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NEW INSIGHTS INTO THE DYNAMICS OF THE TOXIC DINOFLAGELLATE *DINOPHYSIS* EHRENBERG AND DSP (DIARRHETIC SHELLFISH POISONING) EVENTS IN THE ADRIATIC SEA: IMPLICATIONS FOR SAFE MARINE AQUACULTURE PRODUCTION MANAGEMENT

NUOVE INFORMAZIONI SULLE DINAMICHE DEL DINOFLAGELLATO TOSSICO *DINOPHYSIS* EHRENBERG E SUGLI EVENTI DI DSP (SINDROME DIARRHOICA DA MOLLUSCHI) NEL MAR ADRIATICO: IMPLICAZIONI PER UNA GESTIONE SICURA DELLA PRODUZIONE DI ACQUACOLTURA MARINA

Abstract - *Dinophysis* spp. are harmful marine dinoflagellates that produce lipophilic toxins able to accumulate in mussels. Mussel farming is a key activity along the northern Adriatic coast, where *Dinophysis* dynamics, DSP toxicity events, and environmental conditions were monitored from 1998 to 2023. A clear seasonality emerged: *D. acuminata* (Claparède & Lachmann) and *D. sacculus* (F.Stein) occurred in spring–summer, followed by *D. caudata* (Saville-Kent), *D. fortii* (Pavillard), and *D. tripos* (Pavillard) in autumn. DSP toxicity events occurred mainly in autumn–winter, significantly correlated with *D. caudata* ($r_s = 0.84$), *D. fortii* ($r_s = 0.83$), and *D. tripos* ($r_s = 0.66$). Some species showed preferential temperature ranges. Mussel toxicity declined with time, alongside decreasing DIN, phosphate, total phosphorus, and chlorophyll *a*. Nevertheless, in 2015 and 2022, prolonged toxicity events linked to colder surface waters were recorded. This study emphasises the significance of long-term monitoring to understand *Dinophysis* variability and improve aquaculture management.

Keywords: *Dinophysis*, Harmful algal blooms (HABs), Long-term monitoring, Mussel farming, Phytoplankton ecology

Introduction – Harmful Algal Blooms (HABs) occur when microalgae, whether invasive or naturally occurring (e.g., *Dinophysis* spp.) produce toxins, posing a risk to human health and marine ecosystem. *Dinophysis* spp. produce okadaic acid (OA) and dinophysistoxins (DTXs), which accumulate in shellfish causing DSP (Diarrhoetic Shellfish Poisoning), harvesting bans and economic losses (Reguera *et al.*, 2012; Blanco, 2018). The European Union sets the regulatory limit for DSP toxins at 160 µg OA eq kg⁻¹ shellfish tissue. This study examines the long-term dynamics of *Dinophysis* spp. communities and DSP toxicity events along the coasts of Emilia-Romagna and northern Marche, areas of intense mussel farming (EUMOFA, 2022), over the period 1998-2023. Specifically, it investigates the temporal variability of toxic blooms and their association with key environmental variables (temperature, salinity, nutrients, chlorophyll *a*). We hypothesize that (i) DSP toxicity events have declined over time, and (ii) this variability is linked to complex interactions with evolving environmental conditions in the Adriatic Sea.

Materials and methods – The study area is located along the Emilia-Romagna and Marche coasts, where 12 mussel farms and a long-term monitoring station were sampled bi-weekly or monthly depending on the season. Surface seawater and mussels were collected simultaneously at each site. Monitoring data for phytoplankton abundance in seawater, environmental parameters (1998-2023) and chemical analysis on mussel soft tissues (2011-2023) consisted of: quantification of *Dinophysis* spp. in seawater samples, by means of the Utermöhl method, with an inverted microscope (Leitz DIAVERT, Germany and Nikon Eclipse Ti-U, Japan); DSP toxins quantification by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS); environmental parameters as temperature, salinity, chlorophyll *a* (Chl*a*), dissolved oxygen (DO), and nutrients such as dissolved inorganic nitrogen (DIN), including NO₃, NO₂ and NH₃, dissolved inorganic phosphorus or phosphate (PO₄) and total phosphorus (TotP). Data were collected from (i) the EMODnet Chemistry data portal (European Marine Observation and Data Network, URL: <https://www.emodnetchemistry.eu/>; accessed in July 2024); (ii) the Daphne Oceanographic Infrastructure by ARPAE Emilia-Romagna, in collaboration with Consorzio Mitilicoltori Emilia-Romagna (Italy); and (iii) the Marine Ecology Laboratory of the University of Urbino (Italy). Lastly, The Mediterranean Sea Physics Reanalysis (Escudier *et al.*, 2021) was applied to provide a long-term dataset of surface currents, sea temperature and salinity via the E.U. Copernicus MarineService (doi:10.25423/CMCC/MEDSEA_MULTIYEAR_PHY_006_004_E3R1, accessed in July 2024), through the application of the Nucleus for European Modelling of the Ocean (NEMO) hydrodynamic model. Daily values covering the 26-year period from 1998 to 2023 were extracted over an area covering the study site with a 27 × 25 grid at 0.042° (approximately 4 km) spatial resolution.

