Cyanobacteria Toxins, Microcystins and BMAA, in Lake Aerosols from Lower Mill Pond and Cliff Pond, Brewster, MA

Report prepared by

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Abstract

Aerosols from freshwater lakes with toxigenic cyanobacteria pose a potentially serious threat to humans and wildlife. Toxic aerosols are emitted by a wide range of lakes, but the mechanisms of cyanobacterial aerosolization are unknown. In order to further understand how to estimate exposure and health risks, it is necessary to first understand environmental factors that influence aerosol production. This study focused on the environmental drivers that may regulate aerosol production. Physical factors tested in this research include air and water temperature, wind speed and humidity. Field collections of aerosols were made during the summer, 2019, on two lakes, Lower Mill Pond and Cliff Pond by members of the Brewster Ponds Coalition Citizen Scientist Program. The overall goal of this project was to compare air and water conditions with rates of toxic aerosol production in selected lakes. Results of this study contributes to an understanding of the importance of the release of toxic aerosols by lakes and provides a data base that can be used for future risk assessment of cyanobacterial toxin inhalation by humans and wildlife.

Introduction

Cyanobacteria are photosynthetic bacteria formerly known as blue-green algae. Cyanobacteria are unique due to their ability to produce a wide range of cyanobacterial toxins as secondary metabolites, for purposes other than growth and reproduction (Codd 1995). Cyanobacteria are ubiquitous including freshwater, marine and terrestrial ecosystems. In a natural lake ecosystem, excess nutrient availability can lead to a dramatic increase in cyanobacteria concentration within the community. Often, such increased concentrations are visible as cyanobacterial blooms that can be harmful to the ecosystem and pose risks to public health.

Both bloom forming cyanobacteria as well as non-bloom-forming cyanobacteria have been found to produce potent toxins. Clusters or "hot spots" of the neurological disorder amyotrophic lateral sclerosis or ALS have been correlated with poor water quality and cyanobacteria blooms in lakes (Torbick et al. 2017; Caller et al., 2009) suggesting the cyanotoxin BMAA may be an environmental trigger. The link between BMAA and neurological disease has also been supported by experiments examining Vervet monkeys fed a diet of fruit containing BMAA (Cox et al. 2016). Similar statistical associations have been shown between cyanobacterial blooms and non-alcoholic liver disease (Zhang, 2015) indicating a possible effect of the liver toxin microcystin that is produced by bloom-forming cyanobacteria. While there are multiple routes of exposure to cyanotoxins, inhalation of aerosolized toxins poses a likely route of exposure (Stommel et al.

2013). In response to this "aerosol exposure hypothesis", a method was developed at the University of New Hampshire Center for Freshwater Biology to measure the concentration of cyanobacteria toxins released from lakes and rivers (Murby and Haney, 2016; Langley 2019). The current research uses a modified version of above aerosol collector that includes both aerosolized particulate and dissolved toxins.

Toxin Production

Research at the UNH Center for Freshwater Biology currently focuses on the cyanobacterial toxins β-Methylamino-L-alanine (BMAA) and microcystins (MCs) because of their toxicity and prevalence. BMAA is a neurotoxin produced by cyanobacteria that bioaccumulates and biomagnifies (Ibelings and Chorus, 2007). MCs are liver toxins linked to dog deaths due to drinking cyanobacteria contaminated lake water and also to non-alcoholic liver disease in humans (Zhang 2015). While MC may bioaccumulate in the lake food web, there is no conclusive evidence MC biomagnifies up the trophic cascade (Dawson,1997). The World Health Organization (WHO) suggested water treatment guidelines in 2003 limiting microcystin-LR contamination to 1 µg/L of treated drinking water (World Health Organization, 2003). However, in the USA, there are no federal regulatory guidelines for either inhalation of cyanobacterial toxins or the ingestion of BMAA.

Aerosolization

Aerosols are small particles or water droplets suspended in the air. Aerosolization of biological material from water is widely thought to occur through a process of micro-bubble bursting that brings material on the surface of the water into the air (Zhang, 2015). However, the process of aerosolization on inland water bodies is poorly known and may involve other factors, such as evaporation, and climatic variables, including wind speed and direction, temperature differences between lake and air, and humidity (Langley 2019). Aerosols emitted from lake water contain dissolved toxins as well as cyanobacteria cells that may contain toxins. While several studies have documented the presence of cyanobacteria cells and toxins in the air (Langley 2019; Murby and Haney 2016; Wood et al., 2011 and Cheng et al., 2007), little is known about the driving factors that regulate the aerosolization process.

