THE EFFECT OF FORMALDEHYDE AND IODINE AS FIXATIVES FOR PHYTOPLANKTON AND PROTOZOOPLANKTON SAMPLES FROM THE SOUTHERN BRAZILIAN COAST

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INTRODUCTION

The quantitative analysis of phyto- and protozooplankton usually requires sample fixing and storage with chemical fixatives. Glutaraldehyde, osmium tetroxide, Bouin's fixative formaldehyde and iodine are the most commonly used (Wood et al., 1969). Since all these fixatives are selective and no single fixative is suitable for all kinds of organisms, the choice depends on the aim of the investigation (Throndsen, 1978) and on the most important group of the studied region.

The aim of this study was to compare the quantitative analysis of phytoplankton and protozooplankton fixed with both neutralized formaldehyde and acetic iodine. In addition, the effect of storage time was evaluated for both fixatives.

METHODS

Fixatives comparison

Water samples were collected in October 1987 at 16 stations off Rio Grande do Sul State aboard RV "Atlantico Sul" (Fig. 1). From each station, two subsamples were stored in 250 ml ambar flasks, one fixed with borax neutralized formaldehyde (NF) and another with acetic iodine (AI, both diluted with the sample up to 1%). Cell counts were performed within 12 months with an inverted phase contrast Nikon microscope (Utermöll, 1958); 10, 50 and 100 ml settling chambers were used and the counting procedure according to Edler (1979).

The whole settling chamber area was analyzed with 100x magnification for large and less abundant cells. Small centric and pennatae diatoms, naked dinofla-

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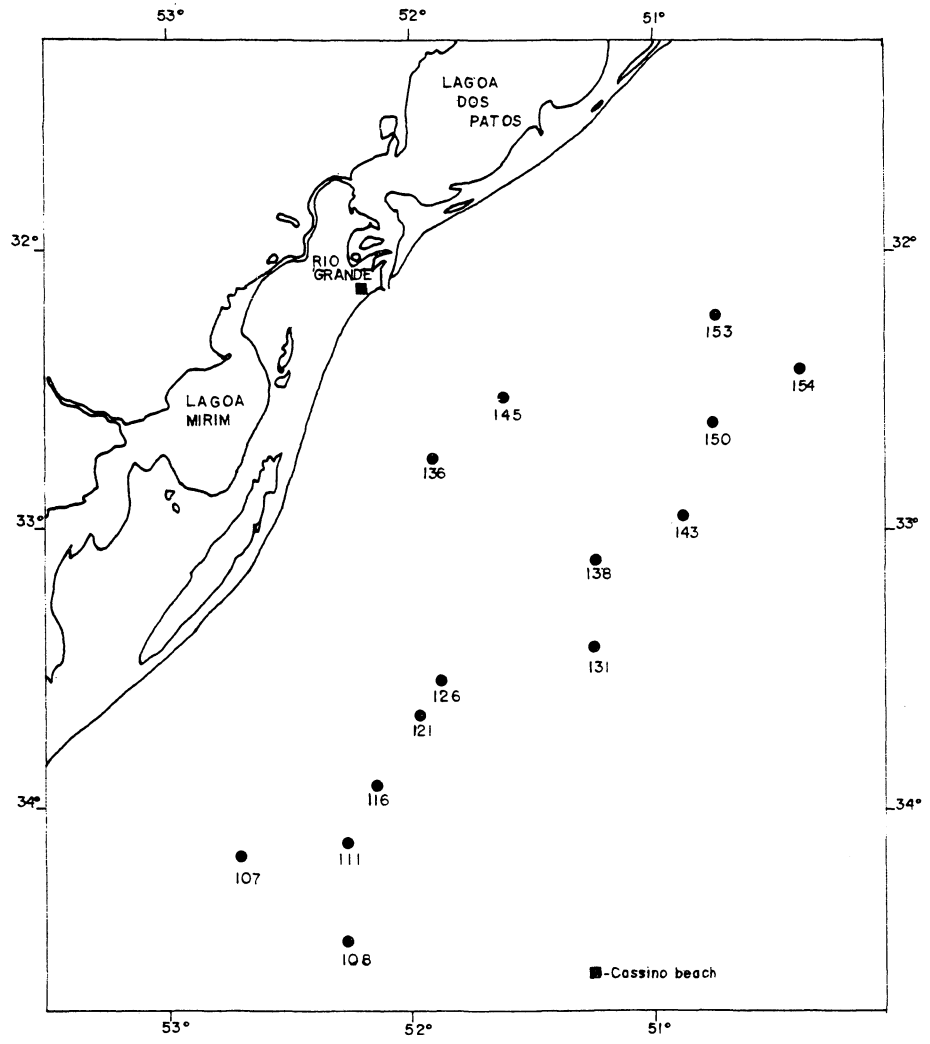

