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Impact of Drought and Wildfires in Recent Trends of Diarrhetic Shellfish Toxins in Cockles from Northwest Portugal and Its Similarities with Sardine Stock Trends in the Period 2001–2022

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Abstract

In Portugal, high levels of diarrhetic shellfish toxins (DSTs) originating from *Dinophysis* spp. are accumulated in bivalves, mainly on the northwest coast (NWC). The occurrence of DSTs in spring and early summer is positively related to precipitation. A decrease in average precipitation after 2003 led to a reduction in DSTs after 2008. However, the accumulation of DSTs in the NWC increased in the period 2020–2022. The hydrological year of 2022 was marked by extreme drought. In cockles from the endemic area of Ria de Aveiro, 37.3% of the weeks tested presented samples above the regulatory limit (RL). The previous record was 31.9% in 2005, also an extremely dry year. The average percentage of weeks above the RL in severe drought years surpassed both the low and high precipitation years. In severe dry years, toxicity was dominated mainly by *D. acuta* toxins. While *D. acuminata* grows after the abundant river discharges in spring, *D. acuta* is capable of growing in summer during upwelling favourable conditions. In the last two decades, extensive forest wildfires were recurrent, but the area burnt reduced sharply after 2017 following tighter fire control measures. Low levels of DSTs or low percentage of weeks with cockles above the RL were related to high burnt areas in the previous year. The recent increase in 2020–2022 of DSTs, a planktonic biomarker in bivalves, was also coincident with the similar temporal increase in either the recruitment or the biomass of *Sardina pilchardus*, a planktivorous fish in decline after 2005/2006. Both low sardine recruitment and biomass were coincident with low precipitation or high burnt areas. Wildfires degrade coastal water quality, as seems reflected both in the cockle's DST and the sardine stock time series.

Keywords Diarrhetic shellfish toxins · Drought · Wildfires · Global change · Ria de Aveiro · Water quality · Sardine recruitment

Introduction

Consumption of bivalve molluscs occasionally presents several risks to the consumer derived from their filter-feeding habits. Acute poisoning risks derive mainly from ingestion in their diet, amongst other noxious microorganisms, of toxin-producing microalgae. The seasonal appearance of toxic microalgae is commonly known as harmful algal blooms (HABs). This terminology includes other phenomena, not necessarily related to ingestion of planktonic microorganisms by bivalves, such as proliferation of some benthic microorganisms ingested by fish or non-toxic microalgae producing high biomass and causing seawater discolorations and/or anoxia, often resulting in fish kills (Hallegraeff et al. 2021). The two most common human syndromes observed in temperate coastal waters around the world have been diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP).

For Western Europe, in the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT), the majority of events recorded were caused by diarrhetic shellfish toxins (DSTs) (Bresnan et al. 2021a, b; Karlson et al. 2021). Western Europe has the highest incidence of DSTs in the world, and this contamination causes prolonged shellfish harvesting bans in several countries (Blanco et al. 2013; Reguera et al. 2014; Bresnan et al. 2021a, b; Fernández et al. 2019).

DSP is a non-fatal acute gastrointestinal syndrome. Major symptoms include diarrhoea, nausea, vomiting and abdominal pain. Their onset might start from half an hour to a few

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hours after ingestion, depending on toxin concentration and amount of flesh ingested, and complete recovery occurs within 3 days (Vale and Sampayo 1999; FAO 2004). DSP was separated for the first time from common microbial aetiologies (salmonellosis etc.) by Japanese researchers in 1976 (Yasumoto et al. 1978). Toxin contamination originates from bivalve predation of microalgae from the genus *Dinophysis* or *Phalacroma* (Reguera et al. 2014). The toxins responsible for DSP are heat-stable polyether analogues of okadaic acid (Reguera et al. 2014). These compounds are potent phosphatase inhibitors, and this property is linked to inflammation of the intestinal tract and diarrhoea in humans (Costas et al. 2022).

Bivalve contamination with DSTs in Portugal derives from accumulation of two parent toxins: okadaic acid (OA) and its analogue, dinophysistoxin-2 (DTX2). Two distinct toxin profiles are recurrent and have been attributed to the oceanic succession in toxin-producing microalgae: *Dinophysis acuminata* producing OA in spring/summer and *Dinophysis acuta* producing OA + DTX2 in summer/autumn (Vale and Sampayo 2000). The seasonal succession in these two *Dinophysis* species and their respective parent toxins is also commonly recurrent on the coasts of Ireland, Spain, the UK and France (Blanco et al. 2013; Dhanji-Rapkova et al. 2018; Fernández et al. 2019; Salas and Clarke 2019; Belin et al. 2021).

The spatial and temporal distributions of marine biotoxins in shellfish from the mainland Portuguese coast were reviewed recently (Vale 2022). Contamination with DSTs is geographically dependent, with bivalves from estuaries and lagoons from the northwest coast attaining higher toxin levels than those in the southwest and south coasts (Vale et al. 2008). On the south coast, toxin levels rarely attain the European regulatory limit (RL) of 160 µg/Kg edible flesh (EFSA 2009), with a few exceptions, namely in blue mussels (*Mytilus* spp.) and wedge clams (*Donax trunculus*) (Vale et al. 2008; Vale 2022).

This north–south gradient in accumulated DSTs has been related to the north–south gradient in precipitation (Vale 2022). As an outcome of this precipitation gradient, a marked N-S nutrient gradient also exists for the macronutrients nitrogen, phosphate and silicate, with steep declines after the Tagus and Sado rivers (Nogueira et al. 2016; Brito et al. 2020). A quantitative approach was found between continental runoff and DSP for the Ares-Betanzes Ria in Galicia (northwest Spain) and Ria de Aveiro in northwest Portugal (Álvarez-Salgado et al. 2011; Vale 2012). In the Ares-Betanzes Ria, the percentage of days closed to harvest blue mussels in summer can be predicted by the average continental runoff of May (Álvarez-Salgado et al. 2011). In the Aveiro Ria, OA accumulated in mussels in June + July was linearly related to precipitation accumulated between January and May or the continental runoff of May (Vale 2012).

The geographical distribution of DSTs for other North Atlantic coasts, such as Spain (Blanco et al. 2013), the UK (Bresnan et al. 2020) and Ireland (Salas and Clarke 2019), is also coincident with nearby high precipitation quotas (Martinez-Artigas et al. 2020; Mayes and Wheeler 2013; Sweeney 2014, respectively). The closure duration of mussel harvesting bans in the worst-affected Galician production areas can vary between 100 and 250 days (Blanco et al. 2013).

According to the Köppen-Geiger climate classification, the climate in mainland Portugal is warm temperate, with the south presenting a dry hot summer and the north a dry warm summer, with a Csa and Csb designation, respectively (Kottek et al. 2006). In recent years, global change has been decreasing precipitation in Mediterranean Europe, and dry regions are becoming drier (EEA 2017). In Portugal, opposite trends have been observed: an increase in temperature and a decrease in precipitation (IPMA 2023a). Between 2001 and 2022, 9 years presented a positive annual temperature anomaly of ≥ 0.5 °C above the 1971–2000 average. Between 2001 and 2022, 8 years presented an annual precipitation negative anomaly ≥ 200 mm below the 1971–2000 average, while only 1 year presented a positive anomaly deviating > 200 mm above the annual average (annual average 879 mm).

Ria de Aveiro is the most productive lagoon area for bivalve molluscs in the northwest coast (DGRNSSM 2022), but is also a high contamination area for DSTs (Vale et al. 2008). In the recent review of the relationship between precipitation and DSTs between 2001 and 2020 at Ria de Aveiro, only 2 years were included that presented extremely low precipitation (Vale 2022). These years were treated ensemble with the remaining 'low precipitation' years. Recently, in the hydrological year of 2021/2022 (starting in October and ending next September), extremely low precipitation was recorded in mainland Portugal (Fig. 1; IPMA 2023a). Despite the recognized trend for lower toxin levels (Vale 2022), in 2022, there were prolonged harvest bans due to DSTs in bivalves from Ria de Aveiro (IPMA 2023b). The occurrence of DSTs during extremely low precipitation years will be reviewed here in detail. Also, DST's accumulation in the common cockle (*Cerastoderma edule*) will be analysed here in detail instead of blue mussel. Despite the highest DSTs maxima are commonly attained in mussels and their wide use as indicator species along the entire coast, often this is the least relevant commercial species. In Portugal, cockles represented a catch of 3921 tonnes in 2021, from which 3709 tonnes (94.5%) came from Aveiro (DGRNSSM 2022).

