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Using mussels to study coastal calcareous nannoplankton associations: Case study from the west coast of Portugal

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Abstract Coccolithophore studies usually involve cruises and the filtration of large amounts of water. With the objective of reducing sampling costs and simplifying the collecting method, we tested *Mytilus galloprovincialis*, a common, local, rocky-shore filtering bivalve, as a biogenic coccolithophore sampler. A small device, named a musseldrome, was developed to harvest the mussels' abundant pellet filaments. Mussel pellets were collected every week, for seven weeks, between May and July of 2014. Abundant coccoliths were found, being dominated by *Gephyrocapsa oceanica*, followed by *Coccolithus pelagicus* and *Coronosphaera mediterranea*. Coccospheres were also present, dominated by *G. oceanica*, frequently present as agglomerates. The results show that the filtering process undertaken by mussels has little effect on coccoliths, coccospheres or even cell clusters. Preliminary results show that mussels can be used as samplers for calcareous nannoplankton community structure studies, since the estimated concentration of each species found were in agreement with results from water-column coccolithophore concentrations from off western Portugal. The advantage of this method over other sampling techniques is that weekly averages of plankton concentrations can be obtained – something that the usual devices used in sampling (phyto)plankton cannot duplicate.

Keywords Coccolithophores, coastal assemblages, mussels, calcareous nannoplankton, sampling methods

1. Introduction

Coccolithophores constitute a major component of planktonic communities throughout the world's oceans (Okada & McIntyre, 1977). They are of remarkable interest, being among the main primary producers, and playing a distinct role in oceanic ecosystems (Field et al., 1998; Balch, 2004). They have a significant role in the global carbon and sulphur cycles (and therefore climate regulation) through direct involvement in ocean–atmosphere gas exchange (Malin & Steinke, 2004), which arises from the fact that they are among the most important pelagic calcifying organisms in the modern ocean (Baumann et al., 2005), accounting for up to 20% of total carbon fixation in some systems (Poulton et al., 2007). For these reasons, studies on coccolithophores have gained importance over the past decade; however, studying modern communities is usually expensive, since boat trips are necessary for sampling. Moreover, coccolithophores are mainly oceanic, and their role in coastal environments is still poorly understood (Ferreira & Cachão, 2005), although some species are known to occur in neritic environments when the conditions are right, such as *Gephyrocapsa oceanica* (Ferreira & Cachão, 2005), or have even become specialised in such environments, such as *Coccolithus pelagicus* (Cachão & Moita, 2000).

With the objective of reducing costs, simplifying the sampling method, and increasing our knowledge regarding coccolithophores in coastal environments, filtering species of coastal bivalves were examined for their role as coccolithophore samplers.

Mussels appear to be the most promising organisms for such an approach, since they filter large quantities of water per day (up to ~30L; Riisgård et al., 2011), and have a particle size selection process (Bougrier et al., 1997; Norén et al., 1999; Riisgård et al., 2011), in which particles >4μm go through a non-selective process (Møhlenberg & Riisgård, 1978), separating suspended particles from algae, the former being expelled with the mucus (Riisgård et al., 2011). Mussels produce two kinds of faeces – intestinal and glandular. When ample food is available, the intestinal faeces constitute the vast majority of the excreted material, but, when little food is present, this decreases considerably in amount. The intestinal faeces consist of less-digested material that is transported directly into the hindgut, bypassing the digestive diverticula. The glandular faeces stem from food that gets into the digestive diverticula, and so is much more thoroughly digested (Riisgard et al., 2011). Both expelled faeces types are deposited beneath

the mussel beds, and contain undigested and rejected material.

A small structure – the musseldrome (Fig. 1) – was developed to collect mussel pellets from a selected set of mussel individuals. The mussel faecal pellets were then prepared and sampled for the identification and counting of calcareous nannoplankton, in the form of (cocco)liths and (cocco)spheres. Coccoliths of calcareous nannoplankton were found to be abundant among the suspended particles, and present a useful proxy for modern community structure studies. Coccospheres were also present, and they provide an indication of the present community structure. Our primary results show that mussels have great potential for use in coccolithophore studies.

2. Material and methods

The study was performed at Peniche (39°N, 9°W), on the western coast of Portugal, using individuals of the species *Mytilus galloprovincialis*.

