
The effect of seawater physical parameters in bivalve farming: could systematic monitoring and early warning prevent negative impacts? A review focused on Vistonikos Gulf, North Aegean Sea

**Ioannis Georgoulis* and
Konstantinos Feidantsis**

Laboratory of Animal Physiology,
School of Biology,
Aristotle University of Thessaloniki,
54124, Greece
Email: georgoim@bio.auth.gr
Email: kfeidant@bio.auth.gr
*Corresponding author

Dimitrios Kouvas

ScientAct S.A.,
Thessaloniki, 54644, Greece
Email: dgk@scientact.com.gr

Athanasios Lattos

Laboratory of Animal Physiology,
School of Biology,
Aristotle University of Thessaloniki,
54124, Greece
Email: lattosad@bio.auth.gr

Georgios A. Delis

Laboratory of Pharmacology,
School of Veterinary Medicine,
Aristotle University of Thessaloniki,
54124, Greece
Email: delis@vet.auth.gr

Alexandros Theodoridis

Laboratory of Animal Production Economics,
School of Veterinary Medicine,
Aristotle University of Thessaloniki, 54124, Greece
Email: alextheod@vet.auth.gr

Basile Michaelidis

Laboratory of Animal Physiology,
School of Biology,
Aristotle University of Thessaloniki, 54124, Greece
Email: michaeli@bio.auth.gr

Ioannis A. Giantsis

Department of Animal Science,
Faculty of Agricultural Sciences,
University of Western Macedonia,
53100 Florina, Greece
Email: igiants@agro.auth.gr

Abstract: Mussel farming in the Vistonikos Gulf (North Aegean Sea) constitutes an activity of high socioeconomic importance. The wider Vistonikos Gulf area consists of three basins (Porto Lagos Lagoon, Vistonikos Bay, and Vistonida Lake) rich in organic material. Oceanographic features, sea currents, meteorological and climatic conditions, and primary productivity of the wider Vistonikos Gulf marine area favour bivalve farming establishment. However, secondary factors associated with climate change may negatively affect these farming establishments. In the present study, historical oceanographic and meteorological data associated with the bivalve's biology and culture is reviewed. The Vistonikos Gulf demonstrates a suitable area for mussels and other bivalves' maintenance and aquaculture development. However, occasional restrictions imposed to mussel farm units in the past are related to meteorological extremes. Thus, monitoring seawater physicochemical properties within farming units may prevent harmful effects (e.g., mortality, heat and oxidative stress) through the establishment of an early warning system indicating the translocation or harvest of the reared bivalves.

Keywords: Aegean Sea; climate change; harmful algal blooms; mussel farming; ocean acidification; temperature; Vistonikos; wetland.

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Biographical notes: Ioannis Georgoulis is a Biologist (Aristotle University of Thessaloniki) and has a Master's degree in Application of Biology, Aquaculture, Innovation and Sustainability. Currently, he is a PhD candidate with the dissertation topic 'Investigation of molecular and biochemical mechanisms for enhancing the thermotolerance 'hardening' of marine species *Mytilus galloprovincialis* and *Sparus aurata*'. He participates in competing research projects related to this subject and he is a co-author in recent publication (2020) in an international scientific journal and he has also publications in national and international conferences.

Konstantinos Feidantsis is a postdoctoral researcher in the Department of Biology of the Aristotle University of Thessaloniki and in the Department of Ichthyology and Aquatic Environment of the University of Thessaly. His research interests include cell and animal physiology and he teaches associated themes on undergraduate and postgraduate courses. He has published more than 35 scientific papers in peer-reviewed journals indexed in Scopus database concerning genetics, physiology and application of molecular markers in animal science. He participates in many national and international research programs and he is a reviewer in many international scientific journals.

Dimitrios Kouvas is the Vice President and Co-owner of the ScientAct S.A. that was established on 1995 from people with business and scientific experience at the field of high technology and its applications, at the fields of environmental research equipment, industrial applications and laboratory equipment. The company was established, with main object the formation of a dynamic and quickly progressive organism. The company with the aim of satisfying and ensuring the trust of customers and fulfilling certain quality standards implements a quality management system in accordance with the requirements of the International Standard ISO 9001: 2015.

