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i) Scaling up experimental ocean acidification and warming research: from individuals to the ecosystem

ii) OAW: from individuals to the ecosystem

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Abstract

Understanding long-term, ecosystem-level impacts of climate change is challenging because experimental research frequently focuses on short-term, individual-level impacts in isolation. We address this shortcoming first through an inter-disciplinary ensemble of novel experimental techniques to investigate the impacts of 14-month exposure to ocean acidification and warming (OAW) on the physiology, activity, predatory behaviour and susceptibility to predation of an important marine gastropod (*Nucella lapillus*). We simultaneously estimated the potential impacts of these global drivers on *N. lapillus* population dynamics and dispersal parameters. We then used these data to parameterise a dynamic bioclimatic envelope model, to investigate the consequences of OAW on the distribution of the species in the wider NE Atlantic region by 2100. The model accounts also

for changes in the distribution of resources, suitable habitat and environment simulated by finely resolved biogeochemical models, under three IPCC global emissions scenarios. The experiments showed that temperature had the greatest impact on individual level responses, while acidification has a similarly important role in the mediation of predatory behaviour and susceptibility to predators. Changes in *Nucella* predatory behaviour appeared to serve as a strategy to mitigate individual level impacts of acidification, but the development of this response may be limited in the presence of predators. The model projected significant large-scale changes in the distribution of *Nucella* by the year 2100 that were exacerbated by rising greenhouse gas emissions. These changes were spatially heterogeneous, as the degree of impact of OAW on the combination of responses considered by the model varied depending on local environmental conditions and resource availability. Such changes in macro-scale distributions cannot be predicted by investigating individual level impacts in isolation, or by considering climate stressors separately. Scaling up the results of experimental climate change research requires approaches that account for long-term, multi-scale responses to multiple stressors, in an ecosystem context.

Introduction

Future oceans will challenge marine organisms with a multitude of ecosystem-level stressors associated with global environmental change (Byrne *et al.*, 2013). Increased atmospheric CO_2 concentrations will both decrease the ocean pH (i.e. ocean acidification) and the saturation of carbonated minerals, disrupting marine carbonate chemistry as well as increasing sea temperature (Doney *et al.*, 2009, Feely *et al.*, 2004, Harvey *et al.*, 2013, Kroeker *et al.*, 2013). Biological responses to Ocean Acidification and Warming (OAW) are thought to depend on a number of physiological and life history attributes at larval, juvenile and adult stages, such as

their dependence on (and type of) calcifying structures, and their ability for acid-base regulation (Kroeker *et al.*, 2013). These responses depend on physiological trade-offs, that is, the transformation and allocation of energy in an organism, determining its demand for resources, and constraining the allocation to vital cellular functions that contribute to organismal performances, survival, and fitness (Brown *et al.*, 2004, Findlay *et al.*, 2011). Predicting long-term ecosystem-level responses of individual species is, however, difficult because experimental climate change research often focuses on single, short-term, species level responses in isolation (Kroeker *et al.*, 2013). What's more, long-term responses are confounded by the ability to adjust and adapt life-history patterns, both of which vary between species and populations (Eliason *et al.*, 2011). Further, inter-specific interactions may regulate high-level impacts of climate change (Harley, 2011), but have received less attention than single-species impacts in the last decade (Wernberg *et al.*, 2012). Individual-based responses of single species alone are thus unlikely to provide a sufficient basis to understand long-term responses in complex ecological environments, where species also interact (Harley, 2011). The response of a population to a changing environment further depends on other processes that operate at different scales, including modifications of behaviour, dispersal and population dynamics (Pörtner & Knust, 2007). These depend also on the availability of habitat and resources necessary to support life (Thomsen *et al.*, 2013),which are driven by environmental conditions varying in space and time.

Biogeochemical ecosystem models are mathematical descriptions of ecosystem processes, that capture essential rates and flows of matter and energy in space and time, and link them to the environment and biota. These can be used to project the bulk properties of ecosystems (Allen *et al.*, 2010) into the future and the past. These models therefore provide a holistic

view of ecosystems where large scale research questions about global climate change can be addressed (Artioli *et al.*, 2014). However, the integration of detailed, species-level experimental information into these macro-scale applications has been limited, because these models operate at much larger spatial and temporal scales and because, for practical reasons, they typically include only very generic descriptions of species (Anderson, 2005). Such integration requires the use of a different type of macro-scale models that can use large-scale environmental patterns, as projected by biogeochemical ecosystem models, and merge it with finer mechanistic descriptions of individual species responses to that environment (Jørgensen *et al.*, 2012). Dynamic bioclimatic envelope modelling (DBEM) enables this approach (Cheung *et al.*, 2011, Fernandes *et al.*, 2013). In DBEMs, the impacts of environmental stressors on important aspects of species ecology like physiology, population dynamics, dispersal, trophic interactions and resource use (i.e. species traits) are considered simultaneously, and can be constrained using experimental or literature derived information gathered at the species-level. This information is complemented by observational species habitat preference data, and macro-scale biogeochemical simulations of environmental conditions and resource availability (i.e. primary production), to project the corresponding changes in macro-scale species distributions (Cheung *et al.*, 2011, Kearney & Porter, 2009). This framework therefore has the potential to overcome the limitations of previous methodologies, and significantly enhance the way in which the necessary, detailed, species-level experimental climate change research is integrated, interpreted, and used in ecosystem level applications.