The musseldrome consists of an adapted, inverted 5L plastic carboy. First the carboy was cut in half. A net with a 0.5cm-diameter mesh was sewn in place, functioning as a barrier between each half of the carboy and, at the same time, unifying the two halves. The capped end of the bottle was used as the pellet collection end; the other half was cut, creating slits for seawater to circulate through. The structure was placed in the water-column, 4.5m above the seafloor, so that the musseldrome was never out of the water (at the low tide of a high-amplitude tide, it stood 1.5m below the water surface). To stabilise it, a 150g fishing weight was tied to the cap. Six specimens, with initial sizes of between 27 and 47mm, were introduced through the slits in the upper half.

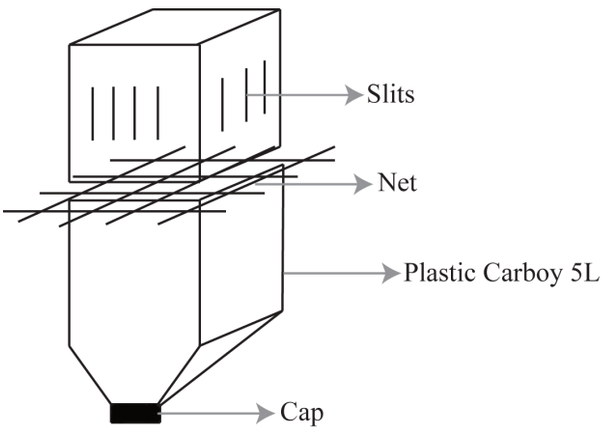

Figure 1: Schematic of the musseldrome. A 5L carboy cut in half, with a net placed between the two halves. The upper and lower parts of the carboy are sewn together with a saltwater-resistant twine to close the musseldrome and keep the mussels inside. Slits are made in the upper part to allow water to flow through and feed the mussels. A fishing weight is tied to the cap to keep the musseldrome fixed in position

Although removed from a nearby mussel bed, the six individuals were left inside the musseldrome for one-month prior to the first faecal filament collection, which were then collected weekly for seven sequential weeks.

Pellet collection was performed by removing the musseldrome from the water, and carefully opening the cap of the collection bottle, so as not to lose any of the sample. After decanting the sample into a bottle, the musseldrome was closed and placed back in the water.

Samples were prepared for observation according to the following four-step method: 1) volume makeup and subsampling; 2) decantation; 3) drying; 4) preparation for microscopy screening. The first step was performed in situ. Filtered water was added to the bottle the pellets were decanted into to make up the volume to 2L. This pellet suspension was homogenised, and three subsamples of 50ml each were prepared. The second step was performed in the lab. Each subsample was subjected to ultrasonic cleaning for 15 minutes (to disaggregate the organic matter) in a P Selecta ultrasonicator, and was then homogenised, after which 1ml was added to buffered water and left to settle in a Petri dish over a coverslip (random settling; Flores & Sierro, 1997). After 24 hours, the preparations were dried at 60°C. Finally, each sample was prepared for microscopic observation. The dried coverslip was removed from the Petri dish and mounted on a slide using a synthetic resin medium (Entellan™), heated to remove air-bubbles, and left overnight to solidify.

The pellet contents were observed under a Zeiss Ortholux II Pol-BK transmitted-light binocular microscope, using polarised light and at a magnification of 1250x.

To calculate the final concentration of liths and spheres per litre, we used the equations developed by Riisgård et al. (2014) for *Mytilus edulis*, since there are no specific equations yet for *M. galloprovincialis*. The equations are dependent on mussel size, which changed due to growth over the seven-week sampling period (see Table 1). We assumed a linear growth over this period, so as to adjust the equations for calculation of the final concentration. The equation used to calculate the amount of litres per hour filtered by each mussel was:

$$F = 0.00135L^{2.088}\ \mathrm{Lh^{-1}}$$

, where L is the mussel length. This equation was devel-

oped for mussels at 12˚C. The same authors estimated an increase of 2.5% in filtration rate for each 1˚C rise in temperature. We adjusted our results to reflect a mean water temperature of 18˚C. The final results can be seen in Table 2.

