

The Life, Work and Scientific Contributions of Claude E. ZoBell

**Tim Gough
BSc Hons Biological Sciences
University of Exeter
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gough_tim@yahoo.co.uk

Abstract

This dissertation explores the life, work and scientific contributions of Claude E. ZoBell, a marine microbiologist who ultimately became a Professor at La Scripps Institution of Oceanography. His pioneering work, before, and whilst at La Scripps are detailed, with a consideration of how this work shaped the field of marine microbiology.

Introduction

Claude E. ZoBell (1904-1989) was a pioneer in the field of Marine Microbiology. During his career, of which there were many highlights, he published nearly 300 research papers, a monograph on marine microbiology, participated in ground-breaking expeditions, and received some of the highest awards a scientist can.

ZoBell studied many different aspects of Marine Microbiology following completion of his doctoral thesis. However, his most important contributions can be grouped into the categories of barobiology, biofilms, petroleum microbiology, microbial physiology and the development of scientific apparatus and techniques.

In order to outline the life and work of ZoBell, I will use these as titles to explore his research. Additionally, I will discuss his Ph. D work on the metabolism of the Brucella group, and look briefly at some of the other aspects of marine microbiology that he studied.

However, I will begin with 'The Life of Claude E. ZoBell'

The Life of Claude E. ZoBell

On August 22nd, 1904, Stella Davis (1881-1958) and Ephraim Andrew ZoBell (1874-1938) had their third of seven children. Claude Ephraim ZoBell became a brother to Ira Deloss (1900-1968), Zelda Marie (1902-1995), Muriel Louise (1907-1988), Henry Davis (1912-), Walter Gee (1913-1914), Elgarda (1915-), and a baby boy, stillborn in 1925.

The family originated in Denmark, and Claude's grandfather, Hans Jorgen ZoBell, and his brother Ole, arrived in America in 1868. The family name was originally Pederson or Hanson (their father's name was Peder Hanson), which was changed when they came to America, as converts to the Church of Jesus Christ of Latter-Day Saints (L.D.S./Mormon). They believed a new start required a new name, and looking through their family ancestry they found a

Zobel. They added an extra l to the name, and became the ZoBells. The capital B was adopted for phonetic purposes.

Hans left home at an early age, and was at sea until the age of 28, as a sailor and merchant seaman. During this time, he travelled extensively, visiting Russia, England, the Mediterranean, Egypt, the Caribbean and South America, and kept logs and journals of all his travels. His love of the sea could have been one of the first influences on Claude, even though Claude himself didn't even see the oceans until he was an adult.

Before the birth of Claude, Stella and Andrew moved from Utah Lake to 40 acres of land in Rudy, Idaho. Stella travelled 250 miles south to the home of her parents in Lakeview, Utah, for the birth of Claude, and stayed for the winter, returning to the rest of the family in early 1905.

Claude spent two years as a student at Rudy Elementary school (1912-13), before the family moved again, to Rigby, Idaho, in 1913. This afforded them a larger house, and three more children. Claude attended Rigby high school, and graduated in 1922 as class valedictorian. Also in 1922, Claude became an elder of L.D.S. During his time at Rigby High School, Claude saved the life of a toddler boy who was in a canal, administering life saving techniques that he had learnt in the scouts. The life saving merit badge enabled Claude to become the first Eagle Scout in the Teton Peaks Council of Boy Scouts, aged 16 (awarded October 1st, 1921).

Claude enrolled at Albion Normal School in 1922, paying tuition fees of twelve dollars per term, which he afforded through part-time work. Claude graduated with a "life certificate" in 1924, allowing him to teach up to 9th grade in Idaho.

Claude took a job as a 5th grade, and only male, teacher and principal of Rigby high school (1924-26). His sister, Elgarda, remembers Claude's passion for aquatic life. She recalls the assistance he gave in building an aquarium for the class, and observing the development of frogspawn, to tadpoles, and then to frogs. Elgarda believes this would have been the very first time that Claude experienced showing science in the water.

Claude resigned in 1926, after "two very enjoyable years". He said "a thirst for knowledge induced me to resign....to continue my education". He attended Utah State Agricultural College, in Logan (now Utah State University). In order to pay for his education, and to recover losses from the failing of the bank where his savings were kept, Claude took a job in the bacteriology department, mainly washing dishes. Hence, Claude became a bacteriologist, and in 1927 gained a

BSc in bacteriology¹, and continued his education to receive his Master of Science in 1929. During his last year at Utah State Agricultural College, ZoBell was also employed as an Instructor in Bacteriology.

Having been awarded the Thompson Scholarship, Claude furthered his education at the University of California at Berkeley, with the Hooper Foundation for Medical Research. His Ph.D thesis was devoted to the metabolism of the Brucella group, and was published in 1931, titled "Cultural Requirements and Metabolism of the Brucella Group". During this time he also developed a synthetic medium for the cultivation of these organisms, which is still used today. Again, whilst here he gained more teaching experience, as a teaching assistant in Bacteriology (1930-31).

During the same period, Claude married for the first time, to Margaret Harding (1908-1994), at the Logan Temple, on June 5th, 1930. During the marriage they had two children, Karl Mark (9th Jan 1932-) and Dean Harding (17 Oct 1934-), both born in La Jolla, California. The marriage was eventually dissolved on March 26th, 1945.

Dean remembers his father taking great pride in his work and spending long hours studying and researching. He sought perfection in all scientific pursuits, as well as using skills learnt as a child to raise chickens for meat and eggs, and to grow and care for an extensive vegetable garden ². Another memory that Dean has of Claude is reading aloud proof manuscripts of his pioneering book "Marine Microbiology", which was published in 1946.

Dean became a physician, specializing in Otolaryngology, and Karl a lawyer³. Both are recently retired.

Having completed his Ph.D. under the watchful eye of Dr. Karl Meyer, Claude moved to La Scripps Institute of Oceanography in La Jolla, California. This is an institution in San Diego which conducts research on nearly all aspects of marine life. It was originally assumed that his stay would be temporary, and that he would eventually return to the medical research group at Berkeley. Luckily for the field of marine microbiology, he did not.

¹ All references quote 1927 as the year that Claude received his degree, even though this was only one year after resigning as principal of Rigby High School. These dates were quoted in the Funeral Address, written by Dean ZoBell.

² Information from a letter from Dean ZoBell

³ Information from letters from Karl and Dean ZoBell.

ZoBell accepted the position of Instructor in Marine Microbiology (1932-1936), and gradually rose through the ranks of Assistant Professor (1936-42), Associate Professor (1942-8), reaching the position of Professor of Marine Microbiology in 1948. During this time he also spent a year as a research associate at the University of Wisconsin (1938), and as a Rockefeller Foundation Fellow studied in Europe in 1947 (including six months at Cambridge University) and Princeton in 1948. He kept the position of Professor until his retirement on 1st July 1972. His work at La Scripps resulted in him becoming Professor Emeritus from 1972 onwards, and he continued working and writing up until eighteen months before his death.

Virtually every year ZoBell was at La Scripps he taught courses on Marine Microbiology (1933-1972), plus courses in Soil Bacteriology and Bacterial Physiology (alternate years 1934-40) and guided many Science Fair winners (1950 onwards). He also supervised around two dozen Ph.D. students, including Richard Morita, Carl Oppenheimer, and Juhee Kim. Richard Morita recalls ZoBell as a research Professor who believed "that the least supervision was the best supervision", however, his door was always open for advice, and would help if a student was headed in the wrong direction. ZoBell expected his students to read all the books and journals in the library in the field of microbiology, and to work in the evenings, and at least Saturday mornings. Morita adds that it was hard to get to know ZoBell as a friend, however, he always respected loyalty. Another of ZoBell's students, Carl Oppenheimer, knew him from 1949-57, and remembers ZoBell as a "great, unrelenting perfectionist".

Whilst at La Scripps, ZoBell also spent time as Assistant to the Director (1936-1952), member of the editorial board of the SIO bulletin (1945-60), as a consultant in Epidemiology (1953-59), and Chairman of the division of Marine Biology (1957-60).

On May 30th 1946, Claude married Jean Switzer (1919-). Jean was born in Knoxville, Tennessee and received a degree from the University of Tennessee in 1940, and in 1943 received an M.A degree from the University of Wisconsin. She became a research assistant at La Scripps under the direction of Claude, in 1944. At this time Claude was also the director of the American Petroleum Institute Project 43A, which involved fundamental research into the origins of oil.

Following the marriage, Jean no longer took paid employment at SIO, but assisted in laboratory and office work, and accompanied Claude on three expeditions: the NAGA expedition to the South China Sea, South Thailand, and Thursday Island, in 1960; the DODO expedition to Philippine and Marianas Trenches in 1964; and the Deepac-X to Japan-Bonin Trench in 1966. Jean also

learnt to read scientific Russian because so many Russian colleagues sent papers to ZoBell.

During his research career, one of the highlights was the Danish GALATHEA round-the-world-deep-sea expedition⁴. This took place between 1950 and 1952, although ZoBell only joined the expedition on July 6th, 1951, in Manila. He remained on board until October 2nd, 1951, when he disembarked at Port Moresby, Papua New Guinea. This leg of the journey involved crossing the deepest parts of the sea, in particular the Philippine Trench. This enabled ZoBell to retrieve organisms from 35,000 feet below sea level, the greatest depth from which viable life had ever been recovered. This confirmed that there was life in the deepest parts of the ocean, with the greatest effects of pressure.

