

## Flow cytometry: characterizing marine particles towards the submicron scale

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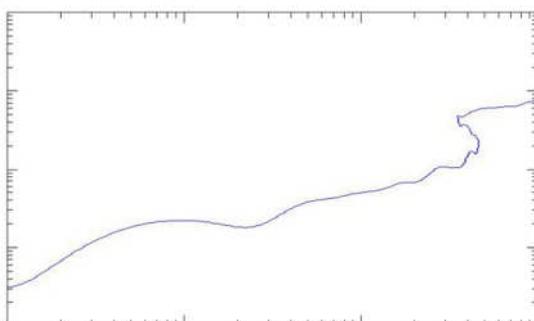
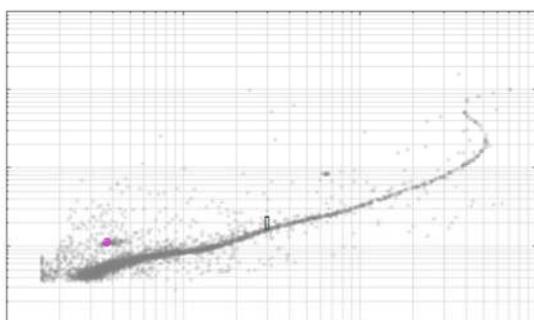
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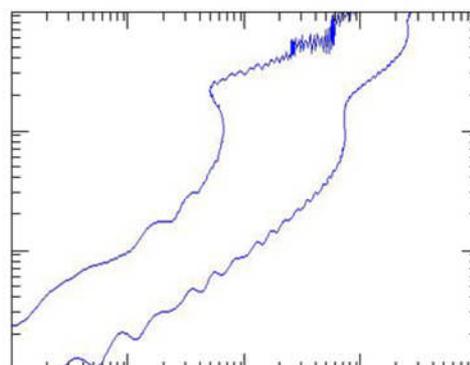
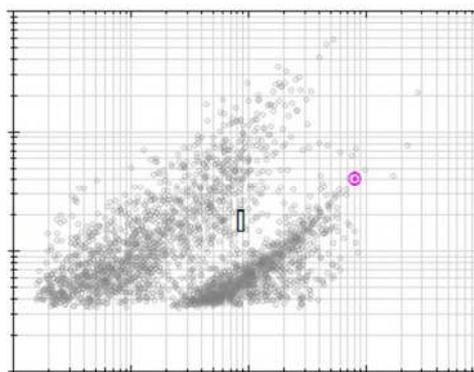
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Modern flow cytometry shows potential for furthering our knowledge of the population composition and size distribution of water-suspended particles down to submicron scales. By combining high-sensitivity laser light scattering and fluorescence pulse shape characterization capabilities with direct imaging of the particles, the CytoSense flow cytometer (CytoBuoy, Woerden, Netherlands) enables resolution of water-suspended particle population variability down to the 1  $\mu\text{m}$  threshold, with the possibility of resolving even smaller diameters in the case of particles with high refractive indices.

In the special case of spherical particles, the accuracy and precision of the instrument can be tested against Mie theory modelling over a range of different refractive indices by analysing air bubbles, polymer beads and approximately spherical phytoplankton samples, with potential for further correction through ray-tracing techniques to account for the finite width of the scanning laser beam.



The successful development of a model to describe the scattering behaviour of particles analysed by the instrument is key to identification of sub-populations within the whole range of sampled particles. By varying size and refractive index parameters used in the modelling, a parameter space can be described within which different sub-populations of particles are expected to occur.



Together with the imaging capabilities of the instrument and the pulse shape characterization of each sampled particle, we gain a wealth of information that has practical applications on a breadth of different fields, from furthering the knowledge of the optical properties of natural waters to species identification and monitoring of phytoplankton populations.

## The genotoxicity of Different Forms of Copper Oxide (NPs & MPs) in *M. modiolus* Mussels

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Nanotechnology has been rapidly explored and of interest all over the world in the last few decades. Nanoparticles are heavily used in different industrial and commercial products, such as cosmetic products, toothpaste and paints.

Copper oxide nanoparticles (CuO NPs) are one example of NPs that are used in many applications, such as batteries, semiconductors and inks. The increased use of CuO NPs will likely result in their release into the environment, especially the marine environment.

