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Trend of *Ostreopsis* cf. *ovata* (Dinophyceae) along the Conero Riviera (northern Adriatic Sea) over two decades

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RUNNING TITLE

Ostreopsis cf. *ovata* trend in N. Adriatic Sea

ABSTRACT

Harmful blooms of the toxic dinoflagellate *Ostreopsis* cf. *ovata* have been a recurrent phenomenon along the Mediterranean coasts in the last decades. Since first recorded, there has been a widespread belief that the extension of this dinoflagellate, well-known in tropical areas, to higher latitudes was due to global warming and the general rise of seawater temperature. Blooms of *O.* cf. *ovata* along the Conero Riviera (northern Adriatic Sea) occur between the end of the summer and the beginning of the autumn since 2006. The *Ostreopsis* cf. *ovata* abundances collected from its first record to today were analysed to better define the interannual trend of this phenomenon and its possible linking to certain climate change predictors. A significant increasing trend in the magnitude of *Ostreopsis* phenomenon was observed up to year 2012, then a stabilization at relatively low values was observed. This trend does not follow the incessant increase in water temperature observed during the last three decades in the Adriatic Sea, but rather recalls patterns seen in invasive species, although the provenience of *O.* cf. *ovata* in the Mediterranean Sea is still unresolved. Even if the *Ostreopsis* bloom in this area seems to slightly lessen in the last decade, *Ostreopsis* abundances still reach values of up to 10^3 cells cm^{-2} which could be harmful to human health.

KEYWORDS

benthic dinoflagellates; global warming; harmful algae; time series; toxic algae

INTRODUCTION

Benthic microalgae of the genus *Ostreopsis* Johs. Schmidt grow in relatively shallow and well illuminated waters, mainly attached to benthic surfaces such as macrophytes, coral rubble, rocks, and sediments. This genus includes species able to produce toxins mostly belonging to palytoxin (PlTX) analogues (Yasumoto *et al*. 2007; Uchida *et al*. 2013; Brissard *et al*. 2015; Accoroni *et al*. 2016; Medina-Pérez *et al*. 2023).

Environmental factors, both abiotic and biotic, are known to influence benthic HAB dinoflagellates in terms of their proliferation, toxin production and accumulation, and their effects on environment and human health. Growth, and abundance of benthic HAB dinoflagellates are largely temperature driven, and the biogeographic distribution is expected to shift in response to climate induced changes as ocean temperature rise provided that other species-specific requirements can be met (Tester *et al*. 2010; Kibler *et al*. 2015). For instance, many studies have highlighted that other environmental factors play a key role, including hydrodynamics, water depth, nutrient (both inorganic and organic) concentrations, substrate availability and allelochemical interactions (Parsons & Preskitt 2007; Richlen & Lobel 2011; Glibert *et al*. 2012; Skinner *et al*. 2013; Accoroni *et al*. 2017a, b; Pichierri *et al*. 2017; Boisnoir *et al*. 2018; Yong *et al*. 2018; Larsson *et al*. 2019).

The species of this dinoflagellate genus were initially reported in tropical areas, and only later in temperate latitudes (Rhodes 2011; Parsons *et al*. 2012). Although in tropical areas they show the highest diversity, the maximum abundances (10^5 cells g^{-1} fw, Delgado *et al*. 2006; Okolodkov *et al*. 2007; Parsons & Preskitt 2007; Faust 2009; Tester *et al*. 2014; Díaz-Asencio *et al*. 2019; Accoroni *et al*. 2020) were lower than at temperate latitudes (up to 10^6 cells g^{-1} fw e.g., Vassalli *et al*. 2018; Mangialajo *et al*. 2011). Especially in the Mediterranean coasts, *Ostreopsis* blooms (mostly caused by *O.* cf. *ovata*, although *O.* cf. *siamensis* and *O. fattorussoi* Accoroni, Romagnoli & Totti 2016, have also been identified, Penna *et al*. 2012; Accoroni *et al*. 2016) are a phenomenon of great concern due to the problems on human health associated with them, mainly due to inhalation of sea water droplets containing aerosolized toxins and/or cells of *Ostreopsis*, or cutaneous contact with cells, causing mainly skin and eye irritations (Berdalet *et al*. 2022). Moreover, although in such area there are no reports of toxicity by contaminated-seafood ingestion (Tubaro *et al*. 2011), PlTX-analogues compounds were detected in shellfish collected from the north Aegean Sea, French Mediterranean coasts, north Adriatic Sea and Gulf of Naples with maximum concentrations ranging from 100 to 600 μg kg^{-1} (Aligizaki & Nikolaidis 2008; Accoroni *et al*. 2011, 2022; Amzil *et al*. 2012; Sardo *et al*. 2020), i.e. concentrations much higher than the threshold of 30 μg kg^{-1} indicated by the European Food Safety Authority (EFSA 2009).

In Mediterranean Sea, *Ostreopsis* blooms appeared as a sudden upsurge around the 2000's, followed by an expansion of the known range for the species in the next years and a relative stability in the following decade, providing the most evident case of range expansion and increased impact over time in this area (Zingone *et al*. 2021). This apparent sudden range expansion and impact was observed even in New Zealand and other temperate areas of the world (Parsons *et al*. 2012). However, no clear increase of species abundances has been reported since the 2000 outburst, while the above-mentioned range expansion has also coincided with a dramatic increase in monitoring programs and research projects focused on benthic microalgae (Zingone *et al*. 2021). Several authors wondered about the link between the climate change and harmful benthic microalgae: although some authors suggest that the predicted higher temperatures and greater number of high temperature days should stimulate more intense *Ostreopsis* blooms in the future (Asnaghi *et al*. 2017; Tester *et al*. 2020), others observed that the trend observed in the Mediterranean Sea recalls that of an invasive species rather than that of a species favored by a temperature increase (Zingone *et al*. 2021).

The northern Adriatic Sea is considered one of the hotspots of Mediterranean HABs, in terms of both occurrence and impacts (Garcés & Camp 2012; Zingone *et al*. 2021), where *Ostreopsis* cf. *ovata* blooms are known since 2006 (Monti *et al*. 2007; Totti *et al*. 2010).