Whilst aboard the expedition ship, Galathea II, ZoBell said, "It is a rare and thrilling experience to participate in such an expedition, where nearly every hour of the night and day new discoveries are being made"⁵. The achievements of Galathea were a huge help in the foundation of the field of barobiology, and indeed ZoBell's first paper published concerning the expedition was given the leading position in 'Science'. ZoBell was awarded the Galathea medal for "recovering living organisms from greatest known ocean depths", and was presented his medal by King Frederik IX, in Copenhagen in 1952.

ZoBell was also ANZAAS lecturer in New Zealand and Australia in 1957, and founded the international 'Geomicrobiology Journal' in 1976, remaining chief editor until 1981. Other highlights of his career were, being awarded the Haiti medal, in 1979, by the Pacific Science Association (comprising 80 nations and territories bordering the Pacific Ocean) for "making the most outstanding contributions to the understanding of the Pacific Ocean" and an honorary D.Sc. from Utah State University, in 1980.

The list of his extramural activities is exhaustive. Some of the more notable, taken from his curriculum vitae, are: member of the San Diego Zoological Society (1934-80); Sponsor of the Eagle Scout Program, San Diego County Council (1935-72); Member of the board of Directors, La Jolla Visiting Nurse Association (1942-52); President of the American Society for Limnology and Oceanography (1949); Counsellor and Judge, Greater San Diego Science Fair, (1949-72); consultant for the International Joint Commission on Oceanography (1952-55) and as a consultant to NASA on environmental extremes and germ-free components for Voyager spacecraft to Mars, (1962-70). He also spent time working for the American Red Cross, as an Instructor in first aid.

⁴ The Galathea Expedition will be discussed further in the next section, along with relevant references.

⁵ From Hadal Bacteria and the "Azoic Zone", Donald J. McGraw, University of San Diego. Unpublished.

Major honours and awards not already mentioned include: Honorary member, Byrd Antarctic Expedition II (1934-5); Oceanography medal, USSR Academy of Sciences for "outstanding studies on the deep sea" (1958); Distinguished Service Award, Utah State University (1961); Distinguished Scientist Award, Gulbenkian Institute, Portugal (1967); A citation from the U.S. Congress for "40 years of outstanding contributions to marine and petroleum microbiology, environmental science, and international co-operation in research," (1972); and, a Distinguished Alumnus Award from the American Association of State Colleges and Universities, "to an alumnus of an AASCU Institution who has achieved national prominence in his or her field, and, thereby, brought honour upon all public higher education" (1987). He received many, many more honours.

ZoBell was retained as a consultant by many companies and organisations, including the Texaco Development Corporation (microbial modification of oil, 1945-55); Bell Telephone Laboratories (biodegradation of submarine cables, 1955-58); Exxon Research (microbiology of oil and marine research, 1969-84); and the General Motors Corporation (microbial transformation of minerals/microbial corrosion of metals, 1968-69). He was also a member of numerous professional and scientific societies, undertook many international lecture tours, and participated in many international conferences. In fact, the 1972 Second U.S.-Japan Conference on Marine Microbiology was dedicated to ZoBell.

During his time at La Scripps, ZoBell wrote nearly 300 papers (a great deal of this research done before government grants), and published a 240 page monograph "Marine Microbiology", an essential document for all marine microbiologists, or as Morita puts it, "the bible and reference cornerstone in the field". Additionally, ZoBell also wrote a book entitled "My Mother - Stella Davis ZoBell - A story of her life and loved ones" (1959).

The greatest achievements of his research career do of course depend on the field of microbiology a scientist is interested in. His work falls under the broad headings of biofilms, microbial physiology, barobiology and petroleum microbiology. Morita considers that ZoBell's greatest achievement was, however, to lay a foundation for the field of marine microbiology.

ZoBell died on March 13th, 1989, of cardiac arrest. He is survived by his wife, two sons, eleven grandchildren, and an ever growing number of great grandchildren. His brother Henry and sister Elgarda Ashliman are also still alive.

His biography has appeared in Who's Who in America, American Men of Science, Leaders in American Education, International Blue Book, Who Knows, and Who's Who in California.

The Work and Scientific Contributions of Claude E. ZoBell.

In to make the work of ZoBell easier to discuss, and follow coherently, I have divided his research into the following categories:

- 1) Work at the Hooper Foundation
- 2) Petroleum Microbiology
- 3) Barobiology
- 4) Biofilms
- 5) Microbial Physiology
- 6) Scientific Methods and Techniques
- 7) Summary of other work

This work will be followed by a consideration of ZoBell's most important contributions to science.

Work at the Hooper Foundation (1929-32)

ZoBell's work at the Hooper Foundation for Medical Research, with Dr. Karl Meyer, University of California, resulted in the publication of his doctoral thesis, "The Cultural Requirements and Metabolism of the Brucella Group". I will discuss ZoBell's work on the Brucella group chronologically, in order of publication.

Before the publication of his thesis, however, ZoBell had his first paper on Brucella published in Science, in 1930⁶. This experimentation, led to the development of synthetic media which made possible the growth of 22 strains of Brucella. Growth improved notably after the fifth generation, showing an adaptation to the protein and peptone free media. Other observations were the role of sodium or ammonium citrate as carbon and energy sources, cystine as an essential nitrogen source, and that cystine and asparagine in combination led to increased multiplication. Additionally it was noted that glycerol, at a concentration of 2 per cent will enhance the growth of all Brucella strains; twenty parts per million of iron (as ferrous or ferric ion) has a stimulating effect; and, the addition of 0.2 per cent agar (resulting in a semi-solid agar) led to accelerated multiplication.

⁶ The metabolism of the Brucella Group in Synthetic Media, Science, 72:176-177 (1930).

Research then focused on the reduction of nitrates⁷ and nitrites. The purpose of this paper was to see if the general belief that the Brucella group lacked the ability to reduce nitrates was correct. For the reasons discussed earlier, a semi-solid media was used for culturing the Brucella. ZoBell and Meyer found that B. abortus and B. melitensis, which grow below the surface of the media, obtained their requirement for oxygen by “disintegration” of nitrates. They also found that of the 425 Brucella strains tested, every strain was able to reduce nitrates. The nitrites do not accumulate, as they are reduced simultaneously. This could explain the belief up until ZoBell’s paper that Brucella could not reduce nitrates, as no nitrites were detected. Additionally, they found that the reduction of nitrates was greatly enhanced when the bacteria were cultured on semi-solid media, as opposed to growth in broth or on agar slants. The presence of succinic, lactic and citric acids, glucose, xylose, galactose and arabinose also accelerated the reduction of nitrates. There was no evidence, however, to suggest that the nitrogen requirement was provided by nitrates, nor could adding increasing amounts of utilizable nitrogen compounds increase reduction.

The work on nitrate and nitrite reduction was furthered⁸. This paper consolidated many of the findings of the paper mentioned earlier, but did expand the work in some areas. The main findings of the paper are:

- (1) the concentration of nitrates in the medium is crucial. Optimal amounts of potassium nitrate or sodium nitrate are between 0.01 and 0.5 per cent. Multiplication is inhibited above 1 per cent, and toxic levels would be reached at 4 per cent;
- (2) Nitrites added at 0.002 per cent completely disappeared after 5 days of incubation, in 425 cultures.
- (3) The *suis* type Brucella could utilise 0.05 per cent potassium in five days, whereas *abortus* and *melitensis* types could not.
- (4) The alkalinity of the medium increases as the incubation period lengthens. This is due to the liberation of base-forming cations, the removal of hydrogen ions, the reduction of intermediate nitrites to ammonium and their reduction to hydroxylamine.
- (5) Any gas evolved is principally nitrogen. Additionally, in media with 0.2 per cent potassium nitrate and potassium iodide, the *suis* type evolve much nitrogen gas, and nitrates and nitrites rapidly disappear.

⁷ Reduction of Nitrates by Representatives of the Brucella Group, Proc. Soc. Exper. Biol. Med., 29: 116-118 (1931).

⁸ Metabolism Studies on the Brucella Group. VI. Nitrate and Nitrite Reduction. J. Infect.Dis., 51: 99-108 (1932).

Another paper regarding nitrite and nitrate reduction was published in 1932⁹. This paper again consolidated some information, although it widened the study of nitrate reduction beyond merely the Brucella group. ZoBell reported that around 600 species of bacteria were found whose ability to reduce nitrates never failed.

The viability of Brucella in aqueous solutions was studied¹⁰. The purpose of this work was to develop a suspending fluid, whose role would be to preserve the viability of suspended Brucella. It was found, quite unexpectedly, that viability was greater in 0.25 per cent salt solution, than it was in 0.85 per cent. Addition of buffers, to make the solution pH7, gave increased viability over solutions of <pH8 and >pH6.6. Additionally, it was found that the proportion of sodium and potassium ions made no difference, but calcium excesses were toxic. 0.02 per cent of cystine or cysteine enhanced the viability. The result of the work was a solution with this composition¹¹:

Potassium monohydrogen phosphate	1.00 Gm.
Cystine	0.20 Gm.
Redistilled Water	1,000.00 cc.
Sodium Chloride	2.50 Gm.
Magnesium sulphate	0.10 Gm.
Potassium dihydrogen phosphate	0.75 Gm.
Calcium chloride	0.01 Gm.

This solution allows Brucella organisms to remain viable for four to five weeks.

The next area of research was the bacteriostatic action of dyes¹². Dyes were used in an attempt to differentiate between different members of the Brucella group. As more strains were added to the genus, categorisation into the three groups, *abortus*, *melitensis* and *suis*, became more difficult, with frequent misclassification. The work by ZoBell and Meyer, enabled the fundamental differences between strains to be duplicated, with an accuracy of 98 per cent.

Three different dye tests were used to distinguish between the three categories of Brucella. The following table shows the growth shown by 444 cultures from

⁹ Factors influencing the reduction of nitrates and nitrites by bacteria in semisolid media. J. Bacteriol., 24: 273-281 (1932).

