Cellular Origin, Life in Extreme Habitats and Astrobiology 19

# Joseph Seckbach J. Patrick Kociolek *Editors*

# The Diatom World



THE DIATOM WORLD

### Cellular Origin, Life in Extreme Habitats and Astrobiology

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# The Diatom World

Edited by

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and

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#### FOREWORD

#### The Diatom World

The impact of diatoms on planet Earth is substantial and far reaching, hence "The Diatom World" is an appropriate title for a book describing the attributes of diatoms, with an emphasis on their ecological roles. The large-scale ecological success of diatoms suggests that they have refined their cellular processes for efficient utilization of nutrients and sunlight to a greater extent than most other unicellular algae. The most visible distinguishing feature of diatoms is their silicified cell walls, and because of its uniqueness, it is reasonable to assume that utilization of silica as a structural material is a valuable adaptation. A rich genomic diversity may also contribute to the diatoms' success; diatom genome sequencing has revealed a roughly equal contribution of plant and animal gene homologs, with a relatively high contribution of bacterial genes.

Diatom research impacts a wide variety of areas. Topics included in this book include morphology, phylogeny/evolution, sexuality/breeding, surface colonization and biofilms, infection and toxicity, bio/nanotechnology, extremophilicity, ecology, and endosymbiosis. Systematics is a major point of emphasis in the book. Two factors contribute to the continuing refinement of diatom systematics: (1) the enormous number of species and (2) distinctions between morphological and genetically based markers. The latter is an especially interesting point because although cell wall structure is ultimately genetically derived, the ability of diatoms to make such a diversity of structures "muddles the message" and there is necessarily no strict correspondence between genetic and structural similarity. Sexuality in diatoms is discussed, which includes an exploration of the concept that classical breeding approaches may be useful for diatom research. Diatoms affect the world as biofouling organisms, both in terms of ecological and economic impacts. The diatom silica cell wall has a universal appeal which encompasses aesthetic beauty coupled with an intellectual fascination about how such structures are made. Several chapters touch upon this subject both in terms of comparisons of the final morphology and the process of structure formation. The value of the silicified cell wall is evident for the diatom, but its usefulness for humans is addressed in terms of a possible source of inexpensive nano-structured materials for nanotechnological applications (with an emphasis on optical properties) and as a bioindicator of water quality. The ability of diatoms to colonize environments with extremes of temperature, pH, and salinity is covered in detail. All is not beautiful in the diatom world, even though they live in protective shells, diatoms can be infected by viruses, and toxin production by diatoms has adverse effects on the ecosystem and human health.

Diatoms impact processes on a wide range of scales, from the nanometer-to-micron range at which their silica structures are formed, through the meters-to-kilometers scale on which diatom communities function, and to the global scale carbon fixation and biogeochemistry. Given their participation in so many processes and at such diverse scales, it is indeed a Diatom World.

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Mark Hildebrand is a Research Professor in the Marine Biology Research Division of Scripps Institution of Oceanography, University of California, San Diego. He received a Ph.D. from the University of Arizona in 1987 and did his postdoctoral work with Benjamin Volcani at SIO. His lab's research is focused mainly on diatoms. in two areas: (1) silicon metabolism and cell wall synthesis and (2) development of diatoms for biofuel production. His work has involved application of molecular techniques to diatoms to clone silicon responsive genes, leading to the identification and characterization of silicon transporters. He contributed to the first determination of a diatom genome sequence (for Thalassiosira pseudonana). His lab has performed a proteomic investigation into cell-wall-associated proteins in T. pseudonana, a thorough examination of silicon transport processes which led to a mechanistic model for transport function and a new understanding of factors influencing uptake kinetics. and developed a synchronized growth procedure that enables monitoring of cellular processes (transcript and protein levels, cell wall formation, etc.) throughout the entire cell cycle. A publication in Journal of Phycology resulting from the latter work was awarded the Provasoli Award for best publication in the Journal for the year 2007 (Hildebrand et al., 2007, J. Phycol. 43:730). Current research in the lab is focused on applying high-resolution imaging techniques (AFM, SEM, TEM, and fluorescence microscopy) to follow the process of diatom cell wall silicification and to couple these processes with identification of the genes responsible for specific aspects of structure formation, which includes transgenic approaches. In terms of the development of diatoms as organisms for biofuel production, the lab is investigating the effect of different triggers for neutral lipid accumulation coupled with "omics" approaches to understand the underlying regulation involved in controlling carbon partitioning between carbohydrates for growth and neutral lipids for energy storage.

