The discovery & history of investigations into gas vacuoles & buoyancy control in cyanobacteria

Geoerge W. Knoecklein

Introduction

Cyanobacteria blooms are regularly featured in the news and many bloom watch forecasting and tracking systems and networks are now in place, with only more to follow. Tracking blooms worldwide may finally answer long-standing questions for lake managers: Are blooms becoming more frequent, longer lasting, and more intense? Blooms could be linked to climate change, with warmer weather, a longer growing season, and higher CO2 all favoring higher numbers of cyanobacteria.

Some cyanobacteria have gas vacuoles – manufactured empty places in the cell consisting of many very tiny hydrophobic cylinders constructed of long chains of nitrogen – carbon molecules. Gas vacuoles decrease the density of the cell. When present, gas vacuoles seemingly guarantee that cells will remain at or near, the surface. While scums do form, the buoyant cyanobacteria genera have an elegant mechanism to generally keep this from happening. The "light:dark cycle", as I refer to it in this article, describes how cells manipulate gas vacuole creation and destruction to maintain a specific density.

This process was discovered in the 1970s based on work initially done in the late 1890s but largely forgotten until recently. The history of the research into what gas vacuoles are, how they function, and what purpose they play is a fascinating one that weaves together years of solid laboratory and in-situ results on how cyanobacteria regulate their own buoyancy. That research will help direct future research into how cyanobacteria with gas vacuoles will respond to changes in their environment.

Historical discoveries

In the late 1800s, cyanobacteria blooms were known as flos-aquae or water blossoms. By that time, there were well documented diel migrations of cyanobacteria up and down in the water column; however, these observations were not yet linked to the organisms' ability to regulate their own buoyancy with gas vacuoles.

Membrane-bound organelles, called vacuoles, were discovered in the 1660s by Antonie van Leeuwenhoek, who conducted the first microscopic examination of living organisms. He referred to the "reflective granules" as "pseudovacuoles", with the prefix "pseudo-" used to distinguish them from the membrane vacuoles that hold fluids and other cellular materials. Klebahn later coined the term "gas vacuoles" for these structures, recognizing their unique composition and function related to buoyancy rather than storage.

Investigating pseudovacuoles in blue-green algae (cyanobacteria were referred to as blue-green algae), Hans Klebahn, a microbiologist in Germany discovered how bizarre these brightly glowing structures were. Working with *Gloeotrichia* cells collected from a surface scum on Lake Plon, magnification revealed that the glowing structures were reflective granules that appeared as gas bubbles. The initial experiments involved attempts to pop the gas bubbles inside the cells. The first experiment involved pumping air into a filled, airtight bottle containing *Gloeotrichia*, thereby increasing the internal pressure several times. This failed to "pop" the gas bubbles, leading him to conclude that they were not "bubbles" after all. He then struck the cork stopper with a hammer (*author note: maybe he was unhappy with this result, or maybe like everything else here on Earth, you have to hit it to make it work*). Klebahn reported the occurrence of three unusual changes to the algae suspension after the hammer strike. First, the algae changed in appearance from milky green to a dark translucent. Second, air bubbles collected at the top under the cork. Third, the algae sank to the bottom of the bottle.

He successfully popped the bubbles that he just concluded didn't exist. Under magnification, he found that the alga had lost the reflective granules that these cells possessed before the experiment and that other, untreated alga from the same bloom still possessed. He concluded that the shock of the hammer somehow succeeded in releasing gas from the granules when increasing the pressure in the fluid didn't. He also surmised that the volume of gas that collected at the top of the jar would be equal to what was in the granules before the strike, and that this volume of gas was what the cells required to remain buoyant, since the algae sank without these granules.

Klebahn reasoned, after numerous experiments, that the gas vacuoles were surrounded by a rigid and impermeable membrane. He believed this because ordinary bubbles disappear in a vacuum, while the cyanobacteria gas vacuole "bubbles" remain unchanged in a vacuum. His reasoning proved incorrect unfortunately, focusing attention on a dead end that persisted for over 50 years.

Cyanobacteria buoyancy in the first half of the 20th Century

The findings of Klebahn seemed to go unrecognized for many years. This is not an exhaustive search but two important

early century limnology textbooks, Paul Smith Welch in 1935, and Franz Ruttner in 1953, have scant information on cyanobacteria (referred to at the time as 'blue-green algae') and neither mention gas vacuoles.

