec and subsequently centrifuged at 5,900 × g for 1 min. This step was repeated using 96% ethanol. The column was dried by centrifugation at 12,000 × g for 2 min, in order to remove the remaining ethanol which would reduce the purity and yield of the elution step. DNA was eluted into a clean 1.5 mL tube by adding 50 µL AE buffer to the centre of the membrane and incubated for 5 min at RT and centrifuging at 5,900 × g for 1 min.

**PCR template amplification.** Universal eukaryote primers (Scholin *et al.*, 1994) targeting the hypervariable D1-D2 domain of LSU rDNA were used to generate fusion primers (454 sequence adaptor A or B; key sequence; MID and D1R-F or D2C-R) for FastStart High Fidelity PCR (Roche, Mannheim, Germany). The reactions were executed in 50 µL volumes containing 1x High Fidelity buffer, 5x High Fidelity Taq polymerase, 200 µM dNTPs, 0.2 µM final concentration of each primer, and 10 ng purified community DNA. The thermal cycling was set up with a denaturation step at 94 °C for 2 min followed by 30 cycles each of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, and finally by a last elongation step at 72 °C for 10 min.

**Amplicon preparation and sequencing.** PCR products were separated in 1% agarose gel at 80 V for 1 h. Larger bands of 700 bp to 900 bp size were excised and purified with the MinElute gel extraction kit (Qiagen, Hamburg, Germany). Contaminants and small fragments were removed with the Ampure Bead PCR purification system (Invitrogen, Karlsruhe, Germany) following the protocol for small fragment removal. The exact size was determined with the Agilent High Sensitivity DNA kit (Agilent, Santa Clara, USA). The 454-amplicon sequencing was carried out on a Roche GS Junior machine (Roche, Mannheim, Germany) following standard protocols.

**Sequencing run post-processing.** The associated 454 Sequencing System Software (v2.7, default parameters) was used for quality control. All sequencing reads featuring inaccurate key sequence, chimeric sequences, biased nucleotides, or unidentified nucleotides were regarded as low quality reads and were discarded. The sequencing data was demultiplexed and cleared from primer fragments (CLC genomic workbench v5.0). Only reads exceeding 200 bp where analysed with MG-RAST v3 (Meyer *et al.*, 2008) with settings off annotation source for LSU, maximum e-value cutoff 1e⁻⁵, minimum % identity cutoff 97 % and minimum alignment
length cutoff 100 for the investigation of contributions of orders and genera and target species (for rarefraction plots for samples see Annex Fig. 4).

LSU sequences of selected HAB groups and species were further examined. The phylogenetic reference tree of Dinophyceae based on concatenated SSU+LSU rDNA sequence data (Kilpert et al., submitted). The tree was optimized for branch lengths – not changing the initial tree topology – using RAxML 7.3.0 (option: -f e) alongside generating the mandatory statistics file for pplacer 1.1 alpha12 (Matsen et al., 2010). The dinoflagellate reference tree was used to place reads from 454 sequencing on it for phylogenetic placement. Read placements on branches were counted for major clades (custom script, Kilpert et al., submitted). Two values, the number of placements within a sub-clade defined by the last common ancestor (LCA) and the number of placements within the entire clade were used to evaluate the species/group diversity.

**Determination of Pseudo-nitzschia spp. by means of qPCR**

A 50 mL water sample was directly collected from the on-board pumping system in a 50 mL centrifuge tube (BD Falcon™) and the plankton community was immediately fixed with Lugol’s neutral solution. The sample was kept cool and in the dark until arrival in the laboratory and cell pellets were prepared for storage on the same day. The pellets were harvested by means of two centrifugation steps: Solid and aqueous phases were separated by centrifugation in the centrifugation tube (centrifuge MR 22i, Jouan, Spain, at 990 × g for 25 minutes at 4 °C in 2010 and at 10 °C in 2011). After discarding the supernatant, undisturbed residuals were transferred to a 1.5 mL safe lock tube (Eppendorf, Hamburg, Germany) and centrifugation was repeated (13,000 × g for five minutes (in 2010 with a centrifuge 5414 D at room temperature, in 2011 with a 5415 D at 4 °C, both Eppendorf, Hamburg, Germany). The cell pellets were stored at −20 °C in a cryo-tube.

A total of 27 samples were selected based on highest cell counts of *Pseudo-nitzschia* spp. by light microscopy and presence of the toxin DA. Selected samples were then processed (following Fawley & Fawley, 2004, Andree et al., 2011) targeting *P. calliantha*, *P. delicatissima* (strain Ra2), *P. arenysensis* (*P. delicatissima*, strain Ra3), *P. fraudulenta*, *P. multistriata*, *P. pungens*, *P. brasiliiana* and *P. galaxiae*. For DNA extraction, cell pellets were thawed and re-suspended in 200 µL lysis buffer (1M NaCl, 70 mM Tris, 30 mM EDTA, pH 8.0) and transferred to a 2 mL cryo-tube containing approximately 50 mg of 0.5 mm diameter zirconium glass beads (BioSpec). Microalgal DNA was collected by adding 25 µL 10% DTAB (dodecyltrimethylammonium bromide) and 200 µL chloroform to the tube. Cells were disrupted for 40 seconds with a BeadBeater-8 (BioSpec) at full speed, and then cooled down on ice. Cell debris and beads were spun down at 2000 × g for 5 minutes in a centrifuge (centrifuge 5415 D Eppendorf, Hamburg, Germany). Then 100 mL of the aqueous supernatant
was transferred to a fresh tube. Of this sample, DNA was extracted by means of a GeneClean Kit (MP Biomedicals, LLC) for genomic DNA isolation following Fawley & Fawley (2004). For the identification of taxa, a hemi-specific assay was used containing one genus-specific 5.8S primer and one species-specific ITS-1 or ITS-2 primer (Andree et al., 2011). Duplicate amplifications were performed on an ABI 7300 in 20 µL volumes extended for 45 cycles following a standard two-step protocol of 94 °C for 30 s followed by primer annealing/extension at 65 °C for 30 s. SYBR Green dye in reaction mixtures was used for amplification detection and melt curve analysis. The thermal profile for melt curve determination started at an incubation of 1 min at 60 °C with a gradual increase in temperature (1°C/15 s) to 95°C, during which time changes in fluorescence were monitored (Andree et al., 2011).

Each species-specific hemi-nested PCR assay included a positive control (either plasmid clone or purified genomic DNA) for each species tested. Samples were only considered positive when the shape and Tm of the melt curve matched the control. Although shape may vary slightly due to point mutations within the amplified region (Andree et al., 2011), when significant deviations from the control were seen these samples were considered as negative for that specific internal transcribed spacer (ITS) PCR assay.

7.2.5 RDA of phytoplankton abundances with respect to environmental data
Several redundancy analyses (RDA; Ter Braak, 1986) were conducted to investigate whether observed heterogeneities of particular taxon cell abundances are related primarily to hydrographic processes or to ambient macronutrient concentrations. To avoid shotgun analysis, RDAs with different combinations of environmental parameters provided detailed analysis of multivariate gradients among combinations of included plankton community and environmental data.

Cell abundances of most taxa were low and in many cases target HAB taxa were absent from the water column; therefore the analyses were carried out with two common genera acting as proxies for alternative ecotypes. From their geo-spatial distribution, it was assumed that *Pseudo-nitzschia* spp. tend to prefer open ocean or coastal waters outside the bays (cf. Lelong et al. 2012 and references therein), whereas *Karlodinium* spp. are more often associated with local freshwater hydrogeochemistry in more enclosed systems (Place et al., 2012).

For all analyses, the community matrix was composed of log(1+x) transformed abundance data of the two taxa. Analyses and visualisations were carried out using the free statistical computing language R and the additional library package vegan (http://cran.r-project.org/web/packages/vegan/vegan.pdf).
7.2.6 Identification and quantification of phycotoxins

Plankton was sampled by collection of 1 L sea water in 2010, and 2 L in 2011 at each station and depth. Water volume was collected in a measurement cylinder directly from the on-board pumping system, and gently transferred to a filter tower with 10 µm Nitex gauze. The filtrate was re-suspended and washed off the filter with 0.2 µm filtered sea water and collected in a 50 mL centrifuge tube (BD Falcon™). The tubes were stored in cooling boxes on ice and in the dark until arrival in the lab. The samples were processed for storage at the same day by condensing cell pellets by centrifugation of the tubes for twenty minutes at 4 °C and 2500 × g (centrifuge MR 22i, Jouan, Spain). The supernatant was discarded with a 25 mL pipette, and the residues were transferred into 1.5 mL safe lock tubes and centrifuged again for five minutes at 12,000 × g (in 2010 with a centrifuge 5414 D, in 2011 with a 5415 D, both Eppendorf, Hamburg, Germany). After removal of the supernatant, the cell pellets were stored in cryo-vials at -20 °C until further processing.

For the extraction of lipophilic toxins, all cell pellets were thawed, suspended in 0.5 mL methanol and homogenized by vortexing. The samples was then transferred to FastPrep tubes with 0.9 g lysing matrix D (Thermo Savant, Illkirch, France) to disrupt cell membranes by reciprocal shaking in a Bio101 Fast Prep instrument (Thermo Savant, Illkirch, France) for 45 seconds at maximum speed (6.5 m s⁻¹). Subsequently, cell debris was spun down by centrifugation in 15 minutes at maximum speed (16,100 × g at 4 °C, Eppendorf 5415 R, Hamburg, Germany). The supernatants were transferred to a spin-filter (nominal pore size 0.45 µm; Millipore Ultrafree, Eschborn, Germany) in a safe lock tube, filtered 0.5 minutes at 800 x g, and then the filtrates were transferred into glass vials and stored at –20 °C until further analysis by liquid chromatography coupled to mass spectrometry (LC-MS/MS). For the extraction of hydrophilic toxins, the remaining cell debris was again suspended in 0.5 mL (in 2010) and 0.3 mL (in 2011) 0.03 M acetic acid and underwent the same Fast Prep and filtration procedure as described above for lipophilic toxins. Samples in glass vials were also stored at –20 °C until analysis of the samples.

Toxin analyses were performed on a LC-MS/MS system consisting of an Agilent model 1100 LC coupled to an ABI-Sciex 4000 Q Trap triple-quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany), equipped with a TurboSpray interface, basically following a multi toxin selected reaction monitoring (SRM) method described in Krock et al. (2008) (Annex Table 1, Table 2). In addition samples were analysed for AZAs with a specific SRM method for all known AZAs as described in Gu et al. (2013).

PSP toxins were assessed by reversed phase ion-pair liquid chromatography with post-column derivatisation and fluorescence detection (LC-FD) as described in (Krock et al., 2007).
7.3 Results

7.3.1 Freshwater contribution and phytoplankton biomass distribution

Clearly, two different regimes were defined by the salinity pattern in the two embayments over depth and time. Regime I was characterised by a distinct low salinity surface layer, separated from the lower water body by a salinity-driven pycnocline at about one meter depth (Fig. 7.2b). This regime prevailed in both embayments during the whole study period in 2011, in Alfacs Bay also in the beginning of May 2010. The second regime, present during all subsequent sampling in Alfacs Bay, and all casts in Fangar Bay in 2010, had a much less pronounced salinity profile. Both regimes were not restricted to the main sampling stations, but also horizontally consistent when compared along transects (Fig. 7.2f, h). Average salinity values were higher in Alfacs Bay (with 34.1 in 2010 and 32.77 in 2011), whereas maximum values were higher in Fangar Bay, exceeding 37 in 2010 and 41 in 2011. Differences in temperature were most prominent over time, rather than over depth and therefore did not follow the described pattern of the two freshwater regimes (Fig. 7.2a). Over the study period, the temperature trend showed an increase from spring towards summer throughout the water column in both years and embayments. Average water temperatures were higher in Alfacs than in Fangar Bay, with maximum temperatures exceeding 27 °C in Alfacs Bay, and 24 °C in Fangar Bay.

