

Research Article

Plankton Resting Stages in the Marine Sediments of the Bay of Vlorë (Albania)

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In the frame of the INTERREG III CISM project, sediment cores were collected at 2 stations in the Gulf of Vlorë to study the plankton resting stage assemblages. A total of 87 morphotypes were identified and produced by Dinophyta, Ciliophora, Rotifera, and Crustacea. In 22 cases, the cyst belonged to a species absent from the plankton of the same period. The most abundant resting stages were those produced by *Scrippsiella* species (Dinophyta). Some calcareous cysts were identified as fossil species associated with Pleistocene to Pliocene sediment, although they were also found in surface sediments and some of them successfully germinated, thus proving their modern status. Total abundance generally decreased with sediment depth at station 40, while station 45 showed distinct maxima at 3 and 8 cm below the sediment surface. The depth of peak abundance in the sediment varied with species. This paper presents the first study of the plankton resting stages in the Bay of Vlorë. The study confirmed the utility of this type of investigation for a more correct evaluation of species diversity. In addition, the varying distribution with sediment depth suggests that this field could be of some importance in determining the history of species assemblages.

1. Introduction

Resting stages produced by plankton organisms in temperate seas accumulate in the bottom sediments of confined coastal areas [1]. Their assemblages represent reservoirs of biodiversity which sustain the high resilience of plankton communities, providing recruits of propagules at each return of favourable conditions, in accordance with the so-called Supply Vertical Ecology model [2]. The existence of benthic stages in the life cycles of holoplankton provides a new key for understanding the role of life cycles in the pelagic-benthic relationship in coastal waters [3, 4]. Consequently, assessments of biodiversity at marine sites should take account of the unexpressed fraction of the plankton community contained in the bottom sediments by performing integrated sampling programs [5, 6]. Despite the proven importance of resting stage banks in coastal marine ecology, the issue of "resting *versus* active" planktoners has commonly been

considered for single *taxa* and only rarely from the whole-community point of view. This is probably due to the great complexity (compositional, functional, and distributional) of resting stage banks. Indeed, it has been demonstrated that at any given moment the species assemblages in bottom sediments (as resting stages) are quite different from the species detectable in the water column (as active stages) [6, 7]. However, the study of such marine "seed banks" (as understood by [3], analogous to terrestrial seed banks in forest soils) is complex on many levels. Resting stages share a common morphological plan despite belonging to organisms from different kingdoms [8]. Consequently, resting stage morphology differs sharply from that of active stages and in some cases their identification is highly problematic. However, it is also true that for some naked dinoflagellates or for thecate ones with a similar thecal plate pattern, cysts are quite different, allowing correct identification without the use of SEM or molecular techniques. Cyst-producing

dinoflagellates differ in the length of their life cycle and/or the timing of cyst production, and the rest capacity of resting stages also varies. They are generally programmed to rest for the duration of the adverse period, but fractions of them can also rest for longer periods, allowing the population to reappear decades later, ([9–11], for copepods, [12, 13], for dinoflagellates). Hairston et al. [14] reported a rest of more than 300 years for a calanoid resting egg, albeit of a freshwater species.

The scarcity of literature on whole resting stage communities encouraged us to describe situations in various parts of the Mediterranean, in order to obtain a rich data set useful for building models and in experimental situations. Here a detailed description of the structure of the marine “seed bank” produced by plankton in the Bay of Vlorë is reported.

The present study also focuses on an Albanian bay that has not been extensively studied from the marine biodiversity point of view. The data from the benthos are compared with analyses of the phytoplankton and microzooplankton [15] in the water column to assess with more precision the biodiversity of the plankton in the Bay of Vlorë.

Moscatello et al. [15] reported that the microzooplankton community of the Bay of Vlorë was composed of more than 200 taxa, of which 97 were classified as “seasonally absent.” The aim of the present paper is to determine whether these absences in the water column correspond to resting stages in the sediments.

2. Materials and Methods

2.1. Study Site. An oceanographic campaign was carried out in the Bay of Vlorë from 17th to 23rd of January 2008 aboard the oceanographic vessel “Universitatis”. This survey was conducted as part of the PIC Interreg III Italy, Albania Project for providing technical assistance for the management of an International Centre of Marine Sciences (CISM) in Albania. The sampling period of the present study (January 2008) coincided with that of Moscatello et al. [15] who investigated active plankton in the water column in the same area.

In order to investigate the presence and distribution of resting stages produced by plankton species in the area, 2 stations were chosen, representing 2 different types of environment: a deep zone (station 40, depth: 54 m), with sediments of terrigenous mud dominated by *Labidoplax digitata* (Holothuroidea) and a shallower site (station 45, depth: 28 m), with sediments of terrigenous mud dominated by *Turritella communis* (Gastropoda) (Figure 1) (for the classification of mud biocenosis in the Bay of Vlorë, see [16]).

2.2. Sampling Procedure. Samples of bottom sediments were collected in three replicates (named 40 a, b, c and 45 a, b, c) using a Van Veen grab with upper windows that allowed the collection of undisturbed sediment cores. At each station, 2 different PVC corers (h: 30 cm, inner \odot 4 and 8 cm) were used in order to obtain 2 different sets of samples. The smaller core was processed to obtain cysts of protists; the larger core was processed to obtain resting eggs of metazoans. This differentiation was necessary because metazoan resting

