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Research Article

Experimental Strategies on Climate Change Impacts: Climate Chamber Approach for Seagrass Meadows

Enes GÖKSAL[1], Büşra KURUOĞLU[1], İnci TÜNEY[1], Esra ÖZTÜRK[1*]

[1]Department of Biology, Faculty of Science, Ege University, 35040 Izmir, Türkiye

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Abstract

Seagrasses are vital to marine ecosystems, providing stability to coastal areas, acting as significant carbon sinks and supporting biodiversity. However, environmental changes, particularly climate change, are threatening seagrass habitats. The global extinction rate of seagrasses has increased significantly, with notable losses in the Mediterranean Sea. This decline is attributed to pollution, climate change, and rising temperatures, which impact seagrass growth, reproduction, and survival.

To study these effects, climate chamber systems simulating future climate scenarios were used. These systems, including aquariums and transparent bags, allow for controlled adjustments of climate variables such as CO_2 concentration, temperature, and pH. Fieldwork conducted in Aliağa, İzmir, involved setting up these systems and collecting samples of the seagrass *Cymodocea nodosa*. The study revealed that aquarium systems were more stable and controllable than bag systems in field conditions.

The findings underscore the importance of climate chamber systems in understanding the ecological impact of climate change on seagrasses. These systems provide valuable insights for developing conservation strategies and managing marine ecosystems. Accurate simulation of future conditions is crucial for predicting and mitigating the effects of global warming on seagrass meadows and marine biodiversity

Keywords: Climate change, IPCC, acclimation, angiosperm, ocean acidification

İklim Değişikliğinin Etkileri Üzerine Deneysel Stratejiler: Deniz Çayırı Yatakları için İklim Çemberi Sistemi

Öz

Deniz çayırları, kıyı bölgelerinde dayanıklılık sağlamaları, önemli karbon yutakları olarak işlev görmeleri ve biyolojik çeşitliliği desteklemeleri ile deniz ekosistemleri için hayati öneme sahiptirler. Ancak, iklim değişimi başta olmak üzere çevresel değişiklikler, deniz çayırlarının habitatlarını tehdit etmektedir. Deniz çayırlarının küresel yok olma oranı önemli ölçüde artmış, Akdeniz'de kayda değer kayıplar yaşanmıştır. Deniz çayırlarındaki bu yok olma durumu, kirlilik, iklim değişikliği ve artan sıcaklıkların deniz çayırlarının büyümesini, üremesini ve hayatta kalmasını etkilemesi ile yakından ilişkilidir.

Bu çalışmada, olası etkileri incelemek için gelecekteki iklim senaryolarını simüle eden iklim çemberi sistemleri kullanılmıştır. Bu sistemler, CO_2 konsantrasyonu, sıcaklık ve pH gibi iklim değişkenlerinin kontrollü olarak değiştirilmesine olanak tanıyan, akvaryumlar ve şeffaf torbalardan oluşan sistemlerdir. Aliağa, İzmir'de gerçekleştirilen saha çalışması, bu sistemlerin kurulmasını ve *Cymodocea nodosa* türü deniz çayırlarının örneklerinin toplanmasını içermektedir. Çalışma, saha koşullarında akvaryumlardan oluşturulan iklim çemberi sistemlerinin, plastik torba sistemlerine göre daha stabil ve kontrol edilebilir olduğunu ortaya koymuştur.

Bulgular, iklim çemberi sistemlerinin iklim değişikliğinin deniz çayırları üzerindeki ekolojik etkilerini anlamadaki önemini vurgulamaktadır. Bu sistemler, koruma stratejilerinin geliştirilmesi ve deniz ekosistemlerinin yönetimi için değerli bilgiler sağlamaktadır. Gelecekteki koşulların doğru bir şekilde simüle edilmesi, deniz çayırlarının ve deniz biyolojik çeşitliliğinin küresel ısınmanın etkilerinden korunmasına ve/veya etkilerinin hafifletilmesine yönelik çözümleri öngörmek için kritik öneme sahiptir.