Mussels	Length (mm) 25/04/2014	Length (mm) 26/07/2014	Difference (mm)	Growth (%)
M#1	32	54	22	68.75
M#2	47	64	17	36.17
M#3	43	60	17	39.53
M#4	27	49	22	81.48
M#5	28	51	23	82.14
M#6	34	58	24	70.59

Table 1: Mussel size variation over the one-month acclimatisation and seven-week sampling period

3. Results

The data correspond not to discrete samples, but to weekly-averaged samples. Some variation was observed over the seven-week period (see Table 3); however the coccolith species distribution remained constant, with a dominance of *G. oceanica*, followed by *G. muellerae* with *Coronosphaera mediterranea*, *Helicosphaera carteri* and *C. pelagicus* as the abundant species. The remaining species were low in abundance/presence, with four species comprising 2% of the coccoliths counted.

Gephyrocapsa oceanica coccospheres were particularly abundant in the mussel pellets, in the presence of agglomerates and *G. muellerae*. Other species showed scarce abundances, usually being absent (see Table 4).

4. Discussion and conclusions

The more surprising results were the presence of coccospheres. Not only were several coccospheres present in the mussel pellets, but, in the case of *G. oceanica*, they were found frequently in the agglomerates (see Figure 2). Also, coccospheres of *C. mediterranea*, a non-placolith-bearing species, were present on the slides. This means that neither the filtering process undertaken by the mussels, nor the ultrasonication step in the sample preparation process, affected the coccospheres.

For comparison of our results with those of other studies on the present-day community structure of coccolithophores along the west coast of Portugal, we used the Guerreiro et al. (2015) study from the Nazaré Canyon and adjacent western continental margins and slopes, which used seafloor sediment surface samples. A close agreement of our results with those of Guerreiro et al. (2015) was found. *Calcidiscus leptoporus*, *C. mediterranea* and *C. pelagicus* showed similar occurrences to those from the Nazaré Canyon. *Gephyrocapsa oceanica* and *H. carteri* showed results similar to the ones from the Extremadura Slope. This could mean that Peniche is under the influence of both oceanographic realms, since it is between the Extremadura shelf slope and the Nazaré Canyon (see Figure 3).

Gephyrocapsa muellerae, with a mean relative abundance of 10%, was the only species found not to agree with the results found in Guerreiro et al. (2015), which were 25% from the Extremadura Slope and 40% at the Nazaré Canyon.

The most probable explanation for this discrepancy regarding *G. muellerae* is the fact that mussel pellets are very rich in organic matter, making it difficult to observe smaller coccolithophore species (see Figure 4). This could explain the lower values for this particular – smaller – species. For this reason, only *Emiliania huxleyi* and *Gephyrocapsa ericsonii* coccospheres were studied.

The other species present in our study were not taken into account in the Guerreiro et al. (2015) analysis, so there is no data available for comparison; however, the species for which we have no data represent less than 2% of our total abundances.

Our preliminary results showed a reasonably good match, in terms of the general structure and proportions of the coccolithophore community, between the data obtained by musseldrome and that from traditional phytoplankton sampling methods.

A study by Moita et al. (2010) found a strong presence of *H. carteri* and *C. mediterranea* in nearshore and coastal samples along the Peniche coast during the summer. This is in agreement with our results, which identified these two species as two of the most relevant in the coccolithophore assemblages. Ferreira & Cachão (2005) showed that *G. oceanica* increased in abundance towards the shore on the Guadiana, Algarve coast, while *G. muellerae* showed the opposite behaviour. This is in accordance with our results, with their strong dominance of *G. oceanica*, and may explain the low presence of *G. muellerae* in our study.

The results from using mussel pellets for coastal calcareous nannoplankton studies are promising. The use of mussels also provides data representing nearshore coccolithophore communities, for which, at present, there are few data. Now that the methodology has been proven to

Week	Filtration rate per mussel with size variation (L/week at 18˚C)						TOTAL (L)
	M#1	M#2	M#3	M#4	M#5	M#6	
5	577.35	1047.24	889.54	436.87	473.07	662.37	4086.44
6	639.27	1111.95	949.06	490.62	531.62	734.91	4457.43
7	704.48	1178.7	1010.58	547.63	593.75	811.39	4846.52
8	773.00	1247.47	1074.13	607.90	659.46	891.80	5253.76
9	844.84	1318.28	1139.7	671.46	728.77	976.20	5679.24
10	920.01	1391.12	1207.29	738.33	801.69	1064.57	6123.01
11	998.52	1466.02	1276.92	808.50	878.25	1156.95	6585.15
12	1080.39	1540.44	1346.23	882.00	958.84	1254.23	7062.14

Table 2: Filtration rate per mussel (L/week), with size variation, at 18˚C. The week number starts at five because the first four weeks correspond to the acclimatisation period. Although this period is not applicable to our results, it is important because mussel growth occurred during this time

