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EDITED BY
Panagiota Katikou,
Veterinary Research Institute Greek Agricultural Organization Demeter, Greece

REVIEWED BY
Živana Ninčević Gladan,
Institute of Oceanography and Fisheries (IZOR), Croatia

*CORRESPONDENCE
Beatriz Reguera
beatriz.reguera@ieo.csic.es

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# *Dinophysis*, a highly specialized mixoplanktonic protist

Beatriz Reguera[1]*, María García-Portela[1], Esther Velasco-Senovilla[1], Pilar Rial[1], Laura Escalera[1], Patricio A. Díaz[2] and Francisco Rodríguez[1]

[1]Centro Nacional Instituto Español de Oceanografía (IEO-CSIC), Centro Oceanográfico de Vigo, Vigo, Spain, [2]Centro i~mar & CeBiB, Universidad de Los Lagos, Puerto Montt, Chile

Several *Dinophysis* species produce lipophilic toxins (diarrhetic shellfish poisoning, DSP and pectenotoxins PTX) which are transferred through the food web. Even at low cell densities (< $10^3$ cell $L^{-1}$), they can cause human illness and shellfish harvesting bans; toxins released into the water may kill early life stages of marine organisms. *Dinophysis* species are mixotrophs: they combine phototrophy (by means of kleptoplastids stolen from their prey) with highly selective phagotrophy on the ciliate *Mesodinium*, also a mixotroph which requires cryptophyte prey of the *Teleaulax/Geminigera* clade. Life cycle strategies, biological interactions and plastid acquisition and functioning in *Dinophysis* species make them exemplars of resilient holoplanktonic mixoplankters and of ongoing speciation and plastidial evolution. Nevertheless, 17 years after the first successful culture was established, the difficulties in isolating and establishing cultures with local ciliate prey, the lack of robust molecular markers for species discrimination, and the patchy distribution of low-density populations in thin layers, hinder physiological experiments to obtain biological measurements of their populations and slow down potential advances with next-generation technologies. The Omic's age in *Dinophysis* research has only just started, but increased efforts need to be invested in systematic studies of plastidic diversity and culture establishment of ciliate and cryptophyte co-occurring with *Dinophysis* in the same planktonic assemblages.

KEYWORDS

***Dinophysis*, toxic HABs, morphological variability, life history stages, plastidic specialist, mixoplankton**

## 1 Introduction

Dinoflagellate species of *Dinophysis* Ehrenberg are widely distributed in tropical, temperate and boreal waters, and in coastal, neritic and oceanic environments (Steidinger and Tangen, 1996). Until the late 1970s, interest in these species was focused on their taxonomy and their marked morphological variability (Jorgensen,

1923; Abé, 1967). Most taxonomists merged *Dinophysis* and *Phalacroma* based on plate tabulation criteria (Hallegraeff and Lucas, 1988) until genetic studies by Jensen and Daugbjerg (2009) supported the recognition of two separate genera. *Dinophysis* attracted special attention in the late 1970's when severe gastroenteritis outbreaks in Tohoku, Japan, unrelated to bacteria, led to the description of a new seafood-borne syndrome, Diarrhetic Shellfish Poisoning (DSP), related to phytoplankton. *Dinophysis fortii* was identified as the source organism of the human illness after consumption of shellfish contaminated with its toxins (Yasumoto et al., 1978; Yasumoto et al., 1980). Ever since, *Dinophysis* species (main cause of shellfish harvesting closures in Europe) have been a target in monitoring programs aimed to safeguard public health and shellfish exploitations (Reguera et al., 2014). *Dinophysis* attracted further scrutiny when Schnepf and Elbrächter (1988) pointed to the orange autofluorescence and ultrastructure of its cryptophyte-like plastids. By the 1990s it was widely documented that initiation of *Dinophysis* blooms was closely related to the onset of thermohaline stratification (Maestrini, 1998) and that observations of ciliate remains in *Dinophysis* digestive vacuoles indicated their mixotrophic nature (Jacobson and Andersen, 1994).