Here, we used a variety of novel techniques to quantify long-term impacts of OAW on species level physiology and trophic interactions of the dogwhelk *Nucella lapillus* (Linnaeus,

1758), a species that exerts strong influence in temperate rocky-shore ecosystems through top-down controls (Trussell *et al.*, 2003 and references therein). *Nucella* preys on barnacles and mussels, foundation species that modify 3D habitat complexity, providing shelter to other species, facilitating the development of algal canopies, and therefore the recruitment of other fauna (Menge & Branch, 2001). *Nucella* predators like the crab *Carcinus maenas* (Leach 1814) also exert indirect controls on the abundance of *Nucella* prey species *via* trophic cascades (Trussell *et al.*, 2003). These are key mechanisms for the maintenance of biodiversity in temperate rocky-shores. Thus, investigating how the predatory activity of *Nucella* and its vulnerability to predators are modified by global stressors is key to understanding and predicting how rocky-shore systems may change in a near-future. In order to do so, we measured *Nucella*'s response to five scenarios of OAW after a 14 month long mesocosm experiment, thus avoiding artefacts caused by shock responses to stressors observed in short-term experiments (Form & Riebesell, 2012). We measured changes in *Nucella*'s resting oxygen consumption (a proxy for metabolic rate in heterotrophs, the energetic cost of living, Brown *et al.*, 2004) and basal activity (i.e. motor activity in the absence of stimuli). These two parameters were used to verify the presence of functional trade-offs, which were expected to be negatively affected by energetic expenditure associated with increased energy cost due to exposure to acidified conditions (Calosi *et al.*, 2013, Parker *et al.*, 2013) and up-regulation of metabolism by warming (Brown *et al.*, 2004). We then investigated how these individual level responses related to the wider ecology of *Nucella*, by measuring trophic interactions relevant at the community level: predatory behaviour and vulnerability to predation. First, we monitored the behavioural response of *Nucella* to a prey mimic made of fresh tissues of a prey species (the mussel *Mytillus edulis*, Linnaeus 1758) using time-lapse photography and digital tracking techniques. Second, as the shell of *Nucella* is its main defence against predators (Crothers, 1985), we used micro-computer-aided

tomography ("microCT") to quantify changes in shell integrity as a proxy for its vulnerability to predation. We complemented these observations with an assessment of the impacts of the long-term experimental treatments on other parameters associated with the wider population dynamics of the species, such as growth and mortality. Finally, we used these results to parameterize, for the first time, a size-spectrum based DBEM (SS-DBEM, Fernandes *et al.*, 2013). This enabled us to scale-up our species-level experimental results, by modelling how the combination of all the ecologically relevant measured responses to OAW may impact on the distribution and abundance of *Nucella lapillus* in the broader NE Atlantic region, by the year 2100. The biogeochemical models used by the SS-DBEM were forced using three global emissions scenarios from the 4th and 5th IPCC Assessment Reports (IPCC, 2007, IPCC, 2013) to simulate three possible degrees of future global change. The projected *Nucella* distributions in each scenario were expected to reflect the local impacts of changing abiotic parameters and resource availability over time, given that the low dispersal potential of this species (i.e. low mobility and direct development, Crothers, 1985) would likely limit its ability to track possible changes in the distribution of suitable habitat. The diversity of data and techniques used here was therefore expected to provide a more complete assessment of how species-level impacts of acidification and warming may propagate across to community and ecosystem scales, than could be predicted from individual-level responses alone.

Materials and Methods

Mesocosm setup and experimental exposures

Nucella lapillus individuals were collected from the low intertidal and sub-tidal fringe of the rocky-shore in Mount Batten, in the Plymouth Sound (N 50° 21' 30.29", E -4° 7' 50.07") in

January 2011. All individuals were immediately transported to the PML Intertidal Mesocosm Acidification System (PML-IMAS) within one hour of collection, where they were initially allowed to acclimate to laboratorial conditions in ambient seawater, pH and temperature, for approximately three weeks. Experimental exposure was initiated in February 2011, and lasted 14 months. A detailed description of the mesocosm setup and monitoring parameters for temperature, salinity, pH, total alkalinity, inorganic nutrients and associated calculated carbonate system parameters can be found in Findlay *et al.* (2013) and Table SI. In summary, the PML-IMAS consists of twenty 1 m^3 mesocosm tanks (700 L of seawater and 300 L of overlying atmosphere) set up in four rows of five. Five experimental treatments were haphazardly allocated between the 20 tanks, with four replicate tanks *per* treatment. The PML –IMAS uses a pump and ballast system to simulate a semi-diurnal tidal cycle that followed the monthly local conditions in the Plymouth Sound during the exposure period. The day-night light cycle was simulated to replicate the average amount of hours for each month. Each tank had an individual recirculating pumping and filtration system.

The experimental treatments used corresponded to three CO_2 concentration treatments at ambient temperature, i.e. the "ambient" treatments: 380, 750 and 1000 ppm; and two CO_2 concentration treatments at ambient temperature plus 2^{o}C, i.e. the "warm" treatments: 380 and 750 ppm. Forty-two individuals were haphazardly allocated to each tank, after the initial acclimation period and once experimental conditions had stabilized. Potential shock effects caused by this non-gradual transition into experimental conditions were expected to have been overcome after more than one year of exposure to experimental conditions, when the measurements were conducted. The mesocosm laboratory is a temperature controlled room that was set so that the seawater temperature in the ambient temperature treatment tanks

followed the average monthly sea surface temperature variability at the Western Channel Observatory L4 station, in the Plymouth Sound (fig. S1). These conditions were seen to be a good representation of the bulk temperature variability at the site where the animals were collected. Warm temperature treatments were further regulated by use of 300 W immersion heaters in individual tanks. The level of acidification in each tank was regulated using a pre-mixed gas system modified from Findlay *et al.* (2008). In brief, the desired atmospheric CO_2 concentration was created by mixing pure CO_2 gas with CO_2-free air using flow meters and mixing vessels, monitored with a closed path CO_2 analyser (820, Li-Cor). Each mesocosm tank was bubbled with the desired air or CO_2-air mix, and the seawater was allowed to reach equilibrium. Loss of CO_2 from the overlying "atmosphere" was minimised by thick PVC covers positioned over each tank and effectively separating the tank atmosphere from the room atmosphere. The pH in the control treatments was maintained as closely as possible to the yearly mean pH at L4 (2008-2012), i.e. pH = 8.08 ± 0.07 (mean ± SD), *via* regulation of the CO_2 concentration as above. During the emersion periods, *N. lapillus* individuals were exposed to the desired CO_2 atmosphere and during immersion the organisms were exposed to sea water which had adjusted its carbonate chemistry in response to the atmospheric CO_2 conditions. Therefore, the experimental treatments exposed our animals to some degree of daily variability in pH and temperature associated with the experimental semi-dial tidal cycles that may be seen as a bulk representation of this variability in a true intertidal rocky-shore. Further variability associated with inter-tidal micro-habitats (Helmuth & Hofmann, 2001) would have been difficult to standardize across replicates, and possibly deter from our ability to test the impact of our main experimental treatments.