The recent increase in the frequency of cockle samples surpassing the regulatory limit (RL) for DSTs was not restricted to 2022, but seemed to be a new trend which started around 2019 (Fig. 2). In face of these unexpected results, other environmental variables were explored here, namely the relation with wildfires. In Portugal, wildfires have increased progressively since the 1970s, but the average area affected annually dropped sharply recently as a consequence of tighter

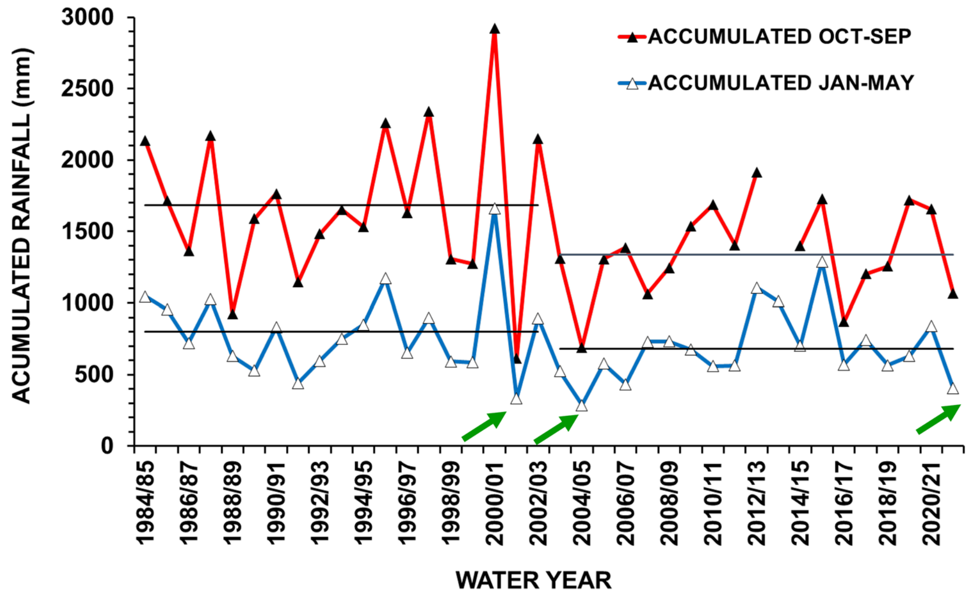


Fig. 1 Precipitation for the water years of 1984/1985 through 2021/22 at Castelo de Burgães station (NW Portugal). Red line depicts annual precipitation for the water year (October–September), and blue line depicts precipitation accumulated only during January–May. Lines represent the respective averages of 1984/1985 through 2002/2003 and 2003/2004 through 2021/2022. Severe drought periods considered here were marked by a green arrow

fire control measures taken after the extensive and tragic wildfires of the 2017 season (Lourenço, 2018).

Wildfires can impact water quality for several months after the fire (Hampton et al. 2022; Raoelison et al. 2023). Ria de Aveiro corresponds to the delta area of Vouga river. The upstream of Vouga river and its subsidiaries are located in abundant forest areas that are also high wildfire risk areas (DGT 2020; ICNF 2020). Although the effects of wildfires in freshwater environments have been studied around the world, its effects in marine environments have been poorly studied so far. Atmospheric nutrient deposition in the ocean might increase after wildfires, in particular during wet days in tropical areas (Sundarambal et al. 2010). Additional nutrients from wildfires, mainly iron, were found to increase phytoplankton production in oligotrophic areas (Liu et al. 2022; Weis et al. 2022).

To improve the knowledge of these opposite trends observed in the cockle DST time series in the twenty-first century, a time series from another filter-feeder was used for comparison: the stock assessment of sardine (*Sardina pilchardus, Walb*.). This planktivorous fish is most relevant for commercial fisheries and its stock assessment records date from the late 1970s (ICES 2022).

Methods

Phytoplankton and Bivalve Sampling

Since 2014, Aveiro Ria is subdivided into four bivalve production areas (RIAV1-4). The areas nearest to the sea connection correspond to production areas RIAV1 and RIAV2

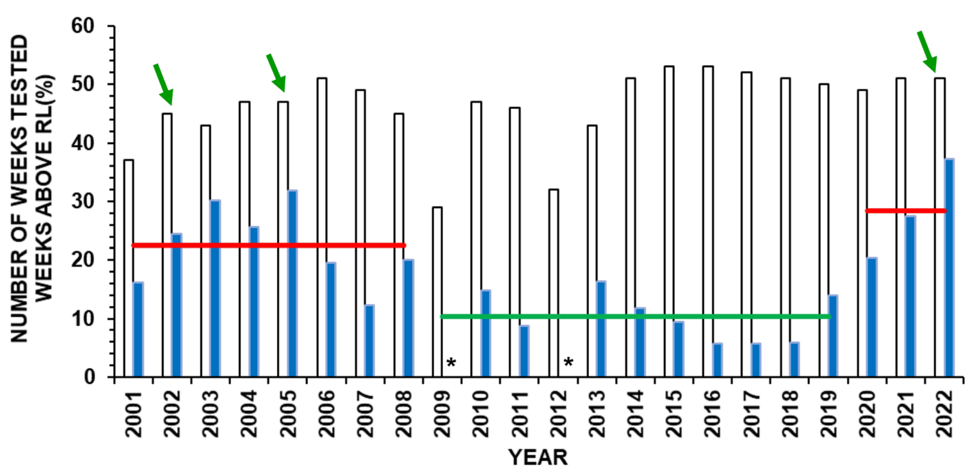


Fig. 2 Number of weeks tested annually for DSTs in Ria de Aveiro's cockle between 2001 and 2022 (white bars) and the percentage of those weeks with samples exceeding the regulatory limit (blue bars). Lines depict interannual average of weeks with cockles above the RL ($N=20$); * excluded from evaluation due to reduced sampling. Dry years marked by green arrows

(Fig. S1). RIAV1 was chosen here because toxin levels in RIAV2 are typically only 85% from RIAV1.

During the 2000's, *C. edule* was sampled once weekly for most of the year, with the major exceptions being the months of December, January and February, when it was sampled only fortnightly or monthly. This translated into an annual sampling rate of circa 42 weeks/year between 2001 and 2011 (Fig. 2). After 2013, with the implementation of the National Bivalve Mollusc Monitoring System (SNMB), sampling was improved and reached an average of 50 weeks/year (Fig. 2).

C. edule sampling was performed from RIAV1 during low tide (IPMA 2023c). Circa 0.5–1.0 kg of bivalves were shucked, and edible parts were homogenized with a common household blender. To uniformise data, when bi-weekly sampling took place during toxic periods, only one weekly sampling was considered. If RIAV1 data was missing, data from RIAV2 was used in replacement.

Water samples for phytoplankton counting's were collected during high tide from RIAV1, preserved with Lugol's iodine and observed by the Utermöhl technique, within 48 h of collection (Karlson et al. 2010). The samples were checked for the presence of harmful species using the IOC list (IOC 2022). Data from RIAV1 presented here for comparison with bivalve toxicity represent the average between the current week's phytoplankton counting and the previous week's counting (IPMA 2023d).

Biotoxin Testing

Although assays for DSTs in Portuguese shellfish by liquid chromatography (HPLC) with toxins coupled to a fluorescent reactive started in 1994 (Vale and Sampayo 1999), more sensitive and selective quantitation started only in 2001 after implementation of liquid chromatography coupled with mass spectrometry detection (Vale 2004). From 2001 until 2012, DST analyses were performed using a single quadrupole instrument (LC–MS) (Vale 2004) and were later upgraded to a triple quadrupole instrument (LC–MS-MS) in 2013, according to the EU-RL-MB protocol (2015).

Briefly, the homogenized tissues were twice extracted with methanol, hydrolysed with sodium hydroxide to recover the parent toxins (freeing these from acyl esters), neutralized with HCl, filtered and injected into the LC system. When using the LC–MS system (2001–2012), toxins were further purified by extraction into dichloromethane and evaporated before injection in the MS (Vale 2004). Toxins were separated in a C18 reversed-phase column with acidified water and acetonitrile and detected in negative ionization mode.