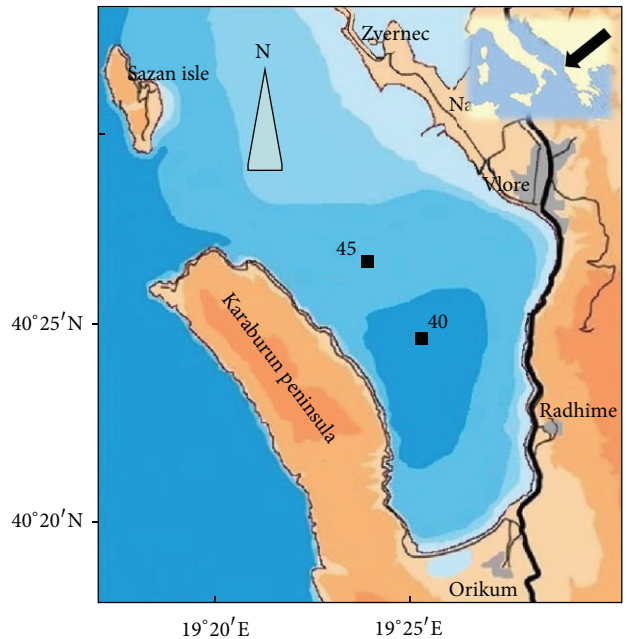


FIGURE 1: Map of study area showing location of two sampling stations (40, 45) in Bay of Vlorë (Albania).

stages are less abundant, so a greater amount of sediment is required. Moreover, their walls are only organic, allowing the adoption of a centrifugation method coupled with filtration to obtain a “clean” sample from a relatively large quantity of sediment. In contrast, protistan cysts are more abundant and have different types of walls (calcareous, siliceous, organic), which complicates the procedure when the whole cyst bank is studied. Thus, the most fruitful method of separating cysts from sediment is filtration through meshes of different sizes.

After extraction, sediment cores were immediately subdivided into 1 cm thick layers, until the 15th cm from the sediment surface. The thickness of 15 cm was chosen because in previous studies we noted that abundances diminished significantly at depths of more than 7–10 cm below the sediment surface [1, 12]. The outer edge of each layer was discarded to avoid contamination from material from the overlaying layer during the insertion of the corer into the sediments. Once obtained, the samples were stored in the dark at 5°C until treatment in the laboratory.

2.3. Protistan Cysts (20–125 μ m). In the laboratory the small-core samples were treated using a sieving technique consisting of the following steps.

- (i) The entire sample is homogenized and then sub-sampled, obtaining 3–5 mL of wet sediment which is passed through a 20 μ m mesh (Endecott’s LTD steel sieves, ISO3310-1, London, England), using natural filtered (0.45 μ m) seawater (Gulf of Taranto).
- (ii) The retained fraction is ultrasonified for 1 min and again passed through a series of sieves (125, 75, and 20 μ m mesh sizes), obtaining a fine-grained fraction (20–75 μ m) containing most of the protistan cysts

and a 75–125 μm fraction with the larger ones (e.g., *Lingulodinium* spp.) and the zooplankton resting eggs. The material retained by the 125 μm mesh was not considered.

No chemicals were used to disaggregate sediment particles, in order to avoid the dissolution of calcareous and siliceous cyst walls.

Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert S100 equipped with a Nikon Coolpix 990 digital camera) at 200 and 320 magnifications. Both full (i.e., probably viable) and empty (i.e., probably germinated) cysts were considered. At least 1/5 of the finer fraction and all of the >75 μm fraction were analyzed.

All the resting stage morphotypes were identified on the basis of published descriptions, ([17–19], for dinoflagellates, [20, 21], for ciliates and germination experiments).

Identification was performed to the lowest possible taxonomic level. As a rule, modern, biological names were used. The paleontological name is reported only for morphotypes whose active stage was not known.

A fixed aliquot (≈ 5 g) of wet sediment from each sample was oven-dried at 70°C for 24 h to calculate the water content and obtain quantitative data for each *taxon* as cysts g^{-1} of dry sediment.

2.4. Metazoan Resting Eggs (45–200 μm). For the analysis of the large-core samples the Onbè [22] method was used, slightly modified by using 45 and 200 μm mesh sizes to obtain a size range typical of mesozooplankton resting eggs.

For each sample a fixed quantity of wet sediment was treated (≈ 45 cm^3).

Only full (i.e., probably viable) resting eggs were counted and quantitative data for each *taxon* are reported as resting eggs $\times 100$ g^{-1} of dry sediment.

2.5. Germination Experiments. To achieve germination, all putative viable cysts of protists isolated from the sediment were individually positioned in Nunclon microwells (Nalge Nunc International, Roskilde, Denmark) containing ≈ 1 mL of natural sterilized seawater. Cysts were incubated at 20°C, 12:12 h LD cycle, 100 $\mu\text{E m}^{-2} \text{sec}^{-1}$ irradiance, and examined on a daily basis, until germination up to a maximum of 30 days. The incubation conditions were chosen on the basis of previous studies [5, 6, 23]. They have proved to be effective for a large number of species.

2.6. Data Analysis. For the 1st cm of the cores, data on resting stage abundance from the 2 sampling stations were obtained by merging the data from the 3 replicates of the 2 sets of samples (those for protistan cysts and metazoan resting eggs). For samples below the first cm, only the 45–200 μm fraction was used, in order to facilitate and accelerate the analysis. From the abundance matrices (*taxa versus* stations and *taxa versus* station and cm respectively) of both surface and deeper sediments, the Bray Curtis similarity measure was calculated

after 4th root transformation in order to allow rare species to become more evident.

The PRIMER “DIVERSE” function (Primer-E Ltd, Plymouth, UK) was used to calculate the taxonomic richness (S), *taxon* abundance (N), Margalef index (d), Shannon-Wiener diversity index (H'), and Pielou’s evenness index (J') for each sample.

The relationships between the samples collected at the 2 stations were analyzed by means of nonmetric multidimensional scaling (nMDS) with superimposed hierarchical clustering with a cutoff at 60% similarity (for surface sediments) and 70% (for the sediment core as a whole), while the SIMPER routine was used to identify relative dissimilarity and the *taxa* that contributed most to the differences.

The statistical significance of the differences between the 2 stations was calculated by means of a 2-way crossed analysis of similarities (ANOSIM) on the Bray-Curtis similarity matrix based on the stratigraphy.

