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Review Article

The current situation and potential effects of climate change on the microbial load of marine bivalves of the Greek coastlines: an integrative review

Aikaterini Zgouridou,[1] Eirini Tripidaki,[1] Ioannis A. Giantsis,[2] John A. Theodorou,[3] Maria Kalaitzidou,[4] Dionysios E. Raitsos,[5] Athanasios Lattos,[1] Apostolia-Maria Mavropoulou,[6] Sarantis Sofianos,[6] Dimitrios Karagiannis,[7] Ilias Chaligiannis,[1,8] Andreas Anestis,[9] Nikos Papadakis,[9] Konstantinos Feidantsis,[1] Dionysia Mintza,[10] Alexandra Staikou[1] and Basile Michaelidis [1*]

[1]*Department of Zoology, School of Biology, Faculty of Science, Aristotle University of Thessaloniki, Thessaloniki, GR-54124, Greece.*

[2]*Department of Animal Science, Faculty of Agricultural Sciences, University of Western Macedonia, Florina, 53100, Greece.*

[3]*Department Animal Production Fisheries and Aquaculture, University of Patras, Messolonghi, Greece.*

[4]*National Reference Laboratory for Marine Biotoxins, Department of Food Microbiology, Biochemical Control, Residues, Marine Biotoxins and Other Water Toxins, Directorate of Veterinary Center of Thessaloniki, Ministry of Rural Development and Food, Thessaloniki, Greece.*

[5]*Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens, Greece.*

[6]*Department of Physics, Section of Environmental Physics and Meteorology, National and Kapodistrian University of Athens, Athens, Greece.*

[7]*National Reference Laboratory for Mollusc Diseases, Ministry of Rural Development and Food, Thessaloniki, 54627, Greece.*

[8]*Hellenic Agricultural Organisation-DEMETER, Veterinary Research Institute of Thessaloniki, Campus of Thermi, 570 01, Thermi, Greece.*

[9]*Laboratory of Hygiene, Social – Preventive Medicine and Medical Statistics, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.*

[10]*Department of Fishery Products, Milk and Other Food of Animal Origin, Ministry of Rural Development and Food of Greece, Athens, Greece.*

Received 15 May, 2021; accepted 4 September, 2021. *For correspondence. E-mail michaeli@bio.auth.g; Tel. 00302310998401.

Summary

Global warming affects the aquatic ecosystems, accelerating pathogenic microorganisms' and toxic microalgae's growth and spread in marine habitats, and in bivalve molluscs. New parasite invasions are directly linked to oceanic warming. Consumption of pathogen-infected molluscs impacts human health at different rates, depending, inter alia, on the bacteria taxa. It is therefore necessary to monitor microbiological and chemical contamination of food. Many global cases of poisoning from bivalve consumption can be traced back to Mediterranean regions. This article aims to examine the marine bivalve's infestation rate within the scope of climate change, as well as to evaluate the risk posed by climate change to bivalve welfare and public health. Biological and climatic data literature review was performed from international scientific sources, Greek authorities and State organizations. Focusing on Greek aquaculture and bivalve fisheries, high-risk index pathogenic parasites and microalgae were observed during summer months, particularly in Thermaikos Gulf. Considering the climate models that predict further temperature increases, it seems that marine organisms will be subjected in the long term to higher temperatures. Due to the positive linkage between temperature and microbial load, the marine areas most affected by this phenomenon are characterized as 'high risk' for consumer health.

Introduction

Marine bivalves, including oysters, clams, cockles and mussels are recognized as a protein rich source for human consumption and offer an economically high-value product for the local communities. As a result, shellfish aquaculture accelerated greatly during the second half of the 20th century, and the demand for increasing bivalve production has remained strong (FAO, 2018; Smaal *et al*., 2018). Occasionally, however, populations of bivalves suffer from severe mortalities, because of several diseases caused by the presence of a wide variety of pathogens. Such pathogens belong to various taxonomic clades, including viruses (Iridolike, Herpes-like virus, Papovaviridae, Togaviridae, Retroviridae, Reoviridae, Birnaviridae and Picornaviridae), bacteria (*Vibrio tapetis*, *Vibrio* spp. and *Nocardia* spp.), protozoans (species of the genera *Marteilia*, *Bonamia*, *Perkinsus* and *Mycrocytos*), metazoan parasites (the species belonging to the copepod *Mytilicola*, among others) and toxic microalgae (Shumway, 1990; Bower *et al*., 1994; Gestal *et al*., 2008; Carella *et al*., 2015; Zannella *et al*., 2017). Additionally, in the context of climate change, outbreaks of infectious diseases are expected to become more frequent (e.g. Burge *et al*., n.d.). Recent studies reported that marine pathogens, boosted by global warming, will impact the immune system of bivalves and weaken their tolerance to extreme temperatures (Ellis, 2015; Turner *et al*., 2016). As a result of physiological malfunction, mass mortalities, and high losses in hatcheries, as well as in natural beds, and spat are expected on a national and global scale (Groner *et al*., 2016; Karagiannis *et al*., 2018; Cao *et al*., 2018a; Avdelas *et al*., 2021). Moreover, the majority of bivalve species grow mainly in estuaries and coastal waters, areas often heavily polluted by urban or agricultural wastes. Consequently, urbanization and pollution in coordination with the rise in surface sea temperature (SST) may accelerate the increase of pathogen contamination, causing infections in the majority of bivalve species (Moullec *et al*., 2019).

The presence of pathogens in aquatic ecosystems is not only a serious biological risk for the aquaculture industry but for public health as well, as bivalve consumption has reportedly increased in the last decades (Elbashir *et al*., 2018). Seafood-borne illnesses include typhoid fever, cholera, infectious hepatitis and many other gastrointestinal infections (Gerba and Goyal, 1978; Rippey, 1994). Several cases of such illness outbreaks have been reported over the last years, posing an especially significant threat to human populations that consume seafood regularly (Elbashir *et al*., 2018; Young *et al*., 2019). The bivalve species which seem to be most vulnerable to pathogen infection are mainly mussels of the genus *Mytilus*, flat oysters (*Ostrea edulis*), clams (*Venus verrucosa*) and to a lesser extent scallops (Family Pectinidae). In the context of climate change, we should prepare for and manage these emergencies through improved surveillance, thereby mitigating the spread of disease and its impacts. Improving surveillance requires fast, accurate diagnosis, forecasting disease risk assessment and real-time monitoring of disease-promoting environmental conditions.

Several commercially important bivalve species are harvested and their geographic distribution has been extensively studied along Greek coasts (Delamotte and Vardala-Theodorou, 2007; Koutsoubas *et al*., 2007). Also, shellfish farming has a major impact on Greece's national economy; it is the second most important aquaculture sector after fish farming (Theodorou *et al*., 2015). However, the almost exclusively farmed shellfish species is the Mediterranean mussel *Mytilus galloprovincialis* and it is farmed mainly in the coastal regions of Northern Greece. In 2017, more bivalve species have been licensed to be farmed in Greece and particularly in the Region of Eastern Macedonia and Thrace, including *Ruditapes decussatus*, *V. verrucosa*, *Aequipecten opercularis*, and *O. edulis*, but the respective farming units' construction has not been completed yet. Apart from these species, a few other edible bivalves were once exploited, but their harvesting is now banned due to either significant declines in their populations (e.g. *Pinna nobilis*) (Theodorou *et al*., 2015, 2017a) or destructive fishing practices and the subsequent enforcement of regulations (e.g. the fisheries of the mussel *Lithophaga lithophaga*) (Katsanevakis *et al*., 2008). Overall, Aegean Sea circulation supports the purification of seawater, positively impacting bivalve farming (Giantsis *et al*., 2014). Despite the great economic importance of bivalve harvesting, the pathogen distribution and contamination of bivalves by Regional Unit of production and harvesting is not clear. On the other hand, more and more investigations are carried out in other to underhand the impacts of pathogens on the bivalve physiological performance in synergy with other drivers of climate change (Turner *et al*., 2016; Zannella *et al*., 2017; Eymann *et al*., 2020; Griffith and Gobler, 2020).

A monitoring program has been established by the Ministry of Rural Development and Food in Greece regarding the health of living bivalve species and the incidences of infection by several pathogen species. The diagnostic methods followed in the National Reference Laboratory for parasite detection strictly follow Standard Operating Procedures issued by the European Union Reference Laboratory for Molluscs Diseases. The present review aims (i) to record, based on the annual reports of the Greek Ministry of Rural Development and Food, the distribution of pathogen species, including parasites, bacteria and toxic microalgae species along the Greek coastlines, where economically important bivalve species are harvested from cultured or natural populations; (ii) to record, based on scientific literature, the physiological impacts (synergistically with other environmental factors) of the detected pathogen on the edible bivalve species; (iii) to analyse and report the past and present climatic data, including SST, *Chl-a* (chlorophyll-a) and salinity; (iv) to record the human poisoning cases attributed to bivalve consumption in Greece; and (v) to integrate the above in an effort to understand the present microbial status and its potential changes to and impacts on bivalve and human health as a result of the holistic effects of various climate change components.

Distribution of edible bivalve species and pathogens along the Greek coastlines

The main commercially important edible bivalve species are reported in Table 1 (Koutsoubas *et al*., 2007). Also, based on the National List of Classified Production Areas (http://www.minagric.gr/images/stories/docs/agrotis/Alievmata/ethnikos_kat_per_dithira160718.htm), Fig. 1 depicts the main Regional Units, including the corresponding marine areas where edible economically important bivalve species are either cultured or harvested from wild populations. As shown, two Regional Units exhibit the highest activity: one located in Thermaikos Gulf and Pieria-Imathia, and the second in the prefecture of Attica (Saronikos Gulf and the nearby islands). Moreover, as shown in Table 1, despite the recent licence provided for culture of more bivalves, only the Mediterranean mussel *M. galloprovincialis* is cultivated in the aforementioned Regional Units. Overall, mussel farming in Greece covers 375.5 hectares (ha) and it is primarily located in the northern part of the country, especially in the Thermaikos Gulf. Specifically, 90% of farms lie in the wider area of the Thermaikos Gulf, representing about 80%–90% of the annual national harvest (Theodorou *et al*., 2011). It should be pointed out that the diversity of bivalve species distributed throughout Greek marine areas has been extensively studied by several investigators (Manousis *et al*., 2010; Zenetos *et al*., 2015). With the exception of the species listed in Table 1, other bivalve species with lower commercial interest, exploited legally, have been reported as well (Koutsoubas *et al*., 2007; Katsanevakis *et al*., 2008). Additionally, Table 1 indicates the preferred temperature

Table 1. List of the main edible and economically important bivalve species distributed in the Marine Regional Units of Greece.

Scientific name	Regional Unit[a]	Cultivated population	Natural population	Sources[b]	Preferred temperature range	Sources[c]
Arca noae (Linnaeus, 1758)	A, B, D, E, F	−	+	1, 3, 5, 10	11.9–17.5°C, mean 15°C	11, 16
Callista chione (Linnaeus, 1758)	B, C, D, E	−	+	1, 2, 3, 5, 10	10.8–21.5°C	11, 13, 17
Cerastoderma glaucum (Poiret, 1879)	A, C, E			2, 3, 8, 7, 9	0–25°C	4, 17, 18
Chamellea gallina	A, B, C, E	−	+	1, 2	9–21°C, mean 10.3°C	11, 13
Chlamys varia (Linnaeus, 1758)	A, B, E	−	+	8, 9, 10	Subtropical, preferred optimum 12°C	11, 13
Donax trunculus	A, B	−	+	1, 10	12.4–21.6°C, mean 19°C	11
Flexopecten glaber (Linnaeus 1758) (*previous name Chlamys glabra*, Linnaeus, 1758)	C, F	−	+	5, 7, 9, 10	13.3–19.7°C, mean 15.6°C	11, 13
Modiolus barbatus (Linnaeus, 1758)	B, C, F	−	+	1, 2, 3, 10	10–20°C, mean 16°C	11, 3, 17
Mytilus galloprovincialis (Lamarck, 1819)	A, B, C, D, E	+	+	1,2, 3, 10	7.1–11.9°C, mean 9.7°C	11, 12, 14
Ostrea edulis (Linnaeus, 1758)	B, D	−	+	1, 2, 3, 10		
Pecten jacobaeus (Linnaeus, 1758)	B, The marine Lake Vouliagmeni	−	+	9, 10	24.4–29.1°C, mean 28.2°C	11
Pinctada maxima	E			1	13.1–16.5°C, mean 14.2°C	11, 13, 15
Ruditapes decussates (Linnaeus, 1758)	A, B, C, D	−	+	1, 2, 3, 10	9.4–21.1°C, mean 18.1°C	11, 12
Venus verrucosa (Linnaeus, 1758)	A, B, D, E, F	−	+	1, 2, 3, 10	8.4–20.1°C, mean 17°C	11, 14, 13

[a]See Fig. 1.
[b]Sources: 1—Hellenic Ministry of Rural Development and Food; 2—Zenetos *et al*. (2005); 3—Koutsoubas *et al*. (2007); 4—Tsotsios *et al*. (2016); 5—Theodorou *et al*. (2011); 6—Leontarakis *et al*. (2008); 7—Tsolakos *et al*. (2020); 8—Lykakis and Kalathakis (1991); 9—Katsanevakis (2005); 10—Mannousis *et al*. (2010); 11—Sealifebase; 12—FAO; 13—EOL (Encyclopaedia of Life); 14—Ocean Biogeographic Information System; 15—Katsanevakis *et al*. (2008); 16—Hrs-Brenko and Legac (1996); 17—Koutsoubas *et al*. (2007); 18—Leontarakis *et al*. (2008).
[c]Refers to the references provided in the table.