Sample	Mean values								
	Go	*Gm*	*Cp*	*Hc*	*Sp*	*Bb*	*Coron*	*Scy* sp.	*Cl*
M# 040614	2.35E+05	1.98E+04	1.23E+04	2.14E+04	5.88E+03	0.00E+00	2.83E+04	0.00E+00	1.07E+03
%	*72.61*	*6.11*	*3.80*	*6.60*	*1.82*	*0.00*	*8.75*	*0.00*	*0.33*
M# 100614	1.96E+05	4.25E+04	1.24E+04	1.47E+04	1.31E+03	6.54E+02	2.78E+04	3.27E+02	4.25E+03
%	*65.36*	*14.16*	*4.14*	*4.90*	*0.44*	*0.22*	*9.26*	*0.11*	*1.42*
M# 180614	1.85E+05	3.13E+04	1.05E+04	1.38E+04	2.10E+03	0.00E+00	2.86E+04	3.01E+02	1.50E+03
%	*67.77*	*11.44*	*3.85*	*5.06*	*0.77*	*0.00*	*10.45*	*0.11*	*0.55*
M# 250614	1.66E+05	2.52E+04	9.15E+03	1.55E+04	2.50E+03	2.77E+02	2.88E+04	8.32E+02	1.11E+03
%	*66.48*	*10.13*	*3.67*	*6.24*	*1.00*	*0.11*	*11.58*	*0.33*	*0.45*
M# 020714	2.30E+05	4.23E+04	1.19E+04	2.23E+04	6.16E+03	0.00E+00	4.42E+04	0.00E+00	1.15E+03
%	*64.23*	*11.82*	*3.33*	*6.23*	*1.72*	*0.00*	*12.35*	*0.00*	*0.32*
M# 090714	2.24E+05	3.14E+04	1.32E+04	1.96E+04	5.00E+03	3.57E+02	3.10E+04	0.00E+00	2.14E+03
%	*68.56*	*9.61*	*4.04*	*6.00*	*1.53*	*0.11*	*9.50*	*0.00*	*0.66*
M# 160714	2.24E+05	2.19E+04	1.29E+04	1.63E+04	2.99E+03	9.96E+02	2.32E+04	3.32E+02	1.99E+03
%	*73.53*	*7.19*	*4.25*	*5.34*	*0.98*	*0.33*	*7.63*	*0.11*	*0.65*
Mean %	***68.36***	***10.06***	***3.87***	***5.77***	***1.18***	***0.11***	***9.93***	***0.09***	***0.62***

Table 3: Lith concentration/L for each sample and species. *Go – G. oceanica*, *Cp – C. pelagicus*, *Gm – G. muellerae*, *Hc – H. carteri*, *Sp – S. pulchra*, *Bb – B. bigelowi*, *Coron – C. mediterranea*, *Scy* sp. – *Scyphosphaera* sp., *Cl – C. leptoporus*

Week		*Go*	*Gm*	*Ge*	*Cp*	*Bb*	*Eh*	*Cl*	*Coron*
26/05 – 1/06	1	4.00E+02	7.51E+01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
2/06 – 8/06	2	2.84E+02	6.67E+01	0.00E+00	0.00E+00	0.00E+00	3.34E+01	0.00E+00	0.00E+00
9/06 – 15/06	3	1.02E+03	8.34E+01	1.67E+01	0.00E+00	1.67E+01	5.01E+01	0.00E+00	0.00E+00
16/06 – 22/06	4	1.15E+03	6.67E+01	3.34E+01	0.00E+00	0.00E+00	8.34E+01	1.67E+01	1.67E+01
23/06 – 29/06	5	1.35E+03	7.51E+01	2.50E+01	0.00E+00	0.00E+00	1.50E+02	0.00E+00	0.00E+00
30/06 – 6/07	6	8.51E+02	2.50E+01	0.00E+00	2.50E+01	0.00E+00	1.25E+02	0.00E+00	2.50E+01
7/07 – 13/07	7	1.03E+03	2.50E+01	0.00E+00	6.66E+03	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Table 4: Mean values of coccosphere concentration/L for each week. *Go – G. oceanica*, *Gm – G. muellerae*, *Ge – G. ericsonii*, *Cp – C. pelagicus*, *Bb – B. bigelowii*, *Eh – Emiliania huxleyi*, *Cl – C. leptoporus*, *Coron – C. mediterranea*

produce results, new campaigns are being prepared to gather further data using mussels as coccolithophore samplers. Future works will aim at comparing mussel pellet data with water-column data collected from the vicintiy of the musseldrome. Such a study will be carried out throughout the year, so that the procedure can be validated/understood for different seasons. This is particularly important, since the Portuguese west coast presents seasonal upwelling, which induces changes in phytoplankton communities. The morphological details of the coccoliths will also be addressed in upcoming sampling campaigns.