Furthermore, there is a lack of understanding of the toxicity of CuO NPs on organisms and human health, particularly benthic filter feeders where data are particularly lacking. Thus, it is crucial to address CuO NPs effects on key marine organisms and compare their toxicity with CuO microparticles (MPs).

Therefore, the aim of the current study was to assess DNA damage and oxidative stress in *M. modiolus* mussels exposed to different concentrations of CuO (NPs and MPs). *M. modiolus* mussels were exposed to a range of nominal concentrations of CuO particles (5, 10, 15 and 20 µg L<sup>-1</sup>) along with the control, for 72 hours. The comet assay was used to assess DNA damage in the gill cells of the mussels, and superoxide dismutase (SOD activity assay) was used to assess oxidative stress.

The horse mussel, *M. modiolus* was selected for this study because of its important ecological role in establishing biogenic reefs, which are increasingly being classified as special marine features and thus require protection, which the understanding of the

sensitivity of *Modiolus* to CuO particulate contaminants will help facilitate.

The results indicated that, both CuO particles (NPs and MPs) have the potential to cause DNA damage in the gill cells of *M. modiolus*, and the level of DNA damage was gradually increased as the concentration of both CuO particle types increased. Similarly, the SOD activity was observed to have increased in the gill cells of *M. modiolus* exposed to both forms of CuO (NPs and MPs) indicating increased oxidative stress, which is the likely cause of the DNA damage. Finally, CuO NPs were more toxic than CuO MPs to *M. modiolus*.

**Keywords:** CuO NPs , nanoparticles, oxidative stress, DNA damage, *M. edulis*, *M. modiolus* mussels.

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## Green algae interacting with single-walled carbon nanotubes affect the feeding behaviour of mussels, mitigating nanotube toxicity

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### Abstract

The increased industrial application of carbon nanotubes has led to a significant interest in their aquatic ecotoxicology and potential for trophic transfer. Green algae (*Tetraselmis suecica*) were exposed in triplicate to 5µg L<sup>-1</sup>, 10µg L<sup>-1</sup>, 50µg L<sup>-1</sup>, 100µg L<sup>-1</sup> and 500µg L<sup>-1</sup> single-walled carbon nanotubes (SWCNTs) for 8 days. Light microscopical observations, confirmed by SEM and Raman spectroscopy, showed that SWCNTs adhered to the external algal cell walls. TEM results suggested SWCNTs may have been internalized by the algae. A direct effect of SWCNT exposure on the algae was a significant (P<0.001) decrease in chlorophyll *a* concentrations, accompanied by a significant decrease (P<0.001) in cell viability by day 7 at concentrations of 500µg L<sup>-1</sup>. We have previously shown that mussels can remove SWCNTs from the water column and reject them as pseudofaeces. In order to study the feeding behaviour of mussels (*Mytilus edulis*) presented with algae in the presence of SWCNTs, known algal concentrations and SWCNT 500µg L<sup>-1</sup> alone and combined were applied to the tanks and mussels left to feed for 10 minutes, after which mussels were transferred to clean seawater and left to depurate for 24h. The control treatment contained algae without mussels, in order to correct for algal cell division. Selective bivalve feeding was observed using a newly developed flow cytometry technique with pseudofaeces as a proxy. Pseudofaeces production increased significantly (P=0.008) under combined algae and SWCNT exposure. DNA damage and oxidative stress were used as ecotoxicological biomarkers of exposure in mussels. A 24h exposure to 500µg L<sup>-1</sup> SWCNTs showed significantly increased DNA strand breaks in both gill cells and haemocytes (P<0.001), and significantly increased oxidative stress, expressed as superoxide dismutase (SOD) activity (P<0.001) and lipid peroxidation in gills (P=0.032). However, when SWCNTs were presented together with algae, DNA damage in haemocytes and gills (P=0.534; P=0.998) or oxidative stress were not significantly increased above control levels (P=0.981; P=0.999). Mussels appeared to largely reject algae containing SWCNTs. However, SWCNT ingested can not be ruled out and we are currently investigating whether the observed SWCNT-algal interaction may facilitate trophic transfer of SWCNTs up the food chain with potential consequences for human health.

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Dr Mark Hartl (my supervisor) for his advice, guidance and support, Dr Lynn Paterson for her guidance in Raman spectroscopy, Mrs Margaret Stobie for her support in aquarium unit, Mr Paul Cyphus for his time and guidance in flow cytometry, Mr James Buchanan for his support and guidance in SEM.