Quantification was performed exclusively with toxin standards acquired from the National Research Council of Canada from 2003 onward. During occasional periods of equipment downtime, quantitative determination of the okadaic acid toxin group was carried out by the commercial colourimetric phosphatase inhibition assay 'Okatest', from Zeulab (Smienk et al. 2013). In these periods, no data is available individually for OA and DTX2.

Environmental Parameters

Ria de Aveiro is the delta of the Vouga river. Monthly precipitation data for the northwest coast was obtained for a nearby meteorological station: Castelo Burgães dam station (code name: 08G/01C; location: 40.853 N, − 8.379 W; 306 m altitude), located in the Vouga's basin (SNIRH 2023). When data were unavailable, precipitation data were retrieved from other nearby stations, namely: Bouçã/Pessegueiro do Vouga (code name 09G/03UG); Campia (09H/01UG) or Ribeiradio (09H/04UG) (SNIRH 2023). The official hydrological year is considered from each October until September of the next civil year, and levels were expressed in mm/m^2. For 2014, data was retrieved from IPMA's Viseu station.

Previously, the best correlation between precipitation and toxins accumulated in blue mussels was not from the full hydrological year (starting in October of the previous civil year), but from the last months of rain before the toxic season which commonly starts in June, and corresponds to the start of the dry summer season (Vale 2012). This includes the months of January through May. For comparison of DSTs with precipitation, the cumulative precipitation between January and May of each year was calculated here also.

Since 2012, the national authority responsible for forest management was reorganized in the 'Instituto da Conservação da Natureza e das Florestas' (ICNF). The total surface of burned areas reported annually is grouped by administrative districts (18 in total). The areas burned in the Aveiro and Viseu districts were retrieved from the progress reports made available annually for the period of 1 January until 15 October, and currently available at: https://www.icnf.pt/florestas/gfr/gfrgestaoinformacao/grfrelatorios/areasardidaseocorrencias. When not available (e.g. years 2000, 2003), areas affected by wildfires were retrieved from ICNF raw data available at: https://www.icnf.pt/florestas/gfr/gfrgestaoinformacao/estatisticas. These districts were chosen due their superposition with the Vouga river basin and upstream forested areas susceptible to soil erosion (Figs. S2 and S3). Figure S2 exemplifies a year when a large percentage of wildfires took place upstream of Vouga's basin.

Sardine and Environmental Parameters

Stock assessment data of *S. pilchardus* is publicly available in the ICES Advice on fishing opportunities, catch, and effort report (ICES 2022). Methods for stock assessment were detailed in Zwolinski et al. (2010). For the comparison of sardine stock with precipitation, the same monitoring stations

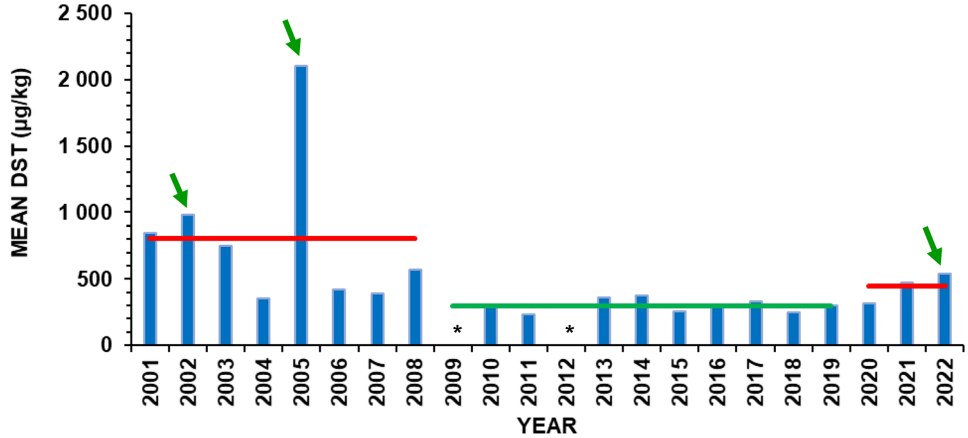


Fig. 3 Average DST concentration in weekly samples above the RL in Ria de Aveiro's cockle. Lines depict interannual averages ($N = 20$). In dry years (green arrows), average annual DST content surpassed the inter-annual average of the respective period. * = not available

were used as described above, as sardine recruitment in zone FAO 9a-Western Iberia is centred off Aveiro. The cumulative precipitation was calculated between March of year N-3 and February of year N, a total of 36 months. This 3-year time span was chosen because yearly surveys start in the months of March/April, and specimens aged older than 3 years represent the minority of the population (ICES 2021).

For the comparison of sardine stock with areas burned by wildfires, the following districts in the NW coast were chosen: Viana do Castelo, covering the Minho and Lima river basins; Aveiro and Viseu, covering the Vouga river basin; Coimbra and Viseu, covering the Mondego river basin (Figs. S2 and S3). For specimens aged 0 years, fires taking place the previous year (N-1) were considered. For specimens aged 1 + years, fires taking place 2 years before (N-2) were considered. These river basins/districts have a large percentage of forest areas in mountain territories, more susceptible to soil erosion after a fire. Douro basin/district was not considered due to its multiple sluices and a large percentage of soil occupation by urbanized areas.

Statistical Analysis

Statistical analyses were carried out in KyPlot, ver. 6.0 (Kyenslab Inc.). The Steel–Dwass Test for pairwise comparisons of one-way layout design was applied. In boxplots, the central line depicts the median, boxes depict the interquartile ranges and the whiskers the min–max ranges. The number of symbols on top of each box-whisker highlights differences at $p < 0.05$, < 0.01 or < 0.001 level, respectively.

Results

DSTs and Drought

Cockle samples surpassing the regulatory limit (RL) for DSTs occurred during 6 to 37% of the weeks tested (Fig. 2). The average toxic content of those samples exceeding the RL was plotted on Fig. 3: it ranged between 230 and 2104 µg DSTs/Kg. Deficient sampling took place along the first quarter of the years 2009 and 2012, and the percentage of toxic samples in those years was omitted from Figs. 2 and 3.

The number of weeks above the RL and their toxin content in the 2001–2022 study period was subdivided into three sub-periods. During 2001–2008, the average inter-annual toxin content of those years was 802 ± 573 µg DSTs/Kg. After 2008, the average toxin content was 300 ± 50 µg DSTs/Kg, and it increased to 445 ± 114 µg DSTs/Kg in the last