Anahtar Kelimeler: İklim değişimi, IPCC, aklimasyon, angiosperm, okyanus asitlenmesi

*Corresponding Author: esra.ozturk@ege.edu.tr
Enes Göksal, https://orcid.org/0000-0002-6368-5161
Büşra Kuruoğlu, https://orcid.org/0000-0002-1792-7721
İnci Tüney, https://orcid.org/0000-0003-0293-6964
Esra Öztürk, https://orcid.org/0000-0001-7115-5323

1. Introduction

Seagrasses are considered critical species of the marine ecosystem due to their contribution to stabilizing the coastal ecosystems [1-3] by stabilizing the sediment, preventing coastal erosion and protecting shorelines. They are significant carbon sinks due to their high productivity, crucial in sequestering a substantial portion of atmospheric carbon dioxide. Moreover, seagrass meadows, which host a variety of species, play a vital role in biodiversity [4- 8]. Seagrass meadow maintenance and management are significant marine ecological challenges. Environmental changes from climate change affect aquatic habitats.

Studies have shown that the global extinction rate of seagrasses was approximately 1% per year before 1940 but has increased to 7% per year in recent times [1, 3, 9]. It has been determined that between 13% and 38% of *Posidonia oceanica* seagrass beds along the Mediterranean coasts have been lost since the 1960s [6]. Many scientific studies have suggested that the cause of this rapid decline is excessive environmental pollution combined with natural conditions and climate change. Furthermore, these studies propose that environmental pollution, which has increased since the industrial revolution, may have a greater impact than climate change [10, 11].

As an effect of climate change, alterations in sea level, salinity, temperature, atmospheric CO_2, and UV radiation can influence seagrass distribution, productivity, and community composition. Rising seawater temperatures, in particular, impact seagrasses in several ways, including changes in growth rates, physiological functions, reproductive patterns, phenology, and seed germination [12- 15]. The loss of seagrasses can lead to decreased productivity and marine biodiversity, which in turn can cause changes in coastal dynamics, a decline in water quality, and the destabilization of sediments.

Studies have shown that the combined effects of temperature increase and eutrophication, significant impacts of climate change, restrict the survival of seagrasses [16].

According to climate scenarios in 2100, critical temperature and pH changes are expected in aquatic ecosystems. RCP (Representative Concentration Pathways), developed by IPCC (Intergovernmental Panel on Climate Change), is an expression used to determine climate change scenarios. RCP scenarios are analyzed in 4 main lines: low-emission scenario (RCP 2.6), medium-low emission scenario (RCP 4.5), medium-high emission scenario (RCP 6.0), and high-emission scenario (RCP 8.5) [17].

Ocean acidification, an increasingly significant scientific topic in the contemporary world, refers to the decrease in pH and increase in H^+ ions in the oceans caused by a rise in CO_2 concentrations. Ocean surface water acidity has increased by 30% since the Industrial Revolution [18]. Today, the pH value of the surface water of the oceans is accepted as 8.1. According to the RCP 2.6 scenario, the pH value of the oceans is expected to decrease to 8.05 in 2100, and the water temperature is expected to increase by 1°C. In the RCP 8.5 scenario, the

pH value of the ocean surface water in 2100 is expected to decrease by 0.3-0.4 units to 7.8, and the water temperature is expected to increase by 3.7°C [3, 17,19, 20]. As a result of the expected 0.3- 0.4 decrease in the pH value of the oceans in the RCP 8.5 scenario, the acidity of the oceans is expected to increase by 151% and will affect all marine life [21].

The climate chamber system being set up in this study is an essential tool to simulate future climate scenarios and learn how particular species and ecosystems will react to these changes. These systems also offer a unique capability to study the natural habitat of critical species like seagrass *Cymodocea nodosa*, providing insights into their response to ocean acidification. By controlling and altering pH levels based on varying CO_2 concentrations, the climate chamber is designed to simulate future climate conditions. It also enables detailed observations and analyses of seagrass responses under projected environmental scenarios. This system improves the repeatability and accuracy of experiments by adjusting climate variables (temperature, CO_2 concentration, light intensity, and nutrient levels), enabling physiological and molecular-level research.

These studies demonstrate ecosystems' adaptive abilities and help to understand how seagrasses, algae, and other marine organisms respond to rising CO_2 levels. It also offers the chance to contrast the results of different climate scenarios, which aids in creating conservation strategies and ecosystem management plans [22].

2. Material Methods

2.1. Site Description and Sampling

The fieldwork was conducted on May 25-26, 2023, in Aliağa/İzmir (38.862355, 27.037297), where the species *C. nodosa* meadow was found (Figure 1). The climate circle system was set up at a depth of 0.5-1 m. Samples were collected in triplicate at specified times.