Athanasios Lattos is a scholarship holder (ELIDEK) PhD candidate in the Department of Biology of the Aristotle University of Thessaloniki with a dissertation topic ‘Seasonal investigation of the microbial load in bivalve molluscs – seasonal and physiological correlations’. He holds two postgraduate diplomas in business administration and aquaculture. He is a student at the postgraduate course of the University of Thessaly ‘Aquaculture – Pathological problems of aquatic farmed organisms’. He participates in five competing research projects related to this subject. He is a co-author of three scientific publications in international scientific journals. He has previously worked for a company in a position to manage aquatic organisms.

Georgios A. Delis is an Assistant Professor of Veterinary Pharmacology, in the School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki. He has also worked as a veterinarian in the Directorate of Rural Development and Veterinary of the Regional Unit of Pella/Region of Central Macedonia. He also conducted private-funded research programs, under the auspices of the Research Committee-Aristotle University of Thessaloniki, in the years 2002–2003, as follows: ‘Bioequivalence study of two albendazole formulations in ‘sheep (research program no. 20978) and ‘Bioequivalence study of two clindamycin formulations in dogs’ (research program no. 21115).

Alexandros Theodoridis is an Associate Professor in the Faculty of Veterinary Medicine, School of Health Sciences, AUTH. He is also a member in HAICTA (Hellenic Association Information Communication Technology in Agriculture, food and environment). His courses in undergraduate and postgraduate studies are of the same interest and among them are biostatistics, entrepreneurship and management of livestock and veterinary enterprises, welfare and pathology of aquatic farmed organisms, economics of animal production. He is the author and co-author of several scientific papers in peer-reviewed journals indexed in Scopus database and he also participated or currently participates in numerous research national or EU research projects.

Basile Michaelidis is a Professor in the Department of Biology of the Aristotle University of Thessaloniki. He is the author and co-author of many scientific publications, while he has supervised several PhDs, master and undergraduate diploma theses. Moreover, he has been and is scientific responsible for many national and international research programs. His main research interests focus on molecular and metabolic responses of marine and land invertebrates and vertebrates to temperature and CO₂-conservation physiology and aquacultures.

Ioannis A. Giantsis is an Assistant Professor in the Faculty of Agricultural Sciences, University of Western Macedonia, Greece. His research interests include animal genetics, marine animal physiology, aquaculture and fisheries. He teaches courses such as aquaculture and fisheries, animal genetics and conservation biology at both undergraduate and postgraduate levels. He has published more than 25 scientific papers in peer-reviewed journals indexed in Scopus database concerning genetics, physiology and application of molecular markers in animal science and particularly in aquatic animals. He has also participated or currently participates in numerous research projects funded by national or EU organisations.

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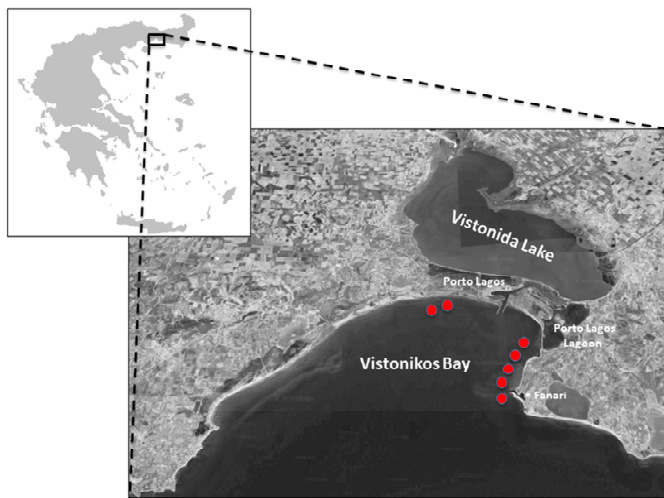
1 Introduction