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## New developments in seawater trace metal analysis at SAMS: Automated preconcentration by SeaFAST Pico

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A vital component of marine research is the trace metal composition of seawater. This measure gives great insight into the cross-disciplinary science studied by partners of MASTS, such as physical dynamics, biogeochemical processes, and chemical pathways within the ocean, including the effects of anthropogenic activities, such as mining and global climate change. Despite the inherent importance of seawater composition to the marine research community, the typically low concentrations of trace metals and the time-consuming sample preparation, as well as stringent laboratory and instrumental requirements present a stumbling block for robust and routine analysis.

SAMS has overcome many of these analytical challenges using the newly available SeaFAST Pico (Elemental Scientific Inc., Nebraska, USA). In combination with the SAMS inductively coupled plasma mass spectrometer (ICP-MS) this system allows the accurate and precise measurement of undiluted seawater trace element concentrations, with no requirement for laborious and expensive pre-treatment. Sample preconcentration within one ultraclean unit dramatically reduces sample processing time and minimises procedural contamination, resulting in higher quality data compared to conventional analytical methods. An additional advantage is the small sample volume required per analysis (2 ml to 12 ml dependent on target element and required precision), obviating large sample volume collection and the increased potential of sample contamination.

The SeaFAST Pico has been designed and extensively tested by scientists involved in the GEOTRACES programme [e.g. 1, 2], widely recognised as the gold standard of seawater chemical analysis. Use of the instrument thus far at SAMS has targeted investigation of the dynamics of iron and manganese discharge and recycling within Loch Etive.

The flexibility afforded by this system has great promise for the measurement of trace metals in other marine matrices, e.g. carbonates and organics. A number of other, novel techniques will be developed

in the near future for use on the SeaFAST, e.g. automated chromatographic separation of small sample neodymium (Nd) for the purposes of isotopic ratio measurements, which will lower levels of sample contamination as well as providing considerable labour savings.

Future applications on the SeaFAST, involving the techniques we are developing, include

- environmental impact analysis of sub-seafloor carbon capture and storage, and the effects on trace metal concentrations in the overlying seawater column;
- metal pathways and ocean dynamics in the NE Atlantic through measurement of dissolved and particulate trace metals in seawater along the Ellett Line;
- environmental monitoring and seawater-carbonate trace metal partitioning by marine carbonate analysis of corals, otoliths, and bivalves;
- ecotoxicology studies to quantify the uptake of polluting metals by urchin larvae exposed to mine tailings.

The distinct advantages of the SeaFAST Pico for SAMS are: (i) improved and cost-effective sample processing and data quality, (ii) increased research output, (iii) broader applications and the development of new research directions, and (iv) increased industrial and academic collaboration. Our poster at the MASTS conference provides further information on our capabilities using the SeaFAST, and future directions of research we would be keen to engage in collaboration with others.

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## Algal toxins in Scottish waters: the first association of domoic acid with the diatom *Pseudo-nitzschia pseudodelicatissima* complex in the North East of Scotland

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Marine Scotland Science operate a coastal ecosystem monitoring site 5 km offshore from the village of Stonehaven in the North East of Scotland (56° 57.8' N, 02 ° 06.2' W). Temperature, salinity, nutrients, phytoplankton and zooplankton have been sampled at this site since 1997 on a weekly basis (weather permitting). Data from this site is being used to understand the variability in the ecosystem associated with climate change and ocean acidification as well as to fulfil the monitoring requirements of the Water Framework Directive and Marine Strategy Framework Directive.

The diatom genus *Pseudo-nitzschia* (Peragallo) is a very common component of the phytoplankton community in Scottish waters. Cells from this genus cannot be identified to species level using routine light microscopy, only divided into two different size classes; '*P. delicatissima* type cells' (< 3µm in diameter) and '*P. seriata* type cells' (>3µm in diameter).

During the phytoplankton growing period, copepods sampled at this site were analysed for the presence of algal toxins. Domoic acid (DA), the toxin responsible for amnesic shellfish poisoning, was recorded in copepods collected on 20 different sampling occasions between June and October. Concentrations ranged from 1 – 19 µg domoic acid per copepod. The highest concentration (19 µg domoic acid per copepod) was associated with a bloom of '*P. delicatissima* type' cells as identified using light microscopy. This bloom reached a maximum cell density of 130,000 cells L<sup>-1</sup> prior to DA being detected in the copepods.

