9. Characterization of the distribution of Dinoflagellates on the Ridge of Santa Croce (*Gulf of Trieste*)

Research unit
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Introduction

The Dinoflagellates are eukariotic unicellular organisms, amongst the main marine and fresh water phytoplanctonic components. They include some 2000 living species belonging to about 130 genera (Taylor, 1987), mainly found in temperate coastal waters and in stability conditions of the column of water. Their size ranges from 10µm a 200µm.

The Dinoflagellates may develop, in case of adverse conditions, a resting form called *cyst* (Matsuoka *et al*., 1989), which may remain viable in the sediment for a long time (Jux, 1968).

In favourable conditions, cyst can remain viable in the sediment for 5-10 years, sometimes even longer, up to the appearance of the factors which will then lead to germination.

Objectives

In accordance with Binder & Anderson (1987) the cyst may be considered as an indicator of changed environmental conditions, of the current trend and of the variations in the sedimentation rate. On this basis the present survey has tried to clarify the correlation between the cyst distribution of Dinoflagellates and the abiotic and biotic factors which develop in the presence of artificial reefs, such as the one submerged in the Ridge of Santa Croce – Gulf of Trieste. As a matter of fact the circulation of the water masses may be changed by the hydrodynamic conditions of the sea bottom energy which generate turbulences or sediment particles deposits.

Methodological notes

The sampling campaign took place in July and December 2005, according to the life cycle of the Dinoflagellates. The pyramids of the Ridge of Santa Croce were identified as the sampling sites (Fig. 9.1).

Date: 26/07/2005

Station	GPS Finding	Depht	Temperature (C°)	Current
D1	45°42.092' N 13°37.381' E	12,4	20	NO
D2	45°42.087' N 13°37.396' E	11,8	20	NO
D3	45°42.068' N 13°37.399' E	12,5	20	NO
D4	45°42.110' N 13°37.382' E	11,8	20	NO
D5	45°42.098' N 13°37.412' E	11,9	20	NO
D6	45°42.077' N 13°37.420' E	12,6	20	NO

Date: 22/12/2005

Station	GPS Finding	Depht	Temperature (C°)	Current
D1	45°42.092' N 13°37.381' E	12,9	10	NO
D2	45°42.087' N 13°37.396' E	12,1	8	NO
D3	45°42.068' N 13°37.399' E	13,0	10	NO
D4	45°42.110' N 13°37.382' E	13,0	10	NO
D5	45°42.098' N 13°37.412' E	12,0	10	NO
D6	45°42.077' N 13°37.420' E	13,0	10	NO

The sampling techniques adapted for the collecting of the marine sediment were core boring with direct method performed during the 12 scuba diving.

In the present research we have taken into consideration the most superficial core sections, as the first layers of the substrate were more involved in the resuspension and redistribution activities due to the sea bottom currents. The cores used were labelled in plexiglass tubes having 4cm in diameter and 30cm long. The cores were infixed in the substrate by means of a little stick up to about 20-25cm deep (Fig. 9.2), sealed on both sides with a rubber stoppers, heaved on board and placed in the fridge submerged in seawater. Three replicas of the sampling for every site were effected in order to have the possibility to choose the most compact corers which was then better preserved during the transportation to the laboratory.

The sampling is kept in the fridge at about 4°C to avoid deterioration. The sampling extrusion of the corer was obtained by means of a manual plunger, which was slid through from the bottom to the top end of the tube. The sample is then cut with a spatula, in sections being 1cm thick. The fractions obtained were put in plastic containers where filtered seawater was added to keep the sample moist. Even the fractions of the sediment samplings were kept in the dark at a temperature of 4°C so as not to interfere with the vitality of the cysts of Dinoflagellates (Anderson, 1980). After the extrusion and preservation, the samples were set ready for the qualitative microscopic analysis.

