The Effects of Anthropogenic Noise Playbacks on the Blue Mussel *Mytilus edulis*

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Marine invertebrates have received scant attention with regard to their responses to anthropogenic noise, despite their pivotal role in marine ecosystems. They are important components of most food webs and perform essential ecosystem services. Preliminary evidence that marine invertebrates can be very sensitive to noise calls for further studies in this context (e.g. reports by the Convention of Biological Diversity and OSPAR).

We conducted a series of carefully controlled laboratory experiments to investigate how short term (up to 6 hours) playback of ship noise affects the blue mussel, *Mytilus edulis*. To help understand whether it is affected by underwater noise, and also how and why, we employed a mechanistic, integrative approach, as set forth by Kight and Swaddle (2011), considering behavioural (valve movement, algal clearance), physiological (oxygen consumption) and biochemical (structural DNA damage, oxidative stress) responses.

Comet assay analysis of haemocytes and gill cells demonstrated significantly higher single strand breaks in the DNA of cells of mussels exposed to ship-noise playback, compared to those kept under ambient conditions. SOD analysis did not identify an excess of superoxide ions, and GSH, and GPx assays showed no increase in either glutathione or glutathione peroxidase. TBAR assays however revealed increased thiobarbituric acid reactive substances, indicating lipid peroxidation in the gill epithelia of noise exposed specimens.

Algal clearance rates of noise-exposed mussels were significantly lower, and oxygen-consumption rates higher than those of control animals, reflecting stress.

Our integrative approach has evidenced that multiple aspects of the biology of the blue mussel can be negatively affected by noise, from DNA integrity, to cell structure/signaling, physiology and behaviour.

This study is the first to show DNA damage in the gills and haemolymph of any marine species in response to anthropogenic noise and highlights that the Comet assay is an adequate tool in noise research. It is also, to the best of our knowledge, the first to use oxidative stress endpoints as a biomarker of the effects of underwater noise in marine organisms. In addition to the negative effects on the level of the organism, which could lead to reduced growth, reproduction, and immune response, the observed reduction in algal clearance rates suggests a reduced ecological performance of the mussels in terms of water filtration. Further work investigating the effects of chronic noise exposure, as well as field trials are currently planned.

Our results highlight the need to investigate the response of animals from all trophic levels, and demonstrate that sessile organisms must be considered before the ecosystemic effects of noise can be understood.

Our integrative approach to noise research can be used as a model for other invertebrate species, and the results generated can be pooled with those obtained for marine vertebrates to inform governments and industry of the effects anthropogenic noise is having in the marine environment.

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The toxicity of differently coated copper oxide nanoparticles towards the marine blue mussel, *Mytilus edulis*.

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Rapid growth in the field of nanotechnology is continually increasing the potential release of nanoparticles (NPs) into the environment. Although copper oxide nanoparticles (CuO NPs) are widely used within numerous industrial and commercial applications (e.g. batteries, electronic circuits, superconductors, solar energy conversion and gas sensors), their toxicity is still poorly understood in comparison with other metal oxide NPs. A key paradigm for NP toxicity is oxidative stress, whereby the production of reactive oxygen species (ROS) by NPs outweighs the antioxidant capacity of biological systems. Where an oxidant/antioxidant imbalance prevails, oxidative stress may cause damage to DNA, lipids and proteins. As filter-filters, and inhabitants of aquatic systems (the main sink for wastewaters and other effluents from human activities), the blue mussel (*Mytilus edulis*) is a species of particular interest in relation to the environmental fate and toxicity of NPs. This study, funded by the FP7 project, NANOSOLUTIONS, aims to investigate the mode of toxicity exhibited by CuO NPs towards *M. edulis* and the effect of surface coatings upon their toxicity. Significant DNA damage (as assessed by the comet assay) was detected in the gill and haemolymph cells of *M. edulis* following 48 hour exposure of relatively low concentrations of CuO NPs (20 μgL^{-1}) to each CuO NP, with different surface chemistries – core (no coating), polyethylene glycol (PEG), nitrate (NO_3^-) and carboxylic acid (COOH). No discernible patterns between DNA damage and CuO NP coatings were observed, however, haemolymph cells (responsible for defence, repair, nutrient and transport) proved to be more sensitive than gill cells. The lysosomal stability of haemolymph cells – which is directly linked to cellular immunity, is currently being investigated to further assess CuO NP mediated damage. Additional assays measuring the activity of the antioxidant enzyme, superoxide dismutase (SOD) and levels of the non-enzymatic antioxidant, glutathione (GSH), will provide further insight to the possible oxidative stress caused by CuO NPs, and whether coating bears any influence.