Progress in understanding the dynamics of subsurface populations of holoplanktonic dinoflagellates (*Dinophysis*, *Karenia*) in the early 2000's was achieved along with increased abilities to observe distributions and processes at different scales (Gentien et al., 2005; Berdalet et al., 2017). The first successful culture of *Dinophysis* (Park et al., 2006) opened possibilities for physiological studies, but maintaining the *Dinophysis-Mesodinium-Teleaulax* food chain in the laboratory has proven to be a cumbersome task undertaken by only a few groups. Measurements required to develop mechanistic models coupled to operational oceanography forecasts are far from being achieved. Targeted sampling of low biomass harmful algal blooms (HABs) of *Dinophysis* and laboratory experiments have revealed species-specific responses to fine scale differences in water column structure and resources (Figure 1J) (Díaz et al., 2013; Díaz et al., 2016; Baldrich et al., 2021; Baldich et al., 2023) (Table 1). But we have barely glimpsed ephemeral life history and feeding processes which occur in very narrow spatio-temporal windows. Here some morphological, life history and feeding behavior peculiarities of *Dinophysis* are analyzed, in particular those which have been controversial or are still unresolved.

## 2 Intraspecific morphological variability in *Dinophysis*

Classification of *Dinophysis* is largely based on the outline of their large hypothecal plates. Size and shape of these plates and their ornaments are affected by vegetative growth, sexual (life cycle) and feeding processes (Figure 1).

### 2.1 Cell cycle related morphological variability in *Dinophysis*

The volume of microalgal vegetative cells between two consecutive generations (a cell cycle) ranges from a minimum in recently divided specimens to a maximum in mitotic cells before division. Dinophysoid thecate dinoflagellates divide by desmoschisis, Von Stosch (1973) which involves sharing the mother theca between the two daughter cells and formation of a new complimentary moiety (Taylor, 1973). Upon cellular fission, wall elements (wings, spines) which were not symmetrically distributed allow recognition and enumeration of mitotic and recently divided cells. These morphological marks together with double nucleated cells, of cell-cycle terminal events can be recognized with light and epifluorescence microscopy, and quantified to estimate *in situ* division rates (Carpenter et al., 1995; Garcés et al., 1997; Gisselson et al., 1999; Reguera et al., 2003). There is only one study involving quantification of cell-cycle phases using synchronized cultures of *D. acuminata* (Jia et al., 2019), and no attempts reported of application of flow cytometry (with cell-sorting) to study cell-cycle response to environmental stressors.

### 2.2 Life cycle related morphological variability in *Dinophysis*

Modelling bloom dynamics of target species is constrained by poor abilities to recognize different life-cycle stages and identify the environmental and intrinsic factors triggering transitions between stages (Garcés et al., 2002). Dinoflagellates are protists with complex, heteromorphic life cycles with transitions between stages with differing ploidy and morphology (Figueroa et al., 2018). Differentiation of vegetative cells into gametes may proceed through a "depauperating division" (Von Stosch, 1973), each mother cell producing two daughter cells with lower biomass, poor pigmentation and distinct swimming behavior (Figures 1E–G). *Dinophysis* species are holoplanktonic with a (presumably haplontic) polymorphic life cycle which includes: small gamete-like cells formed by depauperating division; dimorphic mating gamete pairs connected by their ventral margin with a mating tube, engulfment and gamete fusion to produce a planozygote, which differs from the vegetative cell in having two (instead of one) trailing flagella. A single serendipitous field observation showed that well phased sexual division before sunset (Figure 1 in Mackenzie, 1992) and vegetative division (after sunrise) are triggered by different cues. Current developments in electron microscopy and molecular tools have unveiled frequent cases of small cells misclassified as different taxa. An extreme case is illustrated with field and culture specimens of *Dinophysis caudata* and *D. tripos* (Reguera et al., 2007; Rodríguez et al., 2012). But information is biased by our current focus on a few toxigenic species of *Dinophysis*