The Vistonikos Bay – Porto Lagos Lagoon – Vistonida Lake wetland area extends between the regional unit of Xanthi to the west and Rodopi to the east, and the region of Eastern Macedonia and Thrace, in northern Greece. Specifically, Porto Lagos Lagoon is located in the centre of these three reservoirs and is connected to Vistonida Lake in the north through three canals, and with Vistonikos Bay to the southwest, through one canal (Koutrakis et al., 2005). Vistonida Lake receives the freshwater inflow from the Kosynthos, Kompasos, and Travos streams in its northern part (Tsakoumis et al., 2016). The latter enriches the lake with large amounts of nutrients of agricultural, domestic, and industrial waste water (Markou et al., 2006). The southern part of Vistonida Lake is of higher salinity due to the entering seawater (Koutrakis et al., 2005). Due to its numerous *Posidonia* meadows and other aquatic plants, the wider area is also of high ecological importance, forming an environment suitable for offspring of several bivalve species, including mussels, oysters, cockles, and clams, as well as juvenile fish. This habitat is also recognised as part of the National Park of Eastern Macedonia and Thrace, which is one of the ten Greek wetlands protected by the Ramsar Convention and European Union’s Natura 2000 network (Dimiza et al., 2016). Although there is a lack of climatological data concerning the Vistonikos Gulf, several marine areas of the Mediterranean Sea have been already identified as climate change “hotspots” with effects on marine biodiversity and productivity, respectively (Bethoux et al., 1990; Calvo and Marsh, 2011; Gambaiani et al., 2009; Nicholls and Hoozemans, 1996). Moreover, while climate change exhibits a spatial and temporal heterogeneity, the

frequency and severity of heat waves is expected to increase in the Mediterranean Sea, including the Aegean Sea (Galli et al., 2017).

Mussel *Mytilus galloprovincialis* farming provides employment for the local population, producing a product of high quality and nutritional value, and is therefore of great socioeconomic importance for the area. This farming practice was first developed in the region in 1999, when seven mussel farm units were environmentally and administratively licensed (Decisions DAMT 654-656/24-10-2000, 633/1-11-2001, 640/1-11-2001) (Figure 1). This form of aquaculture is fully compliant with the Ramsar and Natura 2000 Convention protecting the natural environment, since the burden on the marine area is negligible and derives solely from farmed mussels' secretions (Lindahl et al., 2005). In order to avoid genetic pollution, the first mussel farm units were established with native populations, thereby avoiding biological contamination with alien species and at the same time stimulating the local shellfish market. It should also be noted that recently, in 2017, license for the farming of more bivalve species, i.e., *Ruditapes decussatus*, *Venus verrucosa*, *Aequipecten opercularis* and *Ostrea edulis*, has been provided for the first time in Greece; however, the development of the farming units has not been established yet.

Figure 1 The wetland area of the Vistonikos Gulf (see online version for colours)



Note: Red dots indicate the location of mussel aquaculture units.

Water quality in combination with the sea currents and shoreline morphology create a suitable environment for the development of bivalve aquaculture in the Vistonikos Gulf. However, production may be occasionally influenced by secondary factors that are directly dependent on climate. Climate change and ocean acidification are global environmental threats caused by the anthropogenic emissions of carbon dioxide (CO₂) (IPCC, 2013). During the last 40 years, the ocean surface has been continuously warming by > 0.1°C/decade, a fact that is accompanied by changes in salinity (Pörtner et al., 2014), while the accumulation of CO₂ in seawater also leads to a decrease of its pH by 0.1 in the last two centuries (IPCC, 2013). These changes are particularly important and devastating for the environment, especially when considering pre-industrial values (Pörtner et al., 2014). Furthermore, the increase in seawater temperature is responsible for

mass mortality events, increased sensitivities to pathogens, and species invasions (Rodrigues et al., 2015). Seawater quality and, particularly, the presence of pollutants may even cause an alteration in the genetic patterns of bivalve populations in terms of genetic diversity, affecting dramatically the farmed mussels (Giantsis et al., 2012).

