Forty years living with *Dinophysis*: myths and realities

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Abstract

Forty years ago, *Dinophysis forti* was identified as the source of DSP and *Dinophysis* species targeted as potential toxins producers worldwide. Discoveries of their cryptophyte-like pigments, mixotrophic feeding on ciliate prey, and uncertainties about their life cycle made this genus an attractive topic of dinoflagellate biology, phylogeny and ecology. Within the dinoflagellate order, *Dinophysis* species constitute a unique group, the plastidic specialist non constitutive mixotrophs (pSNCM). Only the ciliate *Mesodinium rubrum* fed on *Teleaulax*/*Plagioselmis*/*Geminigera* (TPG) clade cryptophytes has been used to grow *Dinophysis*, but alternative prey are being explored. Strains of each *Dinophysis* species exhibit site-specific functional traits in response to environmental change. Progress in modeling the population dynamics of these selective mixotrophs is hindered by the lack of adapted sampling to *Dinophysis* and their potential prey with a common appropriate spatio-temporal resolution. Are *Dinophysis* low density slow-growing dinoflagellates with no sexual life, unrelated to water discolorations and never the dominant component of the microplankton community? Are all members of the *D. acuminata* complex the same species? Can we have an early warning of *Dinophysis* bloom development? Are DSP events increasing in frequency, intensity and geographic distribution? These and emerging issues are discussed here on the light of past mistakes and recent progress in knowledge.

Keywords: Dinophysis, morphology, life cycle, mixotrophy, ecology, bloom dynamics

Antecedents

Gastrointestinal outbreaks unrelated to bacteria affected 100's of people during shellfish festivals in 1976-77 in Tohoku, Japan and led Prof. Yasumoto *et al.* (1985) to: i) describe a new "Diarrhetic Shellfish Poisoning" (DSP) syndrome; ii) design a mouse bioassay (MBA) to quantify unknown lipophilic toxins (LT) iii) identify *Dinophysis fortii* as the causative organism; iv) elucidate the structure of two groups of toxins: the okadaic acid, OA and its analogs, the dinophysistoxins DTXs (DSP toxins) and the pectenotoxins (PTX), and v) relate *D. fortii* populations $\leq$ 200 cells L^{-1} to the two groups of toxins. Other LT producers may co-occur with *Dinophysis*, but analytical techniques were developed to analyze picked-cells (Lee *et al*., 1989). Nowadays, liquid chromatography coupled with mass spectrometry (LC-MS) (Quilliam *et al*., 1996) is used to determine LT in picked-cells, net tows, shellfish extracts, and even dissolved toxins in seawater with "solid phase adsorption toxin trackers" (SPATT, MacKenzie *et al*., 2004).

Toxic *Dinophysis* distribution and impacts

Dinophysis species, ubiquitous for most of the year often go unreported in monitoring programmes because: i) they occur below detection levels (40–100 cells L^{-1}); ii) they form low biomass HABs (10^3 cells L^{-1}); iii) symptoms of bacterial gastroenteritis-like DSP go unreported; iv) DSP monitoring is lacking in many countries; and v) routine DSP testing is often interrupted (for economic reasons) when closures are already enforced when more dangerous toxins (e.g. PSP) are present. Social awareness of the risks posed by *Dinophysis* is prompted when mass intoxications of shellfish consumers occur. Otherwise, DSP monitoring is required for aquaculture exports (Reguera *et al*., 2014). Under-reporting is obvious when comparing historic distribution of DSP events in Europe, where DSP monitoring started in the late 1980's, with those in the U.S.A. (Fig. 1), with tests prompted after accidental detection of a *D. ovum* bloom in Gulf of Mexico (Campbell *et al*., 2010), and intoxication of consumers in NW U.S.A. (Pacific) coasts (Trainer *et al*., 2013).

