

ZoBell and his contributions to barobiology (piezobiology)

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ABSTRACT

Claude E. ZoBell's work influenced much of marine microbiology and deep-sea microbiology in particular. He summarized briefly the known effects of pressure on bacteria in *Marine Microbiology*, his book published in 1946. Therein, he mentioned themes significant to this day, such as how temperature and nutrition modify pressure action and how decompression sensitivity influences sampling of the deep sea. After the publication of his book, ZoBell entered the field of high-pressure biology during a sabbatical. In 1951, he joined the Danish *Galathea* Expedition. This led to his 1952 article in *Science Magazine* in which he presented evidence based on MPN determinations that bacteria in the deepest parts of the oceans are adapted to high pressure. He also may have been the first to see the growth of bacteria from deep, warm (30°C), high-pressure marine habitats although he did not comment on this aspect of his results. His research done, in his words, "BGG (before government grants)," set the stage for the next generation of deep-sea microbiologists.

Introduction

Prior to the *Galathea* Expedition (1950-52), Spärck states, "we knew nothing of the fauna at depths of 6,000-8,000 metres, we had only the results of two trawls...which revealed the existence of animal life at these depths" and "about the greatest ocean depths, between 8,000 and 10,000 m, we know nothing" [16]. Participants of the round-the-world deep-sea expedition on the Danish research vessel *Galathea* caught animals in Sigsbee trawls and Petersen grabs deployed to the bottom of the Kermadec-Tonga and Philippine Trenches [16]. ZoBell, as a scientist on *Galathea*, showed the presence of pressure-adapted bacteria in sediments collected with a Kullenberg corer [22]. These animals and bacteria comprised the first evidence that life exists at pressures over 100 MPa and is consequently ubiquitous in the sea. By 1960, Piccard and Walsh became the first humans to enter the Mariana Trench aboard the bathyscaph *Trieste* and recorded observations of active animal life [15] in the Challenger Deep, the greatest known ocean depth. In 1953 Soviet scientists began to add to the work done by *Galathea* scientists in three trenches and extended it to eight additional ocean trenches [1]. The *Galathea* Expedition along with ZoBell's work and the dives of *Trieste* are among the singular achievements in the exploration of the deep sea during the twentieth century.

McGraw has examined the work of ZoBell and other scientists on *Galathea* from the perspective of its elimination of the idea of a deep azoic zone in the ocean [12]. Herein I analyze ZoBell's findings themselves and their impact on deep-sea microbiology in the twenty five years following his work on *Galathea*. There are four aspects to this brief

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review: (1) How did ZoBell come to know about the biological problems of high pressure in the deep sea? (2) What preparations did he undertake to deal with these problems? (3) What was his influence on deep-sea biology? (4) Finally, I give a brief conjecture on how the existence of barophilic bacteria came to be doubted in the years following the work of ZoBell and Morita. This development deserves some commentary because it reflects partly on how ZoBell and Morita publicized their work and partly on the implementation of the scientific method by deep-sea microbiologists.

ZoBell's development into a deep-sea microbiologist

ZoBell defended his doctoral dissertation describing studies of *Brucella* on November 25, 1931 at the University of California in Berkeley. His mentor was Karl F. Meyer. ZoBell had little background in marine microbiology [23]. While ZoBell was a graduate student in 1931, T. Wayland Vaughan, the Director of Scripps Institution of Oceanography (SIO), offered him an Instructorship in Marine Microbiology to begin on January 1, 1932 [25]. In 1928, Vaughan had hired Albert Haldane Gee as Assistant Professor of Bacteriology [5]. Haldane Gee asked for a three month leave to be in Toronto, Canada during the fall of 1930 because of his own health problems and those of his father. Subsequent requests by Gee to delay his return were ostensibly the cause of Gee being asked to resign in 1931, effective November 7, 1930. Gee negotiated salary beyond that date to finish writing up work he had done at SIO. The last of the reports he completed thereby exists today only as a bound typewritten manuscript of 125 pages in the SIO Library and is aptly entitled "Marine Bacteriology--Scope and Function. Final Report as Bacteriologist" [7]. Gee proposed that this document serve as the basis for a joint publication with ZoBell and in 1933 wrote that "a recent letter from Dr. ZoBell indicates that we will be able to get together on the publication of a long review" [6]. The collaboration never happened. I speculate that the appearance of the review of Benecke [2] in 1933 may have rendered the Gee--ZoBell collaboration superfluous. Nevertheless, ZoBell must have valued greatly Gee's report since he referenced it several times in *Marine Microbiology*, published in 1946 [20]. Among the papers reviewed by Gee, and not by Benecke, were those on the role of pressure in affecting the distribution, sampling and physiology of marine microorganisms. Gee cited 19th century high-pressure work of Certes and Regnard and early 20th century studies on the inactivation and survival of microorganisms at high pressures. These papers are also cited by ZoBell in his book. Thus, it is reasonable to conclude that ZoBell was keenly aware of the research opportunities in high pressure research almost from the day he first arrived at SIO. He described his responsibilities at SIO thus, "my first job...to determine the extent to which bacteria are active in the open ocean and to assess their possible importance as geochemical agents." [23]. Assisting ZoBell in his early work was Catherine B. Feltham, Gee's former technician [25], with whom ZoBell co-authored several significant papers in marine microbiology.