Bivalve mollusks, such as mussels, oysters, and clams, are among the most vulnerable species to climate change. In the Mediterranean Sea, these species will most likely experience increased thermal stress due to the increased sea surface temperature (SST) (Rodrigues et al., 2015). According to Anestis et al. (2007), *Mytilus galloprovincialis* is already exposed close or beyond its upper critical temperature (25–28°C). Under these increased temperatures, several effects may occur in different life stages of the species (larvae, seed or spat, juvenile, and adult), like survival rate and growth decrease, as well as inability to develop their organic protective layers (Gazeau et al. 2014).

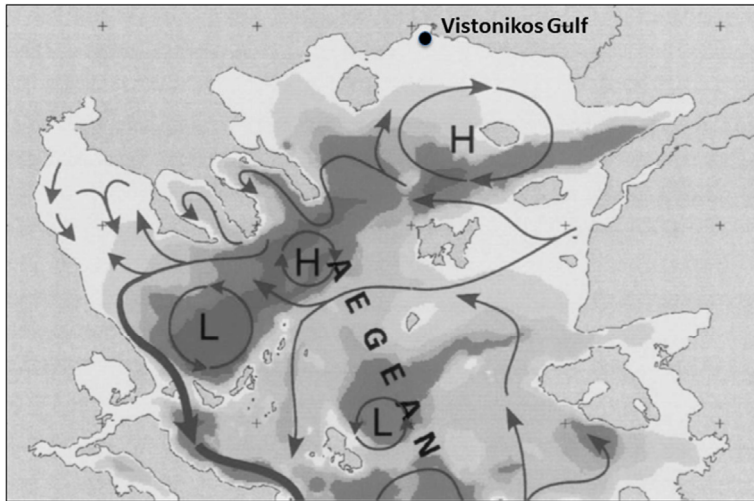
2 Vistonikos area properties

2.1 Oceanography

The Aegean Sea archipelago is characterised by various seabed topologies, encompassing many different depth and relief areas. In contrast, the Vistonikos Gulf exhibits a greater uniformity with lower (not exceeding eight metres) depths (Androulidakis et al., 2017; Koutrakis et al., 2005).

2.2 Sea currents

The mechanisms of water circulation depend on the amount of water and other hydrological factors, such as wind intensity, thermal fluxes, salinity flows, and upstream movement due to river outflows. In the North Aegean, where the circulation of water is cyclonic (Figure 2), the stream flow is strongly influenced by the outflow from the Black Sea, and the additional influx of fresh water from rivers (Olson et al., 2007). These low-salinity inflows are offset by an influx of more saline waters from the eastern Mediterranean, generally balancing the salinity of the Aegean Sea (Poulos et al., 1997). It should be noted that the low salinity water outflow from the Dardanelles is the most important lateral buoyancy force, exceeding that of all rivers (Kourafalou and Barbopoulos, 2003). These streams, having lower temperature and higher nutrient content than the oligotrophic Aegean Sea (Siokou-Frangou et al., 2002), favour the development of mussel aquaculture in the Aegean's northern sections, such as Thermaikos, Vistonikos and the bay of Kavala. In particular, in the Vistonikos region, recorded salinity values are considered normal for coastal waters near estuaries. The lowest values are found in the surface layers of the water column (0 – 4 metres deep). Also, the transfer of fresh water from Lake Vistonida contributes to lower salinity values during winter (Siokou-Frangou et al., 2002).