To date, the presence of OA-related toxins and/or PTXs has been proved by HPLC or LC-MS analysis in picked cells of ten "species" of *Dinophysis* and two of *Phalacroma* as well as toxin production *de novo* in cultures of all *Dinophysis* species except *D. miles*. Field observations support the view that the heterotroph *P. rotundatum* is just a vector of toxins from its ciliate prey (e.g. *Tiarina fusus*) when the latter feeds upon co-occuring *Dinophysis*. There are no reports about cultures of *P. mitra*, which unlike *Dinophysis,* has kleptoplastids of haptophycean origin (Koike *et al*., 2005) Information per species tends to be proportional to their worldwide impact. The *D. acuminata* complex (*D. acuminata*, *D. ovum*, *D. sacculus*), together with (from north to south) *D. norvegica*, *D. acuta*, *D. fortii* and *D. caudata,* are the species associated with all DSP events in the world. Information from rare *Dinophysis* or *Phalacroma* species as well as from more strains of the common species is desirable.

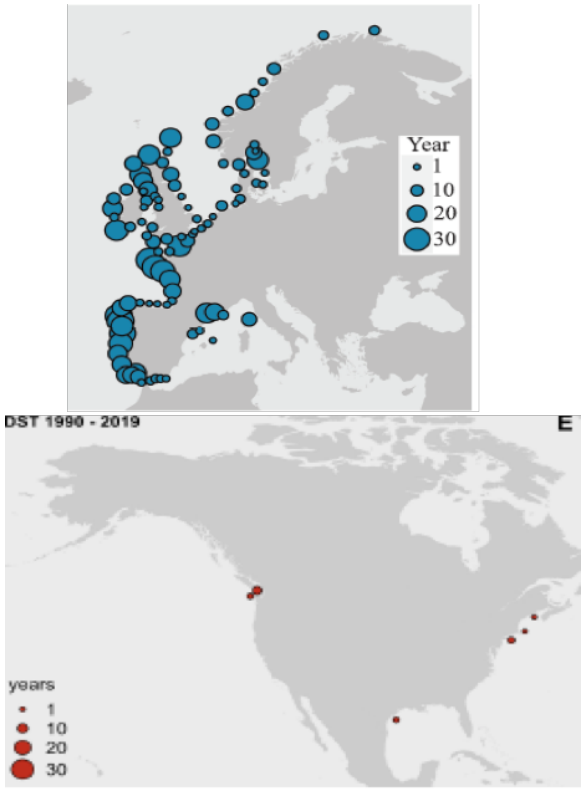

Fig. 1. Reported DSP events in Europe and U.S.A. from 1990 to 2019. IOC HAEDAT database. (in Anderson *et al.,* 2021; Bresnan *et al.,* 2021).

The toxic potential of DSP outbreaks and their impacts are extremely variable and affected by: i) the strain specific toxin profile (OAs : PTXs ratio) and content (cell quota) of the causative organism; ii) interactions of *Dinophysis* blooms and local hydrodynamics, which explain the coexistence of hot spots (retention areas) and "DSP free" areas nearby; iii) the uptake rate and enzymatic transformations by the local commercial species (e.g. mussels accumulate 10 times more toxins than oysters exposed to the same bloom) and iv) the enforced toxin regulations (e.g. PTXs were regulated in Europe until recently; Blanco, 2018).

Historic data need validation in the light of present understanding of the organisms, their toxins and the strength and weaknesses of past and present analytical procedures. A relevant proportion of *Dinophysis* toxins may be released in the water as "dissolved toxins" (in the filtrate through 0.22 µm) and may be adsorbed by organic aggregates and prevail (detected in net tow samples) weeks after *Dinophysis* cells are no longer observed (Pizarro *et al*., 2008).

Dinophysis traits I. Size and shape, cell cycle and life cycle

High intraspecific variability affecting size and shape of *Dinophysis* cells is affected by: i) the cell-cycle, with paired cells united by their dorsal margin and recent division (desmoschisis mode) with asymmetric partition of sulcal lists and spines; ii) a polymorphic life cycle, with small gamete-like cells formed from dimorphic paired cells produced by depauperating division; dimorphic mating gametes connected by their ventral margins with a mating tube: engulfment and gamete fusion, and iii) Mixotrophic feeding (myzocytosis mode of phagocytosis) that results in distorted or deformed specimens full of digestive vacuoles. These forms, first seen during exceptional blooms of *Dinophysis* in New Zealand, Portugal and NW Spain in 1989 are best observed during intensive cell cycle sampling at sea and in cultures (Reguera *et al*., 2012).

