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In situ effects of selected preservatives on total carbon, nitrogen and metals collected in sediment traps

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ABSTRACT

The concentration and chemical composition of preservative or poison to use in sediment trap studies continue to present an important unresolved question. Past laboratory/field experiments designed to answer this question are difficult to interpret, because so-called analogs have been used instead of actual trap materials, which are compositionally complex.

This paper presents our results on the *in situ* effects of formalin, azide and mercuric ion on material collected in MULTITRAPS set at 100 and 300 m for a period of six days in a coastal environment, and at 150 m for a period of 20.6 days in an oligotrophic environment. Effective preservative/poison concentrations used were predetermined from laboratory tests. Parameters tested for relative effects included particulate retention of C, N and selected trace metals, and the effects of the various preservatives/poisons introduced via diffusion chambers or free in solution on *in situ* microbial growth. In addition, the potential contaminating role of large, nonsinking zooplankton (i.e., "swimmers") was investigated.

Substantial differences between treatments were observed. Effects were not uniform, and appeared to be parameter-specific. For example, during the short-term deployment (six days), no significant differences in C flux were observed at 100 m, regardless of preservative used. Traps treated with azide yielded significantly lower N values. At 300 m (short-term deployment), and 150 m (long-term deployment), the azide treatments produced the lowest mean C and N values. Conversely, the formalin traps gave the highest C and N values relative to all treatments at these depths. In terms of metals, >70% of both Cd and Mn were lost to the trap solutions, regardless of oceanic area or time deployed, while most of the Fe tended to remain in the particulate phase. Zinc, largely in association with the particulate phase over the six-day deployment, was lost to the density solution during the 20.6-day deployment, while Pb results were intermediate between these extremes. Results of the diffusion chamber experiment indicate that the formalin and mercuric ion treatments were equally effective regardless of the mode of introduction (i.e., diffusion chamber or free in solution). Azide did not appear as effective when introduced via diffusion.

1. Introduction

During the past decade, the use of particle traps to determine the downward flux of biological and chemical materials has become widespread. Due to the complexity of materials collected by these systems, most investigations have been confined to the

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analysis of selected components which are specifically related to a particular field of interest. Examples include chlorophyll *a* and phaeopigments (Hargrave and Taguchi, 1978; Welschmeyer, 1982), organic C, N and P (Knauer *et al.*, 1979), trace elements (Martin and Knauer, 1980; Knauer and Martin, 1981a), fecal pellets (Honjo and Roman, 1978; Urrere and Knauer, 1981), various lithogenic phases (Honjo *et al.*, 1982), organic nitrogen compounds (Lee and Cronin, 1982; Lee *et al.*, 1983), opal, calcite and aluminosilicates (Wefer *et al.*, 1982), lipids, fatty acids, and wax esters (Wakeham *et al.*, 1980, 1983), and total microbial biomass (Fellows *et al.*, 1981).

Because much of the material of interest is associated with, or derived from, living organisms and appears to be rapidly degraded, preservation is of the utmost importance if the integrity of specific compounds (e.g., organic nitrogen compounds, fatty acids, etc.) is to be maintained during the period of trap deployment. The need for adequate preservation may also be important for inorganic as well as organic compounds. For example, the geochemistry of certain trace metals is strongly tied to organic processes (e.g., Bruland *et al.*, 1978; Ortner *et al.*, 1983), and depending on the primary focus of the field study, appropriate preservation may be imperative. However, as obvious as the need for preservation of trapped material would appear, both the chemical composition and effective concentration of preservative which should be used in sediment trap studies continue to present an important but unresolved problem. For example, a perusal of the sediment trap literature reveals that, although many kinds of preservatives have been employed (Table 1), the reasons for selecting a particular preservative are rarely, if ever, given. Furthermore, the concentrations of the preservative used are not generally reported. Failure to report such basic information makes it difficult in some cases to compare results among the various sediment trap studies.

Although there have been some attempts to evaluate the effectiveness of various preservatives (e.g., Gardner, 1981; Knauer and Martin, 1982; Collier and Edmond, 1982), the results are difficult to interpret, primarily because so-called analogs (e.g., net plankton, squid pens) were used rather than actual trapped materials, which are compositionally more complex. In a recent paper by Gardner *et al.* (1983), it was pointed out that "the effectiveness of poisons in traps has not been adequately determined, because the concentration of poisons in traps has not been monitored." These researchers also showed that the rates of organic material decay inside unpreserved sediment traps can be as high as 1% day^{-1}. Clearly, with the increasing use of sediment traps as a basic research tool, the need to understand decomposition processes in these systems becomes very important.

This paper presents our results on the *in situ* effects of some of the more commonly-used biocides (i.e., "preservatives"; e.g., formalin, azide, and mercuric ion) on total carbon, nitrogen and selected metals collected in MULTITRAPS set in two different ocean regions. The use of the word preservative in this paper is not precise, because poisons such as azide and mercuric ion are not strictly preservatives, as are formalin and glutaraldehyde.

Table 1. Examples of common "preservatives" used in previous trap studies.

Preservative	Concentration	Mode of preservative delivery	Parameter(s) measured	Reference
Mercuric chloride	Not given*	Free in collector	Decomposition of squid pens, lobster tails, shrimp	Gardner, 1981
Mercuric chloride in conc brine	Not given	Free in density solution	Fecal pellets, polycyclic aromatic hydrocarbons	Prahl and Carpenter, 1979
Chloroform	Not given	Released from imploding glass vial	Opal, amino acids, sugars, calcite, aluminosilicates, etc.	Wefer *et al.*, 1982
Chloroform	Not given	Free in collector	Not given	Zeitzschel *et al.*, 1978
None	—	—	Fecal pellets	Lorenzen *et al.*, 1981
None	—	—	C, fecal pellets	Wiebe *et al.*, 1976
None	—	—	Organic carbon, carbonate, fecal pellets	Rowe and Gardner, 1979
Buffered formalin, NaCl	5%, 1.5 M	Free in density solution	Fecal pellets, C, N, P, metals	Knauer *et al.*, 1979; Martin and Knauer, 1980; Knauer and Martin, 1981a; Urrere and Knauer, 1981
Buffered glutaraldehyde	2%	Free in density solution	Fecal pellets	Gowing and Silver, 1983
Paraformaldehyde	5%			
Formalin	10%			
Azide	Not given	Dispensed from diffuser chamber	Organic nitrogen	Lee and Cronin, 1982
Azide + NaCl	Not given	Dispensed from diffuser chamber	Fecal pellets, various organisms, biogenic carbonates	Honjo, 1978
"Rock salt" + oyster shell	Not given	Density solution	Fecal pellets	Dunbar and Berger, 1981
H_3PO_4, NaCl	0.5 M,1.5 M	Free in density solution	ATP, ADP, AMP	Fellows *et al.*, 1981

*Final effective concentrations in trap collectors during/after release unknown.