Samples were taken from young leaves. Epiphytes were quickly removed from the leaves without causing damage, and the leaves were washed with distilled water and blotted with paper towel to remove the excess water. The dried leaves were transferred to 1.5 mL Eppendorf tubes containing RNA preservation solution (RNAlater, Thermo Fischer Scientific). During the fieldwork, the tubes were kept in icebox and transferred to the laboratory under cold chain conditions. All samples were stored at -20°C in the laboratory until analysis.

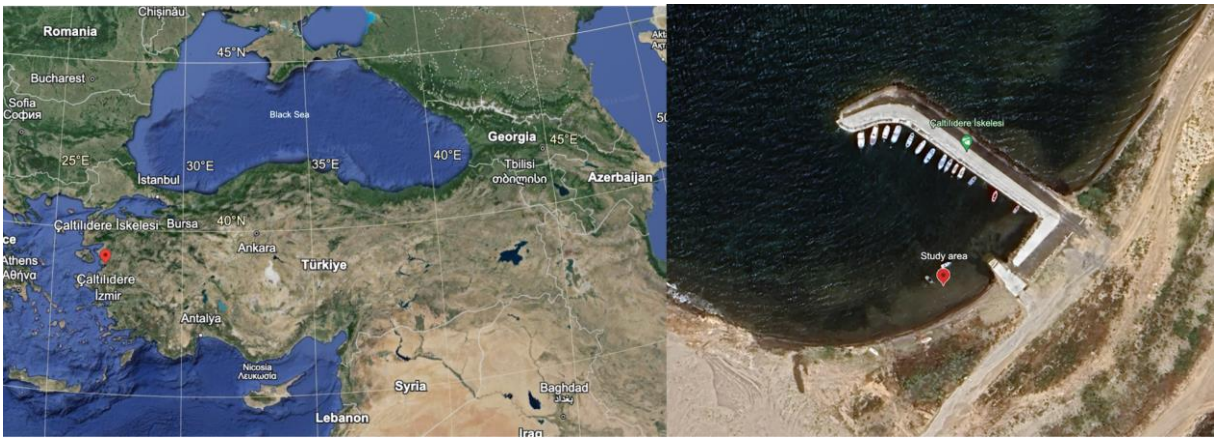

Figure 1. Study area

2.2. Equipment Description

Two distinct climate circle systems were set up using various materials and equipment that were tested. Two distinct systems, aquarium systems (40 x 40 x 20 cm, LxWxH) and transparent bags (26 x 28 cm), were tested as climate chambers, and it was found that both are suitable for field use. CO_2 was added to the sealed bag systems, which were set up independently until the required pH was reached. For added durability, the bag system was designed with two layers. In contrast, the aquarium system was installed inside a single aquarium. Although single-use small CO_2 cylinders can also be used, a 5 L cylinder was used.

2.3. Pre-Lab Testing

Laboratory tests were carried out before the climate circle system was set up to get the desired pH reduction in the seawater added to the bag systems. In these experiments, CO_2 gas was added to seawater samples to reduce the pH by 0.3–0.4 units. The length of time and rate at which CO_2 gas should be supplied were decided upon during this phase. This made it possible to make the required modifications to guarantee the intended pH decrease in the studies conducted in a natural setting.