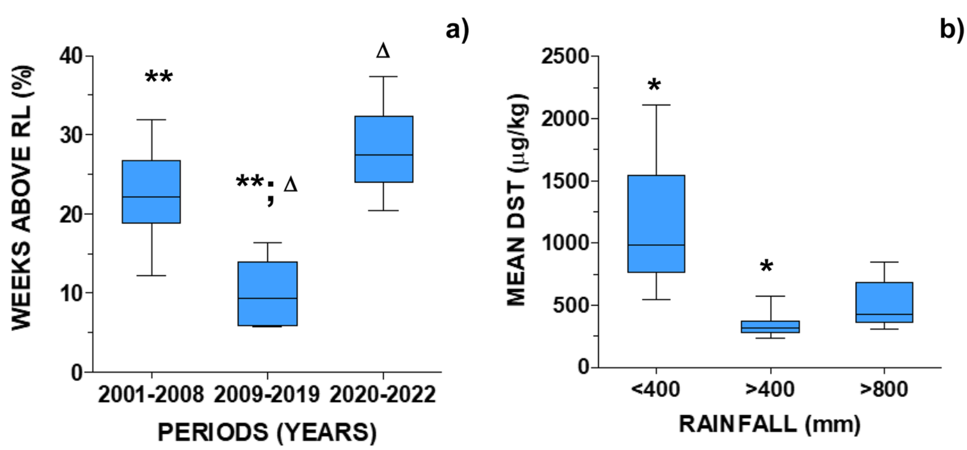
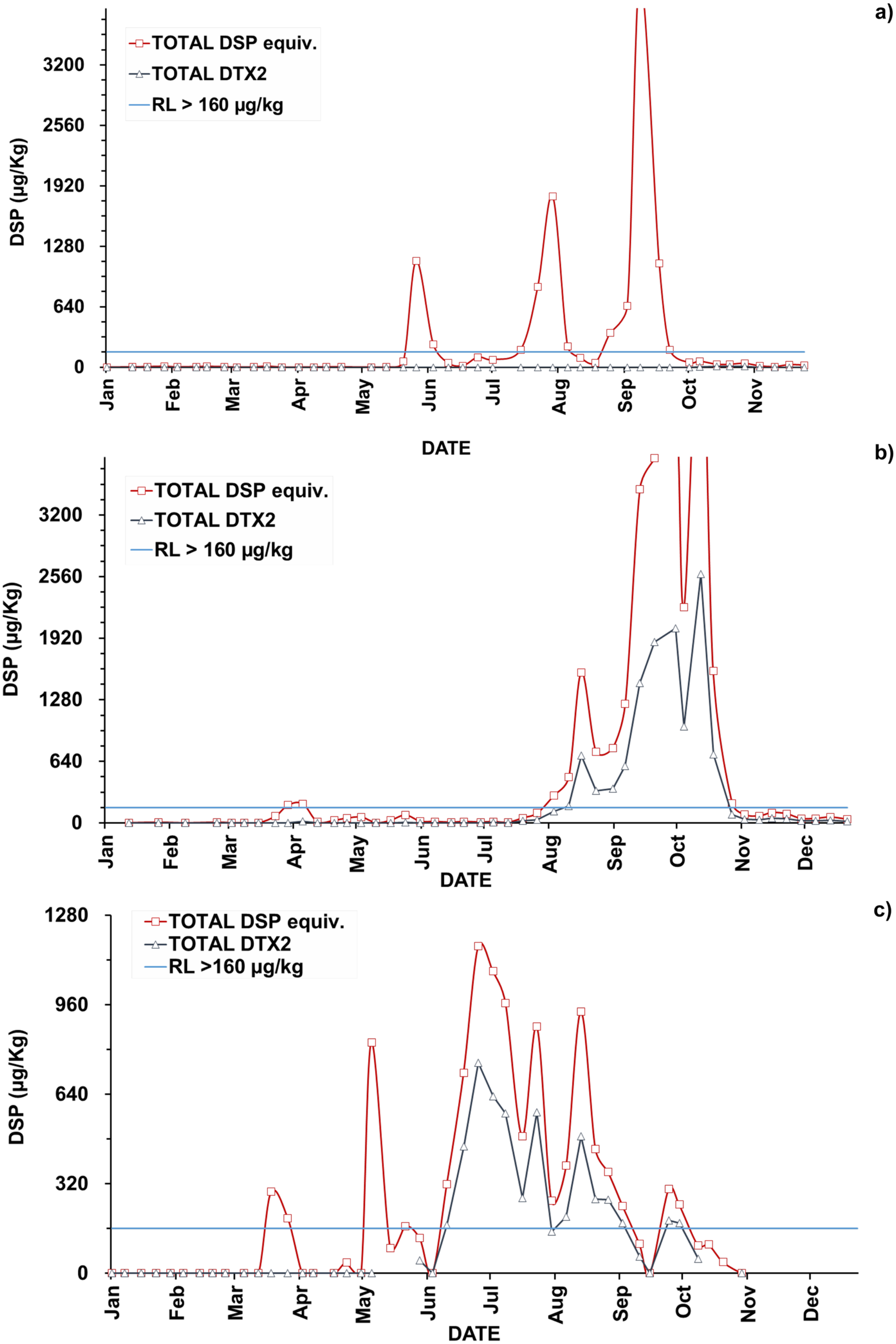


Fig. 4 Relationship between **a** the percentage of weekly samples exceeding the RL for DSTs in Ria de Aveiro's cockle in selected time periods ($N = 20$); **b** the average toxin content with precipitation of the preceding January through May for the years 2001–2022 ($N = 20$). The increasing number of symbols on top of each box-whisker highlights differences at $p < 0.05$ or < 0.01, respectively


a)
TOTAL DSP equiv.
TOTAL DTX2
RL > 160 µg/kg
DSP (µg/Kg)
0
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1280
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Jan
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b)
TOTAL DSP equiv.
TOTAL DTX2
RL > 160 µg/kg
DSP (µg/Kg)
0
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1920
2560
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c)
TOTAL DSP equiv.
TOTAL DTX2
RL >160 µg/kg
DSP (µg/Kg)
0
320
640
960
1280
Jan
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◂**Fig. 5** Evolution in Ria de Aveiro's cockle of total toxicity (in µg AO + DTX2/Kg) and DTX2 concentration (in µg/Kg) alone during the dry years of **a** 2002, **b** 2005 and **c** 2022. The presence of DTX2 in the profile signals the switch from *D. acuminata* to *D. acuta* as the main producing microalga. Blue line depicts the regulatory limit

three years of the 2001–2022 period (Fig. 3). Reduction in average toxicity took place in the second period to just 37% from the first period ($p = 0.0029$). The number of weeks above the RL also reduced from 22.5% during 2001–2008 to 10.3% during 2009–2019 ($p = 0.0057$), increasing again to 28.4% in the 2001–2022 period ($p = 0.0317$; Fig. 4a).

During the 2001–2022 study period, three extremely dry years took place: 2002, 2005 and more recently 2022 (Fig. 1). There was a non-linear relation between rainfall and average toxic content of samples exceeding the RL (Fig. S4). In years with extremely low January–May precipitation, with a precipitation quota below 400 mm, the average DST concentration for cockle samples above the RL was 1209 µg DSTs/Kg ($p = 0.0391$; Fig. 4b). For low precipitation years (400–800 mm), it was 338 µg DSTs/Kg, and for high precipitation years (> 800 mm), it was 518 µg DSTs/Kg (Fig. 4b). The toxicity in cockle samples collected in years with extremely low January–May precipitation was always above the inter-annual average DST concentration for the respective periods, either in the 2001–2008 or the 2020–2022 period, as highlighted in Fig. 3.

Analysing in detail the toxin profiles in these three extremely dry years, most of the samples above the RL were attributed to contamination to a single microalga species throughout the toxic season (Fig. 5), instead of the common succession observed between *D. acuminata* in spring/summer to *D. acuta* in summer/autumn, as can be exemplified recently by the profiles recorded during 2021 (Fig. S5). The presence of DTX2 in the toxin profile signals the blooming of *D. acuta* over *D. acuminata* as the main producing microalga (Figs. 5 and S5).

The average toxin content in samples above the RL in those dry years was quite high: 986 µg DSTs/Kg in 2002, 2104 µg DSTs/Kg in 2005 and 541 µg DSTs/Kg in 2022 (Fig. 3). The number of weeks with toxins above the RL was higher in years with profile dominated by *D. acuta* (31.9% and 37.3%, in 2005 and 2022, respectively; Figs. 2b–c), than in the year dominated by *D. acuminata* (24.4% in 2002; Fig. 2a). The longest consecutive period with samples above the RL was similar in both 2005 and 2022: 13 weeks, while in 2002, it was only 5 weeks. In 2005 and 2022, contamination attributed to *Dinophysis acuminata* also took place during spring (Figs. 5b–c). However, the one or two short-lived episodes observed presented no more than 1–2 weeks above the RL.

Comparison between microalgae occurring in the water column in RIAV1 production area and toxin levels in cockle from the same area was presented in Fig. 6 for the years 2021 and 2022 during the months of May through October. In both years, microalgae ranging between 100 and 500 cells per litre were enough for samples to exceed the regulatory limit for OA-group toxins. In 2022, 100% ($N = 11$) of the samples tested when *Dinophysis* were between 100 and 500 cells/L were above the RL ($p = 0.0131$; Fig. 6b). In the dryer year of 2022, toxin levels increased proportionally with cell counts (Fig. 6b). This was not observed for the rainier year of 2021, when an increase to cell numbers above 500 cells/L did not correspond to an increase in the DST content of cockles (Fig. 6a).

DSTs and Wildfires

With the progressive reduction in precipitation it was expectable the reduction of DST's content in bivalves, which started after 2008, to keep ongoing. The example of severe dry years analysed here seems to be an exception to that expectation. However, by observing the time-series in Fig. 2, it seems the number of weeks with Aveiro cockles above the regulatory started increasing in 2019, and the dry year of 2022 was not an isolated case. Keeping the relevance of nutrients in line of reasoning, the impact resulting from fires was analysed here.