Figure 1. Map showing the position of sampling stations.

gellates and other nanoflagellates were counted under 200x magnification in, at least, 2 and a maximum of 6 diameter transects.

Statistical analyses were performed using the normalized non-parametric Wilcoxon test, at a significance level of 5%. Minimum cell counts for both subsamples were 30 cells. When counts were greater than 30 or equal to zero in only one of the subsamples, the value of 15 was decided for the other subsample.

Time storage effect

In order to test the effect of storage time, surface water was obtained from Cassino Beach (Lat. 32°10'S – Long. 52°10'W, Fig. 1) in March 13, 1989; 8 subsamples were fixed with neutral iodine diluted with the sample up to 1% and another 8 with borax neutralized formaldehyde. Counts were done at the sampling day; weekly, during the 1st month and then monthly until the 4th month. The counting procedure was as described above.

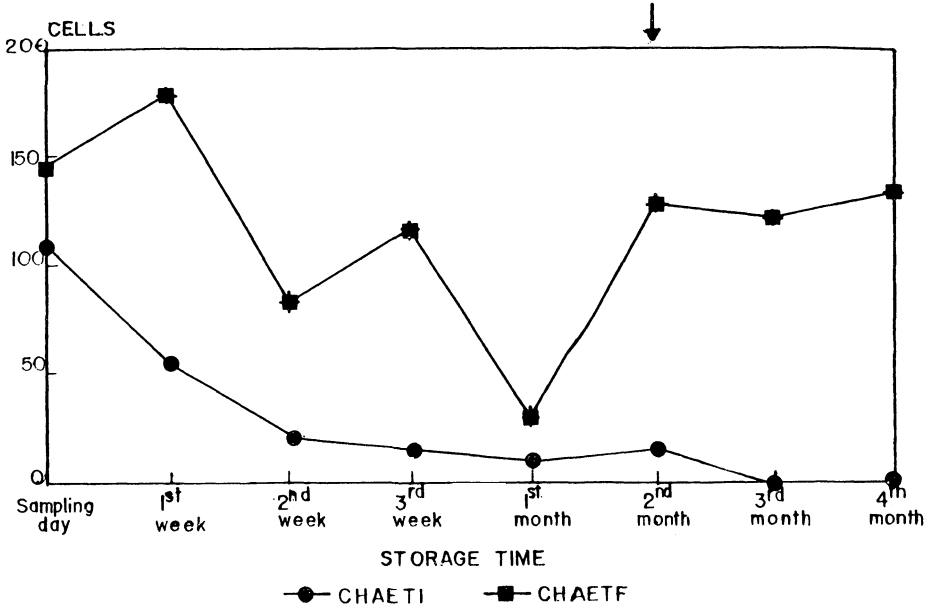

Figure 2. Number of *Chaetoceros* spp. counted in 4 diameter transects, in sub-samples fixed with neutral iodine (CHAETI) and neutral formaldehyde (CHAETF) after some storage times. Counts after arrow were done with the addition of Rose Bengal.

RESULTS AND DISCUSSION

In both tests, particle agglutination was often observed in formaldehyde-fixed subsamples, whereas iodine-fixed were homogeneously dispersed. Smaller cells could thus be hidden within the agglomerates and therefore the counts in the NF subsamples might have been underestimated, specially in seston rich samples. We will only discuss the diatoms data from the storage time test as they were the single group with significant counts.

Diatoms

Large centric and pennatae diatoms presented significant greater counts in NF-fixed subsamples from the shelf stations (tables I and II). The main genera of

this group were *Coscinodiscus, Thalassiosira, Lauderia, Pleurosigma* and *Thalassionema*. However, the smaller diatoms did not show significant differences between subsamples (Tables I and II), being dominated by chain-forming genera (*Chaetoceros, Leptocylindrus, Thalassiosira* and *Nitzschia*).