Activity and predatory behaviour assessment setup

Fourteen months after the beginning of the mesocosm exposures, the basal activity and predatory behaviour of two groups of three individuals from each tank were assessed. The assessment setup consisted of individual 12 x 12 x 40 cm transparent acrylic tanks ("assessment tanks"), enclosing a water layer of approximately 35 cm (0.5 L), and a 5 cm atmosphere (0.07 L). Each tank was placed at one end of a closed 35 x 64 x 90 cm wooden black box, illuminated with an 8 W light. Within each box, at the opposite end, a digital SLR camera (Canon EOS 500 D, 15 MP) was setup to be remotely controlled *via* a PC, using the time-lapse photography software EOS GB time-lapse. The camera enabled the recording of individual behaviour during the assessments (focal distance = 70 cm). Water conditions were manipulated in individual header tanks to reflect those in the mesocosm system in which the individuals had been maintained during the previous 14 months, and supplied to the assessment tanks at approximately 40 mL min^{-1} *via* a peristaltic pump system. In each header tank, regulation of temperature was achieved by use of 100 W immersion heaters. The pH was regulated by gentle bubbling of the desired air (or CO_2-air mix described above) in header tanks and in the assessment tanks, using small aquaria diffusing stones. A closed re-circulation system maintained conditions constant throughout each assessment.

Behavioural assessments

During each assessment (see fig. S2), a randomly selected group of three animals from each tank (n groups $_{total}$ = 40; n groups $_{per\ treatment}$ = 8) was gently lowered to the bottom of the assessment tanks by use of a device made out of nylon mesh and wire, ensuring minimum direct manipulation and disturbance of individuals. Each black box was immediately closed, sheltering individuals from any disturbance related to the presence of observers. Time-lapse

recording of images was initiated immediately, and carried out at five minute intervals for three hours ($n_{images\ per\ assessment}$ = 36). The assessment of individual groups was randomized across treatments over time to avoid confounding of observed behaviours and mesocosm exposure length, as only two groups could be assessed per day. Randomization was achieved using the random number generator package "random" for R (R Foundation for Statistical Computing, Vienna, Austria). Individuals would typically reposition themselves onto the waterline as soon as they were introduced to the assessment tanks, as observed by others (Vadas *et al.*, 1994). Basal activity was therefore measured through the quantification of the overall speed of individuals during their trajectory to the water line at the top of the assessment tank. This behaviour was assessed for 3 hours, as this period has been found to be more than sufficient for individual *N. lapillus* to adjust to the experimental setup and carry out a decision process as to where to place themselves within it (Vadas *et al.*, 1994). Because the presence of one animal in this area appeared to influence the speed and direction of other animals in choosing a location in the tank, "basal activity" henceforth refers to the measurement of the ratio of the distance to time (i.e. "speed") of the first animal to reach the waterline in each assessment. When that animal reached the waterline, movement was recorded only for the time elapsed until then. When all animals failed to reach the waterline during the assessment period, all movements were recorded over the three hours, and basal activity (time and distance, to calculated speed) considered for the individual that initiated movement first. At the end of the activity assessment (3 hours) a prey mimic was gently lowered to the bottom of the assessment tank, using a mesh device as before to minimize interference. The response of individuals to this prey mimic was investigated as a proxy for predatory behaviour. The mimic consisted of a bag of approximately 10 g of fresh live mussels (*Mytillus edulis,* Linnaeus 1758), which were manually crushed and immobilized within a closed double mesh bag immediately prior to the assessment. This standardization of

the prey mimic was required to avoid confounding of the responses associated with a choice of prey based, for example, on prey size (Crothers, 1985). The prey mimic was placed near the diffusing air stone in each assessment tank to maximize the distribution of prey odour cues (fig. S2). "Response time" was recorded as the time taken by the first individual to reach the prey mimic, because the presence of one feeding animal appeared to deter other individuals from approaching the prey mimic. Equally, "foraging distance" was recorded as the overall length of the trajectory covered by the first individual to reach the prey mimic. "Handling time" was calculated as the time during which individual animals were observed directly manipulating the mesh bag containing the prey mimic. When no individuals were able to find the position of the prey mimic, the trajectory considered was that of the most active individual, for all responses to prey. As difficulty in locating food may be an indication of limited chemo-sensory function that has been observed in polychaetes (Schaum *et al.*, 2013), crabs (de la Haye *et al.*, 2012) and fish (Cripps *et al.*, 2011, Dixson *et al.*, 2010, Johannesen *et al.*, 2012) exposed to acidification, we investigated possible mechanisms by which *Nucella* could compensate such potential limitation. To this end, we measured the ratio of foraging distance to prey handling time ("foraging cost") as an indication of the energetic expenditure associated with foraging in relation to the energetic gain associated with feeding. This was calculated to provide an overall energetic cost-benefit metric of predatory behaviour. All assessments were timed to match the introduction of the prey mimic to dusk, when individuals were expected to be most active (Crothers, 1985). Predatory behaviour was assessed for three hours after the introduction of the prey mimic, with images captured at five minute intervals ($n_{\text{images per assessment}} = 36$), as before. At the end of the six hour assessments, all dog whelks were gently removed from assessment tanks, marked, and individual wet weights and lengths recorded, before returning them to the mesocosm system. Prey mimics were euthanized by freezing.