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#### **DIATOMS: GENERAL INTRODUCTION**

Diatoms are microscopic unicellular or colonial (in the shape of filaments or ribbons, or tube dwelling) eukaryotic algae. Their cell walls are with silica shells, and they are ubiquitously distributed in aqueous habitats.

Their name is derived from the Greek words (dia) = "through"+(temnein) = "to cut," since their cells are divided in two halves or two box-like parts (frustules or valves). They are one of the most common types of phytoplankton. One can detect them in marine as well as in fresh water habitats, at high and low temperatures, at different pH values, in hypersaline environments, and in brackish water. Diatoms especially play an important role in the oceans where they fix large amounts of carbon dioxide and synthesize carbohydrates that serve as a chief source of zooplankton food in the marine food chain. Bacteria adhere to and influence diatoms' growth.

There are over 1,250 genera of diatoms in the Class Bacillariophyceae. Diatoms are very responsive to environmental changes, and analysis of diatom communities can be used to study long-term changes.

Their exoskeleton is made of nanometer-sized particles of SiO<sub>2</sub> (silicon dioxide] obtained from their ability to "metabolize" silicic acid  $[Si(OH)_4]$  from the environment and form frustules. These silica "shells" of the cell walls are easily preserved and provide a useful tool for fossil research. Fossil evidence suggests that diatoms originated during, or before, the early Jurassic period (~210 to 144 Mya). It is assumed that these unicellular algae arose from the endocytobiosis of a red alga, which penetrated (or was engulfed) into a single-celled heterotrophic host eukaryotic cell. Their chloroplasts do not accumulate storage carbohydrates, as seen in, for example, the green algae.

Diatoms are often visible to the naked eyes as a golden coating growing on vessels, and they commonly form brown films on aquarium glass or rocks. At higher magnification and especially in the scanning electron microscope, their cells appear very attractive and display extremely beautiful designs and patterns, as shown by several photos in this volume.

Recently, the genome sequences of some diatom species as well as compilations of applied research on diatoms have been published, and the taxonomy of the group has been repeatedly revised in recent years. However, general books on their biology and ecology are few. In the current volume, some of the leaders in diatom research present new information and/or summarize recent research efforts on a wide range of topics, including morphology, nanostructure, morphogenesis, motility, ecophysiology with emphasis on their wide range of habitats and ecological niches, biogeography, taxonomy, molecular evolution and phylogeny, cryptic and endosymbiontic species, toxic species, viruses of diatoms, and more. However, aesthetical aspects are not forgotten.

It is our hope that the *The Diatom World* will foster greater appreciation and research contributions on this incredibly diverse and fascinating group of organisms. We thank all our authors for their contributions and their patience. A special appreciation is due to the anonymous reviewers who evaluated the chapters of this book.

#### Acknowledgments

We thank Professors Aharon Oren, Richard Gordon, and David J. Chapman for revising and improving the above introduction to *The Diatom World*.

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Dr. Seckbach earned his Ph.D. from the University of Chicago (1965) and did a postdoctorate in the Division of Biology at Caltech, in Pasadena, CA. He was appointed to the faculty of the Hebrew University (Jerusalem, Israel) and spent sabbaticals at UCLA and Harvard University and DAAD-sponsored periods in Tübingen, Germany and at LMU, Munich. Dr Seckbach served at Louisiana State University, Baton Rouge, as the first selected occupant of the Endowed Chair for the Louisiana Sea Grant and Technology transfer.

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Stephen S. Nagy, M.D. is an amateur diatomist, microscopist, and photomicrographer, and is a Diplomate of the American Board of Psychiatry and Neurology in Adult Psychiatry. He lives and practices in Helena, Montana, Dr. Nagy obtained his Medical Doctor degree at The Medical College of Pennsylvania in 1977, and passed his Adult Psychiatry Boards in 1982. He became fascinated by diatoms in road cuts when living in Klamath Falls, Oregon and wondered what the chalky deposits around town actually were. He learned to clean diatoms and to make exhibition mounts of them from his mentor, Klaus Dieter Kemp of Somerset County, UK, by telephone and email over many years apprenticeship. From the start his interest was in learning the practical craft of a diatomist, being able to make a selected diatom mount of high quality, and not pursuing this interest as an academic study. He has successfully arranged 300 diatoms on a single microscope slide. Dr. Nagy has been recognized for his photomicrographs of diatoms cleaned and mounted by him, and of other microscopic objects, in the Nikon Small World Photomicrography Competition (placing tenth in 2007), and in the Olympus Bioscapes International Digital Imaging Competition (placing third in 2008). He has had other images judged to be in the top 100 photos of each competition since 2005, and these photos may be viewed online at the respective contest Web sites.