In iodine-fixed subsamples of the storage time test a continuous decrease of *Chaetoceros* spp. (e.g. *C. curvisetum, C. brevis, C. yan-heurcki*) counts were observed. In formaldehyde-fixed subsamples, cell counts also initially decreased with time of storage. However, as we suspected that debris could be hiding the cells we began to use Rose Bengal after the second month of the experiment, in order to distinguish them better. After this, the cells numbers became similar to the initial counts in NF-fixed subsamples.

It has been mentioned that the low pH of acetic iodine is better for diatom's silica conservation (Hasle, 1978b), and for maintaining chain forming species (Harbour apud Hasle 1978b). However, Boalch (apud Throndsen 1978) observed that silica was dissolved by AI as also observed in the present study. In our case siliceous structures of diatoms were disrupted in AI-fixed cells.

Unexpectedly, there were no significant differences of small chain forming diatom concentrations, in samples fixed with both AI and NF. Probably, in AI-fixed samples the breakage of chains produced isolated cells which tended to distribute homogeneously in the chamber. This was not the case of NF-fixed samples. Therefore, the minimum statistically significant number of 30 cells, counted in at least 2 diameter transects as adopted in the present study was only suitable for AI-fixed samples, underestimating the cell numbers in NF samples. The agglutination and hiding of smaller cells observed in NF-fixed samples may also have contributed to this underestimation.

Dinoflagellates

In relation to dinoflagellates, armored (*Ceratium, Protoperidinium* and *Scrippsiella*), larger naked (*Gymnodinium, Gyrodinium* and *Torodinium*) and small naked (unidentified gymnodiniales) had higher counts in AI-fixed samples (Table I, II and III) as also mentioned by Taylor (1978).

Nanoflagellates and oligotrichina ciliates

Nanoflagellates were more abundant in AI than NF-fixed samples (Table I, II and III). However, the flagella were better fixed with NF as also observed by Bloem et al. (1986). In contrast, Throndsen (1978) and Taylor (1978) mentioned that AI is the best fixative for flagella.

We did not detect a significant difference between the Oligotrichina ciliates numbers in AI and NF-fixed samples.

The advantages and disadvantages of iodine and formaldehyde observed in the present study and those mentioned by other authors are summarized in table IV. According to our results we recommend that quantitative samples of phyto- and protozooplankton should be preserved in duplicate with formaldehyde and iodine. Diatoms are better preserved with formaldehyde and in this case the addi-

tion of Rose Bengal is required to distinguish cells within agglomerates. Dinoflagellates and other nanoflagellates should be preserved with iodine.

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ABSTRACT

Formaldehyde and iodine (lugol) were evaluated as fixatives for phytoplankton and protozooplankton samples from the coast of Rio Grande do Sul, Brazil. Diatoms were better preserved with formaldehyde whereas dinoflagellates and other nanoflagellates were better preserved with iodine. No significant differences were observed for ciliates. The use of duplicate samples fixed with both fixatives is recommended.

Key works: Phytoplankton, protozooplankton, countings, fixatives.

RESUMO

O efeito do formaldeído e do lugol como fixadores em amostras de fitoplâncton e protozooplâncton da costa extrema sul do Brasil. Foi comparada a ação do lugol e da formalina na fixação de amostras de fitoplâncton e protozooplâncton obtidas na costa do Rio Grande do Sul, Brasil. As diatomáceas cêntricas e penadas foram melhor preservadas com formalina, enquanto que os dinoflagelados e nanoflagelados foram melhor preservados com lugol. Não foram encontradas diferenças significativas nas contagens de ciliados fixados em ambos os reagentes. Com base nos resultados, recomenda-se a coleta de amostras de fitoplâncton e protozooplâncton em duplicata, utilizando-se o lugol e a formalina.

Palavras-chave: Fitoplâncton, protozooplâncton, contagens, fixadores.

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Table I. Cell counts in NF- and AI-fixed samples obtained from shelf waters off Rio Grande do Sul state.