The method for the preparation of the samples of cysts of vital Dinoflagellates was realised having the least content possible of sediment. The presence of sediment in fact made both the discovery and determination of the organisms difficult. The methodological procedure followed may be summarised in the following points:

1. homogenizing the sample by shaking it slightly;
2. collecting an homogenous sub-sample of sediment (1gr.) and diluting it in filtered seawater;
3. treating the sub-sample with sonication for one minute so as to obtain the separation of the cysts from the sediment in order to examine them more easily. The sonication treatment itself represented potentially a problem, as the effect of the sonication and the heat produced on the physiology of the cysts is not completely known. The ability of the sonication is believed to accelerate the process of excystment – E. Erard, oral communication. This technique is however the only possibility known at the moment to obtain detritus-free cysts;
4. filtering the subsample on nylon filters with decreasing meshes (100µm, 60µm, 40µm, 20µm), assembled on a filtering "Millipore" tower. For the samples with abundance of pelitic component, the filtering was made easy by connecting the system to a vacuum pump and applying a 10mBar depression. The 100 e 60µm mesh size sieves were eliminated as they belonged to a fraction of sediment with a greater particle size measurement compared to the one belonging the cysts. The fraction obtained with 40µm-mesh size sieve contained the majority of the species of cysts; that obtained with the 20µm mesh size sieve was characterized by the

presence of cysts belonging to species of smaller sizes (e.g. *Alexandrium* sp. and *Scrippsiella* sp.);

5. treating with sonication (for 2 minutes) the 40 e 20µm filters placed in a glass containing 10ml filtered seawater and shaking them with circular movements as to create a vortex to separate the sediment components and to have the decantation of heavier material of the sample (sandy components) concentrated at the centre of the container and kept the cysts and the light material suspended;

6. collecting the suspension which was placed in a sedimentation case and observing the sample with an inverted optical microscope ("Leica" model DM IL microscope).

Determination of the species

The determination of the species is a logical process which implies finding the biunique correspondence between objective reality (individual to observe) and the subjective reality (abstract image of the species as it appears to the observer). In the cognitive, logical processes, applied to the world of nature, the objective analysis has been rationalised so as to reach instruments of cognitive survey. Logical processes, the determination keys for example, which help establish a contact between the two parts: the objective and the subjective, separating then an individual of a species from a wider group. Building a model for the determination means optimising the objective observation and reducing as much as possible every subjective interpretation (Bressan, 1986). This logical process has enabled the overcoming the more serious difficulties as in content having the number of determining characters like in the case of the cysts

In the present study some logical processes were used for the identification, described later, which led to the identification of the species as simplified in the original "decoder" suitably realised according to the identification key planned in previous works (Tonzar, 2005).

Identification key

Cellular organization:

1A. FLAGELATE	2.

Habitat:

2A. MARINE SPECIES	3.
2B. FRESH WATER SPECIES	3.

Shape of the body:

3A. SPHERICAL	4.
3B. IRREGULAR GLOBE-SHAPED	4.
3C. OVAL	4.
3D. POLYEDRICAL	4.
3E. PIRIFORM	4.
3F. DROP-SHAPED	4.

Size of the body (bibliographic data):

4A. 20-45µm	5.
4B. 46-55µm	5.
4C. 56-65µm	5.
4D. 66-75µm	5.
4F. 76-90µm	5.

Ornamentations:

5A. PRESENCE OF SPINAS	6.
5B. ABSENCE OF SPINAS	

Morphology of ornamentations:

6A. STRAIGHT SHARP ENDING SPINES	7.
6B. SHARP BENT ENDING SPINES	7.
6C. FUNNEL-SHAPED ENDING SPINES	7.
6D. TRUNCATED ENDING SPINES	7.
6E. IRREGULAR ORNAMENTATION	7.

Size of ornamentations:

7A.1-5µm

7B.6-15µm

7C. 16-25µm

Decoder

Codes							Genus and Species
1A	2A	3A	4C	5B			*Alexandrium pseudogonyaulax*
1A	2A	3A	4A	5A	6B	7B	*Gonyaulax grindleyi*
1A	2A	3F	4B	5A	6C	7B	*Gonyaulax spinifera*
1A	2A	3A	4A	5A	6A	7B	*Lingulodinium polyedrum*
1A	2A	3C	4B	5A	6A	7C	*Protoperidinium conicum*
1A	2A	3A	4A	5B			*Scrippsiella trochoidea*
1A	2A	3C	4B	5A	6C	7C	*Spiniferites* sp.