Analysis using transmission electron microscopy (TEM) revealed that the morphology of the *Pseudo-nitzschia* cells in this bloom were similar to an undescribed species of *Pseudo-nitzschia* within the

*Pseudo-nitzschia pseudodelicatissima* complex reported from the Bay of Fundy (*P. pseudodelicatissima* BOF), the Gulf of Maine (*P. sp. GOM*), the Spanish Atlantic coast (*P. sp. Ner-D6*) and Australia (*P. sp. Hobart5*). This is the first association of domoic acid with this species in Scottish waters.

## The National Marine Biological Analytical Quality Control Scheme

Clare Scanlan<sup>1</sup>, Astrid Fischer<sup>2</sup>, David Johns<sup>3</sup>, Tim Mackie<sup>4</sup>, Myles O'Reilly<sup>5</sup>, Amanda Prior<sup>6</sup>, Claire Mason<sup>7</sup>, Dan Bayley<sup>8</sup>, Matthew Green<sup>9</sup>, Jim Ellis<sup>10</sup>, Joe Silke<sup>11</sup>, Rafael Salas<sup>12</sup>

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### Abstract

Ensuring high quality data is key to good and sustainable management of the environment by ensuring that decisions are based on reliable information. The NMBAQC scheme was set up in 1994 to provide a source of external quality control of biological data for U.K. competent monitoring authorities contributing to UK national or European monitoring programmes. It is also open to consultants and non-U.K. participants. The scheme reports to the Healthy & Biological Diverse Seas Evidence Group under the UK's Marine Monitoring & Assessment Strategy. NMBAQC helps to standardise results and methods by providing training exercises, taxonomic workshops and Best Practice guides. The scheme comprises a number of biological components (e.g. phytoplankton, benthic invertebrates) each with its own set of training exercises and/or assessments. New components are developed as and when required, as determined by monitoring needs.

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## Constraining the effects of Mg:Ca ratio and temperature on non-biogenic CaCO<sub>3</sub> polymorph precipitation

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Ocean chemistry has oscillated throughout Earth history to favour the dominant non-biogenic polymorph of CaCO<sub>3</sub> to be either calcite or aragonite (Sandberg, 1983). Throughout the Phanerozoic these oscillations have occurred to facilitate aragonite-dominant conditions three times and calcite-dominant conditions twice. These aragonite-calcite seas have previously been viewed as a global phenomenon where conditions fluctuate over time, but not in space, and represent the main environmental context in which the evolution of CaCO<sub>3</sub> biomineralisation occurred (Stanley & Hardie, 1998).

The main driving force controlling the precipitation of CaCO<sub>3</sub> polymorphs is the Mg:Ca molar ratio of seawater (Morse et al., 2007). However, other parameters such as temperature, pCO<sub>2</sub>, and SO<sub>4</sub> (Morse et al., 2007) are also known to influence CaCO<sub>3</sub> polymorph formation but are often overlooked in the context of aragonite-calcite seas. Specifically the influence of temperature is important because it is likely to result in aragonite-calcite sea conditions to vary spatially.

Experiments were carried out to quantify the effect of temperature and the Mg:Ca ratio on CaCO<sub>3</sub> polymorph formation. CaCO<sub>3</sub> precipitation was induced by continuously adding bicarbonate to a bulk solution of known Mg:Ca ratio (1,2 or 3), and fixed salinity (35), at 20 and 30°C in still conditions. This set of experiments was repeated with the solution being shaken at 80rpm mimicking more natural marine conditions. The mineralogy and crystal morphology of precipitates was determined using Raman Spectroscopy and Scanning Electron Microscopy.

Preliminary results indicate that aragonite and calcite polymorphs co-precipitate with the ratio of aragonite:calcite increasing with higher temperatures and higher Mg:Ca ratios. The main differences between still and shaking conditions are that more crystals precipitate in still conditions. Further, shaken conditions result in an overall increase of vaterite.

Examining polymorph growth under these experimental constraints allows us to gain a better understanding of how temperature and Mg:Ca together control non-biogenic aragonite and calcite precipitation. Our work indicates that current views on aragonite-calcite seas as spatially homogenous ocean states need to be re-examined to include the effect of temperature on the spatial distribution of CaCO<sub>3</sub> polymorphs. Including temperature in our assessment of aragonite-calcite sea conditions provides a more realistic environmental framework in which to evaluate the evolution of biomineralisation.

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