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# COLLECTING, CLEANING, MOUNTING, AND PHOTOGRAPHING DIATOMS

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#### 1. Introduction

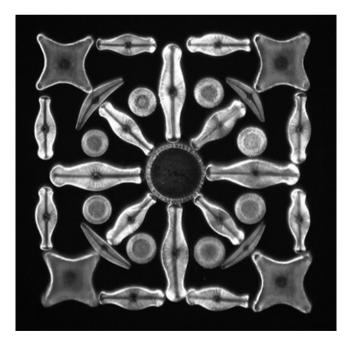
Our beautiful world surrounds us with diatoms, but because they are at or below the limit of resolution of the naked eye, we can literally swim through them and never know that they are there. When people first learn about diatoms, they are amazed that diatoms exist, and then fascinated to find out how to locate and collect them. How can one collect something that one cannot see? One clue to the presence of diatoms is the color of the photosynthetic pigments in live diatoms, which ranges from a golden brown through a brownish olive drab to dark brown, and the typically mucoid appearance of a colony of diatoms. In life, one is essentially looking for golden-brown slime.

The places most collectors look are in freshwater, such as streams or lakes, in brackish or marine waters, and in fossil sites. A problem in collecting is that particles in soils, and silts in marine settings, can approximate the size of diatoms, so that the goal in collecting is to acquire a clean sample without significant contaminants that are difficult to separate. As a consequence, one searches as much as possible for diatoms that are not in contact with mud or silt. Each of these sample types requires a slightly different approach.

#### 2. Collecting in Freshwater

In freshwater settings, the simplest approach to collecting diatoms is to obtain samples of stalked multicellular green algae (or aquatic weeds) that grow on the bottom of the stream or lake. These freshwater green algae are regularly covered with a forest of diatoms, and if the green algae are placed into a zip lock plastic bag and shaken, pummeled, and agitated, the water in the bag will rapidly become a cloudy golden-brown or olive-drab color, as the diatoms are released from the weed and become suspended in the solution. The water is poured into a vertical glass cylinder through a coarse sieve to hold back the green algae, and the diatoms are allowed to settle to the bottom of the cylinder over hours or overnight. This process can be repeated about four or five times to extract additional diatoms from the same sample of green algae with little or no loss of production each time, and there is no damage to the diatom frustules.

There are some other simple collection techniques for freshwater diatoms. Diatoms tend to prefer cold water that is not in direct sunlight, so that the undersides of lily pads, or the damp concrete in the shadow of a bridge, are more likely to have interesting samples than specimens resulting from a search in the sunlight. Some diatoms grow in a sheath around plant stems of rushes, and these diatoms can be stripped off by hand. Other species may grow as a shiny or glossy coating on rocks or on wood exposed to splashing water, appearing like a mucus growth with the characteristic color. Still others may grow in microscopic, essentially colorless tubes, or on the end of tubes, and the whole colony appears like a dirty off-white cotton mop attached to rocks in the river. The most notorious of these is Didymosphenia geminata Lyngbye (Schmidt) which was once thought to be rare and limited to high alpine settings with very clean waters. But this beautiful diatom (Fig. 1) has now been renamed by the public as "Rock Snot" as it has been transported by the felt soles of fly fishermen to streams around the world; it has been found to be a highly invasive species that can grow to cover the bottom of a stream and kill normal insect and plant life. Didymosphenia grows at the end of a colorless stalk, and the colony can have the appearance of a dirty sheep's fleece on rocks under water. Other Cymbella species grow within tiny tubes, and can



**Figure 1.** An ornate square of arranged diatoms by the author, which includes freshwater and marine diatoms. Didymosphenia forms the main spokes of the arrangement and has a shape similar to a classic bottle of Coca-Cola (Photo copyright retained by author).