The aim of this study was to investigate *Ostreopsis* cf. *ovata* abundance trends in an area considered a hot-spot for the blooms, i.e. the Conero Riviera, north Adriatic Sea (Mangialajo *et al*.

2011), gathering data from its first record, 2007, to 2021. The results of this study will help to better define if this phenomenon shows an increasing, stabilizing or decreasing trend, linked or not to certain climate change predictors.

MATERIALS AND METHODS

Sampling

The study was carried out along the Conero Riviera (Ancona, northern Adriatic Sea), at the Passetto station (43°37′09″N, 13°31′54″E) characterized by a rocky bottom and shallow depths. Passetto station is a sheltered site affected by moderate human impact, as the shore is a popular area for summer holidays.

Sampling of *Ostreopsis* cf. *ovata* was conducted weekly from July to November (i.e. covering the entire seasonal bloom period) from 2007 to 2021 mainly on the following macroalgae: *Ulva* cf. *lacinulata* (Kützing) Wittrock (formerly *U. rigida*) (Hughey *et al.* 2021), *Hypnea musciformis* (Wulfen) J.V.Lamouroux (Rhodophyceae) and *Dictyota* cf. *dichotoma* (Hudson) J.V.Lamouroux, *Dictyopteris polypodioides* (De Candolle) J.V.Lamouroux and *Gongolaria barbata* (Stackhouse) Kuntze (formerly *Cystoseira barbata* (Stackhouse) C.Agardh) (Phaeophyceae). Seaweed species were identified by light microscopy following Cormaci *et al.* (2012). Appreciable modification of the benthic macroalgal communities was not observed during this period along the Conero Riviera (Rindi *et al*. 2020). Fragments of macroalgal thalli were collected in three replicates at a depth of 0.5 m following the method described by Totti *et al.* (2010). Briefly, thalli were collected after hooding in plastic bags which were promptly closed underwater, to avoid the loss of epiphytic cells. Samples were then transferred to plastic jars filled with filtered seawater and kept in the dark and at room temperature until transferred to the laboratory.

Surface temperature and salinity were measured with a CTD, Model 30 Handheld Salinity, Conductivity and Temperature System, YSI (Yellow Spring, OH USA) and wave height was recorded according to the Douglas scale. Water samples for nutrient analysis were collected in polyethylene bottles (50 mL) near the sampled substrata avoiding resuspension phenomena, immediately filtered through GF/F Whatman filters (25 mm) and stored in triplicate in 4 mL polyethylene bottles at -22 °C until analysis. The analysis of nitrates, nitrites, ammonium, phosphates and silicates was conducted following the colorimetric method by Strickland and Parsons (1972), using an Autoanalyzer QuAAtro Axflow. Detectability limits were 0.02 µmol l^{-1} for nitrates, nitrites, ammonia and silica and 0.03 µmol l^{-1} for orthophosphates. Dissolved Inorganic Nitrogen (DIN) concentration is intended as the sum of NO_2, NO_3 and NH_4 concentrations.

Ostreopsis sample treatment

In the laboratory, macroalgae were immediately treated following the method described in Totti et al. (2010). Briefly, samples of macroalgae were vigorously shaken in ~100 mL seawater, in wide-necked HDPE sample bottles or plastic bags to dislodge the epiphytic cells. The water samples containing the detached microalgal cells were preserved with 0.8% neutralized formaldehyde (Throndsen 1978) and stored in the dark at 4 °C until microscopic analysis. Finally, the macroalgae were weighed to determine fresh and dry weights, and their area calculated using a conversion factor obtained from the ratio of fresh-weight/surface as described in Accoroni *et al*. (2024).

Microscopy analysis of *Ostreopsis*

Ostreopsis abundances were estimated using an inverted microscope equipped with phase contrast. Sub-samples (1-25 mL) were settled in counting chambers after homogenization, according to the Utermöhl sedimentation method (Hasle 1978). Counting was performed at 200x magnification either on 10–30 random fields, 1–2 transects, or the whole sedimentation chamber, in order to count a representative cell number (at least 300 cells). The identification of *O.* cf. *ovata* was carried out by observing selected samples in epifluorescence after staining with fluorochrome (Calcofluor White, Fritz & Triemer 1985). The identification of *Ostreopsis* cf. *ovata* was confirmed by molecular analysis (Accoroni *et al*. 2011, 2017a, 2020).

The final data were expressed as cells cm^{-2} of macroalga and normalized to *Hypnea musciformis* as this species resulted to be the most available macroalgal species in the study area throughout the blooms in each year. The normalization was conducted following the method described by Accoroni *et al*. (2024). Briefly, macrophyte thalli were weighed to determine the fresh (g fw) and dry (g dw) weight. The area of seaweeds was calculated with image analysis software to obtain the surface area (cm^2) of digitalized images obtained with a scanner. The fresh weight/area and dry weight/area ratios were then calculated for each macroalgal species on a significant number of individuals to obtain a conversion factor, allowing the thallus area to be estimated only by measuring wet or dry weight. Finally, the ratio between (i) *Ostreopsis* abundances on *H. musciformis* and (ii) those on other macroalgal species was calculated on a significant number of individuals. This ratio was adopted as the normalization factor allowing to convert the abundances on a given macroalga to those on *H. musciformis.*

Analysis on *Ostreopsis* cf. *ovata* trend

Data were collected in 2007, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2019, 2021 from August to November (i.e. from 2009 to 2015 each bloom period without interruptions), with a frequency of approximately 14 samples per year.