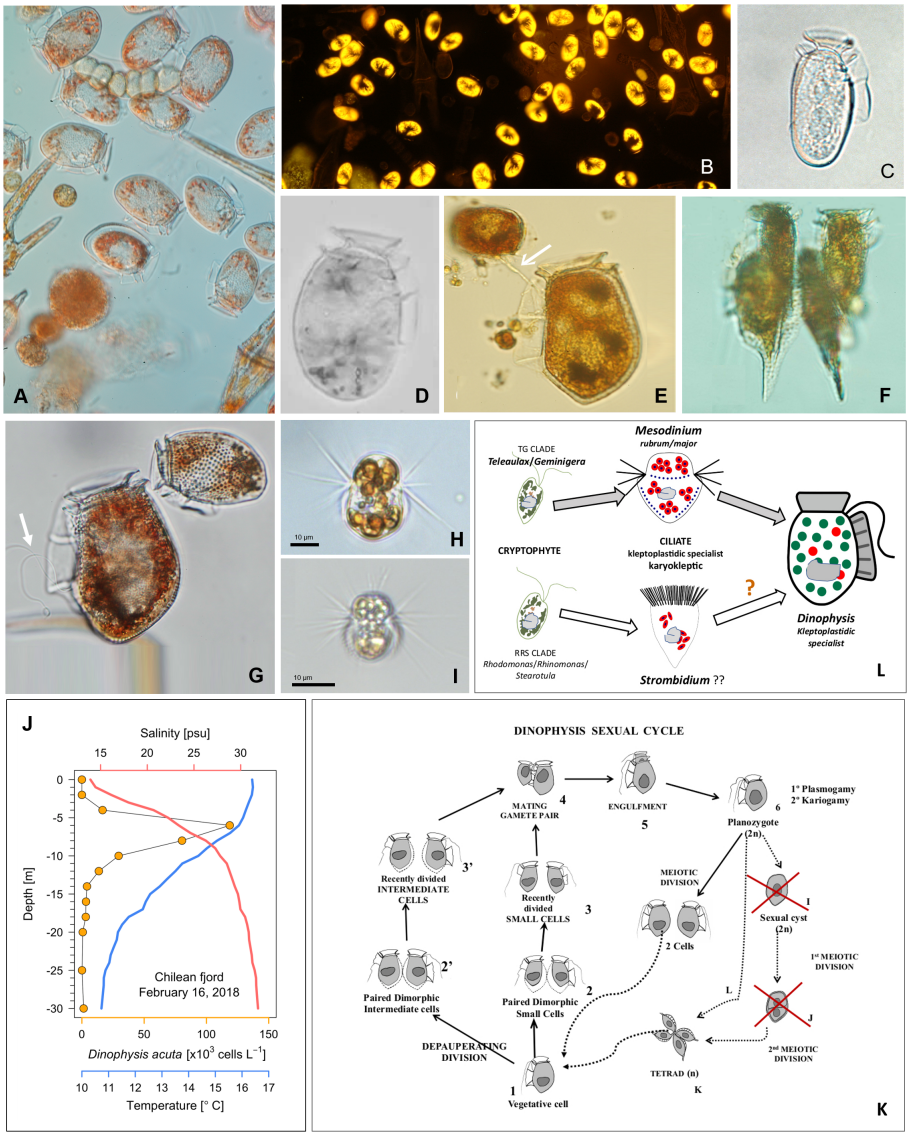


FIGURE 1
**(A–G)** Light micrographs of field specimens of *Dinophysis* spp. from the Galician Rías (NW Spain) **(A)** Net-haul sample of an autumn bloom of *Dinophysis acuminata* bloom (DIC, 400x) and **(B)**. Natural autofluorescence of the same population (excitation 546 nm, emission 585 nm single-pass filter set, specific for phycoerithrin); **(C)** *Dinophysis sacculus* and **(D)** *D. ovum*, two morphospecies of the *D. acuminata* complex; **(E)** *D. acuta* with a towing filament (white arrow) to guide the small (*D. dens*-like) cell before mating; **(F)** A tetrad of small (*D. diegensis*-like) cells of *D. caudata*; **(G)** Planozygote of *D. acuta* with two trailing flagella (white arrow) and *D. acuminata*; **(H)** Live specimen of a *Mesodinium rubrum* strain for Denmark and **(I)** from Southwestern Spain; **(J)** Vertical distribution of *D. acuta*, in a Chilean fjord, aggregated in a thin layer above the depth of maximal density gradient (Díaz et al., 2021); **(K)** Sexual cycle of *Dinophysis* (modified from Escalera and Reguera, 2008) with red crosses marking wrong and dashed lines hypothetical life-cycle transitions; **(L)** Diagram of the cryptophyte-ciliate-dinoflagellate food chain used for mixotrophic cultures of *Dinophysis* (top) and a putative alternative (bottom).

(*D. acuminata* complex, *D. acuta, D. caudata, D. fortii* and *D. norvegica*) which reach bloom numbers (> $10^3$ cells $L^{-1}$) in coastal waters. A thorough revision of the genus with (still inexistent) robust molecular probes would probably lead to a large reduction of the about 100 species recognized in Gómez (2005) checklist. Flow cytometry, a potent tool to monitor DNA changes through the cell cycle (with cell sorter in field populations) has never been applied to study sexual processes (i.e. ploidy, gametogenesis, phased gamete pairing) with *Dinophysis* cultures.