2. Materials and methods

Free-floating MULTITRAPS (described in Knauer *et al.*, 1979) were set in two locations. The first station, occupied in June 1982, was in coastal waters 80 km off Point Sur, California (36°16'N, 122°57'W). The array was set for 6 days with collectors at 100 and 300 m. The second station, occupied during the VERTEX III cruise in October 1982, was in oligotrophic waters 420 km off Manzanillo, Mexico (16°15'N, 107°10'W). This array was deployed for 20.6 days at a depth of 150 m.

a. Selection of preservative concentrations

In order to determine the appropriate preservative concentration to be used in our trap density solutions, we examined the biological effect of various concentrations of formalin, azide, and mercuric ion (added as mercuric chloride) by measuring the incorporation of ^{3}H-adenine into microbial nucleic acids. The decision to use ^{3}H-adenine incorporation was based on the fact that such measurements have been shown to be closely correlated with total microbial growth and cell division (Karl, 1981, 1982), and therefore, should provide an excellent metric for assessing the effectiveness of the respective preservative treatments.

A coastal surface sea water sample was collected 1 km off Waikiki Beach, Hawaii, and was incubated with 33.8 g NaCl, 14.3 g $MgCl_2 \cdot 6H_2O$, 0.93 g KCl (all reagent grade quality), and 0.2 μCi [2,^{3}H]-adenine l^{-1} for 6 hr in the light following the addition of the various preservatives. The effects, relative to the control treatment, are presented in Table 2. Formalin was an effective biocide at all concentrations tested, ranging from 0.01–1.25 M. A similar result was obtained for mercuric ion, over concentrations ranging from 0.05–5.0 mM. Azide, which inhibits only aerobic respiration (Giese, 1979), required additions $\geq$25 mM to produce effects comparable to formalin or mercuric ion (i.e., 3% of control; Table 2). On the basis of these results, we decided to use 25 mM azide and 0.25 mM Hg^{++} as a final effective concentration in the trap density solutions. The formalin used in the short-term deployment was 10 mM, while a 120 mM final concentration was used in the traps deployed for the longer 20.6-day deployment. It should be noted that when trap systems are to be deployed for long periods (i.e., months), higher effective concentrations should be considered, because greater amounts of trapped material could titrate preservatives such as formalin below effective levels (i.e., $[CH_2O]$ in solution is reduced by binding amino groups of adjacent polypeptide chains; Ruthmann, 1966).

b. Individual trap preparation and processing

i. Carbon, nitrogen and metal collectors. All traps used for these measurements were of polycarbonate construction, and were cleaned according to trace metal clean techniques developed for cleaning our polycarbonate primary productivity bottles (Fitzwater *et al.*, 1982). The high-density trap solutions were prepared by adding 55 g reagent grade NaCl l^{-1} to 0.2 μm filtered North Pacific Gyre water, and then passing

Table 2. Effects of selected preservatives on the incorporation of ^3H adenine into total nucleic acids for a surface seawater microbial population.

Treatment	Preservative concentration	^3H-Adenine incorporation nCi l^{-1}	Percent of control
Control (no preservative)	—	29.2	100
Formalin (CH_2O)	0.01 M	1.0	3.4
	0.06	0.36	1.2
	0.60	0.91	3.1
	1.25*	0.0	0
Azide (NaN_3)	0.25 mM	21.5	74
	1.20	13.6	47
	2.5	10.8	40
	12.0	2.44	8.3
	25.0	0.96	3.3
Mercury ($HgCl_2$)	0.25	1.07	3.7
	0.50	1.02	3.5
	2.5	1.16	3.9
	5.0	1.02	3.5

*1.25 M formalin treatment (= 10% formalin) used as adsorption control. Absolute radioactivity incorporated was equivalent to <1% of control sample.

this solution through a Chelex-100 column (Na form) to reduce the concentrations of contaminating metals. The azide and formalin solutions were also passed through Chelex-100 (Na form) before addition to the trap density solutions.

Traps were prepared for testing the effects of mercuric ion on C and N preservation. (Metal collections could obviously not be tested with this preservative, due to potential metal contamination problems.) They were prepared in an identical manner, except the mercuric ion solution was not passed through Chelex. Because of the difficulty removing contaminating "swimmers" (i.e., various zooplankton species that actively swim into the traps and die; Knauer *et al.*, 1979, Knauer and Martin, 1982) from particulates collected during the June deployment when mercuric ion was used, and because we did not want to risk cross-contamination of our other traps during the VERTEX III cruise, this preservative was not used during the October deployment. Appropriate blanks were prepared by filling a number of traps with the various preservative solutions and deploying them sealed along with the other collectors.

Upon recovery, the traps were removed to our trace-metal clean van, and the overlying seawater siphoned off. The gradient was then filtered through acid-washed 0.8 μm Nuclepore filters (placed at the bottom of each trap) into acid-washed, trace metal-clean polyethylene bottles (Bruland *et al.*, 1979). The high-density solution was then acidified with redistilled, quartz-distilled 6 N HCl. Solution blanks were

processed likewise. In the laboratory, the sample and blank solutions were analyzed for metals by both Chelex-100 and organic extraction following the general procedures outlined in Bruland *et al.* (1979). The concentrated metal solutions were then analyzed on a Perkin-Elmer 603 atomic absorption spectrophotometer coupled to an HGA-500 graphite furnace.