The following statistical analyses were performed using the R software (R Core Team 2021), version 4.1.1. Ts (Time-series objects) and decompose functions from the stats package were used to create the time series (with a frequency of 14 samples per year) and to decompose it in its seasonal, trend and irregular components. As the seasonal variation was constant through time, an additive structure was considered for the decomposition model: $\times = Trend\ + Seasonal\ + Random.$

As a shift in the trend was highlighted by the time series decomposition, we performed change point analyses to detect a structural change point, whose presence can affect the time series analysis if not considered (Zeileis *et al*. 2003). Change point detection in time series allows to identify the period defining segments in which the properties of the series differ, suggesting shifts in the processes affecting the data (Zeileis *et al*. 2003; Burg & Williams 2020; Lewin *et al*. 2023). For this purpose, we applied methods and functions belonging to the F statistics and generalized fluctuation tests (Andrews 1993; Andrews & Ploberger 1994; Kuan & Hornik 1995; Zeileis *et al*. 2003). Method of F statistics (Hansen 1992, 1997; Andrews 1993) was used using the Fstats function (which applies F statistic for every potential change point in the series) in combination with breakpoint and sctest functions (to detect the corresponding time and significance) from the strucchange package (Zeileis *et al*. 2002). Generalized fluctuation test, “which fits a model and derives an empirical process that captures the fluctuation either in residuals or in estimates” (Zeileis *et al*. 2003), was applied using the efp function (strucchange package, Zeileis *et al*. 2002) and setting OLS-CUSUM as type for an OLS-based CUSUM test, i.e. a cumulative sum process based on Ordinary Least Squares (OLS) (Ploberger & Kramer 1992; Zeileis *et al*. 2003). Furthermore, cpt.mean from the changepoint package (Killick & Eckley 2014) was used to assess a change point in the mean, setting method as AMOC (at most one change).

As the methods agreed in the detection of the same change point, the trend before and after the estimated change point was assessed fitting a linear model using generalized least squares as this method allows correlation of the error (Pinheiro & Bates 2000). The zeros values were removed for the analysis, in order to assess only the trend of *Ostreopsis* cf. *ovata* non-zero abundances. The GLS (Generalized Least Squares) function from the nlme package was used to fit linear models using generalized least squares (Pinheiro *et al*. 2023).

To assess patterns in the entire series, hierarchical time series clustering was performed using Dynamic Time Warping (DTW) as distance (Wang *et al*. 2013, Aghabozorgi *et al*. 2015). Before clustering, data were standardized using the decostand function (Oksanen *et al*. 2022) and setting method as ‘standardize’ in order to normalize to zero mean and unit variance. Functions tsclust and cvi from the dtwclust package (Sarda-Espinosa 2022) were used for the time series clustering and for the different cluster validity indices (CVIs) computation, respectively. CVIs aim to find the optimum number of clusters (k), characterized by small variance/distance between members of the same cluster and higher variance/distance between members of other clusters. Several CVIs were considered: Silhouette (Wang *et al*. 2013), Dunn (Arbelaitz *et al*. 2013), Calinski-Harabasz (Arbelaitz *et al*. 2013) indices and Score Function (Saitta *et al*. 2007) to be maximized, and COP (Arbelaitz *et al*. 2013), Davies-Bouldin (Arbelaitz *et al*. 2013) and Modified Davies-Bouldin (Kim & Ramakrishna 2005) indices to be minimized. Considering the combination of the different CVIs, a partition of four clusters was chosen for the hierarchical clustering (Table S1).

Analysis on trends of physico-chemical parameters

Data of water temperature, DIN, PO_4 and DIN/ PO_4 were contextually collected during *Ostreopsis* cf. *ovata* sampling. Their time series were decomposed in their seasonal, trend and irregular components, considering an additive structure and the ts and decompose functions of the stats R package. Due to the strong seasonality, the Mann-Kendall tests for the detection of trends were performed on the time series after subtracting the seasonal component, in order to avoid the effect of the seasonality on the trends. The MannKendall function of the Kendall package was used (McLeod 2022).

RESULTS

Ostreopsis bloom

Blooms of *Ostreopsis* cf. *ovata* were detected in all sampling years. Generally, the first cell appearance on benthic substrata occurred at the end of July/early August, the maximum abundances in early autumn (end of September/early October), and the decline of the blooms at end October/early November (Fig. 1).

During summer 2007, the first cells appeared on 20th August, maximum abundances were recorded on 1st October (Table 1) and the bloom declined during the last days of October. During summer 2009, the first cells appeared on 29th July, maximum abundances were recorded on 29th September (Table 1) and the bloom declined during the last days of October. In 2010 the first appearance of *Ostreopsis* cf. *ovata* was recorded on 5th August and the bloom reached the maximum value on 26th August (Table 1) and a second increase of abundances was observed on 16th September, with lower values. The bloom decline occurred in the last week of October. In summer 2011, the first cells were recorded on 5th August, the bloom developed very quickly, reaching the maximum abundances on 31st August (Table 1), much earlier than in previous years. Abundances rapidly decreased although the end of the bloom occurred only in the last days of October. In summer 2012, the trend of the *Ostreopsis* bloom was similar to that of the earlier years of the study, with the first cell appearance on 10th August, maximum abundances on 28th September (Table 1), and the bloom decline occurred during the first part of November. In this year, the bloom was the most intense in terms of abundances recorded and duration. In 2013 the first appearance of *Ostreopsis* cf. *ovata* was recorded on 12th August and the bloom reached the maximum value on 20th August with value 2 order of magnitude lower than the previous years (Table 1). From summer 2014, blooms reached abundances 1 order of magnitude lower than the years up to 2012. In summer 2014, the first cells were recorded on 13th August, the bloom reached the maximum abundances on 16th September (Table 1) and a second increase of abundances was observed on 7th October, with lower values. Abundances rapidly decreased although the end of the bloom occurred only in the first days of November. In summer 2015, the first cell appearance on 1st August, the maximum abundances were recorded on 11th September (Table 1), and the bloom decline occurred during the first part of November. In summer 2019, the first cells were recorded on 13th August, the bloom reached the maximum abundances on 12st September (Table 1) and a second increase of abundances was observed on 24th October, with lower values. Abundances rapidly decreased although the end of the bloom occurred only in the first days of November. In summer 2021, the first cell appearance on 19th July, maximum abundances on 16th September (Table 1), and the bloom decline occurred during the last part of October.

In the period of the study, *Ostreopsis* occurred each summer from 81 to 102 days, with the exceptions observed in summers 2007, 2013 and 2021 when *Ostreopsis* cells were recorded for 56, 53 and 62 consecutive days.