Analysis of time-lapse image sequences

Activity and response to the prey mimic were quantified by digital analysis of the time-lapse image sequences from each trial, using the plugin "Manual tracking", and custom-made scripts, for the open source image analysis software Image J (1.45S, National Institutes of Health, USA). Tracking of each individual trajectory during the assessments enabled the recording of the time and length associated with behaviours here described (see fig. S2 for examples). A total of 36 image sequences were analysed *per* assessment (before and after prey cue addition, $n_{images} = 2596$), excluding cases where software glitches led to image capture failure (4 out of 40 assessments were overall null). Each sequence was analysed three times, to allow the tracking of each individual in the group of three, per assessment. The four outcome variables (basal activity, i.e. speed; response time (to reach prey); foraging distance; foraging cost) were analysed separately using multiple regression and a log-likelihood based stepwise regression analyses for model selection in R. The CO_2 concentration and temperature were considered as main effects and up to first order interaction, and tidal condition at the beginning of each assessment was considered as a covariate. Normality of residuals and homoscedasticity were verified by observation of residual distributions.

Determination of metabolic rates

For heterotrophs, metabolic rate is determined using the rate of oxygen consumption as a proxy. This was measured within two weeks of the behavioural assessments, using stop-flow respirometers (volume 278 mL). Each respirometer contained 20 glass beads (diameter = 1cm) to provide a replica substrate and reducing stress and activity levels. Magnetic stirrers were used to prevent the formation of oxygen partial pressure (pO_2) gradients within the respirometers. The stirrers were separated from the animals by a perforated platform.

Eighteen respirometers were used, and these were divided in to three sets of six; each set was supplied with fully oxygenated seawater from a reservoir, at the desired temperature and CO_2 level matching the respective mesocosm exposure conditions. During the assessment period the temperature was controlled using a recirculating water bath (Grant Cambridge Ltd, Cambridge, UK) monitored using a K type thermocouple inside the respirometers (Omega, HH806AU, Manchester, UK). This provided a water jacket housing the respirometers and cooling coils in the reservoirs. The CO_2 of each reservoir was controlled using the same air and carbon dioxide gas mixes which were used to supply the mesocosm from which the animals had been taken. Sea water was filtered (2.22 µm) and preliminary experiments showed no significant decline in pO_2 within the respirometers in the absence of the animals. Each group of three snails, previously used for the behavioural assessments, was placed in a separate respirometer and allowed to settle under the experimental conditions for 1 h. The respirometers were covered with an opaque plastic sheet to reduce light and disturbance. After 1 h, the flow of sea water though each respirometer was stopped and the decline in pO_2 within each closed respirometer was determined using an OxySense GEN III 5000 series oxygen analyser system (OxySense, Dallas, TX), using the method in Rastrick and Whiteley (2011). Rates of oxygen uptake were calculated as the change in pO_2 h^{-1} from the liner least-squares regression of pO_2 (mbar) plotted against time (h). This was multiplied by the solubility coefficient for oxygen, which was adjusted for salinity and temperature (Harvey, 1955), and the volume of water within each respirometer, taking in to account the volume taken up by each animal. Whole animal values for $\dot{M}O_2$ in μlO_2 h^{-1} were standardised to Standard Temperature and Pressure, Dry (STPD) and expressed as µmol O_2 h^{-1}. Metabolic rates were standardized by biomass, and analysed using multiple regressions and a log-likelihood based stepwise regression analyses for model selection in R, as before.

Susceptibility to predators: analysis of shell integrity

MicroCT scans of shells

To investigate possible shell damage associated with experimental treatments four individuals were randomly selected from the ambient control (380 ppm) and the high CO_2 treatments (1000 ppm) (n=8) at exposure month 14. Individuals were euthanized by immersion in liquid nitrogen, after anesthesia by immersion in an 8% MgCl solution for 12 hours. Specimens were further preserved in dry-ice for air freight, and later stored at -80° C until scanning took place. Images were acquired with a SkyScan 1172 micro-computer tomograph (http://www.skyscan.be/products/1172.htm) at the Hellenic Centre for Marine Research (Crete, Greece). The SkyScan uses a tungsten source and is equipped with an 11 PM CCD camera (4000 × 2672 pixel), with maximal resolution of < 0.8 μm $pixel^{-1}$. Specimens were scanned with a copper and aluminum filter at 100 kV, with a flux of 100 μA, on full 360° rotation and at the highest possible camera resolution. Effective voxel size was 5.5 ± 0.3 μm^3. Projection images acquired during the scanning process were subsequently reconstructed into cross sections (*.png format) with SkyScan's NRecon software which employs a modified Feldkamp's back-projection algorithm. Sections were always reconstructed from the total number of projection images (360°) to maximize detail. Parameters were calibrated between acquired image sets to insure data comparability between individuals – this procedure is hence forth referred to as inter-calibration. The lower limit of the histogram was set at the value of the sample surrounding medium during scans (i.e. air).

Analysis of microCT data

Possible changes in shell density associated with experimental treatments were likely to be more clearly observed in the growing (or newer) edge of shells. Analysis of shell data was therefore primarily centred on image slices corresponding to the upper lip area (top line, fig. S3), where the shell was newer and thinner. This area is henceforth referred to as "lip". Shell damage was also likely to be observed on the surface of shells which, in the absence of a periostracum, were directly exposed to experimental seawater conditions (Rodolfo-Metalpa *et al.*, 2011). Possible changes to the shell surface were therefore investigated focusing on a 0.08 mm deep layer on the surface of each scanned individual. To achieve this, 10 microCT slices corresponding to cross-sections of shells were acquired in the same specific regions of each scanned individual as illustrated in fig. S3. This choice insured a good and comparable coverage of the whole shell between individuals. In each slice, a 15 pixel thick region of interest below the surface of the shell was hand drawn in Image J (pixel size = 5.5 µm). This region is referred to as the "shell surface" in subsequent analysis. The density of the shell surface in each individual was calculated using as a proxy the mean pixel intensity in that region (0 to 255, with high values indicating higher density), across all ten 2D slices, which had been inter-calibrated during reconstruction. The density of the shell in the lip was calculated in the same way, using the whole 2D slice corresponding to that region. Differences in density in each of the parameters (lip and shell surface) between controls and animals from the 1000 ppm CO_2 treatment were compared using one-tailed t-tests. The tests assumed normality and equal variances, as verified *via* Shapiro-Wilk tests and plotting of data dispersion, and used the alternative hypothesis that shell density in the high CO_2 treatment was lower. All data analyses were carried out in R.