A *Dinophysis* life cycle model was proposed by Reguera and González-Gil (2001) and some misinterpretations clarified later (Koike et al., 2006; Escalera and Reguera, 2008) (Figures 1E–G, K). For example, the mating anisogamous gamete pair, 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 guides the small cell to the cingulum to be engulfed. 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, from normal vegetative cells, or from both. All these forms, plus the first remark about the ciliate *Mesodinium*

TABLE 1 Physiological traits in *Dinophysis acuminata* (VGO1349) and *D. acuta* (VGO1065) fed the ciliate *Mesodinium rubrum* (AND0711) fed *Teleaulax amphioxeia* (AND-0710) or *Plagioselmis prolonga* (CR10EHU) (3): 1. Uptake rates of inorganic and organic N sources; 2. Growth response (μ, $d^{-1}$) to light intensity and quality and 3. Tolerance to low, medium and high levels of turbulence (ε, kinetic energy dissipation rate) generated with oscillating grids (*Turbogen*) in 4L cylinders.

| 1. UPTAKE RATES OF NITROGENOUS COMPOUNDS[1] (pmol N $cell^{-1}h^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|
| Species | N source | Antibiotic | FED | | STARVED | |
| | | | AV | SD | AV | SD |
| *D. acuminata* | Urea | +A | 1.28 | 0.09 | 0.52 | 0.10 |
| | | – | 1.04 | 0.03 | 0.62 | 0.08 |
| | Ammonium | +A | 0.84 | 0.22 | 0.32 | 0.08 |
| | | – | 0.99 | 0.32 | 0.56 | 0.06 |
| | Nitrate | +A | 0.05 | 0.01 | 0.03 | 0.00 |
| | | – | 0.05 | 0.01 | 0.02 | 0.02 |
| *D. acuta* | Urea | +A | 2.34 | 0.47 | 1.49 | 0.53 |
| | | – | 1.95 | 0.25 | 1.45 | 0.39 |
| | Ammonium | +A | 2.00 | 0.51 | 1.69 | 0.65 |
| | | – | 2.33 | 0.56 | 1.78 | 0.21 |
| | Nitrate | +A | 0.08 | 0.01 | 0.06 | 0.00 |
| | | – | 0.10 | 0.02 | 0.07 | 0.01 |