¹⁰ Metabolism Studies on the Brucella Group. III. Viability in aqueous solutions (M.H. ZoBell). J. Infect. Dis., 50: 538-541 (1932).

¹¹ Table adapted from C.E.ZoBell and M.H.ZoBell, 1932. (See footnote 10).

¹² Metabolism Studies on the Brucella Group. IV. The Bacteriostatic action of Dyes. J. Infect. Dis., 51: 72-90 (1932).

twenty different countries, when subjected to Fuchsin, Pyronine, and Thionine dye tests¹³.

Dye Test	Growth Shown By Given Type Of Bacteria		
	<i>abortus</i>	<i>melitensis</i>	<i>suis</i>
Fuchsin, 1:50,000	+++	+++	0
Pyronine, 1:200,000	+++	+	0
Thionine, 1:50,000	0	+	+++

They did find, however, some strains which did not conform to this dye testing, for example, three strains of Brucella isolated from Caprine milk in Malta which could not be distinguished serologically.

Another aspect of metabolism studied was the production of hydrogen sulphide¹⁴. Due to Brucella abortus' ability to produce hydrogen sulphide from protein or sulfur-containing amino acids, it was assumed that this could be used in the classification of Brucella isolates (the hydrogen sulphide test). ZoBell aimed to see whether this could in fact give valid classifications, and isolates were first placed into their type, according to reaction in the dye test, mentioned previously. ZoBell confirmed that the majority of *abortus* liberated hydrogen sulphide, but so did *suis*, and some strains of *melitensis* liberated trace amounts after several days of incubation. The difference in amount liberated between *abortus* and *suis* was so small that it could never be used as a means of differentiation. Additionally, when the oxidation-reduction potential of the media was adjusted by adding reducing substances or excluding oxygen, then *melitensis* strains could actively produce hydrogen sulphide.

Another method of classifying Brucella that was investigated was by examining dextrose utilization¹⁵. Earlier work by McAlpine and Slanetz¹⁶ reported that less than 4 per cent of dextrose is utilised by bovine origin Brucella abortus, whereas between 4 and 18 per cent is utilized by Brucella abortus of human and porcine origin, and by Brucella melitensis. Further work saw the classification of 129 cultures on the basis of dextrose utilization¹⁷.

¹³ Adapted from the paper quoted in footnote 12.

¹⁴ Metabolism Studies on the Brucella Group. V. The production of Hydrogen Sulphide. J. Infect. Dis., 51: 91-98 (1932).

¹⁵ Metabolism Studies on the Brucella Group. VII. Dextrose Utilization. J. Infect. Dis, 51: 109-116 (1932).

¹⁶ J.Infect.Dis. 42:73, 1928

¹⁷ Plastridge and McAlpine. J. Infect Dis. 47:478, 1930.

ZoBell and Meyer attempted to see if classification was valid, using dextrose utilization as a determinant. The following table is a summary of some of their findings, using two methods of determination, the copper and ferricyanide methods¹⁸:

Type of Brucella	Method	No. of cultures	Utilization Of Dextrose		
			Minimum	Maximum	Average
<i>abortus</i>	Copper	39	3.6	22.6	10.2
	Ferr'	33	3.5	19.7	9.6
<i>melitensis</i>	Copper	21	1.8	21.5	8.7
	Ferr'	21	2.6	18.4	9.1
<i>suis</i>	Copper	19	3.2	15.4	9.7
	Ferr'	19	4.6	18.4	9.6

The results, although showing that the methods are satisfactory (as the averages for each test are similar), also demonstrate that dextrose utilization is similar for the three types of Brucella. This led ZoBell and Meyer to the conclusion that dextrose utilization was no means of categorizing Brucella.

Whilst studying dextrose utilization, they also found consumption of dextrose could be accelerated by the addition of nitrates, and also that mediums with 1 to 5 per cent peptone led to decreased utilization of dextrose in Brucella abortus. However, this decrease was not sufficiently large to be used for differentiation.

The nutrient requirements of Brucella in synthetic mediums was also examined¹⁹. ZoBell believed that inconsistencies in results obtained by scientists when examining Brucella were caused by the lack of a standard nutrient substratum, which could be used in duplicative experiments. In short, the medium must provide the complete environment for the bacteria. ZoBell considered the following in the construction of a suitable media: carbon or energy requirements, nitrogen, sulphur and phosphorus, accessory growth factors, or vitamins, essential minerals, osmotic pressure, surface and interfacial tension, hydrogen ion concentration, oxidation-reduction potential, gaseous tension, physical consistency, positive and negative catalysis, and temperature and radiations.

The results of the experimentation into nutritional requirements were:

¹⁸ Adapted from the paper quoted in footnote 15.

¹⁹ Metabolism Studies on the Brucella Group. VIII. Nutrient Requirements in synthetic mediums. J. Infect Dis., 51: 344-360 (1932).

- (1) Lactates, citrates, cystine, and asparagine can meet the carbon or energy requirements;
- (2) Nitrogen is provided by ammonium ions and amino acids such as cystine and cysteine;
- (3) Sulphates and thio-amino acids provide the small amount of sulphur needed. Phosphorus is provided by the phosphates
- (4) Magnesium, with either potassium or sodium, are essential mineral requirements;
- (5) Iron is beneficial.

The physicochemical requirements of a synthetic media were also examined²⁰. ZoBell discovered that 2 to 6 atmospheres is the optimal osmotic pressure for Brucella cultivation; the limits to growth between pH 5.8 and 8.7, with optimal growth between pH 6.6 and 7.4; that semisolid media are advantageous²¹; rapid multiplication will only occur if the initial inoculum of cells is large, or if traces of enrichment substance are provided; and that all members of the Brucella group require carbon dioxide or carbonates, and are oxygen-sensitive.

In his early work on the Brucella group, ZoBell hinted at the importance of the growth zones of Brucella in semi-solid media, yet it wasn't until 1937 that he published a paper that dealt specifically with this topic²². He reported that the three types of Brucella grow at different depths on or below the surface of the medium²³:

Type of <u>Brucella</u>	Growth Zone
<i>Brucella abortus</i>	5 to 8 mm (below surface)
<i>Brucella melitensis</i>	2 to 5 mm (below surface)
<i>Brucella suis</i>	Multiplies on surface to a depth of 4 or 5 mm

ZoBell stated that this is not sufficient information to provide a single differential test, but can be used together with other oxidation-reduction activity tests.

He added that these growth zones can be changed by adjusting the pH and the oxidation-reduction potential of the medium.

²⁰ Metabolism Studies on the Brucella Group. IX. Physico-chemical requirements in synthetic media. J. Infect. Dis., 51: 361-381 (1932).

²¹ Reasons for this mentioned earlier

²² Growth Zones of the Brucella in semi-solid Media. J. Bacteriology., 33:44 (1937).

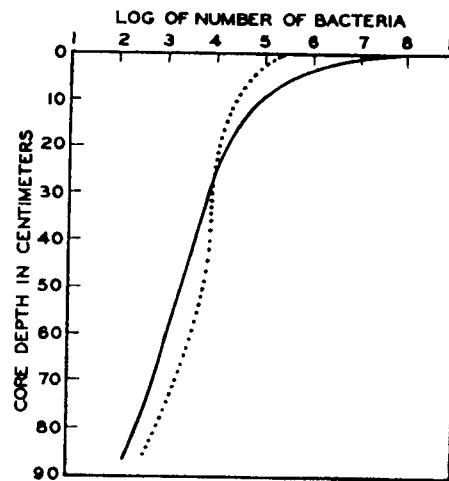
²³ Adapted from paper quoted in footnote 22.

Petroleum Microbiology

The study of petroleum microbiology was one of ZoBell's major research interests, and he was a member of the American Association of Petroleum Geologists, as well as being Director of the American Petroleum Institute Research Project 43A, examining the origins of oil.

When analysing 126 samples collected from marine bottom sediments from the Channel Island Region of the Southern Californian coast, ZoBell discovered bacteria of several physiological types which may have been involved in the diagenesis of marine sediments²⁴. The purpose of this analysis was to quantitatively estimate the bacteria in marine deposits, and to demonstrate the role of these bacteria in geological environment modification.

The experiment involved taking 4 cm diameter cores, between 20 and 90 cm long, from various water depths, from a few metres to over 2,000 metres. This was achieved using a modified Elkman coring device. ZoBell presented evidence which suggested that the bottom-dwelling bacteria multiplied at low temperatures, however, this multiplication was slow, and some bacteria required several weeks to form microscopically visible units when plated on nutrient agar. Another observation was that the highest number of microorganisms occurred in the upper layer of cores, as shown in the graph below:



This graph also shows that the number of aerobic bacteria is greater than the number of anaerobic in the uppermost regions. Both decrease with increasing depth of core, usually with the anaerobic bacteria dominating at core depths over 20 cm.

²⁴ Studies on the bacterial flora of marine bottom sediments. *J. Sed. Petrology.* 8: 10-18 (1938).