A *Dinophysis* life cycle model was proposed in 2001 inspired by field observations and laboratory incubations, and misinterpretations clarified later (Escalera and Reguera, 2008). For example, the small-large (dimorphic) paired cells united by their ventral margin are not undergoing conjugation, i.e., transfer of

nuclear material from a donor to a receptor through a conjugation tube. Instead, a tube from the large cell, similar to the towing peduncle used by heterotrophic protists with their prey, guides the small cell to the cingulum to be engulfed. Some observers interpreted this as an act of cannibalism. Nuclear fusion takes place following engulfment and cellular fusion; planozygotes with two longitudinal flagella can divide without going through a resting cyst stage. It is not known if cells grouped in tetrads result from division of planozygotes or normal vegetative cells. All these forms, plus the first remark about the ciliate *Mesodinium* entangled in mucilage in the bottom of *Dinophysis* culture vessels are well illustrated by Nagai *et al.* (2008). Putative resting cysts turned out to be pellicle cysts of *Fragilidium* after eating *Dinophysis*. A harpoon-like tube has been described for *Dinophysis* to catch prey in addition to a feeding peduncle. How many peduncles do *Dinophysis* species have? After fifteen years with cultures, these and other *Dinophysis* life cycle-related questions have not been resolved.

Controversial "Dinophysis acuminata *complex"*

This complex refers to a group of morphologically similar species of *Dinophysis* difficult to separate when their blooms, with small and intermediate morphotypes co-occur. Sequencing the rDNA-ITS and 5.8S rRNA genes of single cell isolates with a new technique showed a 99% similarity between *D. acuminata* and *D. sacculus*. The apparent success of using the mitochondrial *cox*1 gene to discriminate between *D. acuminata* and *D. ovum* (Raho *et al*., 2008) turned out to be a mistake in the alignment of a *D. acuminata* strain (AM931587). Recently the impossibility to separate *D. acuminata* from *D. ovum* with the available sequences (SSU rDNA, ITS1, ITS2 and *cox*1) was confirmed (Park *et al*., 2019). These sequences are the best to group toxigenic species of *Dinophysis* in several clades, such as the *D. acuminata* complex and the *caudata* group (*D. caudata*, *D. tripos*, *D. miles*).

Field samples with dominance of *D. acuminata*, *D. ovum* or *D. sacculus* are well distinguished by monitoring experts in W Europe on the basis of their size and contours. The first two produce only OA, but *D. sacculus* additionally has DTX1 and PTX2 (Riobó *et al*., 2013). Their distribution shows latitudinal and seasonal differences in Atlantic and Mediterranean coastal waters in Europe as well as in those from Eastern U.S.A. and the Gulf of Mexico (Wolny *et al*., 2020; Sechet *et al*., 2021). It would be unfortunate to group them in routine cell counts as "*D. acuminata* complex", because their life forms are revealing adaptations to environmental conditions, so valuable ecological information would be missed. New portions of the genome or even epigenetic differences should be explored with genomic and transcriptomic tools. In the meantime, we should keep them separated or name them adding the letter *f (form*), followed by the epithet (*acuminata, ovum, sacculus*) that best fits their shape. This is currently done to distinguish morphotypes of *D. caudata* into forms: *abbreviata*, *allieri* and *pedunculata* (Reguera *et al*., 2007).

Dinophysis traits II. Nutritional sources

From Schnepf and Elbrachter's (1988) discovery of the orange autofluorescence of cryptophyte-like plastids in *Dinophysis*