Phytoplankton biomass, estimated by Chl a fluorescence during weekly casts, was heterogeneous over depth, time, and along transects throughout the study period (Fig. 7.2c, e-h). Notably, the upper freshwater-influenced layer during regime I always coincided with lowest algal biomass concentrations (Fig. 7.2c, f, h). Occasionally, a restricted layer directly below the pycnocline in regime I contained an increased Chl a concentration, e.g., in Alfacs Bay on May 11, 2011. These patches were, however, not often horizontally consistent over transect elements ~250 m. A consistent phytoplankton layer along the longitudinal section from the bay entrance towards the key sampling station remained an exception, e.g., in Fangar Bay on 26 May 2011, but was only weakly pronounced (Fig 7.2h).

In the upper water column, increased concentrations of nitrite (> 0.5 µM) and nitrate (> 3 µM) were apparent (Fig. 7.3). Regardless of the regimes, ammonium concentrations were in most cases highest towards the sea floor (> 3 µM), which can be expected due to the vicinity to aquaculture rafts, which were within 5 m at the key station in Alfacs Bay. Also highest silicate concentrations were found in bottom layers (> 15 µM). Low amounts of this nutrient (< 2.5 µM) appeared regardless of the regimes in May 2010 and in June 2011 in Alfacs Bay, and in Fangar Bay during one cast in June 2010 and in May 2011. N:P rations reached from 2 to 540 during the study period.
Fig. 7.2. a-d) Environmental parameters temperature [°C], salinity, density [kg m\(^{-3}\)] and algal biomass estimated by Chl \(a\) fluorescence as derived by weekly CTD casts over depth at the key station in Alfacs and Fangar bays from May to beginning of July in 2010 and 2011. Two different regimes of freshwater influences are visible, with a salinity driven pycnocline that separates a freshwater influenced water surface layer from the lower water body during regime I, and regime II with less defined density and salinity patterns. Temperature and salinity during the missing casts in Alfacs Bay 2011 are inserted as estimated by additional casts retrieved with a Satlantic Hyper Pro II profiling system. The upper meter is not resolved with this system. e-h) Examples for temperature, salinity, and Chl \(a\) fluorescence along transects during both regimes in Alfacs and Fangar bays. Key stations are marked with an arrow, start- and endpoint of transects are denoted in Fig. 7.1.
7.3.2 Occurrence and proliferation patterns of HAB taxa and phycotoxins

7.3.2.1 Presence of harmful species

HAB taxa identified on the basis of morphological features by means of light microscopy mainly comprised regionally known phycotoxin-producing dinoflagellates, such as *Dinophysis acuminata*, *D. sacculus*, *Lingulodinium polyedrum*, *Prorocentrum reticulatum*, *Alexandrium* spp. and *Karlodinium* spp. (Table 7.1). Furthermore, the diatom genus *Pseudo-nitzschia* was heavily represented in field samples. In addition, cells of the potentially toxic dinoflagellate genus *Azadinium* were identified by light microscopy. Different focal plains clearly identified both the presence of an antapical spine (7.4a) and of three stalked pyrenoids visible by a starch shield, all of which were located in the epicome (Fig. 7.4b, c).

---

Fig. 7.3. Distribution of inorganic nutrient concentrations [µM] over depth from May to beginning of July in 2010 and 2011 at key stations in Alfacs and Fangar bays. Samples at each 1m were analysed, with interpolations to half meter depths.

Fig. 7.4. Light microscopic images of an *Azadinium* sp. cell which was detected in a fixed sample from the key station in Alfacs Bay, 2010; a) clearly, a spine is visible, indicated by the arrow, as well as b, c) three stalked pyrenoids in the epitheca, also marked with arrows. d) Focal area is on the sulcus and cingulum.
Subsequent analyses by molecular techniques provided further insights and confirmation of HAB taxa in selected samples (Table 7.1). During the first noted high abundances of *Karlodinium* spp. in Fangar Bay, the species *K. veneficum* (synonym: *K. micrum*) was identified within the 454 amplicon reads. Subsequent phylogenetic placement of LSU rDNA sequences clearly identified *K. veneficum* in these samples. Additional sequences from a putative *Karlodinium* taxon could not be definitively assigned to a given species. Furthermore, the presence of *Alexandrium* taxa, e.g., *A. ostenfeldii* and *A. tamarense*, was confirmed by sequence alignment and phylogenetic placement. The dinoflagellate *Azadinium* was also listed in MG Rast, with a percentage identity of 99.6% and an average alignment length of 253 for the genus, but sequences were not within the reference library of the subsequently conducted phylogenetic placement analysis. In addition, the haptophyte *Prymnesium parvum*, the dictyochophyte *Pseudochattonella farcimen* and the raphidophyte *Chattonella marina* were detected with LSU sequences in MG Rast with a convincing percentage identity of 100%. The latter is consistent with the presence of a *Chattonella* bloom (3 × 10^6^ L^-1^; M. Fernandez-Tejedór, unpublished), leading to discoloured patches on the water surface near the entrance to Fangar Bay in May 2011.

Species diversity of the genus *Pseudo-nitzschia* was targeted by qPCR assays for seven species that are known to occur in the Ebro Delta. Of these, *P. calliantha*, *P. delicatissima* (Ra2), and *P. galaxiae* were identified as potentially toxic members of the genus (Table 7.1). In addition, *P. arenysensis* (*P. delicatissima* Ra3) and an additional *Pseudo-nitzschia* taxon related to the *P. delicatissima* genotypic group was detected by qPCR in certain samples, although given the broad degree of genetic diversity for this species it is difficult to say precisely which sub-species or strain it may have been. In 14 of 27 samples, none of these taxa were identified despite the clear presence of this genus by light microscopy, which indicated that additional taxa are likely present which were not captured by the qPCR assays used in this study (Fig. 7.5f). Supportively, only *P. delicatissima* Ra2 was identified in a surface sample from Fangar Bay in 2011 that contained the highest cell concentrations of *Pseudo-nitzschia* enumerated by light microscopy (7 × 10^6^ cells L^-1^), but with more than 3 orders of magnitude lower estimated abundances by qPCR. Furthermore, the other four potentially toxic members of the genus, *P. brasiliiana*, *P. fraudulenta*, *P. multistriata*, and *P. pungens* were not detected by qPCR in any of the selected samples.
Table 7.1. List of HAB taxa identified in Alfacs and Fangar bays during May – July 2010 and 2011 by inverted light microscopy, and in selected samples from Fangar Bay 2010 by 454 pyrosequencing of LSU sequences, when necessary confirmed with phylogenetic placement. Taxa of the diatom genus *Pseudo-nitzschia* were decomposed by qPCR in selected samples of both bays and years. Bloom forming species that are not considered HABs (such as *Ceratium* spp.) were present, but are not included in the list. *Akashiwo sanguinea* is included as non-toxic but high biomass HAB species.

<table>
<thead>
<tr>
<th>Class</th>
<th>Division</th>
<th>Light microscopy</th>
<th>Molecular methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyceae</td>
<td></td>
<td><em>Pseudo-nitzschia</em> spp.</td>
<td><em>P. delicatissima</em> (Ra2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. calliantha</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. galaxiae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. arenysensis</em> (<em>P. delicatissima</em> Ra3)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. delicatissima</em> (geno-type)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Prymnesium parvum</em></td>
</tr>
<tr>
<td>Haptophyceae</td>
<td></td>
<td><strong>Dinophysales</strong></td>
<td><strong>D. acuminata</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. norvegica</strong></td>
<td><strong>D. norvegica</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. caudata</strong></td>
<td><strong>D. caudata</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. acuta</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. ovum</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. rotunda</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. sacculus</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. hastata</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>D. odiosa</strong></td>
<td></td>
</tr>
<tr>
<td>Prorocentrales</td>
<td><strong>incertae sedis</strong></td>
<td><strong>Prorocentrum lima</strong></td>
<td><strong>P. minimum</strong></td>
</tr>
<tr>
<td>Gymnodiniales</td>
<td><em>Azadinium</em> sp.</td>
<td><em>Amphidinium carterae</em></td>
<td></td>
</tr>
<tr>
<td>Gymnodiniales</td>
<td><strong>Gymnodinium catenatum</strong></td>
<td><em>G. catenatum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Karenia</em> spp.</td>
<td><em>Karenia</em> spp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Karlodinium</strong> spp.</td>
<td><em>Karlodinium</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Gymnodiniales</td>
<td><strong>Karlodinium</strong> spp.</td>
<td><em>K. veneficum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Akashiwo sanguinea</em></td>
<td><em>A. sanguinea</em></td>
<td></td>
</tr>
<tr>
<td>Gonyaulacales</td>
<td><strong>Alexandrium minutum</strong></td>
<td><em>A. minutum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>A. tamarense</strong></td>
<td><em>A. ostenfeldii</em></td>
<td></td>
</tr>
<tr>
<td>Raphidophyceae</td>
<td><strong>Lingulodinium polyedrum</strong></td>
<td><em>L. polyedrum</em></td>
<td></td>
</tr>
<tr>
<td>Dictyochophyceae</td>
<td><strong>Ostreopsis</strong> spp.</td>
<td><em>O. reticulatum</em></td>
<td></td>
</tr>
<tr>
<td>Dictyochophyceae</td>
<td><strong>Chattonella</strong> sp.**</td>
<td><em>Chattonella marina</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Pseudochattonella farcimen</strong></td>
<td><em>P. farcimen</em></td>
<td></td>
</tr>
</tbody>
</table>

* not confirmed by IOC list of toxic species
** not at key stations

7.3.2.2 Distributional and proliferation patterns of harmful taxa

HAB taxa, as identified by light microscopy, were heterogeneously distributed in both vertical and temporal dimensions, but did, in most cases, not persist in the water column in detectible quantities for longer than one week (Fig. 7.5a-f). Alert cell abundances, as defined by the FVO (Food and Veterinary Office) for the region were exceeded in such patches by numerous taxa, amongst them *D. sacculus* and *D. acuminata* (>500 cells L\(^{-1}\)) and *Pseudo-nitzschia* spp. (>200,000 cells L\(^{-1}\)).

Vertical patchiness in species abundances was clearly evident during both salinity–defined regimes. Notable is the appearance of *Dinophysis* spp. abundances in excess of 500 cells L\(^{-1}\) exclusively during phases of regime I in both study periods. In May 2011 high
proliferations of co-occurring *D. sacculus* and *D. acuminata* appeared in both embayments at the same time, with higher abundances of *D. sacculus* in Alfacs Bay, and higher abundances of *D. acuminata* in Fangar Bay (Fig. 7.5a, b). In Alfacs Bay, *D. sacculus* formed patches around the pycnocline in both years, but this positional behaviour did not bear comparison with that in Fangar Bay (Fig. 7.2, Fig. 7.5a). Besides *D. sacculus*, there was no obvious relationship between the abundance of particular HAB taxa and the pycnocline during regime I.

