Standard Niskin and Van Dorn bottles inhibit phytoplankton photosynthesis in Lake Michigan

Introduction

The routine collection of water for primary productivity experiments may result in contamination of the water sample through the introduction of metals or other contaminants (PIETKOWSKI et al. 1982, CHAVES & BROOK 1987, WILLIAM & ROBINSON 1989). These problems were first discovered in the early 1980s, and focused on the possibility of metal contamination (PIETKOWSKI et al. 1982). Other investigations noted reduced photosynthesis rates when water was collected with standard Niskin bottles (CHAVES & BROOK 1987, WILLIAM & ROBINSON 1989), and suggested that the problem may not be metal contamination. WILLIAM & ROBINSON (1989) suggested that the central rubber seal of the Niskin bottle was the source of contamination. Since these studies, it has been customary for many scientists to use modified Niskin (all rubber parts replaced with silicon) or Kelvorn-coated (parts) or Go-Flo bottles for collecting water in primary productivity studies. While these precautions are common in ocean research, many limnologists continue to collect water samples for primary productivity experiments with standard Niskin (e.g., SUITZ et al. 1998, MURPHY et al. 1999) or Van Dorn bottles (e.g., LAVERTY & DURFEE 1999, CALEB et al. 2000). A relatively new text on limnological methods suggests that Van Dorn bottles are well suited for collecting water for primary productivity experiments (WELLMAN & TAYLOR 1991).

The purpose of this study was to compare standard or conventional techniques of water collection with so-called clean techniques that involve the use of Go-Flo or modified Niskin bottles in a freshwater environment.

Materials and methods

Water was collected at two stations (110 m and 45 m) in Lake Michigan, located off Muskegon, MI. Two research vessels, the RV Lauterborn and the RV Windrow, were used to collect water. Since each vessel has a long history of Great Lakes research, and both have large Niskin bottle tanks, Niskin bottles (5 L) on these vessels were used as the standard Niskin bottles (SN). The same Niskin bottles were not used for all experiments; rather, bottles were taken randomly off the racks. Additionally, those Niskin bottles were removed from the RV Lauterborn and modified by replacing the butyl rubber O-rings with silicon O-rings, and the O-ring connected using a Teflon-coated coupling (WILLIAM & ROBINSON 1989). These modifications were made to reduce the contact of the collected sample with rubber parts. These bottles will be referred to as modified Niskin bottles (MNK). Standard Van Dorn bottles (V1) were also used to collect water. On one occasion, a Go-Flo bottle was used to collect water. A Go-Flo bottle (GF) is routinely used to collect contamination-free samples, and is considered the standard for clean water collection.

Because the metal hydride used to suspend sampling bottles may produce metal contamination, Kelvar and nylon sampling lines were used on two occasions. The Kelvar sampling line was used with the Go-Flo sampling bottles in the first experiment, and nylon line was used in a later experiment. Kelvar and nylon lines were spooled on plastic-wrapped hydrostands. A Teflon-coated messenger was used with Kelvar and nylon hydrostands. Standard metal messengers were used with metal hydrostands.

All sampling bottles were cleaned with 10% HCl prior to use, and then rinsed several times with DDW. While the sampling bottles were suspended on the hydrostands, they were allowed to equilibrate with the lake water for several minutes. Immediately after collection, water was transferred to 2- or 4-L polycarbonate bottles that had been cleaned with hydrogen peroxide, washed with 10% DDW, and then rinsed at least twice in DDW. The bottles were then filled with sample water. Water from these polycarbonate bottles was dispensed into 250-ml polycarbonate incubation bottles (duplicate bottles for each treatment) that had been cleaned and stored in the same way as the larger polycarbonate bottles. These incubation bottles were then injected with a clean PO2 stock (prepared as described in FRIEDWATER et al. 1982), and incubated for 4-7 h in a Perimed incubator at ca. 100 mmol photons m-2 s-1. Following this incubation, samples were filtered onto 0.2-μm membrane filters under low vacuum, decontaminated with 50 μl of 0.5 N HCl in scintillation vials (LEAN & BENVENUTI 1979), and then counted in a liquid scintillation counter.

Results

Experiment 1

The purpose of the first experiment was to compare various types of water samplers with the oceanographic standard for primary productivity experiments: a Go-Flo bottle secured on a Kelvar line and tripped with a Teflon-coated messenger (GF). This simple sampling technique eliminates the possibility of metal and rubber contamination. The other three treatments included a modified Niskin bottle on a Kelvar line (MNK), a modified Niskin bottle on a metal hydrostand (MMN), and a standard Niskin bottle on a metal hydrostand (SNM). The only water collection treatment that produced significant differences in photosynthesis was the SNM, which was significantly lower than all other treatments (20% decrease; P < 0.05; Fig. 1). Photosynthetic rates for the GF, MNK, and MNM treatments varied by <5%. Moreover, the similarity between MNK and MMN treatments suggests that the type of hydrostand and messenger did not significantly affect the rate of photosynthesis. As a result of the similarity between modified Niskin and Go-Flo bottles, the modified Niskin bottle replaced the Go-Flo as the control sampling bottle.