until the first culture was established (Park *et al*., 2006) progress occurred in fits and starts over a period of 20 years. Advances in molecular biology were essential for this progress to be made. Final success had to await the cultivation of the ciliate *Mesodinium rubrum* (*Dinophysis* prey) fed cryptophytes. These cryptophytes were the source of kleptoplastids for the phototrophic ciliate *Mesodinium* (Gustafson *et al*., 2000). *Dinophysis* was found to be an obligate mixotroph requiring light and nutrients for photosynthesis using kleptoplastids taken from its ciliate prey (e.g. *Mesodinium rubrum*) which in turn keeps most of its cryptophyte prey (of the TPG clade IV *Teleaulax/ Plagioselmis/ Geminigera)* as an incomplete endosymbiont (Kim *et al*., 2012) In the recent reclassification of planktonic mixotrophic protists (Mitra *et al*., 2016), *Dinophysis* and *Mesodinium* are non-constitutive mixotrophs (NCM), i.e., they lack permanent plastids and need to steal them from their prey. *Dinophysis* eats its prey by piercing it with a feeding peduncle and sucking its content (*myzocytosis*); the eaten prey is dispensed into digestive vacuoles but the plastids are kept as kleptoplastids. Thus, *Dinophysis* is a *plastidic Specialist* NCM (pSNCM), whereas *M. rubrum,* that keeps most of its prey (plastid, nucleus and nuclemorphs) as an incomplete endosymbiont is an *endosymbiont Specialist* NCM (eSNCM). This *Dinophysis-Mesodinium-Teleaulax*) three-link food chain is the only one tested so far for *Dinophysis* cultures (Hernández-Urcera *et al*., 2018). *D. acuminata* prey was estimated to contribute 50% of its daily C intake, but grazing data should be critically reassessed. If the main way *Dinophysis* has to catch *Mesodinium* is with a mucus trap (Nagai *et al*., 2020) most *Mesodinium* losses would be as uneaten specimens disaggregated in the trap.

In addition to live prey, *Dinophysis* needs light and dissolved nutrients to perform photosynthesis. Uptake rates of N^{15} labeled compounds during blooms of several HAB species in a coastal upwelling system were measured (Seeyave *et al*., 2009). *Dinophysis* showed a clear preference for regenerated N sources (ammonium and urea). In contrast with ”high uptake velocity strategists” *Pseudo-nitzschia australis* and *Alexandrium catenella*, *D. acuminata,* a ”high affinity strategist” succeeded in low N environments that were limiting for the other species. These trends have been confirmed in laboratory incubations of *D. acuminata* which yielded very low uptake rates with nitrate, but rapid assimilation of ammonia and urea (Hattenrath-Lehmann and Gobler, 2015). Similar results were obtained with *D. acuta*, a species 3 times the volume of *D. acuminata* that showed uptake rates 2-3 times higher than those shown by *D. acuminata*. Unlike in autotrophic species, starvation did not boost uptake rates, and indeed they were higher in well fed cultures (García-Portela *et al*., 2020). Information on NO_3 reductase membrane transporters from 30 dinoflagellate species (Reference Transcriptome database) showed a paucity of these transporters in *D. acuminata* compared to the amount found in the heterotroph *Noctiluca*. New experimental transcriptomic and isotopic data revealed the central role of NH_4^+ (Hattenrath-Lehmann *et al*., 2021).

Some authors related increased bloom intensity of *Dinophysis* with eutrophication (Hattenrath-Lehmann *et al.*, 2015). After all the above considerations, it seems very

unlikely that *Dinophysis* could outcompete *Pseudo-nitzschia* and other high velocity strategists in nitrogen-richr environments.

Why is it so difficult to cultivate Dinophysis*? Mesodinium rubrum,* grown in the laboratory with *Teleaulax amphioxeia, Plagioselmis prolonga, T. gracilis* and *T. minuta,* was considered to be genus-specific about its selected prey (Peltomaa and Johnson, 2017). *P. prolonga*, only 1 bp different from *T. amphioxeia*, was found to be a haploid stage in the life cycle of the latter (Altenburger *et al*., 2020), so we should change to "TG" the old TPG clade IV. But *Mesodinium* growth rate and yield varied with different prey and optimal results were obtained only if strains of ciliate and its cryptophyte prey had been isolated from the same location (Hernández-Urcera *et al*., 2019). Likewise, *Dinophysis* growth is not the same with different strains of *Mesodinium*.

Matching of *Dinophysis* and *Mesodinium* field populations (spatial-temporal coincidence of two mixotrophs) with different light and nutrient requirements (or a high predator: prey ratio of good quality prey in the laboratory) is the key factor constraining *Dinophysis* growth. This apparent strain-level selectivity of the cryptophyte prey by *Mesodinium* and strain-level preferences of *Dinophysis* for its ciliate prey would explain the fact that only a few laboratories have been able to grow *Mesodinium* from their own locality. In short, *Dinophysis* fed foreign strains of *Mesodinium* and cryptophyte is growing in suboptimal conditions.

