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A serious inhibition problem from a Niskin sampler during plankton productivity studies

Abstract—Low photosynthetic rates and reductions in chlorophyll concentrations were observed in incubations of samples taken with a 30-liter Niskin sampler during productivity studies of oligotrophic waters in the Indian Ocean. By contrast there appeared to be no effect on community respiration. The rates of photosynthesis were 5-fold to 10-fold greater in samples taken with Teflon-lined 10-liter GoFlo bottles, and there was no systematic loss of chlorophyll. The central rubber cord of the Niskin sampler was identified as a potent source of contamination.

The magnitude of primary production in the oceans has been disputed (e.g. Gieskes et al. 1979; Shulenberger and Reid 1981; Jenkins 1982). Discussion and concern initially centered on the ^{14}C technique, but research into its methodology and interpretation has led to consensus that there is no evidence of persistent and gross errors unique to the ^{14}C technique in the measurement of gross production (Fitzwater et al. 1982; Williams et al. 1983; Smith et al. 1984; Bender et al. 1987). Davies and Williams (1984) compared in vitro and in situ photosynthetic rates and found agreement between the two, suggesting that in vitro methods themselves can be regarded as satisfactory. Their study concerned a temperate, coastal population, however, and discrepancies between the methods of measuring production are regarded to be more characteristic of oceanic communities. Thus the question of the accuracy of ocean productivity measurements remains open.

The possibility of introducing toxic metal or organic contaminants via sample collection has been recognized for some time. Carpenter and Lively (1980) and Fitzwater et al. (1982) strongly recommended the use of clean techniques for productivity sampling, especially in oligotrophic situations where ambient metal concentrations may be low.

During a recent cruise in an oligotrophic area of the Indian Ocean we were forced by circumstances to sample with 30-liter Niskin water bottles. We observed low photosynthetic rates and also low chlorophyll-normalized photosynthetic rates. They most likely would have been accepted as typical of the area, were it not for evident and extensive loss of chlorophyll during incubation (*see Table 1*; stations B5, B10, B12). A series of experiments investigated the loss of chlorophyll and low photosynthetic rates.

Except for one experiment, all photosynthetic rates were measured by the oxygen technique (Williams and Jenkinson 1982). The basic procedure was to fill a clean polyethylene container (60 liter) with water that was sampled with a 30-liter Niskin bottle. The resulting sample was well mixed and kept in the dark before dispensing into nominally 125-cm^3 borosilicate glass bottles. For all transfers, silicone tubing was used and care taken to avoid excessive agitation and bubbling. All glassware and silicone tubing were cleaned initially by soaking overnight with cold 10% Decon (a proprietary decontaminating reagent), rinsed five times with distilled water, followed by a further soaking overnight with 10% HCl and three final rinses with distilled water. Once used, the bottles were left between experiments containing the titrated sample; before use, they were rinsed once in hot water and twice in seawater (cf. Williams and Jenkinson 1982).

When setting up the incubation, the bottles were flushed with at least three times their volume of sample and filled in an order that prevented bias. Eight replicates were used for zero times, light and dark samples. Typically one set of samples was incubated from dawn to dusk and a second set to the following dawn. Before dawn, all bottles were placed in a deck incubator in the earlier experiments, and the incubator was cooled with flowing surface seawater. Dark bottles

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Table 1. Chlorophyll concentrations and oxygen-derived photosynthetic and respiration rates in samples taken with a Niskin sampler: the original effect and that of various incubation conditions.