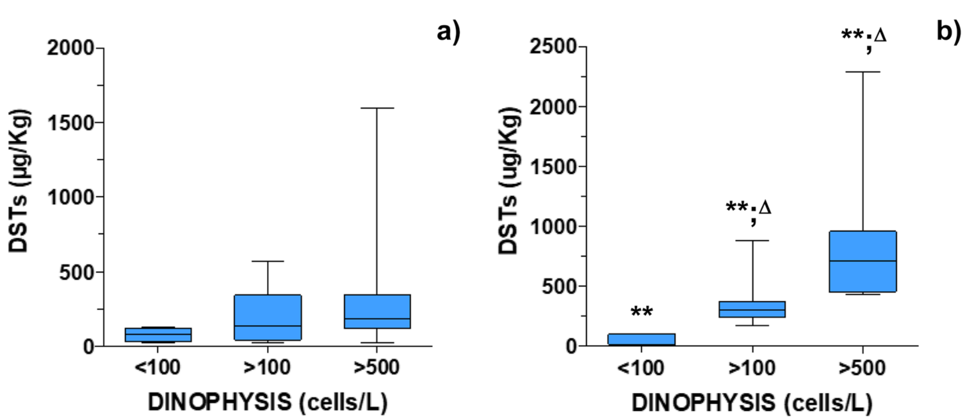

Fig. 6 Relationship between annual average DSTs in Ria de Aveiro's cockles with concentration of toxic *Dinophysis* cells in the water column in **a** 2021 and **b** 2022. The increasing number of symbols on top of each box-whisker highlights differences at $p < 0.05$ or < 0.01, respectively

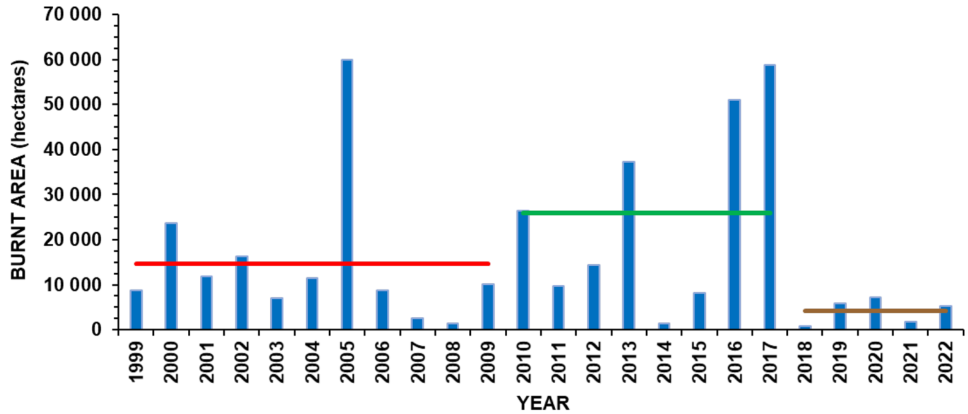

Fig. 7 Total area burned for the districts of Aveiro and Viseu combined. Lines depict interannual averages for selected periods. The average area burned reduced after 2017, due to novel fire control measures in force

Fires have been an increasing summer tragedy in Portugal after the 1970s, but with a sharp reduction after 2017.

The major districts covering the Vouga's river basin are Aveiro and Viseu (Fig. S2). Their soil use and occupation is mostly forests and bushes (> 60%) and agriculture (> 20%) (Fig. S3; DGT 2020). The average interannual area burned in these districts combined in the period 1999–2009 was 14.7 thousand hectares, and increased in the period 2010–2017 to 25.9 thousand Ha (Fig. 7). After 2017, the average interannual area burned reduced to 4.2 thousand Ha, i.e. just 16% from the previous period ($p = 0.0489$; Fig. 8a).

The DSTs accumulated in cockles were related to the area burned the previous year in Aveiro's and Viseu's districts combined. When the area burned exceeded 25,000 hectares, the span of DSTs content was more reduced (interquartile range: 247–377 µg DSTs/Kg) than when the area burned was inferior to that level (interquartile range: 312–661 µg DSTs/Kg) (Fig. 8a). The area burned in the Aveiro district alone was enough to produce the same distinction between toxic levels attained the following year if the area burned was below or above 5000 hectares (data not shown). From another perspective, average toxin levels surpassing three times the regulatory

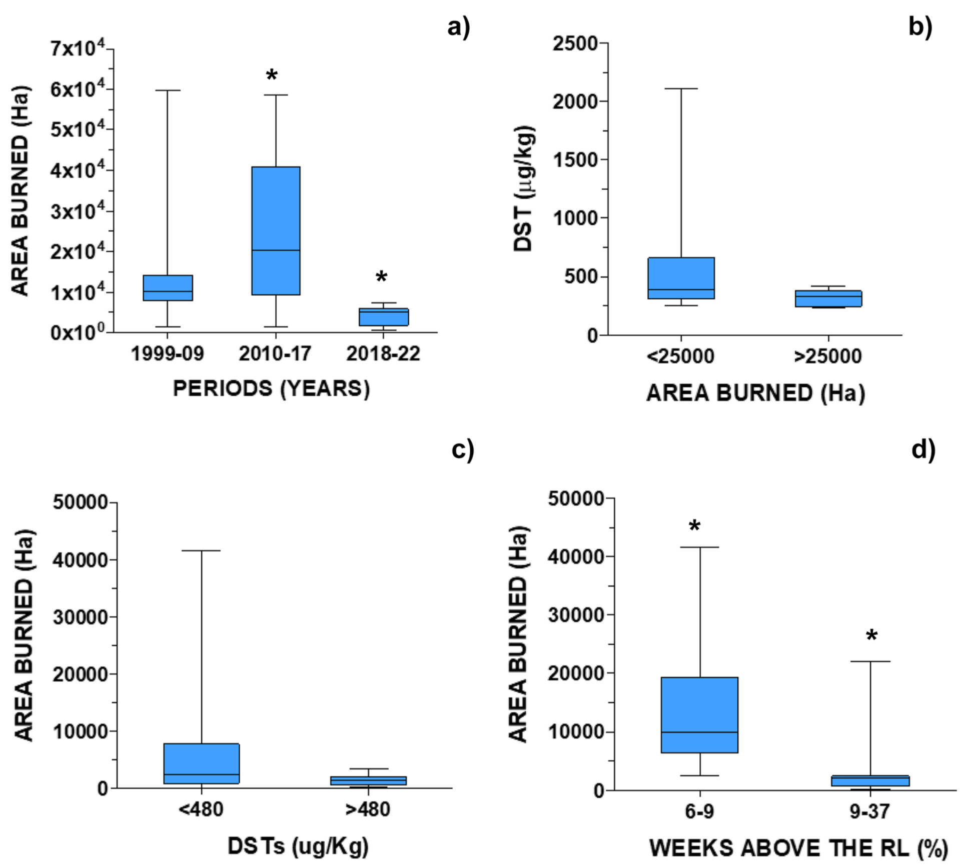

Fig. 8 **a** Distribution of the annual areas burned for the districts of Aveiro and Viseu combined for selected periods; **b** distribution between the annual average DST content in the current year and the area burned in the Aveiro + Viseu districts in the previous year. Distribution of the area burned in the Aveiro district in the year N-1 when **c** average toxin levels exceeded three times the RL; **d** the number of weeks with toxins above the RL exceeded 9%. The symbols on top of each box-whisker highlight differences at $p < 0.05$

limit were restricted to a very low burned area in the Aveiro district in the previous year: interquartile range of 636–2126 Ha (Fig. 8c). The same for the annual number of weeks above the RL higher than 9%: interquartile range of 724–2424 Ha ($p=0.0107$; Fig. 8d).

The Sardine Time Series

The sardine stock time series was analysed next for the same period as the DST time series available: 2001–2022. Sardine juveniles aged 0 years (recruitment) decreased after 2005 (Fig. 9a), while sardines aged 1 year or more (1+) declined after 2006 (Fig. S6). This reduction was more pronounced between 2006–2018 for recruits ($p=0.0051$; Fig. 9a) and 2008–2019 for 1+specimens ($p=0.0008$; Fig. 9b). After these periods, the sardine stock increased in 2019–2022 for recruits ($p=0.0086$; Figs. 9a and S6b) and 2020–2022 for 1+aged individuals ($p=0.0257$; Figs. 9b and S6a).