2.4. Field Testing

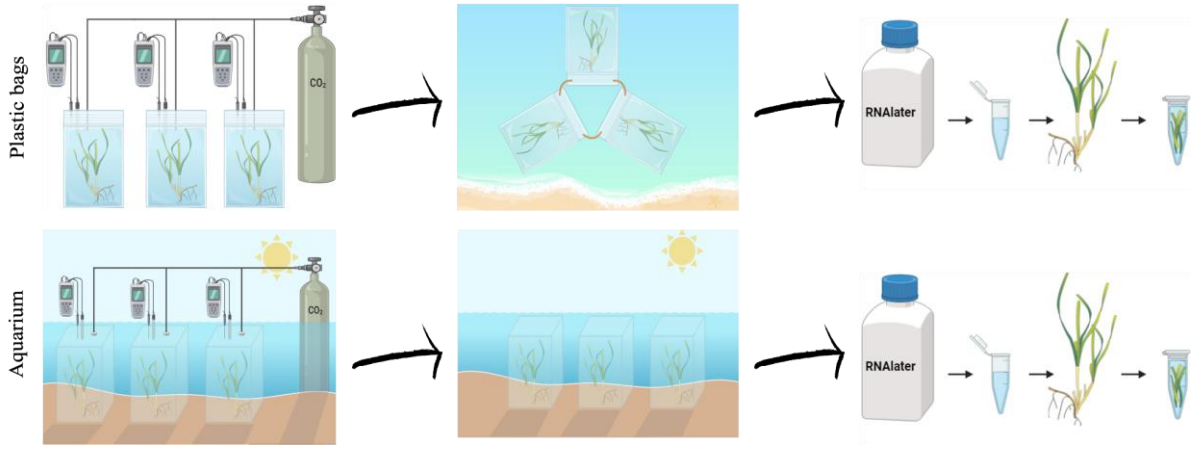

Figure 2. Artificial plastic bag and aquarium climate chamber systems and experiment design

Under field conditions, these enclosed systems, called climate circles, offered a controlled simulation environment. The only independent variable in the system designed in the natural marine environment was the concentration of CO_2. After being removed from their roots, individual *C. nodosa* plants were inserted into light-permeable climate circle systems to create the ideal environment with the right amount of CO_2 gas. Transparent sealed bags and aquarium systems were the two systems that were tested as climate circles. Regular measurements of pH and temperature were taken. It was concluded that both could be used in the field (Figure 2).

3. Results and Discussion

3.1. Results

3.1.1. Differences in Testing Methods at the fieldwork

When compared to the experiment setup in the field, the preliminary laboratory experiments revealed a far more stable environment. The pH reduction experiments were made easier and faster by the stable laboratory conditions and water temperature. However, the field setup's fluctuating water temperature led to a more unstable atmosphere, which had an impact on CO_2 solubility. The solubility of CO_2 was decreased by the rising water temperature, making it more challenging to achieve the appropriate pH levels. In addition, compared to laboratory conditions, there were variations in the duration and rate of CO_2 delivery to the water.

3.1.2. Field Testing

Figure 3. Climate Chamber Systems in the Field

	Plastic Bag	Aquarium
System	Close system	Open system
To reach field	Easy and light	Need space and Heavy
Durable	Sensitive	Less sensitive
Leak	Leaky	Open system
Light intensity	Enough light intensity	Natural light intensity
Set up process	Long set up process	Easy and short set up process
pH check	Not possible	Regularly possible
Progress	Each bag for each time	Same condition for all samples and sampling times

Table 1. Comparison of plastic bag and aquarium chamber systems

When utilized in a field setting, each system has pros and cons of its own. Transporting the closed system climate circle to the field is made simple by its lightweight and compact design, which makes use of plastic bags. Nevertheless, the open system climate circle—which makes use of aquariums—is heavier and requires more room, which makes it challenging to move to the field (Figure 3).

Depending on the duration of the experiment, the bags utilized in the closed system suffered damage as a result of having to stay in the water through the night or during turbulence. Double-layered bags were used to address this problem. In the open system, extra support was needed during the night or turbulent hours to prevent it from toppling over, depending on how long the experiment was going to last, even though it remained stable for extended periods once fixed in the water because of its weight.

In the closed system, the use of transparent bags did not hinder light penetration; however, it should be noted that double-layered bags were used in experiments where the amount of light was crucial. In the open system, natural light penetration continued throughout the entire experimental process.

The experimental process took longer to set up in the field because each bag in the closed system required a different pH adjustment. Since every experimental piece in the open system was housed in the same aquarium, one pH adjustment was adequate in real-world settings. Furthermore, regular pH monitoring was carried out in open systems even though pH control in closed systems was not possible once the experiment started.

Samples were collected by opening each closed system one after the other at the designated time, and storing the samples properly. At the scheduled sampling times in the open system, samples were randomly drawn from the one aquarium and stored under the proper conditions (Table 1).

3.2. Discussion

In selecting the field site, the species' natural habitat to be studied should be considered. The population of *C. nodosa* spreads in shallower and softer substrates. This species also prefers clear and less turbulent waters. Therefore, the field site was selected according to the natural distribution areas of *C. nodosa*. The region should have shallow and non-rocky waters with low turbulence. This selection ensures the conditions necessary for establishing the climate circle system and the successful execution of the study. Thus, both the characteristics of the natural habitat are preserved, and the proper establishment of the system is ensured.

Samples were taken at specific times and kept cold-chained in an RNA preservative solution. Reliable analyses were made possible by this procedure, which guaranteed the preservation of RNA without degradation. Both physiological and molecular techniques were used in the laboratory analysis of the collected samples.