The following table, modified from ZoBell (1938), shows the relative number of different physiological types of bacteria found in the upmost 3 to 5 cm strata of three samples. A '+' sign means that bacteria were present, but not enumerated:

Sediment Sample Number	8160	8330	9309
Depth of Overlying Water	780 metres	505 meters	1322 metres
Sulfate Reduction	1,000	1,000	10,000
Dextrose Fermentation	10,000	100,000	1,000
Starch Hydrolysis	10,000	100,000	10,000
Cellulose decomposition	1,000	+	+
Fat Hydrolysis (lipoclastic)	1,000	+	+
Chitin digestion	100	+	+

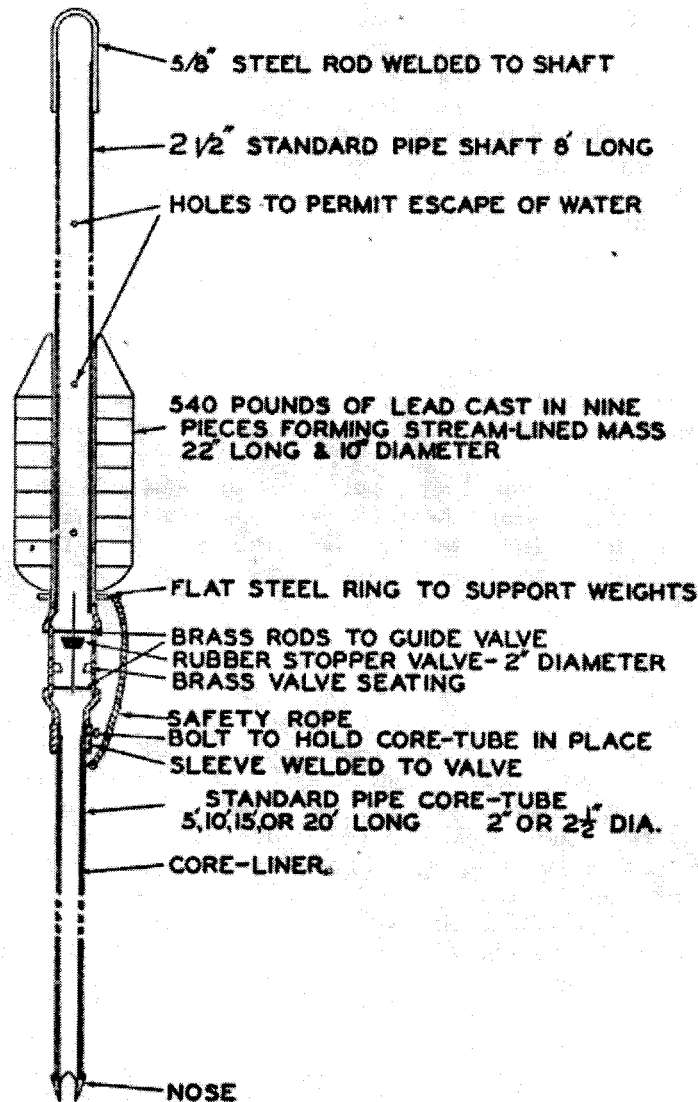
All figures are bacteria per gram of sediment (wet basis).

The most important figures in relation to petroleum formation are those of fat hydrolysis (lipoclastic). ZoBell found that the organisms which liberate fatty acids, and utilize the resulting glycerol, are distributed widely in the marine bottom deposits. The bacteria may create reducing conditions which deoxygenate higher fatty acids, which may be converted to long-chain hydrocarbons. However, in the course of this experimentation, ZoBell found no evidence for this, and he only reported the utilization of lower fatty acids. Even without this proof, ZoBell postulated that unsaturated fats could be converted into saturated hydrocarbons, which are characteristic of petroleum. This would be achieved by the dual activity of bacteria which reduce the oxidation-reduction potential, and the lipoclastic bacteria.

ZoBell's work on sediments was furthered in 1942²⁵, with a hope of discovering if bacterial activity has a role in petroleum genesis. For this experimentation, the coring device used was that described by Emery and Diez (1941), as shown on the next page²⁶:

²⁵ Changes produced by microorganisms in sediments after deposition. *J. Sed. Petrology*, 12: 127-136 (1942).

²⁶ Taken from 'Marine Microbiology, A Monograph on Hydrobacteriology' C.E. ZoBell, 1946.



The advantage of this coring device is that it removes the core without disrupting the stratification of the mud.

This experimentation confirmed ZoBell's earlier observations that the greatest populations of bacteria were found in the uppermost regions of the core, and the number of bacteria found decreases rapidly with increasing core depth, as shown in the following table²⁷:

²⁷ Adapted from ZoBell (1942). See footnote 25

Water Depth	3120 feet	3570 feet	1415 feet
Core Depth in inches	Bacteria per gram (wet)	Bacteria per gram (wet)	Bacteria per gram (wet)
0-1	38,000,000	7,500,000	840,000
1-2	940,000	250,000	102,000
4-5	88,000	160,000	63,000
9-10	36,000	23,000	19,000
14-15	2,400	8,700	1,500
19-20	400	2,100	2,200
29-30	180	600	370
39-40	330	200	190
59-60	250	300	210
79-80	130	100	140
99-100	290	150	140

ZoBell also discovered that the majority of microorganisms retrieved were bacteria, although actinomycetes, yeasts, moulds and algae were also recorded.

It was also confirmed that there was no direct evidence that bacterial activity played an important role in petroleum genesis. ZoBell mentioned the popular theory of the time, that the presence of free hydrogen in the source beds of oil could be produced by bacterial activity. Indeed, ZoBell found hydrogen producing bacteria which produce hydrogen during the anaerobic decomposition of organic matter, in deposits from all depths explored.

ZoBell also studied hydrocarbon production by sulfate-reducing bacteria²⁸. The presence of *Desulfovibrio* species in oil-bearing sands, and the depletion of sulphate in brines suggests these sulfate-reducing bacteria may have been active *in situ*. ZoBell's laboratory experiments aimed to demonstrate the ability of these bacteria to synthesize hydrocarbons from fatty acids.

The media for cultivation that was used was sea water enriched with fatty acids, as the sole source of organic carbon. Following three weeks of incubation, ZoBell was able to recover an oil-like extract, which was a compound consisting of aliphatic hydrocarbons, C₁₀ - C₂₅. Control experiments only yielded small amounts of this mixture.

²⁸ Hydrocarbon production by sulfate-reducing bacteria. J. Bacteriology., 47: 447 (1944).

In 1949, ZoBell examined the occurrence and characteristics of methane-oxidizing bacteria in marine sediments²⁹, fueled by a lack of research in this area. He found that they are a common occurrence in the uppermost layers of marine sediments, and this abundance is increased where free oxygen and methane are present. The following table, adapted from his 1949 paper, shows the number of samples harbouring methane-oxidizers:

Description of material	Total Number of Samples Tested	Number Showing Presence of Methane Oxidizers
SURFACE SAMPLES (topmost 10cm)		
Marine Mud	21	18
Brackish Water Mud	41	36
Paraffin Earth	9	9
Oil- or gas-field Soil	187	139
Beach Sand	9	2
SUBSURFACE SAMPLES (below 10 cm)		
Marine Mud	17	4
Brackish Water Mud	12	1
Beach Sand	3	0

Growth of methane-oxidizing bacteria resulted in the disappearance of methane constituents of the media, in addition to oxygen consumption and carbon dioxide formation. Of the methane oxidized, 10 to 40% of the carbon components were used in the production of bacterial cell substances. The partial pressures which resulted in the most rapid disappearance of methane were,³⁰:

	Partial Pressure
Oxygen	10 - 40%
Methane	Up to 70%
Carbon Dioxide	5-20%

Additionally, ZoBell reported that the methane-oxidizing bacteria studied had optimal activity at 32°C and on mediums of pH 6-7.

²⁹ The Occurrence and Characteristics of Methane-Oxidizing Bacteria in Marine Sediments. *J. Bacteriol.*, 58: 463-473 (1949).

³⁰ Adapted from ZoBell (1949).

ZoBell returned his attention to the subject of petroleum genesis, with one of his most important review papers, 'The Part Played by Bacteria in Petroleum Formation'³¹. In this paper he discussed the processes of bacterial conversion of recent organic sediments into humus and other elements which resemble the elementary composites of crude oil. He also mentioned the formation of methane, hydrogen sulfide, saturated compounds, and other substances that may be present in oil fields. These are formed by anaerobic bacteria which catalyze molecular hydrogen oxidation.

The petroliferous sediments that ZoBell studied rarely contained any aerobes, and the majority of the bacteria were sulfate-reducing. These anaerobes ferment organic matter, which usually results in methane formation. Bacteria can also produce pigmented hydrocarbons, some of which contain the benzene nucleus; they can also degrade proteins to produce the phenol and cresol commonly found in crude oil.

Bacteria can also play a part in the destruction of petroleum hydrocarbons, which can account for the absence of oil in many environments. This could either be by attacking the hydrocarbons, for example, at the oil-water interface, or by increasing the liberation and migration of oil. The most obvious example of this is the bacterial breakdown of the organic matrix of plant bodies containing oil.

Barobiology

It could, and has been said that ZoBell was the founder of barobiology, the study of the effects of pressure on the growth, viability, and metabolism of bacteria in deep sea environments. Indeed, the Galathea Expedition was a great launch for the field, although this was not the only contribution that ZoBell made.

ZoBell and Johnson studied the effects of hydrostatic pressure on terrestrial and marine bacteria³², and other microorganisms, with particular reference to the relationship between temperature and hydrostatic pressure up to 9,000 pounds per square inch (approximately 600 atmospheres). Representatives from the following genera were studied:

³¹ Part Played by Bacteria in Petroleum Formation. *J. Sed. Petrology.*, 22: 42-49.

³² The Influence of Hydrostatic Pressure on the Growth and Viability of Terrestrial and Marine Bacteria. *J. Bacteriol.*, 57: 179-189 (1949).