A comparison of Gee's report with the book of ZoBell shows no indication that ZoBell kept up with new developments on high pressure microbiology in the time interval between 1932 and the publication of his book in 1946. The citations in ZoBell's book include almost only Cattell's classic review [4] as pressure work new since the survey of Gee. Thus, we could reasonably assume that ZoBell developed the intense interest, which compelled him to

vigorously pursue studies of the effects of pressure on bacteria in the sea, sometime in 1946 or shortly thereafter.

There were several key research areas in which ZoBell and his students were working before 1946 that also prepared ZoBell for his deep-sea research on *Galathea*. He worked with the dilution method (most probable number or MPN method) for enumerating bacteria and was concerned that the mathematical basis of the technique could be improved. He enlisted R. D. Gordon, a mathematician, towards this end and wrote a foreword to one of his publications [8]. In the 1930's and 40's, ZoBell was also becoming more and more involved with research on sampling the water column and sediments. The studies of his student, S. C. Rittenberg, on bacteria in cores off the San Diego coast are the most notable.

The transformation of ZoBell into a deep-sea, high pressure microbiologist began presumably during his sabbatical year beginning in June 1947. In a ten-page report written on August 20, 1948 [21], ZoBell gave a detailed accounting of his sabbatical. The first six months were spent in Europe where he stayed one to three weeks at the major microbiological and marine laboratories and he attended a few international scientific meetings. Among the many scientists he visited, the ones who would have the greatest influence on ZoBell's subsequent work were Anton Fr. Bruun, Gunnar Thorson and Ragnar Spärck at the University of Copenhagen. The discussions ZoBell had with these marine zoologists and ecologists must have had a very positive impression on them. These scientists were actively engaged in securing funds for a deep-sea expedition. There is no mention of these plans in ZoBell's sabbatical report. There is an indication, however, in a 1949 letter [3] from Bruun to ZoBell that such discussions, indeed, did take place. In that letter, Bruun informed ZoBell that the Danes procured a ship for their planned expedition "and this means that I am able to invite you onboard on behalf of the Committee for some time" [3]. He also tells ZoBell that unanticipated rising costs for expedition preparations and ship operations will require that ZoBell find his own funding for travel and shipping to and from the ship. McGraw reviews many of the logistic problems ZoBell surmounted [12].

ZoBell spent the first five months of 1948 with Frank H. Johnson at Princeton University for the second half of his sabbatical. This was an outstanding choice of laboratories to visit for someone wishing to enter the field of piezobiology. This can be surmised not only from Johnson's publications in the 1940's but also from the fact that an exhaustive treatise on the effects of temperature and pressure on cells and proteins was published by Frank H. Johnson, Henry Eyring and Milton J. Polissar [11] a few years after ZoBell's visit. Most of the antecedent scientific literature relating to pressure in biological studies can be found in and through its citations. ZoBell published three papers with Johnson. Two were on the inactivation of bacterial spores by high pressure. The other was an experimental survey of the effects of pressure on marine and terrestrial bacteria. In that paper, ZoBell and Johnson coined the word barophilic to describe those bacteria that grow or function preferentially at a high pressure [26]. Their paper, however, does not contain convincing quantitative data to support the existence of such bacteria.

When ZoBell returned to SIO, C. H. Oppenheimer and R. Y. Morita were among his next students. Oppenheimer developed high pressure techniques for the ZoBell laboratory and studied the effects of pressure on the morphology of marine bacteria. Morita became actively engaged in shipboard deep-sea research. He participated first on the Mid-Pacific Expedition. Then, after ZoBell's taking part in the *Galathea* Expedition, ZoBell helped to

arrange for Morita to join the expedition. On *Galathea* Morita obtained sediment cores with a Phleger corer deployed into the Kermadec-Tonga Trench. Sediments were analyzed by Morita for the presence of pressure-adapted bacteria capable of nitrate reduction, starch hydrolysis, and sulfate reduction [27].

The results from the *Galathea Expedition*

The reports of ZoBell [22] and of ZoBell and Morita [27] and Morita's thesis [14] contain the results of their work on the *Galathea Expedition* and are benchmark studies deserving more analysis than is possible here. The first evidence that bacteria are alive in a hadal depth--in the Philippine Trench--was reported by ZoBell [22]. His results are interesting because they are consistent with contemporary findings and are partly enigmatic. Sediment from the deepest parts of the Philippine Trench was collected in 1951 with a Kullenberg corer [22]. ZoBell made serial dilutions with nutrient medium (per the MPN method) of sediment from several cores and incubated them at 2.5°C and both at 100 MPa and 0.1 MPa. Another set of dilutions was incubated at 30°C at the same two pressures. In the absence of a method for plating at high pressures, his choice of the MPN method was excellent. I can find no indication of how long the dilutions were incubated but it is likely that he obtained his results during his 82 days on *Galathea*. Accepting the results at face value, we can state the following from his experiments:

Sediments from 10,400m (probably a slightly overestimated depth) contain bacteria that grow at high pressures whether tested at 2.5°C or 30°C. *Comments:* The high pressure growth at 2.5°C is in accord with today's understanding of deep-sea bacterial growth physiology [17]. The growth observed at 30°C is enigmatic and is discussed further below.