| 2. LIGHT[2] | | *Teleaulax amphioxeia* | *Mesodinium rubrum* | *Dinophysis acuminata* | *Dinophysis acuta* |
|---|---|---|---|---|---|
| QUALITY | INTENSITY (μmol ph. $m^{-2}$ $s^{-1}$) | Specific growth rate μ ($d^{-1}$) | | | |
| white | ~650 | 0.55 | 0.23 | 0.13 | 0.10 |
| | ~200 | 0.56 | 0.39 | 0.06 | 0.12 |
| | ~75 | 0.48 | 0.31 | 0.05 | 0.11 |
| | ~40 | 0.45 | 0.26 | 0.10 | 0.16 |
| green | ~40 | 0.26 | 0.16 | 0.10 | 0.30 |
| | ~10 | -0.01 | 0.03 | 0.07 | 0.12 |
| blue | ~40 | 0.37 | 0.30 | 0.06 | 0.25 |
| | ~10 | -0.08 | 0.17 | 0.07 | 0.15 |

| 3. TURBULENCE[3] ϵ ($m^2$ $s^{-3}$) | Low (L) 0.5 – 8 x $10^{-6}$ | | Medium (M) 0.3 – 4 x $10^{-5}$ | | High (H)0.5 – 4 x $10^{-4}$ | |
|---|---|---|---|---|---|---|
| *Dinophysis acuminata* | CL | L | CM | M | CH | H |
| Growth day 0-6 ($d^{-1}$) | 0.34 | 0.35 | 0.31 | 0.37 | 0.34 | 0.23 |
| Recovery 6-8 ($d^{-1}$) | 0.21 | 0.07 | 0.22 | 0.13 | 0.23 | 0.07 |
| *Dinophysis acuta* | CL | L | CM | M | CH | H |
| Growth day 0-6 ($d^{-1}$) | 0.22 | 0.23 | 0.23 | 0.30 | 0.20 | -0.08 |
| Recovery day 6-8 ($d^{-1}$) | 0.13 | 0.09 | 0.18 | 0.31 | 0.09 | 0.04 |

C- stands for control for each turbulence (L, M, H) treatment.
[1]García-Portela et al., 2020; [2] García-Portela et al., 2018b; [3] García-Portela et al., 2019.

entangled in mucilage in the bottom of *Dinophysis* culture vessels (Nagai et al., 2008) are well illustrated by Nagai et al. (2020). Putative resting cysts turned out to be pellicle cysts of *Fragilidium* after eating *Dinophysis*. A thin and translucid harpoon-like tube, difficult to illustrate, has been described for *Dinophysis* to catch prey in addition to a feeding peduncle (Hansen and Tillmann, 2020). Early observations of the mating process were interpreted as an act of cannibalism. Indeed, the large cell guides the small one to be engulfed in the same fashion as the towing peduncle used by heterotrophic protists (e.g. *Protoperidinium* species) with their prey (Figure 1E). How many tubes do *Dinophysis* cell have? Is *Dinophysis* sexual division a living heirloom of the "cannibal origin of sex" proposed by Sagan and Margulis (1987)?

## 2.3 Identification problems: 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 (Figures 1C, D) (Séchet et al., 2021). Sequencing the ITSrDNA- region of single cell isolates with a new technique showed a 99% similarity between *D. acuminata* and *D. sacculus*, two frequent species of this complex (Marín et al., 2001). Later, 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 *D. caudata* group.

*Dinophysis acuminata* and *D. ovum* are well distinguished in field samples by monitoring experts in Southern Europe on the basis of their size and contour. In eastern USA they also show very different toxic potential. Their distribution shows latitudinal and seasonal differences in Atlantic and Mediterranean coastal waters in Europe (Séchet et al., 2021) as well as those from Eastern USA and the Gulf of Mexico (Wolny et al., 2020; Ayache et al., 2023).