Sample and depth (m)	Incubation conditions	Pigment analyses* (μg liter^{-1}) Chl 0 h / Ph 0 h	Chl 12-h D / Ph 12-h D	Chl 12-h L / Ph 12-h L	Oxygen flux measurements† [μmol liter^{-1} (12 h)$^{-1}$] Photo. 12 h / Resp. 24 h / Resp. 12-h LD	Photo. index‡ (μmol μg^{-1} h^{-1})
B5, 60	Deck, in vitro	0.225	0.131	0.000	−0.02±0.05	—
		0.138	0.116	0.029	0.36±0.08	—
					0.74±0.04	
B10, 55	Deck, in vitro	0.311	0.341	0.044	0.59±0.11	0.190
		0.141	0.155	0.081	0.95±0.09	(1.90)
					0.82±0.12	
B12, 40	Deck, in vitro	0.350	0.272	0.006§	0.15±0.12	0.042
		0.250	0.139	0.035§	1.15±0.11	(0.42)
					0.62±0.12	
B14, 55 (supplemented with 7.5% v/v deep water)	Deck, in vitro	0.277	0.280	0.070	0.21±0.09	0.076
		0.277	0.224	0.147	1.19±0.07	(0.76)
					0.49±0.09	
B16, 60	In situ, in vitro	0.467	0.415	0.259	0.15±0.07	0.032
		0.326	0.267	0.200	0.02±0.07	(0.32)
					0.45±0.08	
B18, 50	In situ, in vitro	0.386	0.332	0.026	0.09±0.08	0.023
		0.213	0.206	0.053	0.71±0.13	(0.23)
					0.75±0.08	

* Chl 0 h, Chl 12 h, Ph 0 h, and Ph 12 h—chlorophyll *a* and pheophytin concentrations, at zero time and after 12 h incubation. L and D—light and dark incubation.

† Photo. 12 h and Resp. 24 h—12-h-determined photosynthetic rates and 24-h-determined respiration rates. Resp. 12-h LD—12-h dark respiration rates determined on samples previously incubated for 12 h in the light.

‡ Photosynthetic index, i.e. the chlorophyll-normalized photosynthetic rate as μmol O_2 (μg Chl *a*)$^{-1}$ h^{-1}, calculated with the initial chlorophyll value and assuming a 10-h light period. Values in parentheses are calculated as μg C (μg Chl *a*)$^{-1}$ h^{-1}, assuming a PQ of 1.2.

§ 24-h value, no data available for 12-h sample.

were placed in black polyethylene bags within the same incubator. Neutral density screens and a cooler-circulator were used when attempting to mimic in situ irradiance and temperature. Temperature control was kept within 2°C. Oxygen concentrations were determined with an automatic Winkler titration described by Williams and Jenkinson (1982). Chlorophyll concentrations of initial and incubated samples were determined by fluorometry on acetone extracts of particulate matter collected on Whatman GF/F filters.

For ^{14}C measurements, samples identical to those used for the oxygen flux measurements were spiked with a solution containing ~4.5 μCi (167 kBq) of $NaH^{14}CO_3$. The isotope, purchased from Amersham International (Berks, England), was not subjected to any purification. The samples were incubated along with the oxygen samples. At the end of the incubation the samples were filtered at ~300 mbar vacuum through a 25-mm-diameter, 0.45-μm Nuclepore filter. The samples were counted by liquid scintillation with Aquasol as a fluor and quench corrected. Dark bottles were incubated along with the light bottles and the radioactivity taken up in the dark subtracted from that taken up in the light bottles. The radioactivity in the dark bottles was about a third that of the light bottles.

Table 1 gives three examples (stations B5, B10, B12) of samples showing low photosynthetic rates plus rapid and extensive loss of chlorophyll in the light, with significantly less chlorophyll loss in the dark. An examination of fluorescence profiles from CTD casts showed no evidence of in situ diurnal change in chlorophyll levels. Thus we reasoned that the decrease in chlorophyll concentrations was an artifact. A number of causes were considered and tested: nutrient exhaustion, incubation conditions, bottle effects, and sampling procedure.

In vitro incubations prevent the replenishment of nutrients by vertical diffusion. A sample was enriched (7.5% v/v) with water taken from the deep nutrient maximum to determine whether this deficiency was a

Table 2. Effect of container on chlorophyll loss.