The dinoflagellates *L. polyedrum* and *P. reticulatum* were primarily present in Alfacs Bay (Fig. 7.5c, d). While *L. polyedrum* was detected in the same casts as *D. sacculus* in 2011, *P. reticulatum* was present at bottom depths in both years, as well as in patches in the upper half of the water column in 2011. Even when exceeding critical abundances for regulatory triggers, cell concentrations of all these HAB taxa accounted for only a small fraction of the overall phytoplankton and dinoflagellate community. One exception is a sample from below the pycnocline of the *D. acuminata* proliferation in Fangar Bay in May 2011, where the species was with 20% the second most abundant dinoflagellate after *Akashiwo sanguinea* (30%).

During regime II, highest abundances of *Karlodinium* spp. were enumerated by light microscopy. These were increasing towards the end of the study period 2010 in both embayments. During this time, highest cell concentrations were found in samples from 3 m depth, with 20,000 cells L\(^{-1}\) in Alfacs Bay and up to 650,000 cells L\(^{-1}\) in Fangar Bay (Fig. 7.5e). The accuracy of the high cell counts in the Fangar Bay sample from 3 m was possibly compromised by the presence of a high load of flocculating particles, yet samples from other water depths of the same cast still contained increased *Karlodinium* cell abundances between 2,900 and 45,000 cells L\(^{-1}\). In any case, cell concentrations of this genus never reached 50% of the total abundance of dinoflagellates, with always higher abundances of *Prorocentrum triestinum* or *Scripsiella* spp. in the respective samples.

Cell concentrations of *Pseudo-nitzschia* spp. were markedly higher in Fangar Bay than in Alfacs Bay and alert levels of 200,000 cells L\(^{-1}\) were frequently exceeded throughout both study periods and hence during both stratification regimes (Fig. 7.5f). There was no apparent relationship to the pycnocline and no formation of thin layers of *Pseudo-nitzschia* was ever observed, based upon microscopic cell enumeration and identification. Maximum abundances of nearly \(7 \times 10^6\) cells L\(^{-1}\) were present on 19 May in a surface sample. After one week (by 26 May), abundances remained critical in patches throughout the water column, with local maxima at 1m, 2m and 4m. The upper patch at 1m was within a Chl a maximum layer as

---

The position of *D. sacculus* abundances in Alfacs Bay was at approximately the same water depth, where the pycnocline was situated before and after the bloom in both embayments. No CTD data were available for the respective casts in 2011.
revealed by CTD casts (see 7.3.1). The described event aligns with data from the regular IRTA monitoring program, where higher cell concentrations of the genus were observed before our study period, from early May and a decline by the end of the month. For the rest of the second study period, *Pseudo-nitzschia* abundances remained far below critical limits. During the study period in 2010, cell abundances in excess of 200,000 cells L$^{-1}$ were detected at multiple depths during three of four casts in Fangar Bay (Fig. 7.5f).

When considering species contribution of this genus, either one, up to four, or none of the species were identified by qPCR assays in selected samples. In Fangar Bay, *P. delicatissima* (Ra2) was the only taxon which was identified in both study periods, and thus during both freshwater regimes. The species was present in the sample with maximum cell concentrations of the genus, according to light microscopic counts, as well as in two patches in the subsequent cast, at 1.5-2 m and 3.5-4 m (26 May 2011). As second species, the distinct *P. delicatissima* genotype was detected at 0.5 m and 2 m in the latter cast. In the study period 2010, also *P. calliantha* and *P. galaxiae* were found in one two adjoined samples (23 June) at 0.5 and 1 m depth, the latter also contained *P. arenysensis*. In addition, *P. delicatissima* (Ra2) was present in both samples, and at 2 m in the same cast. Furthermore, this species was identified in two samples at a prior cast, in one of which in addition, *P. arenysensis* was detected. In Alfacs Bay, only *P. delicatissima* (Ra2) was identified in 2010, while in 2011, only *P. arenysensis* was present. In summary, the distribution of *Pseudo-nitzschia* sp. as determined by means of qPCR was patchy, in mono- or multispecific assemblages. None of the species was assignable to the pycnocline with our reduced dataset. Abundances estimated by means of qPRC did not reflect the microscopic enumeration, especially in the case of highest counted abundances, where the qPCR abundance was three orders of magnitude lower.
Fig. 7.5. a-f) Abundances of major HAB taxa as revealed by identification and enumeration with light microscopy over depth and study time from May to July 2010 and 2011 in Alfac and Fangar bays (cells L⁻³). g) Samples which were additionally selected for 454 pyrosequencing analysis of the whole phytoplankton community, with emphasis on Karlodinium spp., are marked with diamonds. f) Samples selected for qPCR analysis with assays of seven Pseudo-nitzschia spp. are indicated with full circles if any- and open, if none of the taxa was identified. In addition, samples in which the toxin domoic acid was detected are marked with arrows. g-k) Distribution of additional phycotoxins [pg L⁻³] in selected study periods in Alfac and Fangar bays.
Three different RDAs were carried out to investigate hidden effects of freshwater inflow on variations in cell abundances and distribution of the dinoflagellate *Karlodinium*, and the diatom genus *Pseudo-nitzschia*. In the environmental matrix used for the first RDA with salinity and temperature, four main clusters were observed (Fig. 7.6a): Cluster PK, with samples in which both genera were present, cluster K in which only *Karlodinium* and no *Pseudo-nitzschia* spp. were identified, cluster P in which accordingly only *Pseudo-nitzschia* spp. were counted and cluster N with zero counts of both genera. The absence of *Pseudo-nitzschia* spp. (cluster K) was associated to high temperature and low mean salinity (in this single case the median should be considered, as the mean is determined by one low salinity value, Table 7.2). *Karlodinium* spp. were not present in water characterised by higher salinities and cooler temperatures (cluster P), whereas both genera were absent under intermediate physical oceanographic conditions, with lower salinities compared to oceanic waters and lower temperatures (Table 7.2). This situation was also confirmed in a comparative approach, when the key stations in Alfacs and Fangar bays were considered with independent RDAs (Fig. 7.6c-d). When *D. sacculus* and *D. acuminata* were also included in the RDA, variability of both taxa was most strongly related to salinity in Alfacs Bay, whereas in Fangar Bay there was no identifiable relationship to any particular environmental parameter. In any case, the explanatory power of this analysis is questionable for these two species because these taxa were only present in restricted casts, and these were excluded from the analysis due to a lack of corresponding CTD data.
Fig. 7.6. Redundancy analyses with a species matrix of the HAB genera *Karlodinium* spp. and *Pseudo-nitzschia* spp. as identified by light microscopy during the study period from May to beginning of July in 2010 and 2011 in response to environmental parameters. a) Species in response to temperature and salinity were clustered in four groups: low of the variability of abundances of for Alfacs Bay, Fangar Bay, and combined bays. Named in the correct mathematical order these are K (no *Pseudo-nitzschia* spp. cell present in original sample), N (neither of the two taxa present in sample), P (no *Karlodinium* spp. present in sample) and PK (both taxa present in sample). Samples from Alfacs Bay are printed in black, while those from Fangar Bay are displayed in green; b) Species in response to temperature and salinity in Alfacs Bay and d) species in Fangar Bay.

Table 7.2. Physical oceanographic characteristics of the four main RDA clusters defined for Alfacs and Fangar bays, including samples with zero cell counts for *Karlodinium* spp. (P) and (N) or *Pseudo-nitzschia* spp. (K) in response to salinity (PSS-78) and temperature ([°C], ITS-90).

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
<th>Parameter</th>
<th>Description of mean water characteristics</th>
<th>Mean</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>8</td>
<td>Salinity</td>
<td>Low/High (Median)</td>
<td>29.41</td>
<td>35.39</td>
<td>7.76</td>
<td>36.15</td>
<td>11.60</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Temperature</td>
<td>High</td>
<td>23.11</td>
<td>23.73</td>
<td>18.37</td>
<td>25.58</td>
<td>2.36</td>
</tr>
<tr>
<td>N</td>
<td>67</td>
<td>Salinity</td>
<td>Low</td>
<td>32.63</td>
<td>33.77</td>
<td>12.06</td>
<td>36.37</td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
<td>Low</td>
<td>21.10</td>
<td>22.11</td>
<td>17.59</td>
<td>22.21</td>
<td>1.79</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>Salinity</td>
<td>High</td>
<td>34.28</td>
<td>34.87</td>
<td>21.26</td>
<td>41.33</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
<td>Low</td>
<td>21.80</td>
<td>22.12</td>
<td>17.56</td>
<td>25.65</td>
<td>1.88</td>
</tr>
<tr>
<td>PK</td>
<td>171</td>
<td>Salinity</td>
<td>High</td>
<td>33.20</td>
<td>35.20</td>
<td>6.64</td>
<td>37.72</td>
<td>5.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
<td>High</td>
<td>23.36</td>
<td>23.56</td>
<td>17.56</td>
<td>27.99</td>
<td>2.45</td>
</tr>
</tbody>
</table>
7.3.2.3 Distribution of phycotoxins and assignment of toxins to taxa

During the study period in 2011, the phycotoxins domoic acid (DA), gymnodimine (GYM), okadaic acid (OA), pectenotoxin-2 (PTX-2) and its degradation product pectenotoxin-2-seco-acid (PTX-2sa) (Miles et al., 2004), pectenotoxin-11 (PTX-11), 13-demethyl spiroide C (SPX-1), and yessotoxin (YTX) were detected. The presence of these toxins coincided with the period and conditions defined as stratification regime I (Fig. 5g-k). Only GYM, SPX-1 and DA were also detected in plankton samples during regime II in 2010.

Prominent patches are those of PTX-11, PTX-2 and PTX-2sa which were detected in both embayments at the same time in May 2011. In Alfacs Bay, concentrations up to 0.5, 4.5, and 1 ng L\(^{-1}\) were measured of PTX-11, PTX-2, 1 and PTX-2sa respectively, while in Fangar Bay these toxins reached 0.3, 3.8 and 1.9 ng L\(^{-1}\). In June 2011, also OA was found in only one sample at 4 m in Alfacs Bay, with a concentration of 3.7 ng L\(^{-1}\). All these toxins are usually attributable to *Dinophysis* spp. and most of them complied well with maximum abundances of *D. sacculus* and *D. acuminata* in both bays (Fig. 7.5a, b, j). No toxin samples were available for the cast during regime I in 2010, in which *D. sacculus* was also found in Alfacs Bay. In any case, no toxins related to *Dinophysis* spp. or putative degradation products were detected in subsequent casts from 2010. At the same time when highest *D. sacculus* cell abundances and amounts of PTX-2 coincided, also 1 ng L\(^{-1}\) of YTX was measured from the same sample taken at the pycnocline (Fig. 7.5i). This toxin was also present at the subsequent cast at 3 m depth, in a lower amount of 0.8 ng L\(^{-1}\).

The macrocyclic imine toxins SPX1 and GYM were detected during both regimes and in both embayments, with highest amounts in the same surface sample at the end of the study season in 2011 in Alfacs (184 and 40 ng L\(^{-1}\)), and with about 5 ng L\(^{-1}\) for SPX1 also in two bottom samples in Fangar Bay in both years (Fig. 7.5g, h). Both toxins were occasionally also detected with low amounts in additional samples, but rarely reaching 0.5 ng L\(^{-1}\). For both toxins, no clear link to source organisms was evident.

Hydrophilic PSP toxins, were not detected in any sample throughout the study time, despite the confirmed presence of possible producers, including *A. minutum*, *A. ostenfeldii*, *A. tamarense*, and *Gymnodinium catenatum*.