All univariate and multivariate analyses were performed using PRIMER v.6 (Primer-E Ltd, Plymouth, UK).

3. Results

3.1. Total Biodiversity. Resting stages were found at all levels of the 15 cm sediment core columns from the 2 investigated sites in the Gulf of Vlorë.

Merging the data from the 2 sets of samples (20–125 μm and 45–200 μm) and considering both full (probably viable) and empty (probably germinated) forms from each station, 87 different resting stage morphotypes produced by plankton were recognized (Table 1). Most of them (59, belonging to 20 genera) were dinoflagellates, 16 were ciliates (9 genera), 4 rotifers (2 genera), and 5 crustaceans (4 genera), while 3 (1 protistan cyst type and 2 resting eggs) remained unidentified. Station 40 showed higher biodiversity, with 79 morphotypes, 35 of them exclusive to the site. At station 45, 52 morphotypes were observed, 8 of them being exclusive.

Moreover, analysis of the empty forms found among the 20–125 μm fraction led to the recognition of 11 morphotypes, all dinoflagellates.

A total of 36 cyst types were identified as *taxa* missing from the plankton list of the same period (January 2008; [15]). Partly due to nomenclature problems, uncertainty of identification, and differences in examined periods, it was possible to ascertain the contemporaneous presence of species in both pelagic and benthic compartments only in a very few cases.

Identification was frequently impossible due to the presence of previously unreported resting stage morphologies. In such cases, germination experiments allowed the cysts to be attributed to a high level *taxon* at least, as with a *Strombidium* (Ciliophora) cyst, whose morphology is reported here for the first time (see Figure 2).

3.2. Surface Sediments. The analysis of surface sediments (the 1st cm of the cores), that is, those most affected by cyst deposition and resuspension/germination, revealed sharp differences between the 2 analysed stations. In total, 36

TABLE 1: List of resting stage (cyst) morphotypes recovered from sediments of Bay of Vlorë (Albania).

Taxon	St.40	St.45
Dinoflagellates		
<i>Alexandrium minutum</i> Halim	●	● ○
<i>Alexandrium tamarense</i> (Lebour) Balech	●	
<i>Alexandrium</i> sp. 1	●	
<i>Alexandrium</i> sp. 2		●
<i>Bicarinellum tricarinelloides</i> Versteegh	● ○	●
<i>Calcicarpinum perfectum</i> Versteegh		○
<i>Calciodinellum albatrosianum</i> (Kamptner) Janofske and Karwath	● ○	● ○
<i>Calciodinellum operosum</i> (Deflandre) Montresor	● ○	○
<i>Calciperidinium asymmetricum</i> Versteegh		○
<i>Cochlodinium polykrikoides</i> Margalef type 1	●	
<i>Cochlodinium polykrikoides</i> Margalef type 2		○
<i>Diplopelta parva</i> (Abé) Matsuoka		○
<i>Diplopsalis lenticula</i> Bergh	●	○
<i>Follisdinellum splendidum</i> Versteegh		○
<i>Gonyaulax</i> group	● ○	● ○
<i>Gymnodinium impudicum</i> (Fraga and Bravo) G. Hansen and Möestrup		●
<i>Gymnodinium nolleri</i> Ellegaard and Möestrup	●	
<i>Gymnodinium</i> sp. 1	● ○	●
<i>Lingulodinium polyedrum</i> (Stein) Dodge	● ○	● ○
<i>Melodomuncula berlinensis</i> Versteegh	● ○	●
<i>Nematodinium armatum</i> (Dogiel) Kofoid and Swezy	● ○	
<i>Oblea rotunda</i> (Lebour) Balech ex Sournia	●	●
<i>Pentapharsodinium dalei</i> Indelicato and Loeblich type 1	● ○	●
<i>Pentapharsodinium dalei</i> Indelicato and Loeblich type 2	●	●
<i>Pentapharsodinium tyrrhenicum</i> Montresor, Zingone, and Marino type 1	● ○	● ○
<i>Pentapharsodinium tyrrhenicum</i> Montresor, Zingone, and Marino type 2	○	
<i>Polykrikos kofoidii</i> Chatton		○
<i>Polykrikos schwartzii</i> Bütschli	○	
<i>Protoperidinium compressum</i> (Abé) Balech	○	
<i>Protoperidinium conicum</i> (Gran) Balech	● ○	
<i>Protoperidinium oblongum</i> (Aurivillius) Parke and Dodge	●	
<i>Protoperidinium parthenopes</i> Zingone and Montresor		●
<i>Protoperidinium steidingerae</i> Balech	●	
<i>Protoperidinium subinerme</i> (Paulsen) Loeblich III		○
<i>Protoperidinium thorianum</i> (Paulsen) Balech	● ○	○
<i>Protoperidinium</i> sp. 1	● ○	○
<i>Protoperidinium</i> sp. 5		● ○
<i>Protoperidinium</i> sp. 6	●	
<i>Pyrophacus horologium</i> Stein	●	●
<i>Scrippsiella</i> cf. <i>crystallina</i> Lewis	○	
<i>Scrippsiella lachrymosa</i> Lewis	● ○	● ○
<i>Scrippsiella ramonii</i> Montresor	● ○	○
<i>Scrippsiella trochoidea</i> (Stein) Loeblich rough type	●	●
<i>Scrippsiella trochoidea</i> (Stein) Loeblich smooth type	●	● ○
<i>Scrippsiella trochoidea</i> (Stein) Loeblich large type	● ○	●

TABLE 1: Continued.