After the high-density solution had been drained through the Nuclepore filters, the filters were removed from each trap, placed in individual acid-washed Petri dishes, moistened with a small amount of the appropriate density solution, and stored in the clean laboratory for further processing. In the clean laboratory, contaminating swimmers were carefully removed under a dissecting microscope. During the July deployment, the removed swimmers were discarded. However, swimmers removed from the October deployed traps were saved, and analyzed for C and N. It should be emphasized that it was very difficult to remove swimmers from the control, azide and mercuric ion traps, because they had become extremely soft, and tended to disintegrate. Consequently, we were not able to remove all swimmer tissue from those traps. (See Results and discussion.) This problem was not encountered in the traps preserved with formalin, due to its hardening effect. After the swimmers were removed, the Nuclepore filters containing the remaining particulate material were folded and rinsed 3× with pH adjusted (≃8) Milli-Q water to remove salts. (This rinse water was added back to the above gradient solution before analysis.) After rinsing, filters were dried at 60°C in a convection oven. The trapped particulates were then removed from their filters and homogenized in an acid-washed agate mortar and pestle. Separate aliquots for C, N and metal analysis were removed from the homogenate for analysis. Carbon and N analyses were performed on a Hewlett-Packard 185-B CHN analyzer after HCl treatment to remove carbonates. Homogenate aliquots used for trace metal analysis were digested in quartz beakers with redistilled quartz-distilled 16 N HNO_3 (RQ HNO_3), and Ultrex 30% H_2O_2 in a manner similar to that described in Martin and Knauer (1973). After digestion, the samples were evaporated to dryness, and redissolved in 0.25 N redistilled quartz distilled HNO_3. Blank filters from the sealed traps were prepared in a similar manner. The trapped particulate sample solutions were analyzed by graphite furnace (HGA-500) atomic absorption spectrometry (Perkin-Elmer 603).

ii. In situ microbial activity collectors. Traps used for the metabolic activity experiments were of cast acrylic construction. Each trap was cleaned with Micro® and 10% HCl, rinsed with sterile deionized water, cleaned again with 70% ethanol, and finally rinsed with sterile seawater. The density solution used consisted of filtered (0.2 μm) seawater containing 33.8 g NaCl, 14.3 g $MgCl_2 \cdot 6H_2O$, 0.93 g KCl, the respective preservative, and 0.1 μCi [2, ^{3}H]—adenine (15 Ci mmole^{-1}; New England Nuclear Co., Boston, MA) liter^{-1} (ρ = 1.065 g cm^{-3}). Prior to deployment, 1 ml of the solution was removed for determination of total radioactivity, as described by Karl

(1982). Control samples were prepared by placing a subsample of the density solutions into a sterile 500 ml polycarbonate bottle and incubating at the trap depths for the duration of the experiment.

Upon retrieval, the trap density solutions were decanted into clean 2-liter polycarbonate bottles and gently swirled before subsampling. Triplicate 50-ml aliquots were concentrated on GF/F filters and frozen for subsequent determination of ^{3}H-RNA and ^{3}H-DNA (Karl, 1982).

c. Diffusion chamber experiment

In addition to evaluating the performance of homogeneous preservative solutions, we were also interested in the effectiveness of diffusion chambers in our trap systems. This was monitored by placing 30 ml plastic vials with small holes in their caps and containing a given preservative directly into our trap density solutions. Because we had no prior knowledge of the rates of diffusion *in situ*, we used considerably higher concentrations (i.e., 250 mM, 25 mM, and 12.5 M for azide, mercuric ion and formalin, respectively) in the diffusion chambers.

3. Results and discussion

The relative effects of the various preservatives on *in situ* adenine incorporation in terms of both in-solution and diffusion chamber experiments are shown in Figure 1. In general, similar effects were found at both the 100 and 300 m deployment depths during the June 1982 deployment. The least effective preservative was azide when introduced by diffusion. At the 100 m depth, for example, microbial growth (as measured by RNA synthesis) was 22% of the unpreserved control sample. However, both mercuric ion and formalin introduced by diffusion appeared to be as effective as the preservatives placed directly into the trap solutions. The effects of preservatives placed directly into the trap solutions at 100 m showed that, in general, ^{3}H-adenine incorporation into either RNA or DNA was <3% of the control. However, at the 300 m depth, both formalin in diffusion chambers and free in solution showed that adenine incorporation into DNA was relatively high (6–7% of control) compared to the 100 m set. This was tested again during the VERTEX III 20.6-day deployment, except that 120 mM formalin was used. In this latter experiment, adenine uptake into RNA or DNA was <1% of the control.

The effects of the various preservatives on C and N fluxes and C:N ratios for the June and October deployments are shown in Figures 2a and b, respectively. There were no significant differences in the C flux data collected during the June deployment at the 100 m depth (ANOVA, $P < 0.05$), regardless of whether preservatives were used. Although we did not attempt to evaluate the potential impact of swimmers as we did for the C and N flux data collected in October (Table 3), it is our opinion that because of the remarkable replication of all traps (i.e., $\overline{X}$ C at 100 m = 235 mg m^{-2} day^{-1} ± 8%,

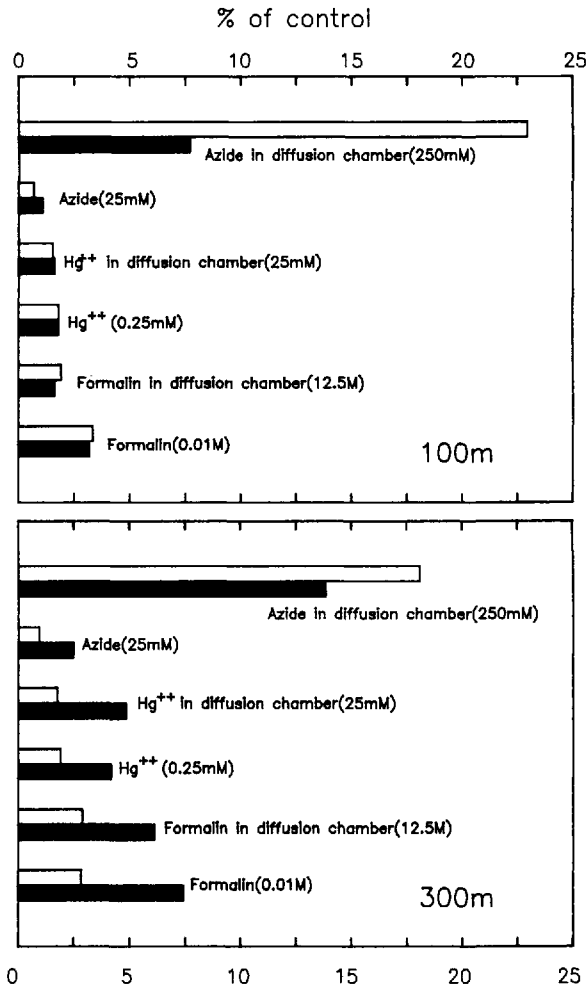

Figure 1. Relative effects of various preservative treatments introduced directly into the trap density solutions prior to deployment, or via diffusion chambers on the uptake of ^{3}H-adenine by microbial populations associated wtih sediment trap collected material. No adsorption correction was made in this experiment. Open bars = incorporation into RNA: solid bars = incorporation into DNA.