Among all measured toxins, only DA was restricted to Fangar Bay and the four samples in which the toxin was found originated from the upper meter of the water column (Fig. 7.5f). Of these, two (in samples from surface and at 0.5 m) were taken in one cast during regime II in 2010 with concentrations of about 200 ng L\(^{-1}\), and the other two of about 5 ng L\(^{-1}\) in subsequent casts (in the surface sample and one week later at 0.5 m depth) during regime I in 2011. High abundances of the DA-producing genus *Pseudo-nitzschia* were observed by light...
microscopy at several depths during all of these casts. Yet, samples containing DA did not coincide with maximum abundances (Fig. 7.5f). For the two completely available depth profiles in which DA was present, the species contribution of *Pseudo-nitzschia* was determined by means of qPCR. Yet, none of the species identified by means of qPCR assays was present in all samples in which the toxin was found. In one of the four samples even taxon was identified.

7.4 Discussion and conclusions

7.4.1 Contribution of freshwater to defining stratification in the two regimes

The typical hydrographic forcing in Ebro Delta bays is strongly influenced by agricultural activities in the Delta basin. Specifically, in the ‘open channel season’, irrigation water is led from the Ebro River through a channel system to the rice fields and freshwater is then released into both embayments. Except a short flood period in winter, established for ecological reasons in compliance with an EU directive in 2001 (Regulation CEE 1257/1999) to provide a habitat for local flora and fauna, this ‘open channel season’ is followed by a ‘closed channel season’. The release of freshwater through the discharge channels is thus considered as the main driver for stratification in Alfacs Bay (Solé *et al.*, 2009). In support of these findings, a strong increase in water stratification in both embayments has been observed in April/May when channels are opened, followed by a period of low stratification between January and May in Alfacs Bay, and between February and May in Fangar Bay (Llebot *et al.*, 2011). Recently, the importance of freshwater inflow for long-term physical behaviour and the importance of wind for short term processes were described by a hydrodynamic model for Alfacs Bay (Llebot *et al.*, in press). Our study period began in May and thereby coincided with the ‘open channel’ season. The apparent freshwater-influenced stratification during freshwater regime I complies well with the above described typical seasonal pattern for both embayments. The second regime is defined by a less stratified water column, with similar conditions over horizontal scales. This may indicate a reduced introduction of freshwater, as neither precipitation nor wind direction and strength differed substantially between years (public meteorological service: www.meteo.cat/). This general regime pattern for both regimes was quite similar over depth and time for both embayments.

Due to fertilization of rice fields, freshwater originating from rice field irrigation introduces considerable inorganic nitrogen into the embayments, on the order of 20 – 100 mmol N m$^{-3}$, whereas the supply of inorganic phosphorus is relatively low (0.5 – 1.5 mmol P m$^{-3}$, Llebot *et al.*, 2010). Nutrient availability in the embayments is strongly dependent on anthropogenic input through freshwater, especially during fertilization of agricultural fields in the delta basin in June until drainage of rice fields in October (Muñoz, 1998), and thus also likely to be linked with stratification of the water column.
Within our study period, the occurrence of the highest concentrations of inorganic nitrate towards the upper water column in nearly all casts of both regimes may reflect the input of excess nutrients from agricultural activities. Considerable nutrient conversion and alterations in the ambient nutrient ratios are expected to be related to in situ metabolic activity within the mussel aquaculture sites. For example, adjacent to the mussel rafts, the concentration of ammonium was high at the bottom, which is expected due to the high loading of mussel faeces and released organic nutrients.

The freshwater influenced upper layer during regime I was clearly characterised by lower algal biomass than in the rest of the water column. This pattern is consistent with observations of low Chl a in the surface layer, as derived from continuous radiometric measurements at the station in Alfacs Bay (Busch et al., submitted). Chl a distribution in the lower water column did not clearly follow salinity or temperature patterns, but was dynamic over temporal (weeks), vertical (0.5 m) and horizontal (< 250 m) scales (Fig. 7.2c, e-h).

7.4.2 Dynamics of the usual suspects and phycotoxins under the two regimes
The majority of HAB taxa and phycotoxins at the stations in the Ebro Delta Bays were consistent with those usually responsible for aquaculture harvesting closures in this region and season, such as *D. sacculus* and *P. reticulatum* (Fernández-Tejedor et al., 2008). Patches of HAB taxa were not restricted to the more saline bottom layer, but also occurred within the upper zone in and above the pycnocline. Despite the relative shallowness of the embayments (≤5 m depth at the key sampling stations) strong vertical patchiness of all HAB taxa was observed. In addition HAB species- and phycotoxin patches were highly dynamic over time. These findings underscore the high variability in proliferations of harmful taxa over spatio-temporal scales in both embayments. Yet, even though phytoplankton variability in either Alfacs or Fangar bays are not expected to be directly dependent on salinity or temperature effects, both have been suggested as proxies for the seasonal behaviour of complex environmental and biotic factors (Llebot et al., 2011). In this study, *D. sacculus*, in association with the dinoflagellates *A. minutum*, *Karlodinium* spp. and *Scrippsiella* spp. have been assigned to the winter-spring season by a principal component analysis with monitoring data from 1990-2003 (Llebot et al., 2011).

7.4.2.1 Dynamics of HAB taxa and phycotoxins under regime I
One of the most prominent patches of HAB taxa during regime I were that of *D. sacculus* and *D. acuminata* in Alfacs Bay and Fangar Bay respectively. Such accumulations of these taxa are common for the season, in particular *D. sacculus* was attributed to multiple aquaculture closures in Alfacs Bay in spring (Fernández-Tejedor et al., 2008). Amongst other species of this genus *D. sacculus* and *D. acuminata* are known to produce OAs, DTXs and PTXs (see review on *Dinopysis* species by Reguera et al., 2012). In both embayments, increased
abundances of their mixed assemblages coincided well with PTX-2 concentrations at the key stations, with *D. sacculus* dominating in Alfacs Bay and *D. acuminata* dominating in Fangar Bay (Fig.7.5 a, b, j). Attributed to the most abundant species, PTX-2 would yield average cell quotas of 24 pg cell$^{-1}$ for *D. acuminata*, and 0.09 pg cell$^{-1}$ for *D. sacculus*. However, PTX cell quotas of 0.09 pg cell$^{-1}$ are unusually low for *Dinophysis*, indicating that there were few cryptic PTX producing *D. acuminata* cells among the dominating non-toxic *D. sacculus* bloom. Furthermore, the hydrolysed degradation product PTX-2sa (Suzuki *et al.*, 2001), was detected at time and depth of highest PTX-2 concentrations. As toxins were measured from particulate material, this may indicate grazing activity on these taxa. In addition the *Dinophysis*-related toxin OA was found during one cast at 4 m in Alfacs Bay. But the low OA level makes it difficult to attribute this toxin to any producing organism.

Population dynamics of *Dinophysis* spp. were characterised by rapid bloom development and decline - critical cell abundances were present for only two weeks in both embayments. During the study period in 2011, *D. sacculus* abundances increased threefold from 220 to 820 cells L$^{-1}$ within a week in Alfacs Bay, which is in the range of estimated local growth rate with a generation time between 2 and 5 days (Garcés *et al.*, 1997). In Fangar Bay, no full depth profile was available for *D. acuminata* in the week before abundances of up to 960 cells L$^{-1}$ were reached in plankton samples. The surface sample of the prior week contained only 20 cells L$^{-1}$ indicating that physical displacement favoured by the hydrodynamic regime rather than growth is the dominant factor in determining cell abundances. Notably, the accumulations of *D. sacculus* and *D. acuminata* occurred in both embayments at the same time.

In Alfacs Bay, both taxa were restricted to depths near the pycnocline, and thereby are most likely related to stratification of the water column. Such aggregations of *Dinophysis* spp. near the pycnocline have also been observed elsewhere, e.g., in the Galician Rías Baixas (Pizarro *et al.*, 2008). In this scenario, physical displacement by upwelling stratification and advection, combined with the presence of its prey organism, the ciliate *Mesodinium rubrum*, is seen as partial key to bloom initiation of *D. acuminata* (Velo-Suárez *et al.*, in press). Hence, an important factor for bloom indication in the Ebro embayments may also be the occurrence of its prey organisms, such as *M. rubrum* triggered by stratification. Yet neither this key species nor other prey taxa were observed in high abundances during the study time. The positioning of *Dinophysis* proliferations at the pycnocline in Alfacs Bay were not mirrored in Fangar Bay. The question of whether or not stratification creates a micro-niche for *D. sacculus* in Alfacs Bay, and why this behaviour was not comparably reflected in Fangar Bay cannot be fully answered with the findings of this study, as detailed information on species distribution over horizontal scales, as well as physical oceanographic characteristics for the respective casts in Alfacs Bay are not available. Yet, also in other geographic regions high cell
abundances of *Dinophysis* spp. are often found to increase under stratified conditions (see review by Reguera *et al.*, 2012). Moreover, the restriction of increased *Dinophysis* spp. abundances during stratified conditions under regime I is by trend indicative for this type species described by Smayda & Reynolds (2001), and hence indicates a habitat preference of this genus.

The toxin GYM was detected in substantial amounts in 2011 during regime I. *Karenia selliformis* is usually responsible for the production of this toxin (Mountfort *et al.*, 2006), but *A. peruvianum* has also been considered as a source in Chesapeake Bay, US east coast (Van Wagoner *et al.*, 2011). In our study, *Alexandrium* spp. were present in the respective sample, but in low abundances of 20 cells L\(^{-1}\), whereas no *Karenia* spp. were identified by light microscopy. Hence, a clear conclusion of the local source organism cannot be draw by our dataset and therefore no statement on associations to water regimes is possible.

### 7.4.2.2 Dynamics of HAB taxa and phycotoxins under regime II

In Fangar Bay, increased abundances of the dinoflagellate *Karlodinium* spp. were reported for June/July 2010 (Busch *et al.*, 2012) during regime II. Up to this time, *Karlodinium* spp. were known to occur in Alfacs Bay in high cell numbers, but not in Fangar Bay, as revealed by the regular monitoring programme (for 1990-2003 see Llebot *et al.*, 2010). Blooms of this genus in the region usually consist of a mixed assemblage of *K. veneficum* and *K. armiger* (Garces *et al.*, 2006). Phylogenetic placement of LSU sequences confirmed the presence of *K. veneficum* in the assemblage in Fangar Bay. The affiliation of additional *Karlodinium* sequences to either *K. veneficum* or *K. armiger* could neither be confirmed nor denied with this dataset. It is therefore not clear if *K. armiger* was also present or if the unidentified taxa also belong to *K. veneficum* or another unknown congener. The study marks the first record that substantial abundances of *Karlodinium* spp. in Fangar Bay are at least partially consisting of *K. veneficum*.

