Seasonal variation of *Emiliania huxleyi* morphology at an ecosystem monitoring site off the east coast of Scotland

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Coccolithophorids are an important component of the phytoplankton community. This calcite-forming group contributes (up to 10%) of marine primary production and plays a key role in global biogeochemical cycles such as the pelagic calcium-carbonate flux. The increase of atmospheric carbon dioxide from anthropogenic activities and the subsequent uptake by the ocean is causing an alteration to the carbonate chemistry, resulting in a decrease of seawater pH (Ocean acidification – OA). Laboratory experiments investigating the impacts of OA on coccolithophores have had mixed results. That aside, laboratory studies are difficult to interpret in the context of Scottish waters as very little is known about the diversity or seasonality of coccolithophores in this region.

Marine Scotland Science operates a coastal ecosystem monitoring programme 5 km offshore from Stonehaven in the north east of Scotland (56º 57.8´ N, 02 º 06.2´ W). Since 1997, samples to record temperature, salinity, nutrients, phytoplankton and zooplankton have been collected weekly, weather permitting. Additional water samples have been collected since 2009 for the determination of the carbonate chemistry parameters; Total Alkalinity (TA) and Dissolved Inorganic Carbon (DIC), from which the pH of seawater at Stonehaven can be determined. From 2010 monthly water samples were collected to investigate the diversity of coccolithophores using scanning electron microscopy (SEM).

Preliminary results reveal that *Emiliania huxleyi* is the dominant coccolithophore at this site and is observed throughout the year. Other species/genera identified include *Syracosphaera corolla*, *Coronosphaera mediterranea*, *Coccolithus pelagicus Braarudii spp.*, and *Helicosphaera carteri* HOL perforate. Two morphotypes of *E. huxleyi* have been identified using morphological criteria, *E. huxleyi* type A and *E. huxleyi* type B. Overcalcified forms of *E. huxleyi* Type A have also been observed, mostly during autumn and early winter. Understanding the seasonal control of *Emiliania huxleyi* calcification will contribute to a better understanding of the biological impact of OA on coccolithophores in Scottish waters.

High performance liquid chromatography (HPLC) and fluorometric analyses of phytoplankton pigments at a Scottish coastal ecosystem monitoring site

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Phytoplankton play a critical role in the food web, harvesting light energy from the sun and passing it up to higher trophic levels. Marine Scotland Science (MSS) operate a number of ecosystem monitoring sites around the Scottish coast. A combination of temperature, salinity, nutrients and plankton are sampled weekly in order to collect base line data to understand functioning of the marine ecosystem, provide a description of seasonal and inter-annual changes and fulfill ministerial commitments to international monitoring as required by the European Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

Measurement of chlorophyll *a* is recognised as an estimate of phytoplankton biomass. Threshold concentrations of chlorophyll *a* have been set by the MSFD and the WFD in order to assess the 'status' of the ecosystem. Fluorometric analysis of chlorophyll *a* is considered a standard method despite being prone to interference from other pigments such as chlorophylls *b* and *c*. The only way to accurately quantify chlorophyll *a* in the presence of other pigments and degradation products is to use a separation technique such as HPLC. In addition to estimating phytoplankton biomass, HPLC analysis can also be used to give an indication of the phytoplankton community composition.

Both methods of pigment analysis have been in operation at the Stonehaven (56º 57.80N, 02º 06.20W) coastal ecosystem monitoring site from 2009. HPLC analysis reveals that fucoxanthin and chlorophyll c_2 are more abundant than chlorophyll *a*. The profile of pigments such as fucoxanthin and peridinin follow the seasonal pattern of diatoms and dinoflagellates. A good relationship between the presence of the pigment gyroxanthin-diester and the harmful dinoflagellate *Karenia mikimotoi* has also been observed suggesting that this pigment can be used as a diagnostic marker for this species in Scottish waters.

A comparison of chlorophyll *a* measurements using both methods shows that, at this site, fluorometric analysis can overestimate chlorophyll *a* compared to HPLC analysis, particularly when chlorophyll c_2 concentrations are high. During this study fluorometric chlorophyll *a* values ranged 50 - 320% of the HPLC result. These data suggest that the chlorophyll *a* determined using the fluorometric method is not true chlorophyll *a* but, at certain times, can be a combination of different chlorophyll pigments. This has implications for the methodology used to monitor pigments to meet the requirements of the MSFD and WFD. The methodology used to calibrate automated sensors such as moorings, gliders and ferry boxes also requires consideration to ensure that the calibration is appropriate.

High Frequency Monitoring of Carbonate Chemistry Parameters (Total Alkalinity and Dissolved Inorganic Carbon) at a Scottish Coastal Monitoring Site to understand seasonal and inter-annual variation.