Taxon	St.40	St.45
<i>Scrippsiella trochoidea</i> (Stein) Loeblich medium type	●	○
<i>Scrippsiella trochoidea</i> (Stein) Loeblich small type	●	○
<i>Scrippsiella</i> sp. 1	●	○
<i>Scrippsiella</i> sp. 4	●	○
<i>Scrippsiella</i> sp. 5	●	○
<i>Scrippsiella</i> sp. 6	●	○
<i>Scrippsiella</i> sp. 8	●	○
<i>Thoracosphaera</i> sp.	●	○
Dinophyta sp. 2	●	○
Dinophyta sp. 7	●	○
Dinophyta sp. 17	●	○
Dinophyta sp. 26	●	○
Dinophyta sp. 30	●	○
Dinophyta sp. 33	●	○
Ciliates		
<i>Codonella aspera</i> Kofoid and Campbell	●	○
<i>Codonella orthoceras</i> Heackel	●	○
<i>Codonellopsis monacensis</i> (Rampi) Balech	●	○
<i>Codonellopsis schabii</i> (Brandt) Kofoid and Campbell	●	○
<i>Epiplocytilis undella</i> (Ostenfeld and Schmidt) Jörgensen	●	○
<i>Rabdonella spiralis</i> (Fol) Brandt	●	○
<i>Stenosemella ventricosa</i> (Claparède and Lachmann) Jörgensen	●	○
<i>Strobilidium</i> sp.	●	○
<i>Strombidium</i> cf. <i>acutum</i> (Leegaard) Kahl	●	○
<i>Strombidium conicum</i> (Lohman) Wulff	●	○
<i>Tintinnopsis beroidea</i> Stein	●	○
<i>Tintinnopsis butschlii</i> Kofoid and Campbell	●	○
<i>Tintinnopsis campanula</i> Ehrenberg	●	○
<i>Tintinnopsis cylindrica</i> Daday	●	○
<i>Tintinnopsis radix</i> (Imhof)	●	○
<i>Undella claparedei</i> (Entz) Daday	●	○
Rotifers		
<i>Brachionus plicatilis</i> Müller	●	○
<i>Synchaeta</i> sp. spiny type	●	○
<i>Synchaeta</i> sp. rough type	●	○
<i>Synchaeta</i> sp. mucous type	●	○
Crustaceans Cladocerans		
<i>Penilia avirostris</i> Dana	●	○
Crustaceans Copepods		
<i>Acartia clausi/margalefi</i>	●	○
<i>Acartia</i> sp. 1	●	○
<i>Centropages</i> sp.	●	○
<i>Paracartia latisetosa</i> (Krizcaguin)	●	○
Unidentified		
Cyst type 1	●	○
Resting Egg 1	●	○
Resting Egg 9	●	○

●: cysts observed as full (i.e., probably viable). ○: cysts observed as germinated (i.e., empty).

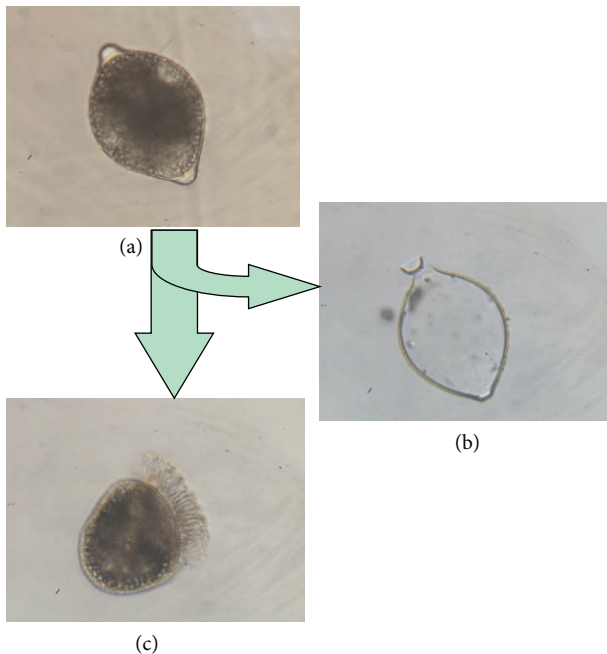


FIGURE 2: Photographs of Ciliophora cyst, with two opposite papulae (a). Its empty shell (hatch occurs from one of two papulae) (b). Germinated active stage, *Strombidium* ciliate (c).

different resting stage morphotypes were observed in this first layer (Table 2), 23 produced by dinoflagellates, 6 by ciliates, 2 by rotifers, 4 by crustaceans, and 1 undetermined. Even considering the small amount of available data, station 40 showed higher biodiversity, in terms of both number of *taxa* and diversity indexes (see Table 3). Total abundances were comparable, however, with 389 ± 127 cysts g^{-1} (average \pm s.d.) at station 40 versus 329 ± 123 cysts g^{-1} at station 45. SIMPER showed 58% dissimilarity between the assemblages of the two sites (Table 4).

The most abundant cyst morphotypes in the surface layers were calcareous cysts produced by species of the Calciadinellaceae family (Dinophyta). At station 40, five cyst morphotypes of this family accounted for 95% of total abundance, while at station 45, 99% was accounted for by just one cyst morphotype, *Scrippsiella trochoidea* medium type, confirming the lower evenness at this station.

The nMDS ordination (Figure 3, stress = 0), with the hierarchical cluster superimposed with a cutoff at 60% similarity, clearly reflects the separation between the samples from stations 40 and 45. Among these, due to its higher diversity, sample 45b is farther from samples 45a and 45c than it is from the samples of station 40.