In 1941, British biologist G. E. Fogg published a critical review of the existing knowledge on what he referred to as the "variously termed gas vacuole" and the so-called "water bloom algae." Fogg concluded that, although it was generally accepted that gas vacuoles were required for cells to float, there was a contrary argument that contended benthic cyanobacteria often have these structures but are never buoyant – suggesting that the role of vacuoles in buoyancy regulation was inconclusive. He goes on to state that no experiments on the effect of external conditions of the formation of gas vacuoles had been carried out.

Basically, at the time of his writing, the gas vacuole was barely understood. G.E. Hutchinson, in *Treatise of Limnology, Volume 2: Introduction to Lake Biology and the Limnoplankton* (1957), lists the cyanobacteria that are buoyant (Table 1) and briefly considers the fraction of the cell needed for gas vacuole space necessary for the cell to become buoyant, reasoning that the empty space inside the cell decreases the cell density. He was able to show that a gas vacuole volume of 0.8 percent of the total cell volume would render the cell neutrally buoyant. He speculated that gas vacuoles function was to provide needed buoyancy to lift plankters out of the anoxic water at the bottom of the hypolimnion. He explains that the sudden appearance of cyanobacteria at the surface of lakes is due to induced gas vacuole formation in populations previously in deeper water.

Table 1. Cyanobacteria genera reported to possess gas-vacuoles by Fogg (1941) and Hutchenson (1957).

Fogg 1941	Hutchenson 1957
Anabaena	*Anabaena*
Anabaenopsis	*Anabaenopsis*
	Anacystis
Aphanizomenon	*Aphanizomenon*
Calothrix	*Calothrix*
Coelosphaerium	
Gloeotrichia	*Gloeotrichia*
	Gomphosphaeria
Lyngbya	*Lyngbya*
Microcystis	
Nostoc	*Nostoc*
Oscillatoria	*Oscillatoria*

Beginning of modern research

In 1965, 70 years after Klebahn's work was published, C.C. Bowen and T.E. Jensen investigated Klebahn's cyanobacteria gas vacuoles using electron microscopy. They found that the vacuoles were composed of many minute hollow cylindrical structures which they called vesicles. Each cylinder was 6 µm wide and at least 200 µm in length with conical end caps. These elements resembled a unit membrane, in that they appeared to be composed of two layers about 2µm wide. These were later confirmed to consist completely of tiny protein molecules with walls only one molecule thick and an hydrophobic inner surface that restricts water molecules from entering the vesicle. Cells that were subjected to sudden pressure were found to be slightly smaller in diameter than control cells. The smaller cells were found to contain, in the place of vacuoles, many short, flat, membranous elements. Cells that were subjected to this pressure treatment and then placed in a freshwater lake were found to recover some gas vacuoles after 9 hours, with extensive recovery after 24 hours. The membranes of the gas vesicles could not be preserved by fixation with potassium permanganate, a method that was typically used to preserve unit membranes, indicating that it was not a typical plasma membrane that is composed of a bilayer of phospholipids.

The Bowen and Jensen paper brought gas vacuoles to the attention of Anthony Edward Walsby who basically picked up where Klebahn had left off. Walsby was a student of G.E. Fogg who three decades earlier stated that no experimental data on the effect of external conditions on the formation of gas vacuoles had been done, began asking those questions. Walsby began publishing papers on gas vacuole research in the mid-1960s and continued until his death in 2024.

Walsby was initially intrigued by the possibility that only the inert gas argon was present inside the vacuole, believing they were rigid impermeable membranes. To solve this, he performed a series of experiments with a Warburg respirometer, which provides critical measurements of changes in gas and heat. Walsby learned five important things: (1) vacuoles are rigid structures but are permeable to any gas; (2) vacuoles are not permeable to water; (3) vacuoles are breathable structures; (4) the volume of gas in the vacuoles is measurable; and (5) vacuoles collapse only under a sudden increase in pressure – if pressure builds up slowly, the pressure in the vacuole maintains equilibrium with outside pressure.

Together, these findings created a paradigm shift from the solidly held belief through the first half of the twentieth century that the vesicle membrane is impermeable, to the reverse reality that the membrane is permeable and breathable to all gases. By the end of the 1970s, the question of what gas was inside the vacuole was no longer of any interest because any ambient gas in the cell could be present in the vacuoles (N2, O2, CO2, COH2, Ar, and CH4 were found to pass through a vesicle).

Walsby subjected cells to a range of artificially increased turgor pressures and was able to measure the necessary pressure to collapse vesicles. Turgor pressure is the internal osmotic pressure caused by diffusion of water into the cell due to increased photosynthetic metabolites. Each vesicle has a critical pressure that, if exceeded, will collapse walls. Walsby found that the pressure inside vesicles was always equal to the hydrostatic pressure on objects in the water column due to the atmosphere (ATM) – a constant 1 ATM to a depth of 10 meters. He found that the internal turgor pressure increases consistently with increasing photosynthetic rate, reaching several ATM – high enough to collapse vesicles and cause loss of buoyancy and cell sinking.