Container	Length of exposure (h)	Chl remaining at the end of incubation (%) Light	Dark
Polythene bags (5 liter)	6	77	86
	12	36	78
Polycarbonate bottles (2 liter)	6	78	86
	12	39	81
Acid-cleaned oxygen bottles	6	76	86
	12	39	76
"Uncleaned" oxygen bottles	6	78	86
	12	37	78

Table 3. Effect of sampler on chlorophyll loss.

Sampler	Length of exposure (h)	Chl remaining at the end of incubation (%) Light	Dark
Niskin	6	47	56
	12	34	83
GoFlo	6	70	65
	12	74	69

cause of the chlorophyll loss. The sample was allowed to stand overnight and then the photosynthetic and respiration rates were determined. Both chlorophyll loss and low rates of photosynthesis continued to be observed (Table 1, B14); thus nutrient exhaustion was not seen to be the prime cause of the observed effect.

By using natural density screens and a water cooler we could approximate the ambient irradiance and temperature. We were also able to confirm that the lid of the Perspex incubator would have attenuated near-UV radiation. We were not, however, able to reproduce light quality, and, in order to eliminate the source of uncertainty, we incubated in vitro samples at their appropriate depths in situ from a drifting array. The loss of chlorophyll and low photosynthetic rates remained (Table 1, stations B16, B18); accordingly, we concluded that incubation conditions were not the root of the problem.

An experiment was set up to follow the loss of chlorophyll in different types and sizes of container to examine the effect of bottle size and cleaning procedure. In addition we extended our conventional cleaning technique by giving the bottles an extra overnight soaking in acidified seawater. No unique effect of bottle size or container material was observed nor did the additional cleaning reduce the effect. Substantial loss of chlorophyll in the light was still recorded in all cases (Table 2).

We were left with the possibility that the contamination was occurring during sampling. Accordingly a rosette sampler was set up with 10-liter GoFlo bottles and water compared with a similar sample collected with the 30-liter Niskin. The samples were incubated in clean, 2-liter polycarbonate containers on deck. Samples collected with the GoFlo bottles showed less chlorophyll reduction (Table 3). Similarly, a set of samples collected with the GoFlo bottles (Table 4) gave much higher photosynthetic rates than previous samples taken with Niskin samplers. The chlorophyll-normalized photosynthetic rates in these samples ranged from 0.28 to 1.23 μmol O_2 (μg Chl a)$^{-1}$ h^{-1} [equivalent to 2–10 μg C (μg Chl a)$^{-1}$ h^{-1}]. Although the chlorophyll data were erratic, marked losses in the light were again no longer evident.

A 30-liter sample was taken with the GoFlo bottles, mixed in a polyethylene container, and half transferred to a Niskin bottle for ~30 min to confirm the toxic effect of the Niskin sampler. From the two subsamples, duplicate sets of bottles were started in a deck incubator at about 0600 hours. At 0730, 1200, and 1900 hours, bottles were removed and inoculated with 4.5 μCi (167 kBq) $NaH^{14}CO_3$ and incubated for 2 h under constant light (200 μEinst m^{-2} s^{-1}). Table 5 (station B24) shows a decrease in the photosynthetic rate preceding what appears to be an accelerating loss of chlorophyll in the samples exposed to the Niskin bottle; in the control sample, neither chlorophyll nor photosynthetic rates showed any systematic decrease.

Finally, in an attempt to locate the source of contamination associated with the Niskin sampler, we incubated a piece of the central rubber cord (10 cm) for 20 min in a 5-liter polythene container with water sampled with the GoFlo bottles. The cord was then removed and the sample, plus a control, incubated on deck. There was marked loss of chlorophyll in the sample that came into contact with the rubber tub-

Table 4. Chlorophyll changes during incubation and oxygen-derived photosynthetic rates in samples taken with a GoFlo sampler (units and details as in Table 1).