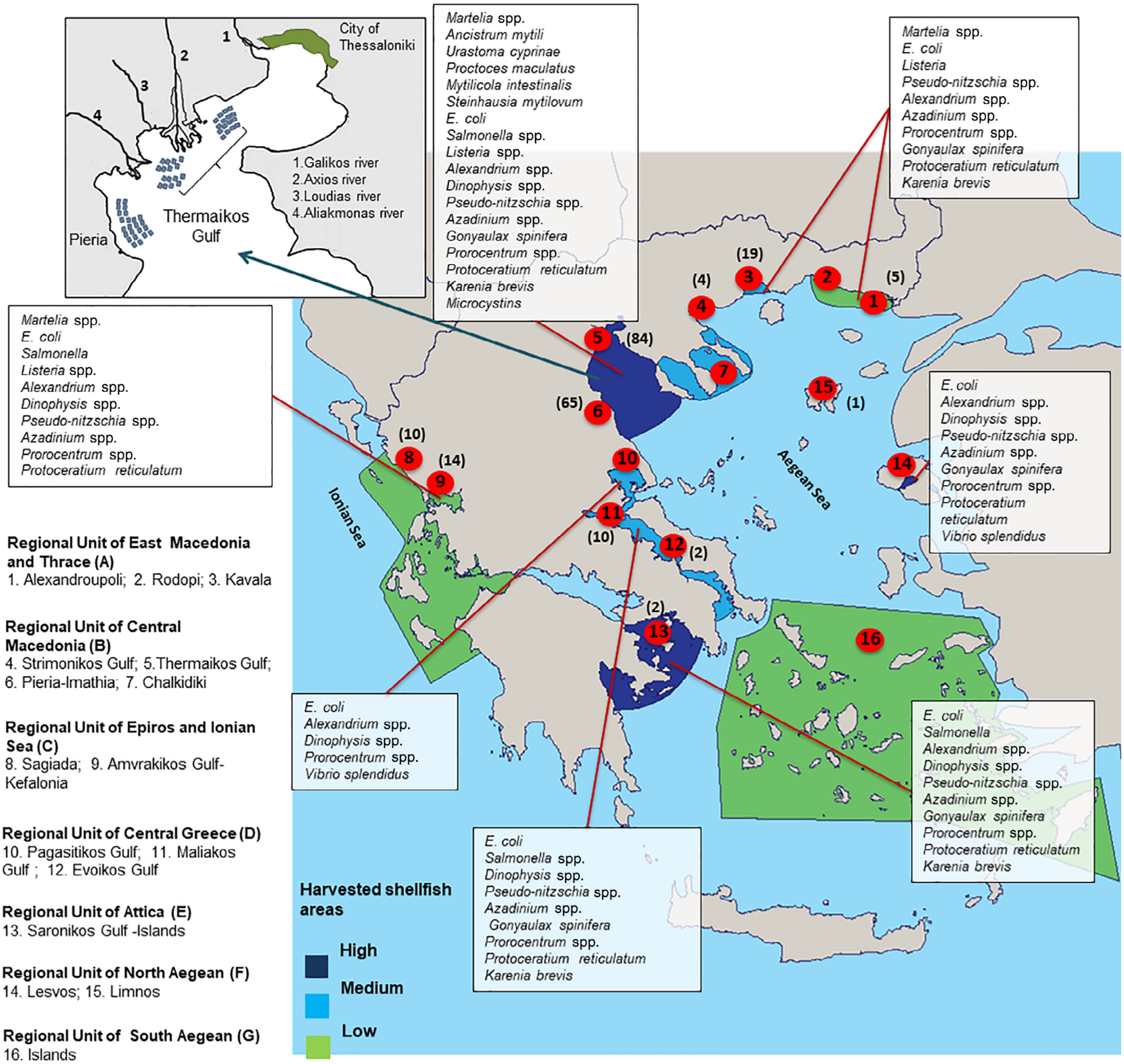

Fig. 1. The Regional Units and the corresponding marine areas (indicated by the red closed circles) where edible bivalve species are either cultivated or harvested from wild population along the Greek coastline. Coloured marine areas indicate differences in the bivalve farming and harvesting and they are characterized as high, medium and low indicated by dark blue, light blue and green colour respectively. The numbers in parentheses indicate the mussels' farming units per marine area. Subfigure depicts mussels' farms in the marine areas Thermaikos Gulf and Pieria-Imathia where Gallikos, Axios, Loudias and Aliakmonas rivers flow. Moreover, this figure depicts the geographic distribution of pathogen species detected in the several Regional Units.

range of the listed bivalve species. However, the temperature range may differ geographically according to the distribution range of the species.

Pathogens

The parasites, bacteria and microalgae species detected in the coastlines of Greece are given in Tables 2, 3 and 4 respectively, while Fig. 1 depicts their geographical distribution per Regional Unit. As shown, several pathogen species are identified in Geek Regional Units of bivalve production and harvesting. Moreover, it becomes clear that there is a great difference regarding the diversity of pathogen species per Regional Unit. Based on the annual reports of the Hellenic Ministry of Rural Development and Food, Fig. 2 summarizes the total percentage of bacteria and microalgae species incidence, reported for the period 2013–2020, in the different Greek marine areas.

Table 2. Parasites species detected in the edible bivalve species distributed in the Greek coastlines.

Species	Host	Regional unit[a]	Sources
Martelia spp.	*O. edulis*	5, 3, 13, 9, 11	Virvilis *et al*. (2003); Balseiro *et al*. (2007)
Martelia spp.	*M. galloprovincialis*	5, 6	Karagiannis and Angelidis (2007); Anestis *et al*. (2010); Karagiannis *et al*. (2013, 2018)
Urastoma cyprinae *Proctoces maculatus* *Mytilicola intestinalis* *Steinhausia mytilovum*	*M. galloprovincialis*	5, 6	Rayyan and Chintiroglou (2003); Rayyan *et al*. (2004)
Ancistrum mytili	*M. galloprovincialis*	5, 6	Rayyan *et al*. (2006)
Cryptosporidium spp.	*M. galloprovincialis*	5	Ligda *et al*. (2020)
Haplosporidium pinnae Mycobacteria	*P. nobilis*	5, 14, 15	Katsanevakis *et al*. (2019); Lattos *et al*. (2020a, 2020b)

[a]See Fig. 1.

Table 3. Bacteria species detected in the edible bivalve species distributed in the Greek coastlines.

Species	Sea water	Host	Regional unit[a]	Sources
Escherichia coli	+	*M. galloprovincialis*, *A. noae*, *V. verrucosa*, *M. barbatus*, *O. edulis*, *C. chione*, *C. gallina*	1, 2, 3, 4, 5, 6, 8, 9,11, 13, 14, 16	Formiga-Cruz *et al*. (2003); Vantarakis *et al*. (2005); Pancucci-Papadopoulou *et al*. (2005); Alexopoulos *et al*. (2011)
Salmonella spp.	+	*M. galloprovincialis*	5, 9, 13	Efstratiou *et al*. (1998, 2009); Economou *et al*. (2013)
Vibrio cholerae *Vibrio parahaemolyticus*	+	*O. edulis*	Crete	Economopoulou *et al*. (2017)
Listeria spp.	+	*M. galloprovincialis*	8, 3, 5	Pancucci-Papadopoulou *et al*. (2005); Economou *et al*. (2013); Soultos *et al*. (2014)
Proteus vulgaris *Proteus mirabilis* *Yersinia enterocolitica* *Staphylococcus aureus* *Pseudomonas fluorescens*		*M. galloprovincialis*	Crete	Pancucci-Papadopoulou *et al*. (2005)

[a]See Fig. 1.

Table 4. Microalgae species detected in the marine areas along the Greek coastlines[a].

	Detection marine area							
Microalgae spp.	Thermaikos Gulf	Alexandroupoli	Kavala	Amvrakikos Gulf	Pagastitikos Gulf	Maliakos Gulf	Saronikos Gulf	Mytilini
Alexandrium spp.	+	+	+	+	+	−	+	+
Dinophysis spp.	+	−	+	+	+	+	+	+
Pseudo-nitzschia spp.	+	+	+	+	−	+	+	+
Azadinium spp.	+	+	+	+	−	+	+	+
Gonyaulax spinifera	+	−	+	−		+	+	+
Prorocentrum spp.	+	+	+	+	+	+	+	+
Protoceratium reticulatum	+	+	+	+	−	+	+	+
Karenia brevis	+	+	+	−	−	+	+	−

[a]Source: Hellenic Ministry of Rural Development and Food.

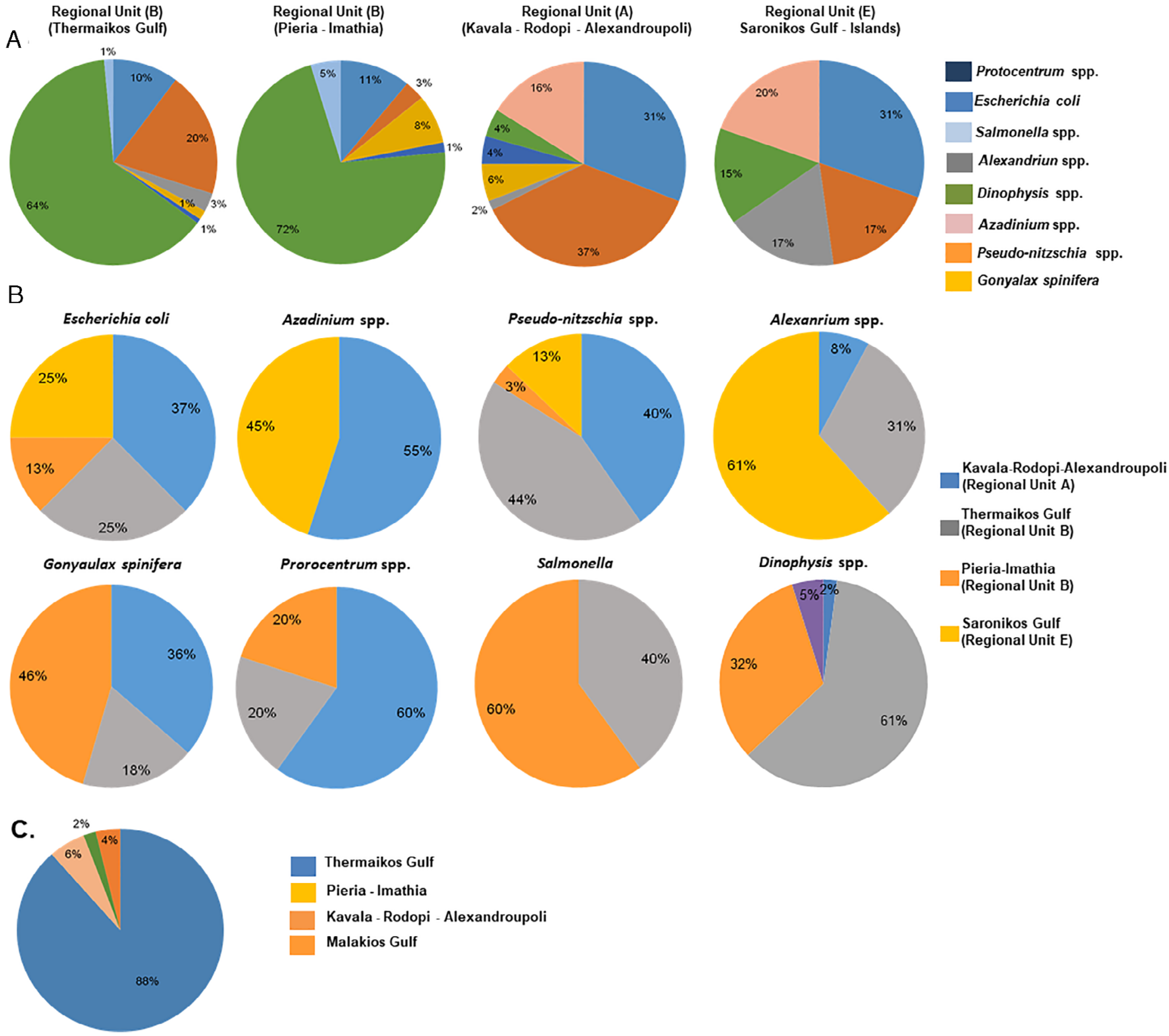

Fig. 2. A. Total percentage of bacteria and microalgae incidence reported for the period 2013–2020 in the different Greek marine areas.
B. Percentage of pathogen species detection in different Greek marine areas.
C. Positive DSP (okadaic acid above regulatory limit – 160 $\mu g\ g^{-1}$) incidence in total (%) in the marine areas Thermaikos and Maliakos Gulf, Pieria-Imathia and Kavala-Rodopi-Alexandroupoli.

Parasites

Several parasite species have been detected across the Greek coastlines (Table 2). Of all these species, however, *Marteilia* spp. seems to have a wider distribution and it is the most examined parasite. In Greece, the first detection of *Marteilia* spp. was reported in samples of *M. galloprovincialis* from the Thermaikos Gulf in October 2004 and July 2005. Further studies showed that it is likely able to complete its life cycle within this area (Virvilis *et al*., 2003; Virvilis and Angelidis, 2006; Karagiannis and Angelidis, 2007). Also, histopathological studies of the infected mussels revealed that plasmodia of *Marteilia* spp. were present in the epithelial cells of the stomach, while sporangia were found in the epithelial cells of the digestive tubules, where sporulation occurred. The increase in infection intensity seems to be synchronized with the gradual increase of ambient seawater temperature (Karagiannis *et al*., 2018). At higher water temperatures, *Marteilia* spp. becomes more active and it is multiplied rapidly (Karagiannis and Angelidis, 2007; Zannella *et al*., 2017).

From a physiological perspective, it has been reported that marteiliosis impacts not only *M. galloprovincialis'* reproduction capacity but also the growth of mussels under field culture conditions (Karagiannis *et al*., 2018).

Specifically, infestation by *Marteilia* can lead to glycogen depletion in mussels as indicated by the emaciation and discolouration of the digestive gland, which in turn leads to a cessation of growth, poor condition index and disruption of the gametogenic cycle, indicative of energy deficiency (Robert *et al*., 1991; Villalba *et al*., 1993a). At the tissue level, marteiliosis leads to formation of granulocytomas, necrosis in the digestive gland (Villalba *et al*., 1993b) and increases in the haemocytes of the haemolymph (Carballal *et al*., 1998). While sublethal effects of marteiliosis are predominantly linked to the activation of immune response, cellular damage and energy deficit, bivalve mortalities appear to be associated with disruption of the digestive tubule epithelia due to sporulation of the parasite (Villalba *et al*., 1993a; Karagiannis and Angelidis, 2007). Moreover, the presence of *Marteilia* spp. impacts the energetics of *M*. *galloprovincialis*, as indicated by the reduction in the efficiency of infected mussels to gain energy from the ingested food, resulting in a significant reduction in scope for growth (Anestis *et al*., 2010).

The impact of marteiliosis on bivalves, however, is not limited only to *M*. *galloprovincialis*. This parasite contributed significantly to the dramatic decline in the natural populations of *O*. *edulis*, observed in Thermaikos Gulf since 1999 (Virvilis *et al*., 2003; Virvilis and Angelidis, 2006). Interestingly, in the same region, *V*. *verrucosa* and *Modiolus barbatus* have not been found to be infected by this pathogen species (Virvilis *et al*., 2003). This finding can probably be attributed to the fact that *M*. *barbatus* inhabits deep waters with lower temperatures, or to the fluctuation of salinity (Pourmozaffar *et al*., 2020). However, the actual loss of bivalve production in the Thermaikos Gulf is difficult to attribute solely to marteiliosis, and only speculations are possible. Overall, environmental conditions and low water quality can weaken the thermal tolerance of bivalves, leading to disease or death of the most vulnerable species (Audemard *et al*., 2001; Carella *et al*., 2018).

Compared to *Marteilia* spp., *Bonamia* spp. have not been detected so far in any bivalve species along the coastlines of Greece. Specifically, the species *B*. *ostreae*, a Haplosporidian protozoan parasite (Carnegie and Cochennec-Laureau, 2004), infects haemocytes of flat oysters *O*. *edulis*, inducing physiological malfunction. Infection is often fatal, depending on host and environmental conditions. In Europe, *B*. *ostreae* was first described in France (Pichot *et al*., 1980) as the causative agent of serious mortalities in this native species. Since then, it has been responsible for a drastic decrease in *O*. *edulis* production in different farming areas along the European Atlantic coast (Carnegie and Cochennec-Laureau, 2004). Although natural populations of *O*. *edulis* species occur in several marine areas of Greece, this parasite has not been detected. However, a recent investigation based on both histopathology and molecular markers revealed the presence of the species *Haplosporidium pinnae*, which belongs in the same family with *Bonamia* spp. (Haplosporidiidae), in *Pinna nobilis* (Lattos *et al*., 2020a). The rest of the parasite species reported in Table 2 have only sporadically been reported, and their impacts on bivalves' physiological performance are unknown.

