Science of the Total Environment 643 (2018) 946–956

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of ocean acidification on the levels of primary and secondary metabolites in the brown macroalga *Sargassum vulgare* at different time scales

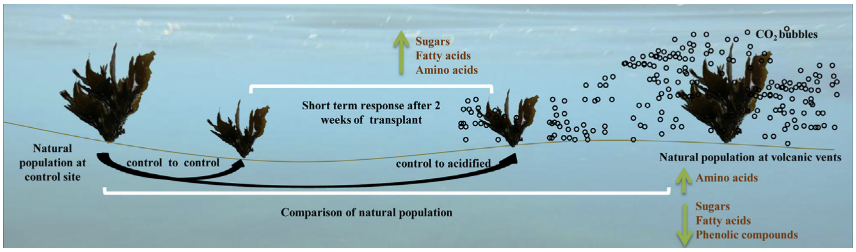
Amit Kumar [a,b,1], Hamada AbdElgawad [c,d,1], Immacolata Castellano [e], Samy Selim [f,g], Gerrit T.S. Beemster [c], Han Asard [c], Maria Cristina Buia [a,*], Anna Palumbo [e,*]

[a] *Center of Villa Dohrn Ischia – Benthic Ecology, Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, P.ta S. Pietro, Ischia, Naples, Italy*
[b] *Centre for Climate Change Studies, Sathyabama Institute of Science and Technology, Chennai, India*
[c] *Integrated Molecular Plant Physiology Research Group (IMPRES), Department of Biology, Groenenborgerlaan 171, University of Antwerp, Antwerp, Belgium*
[d] *Faculty of Science, Department of Botany, Beni-Suef University, Beni-Suef, Egypt*
[e] *Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy*
[f] *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka P.O. 2014, Saudi Arabia*
[g] *Microbiology and Botany Department, Faculty of Science, Suez Canal University, Ismailia P.O. 41522, Egypt*

HIGHLIGHTS

- *Sargassum vulgare* growing at CO_2 vents was compared with those growing at control site.
- *S. vulgare* from control site was transplanted to CO_2 vents for 2 weeks.
- In short-term responses, *S. vulgare* showed increased level of sugars, PUFAs, and EAAs.
- Natural population at vents showed decreased sugars, PUFAs, phenols, and increased EAAs.
- Nutritional values of algae will decrease under acidification in long time scale.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 April 2018
Received in revised form 13 June 2018
Accepted 14 June 2018
Available online xxxx

Editor: Kevin V. Thomas

Keywords:
Macroalgae
Ocean acidification
CO_2 vents
Transplants
Primary and secondary metabolites

ABSTRACT

Most of the studies regarding the impact of ocean acidification on macroalgae have been carried out for short-term periods, in controlled laboratory conditions, thus hampering the possibility to scale up the effects on long-term. In the present study, the volcanic CO_2 vents off Ischia Island were used as a natural laboratory to investigate the metabolic response of the brown alga *Sargassum vulgare* to acidification at different time scales. For long-term effects, algal populations naturally growing at acidified and control sites were compared. For short-term responses, *in situ* reciprocal transplants from control to acidified site and *vice-versa* were performed. Changes in the levels of sugars, fatty acids (FAs), amino acids (AAs), antioxidants, and phenolic compounds were examined. Our main finding includes variable metabolic response of this alga at different time scales to natural acidification. The levels of sugars, FAs, and some secondary metabolites were lower in the natural population at the acidified site, whereas the majority of AAs were higher than those detected in thalli growing at control site. Moreover, in algae transplanted from control to acidified site, soluble sugars (glucose and mannose), majority of AAs, and FAs increased in comparison to control plants transplanted within the same site. The differences in

---

* Corresponding authors.
E-mail addresses: mariacristina.buia@szn.it (M.C. Buia), anna.palumbo@szn.it (A. Palumbo).

[1] Contributes equally.

https://doi.org/10.1016/j.scitotenv.2018.06.176

the response of the macroalga suggest that the metabolic changes observed in transplants may be due to acclimation that supports algae to cope with acidification, thus leading to adaptation to lowered pH in long time scale.

1. Introduction

Over the last few decades, increased atmospheric carbon dioxide (CO_2) levels and temperatures resulting from anthropogenic activities have become a global concern (Meyer et al., 2014). As significant portion of this atmospheric CO_2 is continuously absorbed by the oceans, a shift in seawater carbonate chemistry is occurring resulting in a lowering of pH, a process known as ocean acidification (OA, Sabine et al., 2004). A multitude of marine organisms across different trophic levels are negatively affected by OA (Garrard et al., 2013; Harvey et al., 2014). In general, marine photoautotrophs are believed to benefit from the increased dissolved CO_2 as it is a substrate for photosynthesis. Indeed, growth of several non-calcifying seaweeds and some seagrasses are enhanced by OA conditions (Koch et al., 2013). However, the responses are species specific, even among closely related species due to variations in carbon acquisition strategies from seawater (Wu et al., 2008; Zou and Gao, 2009; Mackey et al., 2015). Moreover, benefits of additional CO_2 are compromised due to subsequent lowering of surrounding seawater pH (Britton et al., 2016). As a result, other physiological and biochemical processes of autotrophs such as reproduction, early life stage development, ion homeostasis, energy demand, nutrient uptake, and enzymatic activities are affected under OA conditions (Roleda et al., 2012; Hofmann et al., 2013; Gutow et al., 2014; Fernández et al., 2015; Nunes et al., 2015; Xu et al., 2015; Kumar et al., 2017a, b; Leal et al., 2017).