Figure 2 The cyclonic flow of streams in the north Aegean

Source: Modified image from Olson et al. (2007)

The precise determination of the hydrodynamic circulation in the Vistonikos Gulf is fraught with difficulties. The currents depend on many factors, most notably winds, water tides, and densities (Manoudis et al., 2005). The tidal effect within the Vistonikos Gulf is generally low. However, it possesses an important role in maritime mass rotation, but also, in conjunction with underground currents, in the transportation of pollution outward into the bay. Combined with the shallow depth, the tidal conditions of the area favour the development of all forms of fishing activities, including aquaculture. Density differences play an important role in the local movement of water (surface – bottom). These differences occur mainly during the hot season when the surface water layers are heated, thus becoming lighter than the deeper layers. As a result, deeper marine masses lack oxygenation. On the contrary, during the winter season, the surface water layers cool down, become heavier, and sink, disturbing water stratification, and thus homogenising the water column, therefore contributing to oxygenation throughout the water column. This is particularly important for the survival of mussels, which are susceptible to low concentrations of dissolved oxygen (Anestis et al., 2007), whereas the general cyclonic water flow of the North Aegean is, among other factors, responsible for the genetic homogeneity of the mussel populations (Giantsis et al., 2014). Although winds are the main cause of water circulation, they are of variable direction and intensity on a short-term scale. Therefore, currents also follow this variable state. The prevailing winds are the northeast, from which mussel cultivation units are protected by Thracian mountain pastures. Surface currents are correlated with the direction of the winds, while the deeper currents typically move in the opposite direction (Manoudis et al., 2005).

2.3 Primary productivity

Primary marine productivity - a property of great importance for the development of mussels – is defined as the production of chlorophyll phytoplankton (Smetacek et al., 2002). These organisms synthesise, via photosynthesis, organic compounds from inorganic salts dissolved in seawater, like nitrogen, phosphoric and silicon salts, and

carbon dioxide. Usually nitrogen and phosphoric salts in the seas are limiting factors for the production of biomass (primary production). On the other hand, silicon does not appear to be a limiting factor except for diatoms (Smetacek et al., 2002).

Concentrations of orthophosphate in the Vistonikos Gulf Bay range from 0 µg/L during summer season, up to the highest recorded concentrations of 75.1 µg/l, which are observed from the end of winter until the middle of spring. As for nitrate concentrations, they represent the highest values during winter and they drop during summer (< 10 µg/l). Also, the concentrations of ammonium salts are higher than those of nitrate and nitrite, with the highest values being observed during winter and spring (Eleftheriadis, 2001). All these above values of inorganic salts can be characterised as suitable for aquaculture, providing ideal nutritional conditions for mussel growth.

3 Variables depending from meteorological-climatic changes

The reasons that contributed to the development of mussel aquaculture in Vistonikos Bay can be summarised:

- a in the presence of natural shellfish populations
- b in the quality of water in combination with the sea currents and shoreline morphology
- c in the presence of the Porto Lagos port and the Fanari fishing harbor.

However, human-induced climate change threatens the Mediterranean bivalve mussel aquaculture, associated with the increase of sea surface temperature, reduction of pH, higher frequency of extreme climatic events, and possible synergies with other non-climatic stressors, such as harmful algal blooms and mollusc diseases (Rodriguez et al., 2015).

3.1 Heatwaves and increased temperatures

The climate of Eastern Macedonia – Thrace could generally be described as typical Mediterranean, with mild winters and a dry, warm summer. While the mean monthly air temperature during summer does not exceed 26°C, instant daily temperature often reaches 40°C (Figure 3). The average seasonal temperature range is between 15°C and 26°C in surface waters in the Aegean (Ciappa, 2019; Feidantsis et al., 2020a) (Figure 4). The maximum critical temperature limit of *Mytilus galloprovincialis* mussels depends on the residence time and has been calculated at 28°C for a few days or at 26°C for a period of 2 weeks (Mavridou et al., 2016).

According to Rodrigues et al. (2015), summer heat waves leading to seawater temperature increase cause significant difficulties in the activity of mussel farms. Marine heat waves (MHWs) can be qualitatively defined as discrete anomalously warm events that last for a prolonged period of time (Galli et al., 2017). The maximal number of consecutive days above 28°C in the Mediterranean Sea could reach 10 or even 20 days in depths of <5m. Although in Vistonikos Gulf that period of consecutive increased temperatures is low, the upcoming predictions until 2050 show an increase of up to 10 days (Galli et al., 2017), while a potential increase of 1–1.5°C in SST of the Eastern Mediterranean, Aegean, and Adriatic Sea is estimated (Lovato et al., 2013).