Park et al. (2019) found that shape in single-cell incubations of *D. acuminata* complex specimens from Korea (all images resembling the *D. ovum* morphotype) changed after weeks of incubation to forms corresponding to descriptions of the two (*D. acuminata* and *D. ovum*) morphospecies. It is important to note within this context that armored dinoflagellate specimens in culture may display a smoother wall texture and their tapered antapical ends lose sharpness (e.g. cells of *D. acuta* in culture may end with the appearance of *D. fortii)*. Some authors group all morphologically close morphotypes and their small cells in their routine cell counts. This is unfortunate, because whether they consider them to be one or two species, different life forms are revealing adaptations to environmental conditions (Margalef, 1978). Valuable ecological information is being missed. In the case of recognizable small cells, e.g. *D. skagii*, the small cell of *D. acuminata*, their detection and quantification provide valuable parameterization for models including life cycle transitions. The continuum of shapes between species of the *D. acuminata* complex may provide a model of ongoing speciation.

New portions of the genome need to be sequenced to develop more robust molecular tools for species identification. In the meantime, we should keep different forms separated or name them adding the letter "f" (form), followed by the epithet (*acuminata, ovum, sacculus*) that best fits their shape, as done in the past to distinguish three morphotypes of *D. caudata*: *abbreviata*, *allieri* and *pedunculata* (reviewed in Reguera et al., 2007).

Recently, promising results have been obtained by comparing transcribed sequences of *D. acuminata* (Atlantic coast of US) and *D. ovum* (Gulf of Mexico) (Gaonkar and Campbell, 2023). *Dinophysis ovum* has some nuclear encoded genes (not present in *D. acuminata*) affecting the synthesis of plastidial pigments (phycoerythrin). These findings have potential to solve the design of a robust probe for the *acuminata* complex. It will also contribute to understand the *Dinophysis* species-specific response to light.

# 3 *Dinophysis* nutritional sources

A large majority of free living dinoflagellates are mixoplanktonic, i.e. they have the ability to combine phototrophy with phagotrophy (Jeong et al., 2010; Hansen and Tillmann, 2020). Photosynthesis is performed either using permanent (constitutive) or temporary (non-constitutive) plastids stolen (kleptoplastids) from a variety (generalist) or from a very selected group of prey (plastidic specialist) (Mitra et al., 2016; Mitra et al., 2023). Most phototrophic dinoflagellates have secondary plastids which contain chlorophyll *c* and peridinin, but some genera (e.g. *Dinophysis, Karenia, Lepidodinium*) contain plastids with pigments other than peridinin. *Dinophysis* and *Amylax* species use second hand cryptophyte-like plastids acquired from ciliate prey *Mesodinium*, which in turn utilize kleptoplastids derived from their cryptophyte prey *Teleaulax* (Figure 1L) (Koike and Takishita, 2008; Kim et al., 2012; Park et al., 2013), Additional haptophyte and cyanophyte-like (presumably from cyanobionts) plastids were found in field specimens of *D. miles* (Qiu et al., 2011). But cultures of this species, and of *Phalacroma mitra*, a pigmented exception among *Phalacroma* species with haptophyte-like plastids (Nishitani et al., 2012), have not yet been established.

## 3.1 Dissolved inorganic/organic nitrogen sources

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 showed *Dinophysis* had a clear preference for regenerated nitrogen (ammonium and urea). Unlike *Pseudo-nitzschia australis* and *Alexandrium catenella*, which are able to take up very fast the nitrates in the upwelled water ("high uptake velocity strategists") *D. acuminata* is a "high affinity strategist", i.e., is able to use very efficiently low concentrations of nutrients that

would be limiting for the other two species (Seeyave et al., 2009). Laboratory incubations of *D. acuminata* yielded very low or even below detection uptake rates of nitrate, but rapid assimilation of ammonia and urea (Hattenrath-Lehmann and Gobler, 2015). *Dinophysis acuta*, a species 3 times larger, exhibited uptakes rates 2-3 times higher than *D. acuminata*. Unlike phototrophic species, starvation did not boost uptake rates which were higher in well fed cultures (García-Portela et al., 2020) (Table 1). Nitrate reductase membrane transporters from 30 dinoflagellate species (Keeling et al., 2014) showed a paucity of these transporters in *D. acuminata* comparable with the amount found in the red *Noctiluca* heterotroph. New experimental transcriptomic and isotopic data revealed the central role of $NH_4$ (Hattenrath-Lehmann et al., 2021).