Data were aggregated as monthly means to investigate seasonality and the relationship among variables through Spearman's rank correlation coefficient (*r_s*, significance level $p < 0.05$), considering the lowest sampling frequency of the biological variables. Relationships between DSP toxins concentration and *Dinophysis* species abundance, as overall temporal trends for environmental and biotic variables on aggregated data were investigated by using generalized linear model (GLM) analysis, with gamma distribution and "log" link function. Temporal trends for *Dinophysis* spp. were assessed using zero inflation analysis, as the count data for *Dinophysis* spp. were zero-inflated. All analyses were carried out using R (ver. 4.2.2; R Core Team, 2021). For each DSP toxicity event, mean environmental conditions during the 15 days preceding the event, from the Mediterranean Sea Physics Reanalysis data, were compared with the 26-year mean.

Results – Eight different *Dinophysis* species were identified in the study period, and those that appeared consistently showed a clear seasonality: *D. acuminata* and *D. sacculus* in spring-summer, followed by *D. caudata* and finally *D. fortii* and *D. tripos* in autumn. Other rare ones were present, particularly *D. hastata* (F. Stein) and *D. parva* (Schiller) (maximum abundance of 40 cells L⁻¹ for both species), and *D. ovum* (Schütt) (data not shown). Generally, *Dinophysis* species abundances were low, with less than 70 (out of 4616) records over 10³ cells L⁻¹. Additionally, DSP toxicity events were mainly recorded from September to January, and correlated with *D. caudata*, *D. fortii* and *D. tripos* ($r_s = 0.84$, $r_s = 0.83$, and $r_s = 0.66$, respectively, $p < 0.05$) (Fig. 1). The GLM model between DSP toxins concentration and *Dinophysis* species abundances indicated

that only *D. fortii* had a significant effect ($p < 0.001$), with DSP toxins levels increasing by 0.90% per unit of *D. fortii*.

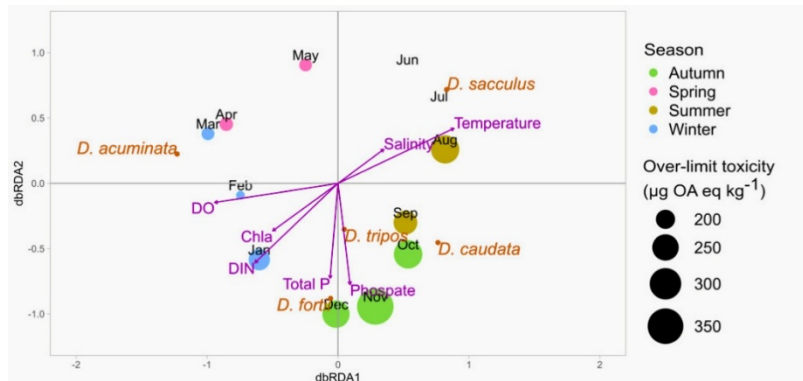


Fig. 1 - The dbRDA of *Dinophysis* species (1998-2023) in relation to environmental variables. Average toxicity values above the limit were superimposed and represented by the size of the dots.
Analisi dbRDA delle specie di Dinophysis (1998-2023) in relazione alle variabili ambientali. I valori medi di tossicità oltre il limite sono stati sovrapposti e rappresentati nella dimensione dei punti.