The sardine stock was compared with the precipitation of the previous 3 years. This time frame was chosen because the majority of the sardine stock (circa 90%) includes specimens aged between 0 and 3 years (ICES 2021). When precipitation increased above 4200 mm, the 0-aged recruits increased 1.7-fold ($p=0.0095$) and the 1+year biomass increased 1.6-fold (Fig. 9c and d, respectively). To arrive at this relation, sardine data between 2011 and 2016 were excluded from data treatment

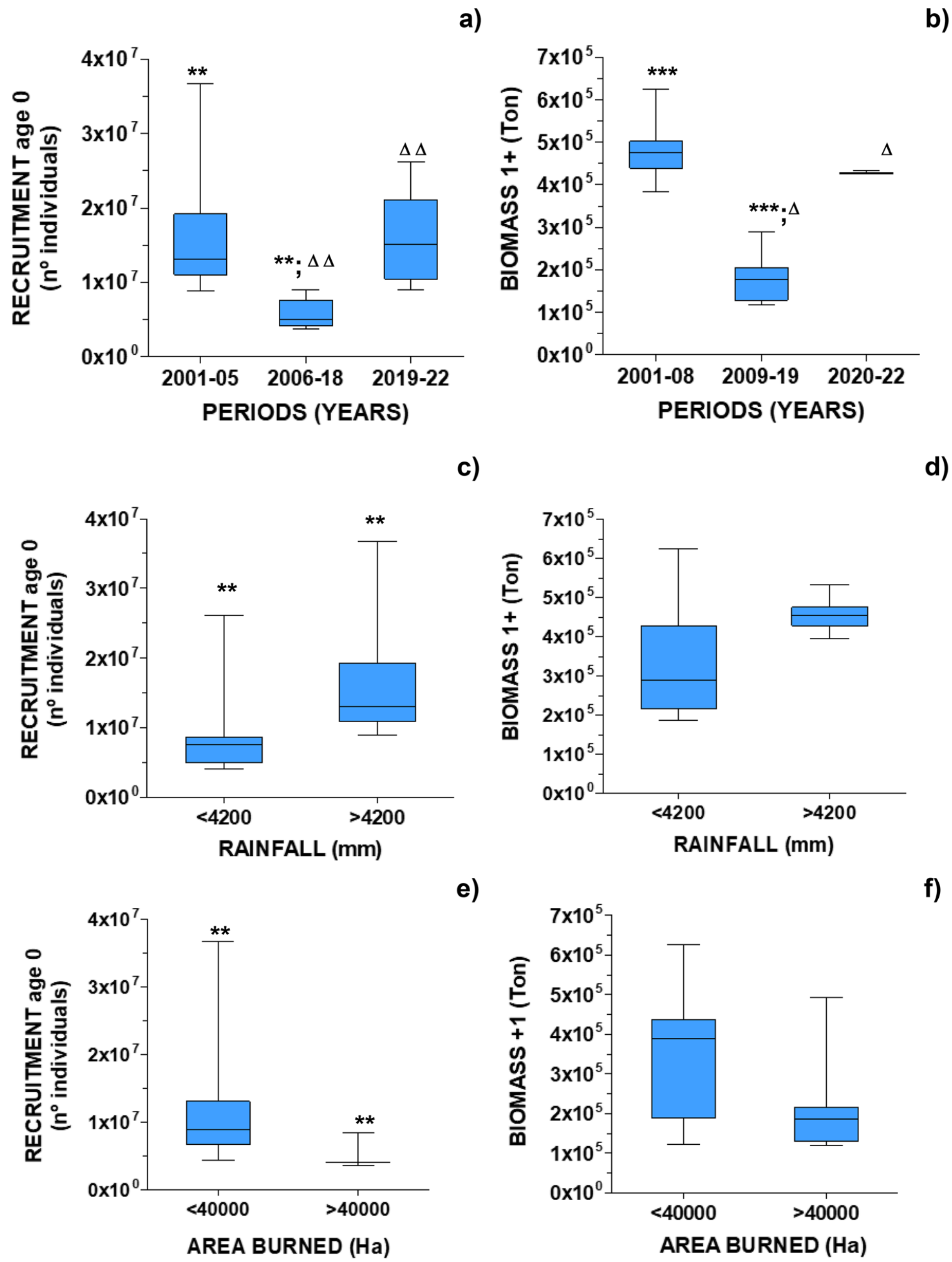

Fig. 9 Sardine **a** recruitment of specimens aged 0 years and **b** biomass of specimens aged + 1 year during selected time periods ($N=22$). **c** and **d** Distribution of recruitment and biomass with accumulated precipitation of the years N, N-1 and N-2. The years 2011–2016 were excluded from data treatment. **e** and **f** Distribution of recruitment and biomass with the area burned in the year N-1 for recruitment and N-2 for biomass. The increasing number of symbols on top of each box-whisker highlights differences at $p<0.05$, <0.01 or <0.001, respectively

(Fig. S7). During these 6 consecutive years, the Iberian sardine stock limit (Blim) was below its biological reference point (BRP) of 196 thousand tonnes (Fig. S6a). Despite precipitation being above 4200 mm, the stock was at its lowest historical levels.

The sardine stock was compared with the areas burned in representative districts covering the FAO 9a Central-North subdivision, covering several selected river basins with upstream forested mountains, such as Arga, Peneda, Montemuro, Caramulo, Açor, Buçaco, Lousã, all high wildfire risk areas (DGT 2020; ICNF 2020; Fig. S8). Both 0-aged recruits and 1 + year specimens decreased when the burned areas exceeded 40,000 hectares (Fig. 9e and f). This reduction in stock was particularly notorious for recruits, with 23% of the low-range concentrations (interquartile range: $4.0–4.1 \times 10^6$ individuals) taking place in years with burned areas above 40,000 hectares ($p = 0.0042$; Fig. 9e).

Discussion

The year 2022 was marked by a severe drought, affecting not only southern Europe, but further north up to the UK (Toreti et al. 2022). A persistent lack of precipitation was followed by a sequence of heatwaves from May onwards, affecting severely river discharges (Toreti et al. 2022). In Portugal, May 2022 was the hottest of the last 92 years (anomaly + 3.47 °C from the 1971–2000 average), with accumulated precipitation at 50% from normal and 97% of the territory in severe drought according to the Palmer drought severity index (IPMA 2022).

A dependence of diarrhetic shellfish toxins with the precipitation patterns has been found previously, both geographically and seasonally (Vale 2012, 2022). Although DST's events take place during the dry season, during low precipitation and river runoff, the severity of toxin accumulation depends on the amount of rain that poured the months before the dry season (up until May), bringing fresh nutrients to coastal ecosystems (Vale 2012, 2022). Orographic precipitation is highest in the northwest coast and reduces towards the south coast, the same occurs with the DSTs maxima observed along the years (Vale et al. 2008; Vale 2022). The progressive decrease in precipitation in Portugal (Fig. 1) has conditioned a reduction in the amount of toxins accumulated in bivalves. The interannual average concentration of DSTs presented a reduction after 2008, either in mussels or in wedge clams from around the Portuguese coast (Fig. 3; Vale 2022).

It was expectable the concentration of toxins remained low, particularly during an extremely dry year, such as 2022. The reverse was observed, with a high number of weekly samples exceeding the regulatory limit in a DST endemic production area, such as Ria de Aveiro. The highest percentage of samples above the RL was observed in the years dominated by *D. acuta* toxins. *D. acuta* blooming is associated with the relaxation of upwelling at the end of summer and northward longshore transport from an epicentre near Aveiro, Portugal, into the Galician Rias (Moita et al. 2006; Escalera et al. 2010; Díaz et al. 2019). The spatial distribution of upwelling pulses along the Portuguese coast is contrasting: northerly winds produce upwelling off the western coast, whereas westerly winds drive it off the southern coast. On the south coast precipitation is low and wind patterns, and upwelling pulses present a seasonality different from the northwest and southwest coasts, with a maximum in May instead of August (Leitão et al. 2019). The distribution of macroalgal species in the Portuguese coast also reflects this latitudinal gradient in environmental conditions (Gaspar et al. 2017).