Optical closure between PSDs and IOPs in natural waters: a Mie-based flow cytometric method

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A flow cytometry (FC) method was developed to retrieve particle size distributions (PSDs) and refractive index (RI) information in natural waters, broadly following an approach previously developed by Ackleson & Spinrad (1988) and subsequently Green et al. (2003). Geometry and signal response of the flow cytometer sensors were characterized to inform a scattering inversion model based on Mie theory. The procedure produces a mesh of diameter and RI isolines where each particle is assigned diameter and RI of the closest node, producing PSD and RI in the 0.5-20+ micron diameter range. Resulting FC PSDs and RIs can then be used as inputs to forward Mie modelling and determination of IOPs, allowing for validation against independently measured scattering and backscattering coefficients.

The method was applied to a set of samples obtained in April 2015 during a research cruise (HE442) in UK waters on board the F.S. Heincke. Each PSD was fitted with a Junge distribution to enable extension of the PSD size range by extrapolation to a minimum diameter of 0.05 µm and a maximum diameter of 500 µm, corresponding to the optically significant range found by Davies et al. (2014) for mid-range values of the PSD slope. Where available, relative RIs as determined by the FC method were used; otherwise, in the Junge extensions values of the real component nr equal to 1.15 (representative of inorganic-like particles) were applied to diameters smaller than 0.5 µm and values of nr equal to 1.05 (representative of organic-like particles) were applied to diameters larger than 20 µm.

Closure could not be reached using the measured PSDs alone; using Junge distributions to extend the size range and include optically relevant, non-detectable particles produced a good fit with measured scattering (linear fit: 0.943x-0.331, adj. R-squared: 0.821; adj. R-squared of 1:1 line: 0.710) (Fig. 1b). The method was also able to resolve subpopulations in the particulate (organic vs. inorganic, fluorescent vs. non-fluorescent) and to determine their individual contribution to the total IOPs (Fig. 1b).

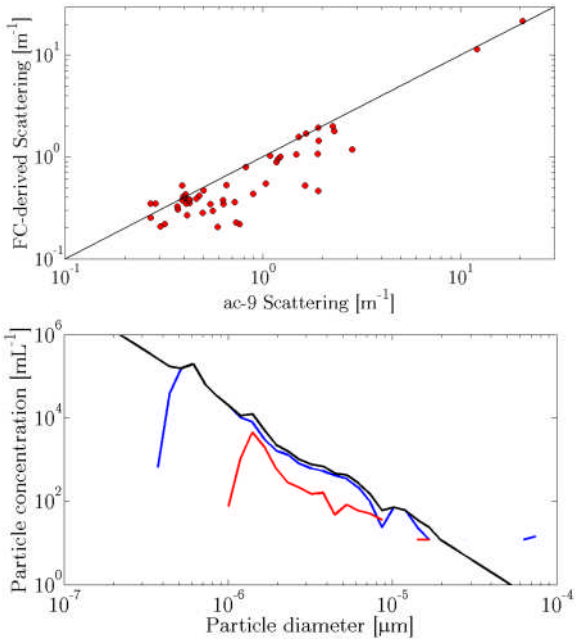

Fig.1: a) Scattering comparison between ac-9 and FC-derived scattering (1:1 line in black); b) Subpopulations in a typical PSD: fluorescing (red), non-fluorescing (blue) and overall PSD with Junge extensions (black).