## 3.2 Difficulties to cultivate highly selective *Dinophysis* and *Mesodinium*

Since Schnepf and Elbrächter (1988) drew attention to the cryptophyte-like plastids in *Dinophysis* until the first culture of *D. acuminata* was established, advances in molecular biology were essential for the final success, preceded by the cultivation of the ciliate *Mesodinium rubrum* (*Dinophysis* prey) fed cryptophytes. These cryptophytes are the source of kleptoplastids for the phototrophic ciliate *Mesodinium* (Gustafson et al., 2000) (Figure 1L). *Mesodinium rubrum*, grown in the laboratory with *Teleaulax amphioxeia*, *T. gracilis*, *T. minuta* and *Plagioselmis prolonga*, is considered to be genus-specific about its selected prey (Peltomaa and Johnson, 2017). *Plagioselmis prolonga*, only 1 bp different from *T. amphioxeia*, was found to be a haploid stage in the diplohaplontic life cycle of the latter (Altenburger et al., 2020), so we should change to "TG" the old TPG clade. 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., 2018). Likewise, *Dinophysis* growth was not the same with different strains of *Mesodinium* (Figures 1H, I) or with different quality and size of the same species/strain (García-Portela et al., 2018; Fiorendino et al., 2020).

Spatio-temporal matching of *Dinophysis* and *Mesodinium* field populations (both mixotrophs with different optimal environmental windows)(Fiorendino et al., 2020) is the key factor constraining *Dinophysis* growth. The apparent strain-level selectivity of the cryptophyte prey by *Mesodinium*, and strain-level preferences of *Dinophysis* for its ciliate prey may explain the fact that only a few laboratories have been able to grow *Mesodinium* from their own locality. Three strains of *Mesodinium* – MBL-DK2009 from Danish waters, AND-0711 from Southwest Spain, and JAMR-2007 from eastern Japan, are the only three shared strains which have been used in most *Dinophysis* experiments carried out to date; they have been fed with *Teleaulax amphioxeia* strains from the same locations, Danish K-0434, Spanish AND-0710 and Japanese JATA, with two exceptions using *Geminigera cryophyla* or a *Teleaulax* strain from the Gulf of Mexico (GoMTA) to feed a foreign *Mesodinium*. There were also Korean strains of ciliate and prey of restrained use under a patent (Yih et al., 2012). In short, a large proportion of research in the last 15 years, with *Dinophysis* fed foreign strains of *Mesodinium* and cryptophyte, has been carried out with cultures growing in suboptimal conditions. 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; Herfort et al., 2017). The latter was the dominant *Mesodinium* species in samples from Argentina and Chile (Johnson et al., 2017). Recently, cultures of *Mesodinium major* fed the same strain of *T. amphioxeia* as *M. rubrum* have been established. The former reached a biovolume 4 times higher and exhibited better adaptations to high light intensities than *M. rubrum*. This is the first case of a *Mesodinium* culture other than *M rubrum*. Most likely, *M. major* will constitute a more appropriate prey for the large sized summer – blooming *D. acuta*. Furthermore, predominance of identical crytophyte plastid sequences belonging to clade V (*Rhodomonas/Rhinomonas/Storeatula)* were found in several species of *Dinophysis*, in ciliates of the genus *Strombidium* and in co-occurring heliozoans in fjordic and oceanic waters off Los Lagos and Aysén, Chile (Díaz et al., 2020). But there are only a few studies where seasonal variability in *Dinophysis*, ciliate and cryptophytes plastids of the same location have been systematically explored.