Time series analysis of *Ostreopsis* cf. *ovata*

The times series of *Ostreopsis* cf. *ovata* showed the typical blooming behaviour of the species in the study area, with bloom initiation, maximum and decline (Fig. 1), as highlighted by the strong seasonal signal (Fig. 3), which remained constant through the series. However, a change in the annual maximum can be observed, as shown by the trend component (Fig. 2). First, an increasing trend can be observed (up to year 2010), followed by a slow decrease and a strong increase in 2012. After this strong peak, a decreasing can be observed, after which the tendency appears more stable.

The structural change point ($p<0.05$) in the *Ostreopsis* cf. *ovata* time series was found on the second sample of October 2012 (15/10/2012), by all the used statistical methods, i.e. F statistics, OLS-based CUSUM process and change in the mean (Figs 5, 6 and 7, respectively). Indeed, the methods agreed in highlighting a shift in the mean (Fig. 7) and in showing a peak around 2012, which exceeded the boundaries indicating a structural shift (Figs 5, 6). This breakpoint partitioned the series in two segments characterized by a mean of 4112.88 ± 1004.99 and 1066.76 ± 253.59 cells cm^{-2} before and after the change point, respectively.

The GLS time-series regression (Figs 8, 9) separated into two partitions, showing a significant increasing trend (148.06 ± 67.84 cells cm^{-2} per sampling period, $p<0.05$) (Fig. 8) and no trend (19.39 ± 19.07 cells cm^{-2} per sampling time, $p>0.05$) (Fig. 9) before (beginning of the series-October 2012) and after the change point (October 2012-end), respectively.

Time series analysis of physico-chemical parameters

The time series decomposition of water temperature, DIN, PO_4 and DIN/PO_4 (Figs S1-16) highlighted the typical strong seasonal behaviour of the physico-chemical parameters and significant increasing and decreasing trends of water temperature (tau = 0.32, $p<0.001$, Mann-Kendall test) and PO_4 (tau = -0.24, $p<0.001$, Mann-Kendall test), respectively. No significant trends were observed for DIN (tau = -0.01, $p>0.05$, Mann-Kendall test) and DIN/PO4 (tau = 0.11, $p>0.05$, Mann-Kendall test).

Cluster analysis

The hierarchical clustering of the different years highlights four clusters. The first cluster grouped the years of 2007, 2011, 2013, 2014, and 2021 (Fig. 10), which had maxima values lower than 10,000 cells cm^{-2} (2007, 2013, 2014, 2021) and/or a series characterized by one peak instead of two (2011, 2013, 2021) (Fig. 11). The years 2009, 2010 and 2012 were characterized by two peaks, with values higher than 10,000 cells cm^{-2} (Fig. 11), clustering in the second group (Fig. 10). Years 2015 and 2019 clustered in two different groups (cluster three and four, respectively) as they were characterized by an irregular trend with values remaining high at the end of the analysed period (Fig. 11).

DISCUSSION

Since its first record in the Mediterranean Sea, there was a widespread belief that the extension of *O.* cf. *ovata* to new areas was a consequence of global warming and a general rise in seawater temperature. However, several records of *Ostreopsis* blooms at low temperature, even down to 7 °C, seems to disagree with this hypothesis (Monti *et al*. 2007; Selina & Orlova 2010; Mangialajo *et al*. 2011; Selina *et al*. 2014). For this reason, some authors suggested that global warming, rather than affecting the extension of the phenomenon, could favour its earlier start, as optimal temperature conditions would be reached earlier in the season (Fricke *et al*. 2018; Drouet *et al*. 2022). While it is not clear if temperature directly affects the maximum abundances reached, some studies (in both field and laboratory) found a positive correlation between abundances and temperature (e.g. Granéli *et al*. 2011; Yamaguchi *et al*. 2012), while others observed negative responses (Cohu *et al*. 2011; Scalco *et al*. 2012; Carnicer *et al*. 2016). These apparently discordant results may be explained considering the different environmental requirements and genetic traits of different strains. In this regard, laboratory studies carried out on strains collected in the Mediterranean Sea showed that the optimal temperature for algal growth was strain-specific, varying between 20 °C and 30 °C depending on the clone (Granéli *et al*. 2011; Pezzolesi *et al*. 2012; Scalco *et al*. 2012; Carnicer *et al*. 2016; Gémin *et al*. 2021).

The bloom of *O.* cf. *ovata* has been constantly reported along the Conero Riviera between the end of the summer and the beginning of the autumn since 2006 (Totti *et al*. 2010; Accoroni *et al*. 2011, 2012). In the Conero Riviera, the role of depth, temperature, nutrients and hydrodynamics on the bloom dynamics of *Ostreopsis* cf. *ovata* blooms has been widely discussed in previous papers. The study area is a shallow site strongly P-limited (Accoroni *et al*. 2017b). Here, calm conditions, temperature > 25 °C and increased availability of inorganic phosphorus, leading N:P to reach the Redfield ratio, have been indicated in each year as optimal conditions to support the *Ostreopsis* bloom onset. Then, blooms can be maintained at temperature values even lower (≈ 20 °C, Accoroni *et al*. 2015, 2022). Once blooms started, *Ostreopsis* cf. *ovata* can efficiently use organic P, as the main source of phosphorus is organic in the study area (Accoroni *et al.* 2017; Ellwood *et al.* 2020). Therefore, the slight decreasing trend of P observed in this study should have affected the bloom onset (that instead occurs each year) rather than bloom intensity and/or duration. In this study, a significant increasing trend in the magnitude of *Ostreopsis* phenomenon was observed from its first record to 2012, then the abundances showed a stabilization at relatively low values. This trend is not following the incessant increasing trends observed in water temperature during the last three decades in Adriatic Sea (and at local scale in this study site), particularly during the warm periods of the year (i.e., during spring and summer, when *Ostreopsis* blooms occur) (Tojčić *et al*. 2023). Therefore, at least for this area of the Mediterranean Sea, the rising of water temperature, linked to the global warming, seems to not be related to an increase of *Ostreopsis* blooms. *Ostreopsis*, in this study, did not even show any earlier bloom time as suggested for other Mediterranean areas (Fricke *et al*. 2018; Drouet *et al*. 2022), as *Ostreopsis* blooms have been observed each year always in late July - August. This could be explained considering that the start of the bloom in this area is linked to the onset of two synchronous optimal conditions, i.e. optimal temperature and nutrient availability. Therefore, even in the case that the global warming could lead to an early bloom start, in this area it is anyway limited by the time of reaching optimal N and P levels which occur in late July – August, therefore, irrespective of the rising temperature observed in the last years. In addition, considering that the highest growth rates for Adriatic *O.* cf. *ovata* strains were recorded at 20 °C (Pezzolesi *et al*. 2014) and the optimal temperature for algal growth of Mediterranean strains do not exceed 30 °C (Granéli *et al*. 2011; Pezzolesi *et al*. 2012; Scalco *et al*. 2012; Carnicer *et al*. 2016), the rising of temperature could even be disadvantageous to *Ostreopsis* bloom in this area, which was seen in the last ten years.