Finally, the several processes by which particles arrive at the mussel colony, and that affect the mussels' uptake of coccolithophores, are still open questions in such a study. The future development of this project will aim to understand those processes and how they impact this method of nearshore coccolithophore community study.

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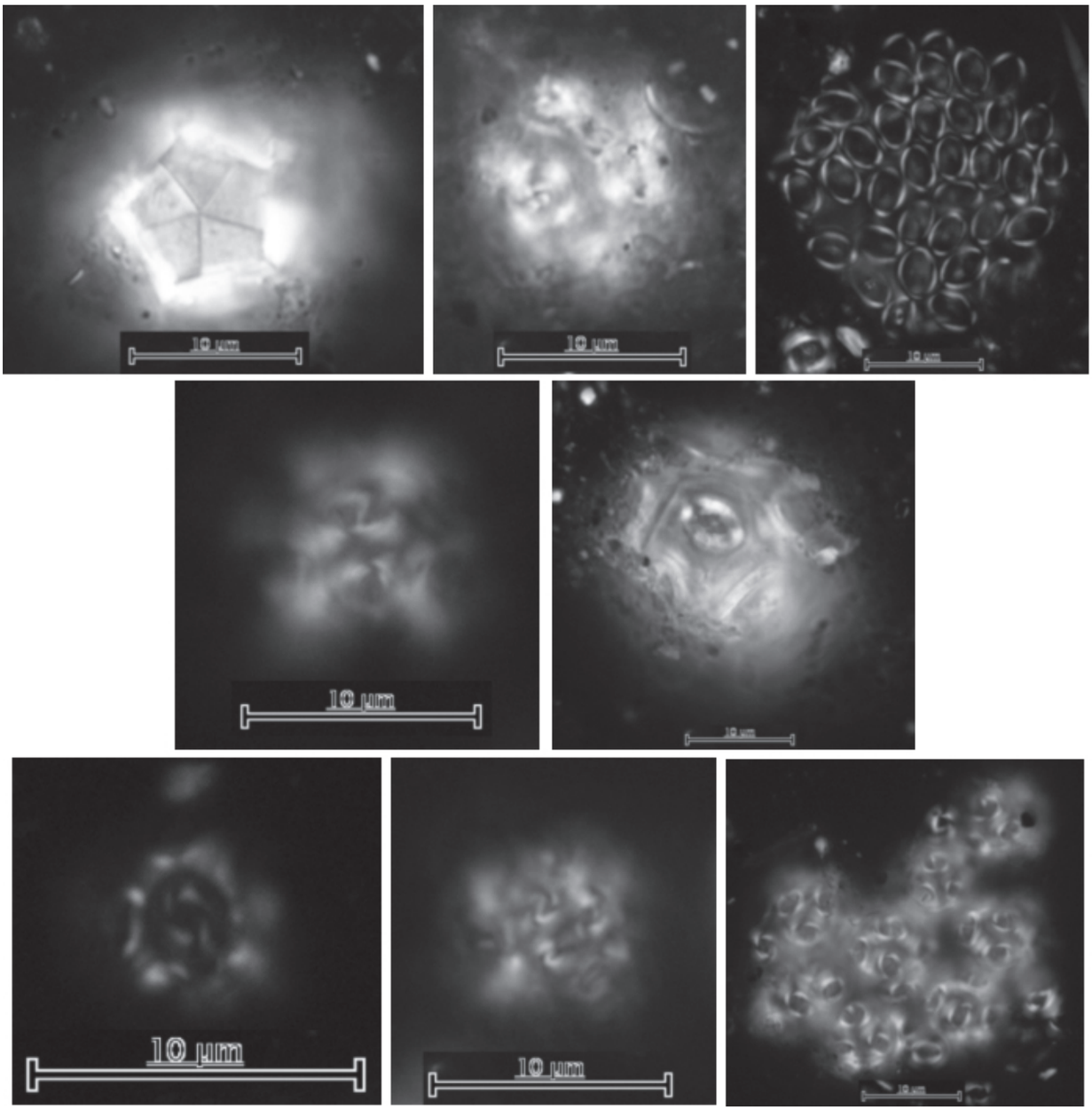