<u>Terrestrial Microorganisms</u>	<u>Marine Microorganisms</u>
<i>Alcaligenes</i>	<i>Achromobacter</i>
<i>Bacillus</i>	<i>Bacillus</i>
<i>Clostridium</i>	<i>Flavobacterium</i>
<i>Escherichia</i>	<i>Micrococcus</i>
<i>Micrococcus</i>	<i>Photobacterium</i>
<i>Mycobacterium</i>	<i>Pseudomonas</i>
<i>Proteus</i>	<i>Vibrio</i>
<i>Pseudomonas</i>	
<i>Sarcina</i>	
<i>Serratia</i>	
<i>Staphylococcus</i>	
<i>Streptococcus</i>	
<i>Hansenula</i>	
<i>Saccharomyces</i>	
<i>Schizosaccharomyces</i>	
<i>Sporobolomyces</i>	
<i>Torula</i>	

The terrestrial organisms were able to grow at 30°C, and all developed within 48 hours, when pressure was normal. There was, however, no noticeable growth under pressure of 600 atmosphere, and this pressure caused sterilization. 400 atmospheres caused retarded growth, and at 300 atmospheres death was still faster than growth.

The marine species predictably coped much better with the effects of pressure. Many species, for example, *Bacillus submarinus*, were able to grow readily at 30°C and 40°C under pressures of 600 atmospheres (their normal habitat being 500 atmospheres). The microflora of muds from the same habitats showed increased growth under pressure, and ZoBell and Johnson coined the term barophile to define these pressure-loving micro-organisms. Additionally, as the depth below the water surface that a micro-organism lives decreases, its sensitivity to pressure increases. Another general finding of the paper was that lower temperature exaggerated the retarding power of high pressure.

The study of hydrostatic pressure was narrowed to *Bacillus subtilis* for Johnson and ZoBell's subsequent two papers³³. They found that at 25°C and in a buffered salt solution of pH7, the spores slowly lost viability, and this could be accelerated

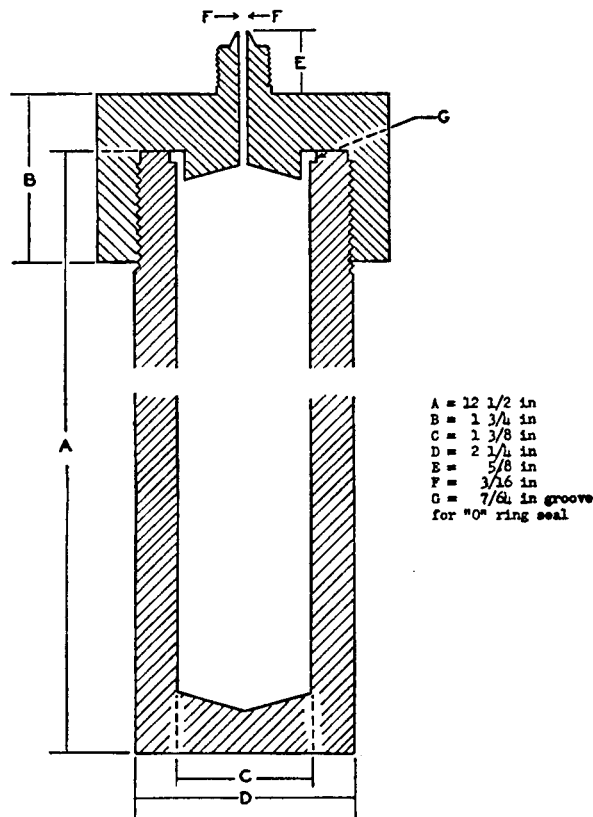
³³ The retardation of thermal disinfection of *Bacillus subtilis* spores by hydrostatic pressure. J. Bacteriol., 57: 353-358 (1949), and,

The acceleration of spore disinfection by urethan and its retardation by hydrostatic pressure. J. Bacteriol., 57:359-363 (1949).

when there is a hydrostatic pressure of 600 atmospheres. At higher temperatures (for example, 92.5°C) spore loss is retarded by increasing hydrostatic pressure.

The experimentation was furthered, using urethan, which resulted in a reversible or irreversible denaturation of some enzymes and proteins. Using 2M urethan at 25°C, they noticed no effect on the viability of spores. However, at 93.5°C, just 0.1M urethan led to an acceleration of spores disinfection, which could be accelerated if the concentration of urethan was raised to between 0.5M and 2M. More relevantly, it was found that this disinfection rate could be retarded by increasing the hydrostatic pressure from 100 to 600 atmospheres.

ZoBell worked with one of his Ph.D. students, Carl Oppenheimer, on the effects of pressure on multiplication and morphology³⁴ of marine bacteria. During their research they developed apparatus and techniques enabling the study of multiplication and viability, at hydrostatic pressures up to 2,000 atmospheres. The diagram below shows a cross-section of the vessel that they used for maintaining microorganisms at high hydrostatic pressures, and each of these could hold 20 to 24 culture tubes:



³⁴ Some Effects of Hydrostatic Pressure on the Multiplication and Morphology of Marine Bacteria. *J. Bacteriol* 60: 71-781 (1950).

The findings of this paper were:

- 1) Pressures between 200 and 600 atmospheres retarded the multiplication of most marine bacteria;
- 2) This pressure combined with a temperature of 30°C was enough to kill many marine bacteria within 2 to 4 days. However, there were some barophilic bacteria, such as *Micrococcus aquivivus*, which could multiply well at 600 atmospheres; and,
- 3) Confirmation of earlier research that higher pressures can be tolerated if the temperature is higher.

A further paper examined sixty-three species of marine bacteria³⁵. This consolidated many of the findings of earlier research, and also detailed some of the morphological variations induced by hydrostatic pressures ranging from 200 to 600 atmospheres after 4 days incubation at 27°C:

Organism	Morphological Variation
<i>Bacillus abyseus</i>	Increase in cell size and spore formation
<i>Bacillus borborokoites</i>	General increase in cell size
<i>Micrococcus aquivivus</i>	General increase in cell size
<i>Sarcina pelagia</i>	Increase in size and marked pleomorphism
<i>Serratia marinorubra</i>	Formation of long filaments and loss of motility
<i>Vibrio phytoplanktis</i>	Longer pleomorphic rods, granular, loss of motility
Culture no. 643	Long filament formation

The Galathea Expedition was a very important point in ZoBell's study of the barophiles, and many publications resulted from this. The main finding of the expedition was that organic life occurs at depths exceeding 10,000 metres, where the temperature borders 2°C, and the hydrostatic pressure is more than 1000 atmospheres³⁶. ZoBell was able to cultivate the bacteria that were retrieved, using barokams to maintain pressure. This was the necessary confirmation that viable life did in fact occur in the deepest parts of the oceans, and that even with

³⁵ The Growth and Viability of Sixty-Three Species of Marine Bacteria as Influenced by Hydrostatic Pressure. J. Mar. Res., 11: 10-18 (1952).

³⁶ 'Meeting Microbes From the Deepest Sea' Hakon Mielche, Aalborg Stiftende, Sunday 29 January, 1967.

the greatest effects of pressure, bacteria could survive. The table below ³⁷ shows the differing physiological types of bacteria found from different depths, and their populations when incubated at 1 or 1,000 atmospheres. It can clearly be seen that there are more bacteria per gram of wet sediment when the pressure is near that of the bacteria's normal habitat:

Water Depth	10,190 m	10,190 m
Incubation Pressure (atm)	1	1,000
Total Aerobes	10 ²	10 ³
Total anaerobes	10 ³	10 ⁵
Starch hydrolyzers	10 ²	10 ³
Nitrate reducers	10 ²	10 ⁵
Ammonifiers	10 ³	10 ⁵
Sulfate reducers	0	10 ²

Following the development of equipment to retrieve micro-organisms from these depths, the amount of research by other scientists in this area increased. The work was mainly on the biochemical adaptations of the barophiles, and the temperature sparing effect on pressure sensitivity.

When studying the effect of pressure on the succinic dehydrogenase system in E. coli ³⁸, ZoBell and Morita found that the system could be inactivated by hydrostatic pressures. This inactivation started at 200 atm at 30°C, and progressed to irreversible inactivation at 1000 atm for 4 hours. Additionally, the effects of inactivation were exaggerated if the temperature was above 40°C or below 8°C.

ZoBell also studied the effects of increased hydrostatic pressures on the growth parameters of Escherichia coli³⁹. The main findings of this study were that:

- (1) Reproduction was retarded by hydrostatic pressures of 200 to 500 atm;
- (2) At 200 atm, a temperature of 40°C resulted in bacterial populations reaching stationary phase within 5 to 10 hrs, whereas at 30°C, it took 10-15 hrs;
- (3) Death rate was promoted over 400 atm;
- (4) Colony count generally decreases more rapidly at 30°C than at 20°C.

³⁷ From Barophilic bacteria in some deep sea sediments. J. Bacteriol., 73: 563-568. (1957).

³⁸ Effect of hydrostatic pressure on the succinic dehydrogenase system in Escherichia coli. J. Bacteriol., 71: 668-672 (1956).

³⁹ Growth, Reproduction, and death rate of Escherichia coli at increased hydrostatic pressures. J. Bacteriol., 84: 1228-1236 (1962).

In a later study⁴⁰ ZoBell studied filament formation in E. coli under hydrostatic pressures. They found that under increasing pressure, filaments were formed in three strains of E. coli, leading to increased cell length.

ZoBell postulated that replication of DNA was repressed, causing inhibition of cell division. The resulting effect of this would be filament formation, a phenotypic response to a changing environment.

ZoBell also studied the RNA content of the three strains of E. coli, and found that as pressure increased, so did the amount of RNA found in cultures. The reason for this increase was unclear, although ZoBell tentatively postulated that this rise could induce filament formation.

Biofilms

Another of ZoBell's research interests was the study of biofilms and the attachment of bacteria to submerged surfaces.

When studying the attachment of marine bacteria to submerged slides⁴¹, ZoBell was particularly interested in the role of micro-organisms in fouling marine structures and navigational equipment. Slides were submerged from the La Scripps Institution pier for between one and seven days, and ZoBell noted the presence of bacteria, and also diatoms and actinomycetes. These would become attached days before other fouling organisms, such as barnacles.