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Ocean acidification (OA) is the decrease in the pH of the earth's oceans as a result of uptake of anthropogenic carbon dioxide (CO_2) from the atmosphere[1]. It has been reported that a third of the anthropogenic CO_2 (from activities such as fossil fuel burning) produced over the past 200 years has been absorbed by the oceans, resulting in a decrease in pH of 0.1 units[2]. By 2100 the pH is predicted to decrease by 0.4 units. Although the input of CO_2 from the atmosphere has only small spatial variation, some marine regions will be more rapidly affected; the susceptibility of water chemistry to change is dependent on the chemical composition and temperature of the water. It is therefore important to establish these natural variations by routine monitoring before changes due to anthropogenic inputs can be assessed. Both the OSPAR/ International Council for the Exploration of Seas (ICES) Study Group on Ocean Acidification (SGOA) and the Global Ocean Acidification Observing Network (GOA-ON) have identified gaps in monitoring of coastal and inshore waters in particular.

Marine Scotland Science (MSS) operate an ecosystem monitoring programme which includes a site on the east coast of Scotland (Stonehaven; 56º 57.80N, 02º 06.20W), with temperature, salinity, nutrient concentrations and plankton abundance being measured on a weekly basis. Baseline data from this site will aid further understanding of the functioning of the marine ecosystem, provide a description of seasonal and inter-annual changes and fulfill ministerial commitments to international monitoring as required by OSPAR, the European Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD). Since 2009 additional discrete water samples have been collected from two depths (1 and 45 m) at the Stonehaven site for the analysis of the carbonate parameters, Total Alkalinity (TA) and Dissolved Inorganic Carbon (DIC) with the aim of providing a baseline for a coastal region in Scottish waters.

An initial assessment of the entire TA/DIC data collected at Stonehaven (2009-2013) has been made. As a consequence of nitrate uptake by phytoplankton cells during the phytoplankton growing season TA concentrations will increase. DIC concentrations would also be expected to follow an annual seasonal cycle, with concentrations decreasing as DIC is used in primary production. As expected a strong TA annual seasonal cycle was observed at Stonehaven with maximum concentrations being between May and July. In contrast, DIC concentrations increased over the winter months to a maximum in March before decreasing to a minimum around July. The winter DIC maximum could potentially be attributed to calcite dissolution in the area. This seasonal pattern in TA and DIC concentrations observed until winter 2011/2012 when the cycle broke down. The seasonal cycle was re-established in 2013.

SGOA and GOA-ON have both identified the need for a commitment to long-term monitoring at sites in coastal and inshore waters to distinguish long-term anthropogenic signals from short-term spatial and temporal variability. The long-term monitoring at Stonehaven highlights this, with the seasonal cycle in TA and DIC breaking down at the site during 2012. To understand the changes that occurred during 2012 it is clear there is a need for integrated monitoring, which includes measurement physical, chemical and biological parameters such as phytoplankton and temperature.

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Can marine renewable installations provide a new niche for priority seabed habitats?

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How marine renewable structures impact the marine environment, both hydrologically and ecologically, is still relatively unknown as installation and development in this sector is still in its infancy. Horse mussels (*Modiolus modiolus*, Linnaeus, 1758) and the calcareous algae Maerl are classified as habitat engineers as they provide refuge for other organisms and have a disproportionate impact on biodiversity relative to abundance. These biogenic reef habitats are recognised as OSPAR priority habitats and included in the list of 'priority marine features' in Scotland, used as MPA search features to prioritise conservation effort (Hirst et al., 2012). Both habitats exist in areas of moderate to strong tidal flows and wave-exposed near shore coastal environments. Currently the renewable industry is testing devices in high flow and wave environments highlighting possible conflict between installation, use and biogenic structures. Large scale changes in tidal, circulation and wave patterns could potentially affect the availability and distribution of biogenic reef habitats but equally structures could also provide new niches.

Protecting biodiversity and priority habitats within 'prevailing climatic conditions' is necessary to achieve EC biodiversity targets however it is very likely that these habitats will be pressured by climate change over the next 100 years (Birkett et al., 1998; Hall-Spencer et al., 2008; Gormley et al., 2013). Therefore a 'win-win' approach where renewable development could facilitate biodiversity enhancement in a changing climate is an exciting prospect which could help to mitigate loss of such habitats.

The hydrological niche of *M. modiolus* and Maerl will be researched. Understanding how energy extraction by tidal and wave marine renewable structures will change hydrodynamics through predictive modelling, at both small and large scales, will also be researched. It is hoped that predictive habitat modelling and environmental envelope analysis will identify where the installation of marine renewables could create new environmental niches for these priority conservation habitats in a changing climate.

Understanding the link between the benthic community and hydrodynamics, as well as the response of biogenic reefs to changes in hydrodynamics will help to inform the most appropriate areas where marine renewable structures could potentially have a positive impact. The intention of this research is to provide evidence to support marine spatial planning, serving to enhance biodiversity and therefore help to achieve various national and European environmental and climate change targets.

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Using AIS to inform Marine Spatial Planning and marine industries

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Automatic Identification System (AIS) was adopted as a requirement aboard certain vessels by the International Maritime Organization (IMO) in 2000. The requirement stated that all ships over 300 gross tonnage on international voyages, all cargo ships greater than 500 gross tonnage, and all passenger ships were required to have AIS fitted by 31st December 2004. AIS provides automatic information on a vessel to other vessels in the area and to shore-based stations in order to increase safety at sea. Smaller vessels and other industries recognized the benefits of having AIS fitted which has resulted in a large quantity of vessel data ranging from large oil tankers to pleasure craft and sailing ships.