Projection of ecosystem-level changes in biogeography

The size-spectrum dynamic bioclimatic envelope model (SS-DBEM) described in Fernandes *et al.* (2013) was used here to project possible changes in biogeography associated with ocean acidification and warming. The SS-DBEM couples the DBEM described by Cheung *et al.* (2011) with a size-spectrum model for resource use based on primary production and temperature (Jennings *et al.*, 2008). The SS-DBEM combines a correlative habitat suitability component with a mechanistic niche component (Kearney & Porter, 2009) to project environmental limits to species distributions, as a result of a transference of the realized species niche (as constrained by the experimental data) to the landscape scale (i.e. the NE Atlantic). Specifically, the correlative habitat suitability component of the model maps out species occurrence to environmental patterns (temperature, depth, substrate type etc.) based on global databases (e.g. sealifebase.org). We complemented this with *N. lapillus* distributional data from the Marine Biological Association of the UK's MarClim project (Mieszkowska *et al.*, *In press*). These were used to define the environmental tolerance range for the species (i.e. its habitat preference profile) based on a set of "filters", including habitat type, depth and latitudinal limits (Close *et al.*, 2006). Current geographic distribution is predicted based these filters. Temperature was not used here as a predictor of current distribution because it was later used to estimate the temperature tolerance and preference of the species (Cheung *et al.*, 2008). On its own, this approach is limited because it does not enable a distinction to be made between direct causality between environment and species distribution, indirect mediation via biotic interactions, and direct response to non-modelled variables co-linear with those considered by the model (Kearney & Porter, 2009, Mac Nally, 2000). Therefore, in addition, the model also includes a mechanistic niche component by which the projected species distribution becomes limited by more factors than just the distribution of suitable habitat. In the mechanistic niche component, change in distribution

and relative abundance (and biomass) caused by changing environmental conditions are simulated by a spatial population dynamic model (Cheung et al. 2011). The spatial and temporal dynamic model is dependent on a set of physiological and ecological response traits, constrained in this case by responses to acidification and temperature observed during the mesocosm experiments, which are used to determine persistence at the meta-population level. In the present study, the model considered changes in resting oxygen consumption (a proxy for metabolic rate), adult mobility (i.e. speed, as a proxy for dispersal potential), growth, length-weight relationship (a proxy for condition), adult and juvenile mortality, and larval dispersal, as measured in response to temperature and acidification. These traits were calculated per treatment level. Change in resting oxygen consumption with temperature (eV) and mobility (i.e. $cm.h^{-1}$) were calculated at 14 months based on the mesocosm measurements already described. Mortality of adults and juveniles (F1 hatched in the laboratory from the same adults described above) was calculated as an overall *% per* treatment, based on the 14 month mesocosm experiments. Larval dispersal was considered to be negligible as *N. lapillus* is a direct developer. Growth rates were calculated as the difference (%) in weight increment ($g\ day^{-1}$) between each treatment and the control (ambient temperature and 380 ppm of CO_2), superimposed on the von Bertalanffy growth equations for *Nucella* in Selin (2010). Growth and length-weight relationships were calculated here using data generated by a parallel 12 month experiment on individuals of the same wild population, which used the same experimental treatment levels and supporting equipment, carried out in the mesocosm facilities of the Marine Biological Association of the UK. A schematic diagram of the model structure and input parameters is illustrated in figure S4.

The environmental forcing for the SS-DBEM (i.e. the environmental parameters, or habitat conditions) was projected for the NE Atlantic region using two spatially and temporally resolved biogeochemical models: the Proudman Oceanographic Laboratory Coastal Ocean Modelling System – European Regional Seas Ecosystem Model (POLCOMS-ERSEM), and the Nucleus for European Modelling of the Ocean – Model of Ecosystem Dynamics, nutrient Utilisation, Sequestration and Acidification (NEMO-MEDUSA 2.0) documented in Artioli *et al.* (2014) and Yool *et al.* (2013). POLCOMS-ERSEM has a track record for performance in regional seas (Allen & Somerfield, 2009, Shutler *et al.*, 2011), while NEMO-MEDUSA is a large-scale global ocean model. Together, they therefore provided a complimentary approach to the simulation of biogeochemical conditions. The two models were parameterized according to three global emissions scenarios (IPCC, 2007, IPCC, 2013) to simulate three possible futures for *Nucella*. The future emissions scenarios considered were: 1) AR4 A1B with a CO_2 equivalent around 700 ppm ("business-as-usual" , IPCC, 2007); 2) AR5 RCP2.6 with a CO_2 equivalent around 400 ppm ("lower emissions" , IPCC, 2013); and 3) AR5 RCP8.5 with a CO_2 equivalent around 1250 ppm ("higher emissions" , IPCC, 2013). In each case, the SS-DBEM was forced for a specific 20 year biogeochemical simulation corresponding to present time (1981-2000) and end of the century (2081-2100). The first five year spin-off period was discarded from further analysis while the subsequent fifteen years were averaged to account for the expected inter-annual natural variability. The biogeochemical model runs simulate not only the landscape-scale habitat conditions (including temperature and pH) but also the resources available in each point in time and space. I.e., primary production (as simulated by the biogeochemical models) and the predicted habitat suitability from other environmental factors were used as a proxy for the carrying capacity of the ecosystem at each point. For a set group of neighbouring points at each specific time point, the SS-DBEM simulates that *Nucella* will use more resources from

primary production where habitat is more suitable. The SS-DBEM was parameterized using the measured changes in *Nucella* traits in relation to the CO_2 concentration and temperature levels observed in the experimental treatments. When no statistically significant differences were found between treatments, model parameters were calculated as the overall mean value for each measured trait.

In each of the three IPCC scenarios used, we ran the SS-DBEM three times, allowing model parameters to vary according to acidification, warming or both effects, using all of our experimental trait data simultaneously. These runs were compared to highlight potentially distinct effects of acidification and warming in the diversity of parameters considered by the SS-DBEM. The final SS-DBEM model grid had a 0.5 ^{o} latitude by 0.5 ^{o} longitude resolution (approximately 56 km^2 depending on latitude). Detailed descriptions of the models used are found in Cheung *et al.* (2011) and Fernandes *et al.* (2013).

