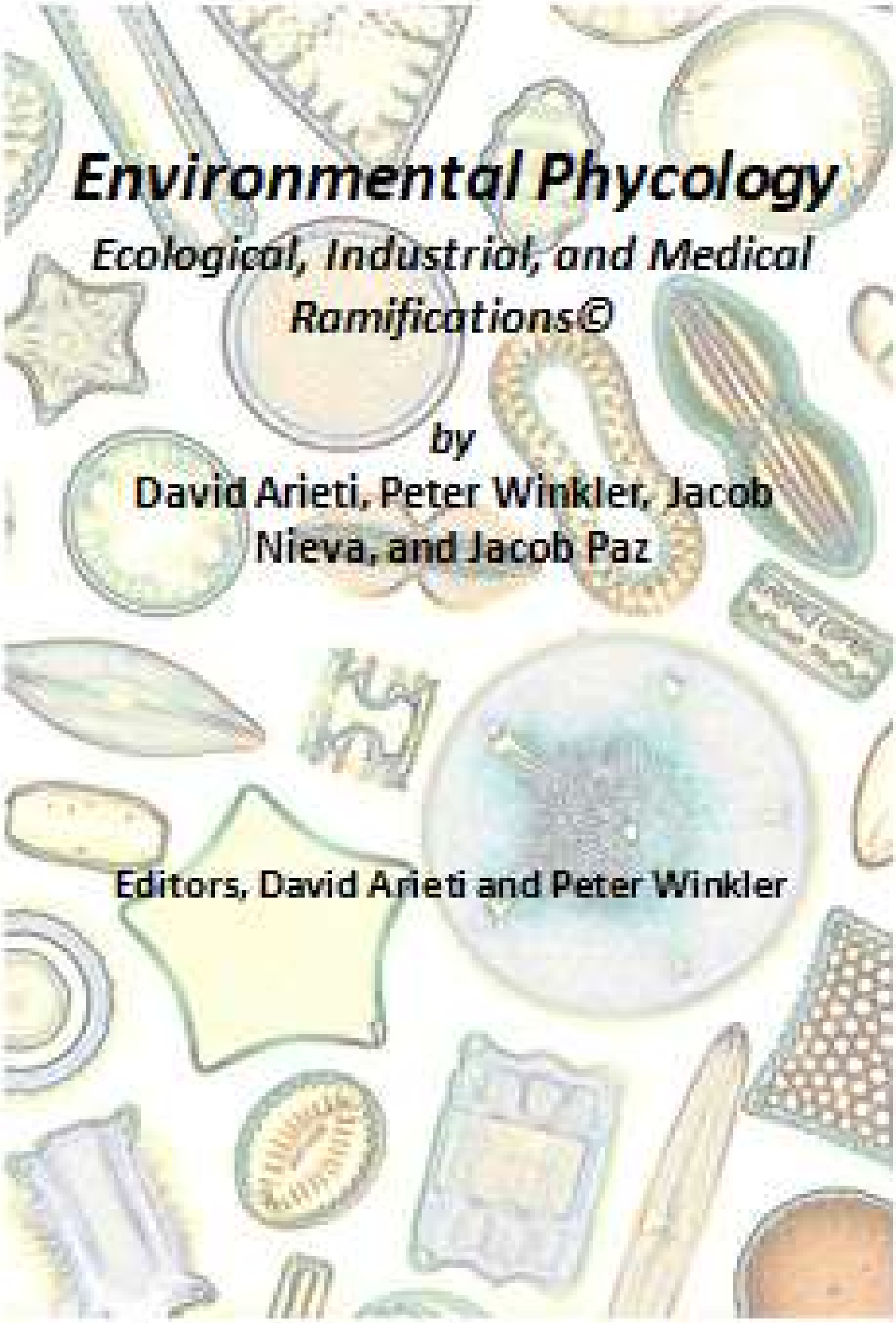


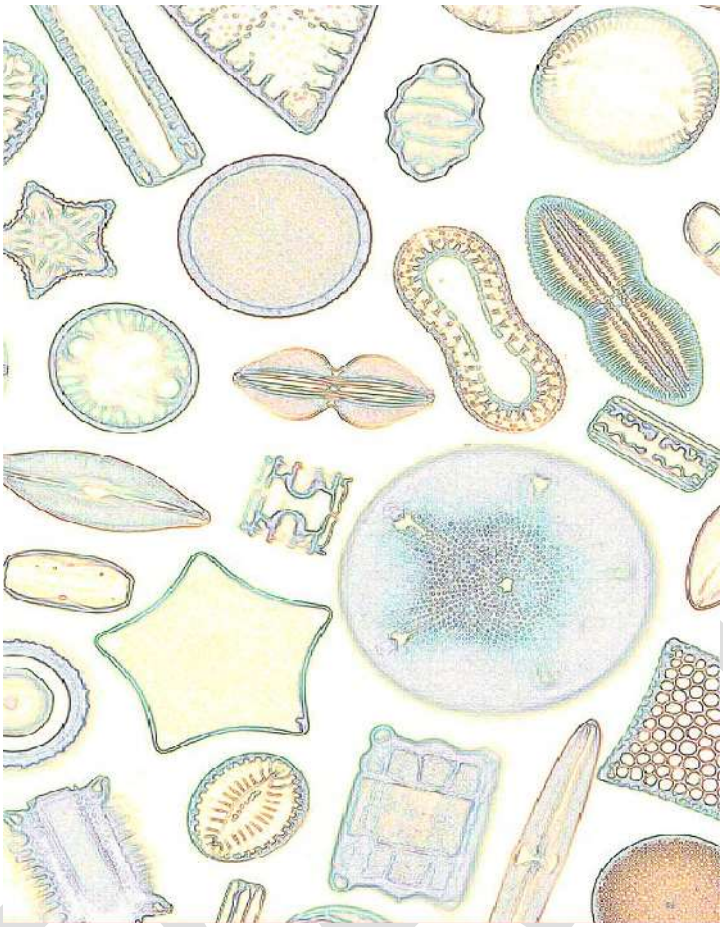
Environmental Phycology

Ecological, Industrial, and Medical Ramifications©

by

David Arieti, Peter Winkler, Jacob Nieva, and Jacob Paz

Editors, David Arieti and Peter Winkler



Cover art: Mixed diatoms

https://commons.wikimedia.org/wiki/File:Diatoms.jpg

Environmental Phycology: Ecological, Industrial, and Medical Ramifications©

by

David Arieti, Peter Winkler, Jacob Nieva, and Jacob Paz

Special Preliminary Draft Edition

Published by David Arieti and Peter Winkler, 2022

FORWARD

This book is essentially a science education book. Phycology, or algology, is a rapidly growing field within the life sciences. As educators, we (the editors) see a need to build the science across a broad spectrum of practitioners, managers, investors, innovators and dreamers. To answer the urgency of needed progress we have decided to make available this limited draft edition of ***Environmental Phycology: Ecological, Industrial, and Medical Ramifications©.***

We find the reasons to publish on a preliminary basis to be numerous:

- To inform colleagues and others about the benefits and detriments of algae.
- To serve as a book proposal for publication of a future expanded edition.
- To interest and recruit other authors, who could benefit professionally from producing published work in their field.
- To help attract young biologists into the field of phycology—a field that will grow in both research and commercialization opportunities.
- To give the "old-hands"—the technology and logistics experts possessing long-term experience, the opportunity to write and share with a broader audience.
- To provide a springboard into research projects with the insights of practitioner authors.

With many years of experience behind us as educators in the biosciences at the college level, we find a great need for an introductory and practical text in algal science. Two reasons in particular are compelling:

- Many persons degreed in life sciences have little botanical training, much less classes in algology.
- Quite a number of professional individuals, at many levels, from many backgrounds, enter the algae industry and would see benefit in becoming conversant in algal science.

Bear with us for this draft edition, as the glossary is not yet included. Much of the terminology in the Chapter One introduction resides at the level of basic life science knowledge. Our readers at an introductory level may need to use a science dictionary, or otherwise, to keep up with the flow of ideas. As for more advanced readers, we believe that our overall message will be clear.