Figure 3 Average (orange) and maximum (red) air temperature values of the four hottest months (June, July, August and September) during the last 20 years (see online version for colours)

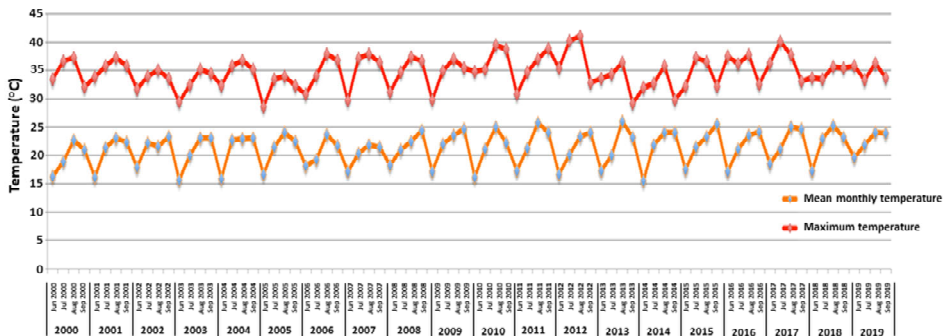
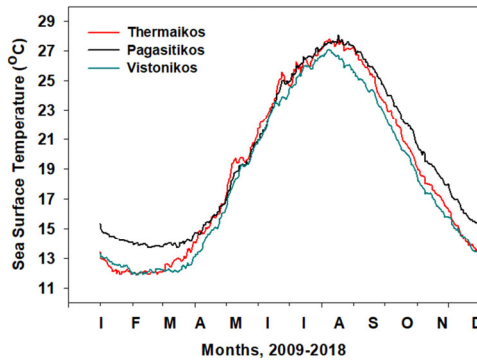


Figure 4 Diagram of average monthly seawater surface temperatures in different areas of the Aegean sea (see online version for colours)



Source: Feidantsis et al. (2020a)

Mussel farm producers pointed out that molluscs were sensitive to temperatures exceeding 28–31°C, leading to mortalities of seed or adult individuals, in some cases reaching up to 100% of the total stock. Furthermore, another observed effect was a decrease in byssus, which affects molluscs' ability for attachment to the production ropes (Rodrigues et al, 2015). Taking into account the increase of temperature, Mediterranean mussels (*M. galloprovincialis*) are regularly forced to encounter lethal temperatures of 25°C, therefore living at the upper limits of their acclimation capacity (Anestis et al., 2007). According to the hypothesis of oxygen and capacity limited thermal tolerance (OCLTT), the increase of temperature beyond the organism's optimum limits (pejus Temperatures, T_p), can lead to a shift from aerobic to anaerobic metabolism, to energy flow, and to body's metabolic capacity deviation from basic maintenance and homeostasis (Pörtner 2001; Sokolova 2013). Recently, Feidantsis et al. (2020b) have shown that exposure of *M. galloprovincialis* at temperatures beyond 22°C increases their *hsp70* and *hsp90* mRNA expression, representing an increase of their thermal stress. Also, both the increased mRNA expression and enzymatic activity of antioxidants indicate the elevation of ROS production and oxidative stress at temperatures beyond 26°C. These results, in combination with the elevated energy demands (due to the

increased antioxidant defense and cell protective activity) and the increased rate of anaerobic metabolism beyond critical temperatures (T_c), seem to disrupt the ATP homeostasis and trigger mussel mortality after long-term heat exposure.