3.3. Whole Sediment Cores. At both the investigated stations, a general decrease in total abundances was observed with depth along the sediment columns. At station 40, higher total abundance and diversity values than station 45 were registered (Figure 4), with a sharp decline between the 6th and 7th centimetres. Beyond this depth, total abundance remained below 100 cysts $100 g^{-1}$. In terms of species, *Codonellopsis*

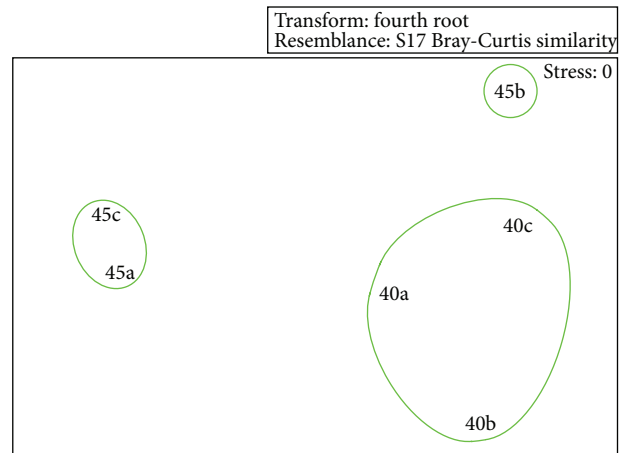


FIGURE 3: nMDS plot of surface sediment samples collected at stations 40 and 45 in Bay of Vlorë. Hierarchical clustering superimposed with cutoff at 60% similarity.

schabii cysts and *Synchaeta* sp. and *Acartia clausi/margalefi* resting eggs were continuously observed along the whole sediment column at both stations. The ciliate *C. schabii* was by far the most abundant *taxon* at station 40 (43% of total abundance), with density highest in the 2nd cm (342 ± 192 cysts $100 g^{-1}$); as with total abundance, a sharp decrease was observed between the 6th and 7th centimetres. Other important species were the copepods *Centropages* sp. (181 ± 50 resting eggs $100 g^{-1}$ at 4th cm) and *Acartia* spp. (67 ± 32 resting eggs $100 g^{-1}$ at 1st cm). At station 45 the most abundant type was *Acartia* spp. (86 ± 57 resting eggs $100 g^{-1}$ at 1st cm) followed by *C. schabii* (70 ± 60 cysts $100 g^{-1}$ at 4th cm) and *Synchaeta* sp. (41 ± 23 resting eggs $100 g^{-1}$ at 5th cm).

In the nMDS ordination (Figure 5, stress = 0.12) with superimposition of the hierarchical cluster with a cutoff at 70% similarity, all the samples from station 45 cluster together, while the samples from station 40 were widely dispersed, a sign of greater variability at this site.

The assemblage structure of the two stations differed significantly at all layers (ANOSIM $R = 0.655$; $P = 0.001$), showing 59% dissimilarity (SIMPER, Table 5).

3.4. Germination Experiments. All putatively viable (i.e., full) protistan cyst types observed were isolated and incubated under controlled conditions to obtain germination. Successful germination generally allowed us to confirm the cyst-based identification, but in some cases it enabled us to go beyond this and discriminate between cysts sharing similar morphology. For example, *Alexandrium minutum* and *Scrippsiella* sp. 1, both have a round cyst with a thin and smooth wall with mucous material attached, *Protoperidinium thorianum* and *Protoperidinium* sp. 1 cysts are both round-brown and smooth, and *Gymnodinium nolleri* and *Scrippsiella* sp. 4 both produce round-brown cysts with a red spot inside. The germination of all these cyst types allowed us to correctly identify these species.

TABLE 2: Abundance (cysts g⁻¹ dw) of probably viable resting stages (cysts) observed in surface sediments of two stations in Bay of Vlorë (Albania). Values from three replicates are reported.

	40a	40b	40c	45a	45b	45c
<i>Calciodinellum albatrosianum</i>	20.1	18.3	35.1	0.0	0.0	0.0
<i>Calciodinellum operosum</i>	0.0	0.0	11.7	0.0	0.0	0.0
<i>Gonyaulax</i> group	20.1	0.0	0.0	0.0	0.0	59.6
<i>Gymnodinium</i> sp. 1	20.1	9.2	0.0	0.0	22.2	0.0
<i>Lingulodinium polyedrum</i>	40.2	0.0	0.0	0.0	0.0	0.0
<i>Melodomuncula berlinensis</i>	40.2	0.0	0.0	0.0	0.0	0.0
<i>Oblea rotunda</i>	0.0	0.0	11.7	0.0	0.0	0.0
<i>Pentapharsodinium dalei</i> type 1	20.1	0.0	0.0	0.0	11.1	0.0
<i>Pentapharsodinium tyrrhenicum</i> type 1	40.2	18.3	23.4	0.0	11.1	0.0
<i>Protoperidinium</i> sp. 1	0.0	9.2	0.0	0.0	0.0	0.0
<i>Protoperidinium</i> sp. 5	0.0	0.0	11.7	0.0	11.1	0.0
<i>Scrippsiella ramonii</i>	0.0	9.2	0.0	0.0	0.0	0.0
<i>Scrippsiella trochoidea</i> rough type	40.2	18.3	46.8	0.0	0.0	0.0
<i>Scrippsiella trochoidea</i> smooth type	0.0	9.2	11.7	0.0	11.1	0.0
<i>Scrippsiella trochoidea</i> medium type	181.0	73.3	105.4	173.1	111.1	238.4
<i>Scrippsiella trochoidea</i> small type	80.5	64.2	58.5	230.8	0.0	0.0
<i>Scrippsiella</i> sp. 1	20.1	0.0	11.7	0.0	11.1	0.0
<i>Scrippsiella</i> sp. 4	0.0	9.2	0.0	0.0	0.0	0.0
<i>Thoracosphaera</i> sp. 1	0.0	0.0	11.7	0.0	11.1	0.0
Dinophyta sp. 2	0.0	0.0	23.4	0.0	0.0	0.0
Dinophyta sp. 17	0.0	18.3	0.0	0.0	0.0	0.0
Dinophyta sp. 26	0.0	18.3	0.0	0.0	0.0	0.0
Dinophyta sp. 33	0.0	0.0	0.0	0.0	11.1	0.0
<i>Codonellopsis schabii</i>	1.0	0.0	0.9	0.6	0.3	0.5
<i>Stenosemella ventricosa</i>	0.1	0.0	0.0	0.0	0.0	0.0
<i>Strobilidium</i> sp.	0.1	0.0	0.1	0.0	0.0	0.0
<i>Strombidium acutum</i>	0.0	0.0	0.0	0.0	11.1	0.0
<i>Tintinnopsis cylindrica</i>	0.0	0.0	0.0	0.0	0.1	0.1
<i>Undella claparedei</i>	0.1	0.0	0.1	0.0	0.0	0.0
<i>Brachionus plicatilis</i>	0.2	0.0	0.1	0.3	0.0	0.1
<i>Synchaeta</i> sp spiny type	0.3	0.2	0.0	0.2	0.0	0.1
<i>Penilia avirostris</i>	0.0	0.0	0.1	0.0	0.0	0.0
<i>Acartia clausi/margalefi</i>	1.0	0.3	0.7	1.5	0.3	0.8
<i>Acartia</i> sp. 1	0.1	0.0	0.1	0.0	0.0	0.0
<i>Centropages</i> sp.	0.3	0.2	0.0	0.2	0.1	0.2
Cyst type 1	0.0	0.0	0.0	57.7	0.0	0.0