$n = 11$) decomposition was not significant over the short, six-day deployment for those traps deployed at 100 m. However, in terms of N collections at 100 m, the azide treatment was found to be significantly lower than the other treatments (Newman-Keuls, $P < 0.005$). The azide treated traps deployed at 300 m yielded the lowest mean C and N flux values relative to the formalin (which gave the highest mean values), control and mercuric ion traps (see values in parentheses, Fig. 2a). However, because only duplicate traps/treatment were used at this depth, statistical differences could not

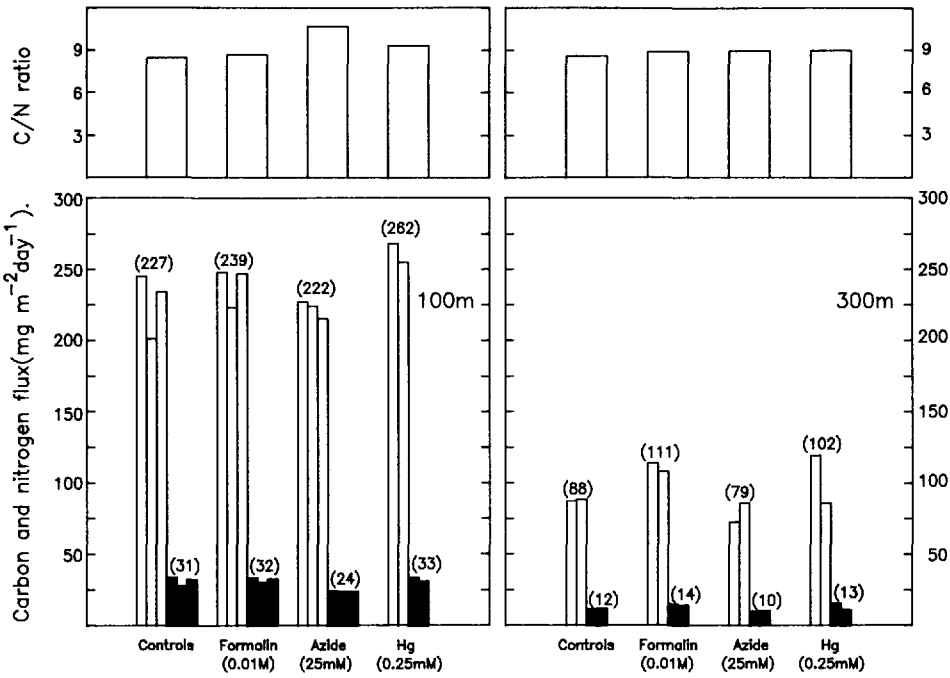

Figure 2a. Carbon (open bars) and N (solid bars) fluxes and C/N ratios obtained for traps deployed at 100 and 300 m off Point Sur, California for a period of 6 days. Numbers in parentheses are mean values.

be demonstrated (ANOVA, $P > 0.10 < 0.25$). Similar results were obtained during the October set (Fig. 2b), in that the formalin traps gave the highest C and N fluxes, while the azide-treated trap yielded the lowest values.

Note that although there were obvious differences in mean C and N flux values between treatments (300 m, June deployment; 150 m, October deployment), these differences were not reflected in the corresponding C:N values (upper panels, Figs. 2a and b). This suggests that at least over the deployment times employed here, the use of C:N ratios may not be a particularly sensitive indicator of decomposition processes.

In a previous study, (Knauer *et al.*, 1979), we reported that failure to remove swimmers from trap-collected material could seriously bias the results, because such organisms could not be considered as part of the passive flux. (Also, see the discussion in Lee and Cronin [1982] relative to biochemical changes from organisms [swimming] falling into traps.) Indeed, Knauer and Martin (1982) showed that swimmer C and N contributions to trap materials collected in California coastal waters between 35–1500 m could be as high as 400%. During the October deployment, we decided to evaluate this same swimmer problem relative to the various preservatives used here. As in the July experiment, we removed as many swimmers as possible from the 150-m

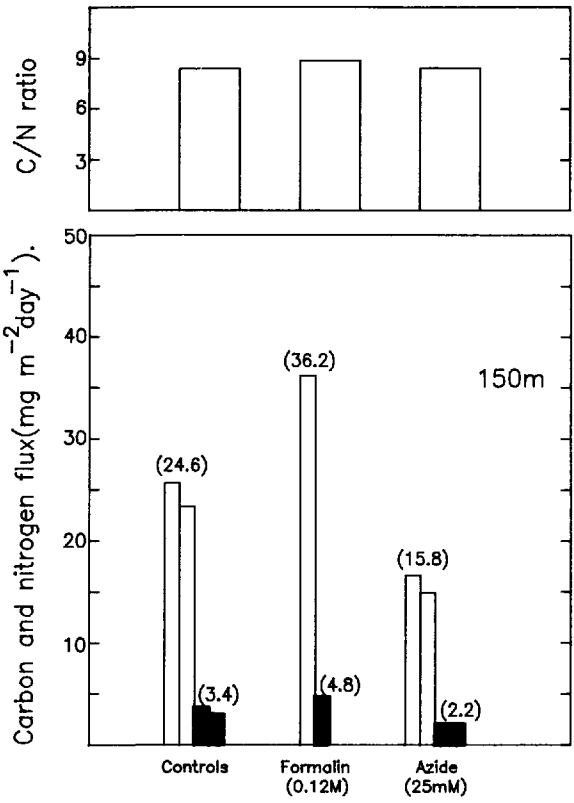

Figure 2b. Carbon (open bars) and N (solid bars) fluxes, and C/N ratios obtained from traps deployed at 150 m off Manzanillo, Mexico for a period of 20.6 days. Numbers in parentheses are mean values.

Table 3. Mean† total C and N in preserved vs. unpreserved trap material after removal of swimmers‡, and total C and N values of removed swimmers.