The populations of *D. acuta* remaining for weeks at low levels are enough to keep bivalves toxic for a prolonged time. This was discovered decades ago by the researchers that confirmed for the first time the connexion between *D. fortii* and DSTs accumulated in Japanese bivalves: 200 cells per litter of this species were enough to render bivalves toxic (Yasumoto et al. 1980). Due to their acute effects targeting the intestinal tract, the okadaites (and also the azaspiracids) are regulated at a much lower threshold than other common marine biotoxins (EFSA 2009). Bivalve contamination has been attributed predominantly to ingestion of intact microalgae cells. But okadaic acid is a very stable toxin in seawater (Blanco et al. 2018). The absorption of dissolved toxins has been demonstrated already for azaspiracid absorption by mussels (Jauffrais et al. 2012), but this aspect has not been addressed for okadaites, and in species other than mussels.

Toxic *Dinophysis* are mixotrophic species and are dependent on certain ciliates (*Mesodinium rubrum*), which are needed to acquire plastids from certain cryptophyte (*Teleaulax* sp.) (Park et al. 2006; Reguera et al. 2014). The dependence on coastal fertilization processes discussed here in relation with precipitation are not related to *Dinophysis* alone but are promoting simultaneously the growth of its food chain preys. The average annual precipitation measured in a meteorological station near the Aveiro lagoon has decreased from 1684 mm in the period 1984/1985 through 2002/2003 to 1382 mm in the period 2003/2004 through 2021/2022, representing an 18% loss (Fig. 1).

During the second half of the twentieth century, there were progressive alterations in land usage, and today, maritime pine forest (*Pinus pinaster*) and the non-indigenous fire-prone eucalyptus (*Eucalyptus globulus*) represent 56% of forest species (Lourenço, 2018; DGT 2020). The total area burned per decade in Portugal was circa 1000 thousand hectares in the 1980s and in the 1990s. In the 2000s, it was 1844 thousand Ha/decade (Ferreira-Leite et al. 2016). The major forest species usually burned are non-native eucalypts, native wild pines and several species of native oaks (*Quercus* spp.) (AFN 2009). A large fraction (60–70%) of the burned areas registered annually originated from large forest fires (LFF), i.e. with burned areas exceeding 100 Ha. The average area burned by LFF was circa 500 Ha (Ferreira-Leite et al. 2016). Wildfires differ from prescribed fires in the temperature achieved, having more negative than positive impacts on forest soils (Johnson et al. 2004; Swindle et al. 2021; Agbeshie et al. 2022). Large forest fires easily result in major gaseous losses of nitrogen due to its low volatilization temperature (200 °C), to be followed by potassium, phosphorus, and sulphur (> 760, > 774, and > 800 °C, respectively), while calcium and magnesium are less easily volatilized (Neary et al. 1999).

The ash layer is very prone to postfire mobilization and export by water, particularly in steep slopes. The forested areas near the Vouga and Mondego basins are located in mountainous territories with steep slopes (Fig. S8), as well as in all other northern river basins, such as Minho, Lima, Cavado and Ave (Almamater 2023). Raoelison et al. (2023) compared the pre-and post-fire freshwater quality data in 44 studies. It revealed that wildfire could increase the concentration of many pollutants by two orders of magnitude. After the wildfire, nutrients, suspended solids and polycyclic aromatic hydrocarbon (PAH) concentrations increased within a year and heavy metals within 1–2 years. Analysis of water courses affected by distinct fires in several regions of central Portugal showed an increase in the total mineralization of water, N and P, the cations Ca, Na, Mg and Mn, and of polycyclic aromatic hydrocarbons (PAHs) (Costa et al. 2014; Ferreira et al. 2016; Mansilha et al. 2019; Basso et al. 2020).

Human fatalities and economical losses have been associated with wildfires sparsely along the years (Bento-Gonçalves 2021). In 2017, a dramatic overturn took place: the national area burned was 428% of the 2007–2016 average, and there were 116 human fatalities and 320 injured in just two large fires (ICNF 2017; Bento-Gonçalves 2021). After 2017, the Portuguese government addressed the issue of agricultural and forestry management with novel and stricter measures. These measures resulted in the effective reduction in the number of human casualties, the number of fires and the total area burned (XXII Governo 2021).

Detailed time series for nutrients along the Portuguese coast are non-existent to test the hypothesis of how leached nutrients arrive at coastal waters during low and high fire years and, in particular, if these are mostly leached during the cold winter season and lost by downwelling, before being available to spring/summer blooms. The same lack of detailed data exists for toxic substances, such as PAHs or metals. Despite the Vouga basin includes the Viseu district, the areas burned in the Aveiro district alone correlated well with DST data. This same approach of using mainly areas burned in districts from the littoral correlated successfully with sardine recruitment at the NW coast. There were areas burned in districts further away from the littoral, as these districts also contain mountainous territories with bushes and forest areas (see Fig. S2). However, the further downstream the further the effects from a wildfire will reduce (Reale et al. 2015), and thus only fires occurring in districts closer to the littoral were chosen for this analysis.

Opposite results were found for the north Australian coast, where additional nutrients from wildfires were found to increase phytoplankton production (Liu et al. 2022). Wildfire impacts were found to be more relevant than warming-induced secondary climate effects, such as the increase in tropical cyclones (Menkes et al. 2016). However, this coastline is oligotrophic and not comparable to the Portuguese coastline. The inorganic macronutrients N and P in the Portuguese NW coast are around 10 μM and 0.5 μM, respectively (Nogueira et al. 2016). On the north Australian coast, these are circa 0.5 μM and 0.25 μM, respectively (Butler et al. 2020).

Megafires were hypothesized to supply iron, often a limiting nutrient, to the ocean (Ito 2011). This was confirmed after the aerosols emitted by the 2019–2020 large Australian wildfires were followed by a widespread phytoplankton bloom in the iron-limited Pacific sector of the Southern Ocean (Weis et al. 2022). This degree of iron limitation found in the Southern Ocean does not apply to the East North Atlantic coastlines (Toulza et al. 2012). Other sources of iron are desert dust and volcanic ash, which might trigger anomalous plankton blooms (Mahowald et al. 2005; Hamme et al. 2010). The Portuguese territories receive regularly dust from the Saharan desert (Mahowald et al. 2005).

Unlike in tropical areas (Sundarambal et al. 2010), nutrients volatilized by combustion are not easily deposited into nearby coastal waters, as wildfires take place during the Portuguese dry warm summer (DGRF 2005). These might enter the global circulation, due to prevailing north winds during the summer (Leitão et al. 2019).

The recruitment of *S. pilchardus* shares striking similarities with the N-S distribution of DSTs (Vale et al. 2008; Zwolinski et al. 2010; Vale 2022). The core areas of juvenile distribution are the Northern Portuguese shelf (centred off Aveiro), the coastal region in the vicinity of the Tagus estuary and the eastern Gulf of Cádiz. The Portuguese northwest coast (i.e. the FAO area 9a Central-North subdivision) is considered to be the main recruitment area, with a secondary area of importance in the Gulf of Cádiz, 9a South-Spain subdivision (ICES 2021).

This small pelagic species is part of the ancient traditional fisheries at the Iberian coast and the most relevant halieutic resource in Portugal, not only for the amounts captured but also for the economic and social implications of its exploitation. The low position in the marine food web, together with a short lifespan and a reproductive strategy of producing large quantities of pelagic eggs over an extended spawning season, render small pelagic fish greatly dependent on the environment (Bakun 1996). Similar to bivalves, sardines are predominantly filter-feeders and efficiently utilize microplankton prey, with small zooplankton and chain-forming diatoms dominating their stomach contents (Garrido et al. 2007).

As both the cockle DSTs and the sardine stock reduced with wildfires, could this be attributable to an improvement in coastal water quality after the reduction of the area burnt annually? Polycyclic aromatic hydrocarbons, although greatly enhanced by fires, are ubiquitous in nature and can be degraded by several bacteria, fungi and algae from terrestrial to marine ecosystems (Ghosal et al. 2016). Impacts from these noxious compounds were observed in the year following the fire for both DSTs and sardine recruits. For the older sardine population, the impacts were observed 2 years after the fire, as older individuals originate from the growth of recruits. The wash away of ashes during the first year post-fire and their natural degradation can allow an increase in plankton growth already in the second year post-fire, as seen for DSTs in 2019 and sardine recruits also in 2019.