Aerosols are easily inhaled by humans and animals that spend time near contaminated water. The concentration of toxins in lake aerosols appears to vary considerably, although the factors contributing are largely unknown. Recent studies have indicated that inhalation of aerosolized cyanobacterial toxins may have a 10-fold increase in toxicity when compared to ingested cyanobacterial toxins (Wood et al., 2011). This suggests that aerosolization of cyanotoxins may be an important route of exposure that is not currently considered in exposure estimates. A first step is to develop a quantitative data base on aerosolized cyanotoxins that can lead to a better understanding of the potential risk of aerosolization cyanotoxins.

Methods

Aerosols were collected biweekly on Lower Mill Pond and Cliff Pond and in Brewster, MA at alternate weeks over a ten-week period from June through August 2019. Cliff Pond aerosol collections were made by Citizen Monitors Chuck Madansky, Sherri Townsend, Gwen Pelletier and Marty Burke. At Lower Mill Pond aerosol collections and water data measurements were made by Nancy Ortiz, Rob and Nancy Condon. Additional air and water quality data were collected by Hailey Carter from UNH who also performed the laboratory sample preparation and toxin analysis. On Lower Mill Pond, each week, an aerosol collector with the attached 3-trap system was used to collect aerosols. During each collection period, weather data was recorded. Weather data included: air temperature, water temperature, wind speed and direction and humidity.

A Compact Lake Aerosol Monitor (CLAM) was assembled and employed for 4-h collections with the CLAM system set up beginning at 8 am and aerosol collection starting by 8:30 am. The CLAM collects both particulates (filters) and dissolved toxins (water traps), separately and in triplicate. During each collection period, the air pumps were run at 2.0 LPM for a total of 480 liters or air per collection. Each CLAM contained three independent aerosol collectors providing three replicates samples for each collection period. The particulate toxins were collected on 25 mm glass fiber filters (Whatman GFF) that were pre-combusted for 1 h at 500° C to create an effective pore size of 0.3 µm. After passing though the GFF filter, air was bubbled in the three water traps to collect the dissolved toxins. The water traps consisted of a tandem interconnected series of three glass traps, each containing 16 mL MilliQ ultrapure water. Toxin tests were carried out with two types of Enzyme-Linked Immunosorbent Assay (ELISA) plates, i.e. Abraxis kits for BMAA and Envirologix High Sensitivity QuantiPlate kits for microcystins.

To enhance detection of the toxins, each liquid trap sample was concentrated ~100x using Thermo Scientific Savant speed vacs. Following concentration, three freeze-thaw-vortex-sonicate cycles were completed on each sample. Three FTVS cycles ensure the cells are broken up and the toxin is released in the solution making it available for detection. Air filters were prepared for analysis by suspending the filters in MilliQ, completing three freeze-thaw-vortex-sonicate cycles then concentrating them by ~7x. Both trap and filter samples were tested for toxins using the standard procedures prescribed for each ELISA kit, using a 4-parameter logistic equation for the standard curves. Once the 996-well plate was read on a Biotek 800TS plate reader, the data were transferred to an Excel spreadsheet and adjusted for sample concentration and for volume of air sampled with the CLAM. Final toxin estimates were expressed as ng BMAA toxin per m^3 and pg MC per m^3. Note that the concentration units used for BMAA (ng) are 1000 times greater than those used for MCs (pg). Graphics, standard curves and regression analyses of results were run using SigmaPlot analysis software.

Aerosolized cyanotoxins at Lower Mill Pond

Microcystins (MCs) were detected in all aerosol samples collected during the summer, 2019 (Figure 1). The two highest concentrations of MCs occurred first on July 5 and again at the end of the sampling season on August 30 with 529.9 and 448.2 pg MC m^{-3}, respectively. The minimum aerosolized MC was 101.7 pg MC m^{-3}. The average summer aerosol MC level was

297.9 pg MC m^{-3}. Most of the aerosolized MC (89.4%) at Lower Mill Pond was in the soluble form, having first passed through the 0.3 µm filters before being captured in the liquid traps.