The origin of *Ostreopsis* cf. *ovata* in the Mediterranean Sea is still unresolved. The absence of a regular monitoring programme of microphytobenthic communities along the Mediterranean coasts does not allow us to evaluate if, and when, these species were introduced in the Mediterranean Sea or if they were already present in the benthic microalgal communities at lower densities (Totti et al., 2010). The increasing trend followed by a stabilization pattern observed in this study suggests that *Ostreopsis* cf. *ovata* in this area may be an alien species. Simberloff and Gibbons (2004) observed that an invasive alien species often shows a phase of sudden increase in the new area followed by a decline, probably due to competition (or other interactions) with other species or by unidentified effects.

Phylogenetic studies, do not resolve this issues, as identical *O.* cf. *ovata* strains found in the Atlantic (Brazil) and Mediterranean Sea are also recorded in Malaysia and Japan (Sato *et al*. 2011; Nguyen-Ngoc *et al*. 2021; Gu *et al*. 2022), suggesting that Western Pacific *O.* cf. *ovata* might have migrated into Mediterranean and/or Atlantic oceans, or vice versa (Sato *et al*. 2011).

If *O.* cf. *ovata* in Mediterranean areas is an invasive alien species, it is not still clear how and when this species was introduced. The first record of *Ostreopsis* dates to 1972 in Villefranche-sur-Mer (France) (Taylor, 1979). Only in 1994 was *O.* cf. *ovata* detected along both the Italian coasts of the Tyrrhenian Sea (Tognetto *et al*., 1995) and in 1997-1998 in Spain (Catalan coast, Vila *et al*., 2001). During the summer of 2005, symptoms of cough, rhinorrea, fever, bronchoconstriction with mild dyspnea, and wheezes were observed in about 200 people exposed to marine aerosols by recreational or working activities in Genoa, Italy (Gallitelli *et al*. 2005; Ciminiello *et al*. 2006). In the Adriatic Sea, *O.* cf. *ovata* was recorded in southern areas in early 2000s in summer (Bottalico *et al*. 2002; Di Turi *et al*. 2003; Ungaro *et al*. 2005), while only in 2006 it has been recorded also along the rocky coasts of the northern Adriatic (Monti *et al*. 2007). Obviously, these "first records" do not necessarily mean that *Ostreopsis* was not there earlier, as it is equally possible that implementation of improved monitoring programs leads to its detection.

In this regard, Walsh *et al*. (2016) proposes two possible scenarios of invasive species population establishment and outbreak timing: the first consists of an invasive species establishment after some number of introduction events and becomes abundant soon after, passing a detection limit. The second describes the establishment and out-break separated in time: as a result, the established population is detected much later. Did *Ostreopsis* bloom soon after (presumably in the same year) it was introduced (if it was) to the Mediterranean Sea, or an existing low-density, but undetected population bloom suddenly due to some sort of trigger occurred later? Fraga *et al*. (2012) suggested that, although an increasing awareness is evident, it is possible that the enhancing of the *Ostreopsis* phenomenon observed in the first years from its first records could has been caused by a phase shift in benthic microalgae communities due to anthropogenic or natural environmental change. Fraga *et al*. (2012) hypothesized that the mass mortalities of benthic invertebrates recorded as a consequence of the exceptional 2003 heat wave (Garrabou *et al*. 2009) could have affect non-identified potential predators of *Ostreopsis*, and a potential cascade effect leading to an increase in *Ostreopsis* populations. If this hypothesis is true, the increasing water temperature could have played two opposite roles on the *Ostreopsis* phenomenon in Mediterranean Sea: an indirect facilitation role in its first establishment, and a disadvantageous role (given the too high values) two decades later.

In conclusion, contrarily to what is generally thought about the relationship between global warming and the *Ostreopsis* occurrence in temperate area, in the northern Adriatic Sea, the two phenomena seem to not be related as neither an increase of abundance nor earlier bloom starts were recorded in this area.

Although the site of origin of *O.* cf. *ovata* in the Mediterranean Sea is still unresolved, this species is considered as an allochthonous species for the Mediterranean Sea (e.g., Corriero *et al*. 2016) and this hypothesis is strongly supported also by the trend of *O.* cf. *ovata* abundances reported in this study. Even if the *Ostreopsis* bloom in this area seems to slightly lessen in the last decade, *Ostreopsis* abundances still reach values up to 10^3 cells cm^{-2} that could be harmful to human health (Accoroni *et al*. 2011, 2022).

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LEGENDS FOR FIGURES

Figs 1–4. Decomposition of the *Ostreopsis* cf. *ovata* time series in its components.

Fig. 1. Time series of the abundances.

Fig. 2. Decomposition of the time series into its trend component.

Fig. 3. Decomposition of the time series in its seasonal component.

Fig. 4. Decomposition of the time series in its random component.