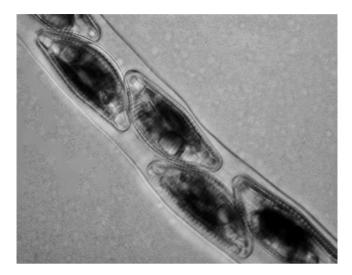


Figure 2. Cymbella prostrata var auerswaldii (Rabenhorst) Reimer growing in a tube (Photo copyright retained by author).

have a similar appearance to the naked eye, that of an off-white or gray mass that appears like a bunch of soft, depigmented green algae (Fig. 2). Under the microscope in a wet mount, the living diatoms line up within these tubes like beans in a pod. Other freshwater diatoms grow as single cells, and can be found between the pebbles on the bottom of slow-moving waters as a fine brown or goldenbrown dust. If there is a river with a constant stream flow, there can be a layer of sediment on the tops or upstream surfaces of underwater rocks, which includes accumulations of single diatoms, and this material can be collected with a turkey baster or other suction device, and allowed to settle in a cylinder as above.

#### 3. Collecting in Brackish and Marine Waters

Diatoms in the ocean are both colonial and free living, and are somewhat more challenging to collect in any quantity than diatoms in freshwater settings. Growth of marine algae that do not have a shiny or smooth surface may be sources of diatoms, similar to the freshwater technique described above. Often these will grow on buoys or on anchor lines, or on the surfaces of wood or plastic that are in contact with the sea for a prolonged period of time. A traditional source of exotic diatoms is washings from the surfaces of marine shells, such as the Conch, and a newly harvested shell can produce extraordinary specimens by simply brushing the surface of the shell with a toothbrush swished in water, and repeatedly rinsed in a collecting bottle as the brush color changes to brown, stained with living diatoms. Free-living marine diatoms can settle onto the surface of rocks or the sand under water, and a collection of sediment or "dust" from the upper surface

of rocks in an area where the water is quiet can provide a lovely marine sample. A sample of sand from the surface of a flat as the tide has receded can include a collection of larger sand granules and smaller diatoms, which need to be rinsed and released from the sand grains. Sediment adhering to a marine anchor can be treated in the same way. Of course, the traditional technique for collecting planktonic species is to tow a very fine funnel-shaped net behind a boat at slow speeds, which is beyond the realm of possibility for most individuals with a casual interest in diatoms. There are other diatoms that grow between the grains of mud or sand, such as Pleurosigma angulatum (Queckett) W. Smith, which finds its way to the surface of the sand at low tide, appearing as a brownish color on moist sand when compared to adjacent areas. These diatoms and some substrate can be gently scraped off of the material underneath using a credit card or other small piece of plastic. The diatoms and the sand can be placed in a shallow dish, moistened with seawater, and covered with a muslin handkerchief, and at the next low tide the *Pleurosigma* will find their way through the handkerchief to the upper surface of the handkerchief, where they can be removed easily with a sable brush.

#### 4. Collecting Fossil Diatoms

There are sites around the world where diatoms fell as sediment out of marine or freshwater bodies of water over time, and formed deep concretions on the bottom. Over time, the organic material decomposed and the diatom frustules were pressed together, resulting in diatomaceous earth, or diatomite. Perhaps, the most famous location to diatomists are the deposits at Oamaru, New Zealand, a marine deposit with extinct and exotically unique forms unlike those found anywhere else in the world. Additional sites of some notoriety include: the freshwater deposits at Terrebonne, Oregon, on the eastern slope of the Cascade Mountains north of Bend, Oregon on the banks of the Deschutes River. These deposits are quite loosely packed, appear as white layers in road cuts near the Deschutes River, and appear to be composed of about 97% unbroken frustules. There are freshwater fossil deposits in Klamath Falls, Oregon, well known to Victorian diatomists as the source of varied freshwater species which form brilliant white, hard chalky deposits throughout the Klamath basin, the site of an ancient lake which preceded the formation of the Cascade Mountains. This diatomite or diatomaceous earth is much more densely compressed and is actually used as chalk by children growing up there to draw hopscotch courts on the pavement of driveways.