Figure 2: **Top row**, left to right – *Braarudosphaera bigelowii* coccosphere, *Calcidiscus leptoporus* coccosphere, *Coronosphaera mediterranea* coccosphere. **Middle row**, left to right – *Gephyrocapsa muellerae* coccosphere, *Coccolithus pelagicus* coccosphere. **Bottom row**, left to right – *Emiliania huxleyi* coccosphere, *Gephyrocapsa ericsonii* coccosphere, *Gephyrocapsa oceanica* eight-coccosphere agglomerate

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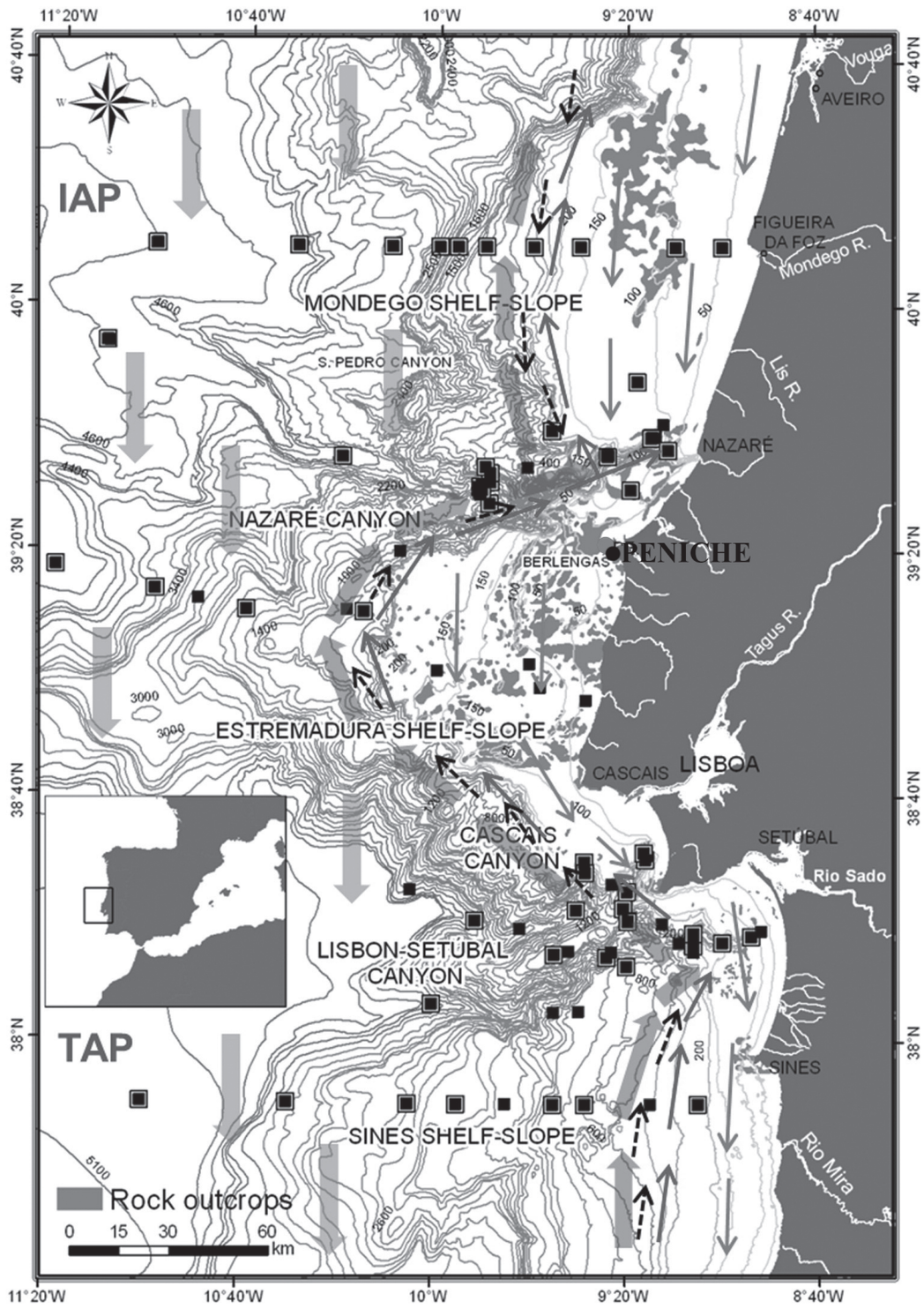