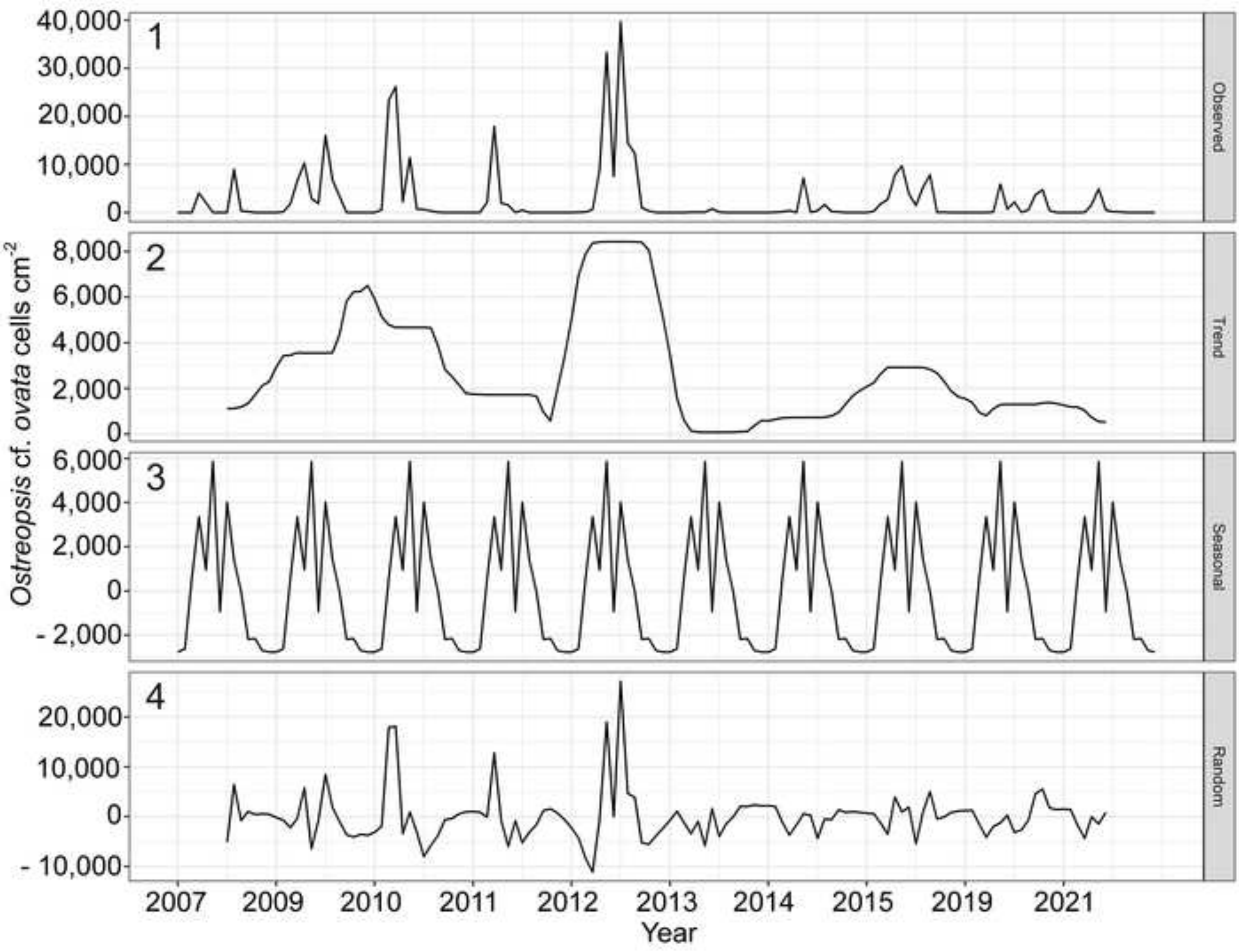
Figs 5–7. Structural change point detection for the *Ostreopsis* cf. *ovata* time series.

Figs 5–6. F statistics and OLS-based CUSUM process with the boundaries at a 5% significance level (red line marks the significant change time).

Fig. 7. Shift in the mean (red lines represent the means).

Figs 8–9. Generalized least squares time series regression for the period before (Fig. 8) and after (Fig. 9) the changepoint (15/10/2012).

Figs 10–11. Hierarchical clustering of the different years in which *Ostreopsis* cf. *ovata* was sampled (Fig. 10) and tendency of the series in each cluster (Fig. 11): cluster one: 2007 (orange), 2011 (dark green), 2013 (light green), 2014 (blue), 2021 (purple); cluster two: 2009 (orange), 2010 (brownish), 2012 (light green); cluster three: 2015; cluster four: 2019.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Figs 5-7