Results

Impacts on resting oxygen consumption and basal activity

Ocean acidification and warming had distinct effects in the resting oxygen consumption of *Nucella* (here used as proxy for metabolic rate). At ambient temperature, resting oxygen consumption (MO_2) decreased steadily with increased CO_2 exposure, but in warm treatments this parameter was significantly higher and invariable with CO_2 concentration: $MO_2 = 16.95 + 6.33 \times \text{temperature} - 0.01 \times CO_2$, $R^2 = 78.49\ \%$ with $F_{2,25} = 45.63$ and $p < 0.01$, fig. 1a. This pattern of impact was only partially mirrored in individual basal activity (i.e. speed, fig.

1b, for which the univariate regression using metabolic rate as a predictor yielded R^2 = 78. 49 %, $F_{1, 9}$ = 4.39 and $p < 0.10$). Acidification in the absence of warming did lead to decreased activity, but under warming conditions *Nucella* was as active as in control treatments (Amb 380, fig. 1b) regardless of acidification levels (fig.1b). A significant amount of variability observed between individuals could not be explained by the experimental treatments (R^2 = 32.15 %, table I and fig. 1b).

Impacts on predatory behaviour

The impact of experimental treatments on the predatory behaviour parameters measured here were significant but complex (table I, fig.1c-f). In the presence of food (prey mimic), foraging time (i.e. "response time", table I, figure 1c) was highly variable, causing no significant impact on the mean responses across individuals. Individuals from the worst acidification scenario were, however, found to cover significantly greater distance to find food in the absence of warming ("foraging distance", table I and fig. 1d), and this variable did not exhibit a clear pattern in other treatments, regardless of temperature. We also found that the amount of time spent feeding ("handling time") appeared to trail the increase in distance covered to find food, despite inter-individual variation. Consequentially, a pattern emerged when foraging cost was calculated (the ratio of handling time to foraging distance). With increased acidification, and independent of temperature, an increase in the amount of time spent feeding exceeded the corresponding increase in distance covered to find prey, leading to a decrease in foraging cost (figs. 1e and f, $p < 0.05$ and R^2 = 52.30 %, table I).

Impact on susceptibility to predation

The analysis of the microCT data revealed profound changes in shell morphology concurrent with acidification (fig.2). Dissolution at the shell apex, irregular definition of whorls and the disappearance of the natural ornamentation pattern with increased acidification (3D reconstructions, fig.2) were consistent with a 20-30% decrease in shell density in the shell lip ($t_6 = -1.80$ and $p < 0.10$) and in the overall shell surface ($t_6 = -2.32$ and $p < 0.05$).

Biogeographical projections for the end of the century

We used a state-of-the-art dynamic bioclimatic envelope model (SS-DBEM) to explore how the mechanisms highlighted by our species-level experimental results scaled through to the ecosystem, considering different emission scenarios and model structure. Overall, higher emissions led to greater reductions in the abundance of *Nucella lapillus* across all areas (fig.3). By 2100, the abundance of *Nucella* in the NE Atlantic shelf coasts would have decreased as an effect of OAW by 66.9 ± 16.8 % (mean ± SD) across all areas (in relation to present day), in business-as-usual and higher emissions scenarios (fig.3 a-d and i-l). Alternatively, abundance could increase marginally in the same period under a lower emissions scenario (1.22 ± 0.78 %, fig.3e-h). The response of the different species traits (i.e. model parameters) to variations in each of the stressors considered over space and time (temperature and CO_2, fig. 3 b-c, f-g and j-k), or of their combination (fig. 3 d, h and l) means that the projected distributional changes are spatially heterogeneous. In the northern UK and Irish coasts, the projected decrease in abundance associated with OAW (in relation to present day) is similar for business-as-usual and higher emissions scenarios for 2100 (by 63.58 ± 4.88 %, fig.3d and l), but in all other coasts abundance may fall by an additional 37.06 ±

4.88 % in the worst scenario (fig.3h). In a future where emissions continue to occur in business-as-usual, the greatest decrease in abundance may occur in the NE coast of the UK (fig. 3d), while in a higher emissions scenario, areas further south would suffer the greatest impacts (fig. 3l). In some areas of the coastline along the English Channel and in the western coast of France, smaller changes in the future distribution of *Nucella* were projected when all model parameters responded to OAW (right column, fig. 3 d, h and l) than when they responded to only one of the individual stressors (second and third columns, fig. 3 b-c, f-g and j-k), at and below business-as-usual emissions levels. In other areas, like the NE of England, the reverse was true, at and above business-as-usual emissions levels (fig. 3). The SS-DBEM projections also indicated that resource availability may be an important factor determining the extent of distributional changes over time. Specifically, the projections indicated that, with the exception of the most extreme higher emissions scenario, *Nucella* would likely be able to meet increased energetic demand associated with OAW in areas with high productivity, such as the German and Dutch coasts (fig.3d and l). However, in less productive areas, like the East coast of England, resource depletion may prevent persistence under OAW.

Discussion

This study shows how environmental stressors impact the ecology of individual species across several layers, and that these are not easy to summarize. Using a diverse range of experimental analyses, we provide an integrated insight into how multi-stressor impacts may be complex and distinct from those expected by the sum of single stressor impacts. Temperature appeared to be the key factor regulating basal physiology and activity, but when

predator-prey interactions were considered, acidification appeared to play an important role too. Furthermore, our macro-scale modelling indicated that the aggregated responses measured at the individual level may lead to substantial change to the future distribution of *Nucella* in the NE Atlantic region by 2100, with concomitant impacts for the dynamics of these rocky-shores. This distributional impact will depend on the magnitude of environmental change (i.e. emissions scenario considered). It will also depend on the future distribution of resources, and on local variation of particular stressor combinations acting on many aspects of *Nucella* ecology together.

