Feeding behaviour of *Fragilidium cf. duplocampanaeforme* and *F. subglobosum* on four *Dinophysis* species

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Abstract

We studied the feeding behaviour of several *Fragilidium* strains of *F. duplocampanaeforme* and *F. subglobosum* from the Atlantic, NW Iberian coast, towards four *Dinophysis* species (*D. acuminata*, *D. acuta*, *D. caudata* and *D. tripos*). LC-MS analyses were performed to follow the fate of toxins in the water or their transference from *Dinophysis* cells to *Fragilidium*. Different feeding behaviour and mechanisms were observed among the *Fragilidium* strains: *F. duplocampanaeforme* fed upon *D. acuminata* and *D. caudata* but not on *D. acuta* and weakly on *D. tripos*. *Fragilidium* engulfed these preys directly through the sulcus as previously described. Allelopathic effects toward certain but not all *Dinophysis* species studied were observed. Frequency of gamete-like small forms of *F. duplocampanaeforme* was much higher (>50%) in cultures preying on *Dinophysis* (*D. acuminata* and *D. caudata*) than in those (<20%) where *Dinophysis* cells were not eaten. Our results are discussed in terms of feeding selectivity, grazing rates, life cycle and toxins transfer through the planktonic food web.

Keywords: Dinophysis, mixotrophy, Fragilidium, okadaic acid, dinophysistoxins

Introduction

Dinoflagellates are marine protists which include photosynthetic and heterotrophic species, but also many mixotrophs (Stoecker 1999). Mixotrophy can be obligatory, like in photosynthetic *Dinophysis* species that “steal” plastsids periodically from the ciliate *Mesodinium rubrum* (Park et al. 2006). However, in many cases mixotrophic dinoflagellates contain plastsids of their own and maximize their growth rates by ingesting other algae (Jakobsen et al. 2000; Stoecker 1999; Jeong et al. 2005). Mixotrophic dinoflagellates can be found in most taxonomic orders such as Gymnodiniales, Prorocentrales, Dinophysiales, Gonyaulacales, etc (Stoecker 1999).

*Fragilidium* is a facultative mixotrophic dinoflagellate. The mixotrophic nature of most *Fragilidium* species has been already reported (Park and Kim 2010). According to former studies, *Fragilidium* feeds only on dinoflagellates but distinct selectivity is observed for each species. Thus, *F. subglobosum* feeds only on *Ceratium* spp., *F. mexicanum* on multiple genera (e.g. *Akashiwo*, *Alexandrium*, *Ceratium*), and *F. duplocampanaeforme* on *Dinophysis* species (Park and Kim 2010, Hansen et al. 2011).

Recently, Park and Kim (2010) described prey specificity and the feeding mechanism in *Fragilidium duplocampanaeforme* isolated in Masan Bay, South Korea. Based on a previous report of *F. duplocampanaeforme* containing *D. acuminata* and *D. caudata* in the French Atlantic coast (Nézan and Chomérat 2009), these authors demonstrated that their isolate fed exclusively on *Dinophysis*. They tested four species: *D. acuminata*, *D. caudata*, *D. fortii* and *D. infundibulus*. Only *D. fortii* was not ingested by *Fragilidium* but allelopathic effects, specifically reduced mobility, were observed in every *Dinophysis* spp.

In the NW Iberian Peninsula, shellfish harvesting closures due to diarrheic poisoning are common in the Galician Rías upwelling coastal system due to the occurrence of *Dinophysis* species, mainly *D. acuminata*, but also *D. acuta* and *D. caudata*. Toxin profiles and cellular content are highly variable between *Dinophysis* species (and between strains of the same species), but all the aforementioned are known to produce okadaates
(okadaic acid and its dinophysistoxins, DTX’s) and/or pectenotoxins (PTX’s) in some degree (reviewed in Reguera et al. 2012).

The occurrence of Fragilidium spp. is well known in Galician waters, but to our knowledge there are no systematic studies on its abundance, diversity and feeding behaviour on Dinophysis spp. or any other dinoflagellates in the area. Amorim et al. (2013) described the presence of F. duplocampanaeforae and F. subglobosum in NW Iberian Peninsula and observed the feeding of both species on Ceratium horridum.