Bacteria that grew at atmospheric pressure were not tested for the ability to grow at high pressure and vice versa. *Comments:* It is unlikely that the bacteria which grew at either of the two test pressures could also grow at the other pressure since no bacterial isolate has been found to date that grows over such a span of pressure. The bacteria that grew at high pressure had to have been different from those that grew at atmospheric pressure. The same can be said, but with less confidence, about the bacterial populations at the two test temperatures! Thus, his experimental results suggest he observed 4 distinct bacterial populations.

Bacteria capable of growth at high pressures at 2.5°C were more abundant than those capable of growth at atmospheric pressure in the samples from 10,400m. *Comments:* Results, using sediments collected from the Kermadec-Tonga Trench, in the *Galathea Report* sometimes show an equal abundance of bacteria at the two pressures. Again, these were probably different populations of bacteria.

Samples from 1,000 and 2,000 m yielded bacteria which grew at atmospheric pressure but not at 1,000 atm. *Comments:* This result is in agreement with contemporary data in at least two ways. First, no upper ocean bacterial isolate has been found that grows over a pressure range of 800 atm. Second, whereas shallow water bacteria are found in the deep sea where they sink attached to or imbedded in sedimenting particles and then find themselves in a state of preservation, deep-sea bacteria are probably far less abundant in the shallow ocean.

Therefore, all of ZoBell's 1952 results, except one, have been validated through work with bacterial isolates beginning in 1979 [17]. His result of bacterial growth at 30°C and

100 MPa may be explained possibly by an unusual heat flow in the Philippine Trench that might be creating a high pressure mesophilic habitat in sediments [18]. At this time, there are no laboratory cultures of bacteria with the property of growing at 30°C and 100 MPa, although such organisms should exist.

Deep-sea microbiology since the *Galathea* Expedition

As accurate as these benchmark publications seem today, nearly a quarter of a century of confusion as to the existence of, and the nature of, pressure-adapted bacteria set in. ZoBell himself wrote [24] that, “barophilic bacteria which grow preferentially, or only, at increased pressures appear to be very rare. Out of hundreds of enrichment cultures from deep sea samples (7,000 to 10,400 m) which grew at 700 to 1000 atm, only a few have yielded bacteria that could be kept growing at these pressures for more than two or three transfers.” Regarding isolates from sediment samples collected from the Philippine Trench and the Mariana Trench, Morita and Becker[13] wrote that, “when these isolates were placed in nutrient medium under the isobaric and isothermic conditions from whence the sediment was taken, the cells expired. This same situation has been noted before with sediment samples taken during the Galathea Deep Sea Expedition.” These and other statements by ZoBell and Morita project insecurity about their initial results and must have contributed to muddling the field. Clearly, the nature of deep-sea barophilic bacteria was open to question by 1970. Gundersen[9], who spent time in ZoBell’s laboratory, believed that, “hydrostatic pressure has not yet been shown to be a general requirement for growth of marine bacteria” and “that the possibility even exists that the deep sea harbours bacteria for which pressure is an absolute requirement; but this will not be known until sampling and cultivation equipment is developed which will permit manipulation of deep-sea samples without interrupting decompressions” Jannasch and Wirsén [10] wrote in a similar vein that, “the final word on whether or not truly barophilic bacteria exist can only be said after we are able to study pure cultures of isolated undecompressed deep-sea strains.”

Deep-sea microbiology in the 1970's became entirely focused on the development of field and laboratory instruments to achieve sampling and sample handling without decompression. Gundersen’s assertions, which can be traced to earlier writings of ZoBell, became the research agenda accepted by all in the 1970’s. The development of pressure retaining samplers was undertaken to find barophilic bacteria regardless of the fact that ZoBell’s 1952 report on bacteria in one of the deepest parts of the ocean was accomplished without any special high pressure sampling equipment! The first barophilic bacterium to be grown in pure culture came from a thermally-insulated pressure-retaining animal trap in which an amphipod, captured alive at 58 MPa, had subsequently died and decomposed at pressure [19]. Follow-up work showed that pressure retention during retrieval from the sea was not necessary for many heterotrophic bacteria including those with an absolute requirement of pressure [17]. Clearly, if any of us working in deep-sea microbiology in the 1960’s and 1970’s had only repeated ZoBell’s initial experiment under the precautions (especially protecting samples from thermal stress) outlined in ZoBell and Morita’s *Galathea Report*, then pressure adapted bacteria would have been obtained immediately and confusion avoided. An expanded analysis of deep-sea biology in the period from 1952 to 1979 may provide additional insights. Nevertheless, ZoBell and Morita through their work on *Galathea* and in subsequent research were enormously influential by being the first to

assert the necessity of treating pressure as an ecological parameter and by employing high pressure laboratory methods in research on marine microorganisms

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