Results

Characteristics of the sediments

A macroscopic estimation of the sediments was carried out by estimating the granulometry of the sediment, according to Udden-Wenthworth (U.S. Standard) (Tab.9.1). The sediment macroscopic evaluation led to the constitution of homogenous grouping of sampling sites:

• pelitic sediment very plastic, light grey, homogenous in the whole corer (pyramid D2);

• dark-coloured pelitic sediment, homogeneous along the whole corer (pyramid D4);

• sediment rougher on the top part of the corer with presence of mussels and shells;

• sediment finer on the lower part of the corer, dark grey colour (pyramids D1, D3, D5 and D6).

Floristic list

Seven taxa were determined, of which only one at the genus level.

Genus and Species	Bibliographic Reference
Alexandrium pseudogonyaulax (Biecheler)	Montresor, 1993
Gonyaulax grindley	Bolch & Hallegraeff, 1990; Blanco, 1989
Gonyaulax spinifera (Claparide & Lachman)	Bolch & Hallegraeff, 1990
Lingulodinium polyedrum (Stein) Dodge	Ruth, 1987
Protoperidinium conicum (Gran) Balech	Bolch & Hallegraeff, 1990; Ruth, 1987
Scrippsiella trochoidea (Stein) Loeblich III	Bolch & Hallegraeff, 1990; Montresor *et al*., 1994
Spiniferites sp.	Blanco, 1989

The data were obtained according to the qualitative vertical distribution of the cysts in the sediment and listed in tables (Tabb. 9.2-9.3) of sampling stations (data of presence/absence) and were elaborated in terms of univariate analysis. In the comparisons percentage quantities were used for the normalization of data.
It was chosen to analyse the data taking as a reference the month of December as, being a study on the cysts of Dinoflagellates, it was preferable to refer the observations at the moment in which the encystment activity was at its highest development.
On this basis some frequency classes of presence were obtained yielded from the empiric analysis of the histograms (Figs. 9.4-9.5).
Albeit four frequency classes were identified:
• I percentage of presence of individuals greater than 25.00% (=dominant cysts);
• II percentage of presence of individuals between 24.99% and greater than 10.00% (=”little present” cysts);
• III percentage of presence of individuals between 9.99% and greater thatn 0.00% (=cysts ± ”rare”);
• IV “absence” of cysts (percentage of presence of individuals 0.00%).
The treatment of the samples led to the analysis of 3 fractions each of one centimetre thick for each corer:
fraction 0.0-1.0cm;
fraction 1.0-2.0 cm;
fraction 2.0-3.0 cm;
The data were obtained keeping also into account the parameter (Tabb. 9.4 – 9.5):
“vitality” (presence of ”eye spot” and/or presence of the cellular content);
“not vitality” (absence of “eye spot” or theca broken).

Analysis of the floristic variation (July)
The percentage of presence of cysts of Dinoflagellates collected in the sediments of the different sampling stations in July was analysed:
• class I (very frequent cysts or even “dominant”: *Lingulodinium polyedrum* (4) with a frequency of presence of 53.30%;
• class II (“little present” cysts): *Alexandrium pseudogonyaulax* (1) with a frequency of presence of 20.00% and *Spiniferites* sp. (7) with a frequency of presence of 13.30%;
• class III (± “rare”cysts): *Gonyaulax spinifera* (3) and *Scrippsiella trochoidea* (6) with a frequency of presence of 6.67%;
• class IV (“absent” cysts compared to December) belong to: *Gonyaulax grindleyi* (2) and *Protoperidinium conicum* (5) with a frequency of presence of 0.00%.

Analysis of the floristic variation (December)

The percentage of presence of species of cysts of Dinoflagellates collected in the sediments of the different sampling stations in December was analysed:

• class I (cysts very frequent or even "dominant"): *Lingulodinium polyedrum* (4) with a frequency of presence of 31.00%;

• class II (cysts "little present"): *Alexandrium pseudogonyaulax* (1) with a frequency of presence of 20.70%, S*piniferites* sp. (7) with a frequency of presence of 13.80%, *Gonyaulax grindleyi* (2) and *Gonyaulax spinifera* (3) with a frequency of presence of 10.30%;

• class III (± "rare" cysts): *Protoperidinium conicum* (5) e *Scrippsiella trochoidea* (6) with a frequency of presence of 6.90%;

• class IV ("absent" cysts): no species.