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Cold-water-corals in a high CO_2 ocean: behaviour, physiology and growth in *Desmophyllum dianthus*

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Increased ocean acidity with accompanying shifts in temperature and aragonite and calcite saturations are expected to be detrimental to marine calcifiers and research to date has painted a bleak outlook for coral species. Colder waters, including polar and deep water are acidifying faster than other water masses, in part because CO_2 dissolves more readily in colder waters, and deep sea corals will need to adapt to ocean acidification faster than shallow species to survive into the future. Here we present results from a 15 month incubation study of the responses of the cosmopolitan deep sea coral *Desmophyllum dianthus* to increased carbon dioxide (750 ppm) and temperature. Skeletal growth was determined using a 136Ba isotope tracer introduced at the start of the incubation. Feeding behaviour was recorded using time-lapse photography after 12 months. An additional 8 month incubation under the same CO_2 treatments at 12 and 15 °C temperature treatments was conducted to examine calcification, respiration and ammonia secretion. Our results show that *D. dianthus* has the ability to withstand elevated pCO_2 (750 ppm) under ambient temperature, however under combined temperature and pCO_2 stress respiration, calcification and metabolic pathways are impacted. Our work highlights the importance of multi stressor experiments to examine the impacts of ocean acidification and suggests that the degree of ocean warming will be a key determinant in the ability of *D. dianthus* to persist in a high CO_2 ocean.

Investigation of the biodiversity and ecology of encrusting epifauna associated with horse mussel *Modiolus modiolus* in the North-East Atlantic

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Key Words: *Modiolus modiolus*; biogenic reefs; epifauna

Biogenic reefs created by the horse mussel *Modiolus modiolus* provide hard substrates in areas that would otherwise be dominated by sediment. Although *M. modiolus* as a species is widespread and common, dense reefs of *M. modiolus* that form a distinctive habitat are much more limited in their distribution. The reefs comprise raised structures, bound together by a matrix of byssus threads which occur in various densities and thicknesses (Walters, 2006). Reefs support a wide range of epibiota, crevice fauna and infauna and are therefore classified as Priority Marine Habitats for conservation in the NE Atlantic (PMH's) (OSPAR, 2010). Reefs physically support a diverse assemblage of suspension feeders, including encrusting species such as barnacles, tube worms, and bryozoans that to date have been poorly documented. The aim of this study is to identify and quantify the encrusting epifauna on different horse mussel shell regions and on shells of different sizes across eight sites throughout the NE Atlantic. The encrusting epifaunal community was recorded, species identity confirmed with selected Scanning Electron Microscope imagery and the species abundance data subjected to standard multivariate analyses to give an understanding of the community complexity of horse mussel shell epifauna, and relate the community of epifaunal organisms to the micro-environmental and biogeographic context.

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The potential impact of engineered nanomaterials (ENMs) on marine ecosystems: investigations using the marine mussel bivalve invertebrate *Mytilus edulis*.

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For many, ENMs are seen as a new class of emerging contaminates with the potential to disrupt many natural ecosystems. However, the precise occurrences, potential hazards, and particular effects of ENMs in marine environments are not sufficiently understood. In fact research focusing on the impacts of ENM contamination in marine environmental sinks lags behind those conducted in freshwater environments. Marine mussel bivalves *Mytilus edulis* act as filters of the sea, pumping large volumes of water and processing various particulates including, if present, ENMs. Therefore they may act as important indicators of ENM contamination. A chronic (21 day) study was performed to represent a low-level, long-term exposure to ENMs in the marine environment. Questions addressed included 1) whether mussels accumulate ENMs during chronic exposure 2) if they can eliminate ENMs from their bodies and at what rate, and finally 3) if accumulation of certain ENMs affects general mussel health (monitored by assessing oxidative stress parameters and haemocyte lysosomal membrane stability) and if levels of accumulation could potentially represent a foodborne hazard to humans. Both TiO_2 and CuO ENMs were included in the chronic study at environmentally relevant levels (0.3 mg/L and 20 µg/L, respectively). The influence of polyethylene glycol, carboxylic acid and ammonium functionalisation on the ENMs behaviour in seawater and on accumulation/excretion kinetics following chronic exposure was also assessed. The ability of *Mytilus edulis* to concentrate large quantities of ENMs in their tissues may lead to deleterious effects for the organism and also act as a foodborne human health concern. Such investigations into the extent to which ENMs accumulate in mussel tissues, and/or are excreted in biodeposits, also provide important information on the transport of ENMs in such environments. For example, high rates of excretion may result in accumulation effects in sediments and direct effects on other marine organisms and the marine ecosystem as a whole.