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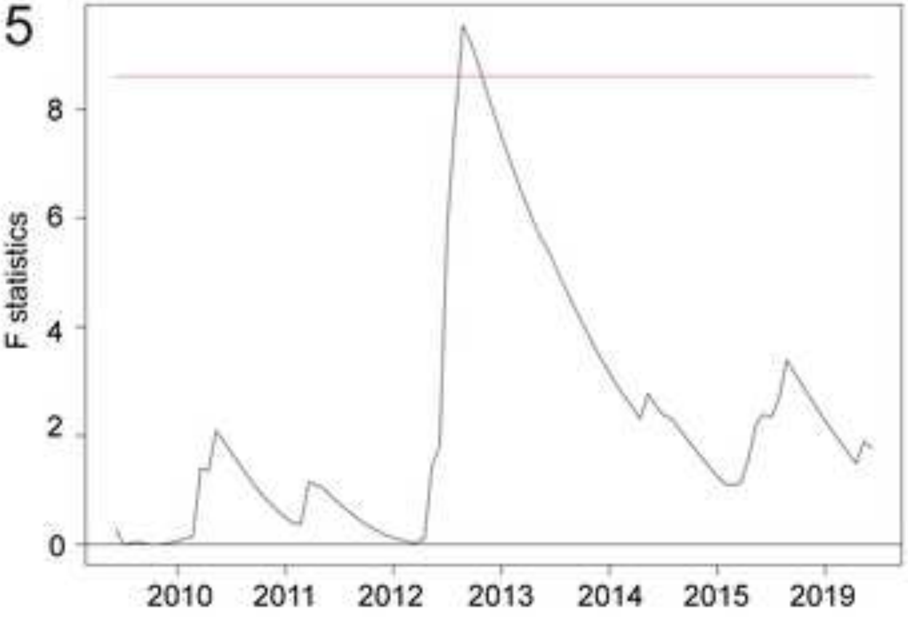
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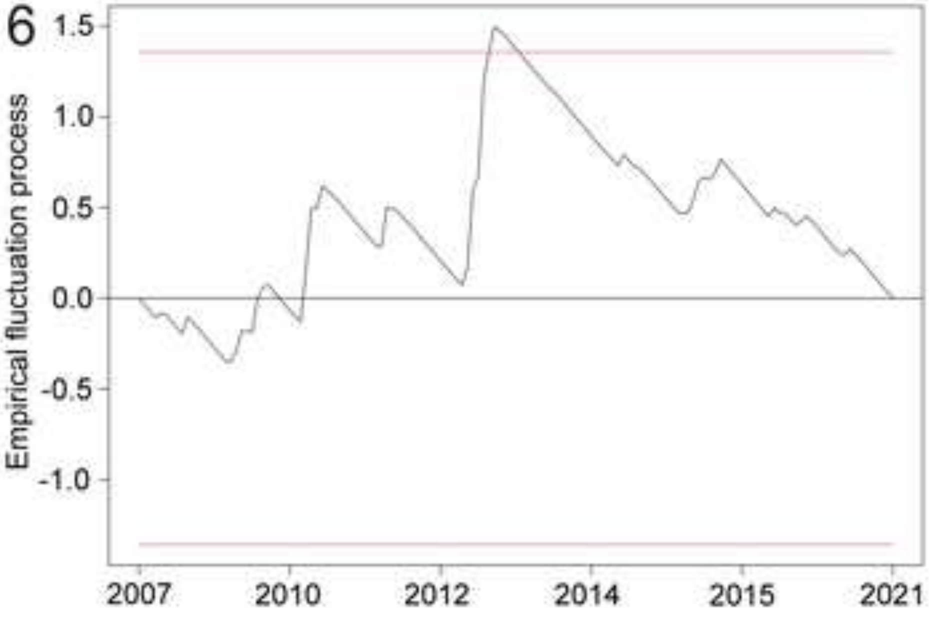
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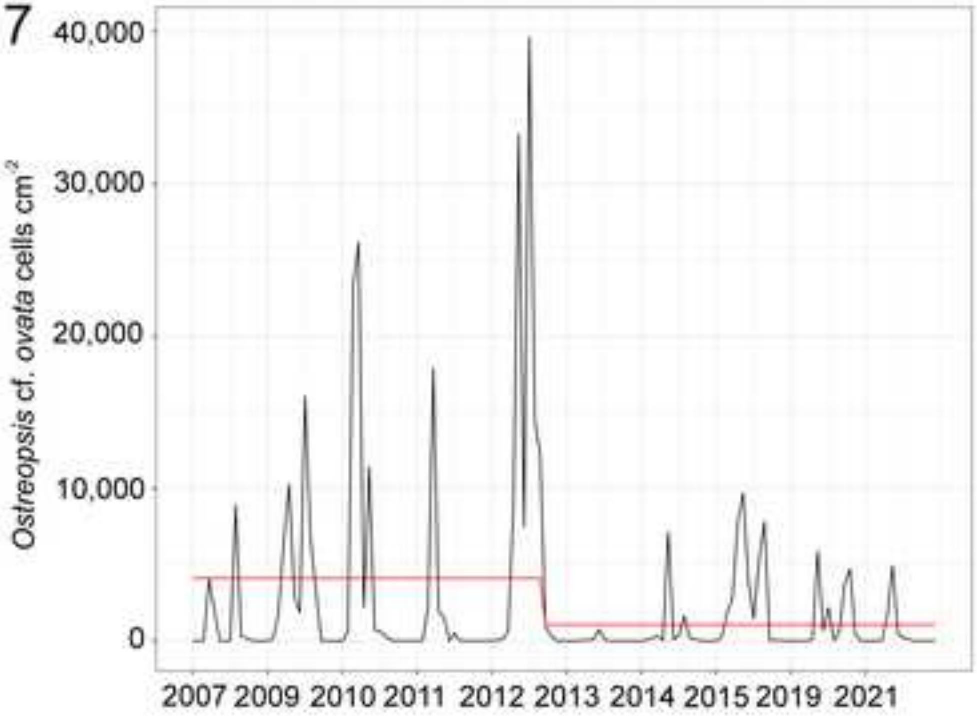
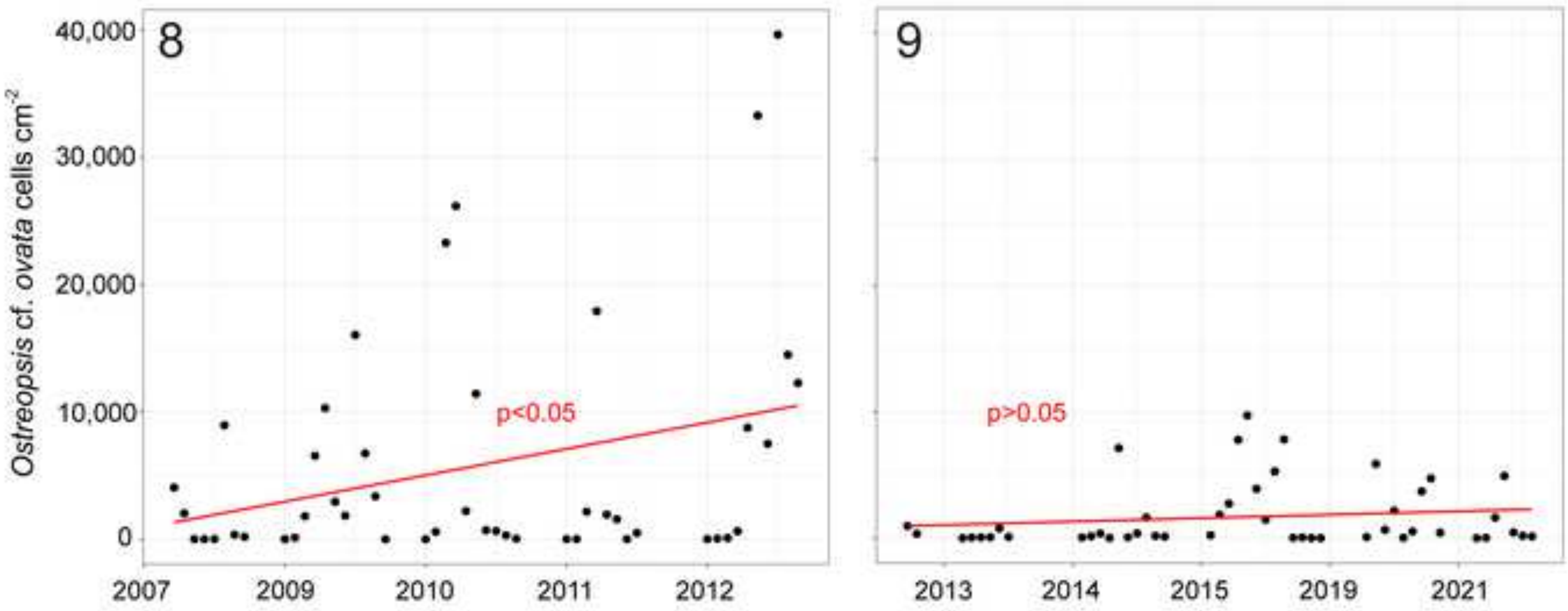
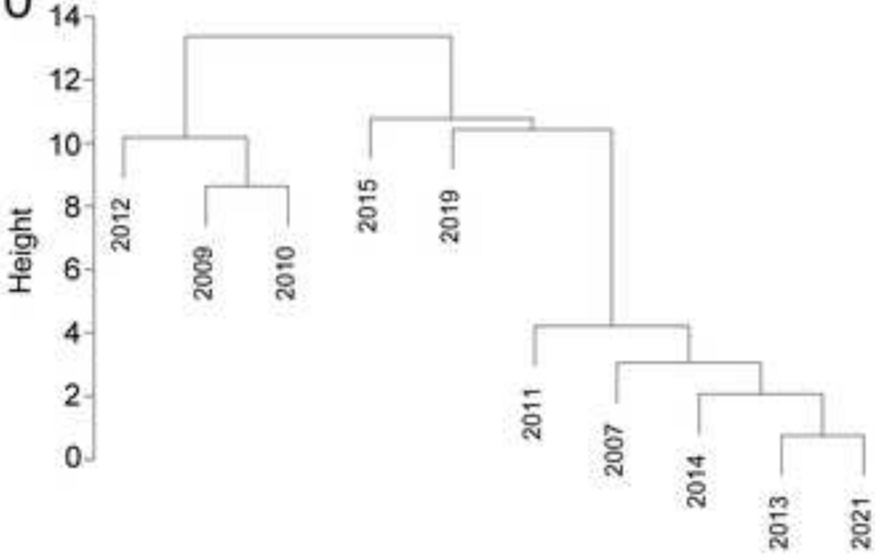
2021