Sample and depth (m)	Incbuation conditions	Pigment analyses (μg liter^{-1}) Chl 0 h / Ph 0 h	Chl 12-h D / Ph 12-h D	Chl 12-h L / Ph 12-h L	Oxygen flux measurements [μmol liter^{-1} (12 h)$^{-1}$] Photo. 12 h / Resp. 24 h / Resp. 12-h LD	Photo. index (μmol μg^{-1} h^{-1})
B22,29	Deck, in vitro	0.213	0.119	0.332	2.63±0.06	1.23
		0.106	0.079	0.133	0.68±0.08	(12.3)
					0.35±0.02	
B21, 68	Deck, in vitro	0.504	0.319	0.525*	1.40±0.02	0.28
		0.231	0.252	0.347	0.59±0.03	(2.8)
					0.80±0.13	
B21, 90	Deck, in vitro	0.326	0.294	0.252	0.97±0.14	0.298
		0.266	0.310	0.399	0.49±0.07	(3.0)
					0.89±0.06	

* 24-h value, no data available for 12-h sample.

ing (Table 5, station B23), confirming it as a source of contamination.

Previous workers (e.g. Marra and Heinemann 1987; Chavez and Barber 1987) have reported a reduction in the photosynthetic rate in samples taken with Niskin samplers. In the case of Chavez and Barber (1987), the reduction in rate was 10-fold, i.e. of the same magnitude as we observed. This finding contrasts with those of Cullen et al. (1986) who, using in vivo fluorescence as an indicator, inferred no inhibition in a set of samples taken from a station in the eastern tropical Pacific. Thus we may expect the effect to be variable. Marra and Heinemann (1987), although they observed inhibition, could not pinpoint its source. Our experiments suggest that the rubber cord is a potent source of contamination, but they do not eliminate others (e.g. the "O" ring and the walls of the vessel). The Niskin bottles came from the NERC Research Vessel Support pool of equipment and, as a consequence, we have little knowledge of their history. The mechanism of the contamination is far from clear. The chlorphyll loss is evidently light-dependent, but the effect does not extend to pheophytin nor is pheophytin the product. The last perhaps is not surprising. The limited time-courses we have suggest an accelerating loss of chlorophyll, reminiscent of free radical reactions.

An interesting and important question is to what extent other components or aspects of the microplankton were affected by the contamination. There is no obvious difference between the respiration rates obtained from water taken by the two samplers (cf. Tables 1 and 3). One can argue, however, that since the effect seems to be light-in-

Table 5. Examination of the source of contamination (units same as Table 1). Sample B23 was taken with a GoFlo sampler.

	Length of exposure to daylight (h)	Chl *a* (μg liter^{-1})	$^{14}CO_2$ fixation rate (μg C liter^{-1} h^{-1})	Photo. index (μmol μg^{-1} h^{-1})
B24, GoFlo	1.5	0.273	0.31±0.04	0.112(1.12)
	6	0.378	0.62±0.11	0.225(2.25)
	13	0.268	0.24±0.05	0.086(0.86)
B24, Niskin	1.5	0.231	0.04±0.06	0.010(0.14)
	6	0.189	0.10±0.05	0.041(0.41)
	13	0.009	0.07±0.05	0.028(0.28)
B23, polythene bag with rubber cord (5 liter)	0	0.295	No data	
	12	0.046	No data	
B23, polythene bag without rubber cord (5 liter)	0	0.277	No data	
	12	0.216	No data	

duced, inhibition might not be expected in respiration samples incubated in the dark.

We routinely incubated a set of replicates, first in the light for 12 h, then in the dark for 12 h. Thus we are able to determine a respiration rate that follows a 12-h exposure to the light. These rates (Resp. 12-h LD, Tables 1 and 4) would have been subject to whatever process inhibited photosynthesis (no overnight recovery of chlorophyll was noted). When compared with the equivalent rate (Resp. 12 h) observed in samples not exposed to light, there are differences, although they are not systematic. We also collected samples with the Niskin bottles for large-volume, long-term, on-deck incubations. These samples were incubated in the light and chlorophyll, ^{14}C uptake, and bacterial and protozoan numbers were monitored over a period of several days. Whereas the chlorophyll concentrations and the ^{14}C-determined photosynthetic rates declined dramatically, heterotrophic microorganisms increased in abundance (J. King pers. comm.). This result suggests that the contamination problem may not extend to the microheterotrophs in this instance.