Individual level responses

Our results on basal activity and resting metabolic rate lend support to the perspective that animals are able to improve survival under adverse conditions caused by acidification alone by reducing metabolism (Calosi *et al.*, 2013, Reipschläger *et al.*, 1997) and specifically during periods of zero energy gain (i.e. rest, Brown *et al.*, 2004). However, temperature appeared to have an overriding effect on both of these parameters, as with concurrent warming, no effect of acidification was apparent. Warming increased mean resting metabolic rates and lead to variable activity levels, regardless of the exposure to different levels of acidification used in this study. After fourteen months of warming, increased metabolic rates in *Nucella* may indicate an increase in energy demand to sustain basic cellular functions, which may lead to trade-offs by which, at this stage, less energy may be available to other non-vital functions (like reproduction). The identification of exactly which of those individual processes are negatively impacted by potential trade-offs would however have required further investigation. Variability in activity (a proxy for overall performance) may reflect inter-individual differences associated with higher maintenance and repair costs, as seen by others, when metabolic rates are high (Calosi *et al.*, 2013). Both results indicate that the individual

level impacts of ocean acidification are significantly different when warming was also considered, illustrating how responses to multi-stressor environments cannot be predicted from the analysis of individual stressor impacts alone.

Good prey, bad predator: bad news for rocky-shore communities

We expected that *Nucella* would require more time to find prey with increased acidification, as individuals were significantly less active in these treatments. However, we found that the time needed to find food did not increase with acidification. Alternatively, we found that far greater distance was covered to find food in the worst acidification scenario, despite of temperature. Greater foraging distance may be indicative of a lowered ability of *Nucella* to locate food when CO_2 concentration was high, consistent with limited chemo-sensory function observed in polychaetes (Schaum *et al.*, 2013), crabs (de la Haye *et al.*, 2012) and fish (Cripps *et al.*, 2011, Dixson *et al.*, 2010, Johannesen *et al.*, 2012) exposed to acidification. We measured foraging cost (the ratio of foraging distance to prey handling time) as a means to determine whether, after 14 months, predatory behaviour had changed to compensate for this possible chemo-sensory limitation. We found that, overall, foraging cost decreased with increased acidification given that a concurrent increase in the observed amount of time spent feeding (i.e. prey handling time) far exceeded the corresponding increase in distance covered to find prey, with and without warming. Thus, the predatory behaviour of *Nucella* did appear to change after 14 months in a way that is consistent with a strategy to cope with a higher energetic expenditure associated with finding food (i.e. greater foraging distance) in acidified conditions, possibly triggered by limited chemo-sensory function. Thus, when predatory behaviour was considered, acidification appeared to be a more important regulatory factor than temperature. Considering only the responses measured in the absence of a prey mimic (i.e. resting metabolic rate and basal activity), we could have

over-looked this potentially significant survival strategy, and the potentially important role of ocean acidification in the overall ecology of *Nucella* in a near-future ocean.

Changes in chemo-sensory function and predatory behaviour have implications also for the susceptibility of *N. lapillus* to predators. This species is known to shorten the amount of time spent foraging outside of refuges, feeding less and choosing to feed on prey in secluded crevices, in the presence of predators (Trussell *et al.*, 2003). This predator avoidance behaviour has also been observed in other gastropods, even when exposed to acidification (Manríquez *et al.*, 2013). Predator avoidance behaviour is, however, not compatible with a need to increase feeding time to compensate the apparent higher energy expenditure associated with finding prey for a chemo-sensorially impaired *Nucella.* It is thus possible that the presence of predators in a community context may inhibit *Nucella* from developing the predatory behavioural modifications we observed in OAW conditions in our experiments. Or, if such modification of *Nucella* predatory behaviour should develop, it may lead to increased mortality by predation. Additionally, the analysis of microCT shell data indicated significantly decreased shell density in acidified treatments, which may be indicative of greater susceptibility to physical damage as a consequence of encounters with predators. Along with shell morphology, shell robustness is a key defence of *Nucella* (and other species, McDonald *et al.*, 2009) against crushing predators like crabs. In fact, stronger shells correlate with higher survival rates in *Nucella* because they require a greater energetic investment and longer handling time for breakage, both of which tend to lead the crabs away, in search of easier prey (Hughes & Elner, 1979). Thus, together, these two results paint a bleak future for *Nucella,* and suggest that significant changes may occur in temperate rocky-shore communities as a consequence of OAW. This is because *Nucella* and its predators exhibit significant influence on the abundance of mussels and canopy forming algae (Trussell *et al.*,

2003 and references therein), both of which are habitat-forming species that have a regulating role in controlling rocky-shore biodiversity (Bulleri *et al.*, 2002, Seed, 1996). Our findings agree with others that also found evidence for the relevance of nervous-system level impacts of ocean acidification for predator-prey interactions in rocky-shores (Watson *et al.*, 2014), but contrast with Landes & Zimmer (2012), who found no change in predator-prey interactions with OAW in a similar ecosystem. Long-term studies of the kind presented here are resource intensive, but are crucial to understand the importance of bottom-up and top-down mechanisms for the propagation of species-level impacts of climate change to community level. However, more conclusive insights might have been obtained in the present study if both prey and predator of *Nucella* had also been maintained in the same exposures, thus unravelling how their own species-level responses to OAW would have modified the predator-prey interactions. Such long-term, multi-stressor, multi-species studies will significantly help drive the field in the future, by helping to elucidate the true impacts of climate change in complex community settings.

Ecosystem-level considerations

Our results, combining individual based measurements, predatory behaviour, susceptibility to predation and modelling, suggest that OAW may lead to substantial, non-additive and complex changes in community dynamics of NE Atlantic rocky-shores within the next 100 years. However, despite its achievements, this study identifies the challenge of predicting ecosystem level climate change impacts based on experimental studies that consider only single responses of individual species in isolation. Different stressors appeared to have greater relevance or impacts in different aspects of *Nucella* ecology, indicating that climate change impacts species across many different levels, but that these responses do not

necessarily follow the same trends. Our results, however, provide a more realistic representation of the true ecosystem level impacts associated with *Nucella*, because we combined a range of species and community level processes simultaneously, and summed effects were estimated using a complex modelling framework.