TABLE 3: Abundance and diversity indices calculated for resting stages in surface sediments at two stations investigated in Bay of Vlorë.

	Abundance cysts g ⁻¹ dw	Total density cysts g ⁻¹ dw	S	<i>d</i>	<i>H'</i>	<i>J'</i>
Station 40	389 ± 127	1167	18 ± 2.7	2.9 ± 0.3	2.2 ± 0.1	0.7 ± 0.1
Station 45	329 ± 123	987	11 ± 4.4	1.8 ± 0.9	0.5 ± 0.2	0.5 ± 0.2

Abundance: average ± standard deviation from three replicates. Total density: sum of cyst abundances observed in three replicates from each station. S: number of taxa identified (average ± standard deviation). *d*: Margalef diversity index. *H'*: Shannon diversity index. *J'*: Pielou's evenness index.

TABLE 4: Results of SIMPER analysis for resting stages from surface sediments at stations 40 and 45 in Bay of Vlorë.

Taxa	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Station 40					
Average similarity: 56.81					
<i>Scrippsiella trochoidea</i> medium type	119.92	21.61	7.45	38.05	38.05
<i>Scrippsiella trochoidea</i> small type	67.73	15.81	6.14	27.83	65.87
<i>Scrippsiella trochoidea</i> rough type	35.13	6.44	2.78	11.34	77.21
<i>Pentapharsodinium tyrrhenicum</i> type 1	27.33	5.18	8.96	9.13	86.34
<i>Calciodinellum albatrosianum</i>	24.53	4.94	7.24	8.69	95.03
Station 45					
Average similarity: 40.37					
<i>Scrippsiella trochoidea</i> medium type	174.21	40.03	5.87	99.16	99.16

Stations 40 and 45

Average dissimilarity = 58.20.

Cysts ascribed to the paleontological *taxa* *Bicarinellum tricarinelloides* and *Calciperidinium asymmetricum* both germinated, thus confirming that they belong to modern *taxa*. The active stages obtained were tentatively identified as scrippsielloid dinoflagellates.

An unknown ciliate cyst, with a papula at both extremities, produced an active stage identifiable as belonging to the *Strombidium* genus (Figure 2).

4. Discussion

The total number of resting stage morphotypes recognized in the present study is particularly high compared with other studies in the Mediterranean. None of these studies gave a number higher than the one reported here, despite being based on a larger geographical area (the whole North Adriatic, in [24]) or a higher number of samples (157 sediment samples in [5]). This richness could be due to our enhanced ability, with the passage of time, to identify cysts from different species, but it could also depend on the consideration of different depths in the sediments. Indeed, the other mentioned studies only reported cysts from the sediment surface, while in the present case the type list grew by more than 60% when below-surface layers were considered.

As a consequence of its richness, the reported list adds 42 morphotypes to the Albanian list and 13 alternative morphotypes to already known *taxa*. This fact clearly demonstrates that the description of cyst assemblages in coastal Mediterranean areas is still far from being exhaustive.

The discovery of differences in the benthic species assemblage with respect to the plankton is partially due to the use in cyst studies of a terminology derived from paleontological studies which has yet to be standardised with reference to modern terminology. However, it is evident that the active stages in the water column assemblage of the Bay of Vlorë [15] differ in number and quality from that of the bottom sediments reported in the present study. By way of example, and only considering the surface sediment layer (i.e., the most affected by recent sinking and/or re-suspension), 4 different

species of *Scrippsiella* (Dinophyta) were isolated as cysts, but only 2 were reported [15] as active stages in the water column for the whole bay. Moreover, in this study 5 different cyst types for the single species *S. trochoidea* were identified, differing in terms of size and wall. This is evidence of great intraspecific diversity, but it could be also a sign of the presence of cryptic species, as discussed by Montresor et al. [25], differing in cyst morphology but not in that of the swimming stage.

The rotifer *Synchaeta* sp. was not found in the water column, but its resting eggs were easily recognizable and abundant, in the sediments.

While the case of *S. trochoidea* confirms that much remains to be discovered about the morphological variability of cysts produced by the same species (see [26], for Dinophyta or [27], for Calanoida), *Synchaeta* sp. is a clear case of a species not detected in the active plankton assemblage but waiting in the sediments for a favourable moment to rejoin the water column.

Also worthy of attention is the observation of a Ciliophora cyst with two papulae on opposite sides (Figure 2), which has never been reported before.

A study of plankton composition was carried out in the same site during the same scientific cruise (January 2008) as the present study [15]. In January 2008, the phytoplankton and the microzooplankton included a total of 178 categories. Considering only the main cyst producers (dinoflagellates and ciliates), examination of the water column at 16 stations gave a total of 76 *taxa* (48 dinoflagellates, 28 ciliates). The present analysis of sediments, from just 2 stations, gave a total of 75 *taxa*. This striking similarity of values was not, however, reflected in the taxonomic composition of the 2 compartments. Indeed, 36 cyst types were identified as *taxa* not present in the plankton list for the same period (January 2008). This number would be even higher if we considered only plankton from stations close to the two used here for the sediments.