Figure 3: Sampling location and oceanographic realm. Black dot represents the sampling area at Peniche. Adapted from Guerreiro et al. (2015)

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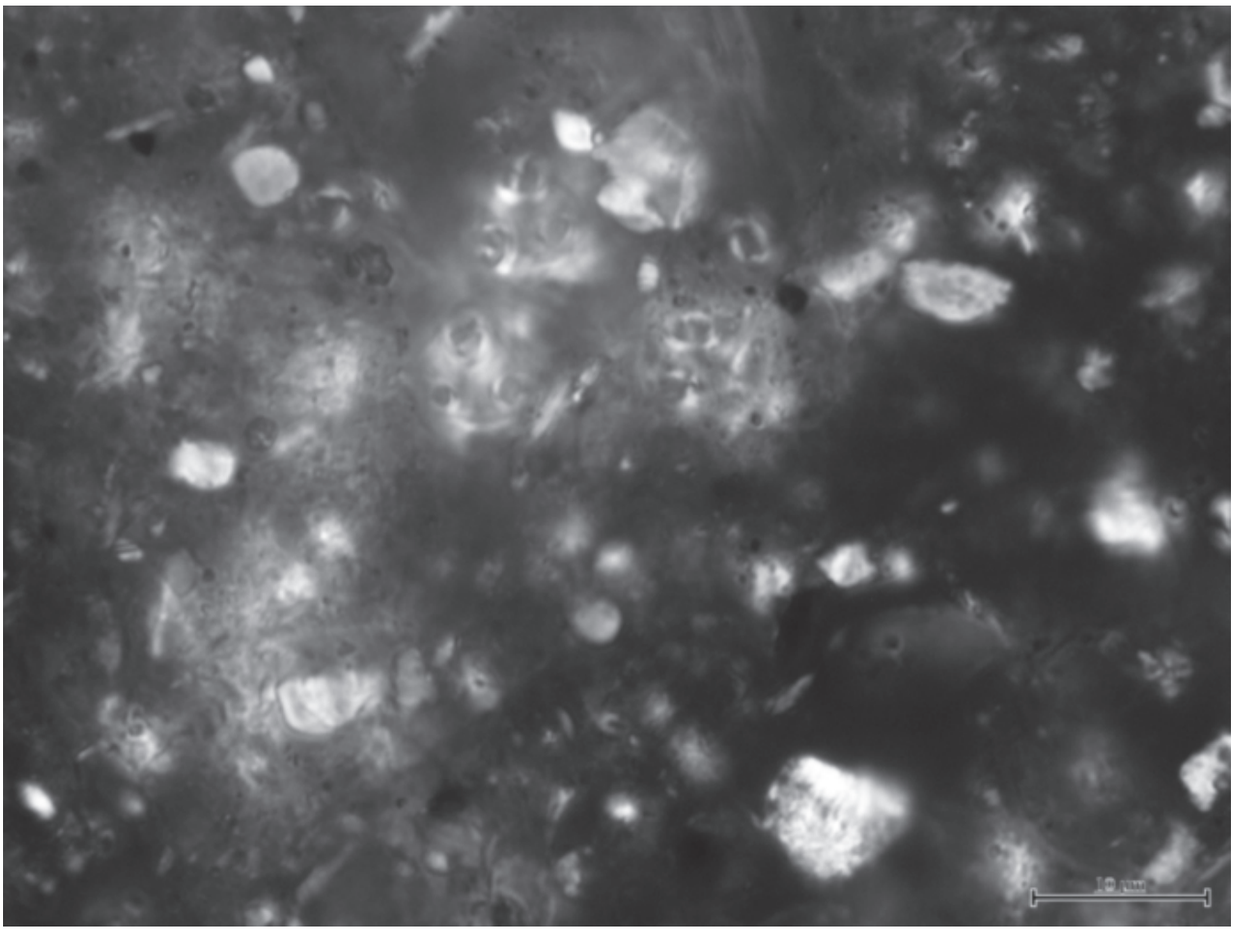

Figure 4: Typical field observation of mussel pellets, with high amounts of organic matter. Three *G. oceanica* coccospheres can be seen close to the centre of the image

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