Seaweed response to OA is quite variable and depends on several factors such as carbon uptake mechanisms and developmental stage as well as the experimental approaches, laboratory scale/*in situ* or free ocean CO_2 enrichment, FOCE (Hurd et al., 2009; Koch et al., 2013; Korbee et al., 2014; Betancor et al., 2014; Celis-Plá et al., 2015; Xu et al., 2015; Cornwall and Hurd, 2015). In this context, natural CO_2 vents (*e.g.* Ischia, Vulcano, Paupa New Guinea, Methana) offer a unique opportunity to investigate the response of seaweeds to OA both in natural populations and in transplants (Porzio et al., 2011; Johnson et al., 2012; Celis-Plá et al., 2015; Kumar et al., 2017a; Porzio et al., 2017; Kumar et al., 2017b). Most studies to date have predominantly addressed either photosynthetic/carbon physiology of seaweeds or were confined to quantitative analysis of small subset of physiological and biochemical parameters (Celis-Plá et al., 2015). Although these studies provided useful insights, a wider approach would be necessary to obtain global insights in changes of metabolic processes of seaweeds under OA. A *de novo* transcriptomic analysis was recently performed on a natural population of the brown alga *Sargassum vulgare* C. Agardh growing at the volcanic CO_2 vents off Ischia Island (Kumar et al., 2017a). This study revealed that at CO_2 vents *S. vulgare* is adapted to live under acidified conditions through increased expression of genes involved in energy metabolism, photosynthetic processes, ion homeostasis, cell wall, carbon storage, and cellular signaling (Kumar et al., 2017a). Moreover, the analysis of physiological and biochemical parameters of the effects of OA on *S. vulgare* at different time scales showed that this species mitigates stress effects by reprogramming its physiology (Kumar et al., 2017b).

Seaweeds undergo metabolomic reprogramming under abiotic stress conditions (Kumar et al., 2016). Metabolites in fact are the end products of cellular regulatory processes, and represent the response to any environmental changes (Fiehn, 2002). They can also trigger gene expression thus regulating responses to stress conditions (Renberg et al., 2010). However, up to date, few studies have looked for variations in the levels of antioxidants, fatty acids (FAs), amino acids (AAs), and phenolic compounds under elevated CO_2 conditions (Figueroa et al., 2014; Chen et al., 2016; Kumar et al., 2017b). In particular, total phenolic content by colorimetric assay has been measured without quantifying the specific phenols (Celis-Plá et al., 2015; Betancor et al., 2014). Changes in the levels of algal metabolites could compromise the health status of the seaweed and its associated communities by altering algal nutritional values and prey palatability (Harley et al., 2012; Arnold et al., 2012; Poore et al., 2013; Poore et al., 2016). Indeed, marine algae are the source of polyunsaturated fatty acids (PUFA) and essential amino acids (EAAs) for many metazoans, and high CO_2/lowered pH may affect their levels with detrimental consequences on the food web (Tsuzuki et al., 1990; Rossoll et al., 2012; Torstensson et al., 2013; Leu et al., 2013; Bermúdez et al., 2015; Bermudez et al., 2016). Here, the levels of primary (carbohydrates, AAs and FAs), and secondary metabolites (phenolic compounds) in the brown alga *S. vulgare* were analyzed. The population naturally growing at the acidified site of Castello Aragonese was compared to that at a control site off Ischia Island at current pH, 8.1. To study short-term responses, these metabolites were also estimated in transplanted samples from control to acidified site and *vice-versa*.

2. Materials and methods

2.1. Study site and sample collection

The brown seaweed *S. vulgare* was collected along the Ischia Island at two locations: Castello Aragonese, acidified site (40°43.87 N, 013°57.78E) and Lacco Ameno, control site (40°45.35 N, 013°53.13E, Fig. 1). Seawater surrounding Castello Aragonese is acidified due to continuous emission of CO_2 from underwater volcanic vents. Venting activities at this site date back to nearly 2000 years (Lombardi et al., 2011), emitting mainly CO_2 (90.1–95.3%) with no detectable harmful sulfur gases. These are not thermal vents, thus seawater temperature at this site is similar to nearby control seawater (Hall-Spencer et al., 2008). *S. vulgare* is growing in the most acidified part of these vents where the pH is constantly around 6.6. The control site is Lacco Ameno, which is located almost 6 km northwest from the acidified site, where another population of *S. vulgare* is growing at current pH value (8.1). Both the sites are sheltered, share similar depth, salinity, temperature, light and other hydrodynamic and physical conditions (Kumar et al., 2017a, b).

In both locations, 9 thalli of similar size (8–10 cm frond length) were haphazardly collected by snorkeling in three different patches along a coastal stretch of about 15 m at similar depth (<1 m) to cover the natural variability of the two local populations. Samples were collected in July 2014 in both acidified and control sites, on the same day at approximately same time (between 11 am and 1 pm). Upon collection, the algae were maintained in seawater of the respective sites onboard and transported to the laboratory within an hour. The tissues were washed with filtered sea water to remove visible epiphytes and immediately stored at −80 °C until further analysis.

2.2. In situ transplant experiment

On the same day of algal collection, *in situ* reciprocal transplants were performed. Five individuals of *S. vulgare* originating from the control site (C) in Lacco Ameno were tied in a net and moved to acidified site (A) in Castello Aragonese (C-A) and *vice-versa* (A-C). In order to evaluate the stress effect due to the transplant itself, other thalli were also transplanted within their respective natural site (C-C and A-A) and were used as controls. After 2 weeks, the algal samples were