In addition to *Martelia* spp., several other parasites have been detected in the cultured mussels. However, little is known regarding their distribution and the physiological impacts on bivalves. The ciliate *Ancistrum mytili* is common in the gills of *M*. *galloprovincialis* from the Thermaikos Gulf and is considered to be more symbiotic than parasitic (Bower *et al*., 1994; Villalba *et al*., 1997). Rayyan *et al*. (2006) reported that the condition index (CI) was not affected, and the tissues of the mussels' hosts were not damaged either. However, the infection could be harmful under unfavourable conditions. The high prevalence of *A*. *mytili* in the Thermaikos Gulf is similar to that found in mussel cultures in north-western Spain (Villalba *et al*., 1997). The *Steinhausia mytilovum* (Field, 1924), a microsporidian parasite, has also been detected in the cultured mussels in the Thermaikos Gulf (Rayyan and Chintiroglou, 2003). In contrast to *A*. *mytili*, however, it infects the cytoplasm of mature mussel ovocytes and the CI of infected mussels, inducing a strong hemocyte infiltration inside affected gonadal follicles. The rate of parasite infection appears to be highly correlated to the pollution in the Thermaikos Gulf. The turbellarian *Urastoma cyprinae* (Graff, 1882) was found to inhabit the mantle cavity between the lamellae of the demibranchs of mussels *M*. *galloprovincialis*. The presence of *U*. *cyprinae* causes pathological reactions in its host, the combined effects of which result in disorganization of the gill filaments. The turbellarian induced a heavy infiltration by blood cells and subsequent necrosis of the gill tissues (Robledo *et al*., 1994; Cáceres-Martınez *et al*., 1998). *Proctoces maculatus* was first reported by Rayyan *et al*. (2004). The prevalence and intensity of these parasites were related to temperature and pollution. Mussels infested with these parasites had significantly lower condition indices than non-infested mussels. *Mytilicola intestinalis* appears to be confined to European waters, including coastal areas of the Adriatic Sea, Mediterranean Sea and North Sea. *M*. *edulis* and *M*. *galloprovincialis* are believed to be the primary hosts for *M*. *intestinalis*. However, other bivalves are known to be infested, including oysters, clams and cockles. Histological evidence indicates that *M*. *intestinalis* causes local metaplastic changes in the gut epithelium, involving the replacement of normal ciliated columnar cells with non-ciliated cuboidal cells. The effects of *M*. *intestinalis* on the CI and biochemical constituents of mussels seem to be strongly related to environmental seasonal variations with exception of the very highest infestations during periods of extreme environmental conditions (Davey and Gee, 1988).

Bacteria and microalgae

Bacteria• Escherichia coli*.* Among the reported bacteria species, *E. coli* is abundant and identified in several marine areas (Table 3). It is a faecal indicator microbe with a life history that cycles between two principal habitats: intestines of endotherms (primary habitat) and environmental water, sediment and soils (secondary habitats), and differs markedly with respect to physical conditions (e.g. temperature) and nutrient availability (Savageau, 1983; Petersen and Hubbart, 2020). *Escherichia coli* is usually found in the lower intestine of endotherms and most strains are harmless, but some can cause severe food poisoning.

The hydrological characteristics of the Thermaikos Gulf, including the water discharges from three local rivers and the effects of urbanization from nearby Thessaloniki, seem to explain the high annual reports of *E. coli* compared to other marine areas (Fig. 3). Mallin *et al*. (2009) reported that faecal coliform bacteria (FCB) concentration was significantly higher during rainfall events compared with drought periods, since increased rainfall causes greater wash-off of FCB colonies from soil surfaces. On the other hand, bacteria populations in surface water have been shown to decrease or be susceptible to inactivation in the presence of increasing temperatures (Schijven and de Roda Husman, 2005; Blaustein *et al*., 2013). Šolić *et al*. (2010) reported that *E. coli* concentration in *M. galloprovincialis* and oysters depended on the concentration of *E. coli* in the surrounding seawater and the rate of filtration of seawater by bivalves, a process that is highly controlled by temperature and salinity. Also, recent investigations suggest that climate change, due mainly to a projected increase in precipitation, may increase *E. coli* concentration in Western European surface waters (Vermeulen and Hofstra, 2014). Morphometrical parameters, lipid peroxidation and oxidative stress have been correlated to chronic exposure of the freshwater mussel *Diplodon chilensis* to *E. coli* (Sabatini *et al*., 2011).

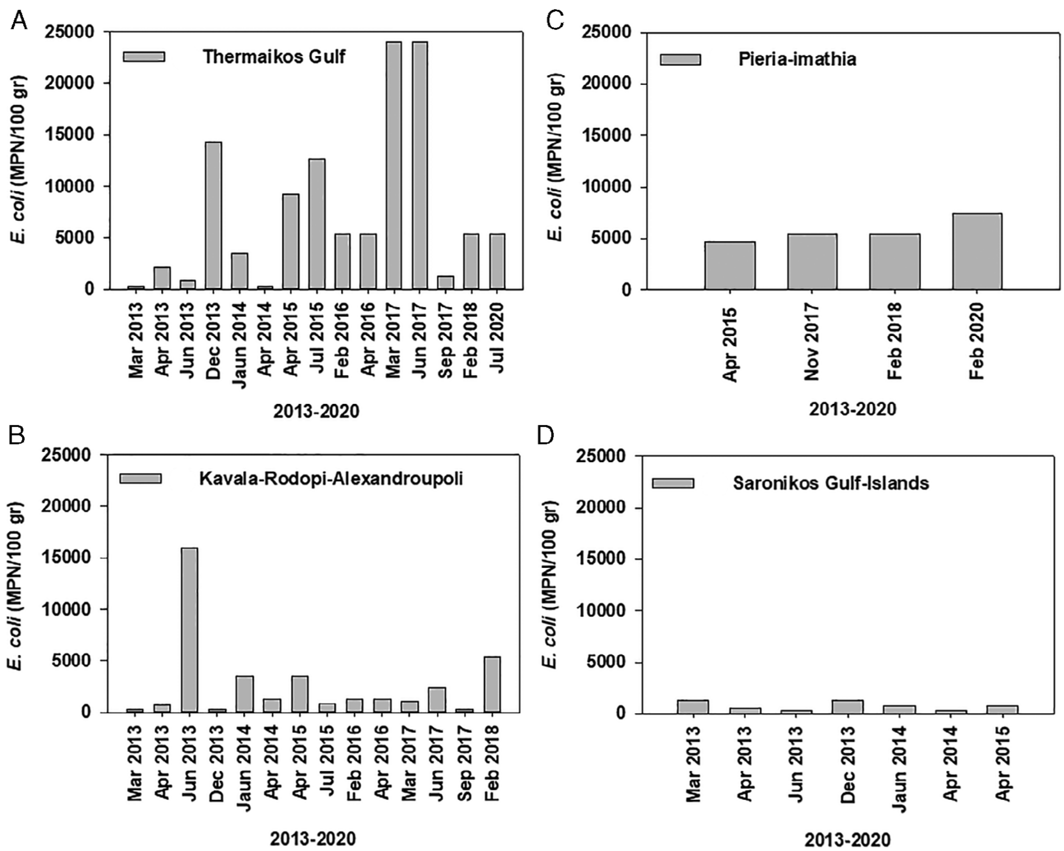

Fig. 3. Most probable number (MPN) of *E. coli* detection incidence in main Regional Units for the period 2013–2020: (A) Thermaikos Gulf, (B) Kavala-Rodopi-Alexandroupoli, (C) Pieria-Imathia and (D) Saronikos Gulf-Islands.

Salmonella *spp.. Salmonella* spp., a genus of Gram-negative bacteria, belongs to the family Enterobacteriaceae and is an important foodborne pathogen worldwide, known for causing a disease called salmonellosis. Low frequency of *Salmonella* spp. has been reported for several Regional Units of Greece. Overall, there is a general burden of the Greek seas with almost constant presence of *E*. *coli* and rarely of *Salmonella* spp. However, according to the infectivity limits of the Ministry of Rural Development and Food, the permissible reference limits of *Salmonella* spp. must be zero due to the infection it causes. Also, it should be noted that the rapid reactions of bivalves to the bacterial load of water indicate that bacteria do not penetrate their tissues but remain in the digestive gland, gills, or on the mantle surface. Their removal from these tissues is based on the process of purification in order to make them suitable for human consumption (Hastings and Heinle, 1995).

Shigella *spp.. Shigella* is a genus of Gram-negative bacteria, facultative anaerobic, genetically closely related to *E*. *coli*. Vantarakis *et al*. (2000) detected *Shigella* species in *M*. *galloprovincialis* by applying a multiplex PCR assay. Several investigations report that the incidence of shigellosis is positively associated with temperature and precipitation, and it is predicted that climate change will increase the incidence of shigellosis in the future. However, further investigations on the association of shigellosis with climate and identification of populations vulnerable to climate change by region are necessary (Song *et al*., 2018).

Vibrio *spp..* Members of the genus *Vibrio* (e.g. *V*. *cholerae* and *V*. *parahaemolyticus*) have been detected recently in some areas of Crete (Economopoulou *et al*., 2017) (Table 3). Furthermore, a recent Next Generation Sequencing analysis of partial 16S rRNA bacterial sequence revealed the presence of various *Vibrio* species in *P*. *nobilis* specimens (Lattos *et al*., 2020b). Vibrio-related infections are increasing worldwide, both in humans and aquatic animals. The ecology of *Vibrio* in coastal waters is intimately related to biotic and abiotic factors. These interactions are also important for *Vibrio* transmission from the aquatic environment to humans. *Vibrio* spp. are associated with protozoa, plankton, plants, bivalves, fishes, birds and a variety of abiotic substrates, such as detrital chitin and inorganic particles (Vezzulli *et al*., 2010). Among other variables affecting such interactions, temperature seems to play an important role in the appearance of *Vibrio* spp. The synergistic effects of pathogen *V*. *splendidus* and seawater acidification impact the bioenergetic status of oysters, which lose their capability to maintain their viability under oxidative stress (Cao *et al*., 2018b).

Listeria *spp. and other bacteria species.* The species *Listeria monocytogenes* is a food-borne pathogen detected in mussels and in various types of seafood, including shrimps, lobsters, crabs and fish (Elliot and Kvenberg, 2000; Rodas-Suárez *et al*., 2006). Soultos *et al*. (2014) demonstrated that *L*. *monocytogenes* is not commonly found in mussels harvested in the North Aegean Sea. However, there the potential for mussels to be contaminated by other *Listeria* species is high. For example, the species *L*. *innocua* has been identified in samples of mussels (Papadopoulou *et al*., 2007).

Apart from the aforementioned bacteria species, mussels may be hosts for other harbouring pathogens bacteria species, such as *Proteus vulgaris* and *Proteus mirabilis*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Pseudomonas fluorescens* (Table 3). Analyses using 16S rRNA gene sequencing showed that cultured oysters are potentially hosts of *P*. *mirabilis* (Fernández-Delgado *et al*., 2007). *Yersinia enterocolitica* is an enteric bacteria responsible for the zoonotic disease yersiniosis. Several species have been reported as potential asymptomatic carriers of *Y*. *enterocolitica*, including rodents, rabbits, cattle, sheep, horses, goats, poultry, seagulls, dogs and cats. Also, the species *P*. *fluorescens* has been studied mainly in dreissenid mussels and there is evidence that the mode of action of the strain Pf-CL145A is intoxication, not infection, and that mussel deaths occur following lysis and necrosis of the digestive gland and sloughing of stomach epithelium (Molloy *et al*., 2013). In Greece, the first report for the presence of the previously mentioned species in bivalves was recorded for *M*. *galloprovincialis* from the island Crete (Papadopoulou *et al*., 2007). However, little is known concerning their distribution along the Greek coastlines, and even less regarding their impacts on bivalve physiological performance.

Microalgae

As reported elsewhere, 61 identified HAB species (toxic, potentially toxic and high biomass producing algae) have spread across the Greek coastline during the last 30 years. Among these, certain algae (16) were associated with the occurrence of important HAB incidents causing damage in the marine biota and the water quality (Koukaras and Nikolaidis, 2004; Nikolaidis *et al*., 2005; Aligizaki and Nikolaidis, 2006; Aligizaki *et al*., 2008; Theodorou *et al*., 2017a, 2017b). Similarly, the analysis of the data obtained in the present work indicates a widespread of *Dinophysis* spp. microalgae along the Greek coastlines (Fig. 1). However, its abundance differs by Regional Unit. Specifically, *Dinophysis* spp. are abundant in the Thermaikos Gulf, Pieria-

Imathia, while the *Pseuonizchia* spp. are abundant in Kavala-Alexandroupoli and Rodopi (Fig. 2).

Dinophysis spp. produces one or two groups of lipophilic toxins: (i) okadaic acid (OA) and its derivatives, the dinophysistoxins (DTXs), and (ii) pectenotoxins (PTXs) (Yasumoto *et al*., 1980; Reguera *et al*., 2014). The OA and DTXs, known as DSP toxins, are acid polyethers that inhibit protein phosphatase and have diarrheagenic effects in mammals. Filter feeding bivalves retain toxic planktonic microalgae and other suspended matter, acting as vectors of the toxins through the food web, and bivalves contaminated with DTXs are a threat to public health. Previous works reported that the Thermaikos Gulf, the Maliakos Gulf in Central Greece and the Saronikos Gulf in South Greece have all been subjected to DSP events caused mainly by *D*. *ovum* and *D*. *acuminata* (Prassopoulou *et al*., 2009; Louppis *et al*., 2010).

In contrast to the Thermaikos Gulf, however, *Pseudo-nitzschia* spp. are abundant in the Regional Unit A (Fig. 1). Consequently, local suspension-feeding bivalves are important vectors of domoic acid (DA), a neurotoxic amino acid produced by diatoms mainly of the genus *Pseudo-nitzschia* spp. and the causative agent of ASP (Trainer *et al*., 2008). Numerous studies have pointed out the physicochemical variables that affect DA production by *Pseudo-nitzschia* species. Most targeted macro- and micronutrients (e.g. Si, N, P, Fe and Cu) involve temperature, irradiance and pH (Bates and Trainer, 2006; Lelong *et al*., 2012; Trainer *et al*., 2012). Additionally, other studies identified salinity as an important factor affecting growth, seasonality, distribution and DA production of *Pseudo-nitzschia* spp. (Lelong *et al*., 2012; Lim *et al*., 2014; Bargu *et al*., 2016). Furthermore, light intensity, photoperiod, river flow, rainfall, or upwelling and factors controlling nutrient availability affect *Pseudo-nitzschia* spp. distribution and bloom dynamics Torres Palenzuela *et al*., 2019). The seasonal variations in *Pseudo-nitzschia* spp. and DA concentration were investigated at three shellfish farms in SW coastal Mediterranean, and the results suggest that temperature, salinity, and inorganic and organic nutrients were significant for the seasonal dynamics of *P*. *seriata* and *P*. *delicatissima* groups (Garali *et al*., 2020).

In addition to the microalgae species mentioned above, other harmful microalgae species have been identified in Greek seas (Table 4), including the dinoflagellates genera *Alexandrium*, *Pyrodinium*, and *Gymnodinium*, and their geographical distribution is shown in Fig. 1. Algae of these species (named 'red tides') produce saxitoxins in shellfish, and consumption of contaminated tissues may lead to PSP and death in humans because of muscle paralysis and cardiorespiratory failure (McGillicuddy Jr. *et al*., 2014). In addition to STXs, tetrodotoxins (TTXs) are compounds that are chemically different from STXs, synthesized by cyanobacteria, and present in freshwater ecosystems that produce a similar paralytic syndrome in cattle, birds and fishes. First detection of TTXs in Greek shellfish was reported by Vlamis *et al*. (2015).