Aerosolized BMAA was detected on all sampling dates at Lower Mill Pond. The two highest concentrations of 303.5 and 73.5 ng m^{-3} were measured midsummer on July 5 and July 19, respectively. The lowest concentration of BMAA (3.6 ng m^{-3}) was recorded on August 16. The average concentration of aerosol BMAA for the sampling period was 21.9 ng m^{-3}. As was found for aerosolized MC, the largest proportion of airborne BMAA was in the soluble form (80.4%) captured in the water traps.

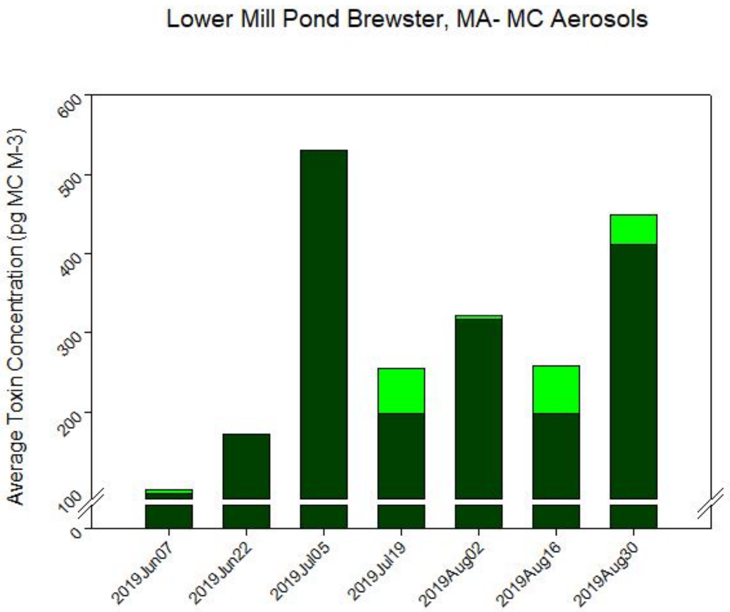

Figure 1. Aerosol concentrations of microcystins (MCs) at Lower Mill Pond, 2019 using the CLAM aerosol monitor. Light bars are particulate MCs collected on filters; dark bars are soluble MCs collected in liquid traps.

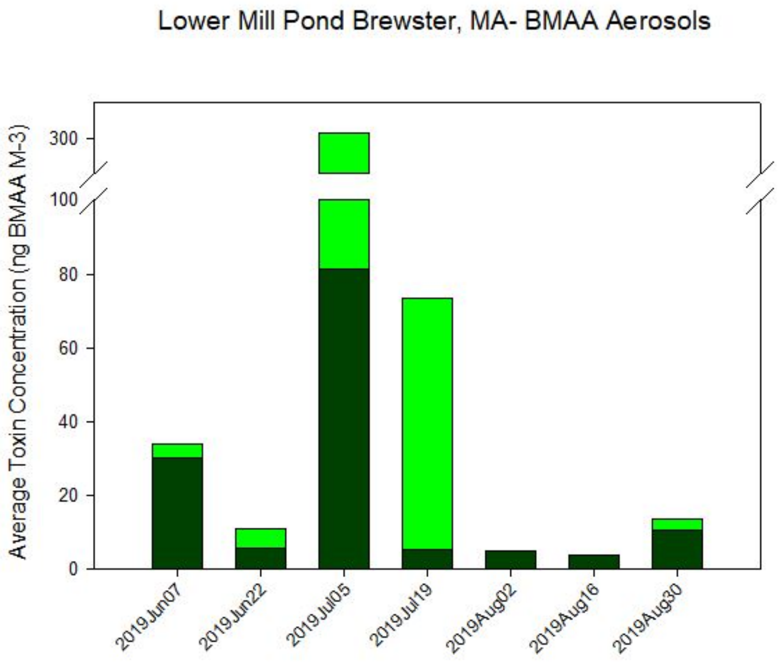

Figure 2. Aerosol concentrations of BMAA at Lower Mill Pond, 2019 using the CLAM aerosol monitor. Light bars are particulate BMAA collected on filters; dark bars are soluble BMAA collected in liquid traps.