In the present study we studied the feeding behaviour of two strains from Fragilidium duplocampanaeforae (VGO1120 from NE Atlantic and VGO692 from Mediterranean Sea) and F. subglobosum IO97-01 (NE Atlantic), on four Dinophysis species isolated in the Galician Rías (D. acuminata, D. acuta, D. caudata and D. tripos).

Material and Methods

Dinophysis species were isolated in NW Spain (D. tripos and D. acuminata (VGO1062 and VGO1063, Oct 2009), Station B1, Ría de Vigo; 42° 21,40’N 8° 46,42’W; D. caudata (VGO1064, Apr 2010), D. acuta (VGO1065, Oct 2010); Station P2, Ría de Pontevedra; 42° 8,22’ N, 8° 51,36’ W). The ciliate Mesodinium rubrum (AND-A0711) fed with the cryptophyte Teleaulax amphioxeia (AND-A0710) was added periodically as prey. Cultures of Fragilidium cf. duplocampanaeforae were isolated during opportunistic samplings at Ría de Vigo, NW Iberian Peninsula (VGO1120; Station B1, July 2009) and Elefsis Bay, Saronikos Gulf (VGO692, July 2003). Fragilidium subglobosum (IO97-01) was established by isolation of single cells from plankton-net samples in Portuguese coastal waters. All cultures were grown in diluted (1/20) L1-Si medium at 19°C, salinity of 32, 12:12 L:D cycle at 150 µmol photons m⁻² s⁻¹ irradiances.

Cultures of Fragilidium and Dinophysis species were mixed in a 3:1 cell:cell ratio in 24-well microplates (Thermo Scientific, NY, USA). Instantaneous rates of increase (r; d⁻¹) were calculated using the equation \( r = \ln (N_t/N_0)/At \), and the ingestion rates (\( I = \text{Dinophysis cells eaten Fragilidium}^1 \) d⁻¹) was calculated following the equations by Frost (1972).

Cultures for toxin analyses were filtered through 1.4 µm Whatman GF/C glass fiber filters. Toxins contained in cells were extracted with MeOH and cell free culture medium was extracted in solid phase (SPE) with Sep-Pak C18 light cartridges (Waters, USA) (Paz et al. 2004).

Samples of cells and cell free culture medium were analyzed by LC-MS for lipophilic toxins (YTXs, SPXs, AZAs, OAs, DTX2 and PTX2) at the Analytical Chemistry Department of the University of Vigo following the conditions proposed by Braña-Magalena et al. (2014). These are based on the EC (2011) method validated by the European Union Reference Laboratory on marine biotoxins (EU-RL) under acidic conditions

Results and Discussion

The Atlantic Fragilidium (VGO1120) fed selectively and heavily upon D. acuminata and D. caudata (Fig. 1A, B). Different sizes typically described in Dinophysis spp. were distinguished and enumerated in this particular experiment. The aim was to detect if feeding of VGO1120 on Dinophysis spp. could discriminate among different cell sizes. But we observed the same trends in their abundance during the experience and only total counts for each Dinophysis species are shown in Fig. 1.

Maximum ingestion rates were observed on D. acuminata (day 1, \( I=2.52±0.48 \) Dinophysis Fragilidium⁻¹ d⁻¹) and D. caudata (day 3, \( I=0.58±0.32 \)). These maximum ingestion rates were similar to those measured in F. subglobosum feeding on the dinoflagellate Ceratium lineatum (Skovgaard 1996; \( I=2.4.5 \)).

The Atlantic Fragilidium grazed only occasionally on D. tripos (<0.10) and not at all on D. acuta. Grazing on D. tripos was confirmed by light microscopy after observing the direct engulfment of a few D. tripos cells, but never on D. acuta. Allelopathic effects (reduced mobility and occasionally cellular death) were observed in all Dinophysis species, excepting D. acuta.