ZoBell studied 73 bacterial cultures, and found that only 24 of these had attachment ability⁴². He also found that some of these could only grow in films on surfaces (thigmotactic). Three of the bacterial cultures formed macroscopically visible films before turbidity was reached in the nutrient broth used.

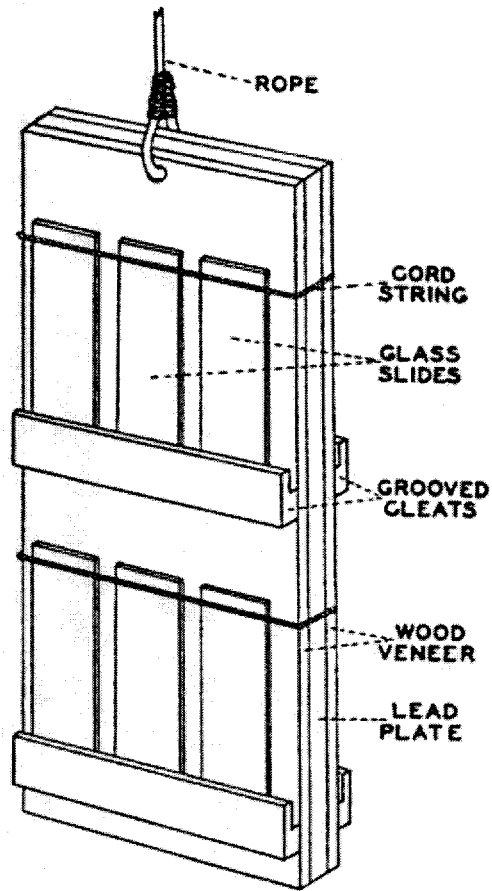
All stages of bacterial development were observed on slides, and many bacteria were evidently multiplying on the slide surface. Additionally, most of the attachment bacteria were encapsulated, and the ability to attach was enhanced by the addition of glucose to the media. He was not able in this experiment to define the nature of the attachment device.

⁴⁰ Filament Formation by Escherichia coli at increased hydrostatic pressures. J. Bacteriology., 87: 710-719 (1964).

⁴¹ Attachment of marine bacteria to submerged slides (E. C. Allen). Proc. Soc. Exper. Biol. Med., 30: 1409-1411 (1933).

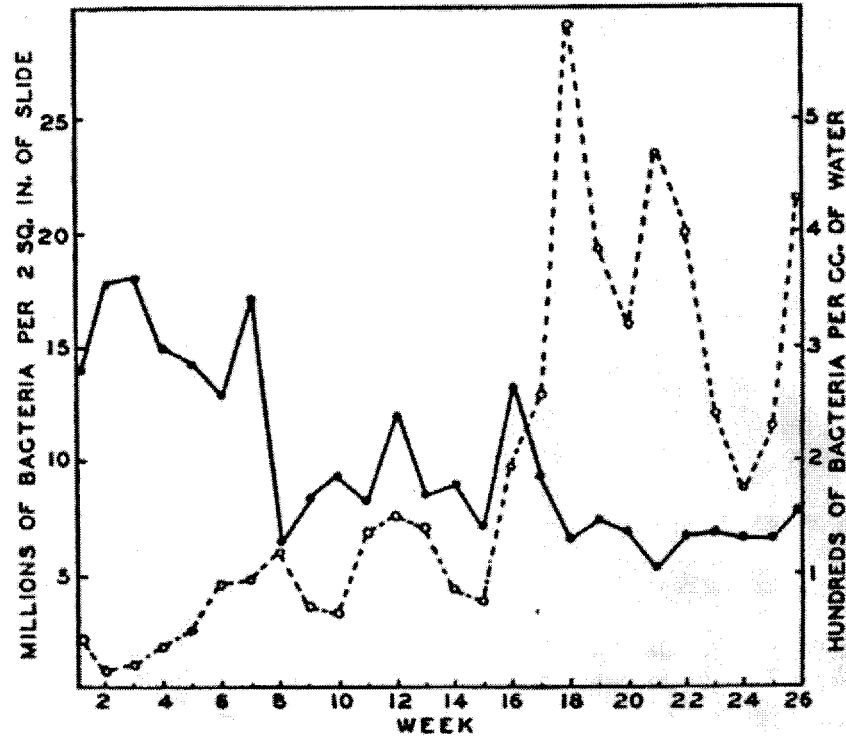
⁴² ZoBell defined these "attachment bacteria".

ZoBell consolidated and furthered this work⁴³. A wood covered lead carrier was developed for holding the glass slides:



The whole structure was coated with paraffin, preventing direct contact between the slides and metal. The following graph shows the weekly average results for bacterial attachment for slides submerged between 6 and 12 metres (depending on tides), for forty-eight hours:

⁴³ The significance of marine bacteria in the fouling of submerged surfaces (E. C. Allen). *J. Bacteriol.*, 29: 239-251 (1935).



(The broken line indicates the number of bacteria per two square inches of submerged slide, and the solid line shows average plate counts per cubic centimetre of sea water).

ZoBell commented that attachment increased once a layer of film formers had colonised the submerged slide. He studied the attachment of bacteria after 24, 48 and 72 hours' submergence, and found the following (bacteria/2 sq. inches):

PERIOD OF SUBMERGENCE	BACTERIA	OTHER M'ORGS	MACROSCOPIC ORGANISMS
24 Hours	2,023,800	2,560	0.3
48 Hours	9,268,200	10,840	1.2
72 Hours	24,115,400	28,310	1.9

Other experiments studied the role of the film in attachment. A comparison of film-coated (with attachment bacteria) and sterile slides was made, and it was found that the film-covered slides did result in a greater level of attachment:

PERIOD OF SUBMERGENCE	MICROORGANISMS	
	Sterile slides	Film-coated slides
24 hours	15	42
48 hours	23	89
72 hours	98	276
120 hours	852	1,257

However, ZoBell noted that bacterial cells rarely occurred in micro-colonies. Cells were most likely to be found singly, or in pairs. ZoBell attributed this finding to the lack of nutrients in sea water, and discovered in laboratory experiments that long chains of cells occurred more frequently when nutrients were added to the sea water.

Observation of the bacteria which attached showed that the 60 per cent were ovoid with a diameter less than 1 micron, and slender bacilli of 1 to 2 microns were also common. The majority of bacteria observed were gram-negative. Actinomyces and Chlamydo bacter were the two types of filamentous forms most frequently noted.

The genera's of diatoms observed were, Grammatophora, Navicula, Licmophora, Fragilaria, Striatella, and Nitzschia.

This experimentation concluded that the primary film-formers are mainly bacteria, which allows subsequent attachment of other fouling organisms. This experiment did not, however, provide evidence that a bacterial film is necessary for macroscopic fouling organisms, such as barnacles, to attach.

ZoBell furthered his study of biofilms by examining the effects of solid or adsorbing surfaces on bacterial activity⁴⁴. This paper examined the sessile nature of some bacteria, methods of attachment, and the effect of solid surfaces.

When examining the sessile behaviour of bacteria, ZoBell used microscopic techniques to study bacteria⁴⁵. ZoBell found that the majority of bacteria in the sea are associated with solid surfaces, and very few are found unattached. Bacteria attach to surfaces of plankton, as these provide food and energy for the bacteria, as by-products of metabolism. Bacterial attachment is also facilitated on plankton, due to the presence of a slimy mucilage.

⁴⁴ The effect of solid surfaces upon bacterial activity. J. Bacteriol., 46: 39-56 (1943)

⁴⁵ Based on work by Cholodny, on the direct microscopic study of soil bacteria.

However, many bacteria were found that were able to attach to clean surfaces. Earlier work by ZoBell and Allen⁴⁶ showed three new species that were able to form films on clean surfaces. These are Achromobacter marinoglutinosus, Achromobacter membraniformis and Flavobacterium amocontactum.

In the 1943 paper, ZoBell amends the names of these bacteria, as they have polar flagella. The first two became classified as Pseudomonads. Other sessile forms that ZoBell discovered include Pseudomonas stereotropis, P. sessilis and Bacillus epiphyticus.

ZoBell found that more bacteria attach to slides during early logarithmic growth phase than in later growth phases, regardless of population density. This led ZoBell to suggest that bacteria attach themselves, rather than arriving there passively.

The mode of attachment was examined, and ZoBell noticed that some bacteria attached by means of a filament or stalk, whose morphology is noticeably different from that of the cell itself. Whether this stalk develops before or after attachment could not be elucidated.

He did not believe, however, that the stalk was the main means of attachment. ZoBell reported the presence of a cementing substance, which is secreted by physiologically active sessile bacteria when they contact solid surfaces. This cement, which stained the slides, had a diameter two or three times the size of the cells. ZoBell could only provide evidence for the adhering qualities of this film, he could not actually determine that it was produced by the bacteria. Additionally, he believed that the film may play a role in nutrient concentration in the bacteria's vicinity.

These were great contributions to the study of biofilms, and much research since has demonstrated the role of extracellular substances, for example polysaccharides, in attachment of bacteria to surfaces.

Another role of the solid surface, in ZoBell's opinion was to retard diffusion of hydrolyzates and exoenzymes away from the cell. The advantage of this is that it would promote nutrient assimilation before digestion.

Microbial Physiology

In this section I will consider ZoBell's work on nitrification and the oxidation of organic matter.

⁴⁶ See footnote 44

Nitrification

The productivity of the sea may be limited by a lack of nitrites, so ZoBell attempted to discover any phenomena which could increase the amount of nitrogen available for plant growth⁴⁷. Research up to this point had shown nitrifiers in soil, marine mud and near the shore. (Later research showed marine nitrifiers in marine bottom deposits, if the oxidation-reduction potential was favourable⁴⁸).

