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Mass mortality of the invasive echinoid *Diadema setosum* (Leske, 1778) in Crete, East Mediterranean Sea

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Abstract

Diadema setosum is an echinoid of Indo-Pacific origin that invaded the Mediterranean Sea in 2006. It is an ecosystem engineer with an important ecological function in its native range, but it can have a detrimental effect on Mediterranean reefs. Recently in 2022 a mass mortality event (MME) affecting this species was recorded in the east Aegean Sea in the eastern part of the Mediterranean basin. We are reporting herein a westward progression of the MME in 2023 affecting established populations in various locations around the island of Crete.

Keywords: *Diadema setosum*; mass mortality event; Crete; Mediterranean Sea.

Introduction

The echinoid *Diadema setosum* (Leske, 1778) is a species of Indo-Pacific origin that is also distributed in the Red Sea. It is believed that it migrated from the Red Sea to the Mediterranean Sea via shipping through the Suez Canal before 2006, when the first record in the Mediterranean Sea occurred, as indicated by the size of their specimens (Yokes & Galil, 2006). Since then, this species has established several populations in the eastern part of the Mediterranean basin (Katsanevakis *et al.*, 2020; Ragkousis *et al.*, 2023) including the Ionian Sea (Dimiatriadis *et al.*, 2023). Currently, the species can be found in high abundance on the Turkish coast, the Dodecanese archipelago and Crete (Southern and Eastern Aegean Sea) (Vafidis *et al.*, 2021; Zirler *et al.*, 2023a).

Sea urchins of the genus *Diadema* are considered to be environmental engineers, with the potential to alter entire benthic communities, in terms of structure and composition (Zirler *et al.*, 2023b). In their native range, diademid echinoids perform an important ecological function, since their grazing controls rampant turf algae growth which in turn inhibits coral reef growth (Sammarco, 1980; Edmunds & Carpenter, 2001). In the eastern Mediterranean basin, a current major issue is the transformation of the once canopy-covered Mediterranean rocky reefs to barren reefs (Vergés *et al.*, 2014). This phenomenon is attributed to over-grazing by invasive herbivorous fish of the genus *Siganus* and to overpopulation of indigenous sea urchins (Vergés *et al.*, 2014; Bevilacqua *et al.*, 2021; Illa-López *et al.*, 2023). Although *Diadema setosum* has been continuously present in the basin for almost two decades, its biology outside the native range, its ecological function, or a possible link to the creation or sustenance of barren reefs in the invaded range is still understudied (Vafidis *et al.*, 2021).

In contrast, the important ecological function of the genus *Diadema* in its native range has been studied extensively. In particular, mass mortality events (MMEs) of *Diadema antillarum* (Philippi, 1845) in the Caribbean Sea and the negative effects of the cessation of algal grazing on coral reef communities have been well documented (Lessios, 2016; Hylkema *et al.*, 2023), indicating the importance of the species in terms of ecosystem function, especially for the proliferation of the coral community (Lessios, 2016). The recent MME affecting *D. antillarum* in the Caribbean Sea since January 2022 has been attributed to a scuticociliate pathogen (Hewson *et al.*, 2023). In 2022 a similar MME of *D. setosum* was recorded in the eastern part of the Aegean Sea affecting populations off the Turkish coast and the Dodecanese islands (Zirler *et al.*, 2023b). This was the first record of an MME affecting *D. setosum* in the Mediterranean and although the cause of this event is as yet unconfirmed, its pathology indicates that most likely it is pathogen driven (Zirler *et al.*, 2023b). Concurrently, an even more extensive

MME started developing in the Red Sea, with the first record of mortalities originating from the Gulf of Aqaba in 2022 and by the end of 2023 expanding to the Gulf of Oman and towards Reunion Island in the Western Indian Ocean (Roth *et al.*, 2024). Furthermore, the presence of a scuticociliate pathogen in infected individuals, identical to the one found in the Caribbean Sea during the 2022 *D. antillarum* MME, was confirmed and for the first time it was reported that other diadematoïd sea urchins were also affected (Roth *et al.*, 2024). The implications of this latest development are alarming, as this MME, already affecting a vast area of great ecological and economical importance, appears very difficult if not impossible to contain, indicating spreading of the waterborne pathogen via shipping and further threatening the diadematoïd populations of the whole Indo-Pacific region (Roth *et al.*, 2024).