Additionally, some *Dinophysis* species exhibited a temperature preference for their growth (Fig. 2). Species as *D. acuminata* and *D. fortii*, appeared to tolerate a broader temperature range, whereas *D. tripos*, along with *D. sacculus* and *D. caudata*, exhibited more distinct niches. Salinity preferences appeared uniform across species.

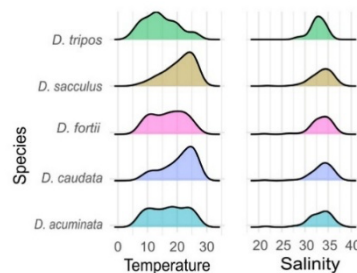


Fig. 2 - Optimum temperature and salinity of *Dinophysis* species retrieved in the study area.
Optimum di temperatura e di salinità per le specie di Dinophysis spp. campionate nell'area di studio.

Furthermore, the analysis of interannual trends revealed a decline in the toxin levels in bivalve mussels, concomitant with a decrease in DIN, phosphate, total phosphorus, and chlorophyll *a* (Table 1).

Tab. 1 - Trend in aggregate data, expressed as percentage change per year.
Tendenza dei dati aggregati, espressi come variazione in percentuale per anno.

Variable	Variation (% Year ⁻¹)
<i>Dinophysis</i> spp. absence	+5.53
OA concentration in mussels	-3.31
Chl <i>a</i>	-1.73
DIN	-1.97
Phosphate	-2.64
Total phosphorous	-3.3

Lastly, the years 2015 and 2022 were distinguished by protracted DSP toxicity events, which occurred in conjunction with colder and marginally saltier surface waters than the mean climatological conditions throughout the entire study period. This was particularly evident in cases where values approached the 10th percentile (ranging from 0.5°C to 1°C below the mean values) (Fig. 3).

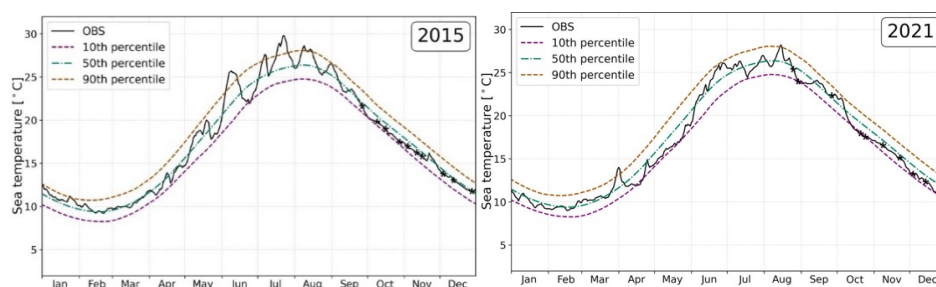


Fig. 3 - Time series of temperature for 2015 and 2021. The observed data are shown, along with the 10th, 50th, and 90th percentiles of the period average (1998-2023). Asterisks indicate detected DSP toxicity events.

Serie temporali della temperatura degli anni 2015 e 2021. Sono riportati i dati osservati, e il 10°, 50° e 90° percentile della media del periodo (1998-2023). Gli asterischi indicano gli eventi di tossicità rilevati.

Conclusions – The present study examined *Dinophysis* dynamics and DSP events in the north-western Adriatic Sea (1998–2023), focusing on mussel aquaculture in Emilia-Romagna and northern Marche. DSP toxins posed a persistent food safety risk, with *D. fortii* showing the most significant toxic potential. A clear seasonality in species occurrence was observed, and some of them seemed to have their own preferred temperature range. However, the mixotrophic nature of *Dinophysis* limited the identification of consistent environmental drivers, especially regarding nutrients. Declines in nutrients and chlorophyll *a*, possibly linked to climate change as suggested by more dedicated studies (Grilli *et al.*, 2020; Ricci *et al.*, 2024), may influence toxicity trends, as they all share the same decreasing trend; however, this relationship remains speculative and a more targeted sampling is required. Only two prolonged toxicity events were recorded in our time series, so their temporal association with colder temperatures is an unexpected but interesting hypothesis that will need to be confirmed by more detailed studies, to provide statistical strength to this possible insight. The occurrence of DSP outbreaks continues to result in substantial economic losses, and these findings highlight both the value and the limitations of long-term monitoring and underline the need for integrated approaches combining biological, environmental, and hydrodynamic data to support sustainable aquaculture management practices.

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