	Trap material with swimmers removed (μg)		Total swimmers removed (μg)		Swimmer C and N as percent of total	
	C	N	C	N		
Unpreserved						
Control	1970	276	870	130	30	32
Formalin	2910	380	1880	405	39	52
Azide	1260	175	1280	270	50	60

†Mean values for unpreserved control and azide trap derived from two replicate traps. Data from only one formalin trap, due to uncorrected loss of material from the replicate trap.

‡See text for definition of swimmers.

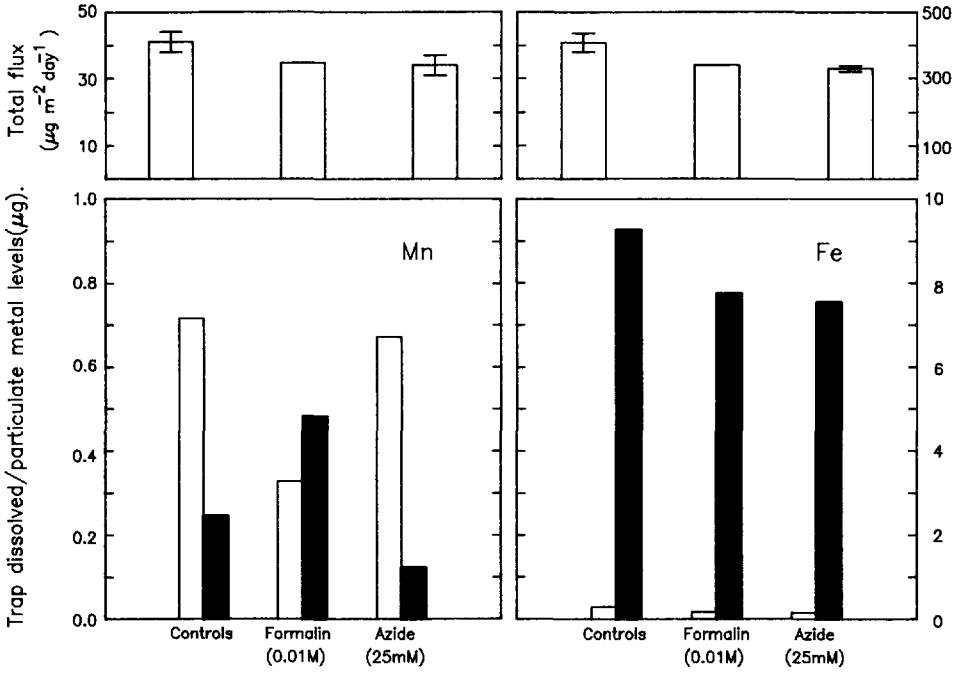

Figure 3a. Trap collected Mn and Fe (June deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total metal fluxes (upper panel). Flux = μg dissolved + μg particulate/0.0039 m^2 × 6 days.

traps, except that we measured their total C and N content. The results are presented in Table 3. Note that although the percent contribution is not as high as we reported in the 1982 report (probably due to low zooplankton biomass in the oxygen minimum waters off Mexico), it is nonetheless significant, ranging from 30–60%. In addition, note that in both the unpreserved control and azide traps, swimmer C and N values are considerably lower than in the formalin trap. In spite of the low number of traps available during the 20-day deployment, we believe this observation to be real, and in our opinion, results from our inability to efficiently remove swimmers from the control/azide traps, due to post-mortem textural changes. The problem of swimmers (which cannot be considered as part of the passive flux) relative to the kind of preservative used is not trivial. For example, both mercuric ion and azide are poisons, and do not bind or harden tissue as do formalin and glutaraldehyde. Consequently, these organisms tend to break apart, making quantitative removal difficult. On the other hand, if no preservative is used, swimmers will still enter the traps. In this case, feeding does occur (G. Knauer, unpubl. observations), which could bias the results. In addition, some of these swimmers, unable to escape, also die, and begin to decompose, again making removal difficult. Thus, losses due to decomposition/dissolution pro-

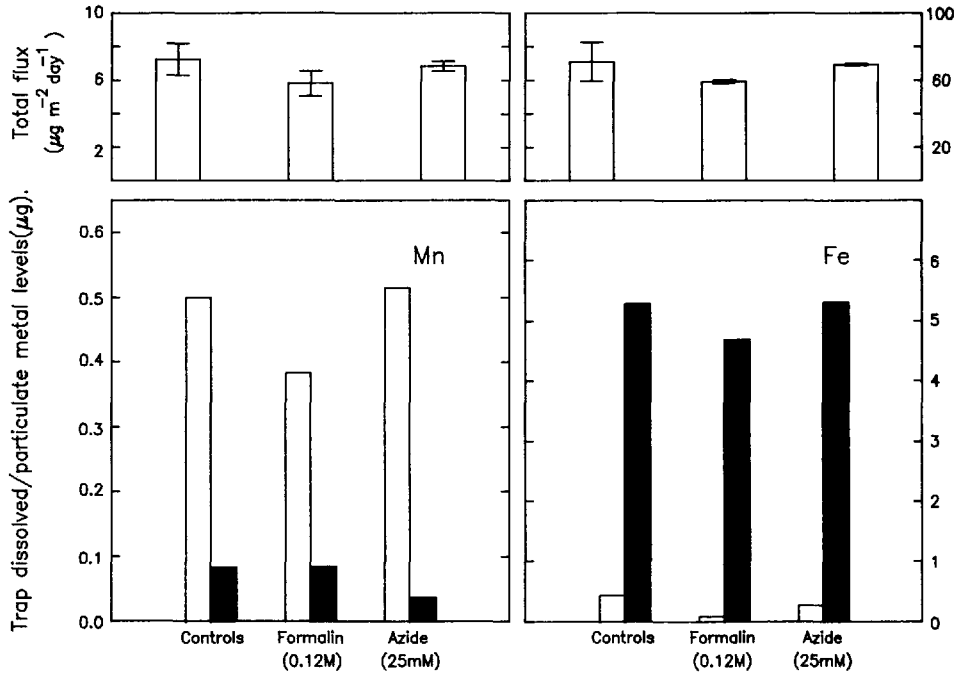

Figure 3b. Trap collected Mn and Fe (October deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total metal fluxes (upper panel). Flux = μg dissolved + μg particulate/0.0039 m^2 × 20.6 days.

cesses may be even greater in the control/azide traps than we were able to resolve here.