7


40,000
30,000
20,000
10,000
0
Ostreopsis cf. *ovata* cells cm^{-2}
2007
2009
2010
2011
2012
2013
2014
2015
2019
2021


8
9
Ostreopsis cf. ovata cells cm-2
40,000
30,000
20,000
10,000
0
p<0.05
p>0.05
2007
2009
2010
2011
2012
2013
2014
2015
2019
2021

10


14
12
10
8
6
4
2
0
Height
2012
2009
2010
2015
2019
2011
2007
2014
2013
2021

11

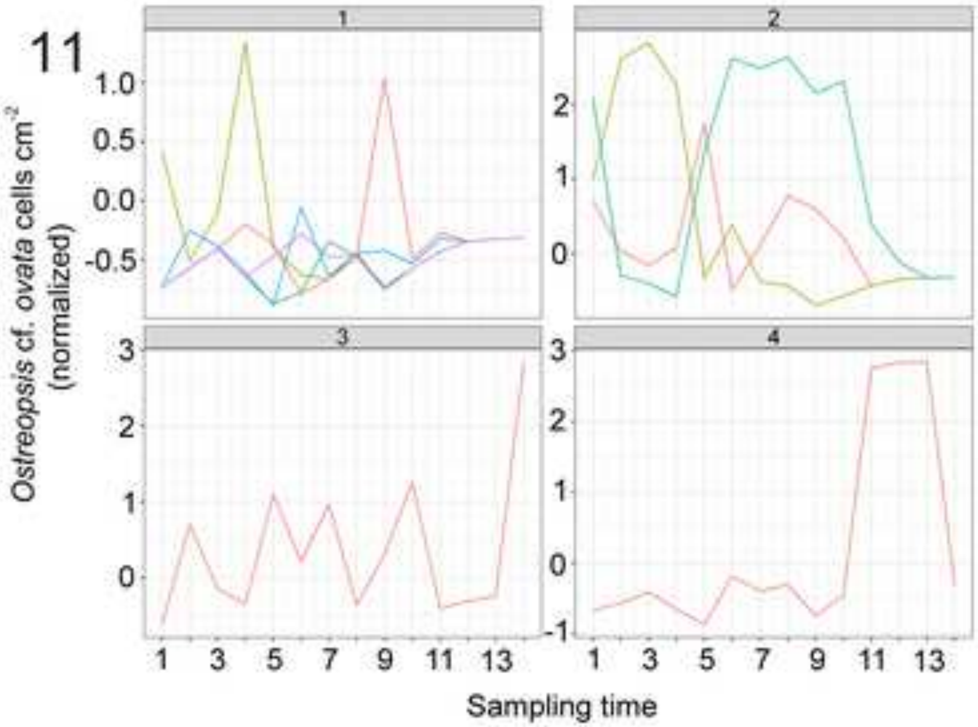

1
2
3
4
1.0
0.5
0.0
-0.5
2
1
0
3
2
1
0
3
2
1
0
-1
1
3
5
7
9
11
13
Ostreopsis cf. ovata cells cm-2 (normalized)
Sampling time

Table 1. Maximum abundances of *Ostreopsis* cf. *ovata* in each year (from 2007 to 2021) in the study site.

Year	**Date**	**Maximum abundance (cells cm^{-2})**
2007	1st October	8,950
2009	29th September	16,052
2010	26th August	2,3293
2011	31st August	17,908
2012	28th September	39,639
2013	20th August	769
2014	16th September	7,132
2015	11th September	11,072
2019	12st September	5880
2021	16th September	4930