Marine deposits at Lompoc, California have long been the site of commercial extraction and production for industrial use for many years. A much smaller deposit of marine diatoms near Dunkirk, Maryland was once a source of diatomite on the US East Coast, featuring exotic extinct diatoms and some diatoms which are still living, perhaps most famous for the large numbers of the beautiful centric form, *Actinoptychus heliopelta* Grunow. Outside of the USA, sites of notoriety include freshwater deposits from Toome Bridge, Ireland, and marine deposits from Szent Peter, Hungary, the exact location of which appears to have



**Figure 3.** The light band is fossil freshwater diatomaceous earth next to Highway 97 at Klamath Falls, Oregon. This diatomite is estimated to be 1.5 million years old. The columnar basalt is thought to have appeared at the time that the Cascade Mountains were formed, ending the life of the lake that produced the diatoms (Photo copyright retained by author).

been lost as national boundaries changed during the Second World War, and which may have been nearly completely extracted, used in the preparation of dynamite during this conflict. Additional marine fossil deposits are located at Mors, Jutland, Denmark, which is so heavily compressed that many valves are fractured, and at additional sites in France and in Russia.

The diatomite in these fossil deposits can range from white to tan-colored to green deposits, which may be found at road cuts as exposed strata, ranging in texture from friable, easily crumbled material to specimens that appear to be quite dense and hard. They are typically compressed to break and form fragments of diatoms, interspersed with whole forms and with mineral deposits that approximate the size of the frustules of the diatoms. If very dense, the diatoms may be cemented together with calcium carbonate and other minerals leached and condensed around the diatoms. It is typically impossible to determine if a deposit is silt or diatoms without looking at a bit of the specimen through the microscope (Fig. 3).

#### 5. Cleaning Diatoms

The goal of cleaning diatom samples is to have as a final product a sparkling white suspension of diatom frustules, valves, and girdle bands in distilled water, free of diatom fragments and contaminating sediments, so that a single diatom valve can be examined accurately. This suspension can be made into a strew slide, placing a drop

onto a glass coverslip or glass slide, which is then allowed to dry without heat, which will tend to make the diatoms clump or form lines. When the drop dries, if the diatoms are clean and without any chemical residue remaining in the solution, they will not be adherent to the glass and may be lifted off to make selected slides. Or, once the strew is made, the slide can be heated to glowing red in order to sinter these small bits of nature's glass onto the surface of the coverslip and then covered with a tiny drop of high refractive-index mountant (Naphrax, Zrax, Styrax, or Hyrax) and coverslip for subsequent examination. In practice, it is sometimes simple and at other times quite difficult to obtain a specimen of this degree of cleanliness, depending upon the extent of contaminants present in the sample.

All fossil deposits must be broken up without damaging the frustules, which precludes a mechanical approach of crushing. The simplest technique is to repeat multiple cycles of freezing and thawing in fresh water until the sample breaks into dust. This may take as few as 3 cycles or as many as 50 or more freeze-thaw cycles.

The next step in cleaning both recent and fossil forms involves the neutralization and removal of carbonates, which is best done with the addition of hydrochloric acid or nitric acid and gentle heat. (Diatoms are remarkably stable in strong acids, but are dissolved by alkalis.) In some cases, such as in cleaning the tubular forms (Cymbella and Didymosphenia) mentioned above, the mass of tubular material simply disappears in contact with this acid with a dramatic "whoosh!" However, in cases of fossil material steeped in carbonates, the diatom suspension may show little if any reaction to the acid, and may require prolonged low heat to extract the carbonates, which typically leach out creating a yellow or tan color in the acid solution. Some fossil samples that I have worked on have required treatment with multiple changes of hydrochloric acid, and heat applied for days before the carbonates are removed adequately. This acid must be washed free and removed completely before any next step can occur.

There have been cold or warm processing techniques described involving solutions of hydrogen peroxide, bleach, or a mixture of dilute sulfuric acid and crystalline potassium permanganate, but each of these has drawbacks. High-concentration hydrogen peroxide can damage the delicate sieve plates present in diatoms (K.A. Kemp, 2005, Scanning electron microscope observation by Frank E. Round of sieve plates cleaned with peroxide, Personal communication), and these techniques are significantly less effective, but also less dangerous, than hot processing with concentrated sulfuric acid using added potassium chlorate.

In the hot-acid technique, the sample is heated in a Pyrex beaker over a burner with concentrated sulfuric acid added to a moist solution of diatoms and water, which will boil until all water is driven from the specimen, leaving the sample in fuming sulfuric acid. The addition of this acid will convert recent samples, and some fossil samples, to a black color, reflecting the oxidation of organic elements to carbon. Then, very carefully, small spatulas of crystalline potassium chlorate, a strong oxidizing agent, are added to the sample under continuing intense heat until the color changes to a dark brown, then tan, and finally to a white or pale yellow color, at which point heat may be discontinued and the sample allowed to cool. When the beaker is no longer hot, a very small amount of distilled water from a