3.2 *Ocean acidification*

Oceans absorb a great amount of atmospheric CO_2 and are responsible for 26% of anthropogenic emissions uptake (Le Quere et al. 2014). This accumulation of CO_2 raises the average surface ocean concentration of H^+ , leading to the decrease of seawater pH and thus to ocean acidification. The pH in ocean surface waters has already decreased by 0.1 (26% increased acidity) since the beginning of the industrial era and additional pH decrease (0.06 – 0.32) is expected until the end of the 21st century (IPCC, 2013). The Mediterranean Sea is a semi-enclosed basin characterised by warm, high- alkalinity waters, with a fast overturning circulation. Therefore, these waters are capable of absorbing higher atmospheric CO_2 levels than the neighboring oceanic region. A dataset from the Mediterranean Sea indicates a pH reduction of approximately 0.0013 pH units per year (Meier et al., 2014) from 1998 to 2005, reaching a 0.05–0.14 decrease in seawater pH since the pre-industrial period (Touratier and Goyet, 2011), while in the next 50 years an increase of 30% in ocean acidity is expected (Ziveri and MedSeA Consortium 2014).

Decreased pH levels are expected to affect the physiology and metabolism of marine organisms through a disruption of intercellular transport mechanisms (Pörtner et al., 2004). Furthermore, the reduction of sea water pH can also lead to a decrease in the carbonate ions (CO_3^{2-}) concentration, one of the building blocks of calcium carbonate ($CaCO_3$), and likely alter the ability of calcifying organisms to precipitate $CaCO_3$ (Gazeau et al., 2007; Meier et al., 2014). Seawater pH levels decrease and the diminished availability of carbonate minerals could hamper the development of the early life stages of mollusks, namely the processes of calcification, growth, byssus attachment, and survival (Gazeau et al., 2013; Kroeker et al., 2013; O' Donnell et al., 2013). Although laboratory research shows that acidification (~0.3 decreased pH) alone does not induce higher mortalities (Gazeau et al., 2014), CO_2 as a stressor can narrow organisms' thermal window by reducing their aerobic performance (Pörtner and Farrell, 2008; Pörtner, 2012). However, the decreased seawater pH in combination with the increased temperatures during summer could amplify the negative stress effects to bivalves.

3.3 *Harmful algal blooms*

Warming and acidification could enhance other harmful stressors of bivalve species (Rodrigues et al, 2015). The increase of temperature during spring, in addition to the elevated nutrient supply from terrestrial sources through eutrophication, could favour the development of harmful algal blooms (Nikolaidis et al., 2005). Harmful algal blooms not only constitute a threat for the mussel farming in Greece but also for human health (Theodorou et al., 2020).

Harmful algal blooms can be characterised, according to Nikolaidis et al. (2005), as toxic, potentially toxic, and non-toxic. In the first group, species-generated blooms can produce toxins which can cause fish/shellfish mass mortalities and human poisoning through contaminated seafood consumption. The second group includes toxigenic potential species that generate toxic blooms when the nutritional status of the local waters

become rich. In the third group, species produce seasonally high biomass blooms, generating anoxic conditions that can kill both fish and invertebrates.

Increased concentrations of harmful microorganisms, such as toxic phytoplankton and toxin-secreting bacteria, are positively correlated with high temperatures (Jay, 2001). In 2012, a series of deaths that were attributed to lipophilic toxins with nerve symptomatology were observed in experimental mice (Vlavis et al., 2015). These toxins (tetrodotoxins), which were also found in mussels from the Vistonikos Gulf, were connected to the presence of *P. minimum* blooms, traditionally occurring in warmer tropical or subtropical waters. Later, in July 2014, when the surface temperature exceeded 30°C, another harmful algal bloom was recorded in Vistonida Lake, causing massive fish deaths in the area. According to Moustaka-Gouni et al. (2016), this event occurred due to the cyanobacterial bloom-forming of *A. favaloroi* and the detection of saxitoxins. Also, from April to September 2019, harmful algal blooms of *Prorocentrum minimum*, *Alexandrium* sp., *Rhizosolenia setigera*, and *Cylindrotheca closterium* occurred in the Vistonikos Bay - Porto Lagos Lagoon - Vistonida Lake area (Stefanidou et al., 2020). Besides posing a threat for bivalves and human health, harmful algal blooms also have an economic impact on the mussel farming units. When these events occur, harvesting areas are closed, and shellfish cannot be harvested (Rodrigues et al., 2015). An early-warning system can soften the impact by monitoring the physicochemical properties of seawater and the early collection or transport of cultivated populations during periods of increased risk (Theodorou et al., 2020).