Overall, the relationship between temperature, growth and toxin production by harmful microalgae is poorly understood. As reported elsewhere, temperature increases (up to 25°C) generally promote the growth of *Dinophysis* spp. and an increase in the production of diarrhetic shellfish toxins (Kamiyama *et al*., 2010). However, for other toxin-producing species, including *Alexandrium* spp., inverse relationships between toxin production and growth rate have been described. Less clear is the impact of warming on toxin production by cyanobacteria including *Microcystis aeruginosa*, *Planktothrix agardhii*, and *Dolichospermum* spp. (formerly *Anabaena* spp.) (Griffith and Gobler, 2020 and references cited herein). Several studies demonstrated that warming might stimulate the production of microcystins, a group of cyclic heptapeptides causing acute and chronic effects derived from their endocellular activity and protein phosphatase inhibition. In a recent study, microcystin-RR and microcystin-LR were detected for the first time in mussel *M*. *galloprovincialis*, harvested from farms in the Thermaikos Gulf (Kalaitzidou *et al*., 2021).

While HABs have been studied mostly in relation to public health, another fundamental issue is their direct effect on filter-feeding bivalves (Shumway, 1990; Oda *et al*., 2001; Landsberg, 2002). Experimental studies indicate that HABs can modulate bivalve–pathogen interactions in various ways, including alteration of immune defence and physiological malfunctions (for an extended review see Lassudrie *et al*., 2020). Previous studies indicated that *Dinophysis* spp. producing PTXs induce hypersecretion of mucus and pseudofaeces, paralysis, alteration of the tissues within the digestive gland, and reduced escape response in adult scallops (Basti *et al*., 2015). McCarthy *et al*. (2014) demonstrated that exposure of adult Pacific oysters and blue mussels to OA resulted in increased DNA fragmentation. Further studies also underlined modified hemocyte functions in Mediterranean mussels (Malagoli *et al*., 2008; Prado-Álvarez *et al*., 2012) and carpet shell clams (Prado-Alvarez *et al*., 2013) when exposed to *Dinophysis* spp. and their toxins. In mesocosm experiments, *Perna viridis* subjected to simulated climate change (warming and/or hyposalinity) and simultaneously exposed to harmful bacteria and/or toxin-producing dinoflagellates indicated significant interactions between the above applied experimental conditions with the metabolic and immunobiological malfunctions. Moreover, stimulated stress responses and detoxification processes were not enough to protect digestive gland cells from the toxicity caused by accumulated STX, as indicated by the increased oxidative stress and immunotoxicity in two oyster species (Cao *et al*., 2018b). Given that the depuration of STX within bivalves can be slowed by warming and acidification, this toxin may be retained for longer periods of time, increasing the risk to bivalve health (Braga *et al*., 2018). Several studies revealed changes in metabolic pathways related to protein folding and stabilization, cytoskeleton structure, gene transcription/translation, feeding, behaviour, and energy balance in mussels after exposure and feeding toxic cyanobacteria (Juhel *et al*., 2006; Oliveira *et al*., 2020).

Although biotoxins in shellfish occur only sporadically in Greece, economic losses and influence on exportations are occasionally detrimental. However, the most affected area is that of the Thermaikos Gulf, with an annual production capacity of approximately 20 000 t of *M*. *galloprovincialis* (Theodorou *et al*., 2011). The first documented outbreak associated with a bloom of *D*. *ovum* in the Thermaikos Gulf during 2000 caused losses worth €5 000 000 to the shellfish industry (Koukaras and Nikolaidis, 2004). In 2017, harvesting and selling of bivalves from the marine area of Mazoma in the Amvrakikos Gulf were forbidden by Official Authorities for more than a month due to a high concentration of biotoxins. More specifically, the total cell number of various microalgae species producing amnesic biotoxins overlapped the limit of 200 000 per litre of seawater.

Fig. 4A depicts the seasonal changes in the values for *Dinophysis* spp. and *Pseudo-nitzschia* spp. at the main Regional Units where they are detected more frequently. As shown, the outbreak of *Dinophysis* spp. is more frequent in the Thermaikos Gulf and during spring and summer. On the contrary, less frequent outbreaks are reported for the *Pseudo-nitzschia* spp. which seems to be detected throughout the year. Conversely, spring and summer represent the two seasons with the higher percentage of reports for *Dinophysis* spp. in Pieria-Imathia. Spring seems to be the main season with the higher incidence of *Pseudo-nitzschia* spp. in the Regional Unit A (Fig. 1). Further analysis of the obtained data revealed that, overall, the highest percentage of outbreaks take place during spring and summer (Fig. 4B).

Bivalve food poisoning incidence

The ability of pathogen species to survive in seawater or to reproduce in the bivalve hosts under particular physiochemical conditions may prove dangerous for human health. On the other hand, the driving forces of evolutionary processes may cause the appearance of strains most detrimental to humans. For example, a strain of *E. coli* producing Shiga toxin (STEC) can cause severe symptoms such as stomach cramps, diarrhoea (often bloody) and vomiting. Recent investigations reported that increased temperatures can result in alterations in *E. coli* gene expression, favouring the occurrence of *E. coli* O157 strain (Philipsborn *et al*., 2016). Also, studies on temperature adaptation, using *E. coli* strains and culture conditions, have shown that mutations in genes affecting a wide array of cellular processes can improve fitness of *E. coli* at elevated temperatures (Deatherage *et al*., 2017; Lenski, 2017).

A recent study estimated that approximately 93.8 million human cases of gastroenteritis and 155 000 deaths occur due to *Salmonella* infection worldwide each year (Hoelzer *et al*., 2011). It is usually characterized by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. Usually, symptoms of salmonellosis are relatively mild, and patients usually recover without specific treatment. However, in some cases, especially in children and elderly patients, dehydration can become serious and life-threatening. Although large *Salmonella* outbreaks usually attract media attention, 60%–80% of all *Salmonella* cases are not recognized as part of a known outbreak and are classified as sporadic cases or not diagnosed at all.

There is consistent evidence that gastrointestinal infection with this bacterial pathogen species is positively correlated with ambient temperature, as warmer temperatures enable more rapid replication and therefore increase rates of *Salmonella* infections (Mills *et al*., 2010). Climate, weather, topology, hydrology and other geographical characteristics of the growing site may influence the magnitude and frequency of transfer of pathogenic microorganisms from environmental sources to humans (World Health Organization Food Safety Report-WHO, 2011). Overall, the transmission of *Salmonella* to humans is a complex ecological process, and illnesses caused by this species are more common when temperatures are higher. Warming, in combination with differences in eating behaviour, may contribute to intestinal infections including *Salmonella* infection (Luber *et al*., 2014; Liu *et al*., 2018). Regression and neural network models were used to determine the correlation between increase in temperature and increase in *Salmonella* outbreaks. Both models showed a strong positive correlation between increase in temperature and *Salmonella* infections.

Waterborne outbreaks of *Shigella* spp. have been reported sporadically for different areas in Greece and it seems to be related to drinking water (Maraki *et al*., 2003; Koutsotoli *et al*., 2006). Shigellosis is usually transmitted from person to person in households, although outbreaks due to contaminated food or water are not uncommon. In most cases, however, the occurrence of poisoning in patients is not studied in such a way as to identify the cause and the infectious agent,

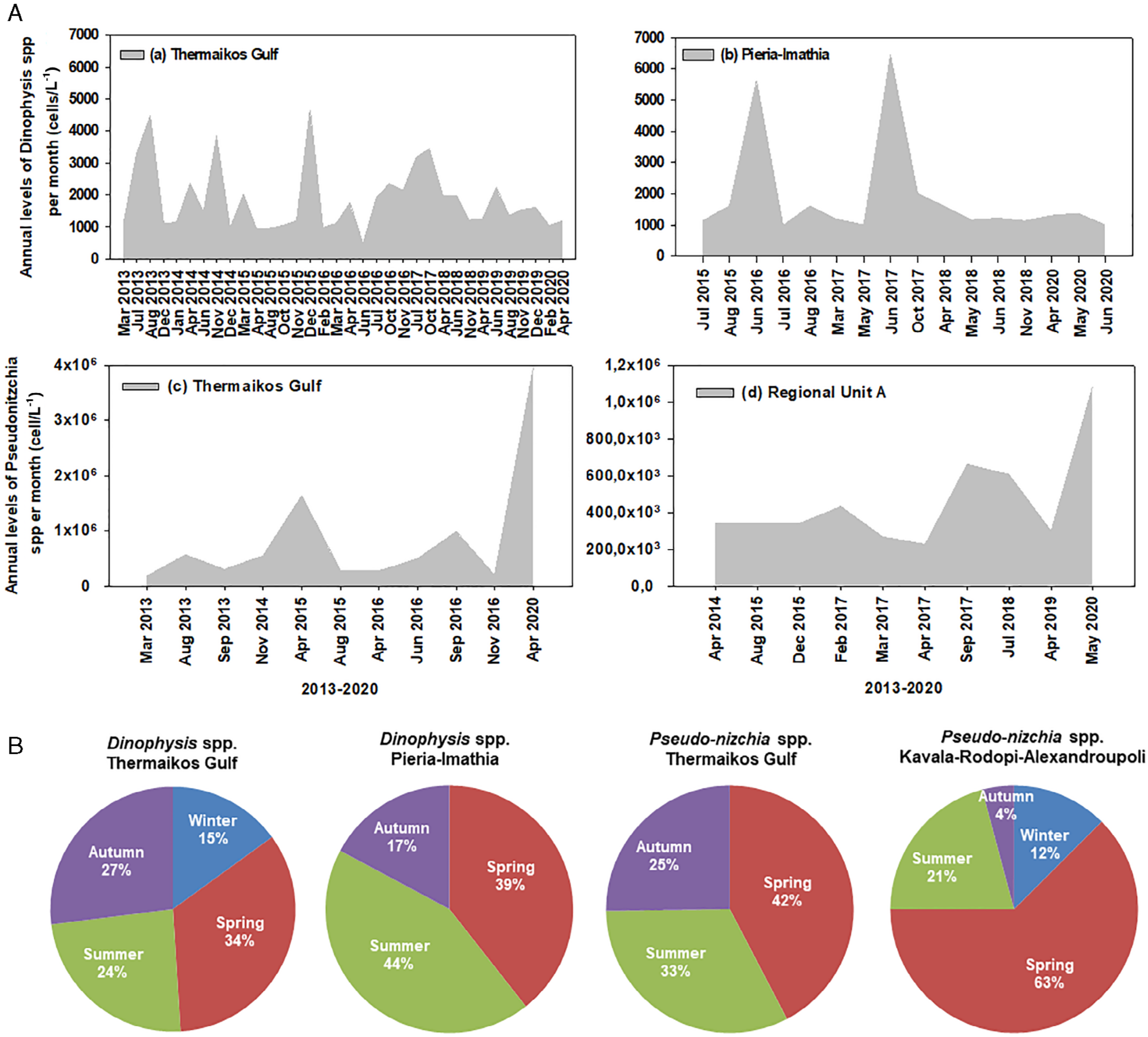

Fig. 4. (A) Monthly outbreaks of *Dinophysis* spp. in the marine areas (a) Thermaikos Gulf and (b) Pieria-Imathia and the *Pseudo-nitzschia* spp. in (c) Thermaikos Gulf and (d) Regional Unit A during the period 2013–2020. Values give the averaged concentrations *Dinophysis* spp. per month. B. Seasonal (Winter, Spring, Summer and Autumn) abundance (%) of *Dinophysis* spp. and *Pseudo-nitzschia* spp. in several marine areas.

while data concerning poisoning cases are kept in hospital files where the patients have been treated but are not published for scientific purposes. Moreover, there is an ambiguity as to the cause of the poisoning cases. For example, although the presence of *Shigella* spp. and the existence of cases of infection after consumption of contaminated food in Greece are positively correlated (Mellou *et al*., 2013), it is not specified whether the food belonged to the category of vegetables or edible bivalve molluscs.

Human poisoning because of *V. parahaemolyticus* outbreak occurred in Spain in 1999 and was associated with the consumption of raw oysters (Lozano-Leon *et al*., 2003). More recently, two new cases of acute gastroenteritis caused by *V. parahaemolyticus* occurred in Central Italy in 2008 (Ottaviani *et al*., 2010), with both patients consuming local mussels.

Listeriosis causes serious symptoms, including septicemia, abortion, liver failure and meningitis, especially among susceptible people like immuno-compromised individuals and pregnant women (Dhanashree *et al*., 2003). Most of the aforementioned bacteria are heat labile and adequate cooking will inactivate them. However, improper handling and cross-contamination or raw seafood eating habits might pose a health hazard, and especially to susceptible populations such as the immune-suppressed, children and the elderly (Papadopoulou *et al*., 2007).

Overall, bivalve molluscs poisoning is minimal in Greece. This could be explained by the low incidence reporting. The region of the Thermaikos Gulf continues to exhibit the highest levels of OA as is indicated by data obtained from the monitoring program for marine biotoxins 2018–2020 (Fig. 2C) (Kalaitzidou *et al*., 2017). Historically, in January 2000, 120 persons of both sexes, aged 8–70 years, visited emergency departments of hospitals in Thessaloniki area with acute non-febrile gastroenteritis after consuming mussels (*M. galloprovincialis*). The predominant symptoms reported by patients were diarrhoea, nausea, vomiting, abdominal cramps and chills, which persisted for more than 24 h. The symptoms were directly connected to the consumption of mussels collected from the Thermaikos Gulf in 2003. The detection of DSP marine biotoxins was conducted by mouse bioassays. The bioassays revealed toxicity to mice from mussel specimens, while large numbers of toxic algae *D. acuminata* were detected in water samples from Thermaikos Gulf. The harvesting of mussels was immediately suspended, and a blooming microalgae monitoring program was established. During the monitoring of mussel toxicity from January 2000 to January 2005, two specimens of mussels positive for DSP were found, one of which was harvested in March 2001 from the event area (Thermaikos Gulf)-western Greece (Amvrakikos Gulf). However, sporadic cases or outbreaks were not reported during this period (Papadopoulou *et al*., 2007). In February 2010, there was a simultaneous increase in gastroenteritis cases in Lemnos, Lesvos and Agios Efstratios (North Aegean islands). A retrospective cohort study on the most isolated island of Agios Efstratios, considering the population under study, showed that the consumption of shellfish introduced into the island from Kavala (Regional Unit A) was significantly correlated with the onset of gastrointestinal symptoms. The fact that clinical and environmental samples were not collected meant that there was no opportunity to link Agios Efstratios incidence with the increased incidence of gastroenteritis in two other islands, Lemnos and Lesvos.

Evidence for future synergistic effects of climate change components and physiological constraints of bivalves

Climate change not only directly impacts marine environments by shifting water temperatures, salinity, pH and dissolved oxygen concentrations but may also indirectly contribute to the emergence of additional ecosystem stressors, such as infectious diseases that affect the health of both aquatic organisms and humans (Sunagawa *et al*., 2015; Borbor-Cordova *et al*., 2019; Bramwell *et al*., 2021). *Perkinsus* spp. outbreaks present a clear example of spatial distribution influenced by global warming, resulting in significant mortality events and depressed production of marine molluscs on the Mediterranean coasts (Villalba *et al*., 2004, 2005). The Mediterranean Sea is now recognized as a hotspot of global change, ranking among the fastest warming ocean regions (Moullec *et al*., 2019). Climate change will continue to intensify within coastal zones throughout this century (Doney *et al*., 2012; IPCC, 2014), while climate projections indicate a potential increase in SST of 1–1.5°C in the eastern Mediterranean, Aegean and Adriatic Seas by 2050, with summer SST regularly surpassing 29°C in the south-eastern Mediterranean (Lovato *et al*., 2013). Analysis of climatic data during the last 25 years indicates a continuous increase in the mean annual temperatures and especially those of summer months (see Fig. 6, plots A and B respectively). Despite the differential distribution of bacteria and microalgae species pathogens along the Greek coastlines, a faster emergence of pathogens outbreaks, especially the thermopile species, is expected. Furthermore, recent investigations showed a general increase in frequency, intensity and depth penetration of marine heat waves in the coming decades along the Greek coasts, up to at least 12 m depths (Galli *et al*., 2017). According to these investigations, toxic microalgae grow not only on the surface but also at deeper levels. Available data indicate that the distribution of most bivalve species ranges between 2 and 15 m depth in the Thermaikos Gulf, strongly indicating future impacts on these species because of climate change (Fig. 6, box D).