In the last couple of decades, the recruitment and the biomass of the Atlantic Iberian sardine suffered a major decline after 2005 and 2008, respectively (Figs. 9a–b; Fig. S6), even despite the tightening of stock control measures in place (ICES 2022). The sardine spawning season is during October–April, its onset coinciding with the main ash discharge period with the first rains. The year 2005 was particularly characterized by both low precipitation and a record number of the area burned (Figs. 1 and 7). Sardine recruits hit their historical minima the following year (Fig. S6b). After this minima, the recruit's populations presented minor recoveries in the years 2007–2009, and again in 2015–2016 (Fig. S6b), immediately following the years with a low burned area of 2006–2008 and 2014–2015, respectively (Fig. 7).

Another important driver for sardine recruitment is water temperature during the spawning season (October–April) around 15 °C or lower (Ferreira et al. 2023). Temperatures were continuously high between 2007 and 2016 (except for 2009), coinciding with a continuous low recruitment. Although temperatures reduced after 2016, there was no recovery in recruits in 2017 and 2018, due to the strong fire season of 2016 and 2017, respectively. Only in 2019, there was a pronounced increase in recruitment, in part due to the sharp reduction in burned areas in the districts of Viana, Aveiro, Viseu and Coimbra combined, from an interannual average of 54.2 thousand Ha (2010–2017) to 7.5 thousand Ha (2018–2022).

Other coincidences between DSTs and sardine biomass might have gone unnoticed in the past. The nearby Galician mussel rope culture expanded during the 1950s and 1960s and reached its maturity in the 1970s (Labarta and Fernández-Reiriz 2019). The occurrence of marine biotoxins presented new challenges to the mussel industry, in an epoch when no preventive biotoxin monitoring was in place. One major contamination episode attributed to PSP took place in 1976, with around 120 people intoxicated (FAO 2004). Another major episode attributed to DSP occurred in 1981, with around 5000 persons intoxicated in Spain alone, followed by other episodes in 1982–1984 (FAO 2004). A particularly long contamination episode was recorded in 1993 (FAO 2004). All the relevant dates related to major DSP episodes coincided with years of high biomass of the Atlantic Iberian sardine stock, such as 1981 or 1993 (Fig. S6a; ICES 2022).

Besides DSTs, paralytic shellfish toxins (PSTs) and amnesic shellfish toxins (ASTs) originate closures of shellfish beds in western Iberia. It is difficult to evaluate the impact of wildfires on the plankton producing these toxins, as these time series present a large interannual variability. PSTs do not occur during the maxima of solar cycle activity (the 11-year sunspot cycle), while ASTs were slightly stimulated by high geomagnetic activity following solar maxima (Vale 2022). Nevertheless, wildfires might help in understanding some of the gaps previously observed.

During the minima of solar cycle 23/24, blue mussels from Ria de Aveiro were above the regulatory limit for PSTs during 4, 0, 5, 7 and 3 consecutive months, respectively, in the years from 2005 to 2009 (Vale 2022). The absence of relevant blooms during 2006 is difficult to explain, but might relate to the intense wildfire season during 2005, which affected the entire north of Portugal, including all coastal districts from Leiria until Viana do Castelo (DGRF 2005; see also Fig. 7 and S2). During the minima of solar cycle 24/25, Aveiro mussels' (northwest coast) were above the RL for 3 months in 2016, but only trace levels of PSTs were found during 2017 and 2018. On the southwest coast, PSTs were above the RL for 4 months during 2018 in mussels from Lisboa (Vale 2022). The appearance of blooms during 2018 on the SW coast but not on the NW coast (where these are commoner; Vale et al. 2008; Vale 2022) might relate to the high burned areas during 2016 and 2017, respectively (Fig. 7; ICNF 2017). The rivers of the SW coast — Tagus and Sado — are less influenced by wildfires, because in the south of Portugal there are less forest and mountain territories where firefighting is more challenging (Fig. S2).

Preventing large wildfires is an increasingly difficult task with the emergence of summers attaining unprecedented fire-prone weather conditions, due to the reduction in precipitation and the increase in air temperature in the Mediterranean area (Carnicer et al. 2022). The year 2022 was the warmest in the period 1991–2020, not only for Portugal, but in many Mediterranean countries, Ireland and UK (IPMA 2023a).

Despite the change in air temperature in the Mediterranean area, the western Iberian ocean has not been increasing in recent decades (WMO 2022). In a review of freshwater cyanobacterial blooming across a wide range of latitudinal habitats in the Americas, the authors found nutrients and not temperature were the key drivers for plankton biomass (Bonilla et al. 2023). The decrease in DSTs associated with the reduction in precipitation seems to have been observed so far only for Portugal. For the remaining Atlantic Europe, high interannual variability has been observed in the number of harmful events per country for individual shellfish toxin syndromes, but evidence for systematic trends is generally inconclusive or absent (Bresnan et al. 2021a, b). For the USA, in the past 30 years, there has been no trend for PSTs, DSTs or NSTs events, while AST's events have increased, mainly on the West Coast (Anderson et al. 2021).

This decrease in a specific toxin biomarker is the opposite of that reported for some southern European coastlines, where the appearance of several 'foreign' HAB species and toxins has been related to water warming. In the Macaronesia region, toxins derived from *Gambierdiscus*, the ciguatoxins, causative agents of ciguatera fish poisoning (CFP), were recognized for the first time in the Canary Archipelago after human outbreaks of fish poisoning in 2004 and in the Madeira Archipelago in 2007 (Pérez-Arellano et al. 2005; Gouveia et al. 2010). In the Mediterranean region, palytoxin-like (PTX) aerosol derived from blooming of *Ostreopsis* caused mild respiratory illness and skin irritation in coastal inhabitants of Italy in 2005 and later throughout the Mediterranean (Gallitelli et al. 2005; Tester et al. 2020).

Conclusions

The presence of PAHs is quite universal in the environment, both from natural and anthropogenic sources. As major accidental oil spills are uncommon on the Portuguese coast and anthropogenic sources are more or less constant year after year, the large intra-annual variability of wildfires seemed to detach PAH's (and other toxicants, such as toxic metals) levels from their current background level, impacting water quality down to marine coastal areas, affecting negatively in the first place the phytoplanktonic species at the basis of the food chain.

These findings were opposite to phytoplankton stimulation by wildfires observed in some oligotrophic waters of the planet. In oligotrophic waters, wildfires can relieve nutrient limitations, overcoming the negative impacts from PAHs in ashes. However, the Portuguese coastline does not present the same degree of nutrient limitations as the examples above.

Two marine filter feeders reflected with high similarity the plankton responses to environmental parameters. In fish, its recruitment or biomass were used as a non-specific planktonic biomarker. In bivalves, DSTs were used as a species-specific planktonic biomarker. While the sardine time series reflects the abundance of a large diversity of plankton species, the cockle time series reflects only a couple of species (only the toxic *Dinophysis*, as other non-toxic *Dinophysis* co-exist).

The consecutive negative anomaly in average precipitation that started in mainland Portugal after 2003 (IPMA 2023a) took circa 3–5 years to be reflected in several time series of marine indicators as reviewed here. Nevertheless, during severe drought, the populations of *D. acuta* were capable of thriving actively during the upwelling season, originating prolonged closures of bivalve production areas. Despite the average reduction in precipitation, the concentrations of DSTs have been increasing recently on the Portuguese northwest coast, and not only during the severe drought years. The combined analysis of the cockle and the sardine time series reinforces that recent marine environmental conditions are indeed favouring plankton growth. These are good news for the fisheries sector, but bad news for the shellfisheries sector due to the prolonged harvest bans imposed by marine biotoxins in bivalves.

The changes in the environmental variables precipitation and wildfire were more relevant for sardine recruits than for older individuals. Older individuals are capable of a more diverse diet, other than exclusively phytoplankton (i.e. zooplankton and ichthyoplankton).

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Data Availability The datasets analysed during the current study are available in the following databases: https://www.ipma.pt/pt/bivalves/; https://www.ipma.pt/pt/publicacoes/boletins.jsp?cmbDep=cli&cmbTema=pcl&idDep=cli&idTema=pcl&curAno=-1; https://doi.org/10.17895/ices.advice.19772455; https://www.icnf.pt/florestas/gfr/gfrgestaoinformacao/grfrelatorios/areasardidaseocorrencias; SNIRH > Dados Sintetizados (apambiente.pt).

Declarations

Conflict of Interest The author declares no competing interests.

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