The effects of formalin and azide on Mn, Fe, Zn, Cd and Pb from the two deployments are presented in Figures 3–5. As we have reported earlier (e.g., Martin and Knauer, 1980; Knauer and Martin, 1981a; Martin and Knauer, 1982), certain trace metals (e.g., Cd and Mn) appear to leave their original carrier phases and enter the density solutions. On the other hand, metals such as Fe tend to remain in the particulate phases. These same general trends were observed in the present study with some apparently time-related differences. Iron (Figs. 3a–b) from both deployments remained primarily in the particulate phase, and neither composition of preservative nor time of deployment appeared to have an effect. Both Mn (Figs. 3a–b) and Cd (Figs. 4a–b) partitioned into solution (i.e., >80% for Mn and >70% for Cd), and again, the type of preservative had little effect.

In terms of Zn, a time-related effect, differences in the kinds of particles trapped, or differences in the chemistry of the water column (e.g., an intense O_2 minimum at the offshore station) may have been operating. Thus, during the six-day deployment, most

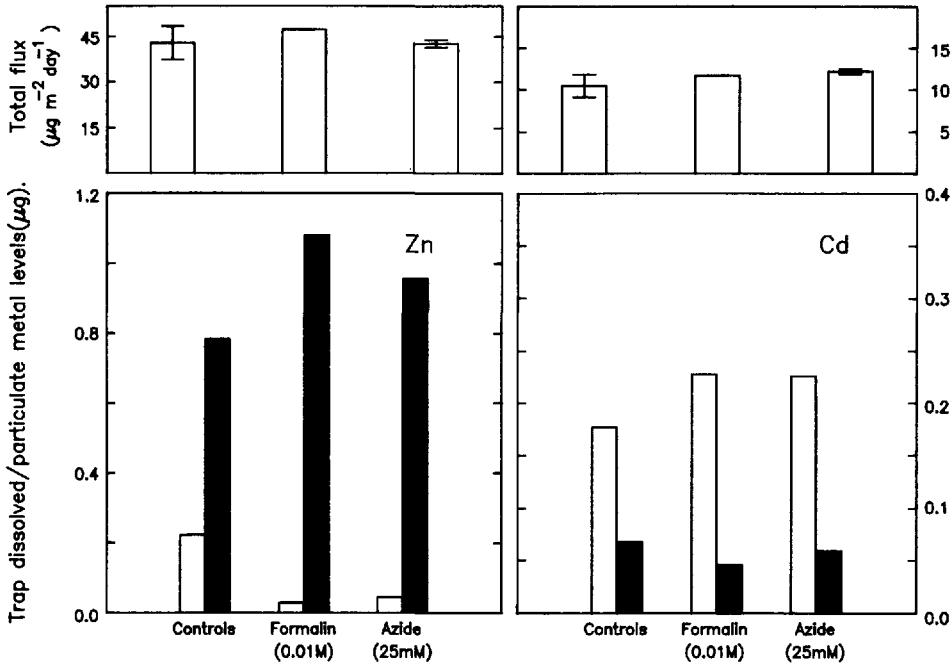

Figure 4a. Trap collected Zn and Cd (June deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total metal fluxes (upper panel). Flux = μg dissolved + μg particulate/0.0039 m^2 × 6 days.

of the Zn remained on particles, with little sign of preservative effect (Fig. 4a). On the other hand, during the 20.6-day set, most of the Zn was found in solution (Fig. 4b).

Lead presents a more intermediate picture, with 40–100% associated with the particulate phase (Figs. 5a–b). However, in this case, formalin does appear to be the preservative of choice. Thus, during the 20.6-day deployment and using a formalin concentration of 120 mM, no Pb was detected in the trap density solutions.

The general picture that emerged in terms of metal-preservative interactions is that, in this system, none of the preservatives tested here completely retarded the loss of a given metal to the soluble phase. In this regard, it is imperative that the surrounding solution (in this case, our density solutions) also be analyzed, if the flux of metals is desired. In this experiment, where metal concentrations in solution are added to the particulate phase, excellent replication relative to *total* metal flux is obtained (see upper panels, Figs. 3–5).

One final point in terms of metal flux measurements and contamination concerns the role of swimmers. If swimmer fragments are left in the particulate phase (as with the control, mercuric ion and azide traps), or if some swimmer-derived metals leach

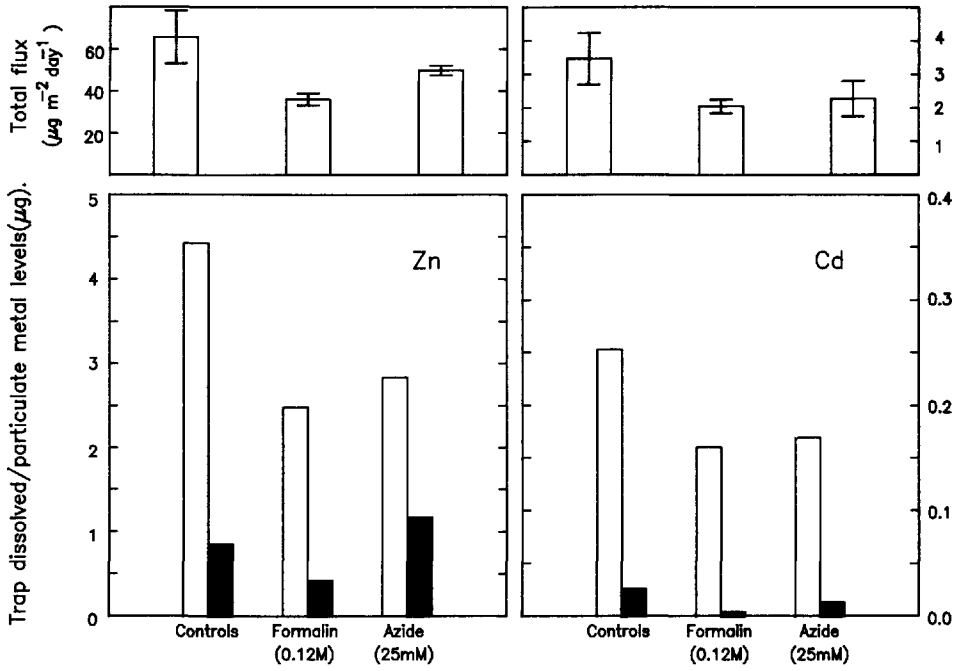

Figure 4b. Trap collected Zn and Cd (October deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total metal fluxes (upper panel). Flux = μg dissolved + μg particulate/0.0039 m^2 × 20.6 days.

into the soluble phase, how serious would this potential source of contamination be? Conservatively, if we assume that *all* of the swimmers removed from the formalin trap contributed their *total* metal burdens (estimated from Martin and Knauer, 1973) to either the particulate or dissolved phases, this would equal only 4, 7, 1, 13, and 35% of the total metal for Mn, Cd, Fe, Zn and Pb, respectively. These numbers are certainly much lower, because we do in fact remove most of the zooplankton intact from our formalin traps. It would appear that, at least for this experiment, (at least in the case of Mn, Cd, Fe and Zn) swimmers, even if not removed, do not pose a serious problem.