Oscillatoria was found to have the highest critical pressure of 0.9 MPa. Microcystis was also found to have strong vesicles, requiring a pressure of 0.75 MPa. Most bloom species, Anabaena, Gloeotrichia, Nostoc, and Aphanizomenon have been found to require a median critical pressure of 0.6 MPa. Walsby (1980) and Walsby and Booker (1983) proposed a buoyancy regulation model in which plankters, Oscillatoria (initially), could either maintain a distinct position in the mid-depth of a water column or move to the surface at night and early morning and descend by noon. The cycle begins when vacuoles are created in the dark, leading to buoyancy. The buoyant cells float up toward the light. The cells experiencing higher light levels photosynthesize at higher rates, creating ballast and increasing turgor pressure. At higher rates, cells replicate, causing vacuole dilution. With both increased turgor pressure and a diluted number of vesicles, the cells sink, ending the cycle.

Buoyancy regulation

The light:dark cycle suggests that cells can regulate their location in the water column by creating and destroying vacuoles. Essentially, cells moving toward the light lose buoyancy while cells moving away from light gain buoyancy, referred to here as the light:dark cycle. *Oscillatoria* has been shown to maintain itself at the most favorable depth in the thermocline (the best light) by adjusting buoyancy to fine-tune its density. The genera use orchestrated sinking when cells get too buoyant and vesicle creation when light levels diminish, returning some buoyancy. Using continuous iterations of this on a small scale allows the cells to maintain position in the thermocline. This light:dark cycle accounts for the observed diurnal migration where cyanobacteria migrate up at night and down in growing daylight. Empirical studies have shown diurnal migrations of *Oscillatoria*, *Microcystis*, *Anabaena*, *Aphanizomenon*, and *Gloeotrichia*. However, *Microcystis*, with the stronger vesicles, was found to not sink as readily as other forms under higher photosynthetic rates.

Bloom formation and climate change

The light:dark cycle describes mid-depth blooms and diurnal migrations but doesn't explain how surface blooms can be sustained in the presence of high CO2 and high light; normally causing sinking, but they do not; instead, they form a surface scum.

It turns out there are at least three short circuits to the light:dark cycle. The first is the distance sinking cells need to go for low light conditions. Initially, this distance is probably measured in meters but increasing cell density in the surface water causes water clarity to decline, shortening the depth cells need to go to find low light. During a dense bloom cell self-shade causes adjacent cells to be in low light conditions, allowing for gas vacuole creation. The second is the ammonia supply; without ammonia, cells in low or no light conditions either don't create gas vacuoles or do so more slowly. Given high ammonia levels cells dramatically increase the rate of vesicle formation. Moreover, high ammonia also allows vesicle formation in higher light and higher CO2 conditions, where vesicle formation would otherwise be prohibited. With high ammonia in the water column, cells can remain buoyant. A third short circuit is higher levels of phosphate in the epilimnion has been shown to allow cells to remain buoyant during high photosynthesis.

Global warming is occurring due to increased levels of CO2 in the atmosphere causing higher CO2 in lake water. Research into gas vacuoles and turgor pressure has shown that high CO2 causes increased rate of photosynthesis in cyanobacteria, suggesting that as carbon dioxide in the atmosphere and hence lake water increases, the presence of cyanobacteria will also increase. Higher ammonia and/or phosphorus will assist in retaining buoyancy. The longer growing season and warmer temperatures result in a longer period of stratification, giving cyanobacteria with gas vacuoles the edge while other phytoplankton sink. Sustained warmer temperatures will allow longer periods of highest growth of cyanobacteria. Combine this with the effects of eutrophication, increased nitrogen and phosphorus, and with anoxic bottom water increasing ammonia and phosphate, both will allow cyanobacteria to remain buoyant in the surface water for longer periods of time.

George Knoecklein earned a doctorate under Peter Rich at the University of Connecticut in 1997. Prior education includes a master's degree from Michigan State University in 1981 and a two-year degree from Unity College in 1973. George is the founder and principal limnologist of Northeast Aquatic Research (NEAR), a Mansfield, Connecticut-based lake management consulting firm founded in 1997. Through NEAR, George has been studying the causes, consequences, and controls of cyanobacteria in regional lakes . His research includes interactions between cyanobacteria and aquatic plants; specifically, how invasive aquatic plants could contribute to cyanobacteria abundance.