Comparatively, the Thermaikos Gulf exhibits higher diversity and more intense outbreaks of pathogens seasonally than the other examined marine areas. It has been suggested that the hydrological regimes and the resulting environmental conditions favour the appearance of the most examined microalgae species in the Thermaikos Gulf (Nikolaidis and Moustaka-Gouni, 1990). Most of the freshwater inputs to the Thermaikos Gulf originate from Axios, Aliakmon, Pinios and Gallikos rivers, occurring between November and early in June (Poulos *et al*., 2000), which coincides thoroughly with *Dinophysis* spp. outbreaks. However, it is worth investigating the role of particular environmental factors by region favouring the abundance of specific microalgae species. The latter is mainly obvious for *Dinophysis* spp. and *Pseudo-nitzschia* spp. in the Thermaikos Gulf and Kavala Bay respectively. Varkitzi *et al*. (2018) reported a close relationship between the frequency of *Pseudo-nitzschia* spp. with silicates and nitrates in the Maliakos Gulf.

Temperature levels of 25–26°C are commonly reached during summer over large areas of the Mediterranean Sea, especially in shallow water coastal ecosystems. These temperatures have been identified, at best, as an upper limit for mussels' normal physiological activities (see Fig. 6, plot E – Anestis *et al*., 2007, 2010; Rodrigues *et al*., 2015; Feidantsis *et al*., 2020, 2021; Lupo *et al*., 2021). According to preferred temperatures for the bivalve species listed in Table 1, it becomes clear that temperatures beyond 22°C will likely trigger physiological constraints in the bivalve species inhabiting the corresponding Regional Units. The present analysis indicated that the Regional Units in Greek coasts, where bivalves are either cultured or harvested from wild

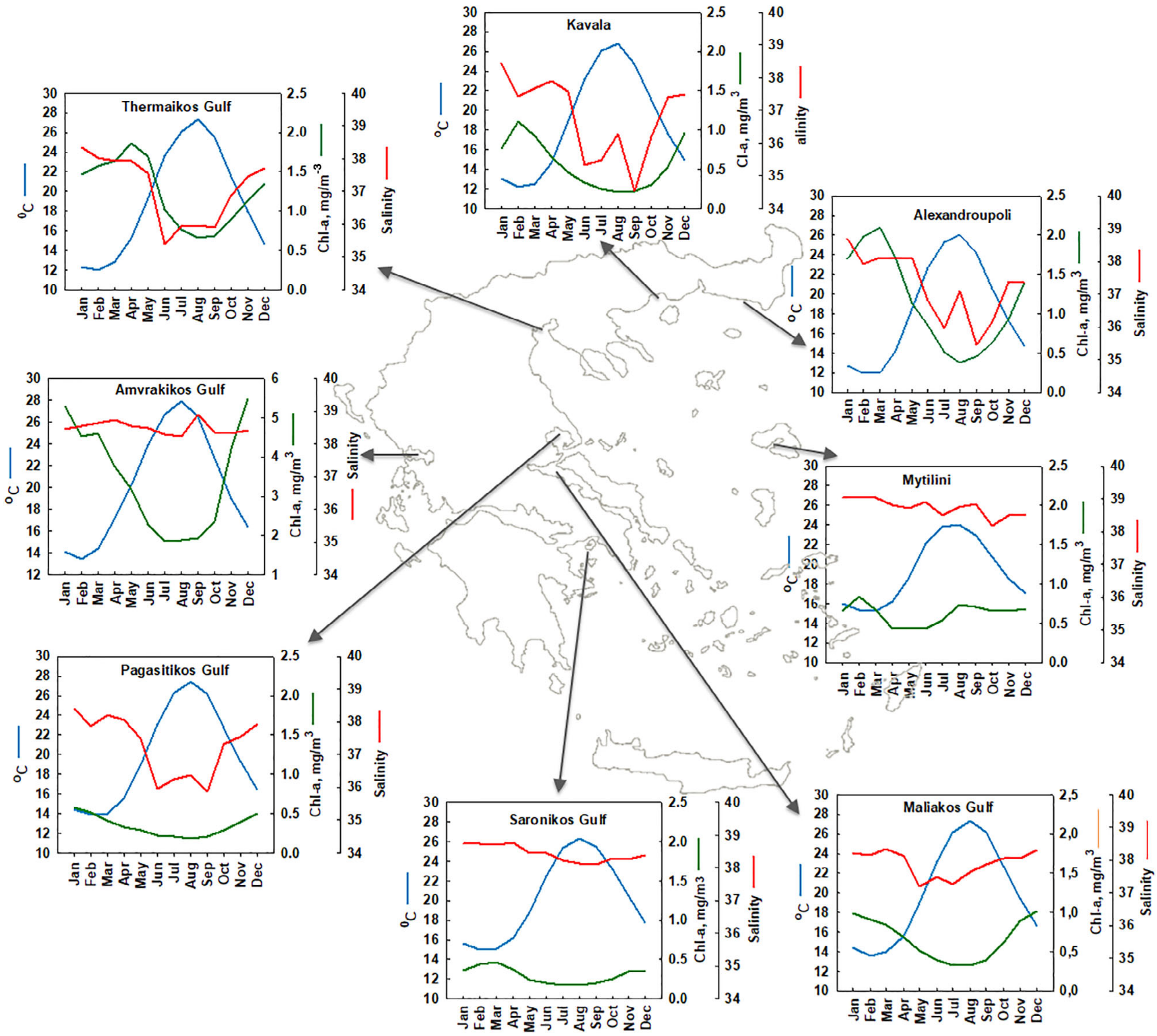

Fig. 5. Monthly temperature (SST), chlorophyll-*a* (*Chl-a*) and salinity variations in the most important marine areas in Greece. The seasonal cycle of SST was calculated based on the available 10 years of observations (January 2009 to December 2020). The seasonal cycle of *Chl-a* was assessed based on 22 years of data (January 1998 to December 2020). The monthly changes in salinity were computed over the time period 1960–2011 to represent the overall long-term seasonal cycle in the areas of interest. In most of the examined areas SST values increases significantly during the summer period, with the highest levels observed mainly in August. In several of these areas, such as Thermaikos Gulf, Kavala and Alexandroupoli, salinity and *Chl-a* levels decreased concomitantly to the SST increase. However, in the Saronikos Gulf, Maliakos Gulf and Mytilini, the decrease of *Chl-a* levels during the summer period was not as intense as in the aforementioned areas. This decrease was also accompanied with milder variations in the salinity levels of these areas. It should be noted though that in Amvrakikos Gulf the decrease in *Chl-a* levels during summer was not accompanied by a decrease in salinity levels which in general remained stable, while in Pagasitikos the decrease in salinity levels was accompanied with fairly stable levels in *Chl-a* levels during the same period.

populations, exhibit SSTs higher than the upper thermal limits of mussels during summer (Fig. 5). Mussels from several Mediterranean Sea production areas, including Regional Unit A (Kavala), B (Thermaikos Gulf) and C (Maliakos Gulf), experienced mortality episodes in recent years as a consequence of summer heat waves (Rodrigues *et al*., 2015). Moreover, mass mortalities of benthic organisms reported in the Mediterranean Sea were attributed to climate-related constraints, as global warming caused hypoxic–anoxic conditions and higher vulnerability to disease (Bally and Garrabou, 2007). Summer 2021 was especially catastrophic for mussel production in the Regional Units of production in Greece as a result of marked mortality of mussels from wild and farmed populations because of the elevation of sea temperature up to 32°C, a temperature that was sustained for 30 days in many marine areas (B. Michaelidis, Personal observations and records).

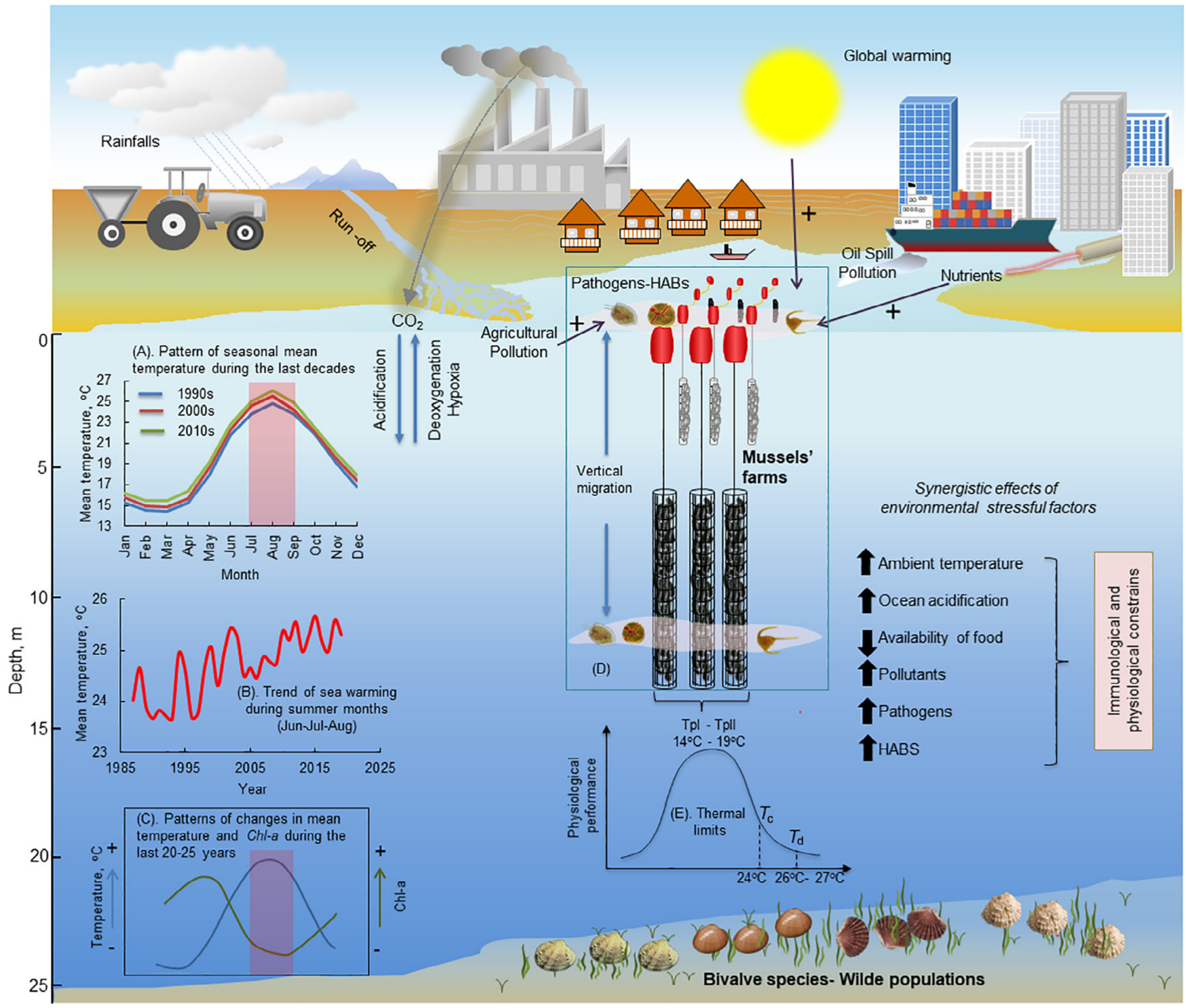

Fig. 6. Proposed model integrating the potential impacts of global warming, pollution, ocean acidification, urbanization, pathogens and food availability on bivalve physiological performance. The plot (A) indicates the seasonal SST values in 1990s, 2000s and 2010s, while the plot (B) indicates the increasing trend in SST values during summers months and for the period 1985 to 2020. Both plots show a shift of annual temperatures towards higher values and a gradual increase in the mean temperatures of summer months respectively. The plot (C) simulates the patterns of seasonal changes in temperature and food availability during the last 20 years. As shown, summer months are characterized by high temperatures and low food availability. The box (D) underlies the presence of pathogens in the sea water and the body of mussels. Urbanization, global warming and agricultural pollution are expected to accelerate the development of pathogens and to favour the appearance of new pathogen strains. Also, conditions favouring the synergistic effects of the above factors will trigger significant immunological and physiological constrains in several bivalve species. The plot (E) simulates the thermal limits and the physiological performance curve for the mussel *M. galloprovincialis* (for further details see Anestis *et al*., 2007, 2010; Feidantsis *et al*., 2020). Tpl and TplI characterize the lower (Tpl) and higher pejus (TplI) temperatures respectively. T_c (critical temperatures) indicates the onset of anaerobic metabolism, while T_d (denaturation temperatures) indicates the onset of heat induced loss of structural integrity at the molecular level (for further explanation see Pörtner, 2010).

However, global warming is only one component of climate change and elevation of temperature is likely to act in tandem with other increasing anthropogenic disturbances such as pollution, eutrophication, overexploitation of resources, habitat modification and destruction, all of which played a major role in altering the structure and functioning of ecosystems such as mussel farms (Pörtner, 2010; Rosa *et al*., 2012). Among these processes, those contributing to the supply or regeneration of biologically important nutrients are particularly influential in determining productivity rates. Longer summers resulting in lower runoffs are expected to influence food availability for bivalves. The pattern of changes in food availability, as estimated by the content of *Chl-a*, show lower energy intake via food during the summer (Fig. 6, plot C). Less food, and therefore energy, intake will further weaken bivalves' defences against stressful conditions (Sokolova, 2013). Moreover, the continuous increase in the magnitude and duration of urbanization, pollution and eutrophication might favour the appearance of new strains harmful for bivalves and human health (Spatharis *et al*., 2007). A recent study revealed the existence of several species that may be potentially toxic in the future for the Thermaikos Gulf (Genitsaris *et al*., 2019). In this context, studying bivalves' mortalities through a metagenomics perspective may largely contribute to understanding the appearance and involvement of new pathogen species in mortality outbreaks by comparing the microbial profiles of animals and seawater in sites where mortality occurs versus sites with a non-mortality context, or before versus during the course of mortality events. Taking into consideration the thermal limits and physiological performance of mussels (Fig. 6, plot E) it becomes clear that climate change will favour the synergistic effect of relative components resulting in significant immunological and physiological constraints on bivalve species, as is presented in Fig. 6.

Overall, a complex pattern of interactions is expected between the organism and several habitat conditions (e.g. temperature, salinity, food availability), which will shape the actual range of temperatures to which the organism is exposed and can tolerate. Investigating these relationships and modelling the environmental control of marine species growth is essential in sustainable management of coastal ecosystems. Recent investigations reported a decline of mussel aquaculture in the European Union and highlighted the economic impacts on mussel farming during the next years because of the synergistic effects of several environmental factors (Avdelas *et al*., 2021). Also Theodorou *et al*. (2020) estimated that continuous closures of harvesting sites due to HABs, lasting more than 4–6 weeks during spring and summer, could be catastrophic for the local economy. Therefore, in the context of both human health and economic loss reduction, as bivalve diseases become emergencies, massive efforts need to be employed for the improvement of the microbiological quality of the product. Accordingly, the development of new practices for sustainable aquaculture becomes urgent as a measure to anticipate the dramatic reduction in the natural harvesting populations (Pernet and Browman, 2021). Decision-making stakeholders and increases in the awareness of the threats posed by marine diseases may lead to policy frameworks facilitating the responses and management that marine disease emergencies require (Groner *et al*., 2016).