It was not possible to correlate cyst abundance along the sediment column with age of deposition, which would require dating of the sediment layers. In any case, our results showed that the total abundance of cysts in the upper layers

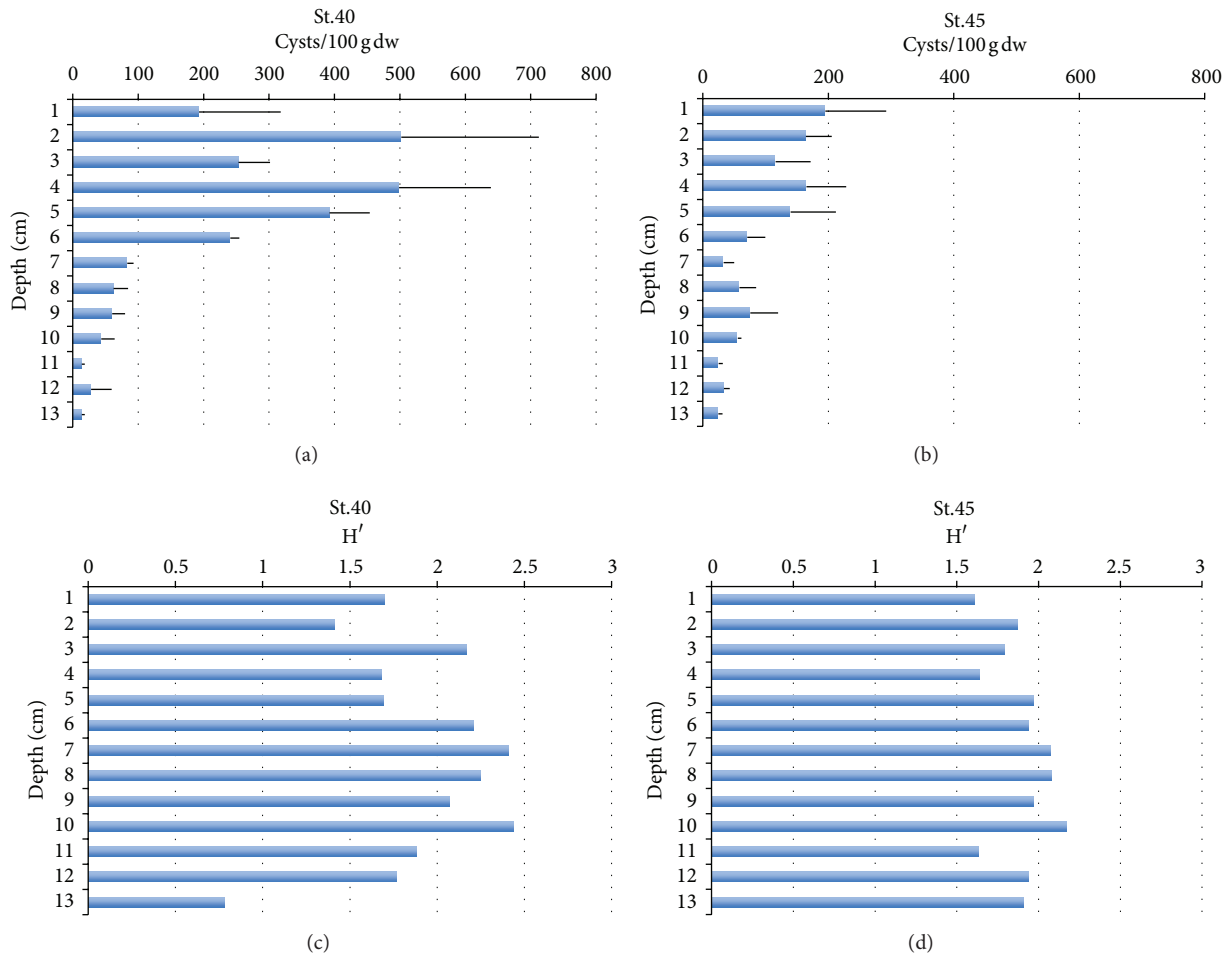


FIGURE 4: Resting stage abundance (average \pm standard deviations) and Shannon's index (H') values recorded for each cm layer of sediment cores collected at two investigated stations in Bay of Vlorë (Albania).

was up to 10 times greater than in lower ones. The sharp decrease in abundance below the 5th cm of depth, at least at station 40, does, however, suggest that an event occurred at a certain moment in the history of the plankton in the Bay of Vlorë, a suggestion that clearly requires further study. Indeed, due to its position, station 40 is a candidate for studies of the history of cyst production (and their arrival in the sediment). Located in a depression on the seabed, the depth of St. 40 (-54 m) probably favours the sedimentation of fine particles and the depletion of oxygen content, and the deposition and accumulation of sinking resting stages can thus be considered undisturbed. In addition, the observed fall in diversity from lower to upper layers could be correlated with the growth of cultural eutrophication (i.e., urban development), as proposed for Tokyo Bay and Daja Bay [28].

This situation at St. 45 (depth 28 m) is not completely identical. It is near the slope of a detritus cone where materials from river Vjosa accumulate and marine currents possibly act at a different rate from those acting on St. 40.

Incubation of encysted forms under controlled conditions to obtain germination is a useful tool for confirming the identification made by observation of the cyst. In some

cases, different species produce very similar cysts, especially when the morphology is very simple, that is, spherical, without processes or wall structures. In the present study, we observed many Dinophyta cysts with the same basic morphology, that is, round body and smooth brown wall with no apparent signs of paratabulation or spines or processes. Their germination allowed us to classify this basic type into at least 6 species. Round brown cysts are typical of *Protoperidinium* species [29, 30], but we also recognized *Diplopsalis lenticula*, *Gymnodinium nolleri*, and *Oblea rotunda*, as well as 3 additional *Protoperidinium* species. In the same way, it was possible to distinguish between *Alexandrium minutum* and *Scripsiella* sp.1, whose cysts are very similar and whose distinctive features are recognizable only after germination.