The importance of local scale forcing on these processes, as revealed by the analysis of the SS-DBEM projections, advises caution about the extrapolation of experimental findings on their own to investigate large scale questions, particularly when studies consider only a small number of individual level responses. For example, it would have been incorrect to assume, based only on the presently observed decrease in foraging-cost for *Nucella* with increased acidification, that the distributional range of this species will expand because average oceanic CO_2 concentrations are and will continue to rise. As we show, large-scale distributional changes will occur as a result of multi-stressor patterns and resources changing locally across the landscape, in a heterogeneous way. Therefore, projection of ecosystem-level consequences of climate change requires a better integration of both macro-scale and local-scale information, about biotic and abiotic drivers, and species ecology. While the SS-DEBM quantifies possible impacts on the use of resources available in the environment primarily as described by size-spectrum theory (Jennings et al. 2008), it does not account for the inter-specific relationship between *Nucella*, its prey and predators explicitly analysed here, and the responses of such relationships to climate change. On the other hand, our experiments indicate that the impact of acidification on the predatory behaviour of *Nucella* could have a significant role also in its ability to acquire food. While the present study represents a significant development in the use of individual level experimental data in an ecosystem level

application, future research may require future model developments that can accommodate such specific information.

The parameterization of the SS-DBEM with experimental data is challenging, requiring expertise in a diversity of subject areas to enable parameter calculation (physiology, behaviour, population dynamics) in addition to that required to run the model. It is the research taking place within those disciplines, with single and multi-stressors, that drives our understanding of the mechanisms of impact that the model aims to capture. Thus, good communication between the modeller and specialists in each of those fields of research is paramount to successful model parameterization, insuring that both model behaviour and assumptions taken are plausible. For example, our projections are based primarily on experimental and observational information gathered within one species population, which is likely adapted or acclimated to a specific set of local environmental conditions (Calosi *et al.*, 2008). We considered whether it was plausible to extrapolate this knowledge to the larger geographical area considered in our simulations. The reason for this is that it is possible that a different population of the same species could have shown some degree of variability in the responses we measured (Findlay *et al.*, 2010 and references therein). Because we measured a large number of ecologically meaningful parameters, it was considered that small differences in specific responses between populations would be diluted in our integrated analysis, and thus that our extrapolation was reasonable. However, for different species and simulations, if those differences are known and sizeable, then they should be considered.

The diversity of data used here is becoming increasingly available, given that the need for long-term, multi-species, multi-stressor experimental climate change research is gaining recognition. Our inter-disciplinary approach integrates this knowledge, providing a more holistic assessment of the effects of OAW than can be derived from assessments carried out within individual disciplines. In doing so, DBEMs also enable the testing of climate impact scenarios on marine species in the context of the ecosystem, at scales that are more relevant to management than those at which empirical and experimental science tend to operate (e.g. decades c.f. a few years). Furthermore, changes in the distribution of individual species (as modelled here for *Nucella*) can be done in a multi-species context, to predict how climate will impact marine biodiversity across the land-scape (Cheung *et al.*, 2009). Biodiversity loss is perhaps an issue more easily communicated to managers and stake-holders of the marine environment than, for instance, the physiological impacts of OAW on specific species. As biodiversity underpins regulating, production, provisioning and cultural ecosystem services (Armstrong *et al.*, 2012, Raymond *et al.*, 2009), this approach may be a successful route to scale experimental climate change research to the wider socio-economic context. Thus, as noted also by others (Metcalfe *et al.*, 2012, Norman-López *et al.*, 2013) it is timely for physiologists, ecologists and numerical modellers to take advantage of such integrative routes to increase the impact of experimental climate change science, beyond speciality fields.

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Tables

Table I: Regression models for responses of activity and predatory behaviour to experimental treatments, after fourteen month long mesocosm exposures to ocean acidification and warming (S.I. 2). Model selection was carried out using a log-likelihood based stepwise procedure. "NA" model structure indicates response variables for which none of the experimental factors and covariate considered provided a better fit than the null model. "df": degrees of freedom.

	Variable	Model structure	df	F	p	R^2 (%)
Basal activity	speed	CO_2 concentration	2, 22	5.21	< 0.05	32.15

	response time	NA	24	0.00	> 0.05	NA
Predatory behaviour	foraging distance	CO_2 concentration x tide	3,23	4.99	<0.01	39.41
	foraging cost	~ CO_2 concentration	2,9	4.93	<0.05	52.30

* Animals only actively sought food at high tide.

Figure Legends

Figure 1: Effects of ocean acidification and warming on individual level responses (a and b) and predatory behaviour (c-f) after fourteen month long experimental exposures.

Figure 2: Micro-CT reconstructions of *Nucella lapillus* shells. Top panel: 3D reconstructions of individuals from control treatments (top row), exhibiting normal, reticulated shell ornamentation. Bottom row shows individuals from the most extreme acidification treatments exhibiting loss of natural ornamentation pattern, worn apex and shallow whorl definition (arrows). Bottom panel: 2D detail of inter-calibrated cross-sections of the lip of the shell of control individuals (top row) and from ambient 1000 ppm CO_2 treatment (bottom), using a 16 colour mask to enhance differences in shell density. Warm colours indicate high density materials (yellow) and cold colours (blue) indicate low density.

Figure 3: SS-DBEM biogeographical projections for *Nucella lapillus* abundance in the present (1986-2000, left, a, e, and i); and future (2086-2100, all other columns), when model parameters are adjusted to respond to changes in temperature (second from left, b, f and j), ocean acidification (third from left, c, g and k) and both (right, d, h and l). The colouring of the plots is the fifteen year average within each cell, indicating abundance standardized relative to the present in scenario A1B (a), varying from 0 (white) to 1 (sky blue). The numbers plotted in red are the % change in *Nucella lapillus* abundance in the future scenarios in relation to the present distribution in each region (red lines), when model parameters respond to acidification and warming simultaneously. Rows correspond to model runs using: POLCOMS-ERSEM 4th IPCC special report emissions scenario A1B "business-as-usual" (top row, a-d); and NEMO-MEDUSA 2.0 using the 5th IPCC special report emissions scenarios AR5 RCP2.6 "lower emissions" (second row, e-h) and AR5 RCP8.5 "higher emissions" (bottom row, i-l).