Herein we report a subsequent *D. setosum* MME from the island of Crete, South Aegean Sea, recorded in 2023, thus confirming the persistence of the etiological factors of the mortality, as well as the geographical expansion of the affected populations. Population densities and mortalities were recorded in different sites in the eastern part of Crete in an attempt to record the progression of this MME.

Methods

Densities, health status and mortality of *Diadema setosum* were recorded in six sites (Fig. 1) of central and eastern Crete (Greece). Three of them were located in north Crete: Alykes, Agia Pelagia (35.415576°N, 24.987757°E), Dianiskari, Elounda (35.256902°N, 25.739842°E) and Atzikiari, Itanos (35.256072°N, 26.222192°E). Two additional sites were in south Crete: Agios Ioannis, Asterousia (34.935932°N, 25.054604°E) and Kalo Nero, Lasithi (35.018226°N, 26.034776°E), and one site located on the north side of Chrysi island, which is situated ~ 8 NM offshore, south of Crete (34.88361°N, 25.69084°E). These surveys were conducted on the 29th of April 2023 (Kalo Nero), on 1st of May 2023 (Chrysi

island), on the 31st of May 2023 (Dianiskari), on the 1st of June (Atzikiari), on the 20th of June 2023 (Agios Ioannis) and lastly on the 8th of June 2023, 26th of July 2023 and 19th of January 2024 (Alykes).

In each site, scuba divers placed four transects of 30 m in length and 2 m in width (i.e., 1 m left and 1 m right of the measuring tape), covering therefore an area of 60 m²/transect, and a total area of 240 m² in each site. The depth range of surveys at the five sites (Dianiskari, Atzikiari, Agios Ioannis, Kalo Nero and Chrysi island) was between 5-10 m, while for Alykes it was between 8-15 m.

Every individual of *D. setosum* that resided within the area of each transect was counted and classified in one of the five following categories, after Hylkema *et al.* (2023): i) healthy (i.e., lacking all signs of illness); ii) lacking spine movement (i.e., slow spine reaction, even after disturbance by the diver); iii) lack of tube feet control (i.e., detached from substrate); iv) presence of epidermal lesions and detached spines, and v) dead (Fig. 2). Lastly, the density of *D. setosum* in each transect and the percentage of moribund urchins (last four categories) were assessed. The numbers of individuals recorded in Alykes were tested using one-way ANOVA in order to investigate statistically significant differences before and after the MME.

Results and Discussion

In Chrysi island, off the south-east coast of Crete, the aggregation of *D. setosum* exhibited the highest density recorded for Crete (1.35 ind/m²), while several individuals of *D. setosum* displaying at least one of the symptoms of illness were observed. This site was the first in which pathologies and mortalities of *D. setosum* were recorded in Crete (Table 1). At the same time, two individuals of *D. setosum* were observed in Kalo Nero, both appearing healthy without any sign of illness. In Atzikiari and Dianiskari, previously established populations of *D. setosum* which had been reported over subsequent years prior to 2020 (Katsanevakis *et al.*, 2020), were completely absent