Is Mesodinium rubrum *the only possible prey for* Dinophysis *species?*
Mesodinium rubrum is the only species tested in laboratory cultures, but a coincidence of plastidic sequences in local *Dinophysis* species with those from *M. rubrum* and *M. major* have been found in the Galician Rias (Rial *et al*., 2015). The latter was the dominant *Mesodinium* species in samples from Argentina and Chile and has recently been established in culture (Drumm *et al*., 2021). Furthermore, predominance of identical crytophyte plastid sequences belonging to *Rhodomonas/ Rhinomonas/ Storeatula* (clade V) were found in several species of *Dinophysis*, in ciliates of the genus *Strombidium* and in co-occurring heliozoans in oceanic waters off Los Lagos, Chile (Díaz *et al*., 2020).

How do Mesodinium *and* Dinophysis *recognize their prey?*
One possibility would be a sympatric coevolution of predator and prey similar to that suggested between planktonic parasites and hosts, i.e. a predator genotype-by-prey genotype interaction.

Dinophysis **Traits III. Specific growth rate μ (d^{-1})**

Dinophysis blooms ($\geq 10^3$ cells L^{-1}) are low biomass HABs; shellfish with DSP toxins above regulatory level (R.L.) have been associated with population densities as low as 200 cells L^{-1} (Yasumoto *et al*., 1985). Nevertheless, there are notable exceptions of blooms reaching cell numbers 1-2 orders of magnitude above the average annual maximum. In these cases, the key question is if the high numbers are either the result of high intrinsic growth rates, or hydrodynamic forcing or a combination of both (physical-biological interactions). *In situ* division rates of *Dinophysis* populations, regardless

of their cell density, can be estimated with model equations based on the mitotic index approach (Carpenter and Chang, 1988) with intensive (36-48 h) monitoring of two successive "terminal events" or stages formed within a discrete time window. The simplified "maximum frequency approach" of McDuff and Chisholm (1982) may be applied (if the circadian rhythms of the local species are known) for comparative purposes. For example, to see vertical distribution of the specific division rate (μ) at depth (μ_z) to identify the μ_{max} depth; to follow up division rates throughout the seasonal growth period. We have learned from these cell cycle studies that: i) *Dinophysis* species have phased-cell division, i.e., all mitotic cells divide within a narrow time window and the onset of light cues the division process; ii) *Dinophysis* are not slow-dividing dinoflagellates. Maximal rates of 0.7 d^{-1} (one doubling d^{-1}) have been observed in different species and regions; iii) In optimal conditions, *Dinophysis* may form quasi monospecific thin layers (10^5-10^7 cells L^{-1}) and; iv) High apparent net growth may be observed in the absence of cell division due to transport (Pizarro *et al*., 2008).

Dinophysis **bloom dynamics and behaviour**

Dinophysis populations follow the common sequence of phases: initiation, exponential growth, maintenance and dissipation or transport (Steidinger, 1975). Bloom dynamics is shaped by the species adaptations and their interaction with the local hydrodynamics affected by geomorphology and climate. Still there are commonalities observed in the same species blooming in different systems with a set of characteristics common to holoplanktonic *Dinophysis*: growth initiation relies on pelagic seeds; development triggered by layering and matching of the populations with that of their ciliate prey. Different morphospecies of the ubiquitous *D. acuminata* complex and the pair *D. acuminata/ D. acuta*, with contrasting traits, are the most studied species and their bloom patterns as model organisms will be summarized here.

Dinophysis acuminata: Unlike *D. acuta*, this species has a very long growth season, from spring to autumn and tolerates a wide range of temperature (T), and the light intensity and turbulence conditions found near the surface (Díaz *et al*., 2016, 2019), i.e., is more stress tolerant and has a wider niche breath than *D. acuta* (García-Portela *et al*., 2018, 2019; Baldrich *et al*., 2021). A conceptual model, based on field observations, was proposed by Velo-Suárez *et al*. (2014) to explain the persistence of *D. acuminata* in an upwelling system (Galician Rías Bajas), leading to up to 9 months of harvesting closures in retention areas: In the model, onset of stratification in spring cues for aggregation of scattered overwintering cells in the pycnocline region. Upwelling pulses enhance stratification and cell entrainment. The growth season starts. Vertical position of the cells is adapted to move offshore with upwelling, inshore with downwelling in a sort of 2D wind-driven conveyor belt. The population boundaries are fixed by the upwelling front. Similar 2D structures are found in other systems where the boundaries are estuarine or tidal fronts and river plumes, and blooms are coupled with the local wind regime.