		GROUPS											
		CENTR DIAT		PENAT DIAT		NAKED DINO		ARMOR DINO		ALOR CILIA		NANOFL	
	STATION	IO	FO	IO	FO	IO	FO	IO	FO	IO	FO	IO	FO
WHOLE	107	34	278	–	–	–	–	–	–	18	39		
	107-25m	147	284	–	–	0	0	–	–	–	–		
	108	0	0	0	0	0	0	•	0	134	109		
	111	756	1295	–	–	64	•	–	–	41	25		
	116	410	748	–	–	127	•	82	68	–	–		
	116-20m	615	1494	30	36	–	–	–	–	–	–		
	121	146	171	–	–	•	0	86	35	–	–		
	126	146	491	–	–	218	37	65	46	–	–		
	131	85	93	–	–	118	25	47	•	38	27		
	136	374	442	•	80	45	51	132	105	22	41		
	138	298	1023	•	47	191	27	103	85	35	•		
	143	–	–	–	–	47	•	38	•	32	•		
	145	234	206	266	504	109	•	66	•	–	–		
	150	54	92	20	39	32	41	57	44	–	–		
	153	–	–	0	•	32	•	–	–	48	•		
	154	115	50	48	76	44	•	–	–	42	•		
TRANS	107	2380	1641	44	93	99	•					169	•
	107-25m	556	783	56	54	52	•					192	96
	108	•	336	–	–	84	0					582	264
	121	219	711	105	99	–	–					–	–
	126	587	1022	253	230	36	0					–	–
	131	•	0	•	0	77	47					–	–
	136	273	242	101	246	223	56					101	•
	138	292	755	81	121	38	0					–	–
	143	–	–	–	–	86	•					•	0
	145	161	119	0	0	33	•					–	–
	150	–	–	0	0	81	32					38	•
	153	•	0	–	–	34	•					–	–
	154	0	•	0	•	80	•					83	•

(–) non-significant counts in both subsamples.
(•) non significant counts in only one of subsamples and considered equal 15.
( ) the group was not evaluated.
TRANS = Smaller and more abundant cell counts, in 6 transects; WHOLE = Larger and less abundant cell counts in the whole chamber; IO = Iodine; FO = Formaldehyde; CENTR DIAT = Centric diatoms; PENAT DIAT = Penatae diatoms; NAKED DINO = naked dinoflagellates; ARMOR DINO = Armored dinoflagellates; ALOR CILIA = Aloricate ciliates; NANOFL = Nanoflagellates.

Table II. Normalized Wilcoxon test results for groups counted in whole chamber surface.

GROUPS	IODINE – FORMALD. + DIFFER.	– DIFFER.	Z	P(z)
Centric Diatoms	2	11	2.59	0.00971*
Penatae diatoms	0	7	2.28	0.02249*
Armored dinoflagellates	10	0	2.75	0.00592*
Naked dinoflagellates	10	2	2.78	0.00535*
Aloricate ciliates	7	2	1.36	0.17307

* indicates significant difference at 5% confidence level.

Table III. Normalized Wilcoxon test results for groups counted in 6 diameter transects.

GROUPS	IODINE – FORMALD. + DIFFER.	– DIFFER.	Z	P(z)
Centric Diatoms	5	6	0.76	0.4498
Penatae diatoms	4	4	0.77	0.4412
Naked dinoflagellates	12	0	3.02	0.0025*
Nanoflagellates	7	0	2.28	0.0224*

* indicates significant diferences at 5% confidence level.

Table IV. Comparison between formaldehyde and iodine as fixatives for quantitative phytoplankton samples.

FORMALDEHYDE		IODINE		REFERENCE
Advantages	Disadvantages	Advantages	Disadvantages	(Year)
Good preservation of coccolithophorids. Longer time of storage.	Thrown off the flagella. Disruption of naked flagellates. Slow sedimentation.	Good preservation of naked flagellates and flagella. Staining. Fast sedimentation.	Bad preservation of coccolithophorids. Short time of storage. Excessive staining.	EDLER (1979) WOOD (1969) THRONDSEN (1978) HASLE (1978a)
		Good preservation of diatoms silica.		HASLE (1978b)
		Good preservation of diatoms chains.		HARBOUR apud HASLE (1978b)
Good preservation of diatom structures and chains. Good preservation of flagella.	Bad preservation of naked flagellates. Agglomerates formation.	Good preservation of naked flagellates numbers.	Bad preservation of diatoms with breakage of chains.	PRESENT STUDY