Despite influences of freshwater during the study period, regime II was characterised by lower stratification than regime I. In Alfacs Bay, *Karlodinium* spp. (*Gyrodinium* spp.) reached densities of 2 – 8 × 10\(^6\) million cells L\(^{-1}\) and caused fish kills of 6 × 10\(^4\) kg and a strong water discoloration in winter blooms in 1994 (Delgado *et al.*, 1995). During the initiation of this bloom, winter water stratification was visible, yet under absence of freshwater inflow from the drainage channels (Garces *et al.*, 1999). From this year onwards, such blooms were regularly observed in Alfacs Bay, but the bloom period shifted from winter to spring in 2000, and to spring-summer in 2003 to 2009 (Fernández-Tejedor *et al.*, 2010). For the time of spring blooms, a positive correlation to stratification was revealed with the long-term dataset on phytoplankton abundances in this embayment (Fernández-Tejedor *et al.*, 2010). Also in other geographic regions, *K. veneficum* blooms have been reported during stratified water conditions...
e.g. in the Neuse River Estuary, North Carolina (Hall et al., 2008) and in the East China Sea (Dai et al., in press). The situation in Alfacs and Fangar bays in 2010 does not confirm a correspondence of Karlodinium spp. abundances with the strongest stratification situation which was observed during regime I. Hence, a correlation between stratification and variability in Karlodinium spp. abundances is not apparent from our dataset. Redundancy analyses, however, revealed a trend of Karlodinium spp. to proliferate at increased temperatures but in less saline waters with respect to water characteristics encountered during our study period. In other regions, salinity values as low as 10 and an optimum temperature of around 25 °C were determined by clustering high K. veneficum abundances from Chesapeake Bay into a temperature salinity plot (see Fig. 2 in Place et al., 2012). In fact both parameters, sea-surface temperature and salinity were successfully applied to a habitat model for the prediction of K. veneficum bloom events in this area (Brown et al., 2013). For the period from January-August 2010, such optimum ranges were also apparent for high genus abundances in Alfacs and Fangar bays. In consideration of these two parameters, high abundances were clustered at similar temperatures around 25 °C, but considerably higher salinity of 32 – 35 (Busch et al., 2012). Hence, these parameters are likely to also serve for environmental models of Karlodinium spp. abundances in the Ebro Delta.

Increased abundances during mixed estuarine conditions that may follow stratification events would also fit the alignment to type species with respect to Smayda & Reynolds (2001). In this concept, Karlodinium spp. would be associated to type species that occur in mixed coastal regions with increased nutrient input. Nutrient input may, in turn, indirectly lead to proliferations of this mixotrophic species, by increasing the availability of prey. This aspect is also included to a scenario with key elements for the development of toxic K. veneficum blooms as proposed by Adolf et al. (2008), which includes co-occurrence of the mixotrophic algae with cryptophytes. This element may also be relevant for the situation in the Ebro Delta. Continuative conclusions or quantifications on the role of cryptophytes for the development of K. veneficum in Fangar Bay are, however, not possible with our dataset.

Besides Karlodinium spp., there was no indication of another HAB species or genus that predominantly occurred during regime II:

7.4.2.3 Dynamics of HAB taxa and toxins during both regimes

Pseudo-nitzschia spp. abundances were not associated to either freshwater regime during the study period; however, this genus was clearly more represented in Fangar Bay, with $7 \times 10^6$ cells L$^{-1}$ during a strong bloom in the study period 2011. According to prior findings, our study was located in the ‘core season’ for blooms in Fangar Bay. In this embayment, blooms of this genus were observed to start in April with cell concentrations of $2.5 \times 10^6$ cells L$^{-1}$, while in Alfacs Bay, abundances above the critical limit often start in summer with a
maximum at $11.5 \times 10^6$ cells L$^{-1}$ (Giménez Papiol et al., 2012). W found considerably higher cell abundances in samples from Fangar Bay than in the study of Giménez et al. (2012). This may partly be explained by differences in the two sampling strategies, as in our study samples at discrete depths were taken, as opposed to integrated samples over the water column. Despite continuative investigations on species-specificity by means of qPCR, our study did not give an indication of a single high biomass producer species. *P. delicatissima* was, however, present in the sample with highest abundances, and belongs, along with *P. calliantha*, to the most abundant taxa along the Catalun coast (Quijano-Scheggia et al., 2008b). Both taxa have also been found to be involved in blooms in Alfac Bay between April 2007 and February 2009 (Andree et al., 2011). Yet, qPCR cell counts of *P. delicatissima* were by far lower than those of microscopic enumeration. Hence, either another species formed high biomasses during the study period, or molecular facets of *P. delicatissima* are yet to be included to qPCR assays.

The independence of this genus from the two described regimes may on one hand be due to the tolerance of many *Pseudo-nitzschia* species towards environmental conditions. For several taxa, optimum salinities below 30 and temperatures between 15 and 35 are reported (cf. review by Lelong et al., 2012). In addition, the strong bloom observed in Fangar Bay persisted even after harsh weather conditions and strong wind and wave action on this day. This flexibility is typical for R strategists as described by Reynolds for freshwater dinoflagellates, a concept that has also been adapted for marine diatoms, e.g., *Pseudo-nitzschia* spp. (Alves-de-Souza et al., 2008). Indeed, the appearance of species in proliferations was heterogeneous over depth and time during our study. Despite the species heterogeneity, statistical analysis revealed the association of high *Pseudo-nitzschia* abundances with increasing salinity and decreasing temperatures. Such water characteristics are typical of bottom waters introduced by the outside Mediterranean waters, indicating possible introduction of *Pseudo-nitzschia* from external origin.

Since the inclusion of DA in routine sampling efforts in 2001, the embayments have been declared an ASP-free zone, but recent investigations of shellfish samples from the years 2008 - 2011 have found DA in several harvested mollusc species within production sites along the Catalan coast, including the Ebro embayments, and have even exceeded regulatory levels (Giménez Papiol et al., 2012). Despite the increase and persistence of *Pseudo-nitzschia* spp. blooms above critical cell concentrations in Alfac and Fangar bays, the study of Giménez-Papiol et al. (2012) did not reveal a relationship of *Pseudo-nitzschia* spp. bloom abundances with DA contaminated molluscs. This is confirmed by our study results, even with the direct comparison of cell abundances to toxin content of particles >10 µm from the same water sampling procedure (to our knowledge, the first detection of DA directly in any particulate
material in the region). This finding is an indication that the *Pseudo-nitzschia* taxa which make up a major portion of the high biomass are not responsible for DA production in the area. It is noteworthy that all DA occurrences were found exclusively in Fangar Bay and here these were restricted to the upper meter. Even though none of the taxa which were captured by qPCR assays could be assigned to all these samples, the location within the upper water column may indicate environmental conditions that are either within habitat preferences of the toxin producer, or initiate toxin production. DA production has been reported in response to various environmental factors for different *Pseudo-nitzschia* taxa, as a single component, or in combination, such as silicate and copper for *P. multiseries* in New England (Fuentes & Wikfors, 2013), or for light or nutrients for *P. multistriata* (Bates *et al.*, 1998 in Lelong *et al.*, 2012). With respect to the conditions during the study period, DA production may be related to higher light intensity, as well as higher nitrate availability in the upper layer.

Our study results indicate the presence of not yet identified taxa of the diatom genus *Pseudo-nitzschia*. By means of qPCR, an additional *Pseudo-nitzschia* taxon related to the *P. delicatissima* genotypic group was detected. This species was sufficiently similar to *P. delicatissima* for the primers to match and amplify something similar but it had some sequence differences which alter its melt curve as compared to the control. Furthermore, there were several occasions, in which high species abundances were confirmed by means of microscopy, but none of the qPCR primers matched to the sequences in the corresponding sample (Fig.7.5f). In addition, one of these samples contained DA, which is in most cases attributable to *Pseudo-nitzschia* spp.. Therefore it is more than likely that the full range of genetic diversity for this genus is not yet fully described. In compliance with these findings, *Pseudo-nitzschia* spp. abundances as derived in Alfacs Bay by the same assays, did generally correlate well with genus counts during the regular monitoring programme over a 2 years period from 2007 to 2009, but did not match during a bloom situation in 2008, when abundances nearly reached $6 \times 10^6$ cells L$^{-1}$ (Andree *et al.*, 2011). Continued investigations on *Pseudo-nitzschia* spp. genetic diversity is highly advantageous for the region, particularly with regard to the necessity to identify the toxin producer/s and triggers for the Ebro Delta embayments.

Additional phycotoxins and taxa which were present during both freshwater regimes were SPX1 and *P. reticulatum*. For SPX-1, no clear link to the source organism was evident. The typical culprit species *Alexandrium ostenfeldii* cannot be readily distinguished from other *Alexandrium* spp. by inverted light microscopy without considering thecal plate features in more detailed investigations that were not part of this study. The presence of this species was, however, confirmed by 454 sequencing and phylogenetic placement in samples of one cast in which the toxin was found. Yet, no samples were analysed with 454 sequencing from these depths and therefore the association cannot be confirmed.
Azaspiracids were not detected in any of the Ebro Delta samples during the study period. However, specimens of the dinoflagellate genus *Azadinium*, an identified source of these toxins (Tillmann et al., 2009) were encountered by light microscopy in several samples of both embayments. The genus was also putatively identified with 454 sequencing as it was listed in MG Rast with high percentage identity and high average alignment. However, the phylogenetic placement of the corresponding LSU sequences failed to assign sequences to a *Azadinium*. This is not surprising considering that the number of newly described taxa of this newly erected genus is increasing rapidly (Tillmann et al., 2012) and has without doubt not yet been fully established for the Mediterranean Sea. Species of *Azadinium* recorded from the Mediterranean Sea so far comprise *A. caudatum* (references in Nézan et al., 2012) and the newly described species *A. dexteroporum*, which is also capable of azaspiracid production (Percopo et al., 2013). Light microscopic observation of *Azadinium* sp. detected in Alfacs Bay samples clearly showed the presence of an antapical spine and the presence of multiple pyrenoids visible by a starch sheath (Fig. 7.4a, b). An antapical spine is also characteristic for *A. spinosum*, *A. polongum*, and for the Mediterranean *A. dexteroporum*. However, the specimen depicted in Fig. 7.4 is clearly different from *A. dexteroporum* by its larger size (ca. 16 × 11 µm, compared to *A. dexteroporum* which is <10 µm and <8 µm in length and width, Percopo et al. 2013). The presence of multiple stalked pyrenoids is up to know are only known for *A. poporum* from the North Sea (Tillmann et al., 2011), which do not have a spine. The presence of a spine and multiple pyrenoids in the epicone is thus a unique combination of features different to all other species of *Azadinium* described so far. In any case, culture strains from the area are needed to perform more detailed morphological studies and to confirm the notion of the presence of a new species. Since the discovery of *Azadinium* spp. as producer of this toxin (Tillmann et al., 2009), more and more congeners have been found (Krock et al., 2012). Therefore, the presence of different azaspiracid analouges which were not included in our analysis is possible.

7.5 Final remarks on HAB dynamics and environmental scenarios

During the study period, the fast dynamics of HAB proliferations over vertical and temporal scales were striking. These were apparent for both, species and associated phycotoxins and it is extremely challenging to select and to cover appropriate scales for routine assessment.

Several taxa were present in such low amounts that their mere presence instead of population dynamics was revealed by this study. Especially interesting is the identification of *Azadinium*, a genus that has not yet been recorded for the area, yet may evolve as new HAB genus of relevance for the region. Furthermore, the consideration of molecular diversity was stressed by this study, especially for taxa which are not easily distinguishable by light microscopy, such as *Pseudo-nitzschia*. The biogeographic specificity of such qPCR assays,
while suffering from their inherent exclusion of non-target organisms, can also point towards the need for broadening the range of assays in use as it becomes clear there exist other taxa, e.g., of *Pseudo-nitzschia* as shown in this study and by Andree *et al.* (2011) for the Ebro Delta embayments. The inclusion of molecular tools is also crucial for the identification of the producer of DA in the region. In addition it was demonstrated that 454 pyrosequencing is a highly valuable tool to address molecular diversity of the entire HAB, or phytoplankton community. It is, however, necessary to conduct continuative investigations for the unambiguous identification of taxa, such as phylogenetic placement of LSU sequences by MG Rast or other BLAST based functions (Kilpert *et al.*, submitted). Even though some sequences may not be aligned in such subsequent analyses, as was the case in our study for *Azadinium* sp., 454 sequencing aids to clarify which not yet identified HAB taxa or groups are present in an aquaculture area and eventually need to be addressed by routine monitoring.