Conversely, analysis of cysts may allow us to identify species whose active stages are indistinguishable, at least by optical microscope. This is the case in the present study for the *Scripsiella* group, which produces active cells that are very difficult to distinguish, although their cysts differ in terms of the type of calcareous covering, colour, and the presence of spines [31, 32].

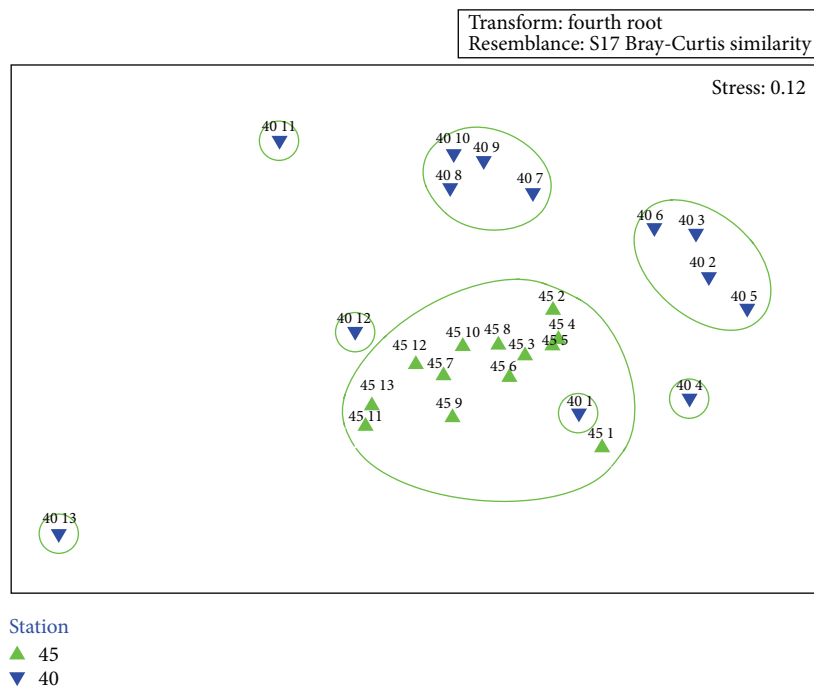


FIGURE 5: nMDS plot of samples from each cm of sediment cores collected at stations 40 and 45 in Bay of Vlorë. Hierarchical clustering superimposed with cutoff at 70% similarity.

TABLE 5: Results of SIMPER analysis for resting stages in sediment cores collected at stations 40 and 45 in Bay of Vlorë.

Taxa	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Station 40					
Average similarity: 44.16					
<i>Centropages</i> sp.	1.77	8.77	0.86	19.86	19.86
<i>Codonellopsis schabii</i>	2.10	7.00	1.31	15.86	35.72
<i>Acartia clausi/margalefi</i>	1.56	6.21	1.22	14.06	49.78
<i>Synchaeta</i> sp. spiny type	1.47	5.31	1.10	12.02	61.81
<i>Penilia avirostris</i>	1.14	4.47	0.98	10.13	71.93
<i>Brachionus plicatilis</i>	0.96	2.90	0.81	6.56	78.49
<i>Stenosemella ventricosa</i>	0.78	1.47	0.55	3.32	81.81
<i>Strobilidium</i> sp.	0.73	1.43	0.51	3.23	85.04
<i>Scrippsiella</i> spp.	0.57	0.95	0.36	2.14	87.18
<i>Gonyaulax</i> spp.	0.54	0.71	0.34	1.60	88.79
<i>Strombidium conicum</i>	0.44	0.69	0.33	1.57	90.36
Station 45					
Average similarity: 52.61					
<i>Acartia clausi/margalefi</i>	1.88	12.13	2.08	23.05	23.05
<i>Synchaeta</i> sp. spiny type	1.73	10.91	1.59	20.74	43.79
<i>Codonellopsis schabii</i>	1.43	6.73	1.12	12.80	56.59
<i>Strobilidium</i> sp.	1.12	6.05	0.92	11.51	68.10
<i>Centropages</i> sp.	1.20	6.05	1.02	11.50	79.60
<i>Brachionus plicatilis</i>	0.84	2.73	0.63	5.19	84.79
<i>Acartia</i> sp.1	0.75	2.57	0.58	4.89	89.67
<i>Lingulodinium polyedrum</i>	0.74	2.39	0.59	4.54	94.22

Groups 40 and 45.
Average dissimilarity = 58.63.

Worthy of special attention here is the recovery during the present study of Dinophyta cysts whose active stages have yet to be identified. As cysts, they are still classified with a paleontological name in accordance with their description from Pleistocene to Pliocene sediment strata in the Mediterranean [33]. Two of these cyst types (*Bicarinellum tricarinelloides* and *Calciperidinium asymmetricum*) were successfully germinated, producing motile forms recognisable as belonging to the Calciodinellaceae family. In any case their frequent observation in surface sediments in other Mediterranean areas [23, 34] and in sediment traps [35] is a clear sign that these species are present in the water column today and need to be better identified.

Conflict of Interests

The authors declare that there is no financial interest or conflict of interests involved.

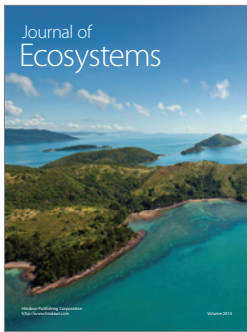
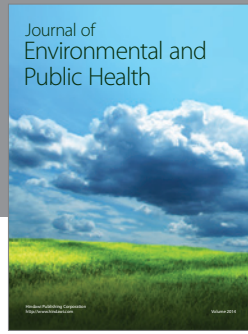
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