Experiment 2

In this experiment there were three treatments: a modified Niskin bottle (MNK), a standard Niskin bottle (SNM), and a standard Van Dorn bottle (VDM). All bottles were suspended on a metal hydrostand. Highest photosynthetic rates were found for the MNK treatment, and significantly lower rates were recorded with the SNM (28% decrease, P < 0.05), and the VDM treatments (6% decrease, P > 0.05; Fig. 2). This experiment verified the results of the earlier experiment, and extended the possible inhibition results to Van Dorn samples.

Experiment 3

A third experiment was conducted in order to further verify the inhibition caused by standard Niskin and Van Dorn samplers, and to eliminate the possibility that metal hydrides were the cause of photosynthetic inhibition. Modified Niskin (MNK for metal hydrostand, MMN for nylon hydrostand), standard Niskin (SNM for metal hydrostand, SNM for nylon hydrostand), and Van Dorn (VDM for metal hydrostand and VDN for nylon hydrostand) bottles were used on metal and nylon hydrostands. The nylon hydrostand replaced the Kelvar line because the Kelvar line was not available. No significant difference in photosynthetic rates was found between the two hydrostands (P > 0.05; Fig. 3), but significant decreases were noted with standard Niskin and Van Dorn bottles on both lines (P < 0.05). In this experiment, standard Niskin and Van Dorn bottles produced similar decreases in photosynthetic rate.
tosynthetic rates, ranging from 40 to 50% of modified Niskin bottle rates (Fig. 3).

Experiment 4
Because all of the earlier sampling involved standard collections of water aboard large limnological vessels where water casts can take several minutes (5–10 min is typical), it was decided to examine the effects of sampling time, at the time that the water spent in the sampling bottles, on photosynthetic rates. Two types of water samplers were used, a modified Niskin (MN) and the standard Niskin (SN). Water samples were left in the individual collection bottles for 1–60 min before they were dispensed into the incubation bottles. Significant decreases were noted for all time periods with the SN treatment as compared to the MNM treatment (P < 0.05; Fig. 4); moreover, the rate of inhibition increased with time. At 1 min confinement, the SN treatment produced a 16% decrease in photosynthetic rate, whereas with a 60-min confinement, the SNM treatment produced an 80% decrease in the photosynthetic rate (Fig. 4).

Discussion
Standard Niskin and Van Dorn bottles should not be used to collect water for phytoplankton photosynthesis experiments in Lake Michigan. In all experiments, water collected with these water samplers exhibited significantly lower photosynthetic rates. The photosynthetic rate decrease was proportional to the amount of time the water spent in the sampling bottles. The present study extends previous work in marine environments (e.g., Williams & Robertson 1989) to a freshwater environment, and suggests that water for all primary production experiments should be collected with Go-Flo or modified Niskin bottles.

Even though the present experiments were limited, the source of contamination appeared to be the rubber found in the O-rings and connecting cord. The latex rubber cords from both sampling bottles and a butyl rubber O-ring from a Niskin bottle significantly reduced the photosynthetic rates, whereas the PVC housing from both bottles did not. This reduction of photosynthesis is similar to that reported by Williams & Robertson (1989), using the latex rubber cords from Niskin bottles with natural communities from the Indian Ocean. Williams & Robertson (1989) placed the latex rubber cord of a Niskin bottle in water collected with a Go-Flo bottle and noted an 80% reduction in the photosynthetic rate.

Some earlier investigators have attributed the decrease in photosynthetic rate to metal toxicity when using standard Niskin bottles (Fitzwater et al. 1982, Markus & Heinmann 1987). However, in the present study, no evidence of metal toxicity was found. This conclusion was based on the similarity of photosynthetic rates from water collected with modified Niskin bottles on metal hydrolines with metal messengers to rates from water collected with Go-Flo or modified Niskin bottles on synthetic lines (Kevlar or nylon) with Teflon-coated messengers. If metal toxicity was significant, it would have been expected that the treatments with metal hydrolines and messengers would have produced lower photosynthetic rates.

The present results likely have much broader
application than simply phytoplankton photosynthetic rates in Lake Michigan. It is likely that photosynthetic rates will decrease in many freshwater environments if water is collected with standard Niskin or Van Dorn bottles. Additional experiments have been performed in smaller Michigan lakes, and in most cases, similar reductions in the photosynthetic rate were observed. Moreover, the negative effects of using standard Niskin or Van Dorn bottles likely extend far beyond photosynthetic rates. Williams & Robertson (1989) noted a decrease in chlorophyll concentrations in water collected with standard Niskin bottles. Price et al. (1986) noted that latex rubber suspended in clean water caused cellular death for many phytoplankton species. This effect was species specific, as not all species were killed. Moreover, phytoplankton are not the only group of planktonic organisms affected by latex rubber. Thymidine incorporation by bacteria was significantly reduced in the presence of latex rubber, and the survival of the crustacean zooplankton, Acartia clausi, was substantially lower when incubated in the presence of latex rubber (exposure lasted for several days; Price et al. 1986). Thus, it is probably a wise practice to use modified Niskin or Van Dorn bottles for all biological sampling.

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