Basic limitation of our sampling regime was clearly the restriction of detailed information on the HAB community in response to physico-chemical parameters on a horizontal scale. Whereas the vertical cross-sections of the water body were sufficiently resolved to capture HAB patches at different depths, such detailed information along transects would have been advantageous to determine if for example *D. sacculus* actually formed a layer over horizontal dimension in Alfacs Bay. Moreover, such taxa often form thin layers in the centimetre scale which would not have been resolved even by our sampling method. To capture the entire water column, integrated samples are recommended by the EU and implemented in the Ebro Delta since 2006 (Fernández-Tejedor *et al.*, 2008). Yet, these would reveal merely the presence of harmful taxa, but not their vertical heterogeneity, including cell concentrations above critical limits, as shown in our study for *Dinophysis* spp. in Alfacs Bay. Therefore, results with hose samplers, despite the recommended measure for HAB monitoring, are to be treated with caution. This holds especially true, as these patches of low biomass HAB taxa do not regularly align with Chl *a* maxima in the water column and are therefore not detected by usual profiling fluorescence measurements, even if these are conducted in a high vertical resolution.

Findings of this study revealed similarity in proliferational patterns during the two regimes. Most of the taxa followed a typical seasonal pattern described for the region. As an example, *D. sacculus* and *Karlodinium* spp. were assigned to the winter-spring season with monitoring data from 1990-2003 (Llebot *et al.*, 2011) and also grouped into a spring-early summer physical scenario as *Karlodinium-Dinophysis* spp. bloom period (Artigas *et al.*, 2008). Both projections share seasonal characteristics to assign taxa to physical scenarios. Our study complies generally well with the mere appearance of both genera from the seasonal point of view, but it was demonstrated here that the season could be differentiated into two
regimes of freshwater influences and mixing. It is more than likely that both regimes display sub-variations with respect to additional environmental parameters, such as organic nutrients, or the nutrient-induced presence of prey for mixotrophic taxa such as *Karlodinium* spp.. These in turn may have complied with habitat preferences of taxa, such as that of *D. sacculus*, which was restricted to time windows within regime I. To outline trends or to assign HAB species to functional groups, and to unambiguously relate these to environmental forcing functions, a long-term dataset is be necessary. Due to a limited availability of data over only two seasons, we do not aim to fully address the question on regulating factors of bloom increases, neither a quantification of these factors. Still, such association to species types with their favoured habitat types is worth following for HAB surveillance in the Ebro Delta and should also include other than HAB species to form typical plankton communities and functional groups.
VII SYNOPSIS AND CONCLUSIONS

The presented doctoral thesis embraces efforts to now- and forecast HABs in an interdisciplinary way, with emphasis on aquaculture zones - in particular, the embayments Alfacs and Fangar in the Ebro Delta system, NW Mediterranean. Such efforts are mandatory for the initiation of adequate warning and mitigation actions and to prevent adverse effects caused by high biomass and/or toxin producing HAB taxa. The study sought to identify how adequate spatio-temporal scales for HAB surveillance could be covered by operational bio-optical sensors and sensor systems, in order to define adaptive strategies or habitat scenarios that may aid HAB forecasting in the Ebro Delta embayments. The specific objectives of this thesis were followed up in three major research lines which were allocated to three theme chapters. Advantages and limitations of bio-optical tools for HAB surveillance were reviewed in Theme Chapter I, with emphasis on operational settings. In Theme Chapter II, the inclusion of a regionally adapted bio-optical system for phytoplankton biomass assessment was examined for a case study in the aquaculture zone of the Ebro Delta. Diversity and vertical patchiness of HAB taxa and phycotoxins with respect to environmental scenarios were addressed in a comparative approach in Theme Chapter III. For the Ebro Delta case study, emphasis was set on the semi-enclosed nature and regional conditions of the two embayments, such as regimes of freshwater inflow due to rice agriculture. In the following, consequences that arise for HAB surveillance, in particular for the Ebro Delta aquaculture sites, are considered in from a synoptic perspective in the context of results of this study.

Alfacs and Fangar bays were identified as optically complex, and especially CDOM dominance challenged the retrieval of Chl $a$ by means of the radiometric sensor system. Algorithms that target the red spectral region and therewith omit the strongest impact of CDOM absorption did, however, fail for algal biomass calculation due to the overall moderate signal of average Chl $a$ concentrations, e.g., below 3 µg L$^{-1}$. Disturbing influences on the biomass proxy signal were effectively solved by regional parameterisation of an analytic OC algorithm. Therewith, Chl $a$ was successfully calculated from remote sensing data and thus allowed to follow algal biomass dynamics over enhanced temporal scales throughout the study time (Theme Chapter II). Major above-water disturbances of the remote sensing signal were boat traffic or anchorage below the sensor FOV, due to fishing activities and aquaculture maintenance. Furthermore, strong surface reflection of the sun impaired the remote sensing signal. A rapid pre-processing procedure was designed to eliminate such non-valid data and to correct the remaining spectra from sun glint (Theme Chapter II).
described adaptations allow an automated processing of large datasets from a long-term data record, and the inclusion to an operational setup for HAB surveillance. Therefore, the radiometric sensor system and described processing techniques serve as proof-of-principle for the automated record of phytoplankton biomass dynamics from sensors with similar spectral ranges, including observations from space. Limitations for the application of the parameterised OC algorithm arise by seasonal changes, e.g. bottom visibility in winter or changes in bio-optical characteristics that would demand a seasonal adaptation of the algorithm.

Phytoplankton dynamics in both embayments were characterised by fast deviations in time and space and both, algal biomass proxies and HAB cell abundances often profoundly changed within one week (Theme Chapter III). The observed patchiness of HAB taxa was expected given with respect to the semi-enclosed nature of the embayments. Weekly assessment can therefore not fully resolve key patterns of bloom development, such as bloom initiation or decline. As an example the increase of algal biomass in June 2010 could not be timed by conventional sampling in Alfacs Bay, while the sampling frequency was sufficiently enhanced by the continuous (excluding dark hours) bio-optical sensor record to define the date (Theme Chapter II). Consequently, such automated records allow the timely initiation of actions to determine whether the biomass increase is due to a potentially harmful species (which was not the case in June 2010, compare Theme Chapter III). In addition the accurate determinate of timing allows to reveal possible drivers, such as the underlying physical environmental conditions.

In consideration of the spatial coverage for HAB surveillance, the inclusion of the bio-optical system to an operational setup is essential, as phytoplankton dynamics were not effectively covered by the radiometric test unit (Theme Chapter II, III). Major limitations the sensor system suffered were first, the coverage of a constricted portion of the surface water by the sensor FOV, while defined patches of increased algal biomass and HAB taxa, in particular Karlodinium spp., were heterogeneously distributed over horizontal scales of kilometres as shown in this study (Theme Chapter III). Second, remote sensing reflectance measurements are per se restricted to the upper layer of the water column, with the given low water transparency even in such shallow embayments as Alfacs and Fangar bays. This upper layer in both bays was defined by low Chl a concentrations, especially during periods of strong stratification, and moreover, also accumulations of HAB taxa did not necessarily extend to this area (Theme Chapter III). Accordingly, these gaps must be closed for an adequate coverage of phytoplankton dynamics, e.g., by multiplication of measurements at strategically relevant locations.
locations of the embayments, and/or by the application of mobile platforms in an environmental observatory. These locations are defined by the semi-enclosed nature and characteristics of both bays and include the bay’s entrance, where HABs jeopardize aquaculture activities via introduction from the surrounding seas, sectors of increased retention time of water, or the vicinity to discharge channels (Theme Chapter II). In the latter area, proliferations of Karlodinium spp. started to reach high abundances in 2010 (Theme Chapter III). In such proposed observation networks, bio-optical sensor systems would therefore not only cover enhanced spatio-temporal dimensions to follow algal biomass dynamics and proliferation patterns, but would also provide long-term synoptic data for now- fore- and hindcasting of bloom events and thereby provide data for dynamic models.

The primary target for the sensor system in Alfacs Bay, was the ubiquitous algal pigment Chl a, which serves as proxy for bloom presence and movement (Theme Chapter I, II). Efforts to detect and track HABs by means of high biomasses and anomalies are useful if regional HAB taxa form high abundances and tend to dominate the algal community during in bloom events (Theme Chapter I). This was the case for one bloom phase of Pseudo-nitzschia as well as for Chattonella during the study period (Theme Chapter III). Karlodinium spp. were not found to dominate algal biomass, but were dominant in prior bloom situations. On the contrary, Chl a-based approaches are not applicable for the detection of other core HAB species of this region that are responsible for closures of the aquaculture sites, namely Dinophysis spp., Alexandrium spp., Prorocentrum lima and Protoceratium reticulatum. These taxa do usually not occur in high magnitudes or to form mono-specific blooms in the area. The case of D. sacculus, which occurred in cell abundances above the critical limit of 500 cells L\(^{-1}\) at the key station in Alfacs Bay (Theme Chapter III) is an example for this limitation of the sensor system, that did not deflect high magnitudes of Chl a at the same time (Theme Chapter II). In conclusion, only a restricted group of bloom forming taxa can be addressed by biomass indicators in the Ebro Delta whereas other groups demand additional measures.

In this thesis the integration of automated bio-optical sensors and sensor systems into HAB assessment was shown to be of high value to address the relevant spatio-temporal scales. For the interpretation of bio-optical data, however, not only detailed knowledge on regional bio-optical characteristics, but also on HAB dynamics are of high importance (Theme Chapter I, II, III). As an example, the practicality of Chl a for the detection of harmful Pseudo-nitzschia spp. accumulations was not supported by results of this study, even though...
this genus fulfils general requirements in being a high biomass bloomer which dominates the algal community during algal blooms. Yet, while critical cell abundances (>200,000 cells L\(^{-1}\)) of this genus were frequently exceeded during the study period, the associated phycotoxin DA did not coincide with highest biomass accumulations. During the study period, *Pseudo-nitzschia* spp. blooms consisted of multiple potentially toxigenic species, but a clear culprit could not be associated with the specific toxin based on results of this study (*Theme Chapter III*). If toxic *Pseudo-nitzschia* sp. make up only a small proportion of the genus abundances, biomass of the genus *per se* would not simultaneously indicate a harmful bloom. Prior to a final conclusion, however, the definition of the culprit taxa and triggers of toxin production and their relation to the overall phytoplankton or genus contribution need to be clearly defined. From a synoptic perspective, the case of *Pseudo-nitzschia* spp. underscores the importance of integrative measures, i.e. combined efforts of large scale areal coverage and HAB species-specific information by microscopic counts, molecular diversity, and the assessment of phycotoxins. A similar situation may arise for *K. veneficum*, of which increased abundances were detected in Fangar Bay for the first time during the study period (*Theme Chapter III*). This is highly unlikely, as mass occurrences of *K. armiger* and *K. veneficum* were responsible for multiple fish kills in Alfacs Bay (Garcés *et al.*, 2006), however, toxicity in samples from the outbreak in Fangar Bay could not yet be confirmed by means of LC-MS/MS in this study. This may be due to simple non-toxicity of the encountered strains, or as well by alterations of the toxin structure in Mediterranean *K. veneficum* strains, but this issue has not yet been elucidated (Place *et al.*, 2012).

