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Biological responses of two marine organisms of ecological relevance to on-going ocean acidification and global warming☆

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ABSTRACT

Recently, there has been a growing concern that climate change may rapidly and extensively alter global ecosystems with unknown consequences for terrestrial and aquatic life. While considerable emphasis has been placed on terrestrial ecology consequences, aquatic environments have received relatively little attention. Limited knowledge is available on the biological effects of increments of seawater temperature and pH decrements on key ecological species, i.e., primary producers and/or organisms representative of the basis of the trophic web. In the present study, we addressed the biological effects of global warming and ocean acidification on two model organisms, the microbenthic marine ciliate *Euplotes crassus* and the green alga *Dunaliella tertiocleta* using a suite of high level ecological endpoint tests and sub-lethal stress measures. Organisms were exposed to combinations of pH and temperature (TR1: $7.9_{[pH]}$, 25.5 °C and TR2: $7.8_{[pH]}$, 27,0 °C) simulating two possible environmental scenarios predicted to occur in the habitats of the selected species before the end of this century. The outcomes of the present study showed that the tested scenarios did not induce a significant increment of mortality on protozoa. Under the most severe exposure conditions, sub-lethal stress indices show that pH homeostatic mechanisms have energetic costs that divert energy from essential cellular processes and functions. The marine protozoan exhibited significant impairment of the lysosomal compartment and early signs of oxidative stress under these conditions. Similarly, significant impairment of photosynthetic efficiency and an increment in lipid peroxidation were observed in the autotroph model organism held under the most extreme exposure condition tested.

1. Introduction

As a consequence of the industrial revolution, the atmospheric concentration of carbon dioxide (CO_2) is constantly increasing (Feely et al., 2004). A significant fraction of anthropogenic CO_2 is absorbed in the oceans (Canadell et al., 2007; Sabine et al., 2004), causing changes in the chemical and physical state of this gas and alterations in a wide range of abiotic and biotic processes. Any increase in the level of atmospheric carbon dioxide shifts the carbon dioxide–carbonate equilibrium in water in the acidic direction. Consequently, the oceans are acidified (for reviews on ocean acidification, see Fabry et al., 2009; Feely et al., 2009). Observations and models indicate that the average pH of the surface ocean has declined from 8.2 by 0.1 units since pre-industrial times as a result of CO_2 emissions and is projected to be approximately pH 7.8 by the end of this century (Raven et al., 2005). The ecological impact of ocean acidification resulting from the uptake of anthropogenic emissions of carbon dioxide is a relatively new concern for the scientific community and has attracted significant and increasing interest. However, while considerable emphasis has been placed on terrestrial ecology consequences, including forest repositories of

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carbon dioxide (Helliwell et al., 2014; Malcolm et al., 2014), the effects on marine environments have received little attention. Studies have primarily addressed reductions in coral calcification (Kato et al., 2014; Movilla et al., 2012), the survival, growth and shell integrity of crustaceans and other invertebrates (Bressan et al., 2014; Punt et al., 2014; Clements and Hunt, 2014) and the induced effects on fitness and/or unpaired development of fishes of economic value worldwide (Bromhead et al., 2015). However, little is known regarding the biological effects of seawater pH decrements on key ecological species, i.e., primary producers and/or organisms representative of the basis of the trophic web (Bermudez et al., 2015; Feng et al., 2017; Liu et al., 2017; Riebesell et al., 2017). Nevertheless, potential negative effects on these organisms may lead to a loss of biodiversity and irreversible changes in ecological structure, functioning and services (Mostofa, 2016; Sweetman and Chapman, 2015; Williamson et al., 2017). Indeed, autotrophic phytoplankton is an important carbon dioxide consumer group (Falkowski, 2012; Field et al., 1998). Since photosynthesis is the major consumer of carbon dioxide, any induced changes of phytoplankton abundance significantly affect the overall carbon dioxide balance. However, the current understanding of the potential effects of acidification on carbon dioxide removal from the atmosphere by phytoplankton is poor (Cermeño et al., 2008).

As well as the potential threat from ocean acidification, seawater temperature increases due to climate change may lead to increases in the metabolic rate of organisms. This aspect further increases the mismatch between primary production and respiration (Hoegh-Guldberg, 2010). The combination of these affects has the potential to generate crucial consequences for the microorganism community, thereby leading to changes in biogeochemical cycles and energy mediation between trophic levels and is therefore worthy of further investigation.

With climate change and ocean acidification becoming major concerns, additional mechanistic studies in aquatic research are needed to address potential interactions between organisms and observed or predicted changes in environmental conditions. These studies are required to increase the general awareness of how environmental parameters in aquatic environments may affect the precious balance of aquatic food webs with far-reaching consequences to the Earth's carbon dioxide level, marine conditions, and aquatic ecosystems. In this context, we assessed the biological effects of combined seawater increments of temperature and decrements of pH on two model organisms.