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References

Alexopoulos, A., Plessas, S., Voidarou, C., Noussias, H., Stavropoulou, E., Mantzourani, I., *et al*. (2011) Microbial ecology of fish species ongrowing in Greek sea farms and their watery environment. *Anaerobe* **17**: 264–266. https://doi.org/10.1016/j.anaerobe.2011.03.003.

Aligizaki, K., Katikou, P., Nikolaidis, G., and Panou, A. (2008) First episode of shellfish contamination by palytoxin-like compounds from Ostreopsis species (Aegean Sea, Greece). *Toxicon* **51**: 418–427. https://doi.org/10.1016/j.toxicon.2007.10.016.

Aligizaki, K., and Nikolaidis, G. (2006) The presence of the potentially toxic genera Ostreopsis and Coolia (Dinophyceae) in the North Aegean Sea, Greece. *Harmful Algae* **5**: 717–730. https://doi.org/10.1016/j.hal.2006.02.005.

Anestis, A., Lazou, A., Pörtner, H.O., and Michaelidis, B. (2007) Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. *Am J Physiol* **293**: R911–R921. https://doi.org/10.1152/ajpregu.00124.2007.

Anestis, A., Portner, H.O., Karagiannis, D., Angelidis, P., Staikou, A., and Michaelidis, B. (2010) Response of *Mytilus galloprovincialis* (L.) to increasing sea water temperature and to marteliosis: metabolic and physiological parameters. *Comp Biochem Physiol A* **156**: 57–66. https://doi.org/10.1016/j.cbpa.2009.12.018.

Audemard, C., Barnaud, A., Collins, C.M., Le Roux, F., Sauriau, P.G., Coustau, C., *et al*. (2001) Claire ponds as an experimental model for *Marteilia refringens* life-cycle studies: new perspectives. *J Exp Mar Biol Ecol* **257**: 87–108. https://doi.org/10.1016/S0022-0981(00)00330-0.

Avdelas, L., Avdic-Mravlje, E., Borges Marques, A.C., Cano, S., Capelle, J.J., Carvalho, N., *et al*. (2021) The decline of mussel aquaculture in the European Union: causes, economic impacts and opportunities. *Rev Aquac* **13**: 1–28. https://doi.org/10.1111/raq.12465.

Bally, M., and Garrabou, J. (2007) Thermodependent bacterial pathogens and mass mortalities in temperate benthic communities: a new case of emerging disease linked to climate change. *Glob Change Biol* **13**: 2078–2088. https://doi.org/10.1111/j.1365-2486.2007.01423.x.

Balseiro, P., Montes, A., Ceschia, G., Gestal, C., Novoa, B., and Figueras Huerta, A. (2007) Molecular epizootiology of the European Marteilia spp., infecting mussels (*Mytilus galloprovincialis* and *M*. *edulis*) and oysters (*Ostrea edulis*): an update. *Bull Eur Assoc Fish Pathol* **27**: 148–156.

Bargu, S., Baustian, M.M., Rabalais, N.N., Del Rio, R., Von Korff, B., and Turner, R.E. (2016) Influence of the Mississippi River on Pseudo-nitzschia spp. abundance and toxicity in Louisiana coastal waters. *Estuaries Coast* **39**: 1345–1356. https://doi.org/10.1007/s12237-016-0088-y.

Basti, L., Nagai, S., Go, J., Okano, S., Nagai, K., and Watanabe, R. (2015) Differential inimical effects of Alexandrium spp. and Karenia spp. on cleavage, hatching, and two larval stages of Japanese pearl oyster *Pinctada fucata martensii*. *Harmful Algae* **43**: 1–12. https://doi.org/10.1016/j.hal.2014.12.004.

Bates, S.S., and Trainer, V.L. (2006) The ecology of harmful diatoms. In *Ecology of Harmful Algae*. Berlin, Heidelberg: Springer, pp. 81–93.

Blaustein, R.A., Pachepsky, Y., Hill, R.L., Shelton, D.R., and Whelan, G. (2013) *Escherichia coli* survival in waters: temperature dependence. *Water Res* **47**: 569–578. https://doi.org/10.1016/j.watres.2012.10.027.

Borbor-Cordova, M.J., Torres, G., Mantilla-Saltos, G., Casierra-Tomala, A., Bermúdez, J.R., Renteria, W., and Bayot, B. (2019) Oceanography of harmful algal blooms on the ecuadorian coast (1997–2017): integrating remote sensing and biological data. *Front Mar Sci* **6**: 13. https://doi.org/10.3389/fmars.2019.00013.

Bower, S.M., McGladdery, S.E., and Price, I.M. (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Ann Rev Fish Dis* **4**: 1–199. https://doi.org/10.1016/0959-8030(94)90028-0.

Braga, A.C., Camacho, C., Marques, A., Gago-Martinez, A., Pacheco, M., and Costa, P.R. (2018) Combined effects of warming and acidification on accumulation and elimination dynamics of paralytic shellfish toxins in mussels *Mytilus galloprovincialis*. *Environ Res* **164**: 647–654. https://doi.org/10.1016/j.envres.2018.03.045.

Bramwell, G., Schultz, A., Sherman, C., Giraudeau, M., Thomas, F., Ujvari, B., and Dujon, A.M. (2021) A review of the potential effects of climate change on disseminated neoplasia with an emphasis on efficient detection in marine bivalve populations. *Sci Total Environ* **775**: 145134. https://doi.org/10.1016/j.scitotenv.2021.145134.

Burge, C.A., Burge, C.A., Eakin, C.M., Friedman, C.S., Froelich, B., Hershberger, P.K., *et al*. (2014) Climate change influences on marine infectious diseases: implications for management and society. *Ann Rev Mar Sci* **6**: 249–277. https://doi.org/10.1146/annurev-marine-010213-135029.

Cáceres-Martınez, J., Vásquez-Yeomans, R., and Sluys, R. (1998) The Turbellarian *Urastoma cyprinae* from edible mussels *Mytilus galloprovincialis* and *Mytilus californianus* in Baja California, NW México. *J Invertebr Pathol* **72**: 214–219. https://doi.org/10.1006/jipa.1998.4754.

Cao, R., Liu, Y., Wang, Q., Yang, D., Liu, H., Ran, W., and Zhao, J. (2018b) Seawater acidification reduced the resistance of *Crassostrea gigas* to *Vibrio splendidus* challenge: an energy metabolism perspective. *Front Physiol* **9**: 880. https://doi.org/10.3389/fphys.2018.00880.

Cao, R., Wang, D., Wei, Q., Wang, Q., Yang, D., Liu, H., *et al*. (2018a) Integrative biomarker assessment of the influence of saxitoxin on marine bivalves: a comparative study of the two bivalve species oysters, *Crassostrea gigas*, and scallops, Chlamys farreri. *Front Physiol* **9**: 1173. https://doi.org/10.3389/fphys.2018.01173.

Carballal, M.J., Villalba, A., and López, C. (1998) Seasonal variation and effects of age, food availability, size, gonadal development, and parasitism on the hemogram of *Mytilus galloprovincialis*. *J Invert Pathol* **72**: 304–312. https://doi.org/10.1006/jipa.1998.4779.

Carella, F., Aceto, S., Mangoni, O., Mollica, M.P., Cavaliere, G., Trinchese, G., *et al*. (2018) Assessment of the health status of mussels *Mytilus galloprovincialis* along the Campania coastal areas: a multidisciplinary approach. *Front Physiol* **9**: 683. https://doi.org/10.3389/fphys.2018.00683.

Carella, F., Feist, S.W., Bignell, J.P., and De Vico, G. (2015) Comparative pathology in bivalves: aetiological agents and disease processes. *J Invert Pathol* **131**: 107–120. https://doi.org/10.1016/j.jip.2015.07.012.

Carnegie, R.B., and Cochennec-Laureau, N. (2004) Microcell parasites of oysters: recent insights and future trends. *Aquat Liv Res* **17**: 519–528.

Davey, J.T., and Gee, J.M. (1988) *Mytilicola intestinalis*, a copepod parasite of blue mussels. Diseases in marine bivalve molluscs. *Am Fish Soc Spec Publ* **18**: 64–73.

Deatherage, D.E., Kepner, J.L., Bennett, A.F., Lenski, R.E., and Barrick, J.E. (2017) Specificity of genome evolution in experimental populations of *Escherichia coli* evolved at different temperatures. *Proc Natl Acad Sci U S A* **114**: E1904–E1912. https://doi.org/10.1073/pnas.1616132114.

Delamotte, M., and Vardala-Theodorou, E. (2007) *Shells from Greek Seas*. Athens, Greece: Goulandris Natural History Museum.

Dhanashree, B., Otta, S.K., Karunasagar, I., and Goebel, W. (2003) Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. *Food Microbiol* **20**: 447–453. https://doi.org/10.1016/S0740-0020(02)00140-5.

Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., *et al*. (2012) Climate change impacts on marine ecosystems. *Ann Rev Mar Sci* **4**: 11–37. https://doi.org/10.1146/annurev-marine-041911-111611.

Economopoulou, A., Chochlakis, D., Almpan, M.A., Sandalakis, V., Maraki, S., Tselentis, Y., and Psaroulaki, A. (2017) Environmental investigation for the presence of *Vibrio* species following a case of severe gastroenteritis in a touristic Island. *Environ Sci Pollut Res* **24**: 4835–4840. https://doi.org/10.1007/s11356-016-8231-7.

Economou, V., Gousia, P., Kansouzidou, A., Sakkas, H., Karanis, P., and Papadopoulou, C. (2013) Prevalence, antimicrobial resistance and relation to indicator and pathogenic microorganisms of *Salmonella enterica* isolated from surface waters within an agricultural landscape. *Int J Hyg Environ Health* **216**: 435–444.

Efstratiou, M.A., Mavridou, A., and Richardson, C. (2009) Prediction of Salmonella in seawater by total and faecal coliforms and Enterococci. *Mar Pollut Bull* **58**: 201–205. https://doi.org/10.1016/j.marpolbul.2008.10.003.

Efstratiou, M.A., Mavridou, A., Richardson, S.C., and Papadakis, J.A. (1998) Correlation of bacterial indicator organisms with *Salmonella* spp., *Staphylococcus aureus* and *Candida albicans* in sea water. *Lett Appl Microbiol* **26**: 342–346.

Elbashir, S., Parveen, S., Schwarz, J., Rippen, T., Jahncke, M., and DePaola, A. (2018) Seafood pathogens and information on antimicrobial resistance: a review. *Food Microbiol* **70**: 85–93.

Elliot, E.L., and Kvenberg, J.E. (2000) Risk assessment used to evaluate the US position on *Listeria monocytogenes* in seafood. *Int J Food Microbiol* **62**: 253–260. https://doi.org/10.1016/S0168-1605(00)00344-5.

Ellis, E.C. (2015) Ecology in an anthropogenic biosphere. *Ecol Monogr* **85**: 287–331. https://doi.org/10.1890/14-2274.1.

Eymann, C., Gotze, S., Bock, C., Guderley, H., Knoll, A.H., and Lannig, G. (2020) Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758) – explaining ecological findings under climate change. *Mar Biol* **167**: 17. https://doi.org/10.1007/s00227-019-3620-3.

FAO. (2018) *The State of World Fisheries and Aquaculture (SOFIA) – Meeting the Sustainable Development Goals*. Rome, Italy: Food and Agriculture Organization, p. xiii +210.

Feidantsis, K., Giantsis, I.A., Vratsistas, A., Makri, S., Pappa, A.Z., Drosopoulou, E., *et al*. (2020) Correlation between intermediary metabolism, Hsp gene expression, and oxidative stress-related proteins in long-term thermal-stressed *Mytilus galloprovincialis*. *Am J Physiol* **319**: R264–R281. https://doi.org/10.1152/ajpregu.00066.2020.

Feidantsis, K., Michaelidis, B., Raitsos, D.E., and Vafidis, D. (2021) Seasonal metabolic and oxidative stress responses of commercially important invertebrate species correlation with their habitat. *Mar Ecol Prog Ser* **658**: 27–46. https://doi.org/10.3354/meps13565.

Fernández-Delgado, M., Contreras, M., García-Amado, M. A., Gueneau, P., and Suárez, P. (2007) Occurrence of *Proteus mirabilis* associated with two species of Venezuelan oysters. *Rev Inst Med Trop Sao Paulo* **49**: 355–359.

Formiga-Cruz, M., Allard, A.K., Conden-Hansson, A.C., Henshilwood, K., Hernroth, B.E., Jofre, J., *et al*. (2003) Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas. *Appl Environ Microbiol* **69**: 1556–1563. https://doi.org/10.1128/AEM.69.3.1556-1563.2003.

Galli, A., Iha, K., Halle, M., El Bilali, H., Grunewald, N., Eaton, D., *et al*. (2017) Mediterranean countries' food consumption and sourcing patterns: an ecological footprint viewpoint. *Sci Total Environ* **578**: 383–391. https://doi.org/10.1016/j.scitotenv.2016.10.191.

Garali, S.M.B., Sahraoui, I., de la Iglesia, P., Chalghaf, M., Diogène, J., Ksouri, J., and Sakka Hlaili, A. (2020) Factors driving the seasonal dynamics of *Pseudo-nitzschia* species and domoic acid at mussel farming in the SW Mediterranean Sea. *Chem Ecol* **36**: 66–82. https://doi.org/10.1080/02757540.2019.1676417.

Genitsaris, S., Stefanidou, N., Sommer, U., and Moustaka-Gouni, M. (2019) Phytoplankton blooms, red tides and mucilaginous aggregates in the urban Thessaloniki Bay, eastern Mediterranean. *Diversity* **11**: 136. https://doi.org/10.3390/d11080136.

Gerba, C.P., and Goyal, S.M. (1978) Detection and occurrence of enteric viruses in shellfish: a review. *J Food Protect* **41**: 743–754. https://doi.org/10.4315/0362-028X-41.9.743.

Gestal, C., Roch, P., Renault, T., Pallavicini, A., Paillard, C., Novoa, B., *et al*. (2008) Study of diseases and the immune system of bivalves using molecular biology and genomics. *Rev Fish Sci* **16**: 133–156. https://doi.org/10.1080/10641260802325518.

Giantsis, I.A., Mucci, N., Randi, E., Abatzopoulos, T.J., and Apostolidis, A.P. (2014) Microsatellite variation of mussels (*Mytilus galloprovincialis*) in central and eastern Mediterranean: genetic panmixia in the Aegean and the Ionian seas. *J Mar Biol Assoc UK* **94**: 797–809. https://doi.org/10.1017/S0025315414000174.

Griffith, A.W., and Gobler, C.J. (2020) Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae* **91**: 101590. https://doi.org/10.1016/j.hal.2019.03.008.

Groner, M.L., Maynard, J., Breyta, R., Carnegie, R.B., Dobson, A., Friedman, C.S., *et al*. (2016) Managing marine disease emergencies in an era of rapid change. *Philos Trans R Soc Lond B Biol Sci* **371**: 20150364. https://doi.org/10.1098/rstb.2015.0364.