Obviously, the choice of preservative is not a trivial matter, and necessarily involves many questions, such as the time scales over which the traps will be deployed, as well as the nature of the compound or reaction to be measured. For example, because azide is a weak base, it *may* be preferable to unbuffered formalin, if preservation of $CaCO_3$ is desired. On the other hand, azide only inhibits aerobic respiration, and CO_2 can still be produced anaerobically. This could create the undesirable effect (relative to $CaCO_3$ dissolution) of lowering the pH, if traps are deployed over long time periods (e.g., months to years).

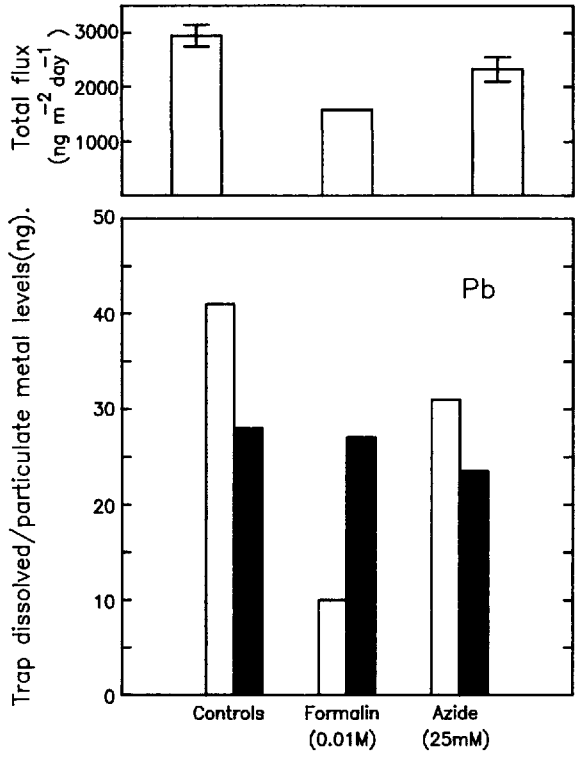

Figure 5a. Trap collected Pb (June deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total Pb fluxes (upper panel). Flux = ng dissolved + ng particulate/0.0039 m^2 × 6 days.

It also appears that decomposition/dissolution losses (based on total C and N) of organic compounds can occur. In addition, some compounds, depending on molecular weight, are probably lost to the surrounding trap solutions, regardless of the type of preservative used as a result of cell lysis. Clearly, because of the potential complexities of trapped material, each investigator should evaluate the effects of a given preservative on their system as suggested by Brock (1978), in his discussion of the use of poisons in biogeochemical investigations.

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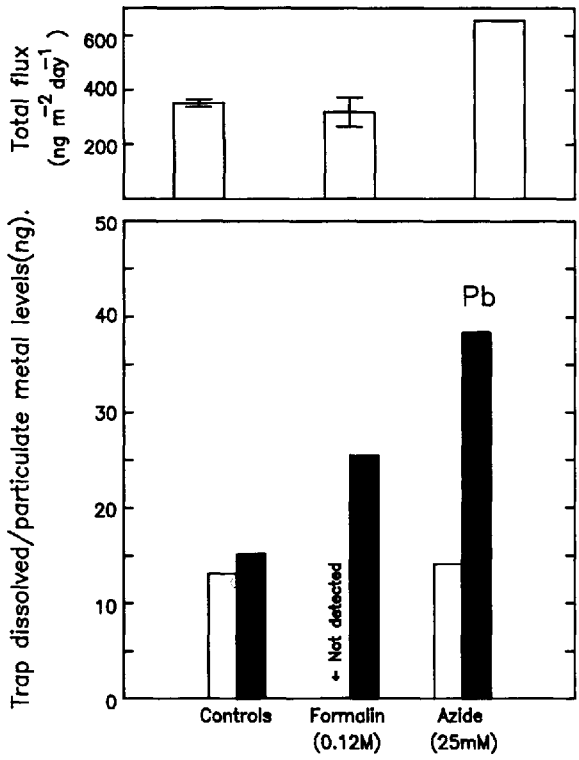

Figure 5b. Trap collected Pb (October deployment) in the dissolved (open bars) and particulate (solid bars) phases, together with total Pb fluxes (upper panel). Flux = ng dissolved + ng particulate/0.0039 m^2 × 6 days.

REFERENCES

Brock, T. D. 1978. The poisoned control in biogeochemical investigations, *in* Environmental Biogeochemistry and Geomicrobiology, W. E. Krumbein, ed., Vol. 3, Ann Arbor Science, 717–726.

Bruland, K. W., G. A. Knauer and J. H. Martin. 1978. Cadmium in northeast Pacific waters. Limnol. Oceanogr., *23,* 618–625.

Bruland, K. W., R. P. Franks, G. A. Knauer and J. H. Martin. 1979. Sampling and analytical methods for the determination of copper, cadmium, zinc and nickel at the nanogram per liter level in sea water. Anal. Chim. Acta, *105,* 233–245.

Collier, R. W. and J. M. Edmond. 1982. Plankton composition and trace element fluxes from the surface ocean, *in* Trace Elements in Seawater, C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton and E. D. Goldberg, eds., Proceedings of NATO, ARI, Erice, Italy, Plenum Press, 920 pp.

Dumbar, R. B. and W. H. Berger. 1981. Fecal pellet flux to modern bottom sediment of Santa Barbara Basin (California) based on sediment trapping. Geol. Soc. Am. Bull., *92,* 212–218.