Higher growth rates and cell densities of the Atlantic Fragilidium were measured in D. acuminata and D. caudata treatments at the end of the exponential phase, reached in <10 days (0.34 d⁻¹ in both cases), with lower values if cultured with D. acuta and D. tripos (0.22 d⁻¹ and 0.19 d⁻¹, respectively). The Atlantic Fragilidium grew faster when feeding actively on Dinophysis during the first week of the experiment relative to the control
treatment. After that, cultures reached the stationary phase at day 9. The control treatment of the Atlantic Fragilidium reached higher cell densities and the stationary phase at day 13, but corresponding growth rates were lower (0.17 d⁻¹). At the end of the experiment, dominance of small sized cells in VGO1120 was observed in D. acuminata (59.5% ± 3.9) and D. caudata (49.8% ± 10.6) treatments, relative to D. acuta (19.4% ± 2.7), D. tripos (14.9% ± 0.8) and the control treatments (34.1% ± 3.5).

Hansen (2011) recently described Fragilidium mixotrophic behaviour as “type-1”: “addition of prey results in large increases in growth rates, at least at low irradiance”.

In our study a faster initial growth rate was measured in F. duplocampanaeforme fed on Dinophysis, as reported in F. subglobosum fed on Ceratium lineatum and C. tripos (Skovgaard 1996; Hansen et al. 2000). Nonetheless, these growth rates were not markedly high in comparison with control treatments (autotrophic growth). The differences between mixotrophic and autotrophic growth rates in the Atlantic Fragilidium could have been larger if higher prey:predator ratios or lower irradiances were assayed.

None of the D. acuta cells were ever seen ingested either by the Atlantic isolate VGO1120, or by any of the other two Fragilidium strains in this study. Moreover, we could not find any evidence under the light microscope for the feeding of the Mediterranean isolate of F. duplocampanaeforme (VGO692) or F. subglobosum on any Dinophysis species. The only apparent effects were allelopathic, as a higher proportion of D. acuminata cells that looked unhealthy and probably dead appeared with VGO692 or IO97-01 relative to the control treatments.

Lipophilic-toxins were also analyzed in the cells and clarified culture medium to follow the fate (i.e. the potential transference and transformation) of these compounds from Dinophysis spp. to Fragilidium (specifically VGO1120). YTXs, SPXs and AZAs were not detected in Dinophysis or in Fragilidium species used in this work. Moreover, DSP-toxins (OAs, DTXs and PTXs) were not found in control cultures of Fragilidium. Fragilidium cells that had been preying on D. acuminata and D. caudata showed detectable values of okadaic acid (OA) and pectenotoxin-2 (PTX2), in each case (Table 1).

Table 1. DSP toxins in clarified medium and cells of Fragilidium. D. acuta and D. tripos were not cleared out by Fragilidium and their cells also contributed to the toxin amount per cell.
The clarified medium contained higher amounts of these toxins but also different compounds released from the *Dinophysis* cells that could not be detected in *Fragilidium*. In the case of *D. tripos* and *D. acuta* treatments, similar results were obtained both in the clarified medium and in the cells.

Our results suggest that *F. duplocampanaeforme* fed on toxic *Dinophysis* spp. accumulates DSP-toxins. These toxins seem to be quickly metabolized and the cellular levels are very much reduced in *Fragilidium* a few days after all *Dinophysis* were removed.

We do not know if transformations of DSP-toxins in *Fragilidium* take place, following a similar scheme to that in filter-feeding mollusks; this needs to be addressed in larger scale experiments to detect minor levels of DSP-toxin derivatives. Finally, we suggest that some prey recognition mechanism, rather than toxicity, could explain the selectivity of the Atlantic and Mediterranean *F. cf. duplocampanaeforme* isolates toward the *Dinophysis* species tested in this study.

**Acknowledgements**

We thank Ana Gago and José M. Leao (University of Vigo) for help in LC-MS toxin analyses. Funded by projects DINOPOL, Plan Nacional I+D+I (CTM2009-12988-C02-01) and “Aislamiento y purificación de espirólidos a partir de cultivos de *Alexandrium ostenfeldii*” CSIC -PIE- 201270E032.

**References**