The field site was perfect for *C. nodosa*'s natural habitat because of its shallow, sandy substrate and low turbulence waters.

We choosed for the aquarium system due to its enhanced stability and simplified pH control in field settings. Unlike the closed system, which required individual pH adjustments for each bag and often suffered damage from prolonged water exposure and turbulence, the aquarium system allowed for a single pH adjustment and maintained stability due to its weight. Additionally, the aquarium system ensured consistent natural light penetration throughout the experiment, reducing the need for double-layered bags. This setup facilitated easier sample collection and regular pH monitoring, making it more efficient and reliable for our research objectives.

The study's successful setup and execution allowed for accurate and trustworthy results. This was made possible by the field site selection and the use of the aquarium climate circle system.

Previous CO_2 climate enrichment studies have used closed-loop systems to regulate gene expression levels for about a month before further analyses [23, 24]. A study demonstrated the potential of *in situ* carbon enrichment systems for long-term climate change research in nearshore coastal communities, highlighting their viability and cost-effectiveness. The study underscored the importance of understanding how elevated pCO_2 levels impact marine ecosystems [23].

We similarly established an aquarium-based climate circle system. However, unlike the fully closed systems used in CO_2 climate enrichment studies, our setup examined short-term responses to climate change. While our aquarium system proved to be successful, it is not suitable for long-term climate change studies as described in similar research. This indicates that while our approach is effective for short-term analysis, modifications would be necessary for it to be applicable in long-term studies.

The incubation chambers designed in a study consist of gas-tight polyethylene plastic bags with a port for drawing water samples. These bags are 30 x 20 cm in size and have a capacity of approximately 2.5 liters. The chambers are sealed with a PVC clamp and incubated at a depth

of 2 meters on a steel bar. The flexible nature of the plastic bags allows them to prevent external turbulence from affecting the boundary layers inside [22]. However, we used 26 x 28 cm plastic bags for this study. We sealed the two layers bags using their own locking mechanisms, not PVC clamps. Despite this variation, the samples remained stable during the incubation period, indicating that our results were successful. This implies that the more basic bag mechanism can function just as well, providing a more direct and possibly economical method without sacrificing the samples integrity and stability. However, this system is also unsuitable for long-term research.

Compared to conducting experiments in a laboratory setting, setting up a climate chamber system in the field is more difficult and expensive. A more stable environment and improved reproducibility can be achieved by moving seagrass specimens from the field into the laboratory in seawater and then arranging the experiment after 7 days incubation. Seagrass specimens will have a higher chance of survival if they are placed in filtered and sterilized seawater (by autoclaving or UV light). The collected seawater's salinity and temperature should also be continuously controlled in the lab. This kind of setup has been used in earlier aquarium studies, which have recorded survival rates during the incubation and experimental phases [15, 25].

Ecologically significant gene-physiology interactions can be discovered and how organisms react to environmental stresses at various scales can be comprehended by combining the use of molecular and physiological techniques. Compared to laboratory settings, controlling pH in the field can be far more difficult. Consequently, it is thought that by reducing pH fluctuations, utilizing a single aquarium system in the field as opposed to several CO_2-enriched bag systems as in the study would produce more accurate results.

Conclusion

In conclusion, climate chamber systems are essential resources for comprehending and mitigating the ecological and biological effects of global warming. To create sustainable management plans and help researchers and policymakers be better prepared for potential future climate scenarios, they offer insightful information. By using these systems, it might be possible to safeguard biodiversity from climate change and build resilient ecosystems. The other important thing is that preference for using aquarium systems as climate chambers is also important for addressing plastic pollution and sustainability.

Ethics in Publishing

There are no ethical issues regarding the publication of this study. Sampling permissions received from the Ministry of Agriculture and Forestry General Directorate of Nature Conservation and National Parks number E-21264211-288.04-7929183.

Author Contributions

All authors contributed equally to the writing of this manuscript.

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References

[1] Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., et al. (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems, Proceedings of the National Academy of Sciences of the United States of America, 106(30), 12377-81.

[2] Schliep, M., Pernice, M., Sinutok, S., Bryant, C. V., York, P. H., Rasheed, M. A., and Ralph, P. J., (2015) Evaluation of Reference Genes for RT-qPCR Studies in the Seagrass *Zostera muelleri* Exposed to Light Limitation, Scientific Reports, 5:17051.

[3] Koch, M., Bowes, G., Ross, C., and Zhang, X-H., (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae, Global Change Biology, 19, 103-132.