How *Mesodinium* and *Dinophysis* recognize their local (optimal) prey is not known. One possibility may be a sympatric coevolution of predator and prey similar to that suggested between planktonic parasites and hosts, i.e. interactions between the predator and prey genotypes.

## 3.3 *Dinophysis* plastids

Following the first mixotrophic culturing of *D. acuminata* (Park et al., 2006), there was a controversy as to whether *Dinophysis* performed photosynthesis with its own (constitutive) plastids or with those kept from prey (García-Cuetos et al., 2010). Kim et al. (2012) showed that after ingestion of *Mesodinium*, the retained plastids in *D. caudata* suffered some transformation and lost two of the four surrounding membranes. Thus, *Dinophysis* reproduces in a few hours a process which may have taken years of evolution for ancestral heterotrophic dinoflagellates to acquire permanent plastids.

Further questions were raised about the potential control of *Dinophysis* over its kleptoplastids, which in *D. acuta* cultures were found to display photoregulation (Hansen et al., 2016). Since *Dinophysis* do not keep the nuclei of their prey, this observation suggested some role for the host in controlling the kleptoplastid physiology. Rusterholz et al. (2017) demonstrated that *D. acuta* and *D. acuminata* were able to divide their kleptoplastids during cell division; decreases in total kleptoplastid volume and their number were not associated with dilution by cellular divisions. To date, *Dinophysis* represents the only kleptoplastidic protist showing plastidial division in the absence of the prey nucleus. Is this an example of ongoing evolutionary transition towards permanent possession of chloroplasts of cryptophyte origin?

Transcriptomic analysis showed that products of some nuclear encoded genes transported by kleptoplastids are acquired in *D. acuminata* by Lateral Gene Transfer (LGT), a process which does

not involve vertical genetic inheritance from fucoxanthin dinoflagellates, haptophytes and cryptophytes (Wisecaver and Hackett, 2010). More recently, Hongo et al. (2019) studied the origin of genes encoding *D. fortii* proteins involved in photosynthesis (including the biosynthesis of porphyrins, chlorophylls and isoprenoids). A total of 58 proteins involved in these processes were identified, 30 of which were traced to peridinin dinoflagellates, 21 to other groups/species (from fucoxanthin dinoflagellates, haptophytes, chlorarachniophytes, cyanobacteria and cryptophytes) as a result of LGT, and 7 from unknown sources.

An interpretation of these results is that since the ancestral *Dinophysis* engulfed haptophytes and/or fucoxanthin containing dinoflagellates, the original peridinin plastid has been reduced (as in *D. acuminata)* (Janouškovec et al., 2017). During evolution, photosynthetic species of *Dinophysis* began feeding on *M. rubrum* and using its derived plastid, with a more recent transition to retention of plastids obtained from cryptophytes.

The OMICs age in *Dinophysis* research has only just started. Molecular tools unblocked the bottleneck (identification of the prey) that slowed progress in knowledge of *Dinophysis* physiology. Present challenges include prediction of the response of individual HAB species to environmental change. New breakthroughs can be expected with the help of OMIC technologies and *in situ* imaging platforms. BUT before, special efforts need to be invested in modest artisanal activities aimed to enlarge the meagre list of ciliate prey strains available and to carry out systematic observations on plastidial diversity in local populations of the ciliate and cryptophyte communities supporting *Dinophysis*.

## Author contributions

BR: Conceptualization, Writing – original draft, Writing – review & editing. MG: Investigation, Methodology, Writing – review & editing. EV: Methodology, Writing – review & editing. PR: Methodology, Writing – review & editing. LE: Methodology, Writing – review & editing. PD: Writing – review & editing, Project administration. FR: Conceptualization, Writing – review & editing, Project administration.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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