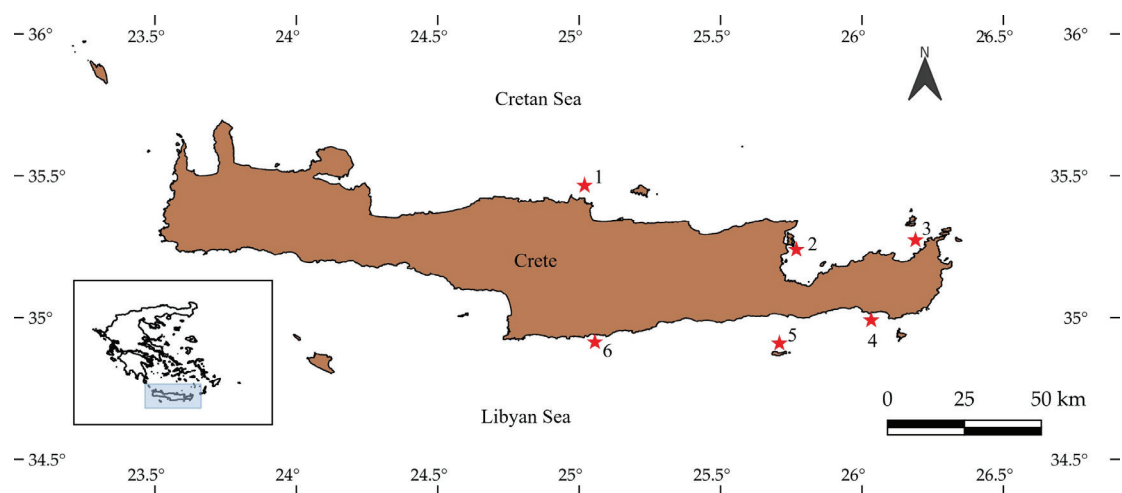


Fig. 1: Map of Crete showing the six sites of this study: 1) Alykes (35.416551°N, 24.987420°E), 2) Dianiskari (35.256665°N, 25.740725°E), 3) Atzikiari (35.254234°N, 26.224514°E), 4) Kalo Nero (35.018702°N, 26.034799°E), 5) Chrysi (34.878092°N, 25.711922°E) and 6) Agios Ioannis (34.938220°N, 25.053236°E).

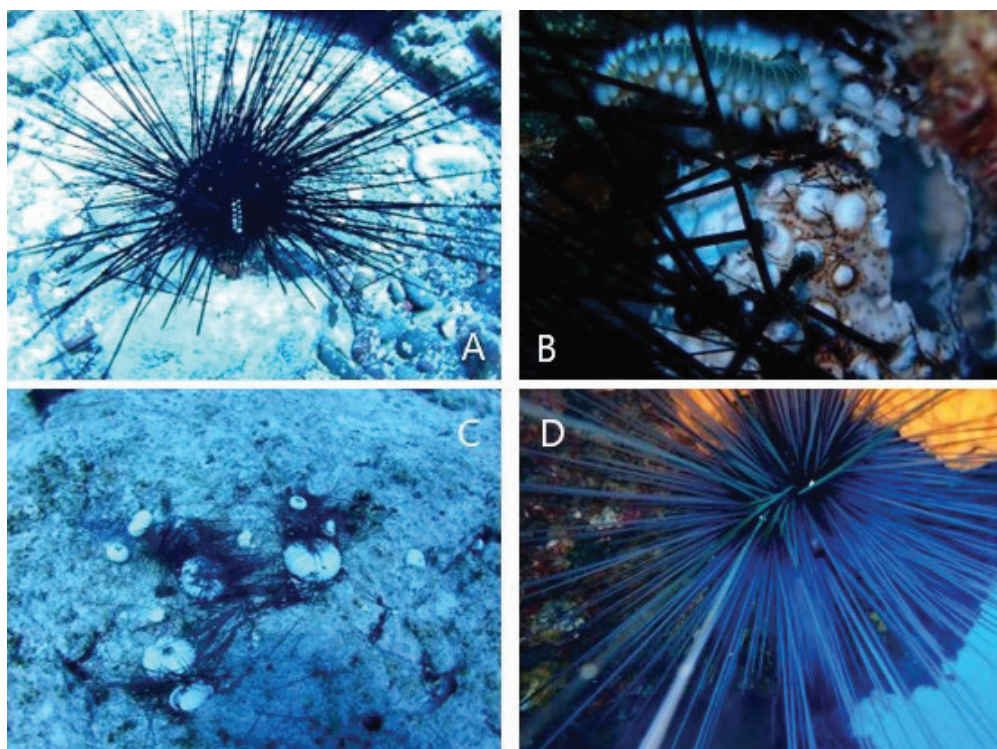


Fig. 2: *Diadema setosum* showing different health status: A) an individual with epidermal lesions and detached spines, B) a deceased individual being consumed by a *Hermodice carunculata* fireworm, C) an aggregation of deceased *D. setosum* and D) a healthy individual in an overhang.