[4] Duarte, C.M., Middelburg, J.J., and Caraco, N., (2005) Major role of marine vegetation on the oceanic carbon cycle, Biogeosciences, 2, 1–8.

[5] Piazzi, L., Balata, D., and Ceccherelli, G., (2015) Epiphyte assemblages of the Mediterranean seagrass *Posidonia oceanica*: an overview, Marine Ecology, 37, 2- 41.

[6] Marbà, N., Díaz-Almela, E., and Duarte, C.M., (2014) Mediterranean seagrass (*Posidonia oceanica*) loss between 1842 and 2009, Biological Conservation, 176, 183- 190.

[7] Mazella L., Scipione, M.B., Gambi, M.C., Buia, M.C., (1993) The First International Conference on the Mediterranean Coastal Environment, MEDCOAST 93, At: Antalya, Turkey, 2-5 November, 103-116.

[8] Cancemi, G., Buia, M.C., and Mazella, L., (2002) Structure and growth Dynamics of *Cymodocea nodosa* meadows, Scientia Marina, 66(4), 365- 373.

[9] Cressey, D., (2009) Vital marine habitat under threat. Nature.

[10] Koch, M.S., Schopmeyer, A., Kyhn-Hansen, C., & Madden, C.J., (2007) Synergistic effects of high temperature and sulfide on tropical seagrass. Journal of Experimental Marine Biology and Ecology, 341, 91–101.

[11] Hoffle, H., Thomsen, M.S., & Holmer, M., (2011) High mortality of *Zostera marina* under high temperature regimes but minor effects of the invasive macroalgae *Gracilaria vermiculophylla*. Estuarine. Estuarine, Coastal and Shelf Science, 92, 35–46.

[12] Marbà, N., Duarte, C. M., Cebrián, J., Gallegos, M. E., Olesen, B., and Sand-Jensen, K., (1996) Growth and population dynamics of *Posidonia oceanica* on the Spanish Mediterranean coast: elucidating seagrass decline, Marine Ecology Progress Series, 137, 203- 213 s.

[13] Short, F.T. & Neckles, H.A., (1999) The effects of global climate change on seagrasses, Aquatic Botany, 63,169-196.

[14] Marba, N. and Duarte, C.M., (2010), Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality, Global Change Biology, 16: 2366-2375.

[15] Nguyen, H. M., Kim, M., Ralph, P.J., Marín-Guirao, L., Pernice, M., and Procaccini, G., (2020) Stress Memory in Seagrasses: First Insight Into the Effects of Thermal Priming and the Role of Epigenetic Modifications, Front. Plant Sci., 11:494.

[16] Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J.M., Romero, J., Pérez, M., (2019) Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass, Marine Environmental Research, 145, 27-38.

[17] IPCC, (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)], Geneva, Switzerland, 151 pp.

[18] Doney, S.C., Fabry, V.C., Feely, R.A., and Kleypas, J.A., (2009) Ocean Acidification: The Other CO_2 Problem, Annual Review of Marine Science, 1:169–92 s.

[19] Orr, J.C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G. K., Rodgers, K.B., Sabine, C.L., Sarmiento, J. L., Schlitzer, R., Slater, R.D., Totterdell, I. J., Weirig, M.F., Yamanaka, Y., and Yool, A., (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature, 437, 29.

[20] Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M., Gattuso, J., & Hall-Spencer, J., (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. Biology Letters, 4, 689–692.

[21] IPCC, (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change

[22] Olivé, I., Silva, J., Lauritano, C., Costa, M.M., et al. (2017) Linking gene expression to productivity to unravel long- and short-term responses of seagrasses exposed to CO_2 in volcanic vents, Scientific Reports, 7: 42278.

[23] Campbell, J.E., & Fourqurean, J.W., (2011) Novel methodology for in situ carbon dioxide enrichment of benthic ecosystems. Limnology and Oceanography:Methods, 9, 97–109.

[24] Campbell, J.E., & Fourqurean, J.W., (2013) Effects of in situ CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum.* Marine Biology, 160,1465-1475.

[25] Oscar, M.A., Barak, S. and Winters, G., (2018) The Tropical Invasive Seagrass, *Halophila stipulacea*, Has a Superior Ability to Tolerate Dynamic Changes in Salinity Levels Compared to Its Freshwater Relative, Vallisneria americana. Front. Plant Sci. 9:950. doi: 10.3389/fpls.2018.00950