Toxic effects of tubing have also been reported by Price et al. (1986). They examined latex, Tygon, and silicone, but unfortunately not neoprene tubing. Their experiments were over longer intervals. They found latex rubber to be particularly and persistently toxic to marine algae and to reduce bacterial activity of seawater samples. The results suggest that the mechanism of toxicity is different in the cases of latex and neoprene, which is not surprising.

Our general findings are in line with the conclusions of Marra and Heinemann (1984, 1987) that given normal good practice (e.g. clean glassware) the problem of contamination in oligotrophic waters lies more with sampling than with the incubation step. Our results suggest that unless clean sampling techniques are adopted, low photosynthetic rates and photosynthetic indices must be treated with caution if not suspicion, and when encountered it would be prudent to measure chlorophyll both at the beginning and the end of the incubation to check for adverse effects. It appears (present work; Williams unpubl.; Williams et al. 1983; Williams and Jenkinson 1982; Marra and Heinemann 1987; Laws et al. 1987) that when clean techniques are used it is common to encounter photosynthetic indices close to or above the "theoretical maximum" as defined by Falkowski (1981). This finding contrasts with our previous notions of oligotrophic communities gained from observations made before our understanding of the need for clean sampling techniques. We do not know to what extent the earlier lower rates were a consequence of contamination effects, but it is obviously important that in the future every effort be made to avoid bias due to contamination. In the case of Niskin bottles, if it is not possible to replace them, it is essential to replace the central cord of neoprene or latex rubber with silicone or epoxy-coated stainless steel springs and the neoprene "O" rings with their silicone equivalents.

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Carbon isotopic compositions of estuarine bacteria

Abstract—A bioassay was developed to assess the stable carbon isotopic compositions of planktonic bacteria from the Parker River estuary, Massachusetts. A small inoculum of natural bacteria was added to filtered estuarine water, then incubated for 24–48 h until bacteria reached the end of log-phase growth. Bacteria harvested at the end of these bioassays exhibited a wide range of $\delta^{13}C$ values from −11.5‰ (near the −13‰ value of *Spartina*) to −27.4‰ (near the −29‰ value of upland C-3 plants). This wide range of $\delta^{13}C$ values suggests that bacteria in the estuary use substrates from a variety of primary producers. Experiments with glucose and dissolved organic carbon leached from oak and *Spartina* leaves showed that bacteria had $\delta^{13}C$ values within ±2‰ of their growth substrates. The results suggest that carbon isotopic measurements are useful for tracing the linkage between bacteria and the plant sources of substrates that support bacterial growth.

Planktonic bacteria are important components of microbial food webs in estuaries (Wright et al. 1987; Rublee et al. 1984). Organic matter supporting the growth of estuarine bacteria may be derived from several major sources: upland vegetation, salt marsh plants, or phytoplankton (Rublee et al. 1984; Ducklow and Kirchman 1983; Gallagher et al. 1976; Turner 1978; Coffin and Sharp 1987). The relative contributions of these substrate sources and the factors that control their rates of supply have not been established because of the complex physical characteristics of estuaries. An experimental approach was developed with stable carbon isotopic measurements to determine the origin of organic matter assimilated by estuarine bacteria.

Stable carbon isotopic ratios of upland vegetation, *Spartina*, and phytoplankton differ because they derive CO_2 from sources with different $\delta^{13}C$ values (aquatic or atmospheric) and because different pathways for carbon fixation (C-3 or C-4 metabolism) yield different $\delta^{13}C$ values. As a result, the $\delta^{13}C$ values are about −29‰ for upland C-3 plants, −13‰ for *Spartina*, and −21‰ for phytoplankton (Peterson et al. 1985). These large differences in stable carbon isotope ratios may provide one possible means for determining which estuarine dissolved organic matter (DOM) sources support bac-

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