Aerosolized Cyanotoxins at Cliff Pond

At Cliff Pond Microcystins (MCs) were detected in all aerosol samples collected during the summer, 2019 (Figure 3). Similar to Lower Mill Pond the highest concentration of MCs occurred first on July 15 with 459.4 pg MC m^{-3}, respectively. The minimum aerosolized MC was 186.1 pg MC m^{-3}. The average summer aerosol MC level was 255.7 pg MC m^{-3}, only slightly lower that the average of Lower Mill Pond (297.9 pg MC m^{-3}; see Table 1). Soluble MCs dominated to Cliff Pond aerosols, with an average of 97.1% of the MCs captured in the post-filter liquid traps.

Whereas aerosol BMAA in Lower Mill Pond had the highest concentrations in the first two weeks of July, BMAA aerosols in Cliff Pond were highest in the first week of August (38.0 ng BMAA m^{-3}). Through much of the summer, BMAA levels were much lower and relatively constant. The lowest air BMAA was 11.1 ng BMAA m^{-3} and the mean aerosol BMAA for the sampling period was 22.3 ng BMAA m^{-3}. Excluding the two outliers, the toxin captured on the filters accounted for an average of 31.14% of the total toxin measuerd. All of these statistics exclude the two outlier filters, but are shown in the graph in Figure 4.

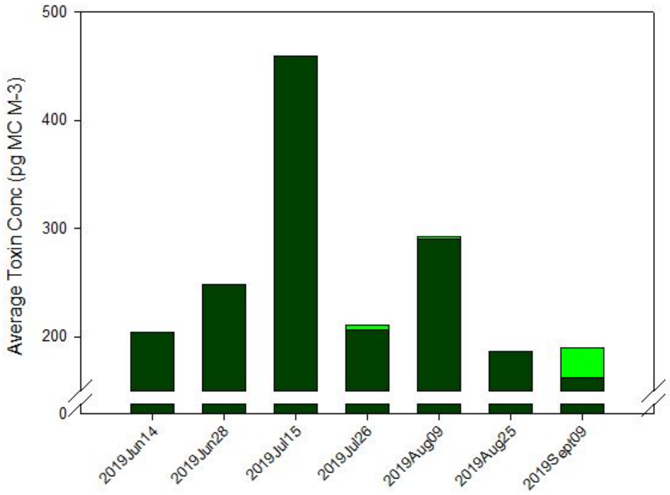

Figure 3. Aerosol concentrations of microcystins (MCs) at Cliff Pond, 2019 using the CLAM aerosol monitor. Light bars are particulate MCs collected on filters; dark bars are soluble MCs collected in liquid traps.

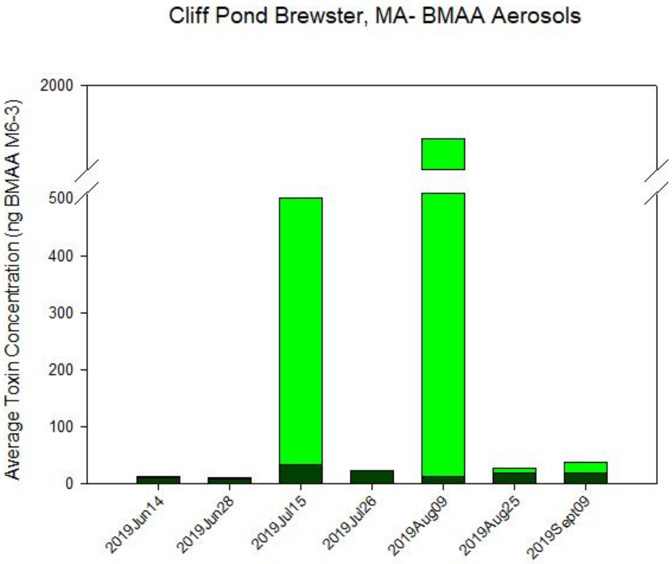

Figure 4. Aerosol concentrations of BMAA at Cliff Pond, 2019 using the CLAM aerosol monitor. Light bars are particulate BMAA collected on filters; dark bars are soluble BMAA collected in liquid traps. Note that the high values on July 15 and August 9 were due to a single filter on each date with exceptionally high values. Since it is suspected this was due to the presence of large bloom forming cyanobacteria not normally present in aerosols, these “outliers” were not used in the calculation of average values for the dates.