Hastings, R.W., and Heinle, D.R. (1995) The effects of aquaculture in estuarine environments. *Estuaries* **18**: 1.

Hoelzer, K., Switt, A.I.M., and Wiedmann, M. (2011) Animal contact as a source of human non-typhoidal salmonellosis. *Vet Res* **42**: 1–28. https://doi.org/10.1186/1297-9716-42-34.

Hrs-Brenko, M., and Legac, M. (1996) A review of bivalve species in the eastern Adriatic Sea: II. Pteromorphia (Arcidae and Noetidae). *Nat Croat* **5**: 221–247.

Intergovernmental Panel on Climate Change. (2014). IPCC 2014 climate change.

Juhel, G., Davenport, J., O'Halloran, J., Culloty, S.C., O'Riordan, R.M., James, K.F., *et al*. (2006) Impacts of microcystins on the feeding behaviour and energy balance of zebra mussels, *Dreissena polymorpha*: a bioenergetics approach. *Aquat Toxicol* **79**: 391–400. https://doi.org/10.1016/j.aquatox.2006.07.007.

Kalaitzidou, M., Filliousis, G., Petridou, E., Economou, V., Theodoridis, A., and Angelidis, P. (2017) Cyanobacterial harmful algal bloom in the delta of Axios River in the northern Greece: impacts and monitoring. *CEUR Workshop Proc* **2020**: 28–33.

Kalaitzidou, M., Papageorgiou, K., Theodoridis, A., Economou, V., Filiousis, G., Kritas, S., and Petridou, E.

(2021) Experimental exposure of the Mediterranean mussels *Mytilus galloprovincialis* to potentially toxic Cyanobacteria (Synechocystis sp.) and detection of microcystins. *Acta Vet Eurasia* **47**: 10–19.

Kamiyama, T., Nagai, S., Suzuki, T., and Miyamura, K. (2010) Effect of temperature on production of okadaic acid, dinophysistoxin-1, and pectenotoxin-2 by *Dinophysis acuminate* in culture experiments. *Aquat Microb Ecol* **60**: 193–202. https://doi.org/10.3354/ame01419.

Karagiannis, D., and Angelidis, P. (2007) Infection of cultured mussels *Mytilus galloprovincialis* by the protozoan *Marteilia* sp. in the Thermaikos Gulf (N Greece). *Bull Eur Assoc Fish Pathol* **27**: 131–141.

Karagiannis, D., Michaelidis, B., Theodoridis, A., Angelidis, P., Feidantsis, K., and Staikou, A. (2018) Field studies on the effects of Marteilia sp. on growth of mussel *Mytilus galloprovincialis* in Thermaikos Gulf. *Mar Environ Res* **142**: 116–123. https://doi.org/10.1016/j.marenvres.2018.09.027.

Katsanevakis, S. (2005) Population ecology of the endangered fan mussel *Pinna nobilis* in a marine lake. *Endanger Species Res* **1**: 51–59. https://doi.org/10.3354/esr001051.

Katsanevakis, S., Lefkaditou, E., Galinou-Mitsoudi, S., Koutsoubas, D., and Zenetos, A. (2008) Molluscan species of minor commercial interest in Hellenic seas: distribution, exploitation and conservation status. *Mediterr Mar Sci* **9**: 17–32. https://doi.org/10.12681/mms.145.

Katsanevakis, S., Tsirintanis, K., Tsaparis, D., Doukas, D., Sini, M., Athanassopoulou, F., et al. (2019). The cryptogenic parasite Haplosporidium pinnae invades the Aegean Sea and causes the collapse of Pinna nobilis populations. *Aquat Invasions* **14**(2): 150–164. https://doi.org/10.3391/ai.2019.14.2.01

Koukaras, K., and Nikolaidis, G. (2004) Dinophysis blooms in Greek coastal waters (Thermaikos Gulf, NW Aegean Sea). *J Plankton Res* **26**: 445–457. https://doi.org/10.1093/plankt/fbh042.

Koutsotoli, A.D., Papassava, M.E., Maipa, V.E., and Alamanos, Y.P. (2006) Comparing Shigella waterborne outbreaks in four different areas in Greece: common features and differences. *Epidemiol Infect* **134**: 57–162. https://doi.org/10.1017/S0950268805004747.

Koutsoubas, D., Galinou-Mitsoudi, S., Katsanevakis, S., Leontarakis, O., Metaxatos, A., and Zenetos, A. (2007) Chapter: II.5: Bivalve and gastropod molluscs of commercial interest for human consumption in the Hellenic seas. In *State of the Hellenic Fisheries*: Greece: HCMR.

Landsberg, J.H. (2002) The effects of harmful algal blooms on aquatic organisms. *Rev Fish Sci* **10**: 113–390. https://doi.org/10.1080/20026491051695.

Lassudrie, M., Hegaret, H., Wikfors, G.H., and da Silva, P.M. (2020) Effects of marine harmful algal blooms on bivalve cellular immunity and infectious diseases: a review. *Dev Comp Immunol* **108**: 103660. https://doi.org/10.1016/j.dci.2020.103660.

Lattos, A., Giantsis, I.A., Karagiannis, D., and Michaelidis, B. (2020a) First detection of the invasive Haplosporidian and Mycobacteria parasites hosting the endangered bivalve *Pinna nobilis* in Thermaikos Gulf, North Greece. *Mar Environ Res* **155**: 104889. https://doi.org/10.1016/j.marenvres.2020.104889.

Lattos, A., Giantsis, I.A., Karagiannis, D., Theodorou, J.A., and Michaelidis, B. (2020b) Gut symbiotic microbial communities in the IUCN critically endangered *Pinna nobilis* suffering from mass mortalities, revealed by 16S rRNA amplicon NGS. *Pathogens* **9**: 1002. https://doi.org/10.3390/pathogens9121002.

Lelong, A., Hégaret, H., Soudant, P., and Bates, S.S. (2012) Pseudo-nitzschia (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. *Phycologia* **51**: 168–216. https://doi.org/10.2216/11-37.1.

Lenski, R. (2017) Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *ISME J* **11**: 2181–2194. https://doi.org/10.1038/ismej.2017.69.

Leontarakis, P.K., Xatzianastasiou, L.I., and Theodorou, J.A. (2008) Biological aspects of the lagoon cockle, Cerastoderma glaucum (Poiret 1879), in a coastal lagoon in Keramoti, Greece in the northeastern Mediterranean. *J Shellfish Res* **27**: 1171–1175.

Ligda, P., Claerebout, E., Casaert, S., Robertson, L.J., and Sotiraki, S. (2020) Investigations from northern Greece on mussels cultivated in areas proximal to wastewaters discharges, as a potential source for human infection with *Giardia* and *Cryptosporidium*. *Exp Parasitol* **210**: 107848. https://doi.org/10.1016/j.exppara.2020.107848.

Lim, H.C., Lim, P.T., Teng, S.T., Bates, S.S., and Leaw, C.P. (2014) Genetic structure of *Pseudo-nitzschia pungens* (Bacillariophyceae) populations: implications of a global diversification of the diatom. *Harmful Algae* **37**: 142–152. https://doi.org/10.1016/j.hal.2014.06.004.

Liu, H., Whitehouse, C.A., and Li, B. (2018) Presence and persistence of Salmonella in water: the impact on microbial quality of water and food safety. *Front Public Health* **6**: 159. https://doi.org/10.3389/fpubh.2018.00159.

Louppis, A.P., Badeka, A.V., Katikou, P., Paleologos, E.K., and Kontominas, M.G. (2010) Determination of okadaic acid, dinophysistoxin-1 and related esters in Greek mussels using HPLC with fluorometric detection, LC-MS/MS and mouse bioassay. *Toxicon* **55**: 724–733. https://doi.org/10.1016/j.toxicon.2009.10.026.

Lovato, T., Vichi, M., and Oddo, P. (2013) High-resolution simulations of Mediterranean Sea physical oceanography under current and scenario climate conditions: model description, assessment and scenario analysis, CMCC Research Papers RP0207.

Lozano-Leon, A., Torres, J., Osorio, C.R., and Martinez-Urtaza, J. (2003) Identification of tdh-positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. *FEMS Microbiol Lett* **226**: 281–284. https://doi.org/10.1016/S0378-1097(03)00604-9.

Luber, G., Knowlton, K., Balbus, J., Frumkin, H., Hayden, M., Hess, J., *et al*. (2014) Ch. 9: Human health. In *Climate Change Impacts in the United States: The Third National Climate Assessment*, Melillo, J.M., Terese (T.C.) Richmond, and Yohe, G.W. (eds): U.S.A.: U.S. Global Change Research Program, pp. 220–256. https://doi.org/10.7930/J0PN93.

Lupo, C., Bougeard, S., Le Bihan, V., Blin, J.L., Allain, G., Azéma, P., *et al*. (2021) Mortality of marine mussels Mytilus edulis and M. galloprovincialis: systematic literature review of risk factors and recommendations for future research. *Rev Aquacult* **13**: 504–536. https://doi.org/10.1111/raq.12484.

Lykakis, J., and Kalathakis, M. (1991) Fisheries and aquaculture: Greece. In *Developments in Aquaculture and Fisheries Science, Vol. 21. Scallops: Biology and Aquaculture*, Shumway, S. (ed). New York: Elsevier, pp. 795–808.

Malagoli, D., Casarini, L., and Ottaviani, E. (2008) Effects of the marine toxins okadaic acid and palytoxin on mussel phagocytosis. *Fish Shellfish Immunol* **24**: 180–186. https://doi.org/10.1016/j.fsi.2007.10.012.

Mallin, M.A., Johnson, V.L., and Ensign, S.H. (2009) Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. *Environ Monit Assess* **159**: 475–491. https://doi.org/10.1007/s10661-008-0644-4.

Manousis, T., Mpardakis, G., Paraskevopoulos, C., and Galinou-Mitsoudi, S. (2010) The Bivalvia Mollusca of Thessaloniki & Thermaikos Gulfs (North Aegean Sea, Greece) with emphasis on new species for Hellenic waters. *J Biol Res-Thessalon* **14**: 161–179.

Maraki, S., Georgiladakis, A., Tselentis, Y., and Samonis, G. (2003) A 5-year study of the bacterial pathogens associated with acute diarrhea on the Island of Crete, Greece, and their resistance to antibiotics. *Eur J Epidemiol* **18**: 85–90. https://doi.org/10.1023/A:1022528205793.

McCarthy, M., O'Halloran, J., O'Brien, N.M., and van Pelt, F. F. (2014) Does the marine biotoxin okadaic acid cause DNA fragmentation in the blue mussel and the pacific oyster? *Mar Environ Res* **101**: 153–160. https://doi.org/10.1016/j.marenvres.2014.09.009.

McGillicuddy, D.J., Jr., Brosnahan, M.L., Couture, D.A., Hed, R., Keafer, B.A., Manning, J.P., *et al*. (2014) A red tide of *Alexandrium fundyense* in the Gulf of Maine. *Deep Sea Res II* **103**: 174–184.

Mellou, K., Sideroglou, T., Potamiti-Komi, M., Kokkinos, P., Ziros, P., Georgakopoulou, T., and Vantarakis, A. (2013) Epidemiological investigation of two parallel gastroenteritis outbreaks in school settings. *BMC Public Health* **13**: 1–7. https://doi.org/10.1186/1471-2458-13-241.

Mills, J.N., Gage, K.L., and Khan, A.S. (2010) Potential influence of climate change on vector-borne and zoonotic diseases: a review and proposed research plan. *Int J Health Geogr* **9**: 54–1514. https://doi.org/10.1289/ehp.0901389.

Molloy, D.P., Mayer, D.A., Giamberini, L., and Gaylo, M.J. (2013) Mode of action of *Pseudomonas fluorescens* strain CL145A, a lethal control agent of dreissenid mussels (Bivalvia: Dreissenidae). *J Invertebr Pathol* **113**: 115–121. https://doi.org/10.1016/j.jip.2012.12.013.

Moullec, F., Barrier, N., Drira, S., Guilhaumon, F., Marsaleix, P., Somot, S., *et al*. (2019) An end-to-end model reveals losers and winners in a warming Mediterranean Sea. *Front Mar Sci* **6**: 345. https://doi.org/10.3389/fmars.2019.00345.

Nikolaidis, G., Koukaras, K., Aligizaki, K., Heracleous, A., Kalopesa, E., Moschandreou, K., *et al*. (2005) Harmful microalgal episodes in Greek coastal waters. *J Biol Res – Thessalon* **3**: 77–85.

Nikolaidis, G., and Moustaka-Gouni, M. (1990) Phytoplankton of a warm Monomictic Lake–Lake Vegoritis, Greece. *Arch Hydrobiol* **4**: 19.

Oda, T., Sato, Y., Kim, D., Muramatsu, T., Matsuyama, Y., and Honjo, T. (2001) Hemolytic activity of *Heterocapsa circularisquama* (Dinophyceae) and its possible involvement in shellfish toxicity. *J Phycol* **37**: 509–516. https://doi.org/10.1046/j.1529-8817.2001.037004509.x.

Oliveira, F., Diez-Quijada, L., Turkina, M.V., Morais, J., Barreiro Felpeto, A., Azevedo, J., *et al*. (2020) Physiological and metabolic responses of marine mussels exposed to toxic Cyanobacteria *Microcystis aeruginosa* and *Chrysosporum ovalisporum*. *Toxins* **12**: 196. https://doi.org/10.3390/toxins12030196.

Ottaviani, D., Leoni, F., Rocchegiani, E., Canonico, C., Poteziani, S., Santarelli, S., *et al*. (2010) *Vibrio parahaemolyticus*-associated gastroenteritis in Italy: persistent occurrence of O3: K6 pandemic clone and emergence of O1: KUT serotype. *Diagn Microbiol Infect Dis* **66**: 452–455. https://doi.org/10.1016/j.diagmicrobio.2009.11.015.

Pancucci-Papadopoulou, M.A., Zenetos, A., Corsini-Foka, M., and Politou, C.Y. (2005) Update of marine alien species in Hellenic waters. *Mediterr Mar Sci* **6**: 147–158. https://doi.org/10.12681/mms.188.

Papadopoulou, C., Ecoomou, E., Zakas, G., Salamoura, C., Dontorou, C., and Apostolou, J. (2007) Microbiological and pathogenic contaminants of seafood in Greece. *J Food Qual* **30**: 28–42.

Pernet, F., and Browman, H.I. (2021) The future is now: marine aquaculture in the anthropocene. *ICES J Mar Sci* **78**: 315–322. https://doi.org/10.1093/icesjms/fsaa248.

Petersen, F., and Hubbart, J.A. (2020) Physical factors impacting the survival and occurrence of *Escherichia coli* in secondary habitats. *Water* **12**: 1796. https://doi.org/10.3390/w12061796.

Philipsborn, R., Ahmed, S.M., Brosi, B.J., and Levy, K. (2016) Climatic drivers of diarrheagenic *Escherichia coli* incidence: a systematic review and meta-analysis. *J Infect Dis* **214**: 6–15. https://doi.org/10.1093/infdis/jiw081.

Pichot, Y., Comps, M., Tige, G., Grizel, H., and Rabouin, M. A. (1980) Recherches sur *Bonamia ostreae* gen. n., sp. n., parasite nouveau de l'huître plate *Ostrea edulis* L. *Rev Trav Inst Pêches Marit* **43**: 131–140.

Pörtner, H.O. (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J Exp Biol* **213**: 881–893. https://doi.org/10.1242/jeb.037523.