Table 1. Dates of surveys, number of transects per site, average densities and number of healthy and infected individuals in the six sites of this study. Asterisks denote statistically significant difference ($p < 0.05$) of the numbers of *D. setosum* in Alykes before and after the MME.

Site	Date of survey	No of transects/site	Average density (ind/m ²)	No of healthy individuals	No of infected individuals
Kalo Nero	29/4/2023	4	n/a	2 (out of transects)	0
Chrysi	1/5/2023	4	1.35	214	110
Dianiskari	19/5/2023	4	0	0	0
Atzikiari	1/6/2023	4	0	0	0
Alykes	8/6/2023	4	0.3	72	0
Agios Ioannis	20/6/2023	4	n/a	3 (out of transects)	0
Alykes	26/7/2023	4	0.5	119 *	0
Alykes	19/1/2024	4	0.03	6 **	0

at both sites in the current surveys (conducted during 2023). Both sites are located on the northeastern coast of the island and no surviving individuals of *D. setosum* nor traces of a recent MME could be identified. In contrast, in Alykes, located further along the north-western coast of Crete, a healthy population of *D. setosum* was observed over two subsequent surveys in summer 2023 (June and July), with an average density of 0.4 ind/m². However, several months later, in December 2023, this population suffered from the same pathology witnessed in Chrysi with almost no individuals of *D. setosum* surviving. Since this event, the average density of *D. setosum* has decreased significantly from 0.5 ind/m² to 0.03 ind/m² in Alykes (Table 1). Previously, a few healthy individuals were recorded in Agios Ioannis on the south coast. In all

surveyed sites, the native sea urchins *Arbacia lixula* (Linnaeus, 1758) and *Paracentrotus lividus* (Lamarck, 1816) were present and seemingly unaffected by the pathology of *D. setosum*. Lastly, it is worth noting that both MME events witnessed in Chrysi and Alykes occurred during periods of low ambient seawater temperature, which at both locations was approximately 18 °C. However, in order to link temperature with the development of the MME more extensive temporal and geographical datasets are required.

The data collected during May and June of 2023 and January of 2024 suggest that the recent MME (Zirler *et al.*, 2023b) affecting *Diadema setosum* in the eastern Aegean Sea is still ongoing, and progressing through successive years (i.e., 2022 to 2024). The northeastern part of the is-

land of Crete was presumably already affected, since the previous existence of *D. setosum* (Katsanevakis *et al.*, 2020) has not been evidenced in the site of Atzikari during this study. The presence of a population in Dianiskari has been recorded twice (Katsanevakis *et al.*, 2020; Ragkousis *et al.*, 2023) while it was observed to be unaffected until December of 2022, thus leading to the suspicion that the MME affected this particular site probably during the first half of 2023. Remaining on the north coast of Crete while moving to the west, the recent outbreak of mortality of the invasive sea urchin recorded in Alykes indicates that the MME is still ongoing along the coastline of Crete. It appears that coastal areas of Crete with high densities of the invasive urchin are more susceptible to the MME, as the two sites with the highest densities (Chrysi and Alykes) have been affected, while no evidence of the MME was recorded in sites where the presence of *D. setosum* is inherently scarce (Agios Ioannis and Kalo Nero).

In conclusion, the condition of *Diadema setosum* populations around the island of Crete needs to be investigated closely in order to monitor the progression of the MME in the future. Recurrent surveys along a geographically representative set of locations in different seasons, combined with continuous monitoring of the seawater temperature, will enhance our understanding of the progress of this invasion which strongly affects native habitats, and potentially inform managerial decisions towards its mitigation.

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