Discussion

Comparison of Aerosolized Cyanotoxins in the Two Study Lakes

Lower Mill Pond and Cliff Pond had surprising similarities in their MC aerosols, although direct comparisons are difficult, since the lakes were sampled on alternate weeks. In both lakes the highest aerosol MCs were recorded in July, with Lower Mill Pond having the highest MC emission levels of roughly 530 pg MC m^{-3}, versus 459 pg MC m^{-3} in Cliff Pond. Similarly, average summer MC aerosols were roughly 14% higher in Lower Mill Pond, 297.9 versus 255.7 pg MC m^{-3} in Cliff Pond. Conversely, the late August peak in MC aerosol seen in Lower Mill

Pond was not found in Cliff Pond, suggesting some differences in seasonal patterns of aerosolization.

BMAA aerosol patterns differed noticeably between the two lakes. In Lower Mill Pond there was a more consistent release of BMAA through the summer, beginning in June, whereas in Cliff Pond, aerosolization of BMAA was consistently at relatively low levels, except for two midsummer dates when BMAA concentrations were high (Figure 4). These punctuated peaks of BMAA aerosols were caused in part by exceptionally high BMAA concentrations on some of the filters. Because of their disproportionate influence on mean values, these high values were considered "outliers" and not used for calculating summer averages. This strongly suggests that in midsummer there may have been an episodic release of BMAA-rich cyanobacteria cells (particulate), causing the high variability in the filter toxins at that time. Such events of particulate toxin release were not noted in Lower Mill Pond, indicating possible differences in the composition of the toxic cyanobacteria in the two lakes. The potentially important "spiking" of aerosolized toxins released by the lake should be examined in future studies.

Comparison of Results with Other Studies

Few studies have measured the concentrations of aerosolized cyanobacteria toxins near lakes containing cyanobacteria. In a detailed study of eight low productivity lakes in New England, Langley (2019) found that even very clear, oligotrophic lakes release microcystins into the air. The maximum concentration in her study was 3.8 pg MC m^{-3}. In a study of largely mesotrophic New Zealand lakes, Wood and Dietrich (2011) measured MC aerosol concentrations that ranged from 0.2-16.2 pg MC m^{-3}, whereas Backer et al. (2010) in a study of aerosols using a shoreline aerosol collector during bloom conditions in two highly eutrophic California lakes found higher average concentrations of 52 pg m^{-3}, although many aerosol samples yielded below detectable levels. Unfortunately, at this time there are no published reports of the concentrations of BMAA in lake-generated aerosols to compare with our study.

The present study detected both MCs and BMAA in the aerosols from Lower Mill Pond and Cliff Pond and were often higher than seen in previous studies of comparable lakes. One might assume that the higher values are either due to the particular features of the study lakes or to differences in the methods employed. The above-mentioned studies collected the aerosols on filters, whereas the CLAM used in our study uses both filters followed by a series of three liquid traps that can absorb the water-soluble BMAA and microcystins (both water and lipid soluble). Since in the Brewster Ponds study the liquid traps collected more than 80-90% of the aerosolized MC and BMAA in both lakes (with the exception of two "outlier" samples from Cliff Pond), it is likely that the higher concentrations of cyanotoxins reported in this study are due in part to the improved method of collection.

Environmental Regulation of Aerosol Emission

Comparisons were made to find possible correlations between the emission rates of aerosols and environmental conditions at the lakes. Langley (2019) found that a strong predictor of lake MC aerosols was the difference between the air temperature and water temperature. Comparisons of MC aerosols from both lakes combined suggested a general pattern of control (Figure 5) that was not statistically significant (p>0.05). This is not surprising considering the low number of observations and high variability of the aerosol samples. However, using particulate MC data from Cliff Pond and Lower Mill Pond when lake water was warmer than the air, a significant trend (p=0.02) was observed (Figure 6), i.e. aerosols were higher when the water temperature was greater than the air temperature. The robustness of this linear model should be further tested with other lakes with different levels of MC toxicity. No significant correlations were found for BMAA and temperature suggesting the aerosolization of this toxin, a small amino acid about 10% of the mass of the larger MC heptapeptide molecule, may regulated by factors other than temperature or by combinations of environmental factors.