Poulos, S.E., Chronis, G.T., Collins, M.B., and Lykousis, V. (2000) Thermaikos Gulf Coastal System, NW Aegean Sea: an overview of water sediment fluxes in relation to air–land–ocean interactions and human activities. *J Mar Syst* **25**: 47–76. https://doi.org/10.1016/S0924-7963(00)00008-7.

Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., *et al*. (2020) The role of salinity in physiological responses of bivalves. *Rev Aquac* **12**: 1548–1566. https://doi.org/10.1111/raq.12397.

Prado-Alvarez, M., Flórez-Barrós, F., Méndez, J., and Fernandez-Tajes, J. (2013) Effect of okadaic acid on carpet shell clam (Ruditapes decussatus) haemocytes by in vitro exposure and harmful algal bloom simulation assays. *Cell Biol Toxicol* **29**: 189–197. https://doi.org/10.1007/s10565-013-9246-1.

Prado-Álvarez, M., Romero, A., Balseiro, P., Dios, S., Novoa, B., and Figueras, A. (2012) Morphological characterization and functional immune response of the carpet shell clam (*Ruditapes decussatus*) haemocytes after bacterial stimulation. *Fish Shellfish Immunol* **32**: 69–78. https://doi.org/10.1016/j.fsi.2011.10.019.

Prassopoulou, E., Katikou, P., Georgantelis, D., and Kyritsakis, A. (2009) Detection of okadaic acid and related esters in mussels during diarrhetic shellfish poisoning (DSP) episodes in Greece using the mouse bioassay, the PP2A inhibition assay and HPLC with fluorimetric detection. *Toxicon* **53**: 214–227. https://doi.org/10.1016/j.toxicon.2008.11.003.

Rayyan, A., and Chintiroglou, C.C. (2003) *Steinhausia mytilovum* in cultured mussel *Mytilus galloprovincialis* in the Thermaikos Gulf (north Aegean Sea, Greece). *Dis Aquat Organ* **57**: 271–273.

Rayyan, A., Damianidis, P., Antoniadou, C., and Chintiroglou, C.C. (2006) Protozoan parasites in cultured mussels *Mytilus galloprovincialis* in the Thermaikos Gulf (north Aegean Sea, Greece). *Dis Aquat Organ* **70**: 251–254.

Rayyan, A., Photis, G., and Chintiroglou, C.C. (2004) Metazoan parasite species in cultured mussels *Mytilus galloprovincialis* in the Thermaikos Gulf (North Aegean Sea, Greece). *Dis Aquat Organ* **58**: 55–62. https://doi.org/10.3354/dao058055.

Reguera, B., Riobó, P., Rodríguez, F., Díaz, P.A., Pizarro, G., Paz, B., *et al*. (2014) Dinophysis toxins: causative organisms, distribution and fate in shellfish. *Mar Drugs* **12**: 394–461. https://doi.org/10.3390/md12010394.

Rippey, S.R. (1994) Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev* **7**: 419–425. https://doi.org/10.1128/CMR.7.4.419.

Robert, R., Borel, M., Pichot, Y., and Trut, G. (1991) Growth and mortality of the European oyster *Ostrea edulis* in the bay of Arcachon (France). *Aquat Living Resour* **4**: 265–274. https://doi.org/10.1051/alr:1991028.

Robledo, J.A.F., Santarém, M.M., and Figueras, A. (1994) Parasite loads of rafted blue mussels (*Mytilus galloprovincialis*) in Spain with special reference to the copepod, *Mytilicola intestinalis*. *Aquaculture* **127**: 287–302.

Rodas-Suárez, O.R., Flores-Pedroche, J.F., Betancourt-Rule, J.M., Quinones-Ramirez, E.I., and Vázquez-Salinas, C. (2006) Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. *Appl Environ Microbiol* **72**: 7410–7412. https://doi.org/10.1128/AEM.00956-06.

Rodrigues, L.C., van den Bergh, J.C.J.M., Massam, F., Theodorou, J.A., Ziveri, P., and Gazeau, F. (2015) Sensitivity of Mediterranean bivalve Mollusc aquaculture to climate change and ocean acidification: results from a producers' survey. *J Shellfish Res* **34**: 1161–1176. https://doi.org/10.2983/035.034.0341.

Rosa, R., Marques, A., and Nunes, M.L. (2012) Impact of climate change in Mediterranean aquaculture. *Rev Aquac* **4**: 163–177. https://doi.org/10.1111/j.1753-5131.2012.01071.x.

Sabatini, S.E., Rocchetta, I., Luquet, C.M., Guido, M.I., and De Molina, M.D.C.R. (2011) Effects of sewage pollution and bacterial load on growth and oxidative balance in the freshwater mussel *Diplodon chilensis*. *Limnologica* **41**: 356–362. https://doi.org/10.1016/j.limno.2011.04.004.

Savageau, M.A. (1983) *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. *Am Nat* **122**: 732–744. https://doi.org/10.1086/284168.

Schijven, J.F., and de Roda Husman, A.M. (2005) Effect of climate changes on waterborne disease in The Netherlands. *Water Sci Technol* **51**: 79–87. https://doi.org/10.2166/wst.2005.0114.

Shumway, S.A. (1990) Review of the effects of algal blooms on shellfish and aquaculture. *J World Aquacult Soc* **21**: 65–104. https://doi.org/10.1111/j.1749-7345.1990.tb00529.x.

Smaal, C., Ferreira, J.G., Grant, J., Petersen, J.K., and Øivind, S. (2018) *Goods and Services of Marine Bivalves*: The Netherlands: Springer ISBN 978-3-319-96776-9.

Sokolova, I.M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr Comp Biol* **53**: 597–608. https://doi.org/10.1093/icb/ict028

Šolić, M., Jozić, S., and Krstulović, N. (2010) Interactive effects of temperatures and salinity on the rate of concentration of *Escherichia coli* in mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*). *Fresen Environ Bull* **19**: 1634–1639.

Song, Y.J., Cheong, H.K., Ki, M., Shin, J.Y., Hwang, S.S., Park, M., *et al*. (2018) The epidemiological influence of climatic factors on shigellosis incidence rates in Korea. *Int J Environ Res Public Health* **15**: 2209. https://doi.org/10.3390/ijerph15102209.

Soultos, N., Iossifidou, E., Ambrahim, A., Psomas, E., Tzavaras, I., Koutsopoulos, D., and Lazou, T. (2014) *Listeria monocytogenes* in mussels (*Mytilus galloprovincialis*) harvested from North Aegean coastal area. *Turk J Vet Anim Sci* **38**: 50–53. https://doi.org/10.3906/vet-1212-36.

Spatharis, S., Danielidis, D.B., and Tsirtsis, G. (2007) Recurrent *Pseudo-nitzschia calliantha* (Bacillariophyceae) and *Alexandrium insuetum* (Dinophyceae) winter blooms induced by agricultural runoff. *Harmful Algae* **6**: 811–822. https://doi.org/10.1016/j.hal.2007.04.006.

Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., *et al*. (2015) Structure and function of the global ocean microbiome. *Science* **348**: 1261359. https://doi.org/10.1126/science.1261359.

Theodorou, J.A., James, R., Tagalis, D., Tzovenis, I., Hellio, C., and Katselis, G. (2017a) Density and size structure of the endangered bivalve *Pinna nobilis*, (fan mussel) in the shallow water zone of Maliakos Gulf, Greece. *Acta Adriatica* **58**: 63–76.

Theodorou, J.A., Leech, B.S., Perdikaris, C., and Hellio, C. (2017b) Summer re-immersion handling of the Mediterranean mussels. In: *13th MEDCOAST 2017 Congress Proceedings*. Mellieha, O.E. (Ed.). Malta, 31 Oct–04 Nov 2017, pp. 363–368.

Theodorou, J.A., Moutopoulos, D.K., and Tzovenis, I. (2020) Semi-quantitative risk assessment of Mediterranean mussel (*Mytilus galloprovincialis* L.) harvesting bans due to harmful algal bloom (HAB) incidents in Greece. *Aquac Econ Manag* **24**: 273–293. https://doi.org/10.1080/13657305.2019.1708994.

Theodorou, J.A., Perdikaris, C., and Filippopoulos, N.G. (2015) Evolution through innovation in aquaculture: the case of the hellenic mariculture industry (Greece). *J Appl Aquac* **27**: 160–181.

Theodorou, J.A., Viaene, J., Sorgeloos, P., and Tzovenis, I. (2011) Production and marketing trends of the cultured Mediterranean mussel *Mytilus galloprovincialis* Lamarck 1819, in Greece. *J Shellfish Res* **30**: 859–874. https://doi.org/10.2983/035.030.0327.

Torres Palenzuela, J.M., González Vilas, L., Bellas, F.M., Garet, E., González-Fernández, Á., and Spyrakos, E. (2019) *Pseudo-nitzschia* blooms in a coastal upwelling system: remote sensing detection, toxicity and environmental variables. *Water* **11**: 1954. https://doi.org/10.3390/w11091954.

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., and Trick, C.G. (2012) Pseudo-nitzschia physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae* **14**: 271–300. https://doi.org/10.1016/j.hal.2011.10.025.

Trainer, V.L., Hickey, B.M., and Bates, S.S. (2008) Toxic diatoms. In *Oceans and Human Health: Risks and Remedies from the Sea*. New York: Elsevier Science Publishers, pp. 219–237.

Tsolakos, K., Katselis, G., and Theodorou, J.A. (2020) Taphonomy of mass mollusc shell accumulation at Amvrakikos gulf lagoon complex sandy barriers (NW Greece). *Oceanologia* **63**: 179–193. https://doi.org/10.1016/j.oceano.2020.11.004.

Tsotsios, D., Tzovenis, I., Katselis, G., Geiger, S., and Theodorou, J.A. (2016) Spat settlement of the smooth scallop *Flexopecten glaber* (Linnaeus 1758), and variegated scallop *Chlamys varia* (Linnaeus 1758) in Amvrakikos Gulf, Ionian Sea (NW Greece). *J Shellfish Res* **35**: 467–474. https://doi.org/10.2983/035.035.0219.

Turner, L.M., Alsterberg, C., Turner, A.D., Girisha, S.K., Rai, A., Havenhand, J.N., *et al*. (2016) Pathogenic marine microbes influence the effects of climate change on a commercially important tropical bivalve. *Sci Rep* **6**: 32413. https://doi.org/10.1038/srep32413.

Vantarakis, A., Komninou, G., Venieri, D., and Papapetropoulou, M. (2000) Development of a multiplex PCR detection of Salmonella spp. and Shigella spp. in mussels. *Lett Appl Microbiol* **31**: 105–109. https://doi.org/10.1046/j.1365-2672.2000.00797.x.

Vantarakis, A.C., Tsibouxi, A., Venieri, D., Komninou, G., Papapetropoulou, M., and Athanassiadou, A. (2005) Evaluation of microbiological quality of coastal waters in Greece. *J Water Health* **3**: 371–380. https://doi.org/10.2166/wh.2005.045.

Varkitzi, I., Markogianni, V., Pantazi, M., Pagou, K., Pavlidou, A., and Dimitriou, E. (2018) Effect of river inputs on environmental status and potentially harmful phytoplankton in a coastal area of eastern Mediterranean (Maliakos Gulf, Greece). *Mediterr Mar Sci* **19**: 326–343. https://doi.org/10.12681/mms.14591.

Vermeulen, L.C., and Hofstra, N. (2014) Influence of climate variables on the concentration of Escherichia coli in the Rhine, Meuse, and Drentse Aa during 1985–2010. *Reg Environ Change* **14**: 307–319. https://doi.org/10.1007/s10113-013-0492-9.

Vezzulli, L., Pruzzo, C., Huq, A., and Colwell, R.R. (2010) Environmental reservoirs of *Vibrio cholerae* and their role in cholera. *Environ Microbiol Rep* **2**: 27–33. https://doi.org/10.1111/j.1758-2229.2009.00128.x.

Villalba, A., Mourelle, S.G., Carballal, M.J., and Carmen Lopez, M. (1993b) Effects on infection by the protistan parasite *Marteilia refringens* on the reproduction of cultured mussels *Mytilus galloprovincialis* in Galicia (NW Spain). *Dis Aquat Organ* **17**: 205–213.

Villalba, A., Mourelle, S.G., Carballal, M.J., and Lopez, C. (1997) Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rias of Galicia (NW Spain). *Dis Aquat Organ* **31**: 127–139. https://doi.org/10.3354/dao031127.

Villalba, A., Mourelle, S.G., Lopez, M.C., Carballal, M.J., and Azevedo, C. (1993a) Marteiliasis affecting cultured mussels *Mytilus galloprovincialis* of Galicia (NW Spain). I: Etiology, phases of the infection, and temporal and spatial variability in prevalence. *Dis Aquat Organ* **16**: 61–72.

Villalba, A., Reece, K.S., Ordás, M.C., Casas, S.M., & Figueras, A. (2004). Perkinsosis in molluscs: a review. *Aquat Living Resour*, **17**(4): 411–432. https://doi.org/10.1051/alr:2004050

Villalba, A., Casas, S.M., López, C., & Carballal, M.J. (2005). Study of perkinsosis in the carpet shell clam Tapes decussatus in Galicia (NW Spain). II. Temporal pattern of disease dynamics and association with clam mortality. *Dis Aquat Org*, **65**(3): 257–267. https://doi.org/10.3354/dao065257

Virvilis, C., Angelidis, P., and Photis, G. (2003) Presence of the parasite *Marteilia* sp. in the shellfish of the Thermaikos Gulf in northern Greece. *Bull Eur Ass Fish Pathol* **23**: 157–161.

Virvilis, C., & Angelidis, P. (2006). Presence of the parasite Marteilia sp. in the flat oyster (Ostrea edulis L) in Greece. *Aquaculture*, **259**(1-4): 1–5. https://doi.org/10.1016/j.aquaculture.2006.05.008

Vlamis, A., Katikou, P., Rodriguez, I., Rey, V., Alfonso, A., Papazachariou, A., *et al*. (2015) First detection of tetrodotoxin in Greek shellfish by UPLC-MS/MS potentially linked to the presence of the dinoflagellate *Prorocentrum minimum*. *Toxins* **7**: 1779–1807. https://doi.org/10.3390/toxins7051779.

World Health Organization (WHO) – Food Safety Report. (2011) *Food safety*. URL http://www.who.int/foodsafety/en/.

Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., and Fujita, N. (1980) Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. *Bull Jpn Soc Sci Fish* **46**: 1405–1411. https://doi.org/10.2331/suisan.46.1405.

Young, N., Robin, C., Kwiatkowska, R., Beck, C., Mellon, D., Edwards, P., *et al*. (2019) Outbreak of diarrhetic shellfish poisoning associated with consumption of mussels, United Kingdom, may to June 2019. *Eurosurveillance* **24**: 1900513.

Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, V., Galdiero, M., *et al*. (2017) Microbial diseases of bivalve mollusks: infections, immunology and antimicrobial defense. *Mar Drugs* **15**: 182. https://doi.org/10.3390/md15060182.

Zenetos, A., Arianoutsou, M., Bazos, I., Balopoulou, S., Corsini-Foka, M., Dimiza, M., *et al*. (2015) ELNAIS: a collaborative network on aquatic alien species in Hellas (Greece). *Manag Biol Invasions* **6**: 185–196. https://doi.org/10.3391/mbi.2015.6.2.09.

Zenetos, A., Çinar, M.E., Pancucci-Papadopoulou, M.A., Harmelin, J.G., Furnari, G., Andaloro, F., *et al*. (2005) Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. *Mediterr Mar Sci* **6**: 63–118. https://doi.org/10.12681/mms.186.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information.