Dinophysis acuta: has a short growth season, from late summer to early autumn and is more neritic than *D. acuminata*. Blooms of *D. acuta* in SW Ireland (Raine *et al*., 2018) and Galicia (Escalera *et al*., 2010) initiated in mid-shelf

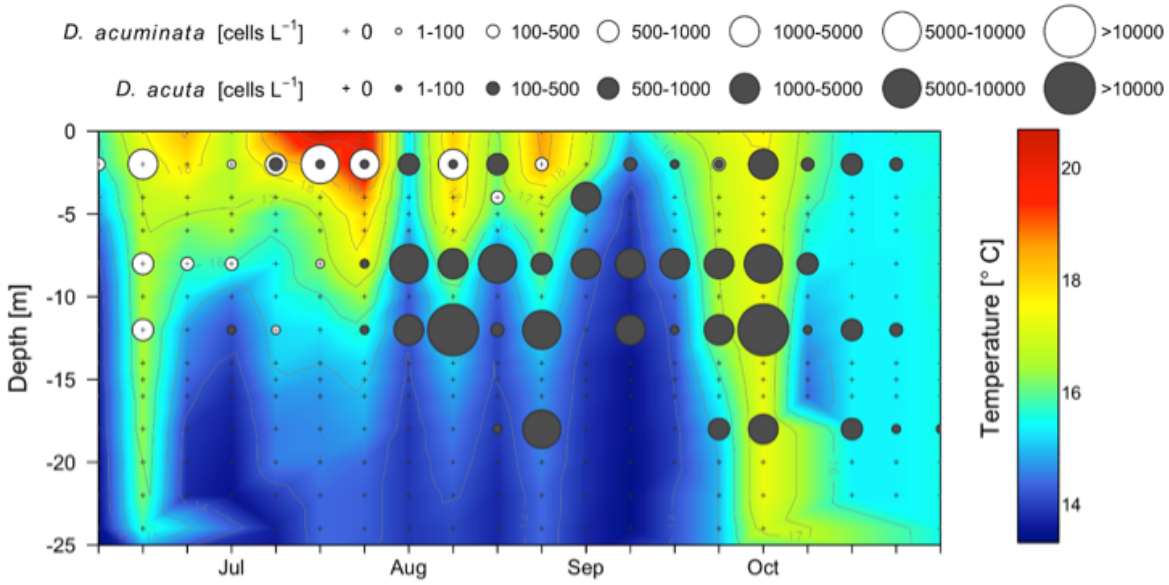

Fig. 2. Vertical segregation of *D. acuminata* and *D. acuta* cell maxima when their blooms co-occur. Ria de Pontevedra, summer-autumn 1990 (modified from Reguera *et al*., 1993).

waters, share common physical environment requirements: persistent thermal stratification (~2 month) and water column stability met in late summer. Cell maxima and thin layers are formed in the pycnocline region above the *chl*-maximum. A 3D dimension due to long-shore transport (coastal jet in Ireland and Portuguese coastal current in Galicia) adds complexity to bloom forecasting. Wind reversal and transport of shelf population to the aquaculture sites explains high net growth of *D. acuta* in the Scottish lochs (Whyte *et al*., 2014) and Galician Rías (Escalera *et al*., 2010).

Aggregation, patchy distribution and thin layering, commonly observed in *Dinophysis* populations are key behavioural traits which interact with physical stratification, and allow for population development (quorum sensing), feeding upon ciliate populations, mating encounters, allelopathic interactions, etc. Otherwise these low density holoplanktonic mixotrophs would never reach density thresholds required for these processes.

Some hints for *Dinophysis* blooms forecasting

A good starting approach is to analyse conditions associated with exceptional blooms in terms of intensity, distribution and phenological changes, as well as years of absence.

Increased stratification enhanced by climatic anomalies, and unusual wind patterns transporting blooms to exceptional distances are the most frequently reported physical conditions. Simplistic interpretations of bloom densities and T correlations often ignore that sea surface temperature (SST) is

sometimes a proxy for thermal stratification, that temperature salinity (TS) values are signature of water masses and that TS and light intensity co-vary with depth.