One of the most noteworthy issues to consider with respect to bio-optical HAB surveillance in the Ebro Delta embayments is the presence of taxa that contain the pigment gyroxanthin-diester which is only present in few algal species (*Theme Chapter I*, compare Table 2.3). Besides *Karlodinium* spp., only *Karenia* spp. and *Prymnesium parvum* were analysed during the study period (*Theme Chapter III*). In addition, only species of the dinoflagellate *Takayama* and the colony forming, foam generating haptophyte *Phaeocystis* have been identified from the species listed, only the latter has occasionally reached higher biomasses which are usually accompanied by the generation of organic foam (M. Fernandéz-Tejedor, pers. comm.). Therefore, the use of gyroxanthin-diester as regional marker for *K. armiger* and *K. veneficum* in Alfacs and Fangar bay is most likely. Bio-optical techniques rely on distinct absorption characteristics of the pigment suite and have been successfully applied with directly retrieved absorption, as well as with inverted hyperspectral remote sensing spectra for *K. brevis* in Florida (*Theme Chapter I*). The inversion of hyperspectral remote sensing data to phytoplankton absorption spectra in the Ebro Delta embayments by means of the QAA (see Annex Fig. 3) or other algorithms that deconvolute
the remote sensing signal to said IOPs therefore holds the potential to discriminate high cell densities of *Karlodinium* spp. in the Ebro Delta embayments.

During the study period, multiple HAB taxa were determined in Alfac and Fangar bays, of which most species are regionally known to be causative for closures of the aquaculture sites. Yet, a putative new toxigenic species of *Azadinium* was also detected by means of light microscopy as described in *Theme Chapter III*. The use of alternative approaches for HAB detection, e.g., the screening of the overall phytoplankton community by means of 454 pyrosequencing and the subsequent confirmation of HAB taxa with phylogenetic placement proved to be highly useful for the discrimination of species that were not distinguished by light microscopy, such as *A. ostenfeldii* or *A. tamarense*, or present in only low amounts. In addition, also the case of *Pseudo-nitzschia* demonstrates the need to include molecular tools for describing cryptic diversity of species assemblages, in this case revealed by means of qPCR.

But which factors trigger the observed patchiness and which factors control the population dynamics of the diverse HAB taxa in the Ebro Delta embayments? A definite assignment to environmental forcing functions of HAB development require long-term datasets and were therefore not within the scope of this thesis. Nevertheless, a clear trend which was observed during our study period was the separation of a general seasonal bloom period with typical taxa into two different freshwater influenced stratification regimes that apparently have complied with habitat preferences of taxa. As an example, critical cell abundances of *Dinophysis* spp. (> 500 cells L\(^{-1}\)) were observed in the regime with high stratification, while *Karlodinium* spp. abundances increased under less defined stratification conditions (*Theme Chapter III*). These findings may link to the different species- and habitat types described by Smayda & Reynolds (2001, see Fig. iii.2). In this scenario, *Karlodinium* spp. would be assigned to species type I- equivalent to the situation found in our study with a respectively low level of stratification, and *Dinophysis* spp. assigned to species type VII, usually found in more stratified areas. Such changes in habitat type in Alfac and Fangar bays, as indicated by the two freshwater inflow regimes may be a step towards description of habitat preferences and thereby predictive models for HAB forecasting activities.

The findings of this thesis lay the foundation for the successful coverage of appropriate spatio-temporal scales for selected HAB taxa by means of a bio-optical sensor system in the Ebro Delta. Of these, especially *Karlodinium* spp. belong the major target group of such sensor systems, as the extraordinary pigment suite of this species may allow a delineation of the genus from the bulk optical signal in this region, which may even be applicable with remote sensing reflectance measurements, given that these are in hyperspectral resolution. Regarding forecasting of HABs by making use of adaptive strategies or habitat preferences,
findings indicate differences for different HAB types, yet long-term data records are necessary for statistically sound conclusions. The inclusion of integrated bio-optical and conventional sampling for HAB surveillance allows to effectively monitor the presence and movement of HABs and to gain insights on situations/environmental scenarios that make their appearance likely. This in turn holds the opportunity to optimise and direct public and private responses to the harmful event and to reduce undesired impacts.
VIII FUTURE PERSPECTIVES

The study has provided insights on the inclusion of bio-optical tools and phytoplankton dynamics to HAB now- and forecasting, in particular to support surveillance in the aquaculture zone of the Ebro Delta embayments. These findings benefit a spatio-temporally enhanced survey to assure food safety issues that arise due to harmful algal taxa and phycotoxins, but also show up needs for future key activities and research strategies.

SETUP OF AN ENVIRONMENTAL OBSERVATORY. One major prospect derived from this study is the establishment of an environmental observation system in the Ebro Delta embayments. Hyperspectral radiometric sensor systems are capable to serve as key elements in such a setup. As direct consequence of these findings, a number of demands were encountered which need to be considered for the extension of a radiometric- to an observation system. These arise because of the semi-enclosed nature and corresponding bio-optical complexity and patchiness of HABs in the bays, as well as their ecosystem services, as aquaculture and recreational area.

In respect to spatio-temporal coverage, key demands on an environmental observatory for HAB surveillance raised in this thesis are: 1) the necessity to provide fully continuous measurements which accord to highly dynamic temporal dimensions of phytoplankton; and 2) the extension to horizontal and vertical m that correspond to proliferation behaviour and movement of HABs. Accordingly, future research should concentrate on the following aspects. First, the selection of sensor elements and platforms needs to be addressed. Second, strategic locations for bio-optical network elements, such as the previously cited areas of increased water retention times need to be evaluated. In consideration of the uptake of harmful phytoplankton and accumulation of potential phycotoxins by mussels, the placement of sensors directly within and outside of the mussel culture transects should be considered. Furthermore, to gain knowledge on phytoplankton dynamics and origin, also a lengthwise cross-section in between the two strategic zones of the entry of the bays to the Mediterranean and the inner part of the bays would be beneficial. These measures would not only enable now-casting activities, but also enhance knowledge on the origin and their triggering forcing functions, which provide input data to ecosystem models. Last, technical and budgetary limitations for long-term sensor setups must be taken into account. Investment, SWaP factors, maintenance and prevention of bio-fouling imply a trade-off between optimal spatio-temporal coverage for HAB surveillance and actual feasibility.

Sensors on aerial platforms, such as satellites, aircrafts or unmanned vehicles form another component that would strongly support the described elements of a future environmental observatory. Albeit the reduced temporal resolution of overflight times does not comply to the high temporal and spatial HAB dynamics in the Ebro Delta bays, yet both
approaches complement a continuous monitoring system in a valuable way. Such observations allow tracking the transport of large water masses or algal surface patches from the outside southward current towards the entrance of the embayments. Here, such massive inoculates may develop into a HAB of large dimensions due to the semi-enclosed nature of the embayments.

Long-term environmental data provide a climatology of typical seasonal situations and can be used to detect algal biomass anomalies on a large scale. Also, such seasonal patterns may mark environmental scenarios to which typical HAB types are associated. Future work should concentrate on two aspects: First, the calculation of relevant bio-optical parameters from sensor data, and second, the determination of typical seasonal parameters. Seasonal ground data on IOPs are of high value for validation of sensor data and also for models that rely on initial input parameters of specific IOps. The assessment of typical specific IOps for different seasons is therefore an important future task.

INCLUSION OF THE GENERAL PUBLIC. One additional method which is becoming more and more prominent by the increasing availability of mobile networks and smartphones is the inclusion of the general public in conducting environmental observations. These crowdsourcing measurements are generally compromised by lower quality but they still have a high value due to the multiple input of interested citizens over increased spatio-temporal scales. By this means, environmental awareness towards the HAB topic is also raised. These even influence policy levels if indications for anthropogenic induction of HABs are uncovered. Future efforts should be directed towards measurements that the public can supply to support HAB surveillance and on the recruitment and training of the public to conduct measurements.

APPROACHING SPECIES-SPECIFICITY WITH BIO-OPTICAL COMPONENTS. As target for the detection of HAB taxa by means of bio-optical instrumentation, the ichthyotoxic dinoflagellates *K. veneficum* and *K. armiger* deserve particular attention. Future studies should concentrate on whether or not the pigment gyroxanthin-diester can reliably serve as species-specific marker for *Karlodinium* blooms in the Ebro Delta embayments, as indicated in this study. Subsequently, absorption characteristics for the distinction of *Karlodinium* spp. need to be addressed by laboratory studies, such as the filter pad method (as described in *Theme Chapter II*) or enhanced absorption signals, e.g., with the PSICAM or liquid capillary waveguides. Eventually, such methods should be tested and validated in the field with enhanced datasets. There are inherent limitations on species-specificity of bio-optical sensors and on the detection of harmful taxa that do not usually dominate the algal community nor produce high biomasses. Some examples are described in *Theme Chapter I* and comprise
optical principles such as operational flow cytometry combined with imaging that is applicable for algae with typical morphological features in the Ebro Delta, such as *Dinophysis*.

**DETERMINATION OF TAXA AND PHYCOTOXINS.** Findings of this thesis strongly underscored the necessity to back up bio-optical approaches with information on HAB taxa and phycotoxins. The determination of both taxa and toxins is therefore crucial to initiate adequate warning measures. As relevant to results of this study, core areas of future research should address primarily toxicity and molecular diversity of three genera: the dinoflagellates *Karlodinium* and *Azadinium*, and the diatom *Pseudo-nitzschia* spp.. With respect to Mediterranean strains of *Karlodinium veneficum*, routine measurement of karlotoxins needs to be established, allowing the detection of this toxin in field samples. This implies determining the chemical structures of the toxins and the subsequent establishment of a robust LC-MS/MS method. Regarding *Azadinium*, there is not much known on the molecular diversity of this species in the Mediterranean, and nothing at all for the Ebro Delta embayments. Here, the identification of *Azadinium* or and the determination of the toxicity, and thereby the inclusion with HAB taxa for the region, needs to be clarified. To unambiguously assign toxins to taxa, however, isolation and culture of the respective species is crucial. This holds also true for the identification of the DA producer amongst several potentially toxic *Pseudo-nitzschia* species. From an operational point of view, there are examples of *in situ* ‘ecogenomic sensors’ which base on molecular diversity signatures (*Theme Chapter I*). In the Ebro Delta, these would be especially advantageous to track *Pseudo-nitzschia* species along depth profiles or transects, yet with focus on research. Especially with respect to quality control aspects in aquaculture, all measurements for molecular diversity or toxicity of plankton samples need to comply with official regulation and need to be developed to a point that allows certification.

The use of 454 pyrosequencing for metagenomic studies has provided important insights on species diversity. Even though certain taxa could not be unambiguously identified by this method and subsequent phylogenetic placement, this method is of high value to show up future areas of research for putative new HAB taxa and their genetic diversity.

**KNOWLEDGE ON POPULATION DYNAMICS AND HABITAT TYPES.** Certainly, the alignment of HAB taxa to species types and assign these to a habitat and life strategy complex (compare Fig. iii.2) deserves more attention for the Ebro Delta embayments. The here presented thesis only scratched the surface of this approach and the potential should be further exhausted. The question, if the alignment of Ebro Delta HAB- and non-HAB taxa into the C-S-R grid is possible is a start on this comprehensive topic. If so, this may explain why one species dominates over another and which triggering forcing functions were responsible for a shift in species assemblages. This knowledge can then be transferred to predictive models of HABs.
In addition, species assemblages may retain optical key indices that are detectable with bio-optical means.