Analysis of the floristic variation (July-December).

A comparison of the percentage of species of Dinoflagellates cysts was collected in the sediments of the different sampling stations in July and December (Fig. 9.6) and was analysed:

From the histogram analysis a difference of distribution of species was highlighted in the two different samplings. Therefore, three trends may be identified:

Species which in June had a presence quantity higher than the month of November: *Lingulodinium polyedrum* (4);

Species which in December had a presence quantity higher than July: *Gonyaulax spinifera* (3), *Gonyaulax grindleyi* (2) and *Protoperidinium conicum* (5);

Species which had the same presence quantity in both seasons: *Alexandrium pseudogonyaulax* (1), S*piniferites* sp. (7) and *Scrippsiella trochoidea* (6).

Comparison of the analysis of the floristic evaluation in the fractions (July)

A percentage of species of Dinoflagellates cysts collected in the sediments of the different fractions (0.0-1.0cm, 1.0-2.0cm and 2.0-3.0cm) of the 6 sampling stations of July was analysed (Fig. 9.7):

D1 showed a greater distribution of cysts in the fractions 1.0-2.0cm and 2.0-3.0cm;

D3, D4 and D6 showed a greater distribution of cysts in the fraction 1.0-2.0cm;

D2 and D5 showed a greater distribution of cysts in the fraction 0.0-1.0cm.

Comparison of the analysis of the floristic variation in the fractions (December).

A percentage of species of Dinoflagellates cysts collected in the sediments of the different fractions (0.0-1.0cm, 1.0-2.0cm and 2.0-3.0cm) of the 6 sampling stations of December was analysed (Fig. 9.8): in all the stations a greater distribution of the cysts in the fractions 0.0-1.0cm and 1.0-2.0cm was noticed with gradual decrease in the following fraction. A similar distribution in all three fractions was found only in the station D1.

Distribution of cysts of Dinoflagellates in the different stations (July-December).

A percentage of species of Dinoflagellates cysts collected in the sediments of the different stations in July and December was analysed (Fig. 9.9).

The histogram analysis showed a greater presence of cysts in the stations D1, D2 and D6. The highest concentration of cysts was found in the station D6 both in July and December.

Conclusions

From the analysis of the distribution of the cysts in the two months object of study in the 6 sampling stations, with reference to the species present, the following was found:

• *Lingulodinium polyedrum* (4), is an ubiquitous species having a percentage distribution higher both in July and in December;

• the species *Alexandrium pseudogonyaulax* (1), S*piniferites* sp. (7), *Gonyaulax grindleyi* (2), *Gonyaulax spinifera* (3), *Protoperidinium conicum* (5) and *Scrippsiella trochoidea* (6) are species having a percentage distribution higher in December.

Notes on the sampling stations:

The stations D6 and D1 were found to have the highest percentage of presence of cysts, with no significant differences connected to the seasonality;

The station D2 was found to be the stations with the highest distribution of cysts in July compared to December;

The stations D3, D4 and D5 were found to be the stations with the highest distribution of cysts in December compared to July.

Notes on the fractions:

In December a higher percentage of distribution of cysts in the factions 0.0-1.0cm and 1.0-2.0cm was found.

In July a decrease of percentage of distribution of cysts in the fraction 0.1-1.0cm was recorded.

The percentage of distribution of cysts was found to be very low in both sampling months in the fractions 2.0-3.0cm.

As far as the difference of presence of cysts is concerned in both months, we can suppose an excystment after July. This supposition came in relation to the different distribution of cysts between July and December.
For the stations of the present study there was a degrading distribution of cysts in the lower fractions. This observation, based on the above preliminary observations, could be related with the hypothesis of a low resuspension due to the presence of the artificial reefs, which could alter the hydrodynamic conditions of the sea bottom energy.
The preliminary results must be considered carefully in relation to the monitoring of the presence of species which are potentially toxic to human health (such as *Gonyaulax grindleyi* Reinecke which is an organism producer of toxins which induce the DSP syndrome into man), which may damage the fish resources as well as alter the benthonic component of origin.
As far as the analysis of the results obtained so far, an evaluation of an environmental impact is currently under investigation, making a continuous monitoring of the site necessary. It is therefore necessary to continue the present research even extending the survey area radially.