The green alga *Dunaliella tertiocleta* was selected as a representative species of marine primary producers, while the marine ciliate *Euplotes crassus* was selected for its key role as a heterotroph species in microbenthic environments. Growth rate, PSII efficiency and oxidative stress were investigated in the autotroph *D. tertiocleta*. In the eukaryote *E. crassus,* measurements of cell viability and replication rate were combined with the stress biomarkers, lysosomal membrane stability, lipofuscin accumulation and phagocytosis rate (Moore et al., 2006).

2. Materials and methods

2.1. Cell culture

Cells of *Euplotes crassus* were isolated from samples collected in the Tyrrhenian Sea (Italy) and grown under controlled laboratory conditions. Organisms were cultured in standardised artificial seawater (ASTM D1141-98; 34‰, pH = 8.1, 24 ± 0.5 °C) in an incubator chamber FTC 90 (VELP Scientifica Spa, Milan, Italy). Cultures were grown under dark conditions with an oxygen concentration ≥8.5 mg/L and fed weekly with *D. tertiocleta* (900 cells/mL). The analyses on ciliated protozoa were then performed during the logarithmic growth phase.

D. tertiocleta cells were isolated from water samples from the same geographical area and subsequently identified according to Preisig (1992). The obtained axenic algal culture was grown in ASTM artificial seawater (ASW, ASTM, 2013) enriched with a sterilised liquid nutrient medium according to Lain (1991). Cultures were renewed weekly by inoculating 1 mL of algae culture into 250 mL of freshly prepared growth medium. The flasks were maintained in a growth chamber (FTC 90E, VELP Scientifica, Italy) at 22.0 ± 0.5 °C. To assure optimal culture growth conditions, continuous uniform illumination with a quantum flux of 0.35 × 1020 photons/m2/s ± 20 per cent in the spectral range 400–700 nm was provided by cool white fluorescent lamps.

2.2. Experimental design

To simulate scenarios of the interconnected effects of climate change and ocean acidification experienced by aquatic organisms within the end of this century, two different combinations of temperature and pH were set up according to the projections of the climatological model developed under the European Project on OCean Acidification project (EU-EPOCA, Gattuso et al., 2011). The CO_2 calculation routine CO_2SYS was used to calculate the parameters of the seawater carbonate system and assist the design of ocean acidification perturbation experiments (Bellerby et al., 2005, 2012; Lavigne and Gattuso, 2011, Table 1). The pH scale was considered as the total scale (mol/kg-SW), and carbonic acid constants were considered according to Mehrbach et al. (1973) and refit according to Dickson and Millero (1987). KSO_4 was calculated according to Dickson (1981). The results of carbonate system simulation are provided as Supplementary Electronic Material (SEM).

Two different combinations of pH and temperature were tested within the experimental phase to simulate the predicted trend of ocean acidification and warming: $7.9_{[pH]}$ @ 25.5 °C (TR1) and $7.8_{[pH]}$ @ 27.0 °C (TR2). These parameters were selected according to the outcomes of the climatological model established to simulate average changes of oceanographic chemical-physical conditions of the north west Mediterranean basin within the next century. Control organisms were maintained at $8.1_{[pH]}$ and 24 °C, as conditions typically recorded in the sea surface temperature of the Tyrrhenian basin during the summer (Brunetti et al., 2014; Macias et al., 2013). All glass and plastic ware used within the experiments were cleaned prior to use by overnight soaking in 1 M HNO_3 followed by rinsing three times in MilliQ, dried in laminar flow hood and treated with UV light for 2 h.

Experiments were performed in an open unsterile system comprising a 6-L polyethylene tank filled with ASW, which was maintained in continuous agitation using two stirring bars (Fig. 1). An integrated pH/thermometer was remotely connected to open a line of analytical grade CO_2 using an electro-valve set up when the ASW pH exceeded the setup value (Fig. 1, NESA Srl, Italy). The pH/thermometer probe was calibrated prior to each test according to Bresnahan et al. (2014) and Dickson et al. (2007). The entire system was placed in an incubator provided with internal light 60 μE/m2/s (PAR) to perform tests with algae. System status, temperature and pH profiles were continuously recorded using internal data loggers to provide accurate data for QC/QA purposes. The entire data set is available as SEM. Test organisms were placed in modified 50-mL PP Falcon tubes (Corning, USA). Briefly, the conical bottom was removed and replaced with a PTFE membrane disc filter (Pall, USA) of two different pore sizes, to maintain confined protozoa (10 μm) or algae (0.1 μm). This design enabled the optimal medium circulation inside all tubes. Both ciliated protozoa and algae were previously acclimatised for 7 days to each testing pH and temperature combinations by first increasing the medium pH and temperature