Goals of the study and risks related to Toxic Aerosols

It has been suggested that correlations between cyanotoxins and liver disease and neurological disorders may be due to inhalation of aerosolized toxins (Stommel et al. 2012). One of the major goals of the current study was to determine whether cyanobacteria toxins were released into the air and if so, what were the concentrations of these toxins. Secondly, by measuring the aerosols from two different lakes we could compare their differences and similarities. Finally, the study provided a unique opportunity to test whether the newly designed CLAM aerosol collector could be employed by citizen monitors. The goals of detection and quantification of the concentrations of two cyanobacteria toxins were successfully accomplished in this study. Comparison of the two study lakes revealed both similarities in the seasonality and concentrations of microcystin aerosols, but also differences in the neurotoxin BMAA. Operation of the CLAM collector and handling of samples was carried out successfully and in a highly professional manner by the citizen monitors on both Cliff Pond and Lower Mill Pond. In other words, thanks to the efforts of the many persons involved, this study can be considered successful in accomplishing its objectives.

At this time, it is too early to speculate on the health risks that aerosolized cyanotoxins may represent. Although limits for ingestion of microcystins have been recommended for drinking water and recreation, no comparable limits have been proposed for microcystins in air, in part, because of the paucity of information available on the concentrations of aerosolized cyanotoxins near lakes. In this regard, it is likely that the quantitative measurements made in the current study represent an important step leading a better understanding of the risks posed by toxic aerosols.

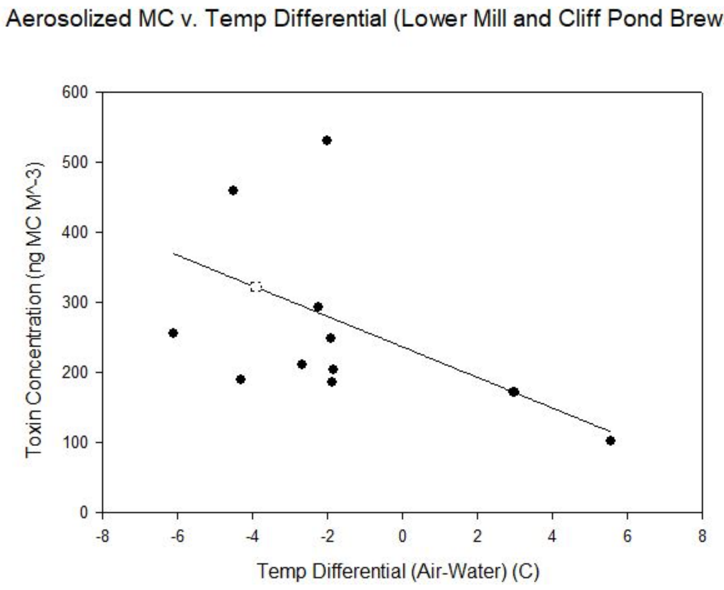

Figure 5. MC aerosol concentrations (particulate and dissolved) at Cliff Pond and Lower Mill, 2019 using the CLAM aerosol monitor (no significance).

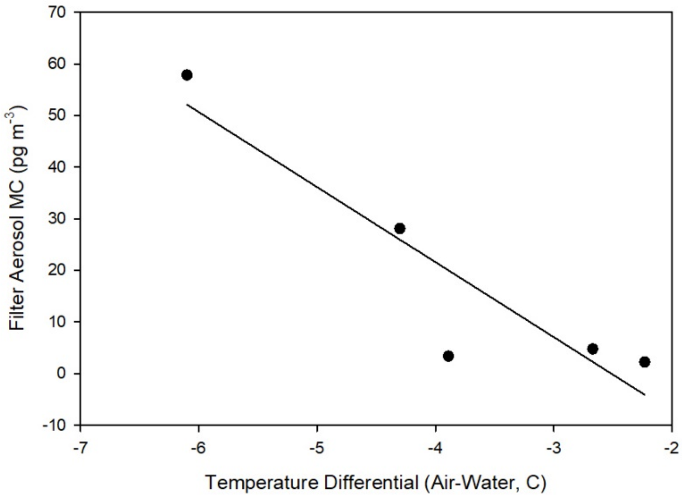

Figure 6. MC aerosol concentrations (particulate only) at Cliff Pond and Lower Mill, 2019 using the CLAM aerosol monitor (R^2 = 0.79, P=0.02).

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