The goals of the final expanded edition as we visualize it, will appeal to wide range of readers. Increasing numbers of biologists will learn about algae in greater depth and will consider the career opportunities in the field. Business mangers and entrepreneurs will consider algal options for new solutions. Thinking people, everywhere, do appreciate the physiological need to breathe and the benefits of fresh, healthy air. None the less, the origin of all atmospheric oxygen (O_2) tends to be unknown by the average person and unappreciated by society at large. Fortunately, the trained

biologist, botanist, or ecologist knows, understands, and beholds that all of the O_2 needed by breathing people and, indeed, all other life, originates from photosynthesis: The use of sunlight, water, and carbon dioxide to make food as done by all of the broad spectrum of plant life. Of all global photosynthesis, as much as 70% originates from algae. Algae? Green pond scum? Seaweed? The average person, at this point, pauses, takes a deep breath, and asks, "does this really matter to me?" In a world in where resource utilization, climate change, and public health topics appear daily in the news, often in truly apocalyptic specter, an understanding of algal life becomes increasingly important. Much more so for those who seek to:

- Influence Public policy;
- Utilize resources to a beneficial and prosperous extent on a sustainable basis;
- Plan to avoid calamitous threats to the health and welfare of people and ecosystems.

All posed by the scientifically estimated 75,000 species of algal life (see Chapter 1), a conservative, systematic view of the myriad of algal life. Certainly a large number for the mind to contemplate, as well as for the algologist, or phycologist, to explore and interpret for intrinsic value. The future of humanity on Planet Earth will include an increasing knowledge and functional capacity to recognize the opportunities, the hazards, and the limits that define our relationship with algal life and our planet.

You might ask, isn't compiled information available online? Yes, much is, and too much in some respects. Baseline reviews of algal subject areas with basic explanations by skilled practitioners are needed in a single source to satisfy the demand for learning in a growing field. To propose to be a practitioner desk reference in this case is not to rival internet sources, which are regularly updated and expanded. but to provide perspective and explanations to frame learning in algal topics of interest.

How extensive can algal topics be? A cursory view of the benefits and detriments of algae does indeed reflect the importance of algal science, or phycology. Most fundamentally, we benefit from algae in aquatic food chains as the base food support for all animal life, including edible harvests of fish and shell fish. We humans also eat algae as food or food ingredient. Coastal dwelling people have harvested seaweeds for millennia, as a food staple. Conversely, some algal forms are toxic. They contaminate food chains, resulting in poisoning of people by consumption of tainted fish and shellfish. When toxic algal species proliferate into vast blooms that render large swaths of coastal waters unfit for recreational or commercial usage the outcomes are catastrophic for local economies. Yet on the positive side, selected species of algae are now being cultivated for biofuels, medicines, fertilizers, and water purification processes. Again conversely, the book describes the mounting costs due to the detrimental algal fouling of potable waters, and water treatment systems, including agricultural systems. The great amounts of algaecide applied to water systems, and to freshwater habitats has a cost in both financial and ecological terms, as well as public health. This book lays out the need to distinguish between investments and costs when considering sustainability of water resources. This book poses the questions of investing in a sustainable balance with algal life, as opposed to simply

paying the costs of short-cut solutions without sustainable progress. This book will elaborate on the differences and the options of policy, public and corporate, to seek a sustainable balance of algal life. Clearly, the diversity of algal forms and health of algal populations are among the best of resistances to global warming, as photosynthesis directly counteracts it.

The economics of resource use and of property values in coastal communities and along shorelines are sure to revolve around algal species and populations. In some cases directly due to algae where they may be harvested as food. Directly, too, with a negative impact where toxic or noxious algae are contaminating the fisheries, or the water supplies. In many other cases, are an indirect outcome, a symptom, not a cause of underlying ecological degradation, and properties decline in value to people. ***Environmental Phycology: Ecological, Industrial, and Medical Ramifications*** is intended to be an important resource in the understanding of a broad and diverse biological field. This title is meant to serve an eclectic assemblage of people within modern algal operations: Biochemists, chemists, chemical engineers, agronomists, physicians, clinicians, nutritionists, pharmacologists, business managers, public policy workers, and educators, to name some. It may be best described as a professional primer, a practitioner desk reference, and a policy maker/educator manual of background information. The book represents the best use of science for those who are tasked with determining the opportunities and limitations that will define sustainability of resources and quality of life for future generations.