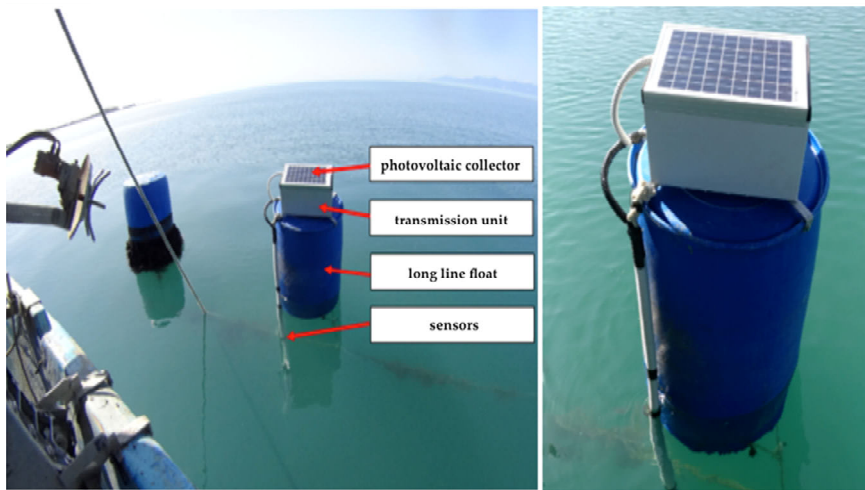
4 Monitoring

Mussel aquaculture is a particularly lucrative form of primary production in Greece (Theodorou et al., 2011). The oceanographic and seawater physicochemical characteristics, and the meteorological and climatological data of the Vistonikos Gulf, as summarised above, demonstrate the suitability of the area for the development of bivalve aquaculture. However, production may be occasionally influenced by secondary factors that are directly dependent on the climate. Therefore, accurate monitoring of the temperature and physicochemical characteristics of seawater in mussel farms may be a valuable solution to such limitations, thus creating new tools for the rational management of mussel aquaculture.

In summer 2020, a telemetric station was established within a mussel farm in the Vistonikos Gulf, measuring various qualitative seawater parameters, i.e., temperature, salinity, and dissolved oxygen at real time scale. Also, an online web application was created for telemetric data download. Notably, three sensors were installed at three different depth points (2, 6 and 8 m from the surface), wirily attached to a temporary recording and telemetry transmission unit (Figure 5). Measurement and recording of parameters are performed every 30 minutes, and are simultaneously transmitted on the online platform. Energy power of the whole station is supplied via a photovoltaic collector through a rechargeable battery.

Taking into consideration the mussels' capacity to close their valves and be translocated alive to other habitats, thereby avoiding potential deleterious environmental factors for some time, the early warning system may prevent harmful effects, such as mortality, heat, and oxidative stress caused by seawater extremes.

Figure 5 Online web application for telemetrical sea water data (i.e., temperature, salinity and dissolved oxygen) download (see online version for colours)



Notes: Red arrows point at photovoltaic collector, transmission unit, long line float and sensors.

5 Conclusions

The majority of the Vistonikos seabed is sandy, hosting many coastal areas with *Posidonia* meadows, while there are fewer rocky areas (Dimiza et al., 2016). At Fanari Cape, an artificial reef system was constructed and installed in 1999, consisting of a protective zone (240 m³) and a core (nine Italian and nine French artificial reefs) exceeding length of 25 km at an approximate depth of 6 m (Manoudis et al., 2005). The construction of these artificial reefs is a measure of great importance for the management of coastal marine ecosystems and has also proven to be very effective in enhancing fisheries (Pickering et al., 1998). They can also play an important role in the marine area of the coastal zone, including protection against the mechanical impacts of fishing gear, such as trawling, habitat restoration, increased territorial heterogeneity, and substrate diversity in deep seabeds (Manoudis et al., 2005).

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