Fellows, D. A., D. M. Karl and G. A. Knauer. 1981. Large particle fluxes and the vertical

transport of living carbon in the upper 1500 m of the northeast Pacific Ocean. Deep-Sea Res., *28A,* 921–936.

Fitzwater, S. E., G. A. Knauer and J. H. Martin. 1982. Metal contamination and its effect on primary production measurements. Limnol. Oceanogr., *27,* 544–551.

Gardner, W. D. 1981. Organic matter decomposition experiments, *in* Sediment Trap Intercomparison Experiment, R. F. Anderson, and M. P. Bacon eds., Tech. Memo. No. 1–81, W.H.O.I., Woods Hole, MA, 120 pp.

Gardner, W. D., K. R. Hinga and J. Marra. 1983. Observations on the degradation of biogenic material in the deep ocean with implications on accuracy of sediment trap fluxes. J. Mar. Res., *41,* 195–214.

Giese, A. C. 1979. Cell Physiology. 5th ed. W. B. Saunders, Philadelphia, PA, 609 pp.

Gowing, M. M. and M. W. Silver. 1983. Origins and microenvironments of bacteria mediating fecal pellet decomposition in the seas. Mar. Biol., *73,* 7–16.

Hargrave, B. T. and S. Taguchi. 1978. Origin of deposited material sedimented in a marine bay. J. Fish. Res. Board Can., *35,* 1604–1613.

Honjo, S. 1978. Sedimentation of material in the Sargasso Sea at a 5367-m deep station. J. Mar. Res., *36,* 469–492.

Honjo, S., S. J. Manganini and J. J. Cole. 1982. Sedimentation of biogenic matter in the deep sea. Deep-Sea Res., *29A,* 609–625.

Honjo, S. and M. R. Roman. 1978. Marine copepod fecal pellets: production, preservation and sedimentation. J. Mar. Res., *36,* 45–57.

Karl, D. M. 1981. Simultaneous rates of ribonucleic acid and deoxyribonucleic acid synthesis for estimating growth and cell division of aquatic microbial communities. Appl. Environ. Micro., *42,* 802–810.

——1982. Selected nucleic acid precursors in studies of aquatic microbial ecology. Appl. Environ. Micro., *44,* 891–902.

Knauer, G. A. and J. H. Martin. 1981a. Phosphorus-cadmium cycling in northeast Pacific waters. J. Mar. Res., *39,* 65–76.

——1981b. Primary production and carbon-nitrogen fluxes in the upper 1500 m of the northeast Pacific. Limnol. Oceanogr., *26,* 181–185.

——1982. Studies on the biological transport of materials from surface to deep ocean waters. I. Fluxes of carbon, nitrogen and phosphorus. II. Fluxes of trace elements, *in* Second US-USSR Symposium on Biological Effects of Pollutants on Marine Organisms. Terskol, USSR. June 4–9, 1979. EPA-600/3-82-034, 145–165.

Knauer, G. A., J. H. Martin and K. W. Bruland. 1979. Fluxes of particulate carbon, nitrogen and phosphorus in the upper water column of the northeast Pacific. Deep-Sea Res., *26A,* 97–108.

Lee, C. and C. Cronin. 1982. The vertical flux of particulate organic nitrogen in the sea: decomposition of amino acids in the Peru upwelling area and the equatorial Atlantic. J. Mar. Res., *40,* 227–251.

Lee, C., S. G. Wakeham and J. W. Farrington. 1983. Variations in the composition of particulate organic matter in a time-series sediment trap. Mar. Chem., *13,* 181–194.

Lorenzen, C. J., F. R. Shuman and J. T. Bennett. 1981. *In situ* calibration of a sediment trap. Limnol. Oceanogr., *26,* 580–584.

Martin, J. H. and G. A. Knauer. 1973. The elemental composition of plankton. Geochim. Cosmochim. Acta, *37,* 1639–1653.

—— 1980. Manganese cycling in northeast Pacific waters. Earth Planet. Sci. Lett., *51,* 266–274.

—— 1982. Manganese cycling in northeast Pacific equatorial waters. J. Mar. Res., *40,* 1213–1225.

Ortner, P. B., C. Kreader and G. R. Harvey. 1983. Interactive effects of metals and humus on marine phytoplankton carbon uptake. Nature, *301,* 57–59.

Prahl, F. G. and R. Carpenter. 1979. The role of zooplankton fecal pellets in the sedimentation of polycyclic aromatic hydrocarbons in Dabob Bay, Washington. Geochim. Cosmochim. Acta, *43,* 1459–1972.

Rowe, G. T. and W. D. Gardner. 1979. Sedimentation rates in the slope water of the northeast Atlantic Ocean measured directly with sediment traps. J. Mar. Res., *37,* 581–600.

Ruthmann, A. 1966. Methods in Cell Research. Translated from German in 1970. Cornell Univ. Press, 128 pp.

Urrere, M. A. and G. A. Knauer. 1981. Zooplankton fecal pellet fluxes and vertical transport of particulate organic material in the pelagic environment. J. Plank. Res., *3,* 369–387.

Wakeham, S. G., J. W. Farrington, R. B. Gagosian, C. Lee, H. DeBaar, G. E. Nigrelli, B. W. Tripp, S. O. Smith and N. M. Frew. 1980. Organic matter fluxes from sediment traps in the equatorial Atlantic Ocean. Nature, *286,* 798–800.

Wakeham, S. G., C. Lee, J. W. Farrington and R. B. Gagosian. 1983. Biogeochemistry of particulate organic matter in the oceans: Results from sediment trap experiments. Deep-Sea Res., (in press).

Wefer, G., E. Suess, W. Balzer, G. Liebezeit, P. J. Muller, C. A. Ungerer and W. Zenk. 1982. Fluxes of biogenic components from sediment trap deployment in circumpolar waters of the Drake Passage. Nature, *299,* 145–147.

Welschmeyer, N. A. 1982. The dynamics of phytoplankton pigments. Implications for zooplankton grazing and phytoplankton growth. Ph.D. dissertation, Univ. of Washington, 176 pp.

Wiebe, P. S., H. Boyd and C. Winget. 1976. Particulate matter sinking to the deep sea floor at 2000 m in the Tongue of the Ocean, Bahamas with a description of a new sediment trap. J. Mar. Res., *34,* 341–354.

Zeitschel, B., P. Diekmann and L. Uhlmann. 1978. A new sediment trap. Mar. Biol., *45,* 285–288.

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