Such models provide the opportunity to not only now-cast HABs, but also to determine why and when proliferation occurs. This in turn would allow reducing negative impacts on aquaculture and to initiate mitigation efforts that prevent harm by the optimisation of public and private response. In further, causes of HABs may be identified, and this would aid to control or even prevent certain HAB types in an area. In this sense, these future steps may advance towards preventive measures rather than mitigation actions to deal with this environmental hazard.
ACKNOWLEDGMENTS

This PhD mission has accompanied me through employments in projects from bio-optics to citizen science at the University of Applied Sciences, IMARE/IMARE GmbH, AWI, and the ICBM at the Carl von Ossietzky University in Oldenburg. In addition, intensive field work at the IRTA in Sant Carles de la Ràpita in Spain formed an important part of my thesis.

The work was conducted within the framework of the SCOR/IOC GEOHAB Core Research Project on HABs in Fjords and Coastal Embayments, yet it was an independent project and all phases of my thesis would not have been possible without partial funding, provision of working environment, and flexibility of multiple institutions and, in particular, the people therein. I am very thankful to these people and I highly acknowledge support from, in temporal order, the IMARE/IMARE GmbH and the European Regional Development Fund (ERDF) funding the IMARE, AWI, IRTA, TriOS and the ICBM. Especially the travel grant for the field phase in Spain from my Helmholtz graduate school POLMAR is highly appreciated.

For a well-balanced supervision between scientific support & guidance when needed and inspiring trust and confidence to let me grow I sincerely thank my supervisors Allan Cembella and Oliver Zielinski. Your collective supervision has certainly helped to keep me on track and I think it speaks for itself if I say that I will enjoy our future projects. This work did not end with the submission of this document.

Much obliged is also the effort and support from my Doc Team composed of my two supervisors, Marcel Wernand (NIOZ), Astrid Bracher (AWI) and Jorge Diogène (IRTA) who regularly discussed progress of my work and also helped to focus to essential aspects. Extra credit goes to Marcel for regularly coming all the way from Texel to Bremerhaven for these events. I also thank the POLMAR team for framing this PhD with scientific courses, social activities and good advice whenever it was dearly needed.

There are many people in my working groups that I thank for their scientific support, advice and practical help. From the spatial perspective, I start with the German connection in which two groups formed the topical pillars of my thesis work: The Marine Sensors team oscillating around Oliver and the Ecological Chemistry group at AWI. People in both groups have kindly adopted me into a great and supportive working environment and I thank present and former members for the good time, pleasant atmosphere, and recently also chanterelles and bay boletes. Especially acknowledged is the support from Daniela & Rohan for help in the sensor system setup before and during the field work, Moni, Marta, Daniela² & Imke for CDOM measurements, Moni for the self-made nutrient-tube holder, Björn, & Hauke for first
aid in programming, Jan for statistics and two already mentioned untiring drivers to Spain and back. Also unforgotten are voluntary movers who transferred my office five times during the course of the PhD. At the AWI I am particularly indebted to Bernd for sharing Priscilla and time, Urban for patience in the microscopy room (and also for the schematic algal cells in Fig.iii.1), Uwe for 454 sequencing, Anne & Wolfgang for unbeatable support in lab- and logistics, Justus & Tatyana for nutrient analysis, the MARMICs Greta & Beatriz, and finally the AWI Phytooptics group, in particular Astrid, Bettina and Sonja for help in lab work and two valuable excel sheets.

Field work in the Ebro Delta formed an essential part of my PhD project and I am very thankful when thinking back to the inspiring and beautiful time I had with the Spanish connection. Moltes gràcies to IRTA staff, in particular my guest working group and flatmates in Sant Carles de la Ràpita that made me feel being at home during my stay. Special thanks go here to Nuria for her hospitality in Amposta. I thank Marga and Jorge for the cooperation, scientific support and infrastructure, Karl for qPCR, my favourite captains Josep Ma. & Xavi for unfailing navigation and help with sampling, numerous colleagues and students who accompanied me on the boat, Raphael and his super-flexible taller people for technical support and solutions before problems arose. Gràcies also to Pepe who trustingly left me his aquaculture caleta #84 in Alfacs Bay for the installation of my sensor system and even sponsored a complete door for the shed on the raft.

Furthermore, I want to express my gratitude to numerous external scientists who work in the Delta and here I especially thank Elisa, Mireia, and Norma from ICM, and Jaume, Elena, and Oliver from CSIC for sharing boats, equipment, thoughts and tapas. Further credit goes to the Ebro Delta for providing scenic views, flamingos, and a source for a nutritional basis with tasty shrimps, mussels, fish, and sea-salt that sweetened my life in Sant Carles de la Ràpita.

For partial proof-reading of the document I thank Bernd, Anna & Silke, and for Abstract translation Erika & Jaume.

This leaves my family and friends, and I am most thankful to my parents, my sister and my dearest grandaunt Erna for never-ending trust and support throughout my career. With all my heart, I thank Thomas for keeping faith and love, and for being responsible that these PhD years can be added to the best of my life.

The mission is accomplished, and I am both at the same time: glad to finish and have this document printed, and keen to continue with old and new, colleagues and friends.
REFERENCES


REFERENCES
References


REFERENCES


196
REFERENCES


Kilpert F, Meyer JM, Töbe K, Gottschling M, Tillmann U, Cembella AD & John U (submitted to *Environmental microbiology*) Molecular diversity of dinoflagellates from the North Sea: Detecting new clades of Amphidomataceae by OTU clustering and phylogenetic placement of LSU rDNA sequences.


REFERENCES


Annex 1 Fig.1. Original figure & caption for Supplementary Fig. 1 in Bargu et al., 2012. Reconstruction of Santa Cruz Sentinel front page from August 51 18, 1961. The text was reset to make it readable and the images reprinted from original 52 negatives (Photos from Covello and Covello Photography with permission).
Annex 2. Original figures & captions for Fig. 5 Smayda & Reynolds, 2001. A) Schematic matrix of pelagic marine habitats along an onshore–offshore gradient separating deep-mixed and well-stratified, but nutrient-deficient systems. $I^*$ refers to irradiance level received by cells within water column; $H_m$ represents depth of mixed-layer. Overlap of types within the habitat template schema does not always imply their contiguity. The diagonal approximates the main successional sequence depicted in Margalef et al. (Margalef et al., 1979). b. Predominant dinoflagellate life-form Types (from Figure 4) associated with the turbulence-nutrient matrix (from Figure 5a) along an onshore–offshore continuum characterising pelagic habitats. Type I = gymnodinioids; Type II = peridinioids and prorocentroids; Type III = ceratians; Type IV = frontal zone species; Type V = upwelling relaxation taxa; Type VI = coastal current entrained taxa; Type VII = dinophysoids; Type VIII = tropical oceanic flora; Type IV = tropical shade flora. Consult text for Type species.
Annex Fig. 3. Pigment absorption at selected wavelengths as derived by the quantitative filter pad technique (QFT) from 11 Alfacs Bay surface samples in 2010 is set against corresponding pigment absorption derived from remote sensing spectra by a) the Quasi-Analytical-Algorithm (QAA) by Lee et al., 2001, and b) by the spectral optimisation approach (Opt) by Lee et al., 2010. The data points with zero absorption in b) origin from two of the eleven spectra where the approach failed to invert $R_s$ spectra.
Annex Fig. 4. Species richness for 454 pyrosequencing, analysed in MG Rast (Meyer et al., 2008), is shown in rarefaction plots for the three samples taken at the key station in Fangar Bay: a) from surface sample and b) at 3.5 m depth on 22 June 2010, and c) surface sample on 1 July 2010. The flattened curves to the right demonstrate that a sufficient number of reads have been taken, as only few additional species would be obtained by increasing number of reads.
Annex Table 1. Mass spectrometric parameters used for the multi-phycotoxin method (adapted from Krock et al., 2008).

<table>
<thead>
<tr>
<th>Period I (0 – 8.75 min)</th>
<th>Period II (8.75 – 11.2 min)</th>
<th>Period 3 (11.2 – 18.0 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curtain gas [psi]</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Ion-spray voltage [V]</td>
<td>5500</td>
<td>5500</td>
</tr>
<tr>
<td>Temperature [°C]</td>
<td>275</td>
<td>Ambient</td>
</tr>
<tr>
<td>Nebulizer gas [psi]</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Auxiliary gas [psi]</td>
<td>50</td>
<td>Off</td>
</tr>
<tr>
<td>Interface heater</td>
<td>On</td>
<td>On</td>
</tr>
<tr>
<td>Declustering potential [V]</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Entrance potential [V]</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Exit potential [V]</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>MRM transitions</td>
<td>312 → 161 (39)</td>
<td>508 → 490 (40)</td>
</tr>
</tbody>
</table>

Annex Table 2. Mass transition and retention times of toxins for multi-phycotoxin method (adapted from Krock et al., 2008).

<table>
<thead>
<tr>
<th>Toxin*</th>
<th>Mass transition [m z⁻¹]</th>
<th>Retention time [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domoic acid (DA)</td>
<td>312 → 266; 312 → 161</td>
<td>7.58</td>
</tr>
<tr>
<td>Gymnodimine (GYM)</td>
<td>508 → 490</td>
<td>9.83</td>
</tr>
<tr>
<td>Spiroside A</td>
<td>692 → 150</td>
<td>10.42</td>
</tr>
<tr>
<td>Spiroside B</td>
<td>694 → 150</td>
<td>10.72</td>
</tr>
<tr>
<td>13-Desmethylspirolide C (SPX-1)</td>
<td>692 → 164</td>
<td>12.07</td>
</tr>
<tr>
<td>Spirolide G</td>
<td>692 → 164</td>
<td>11.85</td>
</tr>
<tr>
<td>13-Desmethylspirolide D</td>
<td>694 → 164</td>
<td>11.85</td>
</tr>
<tr>
<td>Spirolide C</td>
<td>706 → 164</td>
<td>11.93</td>
</tr>
<tr>
<td>20-Methylspirolide G</td>
<td>706 → 164</td>
<td>12.12</td>
</tr>
<tr>
<td>Okadaic acid (OA)</td>
<td>822 → 223</td>
<td>12.30</td>
</tr>
<tr>
<td>DTX-1</td>
<td>836 → 237</td>
<td>12.30</td>
</tr>
<tr>
<td>DTX-2</td>
<td>822 → 223</td>
<td>12.33</td>
</tr>
<tr>
<td>PTX-1</td>
<td>892 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-2</td>
<td>876 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-2sa</td>
<td>894 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-4</td>
<td>892 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-8</td>
<td>892 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-11</td>
<td>892 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-12</td>
<td>874 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-13</td>
<td>892 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>PTX-14</td>
<td>874 → 213</td>
<td>12.40</td>
</tr>
<tr>
<td>AZA-1</td>
<td>842 → 824</td>
<td>13.88</td>
</tr>
<tr>
<td>YTX</td>
<td>1160 → 965</td>
<td>12.45</td>
</tr>
</tbody>
</table>

*DTX= dinophysistoxin, OA-d8 = okadaic acid C8 diol ester, PTX =pectenotoxin, sa = seco acid, AZA = azaspiracid, YTX = yessotoxin