Exceptional *D. acuta* blooms have been associated with positive SST anomalies in the Galician Rias, and produced some early conclusions, that it was a stenotherm requiring higher T for optimal growth. But *D. acuta* is a temperate-cold-temperate species. Samplings with adequate vertical resolution showed that when *D. acuminata* and *D. acuta* co-occur, cell maxima of *D. acuta* are in colder deeper water with lower light intensity and higher stability (Fig. 2). Appropriate water column structure must be accompanied by good inoculum for initiation in shelf waters without prey limitation. Strong upwelling dispersal after bloom decay was the hypothesis to explain absence of the population the next year despite growth favourable environmental conditions (Moita *et al*., 2016). Record densities of *D. acuta* (67 x 10^4 cells L^{-1}) in a thin layer were found in Puyuhuapi (~41° S), a Chilean fjord with high water residence time, and good connection with oceanic waters, in summer 2017-18. Mesoscale climate anomalies led to extra precipitation and enhanced salinity gradients in the spring preceding summer drought with high positive SST (+2 °C) anomaly (Díaz *et al*., 2021).

Exceptional densities of *D. acuminata*, two orders of magnitude denser and over two months earlier than the 20 year mean occurred in early spring 2012 in two distant places in what appeared to be a mesoscale event affecting Western Iberia (Galician Rias Bajas) and the Bay of Biscay (Arcachon, France). Anomalous winter wind patterns and positive anomalies (+2 °C) promoted: in Galicia, upwelling dominance in winter and in the SE Bay of Biscay, reversal of the Gironde River plume bringing increased haline stratification to Arcachon Bay. In both cases, the result was an early diatom spring bloom and stratification preceding *Dinophysis* development (5.3 × 10^4 cells L^{-1} in integrated 0-5 m samples in Galicia) (Díaz *et al*., 2013). Conversely, years of late and poor *D. acuminata* bloom development in the time series (e.g. in 1996) coincided with late onset of the upwelling season. Detection levels of overwintering cells in the rías (after milder autumn conditions) and prediction of the forthcoming upwelling season seem the best tools to predict early spring blooms of *D. acuminata* in NW Iberia.

Another scenario for cell-maxima, in the case of terminal populations of *Dinophysis*, is wind-stress relaxation and downwelling. That was the case with shelf populations in two upwelling systems: *D. fortii* (~10^5 cells L^{-1}; Pitcher and Calder, 2006) in the Benguela, South Africa, and *D. acuminata* (~10^7 cells L^{-1}; Imarpe, 2017) in the Humboldt, Perú, upwelling system. Longshore transport in buoyant river plumes is another mechanism to advect dense mature populations of *Dinophysis*. The most intense and spread *D.* cf *ovum* bloom in S Brazil (58 x 10^4 cells L^{-1}) occurred in autumn-winter 2016. It was associated with exceptionally intense southwesterly winds, which induced an inflow of cold (-4°C anomaly) low salinity buoyant plume from La Plata River up to Paraná, 1100 km north (Proença *et al*., 2018). The same species had beaten records on the Uruguayan coast, northern margin of La Plata estuary during a hot and dry spring 2015 associated with the warm (> 20°C) saltier (>31

psu) waters of the Brazil Current (Méndez *et al*., 2018). These conditions probably strengthened the front between the estuarine plume and the Brazil Current, moved the front closer to the coast, and favoured spring phytoplankton growth.

With the exception of a few EU or nationally funded cruises where *Dinophysis* species were the target organism, most information on *Dinophysis* dynamics is based on monitoring data collected within the aquaculture sites. Research on *Dinophysis* species suffers from a lack of information from shelf stations where important processes on initiation and dispersal occur. Recurrent questions about increasing trends in US and other countries cannot be answered until an appropriate multidecadal time series of observations is gathered

Recommendations

- Long term (seasonal and annual) studies on dinoflagellate–ciliate prey relations: rDNA and plastid sequencing of *Dinophysis* and co-occurring endosymbiont-containing ciliates.
- Mesoscale surveys on shelf waters. Data to validate ongoing operational models, in particular transport models; observations in thin layers.
- To monitor *Dinophysis* and potential prey with the same spatio-temporal resolution, explore the poorly studied: neuston and benthic boundary layers.
- Increasing or decreasing trends need decadal time series of data collected with identical methods.

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