The NAFC Marine Centre has collated, processed, and analyzed AIS data since December 2012 for the waters around Shetland. AIS data is exported monthly from the on-site database, linked to a second vessel database, processed, quality controlled, and analyzed, prior to mapping in ArcGIS. Within ArcGIS, AIS pings are transformed into vessel tracks, density maps, and interpolations. During 2013, over 2000 vessels fitted with AIS were recorded around Shetland. Within the 12 nm limit around Shetland, 48 different registered countries were recorded with vessels originating from ports of 26 different countries. When examined by ship type, 29% were oil related. Vessels leaving the 12 nm limit recorded 23 different destinations ranging as far away as West Africa, and South and North America.

Outputs from the AIS data have been used in the Shetland Marine Spatial Plan to more accurately plot shipping routes around the islands and to examine the global connectivity of Shetland as an indicator of potential vector pathways for non-native species. Additional uses include aspects such as detailed mapping of fishers' activity, pollution risks, and noise impacts, to name a few. End users of the information would vary depending on their requirements but may include Marine Spatial Plans, marine developments to satisfy EIA requirements, MPAs or areas requiring monitoring of specific ship types, or researchers.

Comparing manual and computer-assisted benthic cover estimations from underwater imagery for offshore wind farms impact assessment

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Large scale offshore wind energy generation has the double advantage of drawing on renewable resources and of helping countries decrease their dependency on nuclear power. Offshore wind farms are typically sited at marine locations characterized by soft substrata but a few future wind farms are planned on rocky sea beds, for example, the first consented full-scale offshore wind farm Havsul, near Alesund in Norway.

Studying the environmental impacts of wind farms in such ecosystems requires the ability to monitor benthic cover over time in order to assess changes potentially due to the wind farm. In this study, the analysis of percentage benthic cover was carried out manually from benthic videos collected by ROV and by automatically extracting data from video mosaics of the video transects. Eighty mosaics were analysed and benthic cover was estimated by using a computer algorithm that was optimized manually using a training set of colours representing various types of benthic cover. The computer estimation was equally or more consistent than the human analysis and also more effective in terms of manpower required, decrease of analysis error and high reproducibility. Computerized image analyses of video mosaics are therefore a valid way to measure changes in benthic cover at offshore wind farms, especially those on rocky reefs.

Investigating the use of Fast Repetition Rate Fluorometry (FRRf) to study phytoplankton physiology in optically complex oceans

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Phytoplankton form the base of the marine food chain and are responsible for up to half of the Earth's global primary productivity (GPP). As such, it is important to be able to monitor any changes in phytoplankton physiology in order to establish response to changes in environment and observe population dynamics. It is possible to monitor changes in algal physiology optically using variable fluorescence techniques (Oxborough et al, 2012). FRRf gives us the capability to probe the efficiency of the algal photosynthetic apparatus thus making it possible to explore the apparent "health" of these organisms.

Although variable fluorescence techniques are relatively well established, recent studies have suggested limitations in the ability to interpret FRRf data from mixed populations of phytoplankton (Suggett et al, 2009). It is suggested that physiological information is sometimes masked by signals associated with taxonomic composition. As well as this, concerns have been expressed about potential instrument performance limitations in the presence of strong concentrations of coloured dissolved organic matter (CDOM) and sediment, as can occur in coastal waters.

We propose to tackle the issue of instrument performance in optically complex waters by carrying out rigorous characterisation of the instrument under a series of controlled lab experiments. Performing this basic characterisation will provide a strong evidence base on which to justify our interpretation of the instrument performance under a variety of natural conditions. This is particularly important for applications in coastal waters where the influence of non-algal materials will be greatest.

The influence of taxonomic variability on the outputs produced by the FRRf will be explored through investigation of spectral fluorescence characteristics through construction of a unique lab-built FRRf using a tunable supercontinuum laser as the excitation source. This will provide new capabilities to distinguish the effect of different pigment groups and changes in pigment function. Ambiguities in the underlying physiological model(s) will be addressed through direct measurement of fluorescence lifetime properties.

Following these preliminary, but fundamentally important, investigations into the operation and interpretation of FRRf data, further work will focus on establishing algal absorption efficiencies via measurement of total absorption cross-sections and Photosystem II absorption cross-sections. This will be accomplished using laboratory cultures and data collected from a forthcoming cruise with partners from HZG (Germany) across the Northwest European shelf, scheduled for 2015. In this case we will exploit recent advances in instrumentation for directly measuring absorption coefficients for turbid water samples and the recent availability in our group of an imaging flow cytometer to count, size and identify algal particles in mixed populations.

FRRf is by no means a new technology; however, there are still important areas of instrument characterisation and physiological model development that must be addressed. There is also scope to further develop and improve the ability to measure phytoplankton physiology with important applications in detection of harmful algal blooms, biofuels and response to climate change.

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