Nitrosomonas from soil cultivated in sea water did not produce any nitrites. ZoBell's conclusion was that either cultivation methods used were inappropriate or that nitrifiers in the sea differ from those found in soil.

Further experimentation suggested that sunlight favoured nitrification in a sample of sea water and ammonium sulfate. Exposure to sunlight for two weeks resulted in an increase in nitrite and nitrates, and a decrease in ammonium sulfate concentration. The photochemical nitrifying properties were attributed to oxidizing substances or organic catalysts, however, this could only occur a few millimetres from the water surface, as light penetration through water is low.

Later, the methane-oxidizing bacteria were shown to have nitrite producing ability⁴⁹, and could utilise NH_4Cl , peptone, glutamic acid, or KNO_3 as nitrogen sources.

ZoBell also demonstrated that nitrites could be produced in bacteria-free cultures, when he discovered the assimilation of ammonium nitrogen by the phytoplankton Nitzschia closterium⁵⁰.

Organic Matter

When studying the oxidation of organic matter by sea-water bacteria, ZoBell was interested to see if oxygen tension would have any effect⁵¹.

The concentration of oxygen studied ranged from 0.3 to 12.74 cc./l. and it was found that the concentration didn't influence the rate of oxidation of organic

⁴⁷ Photochemical nitrification in sea water. *Science*, 77:27-28 (1933)

⁴⁸ Oxidation-Reduction potentials and the activity of marine nitrifiers. *J. Bacteriol.*, 29: 78 (1935).

⁴⁹ Production of nitrite from ammonia by methane oxidizing bacteria. *J. Bacteriol.*, 65: 216-219 (1953).

⁵⁰ The assimilation of ammonium nitrogen by Nitzschia closterium and other marine phytoplankton. *Proc. Nat. Acad. Sci.*, 21: 517-522 (1935).

⁵¹ The effect of oxygen tension on the rate of oxidation of organic matter in sea water by bacteria. *J. Mar. Res.*, 3: 211-218. (1940).

matter. Oxygen only became a limiting factor when its concentration fell below 0.3 cc. per litre.

The oxygen consumption of bacteria was found to fall, however, following two days of incubation, and this was due to organic matter having been utilised. Additionally, when sea water was enriched with asparagine and glucose, the rate of respiration increased.

Lignoprotein could also be oxidized, yet slowly, and is not oxygen tension dependent.

The work on utilization of organic matter was continued⁵². ZoBell was concerned that most experimentation had focussed on high concentrations of organic matter, which were greater than that found in natural environments. He attempted to rectify this situation by using lower concentrations in his experimentation. It was found that Escherichia coli, Staphylococcus citreus, Bacillus megatherium, Proteus vulgaris and Lactobacillus lactis could grow and multiply in media containing either peptone or glucose at 0.1 mgm./l, and he postulated that bacteria could grow at lower levels. Experimentation was limited by the difficulty of preparing nutrient solutions of concentrations lower than this.

ZoBell found that utilization of food was increased by adding a greater concentration of nutrients or by increasing the inoculum size.

This study also confirmed that excess food may be converted into basal protoplasm, and experimentation showed that between 30 and 40 per cent of nutrients added were so converted.

Scientific Methods and Techniques

In many of his research papers, ZoBell described special techniques and methods that facilitated his experimentation. However, he also wrote some papers which made direct reference to the development of techniques. These are hugely important contributions to marine microbiology, and these methods allowed knowledge of marine microbes to be extended.

Two of ZoBell's most important contributions were, the cultural requirements of heterotrophic aerobes, and the development of apparatus for collecting water samples from different depths for bacteriological analysis, and these are both considered benchmark papers.

⁵² Bacterial Utilization of low concentrations of organic matter. J. Bacteriol., 46: 39-56 (1943).

The cultural requirements of heterotrophic bacteria⁵³

ZoBell examined the cultural requirements of heterotrophic bacteria because he found that quantitative results from researchers across the world were not comparable. He believed that the most likely reason for this was the lack of a consistently utilised media. The aim of the experimentation was to prepare a nutrient medium which as well as providing maximum counts, would also be reproducible.

Salinity Requirements

ZoBell attempted to resolve the contradictions that had appeared in earlier research regarding salinity requirements. To do this he prepared media which were consistent, apart from the ratio of sea water to distilled water. He found that a maximum growth index was achieved if the media contained 100 per cent sea water, and decreased to a growth index of eight if 100 per cent distilled water was used. This data is shown in the following table:

Per cent sea water	100	75	50	25	10	5	0
Per cent distilled water	0	25	50	75	90	95	100
Average number of bacteria	224	200	141	84	36	26	17
Growth Index	100	89	63	41	16	12	8

Further experimentation revealed that synthetic sea water is not satisfactory as a substitute.

Effect of Nitrate

In this experimentation, ZoBell was aiming to discover if the addition of nitrates to media was justified. Indeed, most researchers added nitrate as a matter of course, without examining whether this addition was merited. In his experimentation, ZoBell used a concentration of potassium nitrate between 0 and 0.10 per cent, and found that there was no beneficial effect in increasing the concentration. At the highest concentrations, the addition of potassium nitrate was found to be detrimental. ZoBell concluded that nitrates need only to be added if the experiment is to test the ability of bacteria to reduce nitrates.

Phosphate

⁵³ Studies on marine bacteria. I. Cultural Requirements of Heterotrophic aerobes. J. Mar. Res., 4:42-75 (1941)

It was found that sea water generally contains sufficient potassium for bacterial multiplication, however, where this is untrue, the addition of 0.01 per cent dibasic potassium phosphate is beneficial.

Iron

Plate counts increased from 18 to 76 per cent if a trace amount of iron was added to the media.

pH

A pH of between 7.5 and 7.8 resulted in the best growth. This was surprising as the pH of surface sea water ranges between 7.8 and 8.8, and decreases with depth. The bacteria used in the experiment were from sea water samples ranging from pH 8.0 to 8.3.

Solidifying Agents

Bacto-agar at concentrations of 1.2 to 1.5 per cent was found to be the most suitable solidifying agent. Gelatin and silica gel resulted in lower plate counts.

ZoBell recommended medium 2216, which contains 0.5 per cent Bacto-peptone, 0.01 per cent ferric phosphate and 1.5 per cent Bacto-agar. This is dissolved in sea water, and has a pH of 7.6 following sterilization. This media is still widely available.

This paper was the result of eight years of study, and is important because it demonstrates that nutrients only need to be added to media in small quantities. This showed that low nutrient levels could be utilized by bacteria, which has been repeatedly confirmed since.

This media is still being used in research⁵⁴.

Apparatus for collecting water samples⁵⁵

ZoBell aimed to develop apparatus that would be able to retrieve bacteria from varying depths, whilst being easily sterilized and able to work under high hydrostatic pressure. ZoBell was not the first scientist to attempt to construct a

⁵⁴ For example, Nair et al, Culturable heterotrophic bacteria from the euphotic zone of the Indian Ocean during the summer monsoon. *Oceanologica Acta*, 1994. 17(1). 63-68.

⁵⁵ Apparatus for collecting water samples from different depths for bacteriological analysis. *J. Mar. Res.*, 4: 173-188 (1941).

suitable device, and his work furthered that by, for example, Johnston (1892)⁵⁶, who developed a device for stopper removal whilst underwater.

ZoBell also had to consider which materials the container should be made of. Metals such as bright brass, bronze, or other alloys containing zinc, tin, nickel, or copper could not be used as they were found to be bactericidal. Glass could be used without detriment, and the device developed, called the J-Z Sampler, is shown on the following page:

⁵⁶ On the collection of samples of water for bacteriological analysis. Canadian Rec. of Sci., 5: 19-28. (1892)