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How do SCUBA divers value coral reef protection?

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While ecological links between terrestrial and marine ecosystems have been long recognised, management rarely crosses ecosystem boundaries. Coral reefs are particularly susceptible to damage through terrestrial sediment run-off, as this reduces light levels, leading to decreased growth rates, as well as altering reef structure and coral species composition (Fabricius 2005). Failing to account for these impacts within management therefore threatens reef protection.

Connecting funding between the terrestrial and marine ecosystems can promote coordinated conservation efforts. User fees are employed to provide funding within both marine and terrestrial protected areas, but funds are typically tied to the resource under direct use. Implementing user fees across boundaries requires an understanding of how users value the environmental resource, and how they would benefit from an improvement in ecosystem quality. Stated preference methods are frequently used to estimate the willingness of groups of users to fund improvements in such contexts. Among the set of stated preference methods, choice experiments provide the possibility to value ecosystem attributes, enabling direct links to be drawn between willingness to pay and environmental change (Train, 2009).

In this study we investigate the potential to utilise user fees across ecosystem boundaries. In order to quantify the extent to which coral reef users are willing to support management actions to improve ecosystem quality, we conducted a choice experiment with SCUBA divers on the island of Bonaire, Caribbean Netherlands. Specifically, we estimated their willingness to pay to reduce terrestrial overgrazing as a means to improve reef health. Willingness to pay was estimated using the multinomial, random parameter and latent class logit models.

Willingness to pay for improvements to reef quality was positive for the majority of respondents, though the latent class model identified one class with zero willingness to pay (class share: 0.16). Estimates from the latent class model determined willingness to pay for reef improvements of between $34.10 - $109.58/year, dependent on class membership. This represents a significant source of funding for terrestrial conservation, and illustrates the potential for user fees to be applied across ecosystem boundaries.

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The sensitivity of modelled *Lophelia pertusa* larval dispersal and population connectivity to climate variability

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Is the existing *Lophelia pertusa* network in the North Atlantic robust to climate change? Dispersal of larvae from sub-populations of *L. pertusa* and connectivity between sub-populations are crucially dependent on the atmospheric state. Laboratory observations have revealed that *L. pertusa* larvae have sufficient vertical swimming ability to reach near-surface waters, exposing the larvae to stronger and more variable currents than at depth. When this behaviour is introduced into a larval particle tracking model, the resulting dispersal of larvae from sub-populations in the NE Atlantic shows strong correlation with the North Atlantic Oscillation, the dominant mode of inter-annual atmospheric variability in the region. Positive NAO state is correlated with a more connected, though directional west to east, network. Negative NAO results in a less connected network, though with more larvae transported from east to west. A long-term shift towards either more positive or more negative NAO state would result in loss of connections, potentially isolating sub-populations.

The *L. pertusa* network connectivity is also threatened by reduced larval lifespan through rising ocean temperatures, and the impact of ocean acidification shallowing the aragonite saturation horizon exposing reefs to potentially corrosive waters. The larval dispersal and network connections are modelled under these scenarios, demonstrating increasing fragmentation of the network.

These results pose challenges for management of vulnerable deep sea coral populations. Increasing fragmentation of the networks, and with it decreasing resilience, may be inevitable without significant intervention.

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