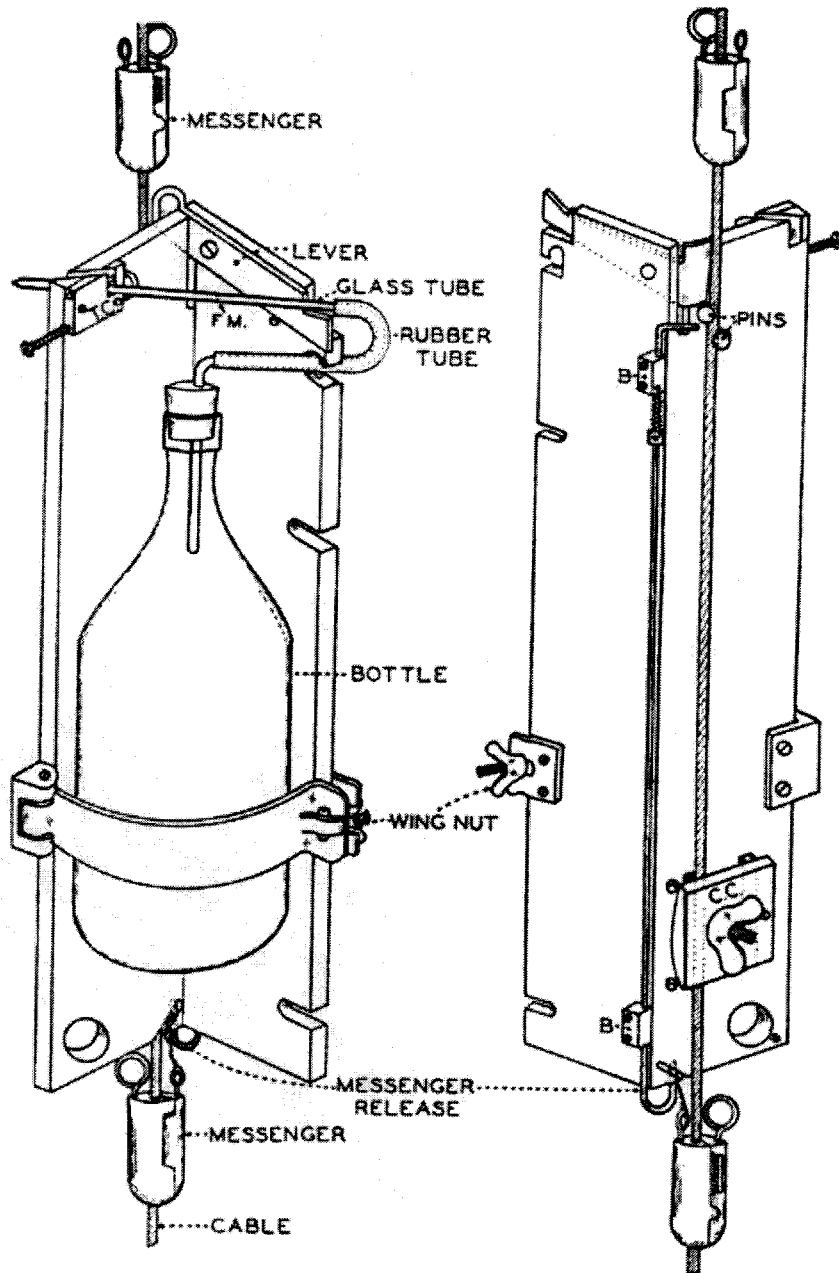


Figure 37. Front and back view of the J-Z bacteriological sampler.

This diagram shows the glass bottle clamped into a brass carrier, which is lowered into the sea. The messenger is dropped when the container reaches the required depth, allowing a sample to be taken.

This piece of equipment is suitable for collection of samples to 200 metres below the sea surface. For depths greater than this, where there is a greater hydrostatic

pressure, the bottle is replaced by a pressure-resistant collapsible rubber bottle. However, the sample cannot be kept in the rubber bottle for very long, as certain marine bacteria will attack it.

This apparatus was an important contribution, as it was easily constructed, and reusable (apart from the inlet tube). These devices are still available today, with only minor modifications.

What made this a benchmark paper however were the findings on the toxicity of metal bottles to marine bacteria. In continuing work by the likes of Raadsvald⁵⁷, ZoBell demonstrated that toxicity to bacteria would occur only 5 minutes of exposure to metal Nansen bottles that were frequently used in sampling.

There was just one criticism of ZoBell's apparatus, and that was that the capacity of the storage bottle was too small.

Summary of Other Work

Of the nearly 300 research papers that ZoBell had published during his life, he covered many aspects of marine microbiology. It has not been possible to mention all of this research in this document. However, of his other studies the following areas were important:

- 1) Microbial infections of marine fish
- 2) Occurrence of bacteria in higher marine organisms
- 3) Coliform bacteria in water supplies, sea water and beaches
- 4) Bacteria in Great Salt Lake
- 5) Aerial transport of living microbes
- 6) The role of sulfate-reducing bacteria and hydrogen sulphide production
- 7) Oxygen tension and the oxidation of organic matter
- 8) Bacteria as food for marine animals
- 9) Bacterial release of oil from oil-bearing materials

The Most Important Contributions of Claude E. ZoBell

ZoBell wrote nearly three hundred papers during his research. It is therefore very hard to summarise his most important contributions.

To see how ZoBell shaped marine microbiology, a brief consideration of the history and status of the field is necessary. The first work on marine bacteria began in the late nineteenth century, however, this work was still always trying

⁵⁷ Raadsvald, C. W. 1934. The oligodynamic effects of metals and metal salts. Chem. Weekbl. 31: 497-504.

to find links with medical microbiology. For example, many related their studies to Cholera vibrios distribution.

Soon after, marine bacteria were isolated from oceans, up to depths of 300 metres⁵⁸. In the same period, Prince Albert of Monaco gave support to marine microbiology, and the German Plankton Expedition of 1889 stimulated interest in micro-organisms of the sea.

Britain followed Germany as a centre for research (who were mainly concerned with luminescence and the nitrogen cycle), with benchmark papers by Drew, on denitrifying bacteria, and Lloyd, on the Clyde Sea area. The amount of work on light production and physiology was high in Europe up to the 1930s.

In America during this period, Harvey and Johnson were also studying luminescence, and in the Soviet Union scientists were studying bacteria of the Arctic Ocean and Caspian Sea.

Work began by ZoBell and Waksman, both at La Scripps, in the 1930s. Hence began a huge research effort into many aspects of marine microbiology.

There are many of ZoBell's contributions which are outstanding, and are considered benchmark papers. Regarding biofilms, ZoBell's observations on the attachment of bacteria to surfaces were a great achievement. This work was a foundation for this much studied subject, and ZoBell is often quoted in scientific papers, as an introductory reference to the subject.

In the field of barobiology the work aboard Galathea II was a huge advancement, especially the provision of evidence for viable life in the deepest parts of the ocean. This was a major contribution, and even though in the following years micro-organisms were found at deeper ocean depths, ZoBell provided the foundation for the study of the barophilic bacteria, and showed that many bacterial species required hydrostatic pressure in order to function physiologically. His work on barobiology was also very important in providing details of techniques that could be employed to retrieve and culture bacteria requiring up to 1,000 atmospheres of pressure. ZoBell always was a scientist 'in the field' and as much of his work as possible was conducted in natural environments and he understood the limitations of laboratory based results. The work on Galathea is a prime example of this, and the fact that the first article he published on this gained the leading position in 'Science' (America) demonstrates what an important contribution this was.

⁵⁸ Certes (1884) On the culture, free from known sources of contamination, from waters and from sediments brought back by the expeditions of the Travailleur and the Talisman. C. R. Hebd. Seances Acad. Sci., 98, 690-693.

Work of this nature was groundbreaking in a scientific community whose attentions were focused primarily on medical microbiology. ZoBell's research career did originally start at the Hooper Foundation for Medical Research, and his stay at La Scripps was meant to be brief, before his return to studying medical microbiology. His work on the metabolism of Brucella may not be what he is most remembered for, but it is important in describing the nutrients and physico-chemical requirements a bacteria needs. It highlights the quantity of research that is needed when studying the cultivation prerequisites of a bacterial genera.

ZoBell's break from medical microbiology, in hindsight, can be seen as fortuitous for the field of marine microbiology. His research helped to lay a foundation for this area, and established it as a subject which had a great potential for research.

Another benchmark paper that ZoBell published listed sixty new marine species⁵⁹. Classification is a necessity for any study of organisms, and as marine bacteria are so different from 'medical bacteria', this is particularly true. ZoBell's work had an additional advantage. The paper actually described morphological details, biochemical characteristics, and the effect of temperature and salinity on growth. Together with the book, *Marine Microbiology*, this work provided a foundation for further studies on classification, including that by Shewan et al., (1960)⁶⁰ on identification of gram-negative bacteria, and Colwell and Liston (1961) on taxonomic relationships between the pseudomonads⁶¹.

ZoBell also provided information regarding the possible role of bacteria in the formation of petroleum products. His research aim was to provide the mechanism by which this was done, although he never achieved this. However, his importance in the field of petroleum microbiology is demonstrated by his position as Director of the American Petroleum Institute Research Project 43A, regarding the origins of oil.

Another important contribution was the publication in 1946 of 'Marine Microbiology', a 240 page textbook. This work collated information on all aspects of marine microbiology, and included chapters on microbiological techniques. It was, at the time, the essential publication for anyone interested in marine microbiology, and its importance cannot be underestimated.

⁵⁹ A List of Marine Bacteria Including Descriptions of Sixty new Species. Bull. Scripps Inst. Oceanogr. Univ. Calif, 5 (2) 1944.

⁶⁰ A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the Pseudomonadaceae. J. Appl. Bacteriol., 23 (3), 379-390 (1960).

⁶¹ Taxonomic relationships among the Pseudomonads. J. Bacteriol., 82, 1-14 (1961).

Furthermore ZoBell's work is very important because his work always involved the design and construction of novel equipment for research, and the development of media for cultivation. This work was discussed earlier, and the medium for culturing heterotrophic bacteria is still used today, approaching sixty years after development.

To conclude, one of ZoBell's p.H. D students, Richard Morita, said that ZoBell's greatest achievement was "laying the foundation for all marine microbiology".

Appendices

1. Papers' of C. E. ZoBell Consulted and Cited.
2. Additional References
3. Acknowledgements
4. Letters received
5. Family Records

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The staff at Bristol University Library.

Letters

1. A typed version of a handwritten letter from Henry Davis ZoBell
2. From Jean S. ZoBell

3. From Karl ZoBell
4. From Dean ZoBell
5. From Elgarda Ashliman

From Henry Davis ZoBell

Ogden, Utah
Sept 15, 1997

Tim Gough,

In answer to your letter of Aug 28, I am sending to you a few items I remember about my brother Claude E. ZoBell. The major things you probably have received from his wife Jean and sons Dean and Karl.

Claude was one of the first in Idaho to receive the Eagle Scout Award.

He was the catcher on the school baseball team and seldom could a runner steal a base on him.

Claude and his older brother were experts in dynamite. On the 4th of July they would go to the church centre at day break and ignite several sticks of this explosive. The sound could be heard for 2-3 miles. This was the start of the days celebration.

Claude was the valedictorian of his Rigby High School graduating class. In his late teens he was principal of the Rigby High School.

As a younger brother I always looked up to him and his many accomplishments. He gave me advise and suggestions when asked for such things.

Sincerely, Henry D ZoBell.