We look forward to working with our collaborators as we complete the tentative proposed chapters. If you want to inquire further about collaboration, or if you have questions or comments, you can contact the editors:

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Author Biographies

David Arieti

David Arieti has been involved in environmental issues for almost fifty years. He went to the University of Denver for his BA degree in Science Area Major. He worked on The Sea of Galilee where he did algal research. He returned to the US where he earned his MS degree in Marine Science from Long Island University. He worked studying the effects of chlorine produced oxidants on Chesapeake Bay in Maryland. He then worked at consulting firms in Washington D.C. where he worked on projects that dealt with the fate of pesticides in the soil; health effects on people working with the dyeing and finishing of textiles, and food additives. He then worked at the Baltimore Environmental Center as research director where he worked on hazardous waste issues. He also worked on the Hudson River studying the fish that were prevented from entering the waterways used to cool the condensers from the Indian Point Nuclear Power Plant in New York.

David also began teaching Environmental Science and Biology as an adjunct professor at various colleges in Maryland and Illinois, where he is today. He has been the recipient of three best Teacher-of-the-Year awards at three different colleges. In 1996 he won at Columbia College, in 2002 he won at Oakton Community College, and in 2005 he won at Daley College; all three colleges are in the Chicago vicinity.

He wrote his first book, entitled *The Earth is My Patient* in 2005. He has gathered information through newspapers, books, and magazines, as well as the electronic media (radio and TV) and witnessed in real life the havoc that humans have wrought on the planet. In his first book, he lists the real causes of environmental pollution and its effects on the planet, as well as possible solutions. He published his second book, *Prognosis Disaster*, in 2011. Jacob Nieva was a co-author of both books. **Email: darieti@comcast.net**

Peter Winkler

Early in his career Peter Winkler worked as a research biochemist, with some short stints as an ecological field researcher. He progressed into an Environmental Inspector position with The County of Lake (Illinois), specializing in stormwater infrastructure, including management, and maintenance, as well as resource conservation and planning. The regulation and enforcement elements of that role provided deep experience to Peter regarding negotiating, consensus building, and integrating rules to reality. Exiting the agency realm for a small consulting business serving civil engineers, real estate professionals, and property owners and buyers, he succeeded to merge his business into a larger provider of ecological services. Peter joined Integrated Lakes Management as an Aquatic and Terrestrial Service Manager, a position he describes as his favorite, because of "getting to do it all," from desk to boat. And for the entry into algology with further botanical learning in the process of serving a clientele needing improved solutions. Peter next shifted his science capabilities to a "big pharma" corporation in the position of Validation Scientist. In that capacity he was able to learn about a broad spectrum of biological and manufacturing technologies, such as cell cultures and bioreactors. He gained key knowledge and insight to the role of validation in science, engineering, and manufacturing.

Regarding Peter's educational background, it is important to note that for most of his career he also filled an adjunct faculty position and taught college level classes in General Biology, Environmental Biology, Microbiology, and Evolution. He did the bulk of his teaching work at Oakton Community College, of Des Plaines, Illinois. He holds a B.S. degree in Biology from Northeastern Illinois University, Chicago; and M.S. degree in Environmental Biology from Eastern Illinois University, Charleston. **Email: pwinklerbio@gmail.com**

Jacob Nieva

Jacob O. Nieva, M.D. is a medical doctor who specialized in Internal Medicine in the Philippines. He graduated from the University of Santo Tomas, Faculty of Medicine and Surgery in 1980. He took five years residency training in Internal Medicine in the Rizal Medical Center, Pasig, Metro Manila, after which he practiced for 18 years before coming to the United States. He has been an adjunct professor at Oakton Community College, Des Plaines, Illinois, teaching Human Anatomy and Physiology since 2004.

Being from the Philippines, Jacob knows firsthand about rainforest destruction and its effects on disease transmission. Jacob has written the bulk of the chapter (12) dealing with algal toxicity diseases in this book, as well as chapters on diseases in David Arieti's earlier books.

Jacob Paz

Jacob Paz has over 40 years of experience in industrial hygiene, environmental science, environmental engineering, and research in those disciplines. He has acquired expertise in: EPA, OSHA, ANSI, and ACGIH standards and requirements, along with corporate and agency compliance requirements. Notably, Jacob was cited in a letter by the Nuclear Regulatory Commission to ensure compliance with the U.S. Environmental Protection Agency on the cumulative effects of health risk associated with metals and radionuclide regards to the Environmental Impact Statement at Yucca Mountain Project; resulting in a request for a Supplemental Environmental Impact Statement (March 2009). Several issues of which may even reach the US Supreme Courts.

His far-reaching scope of operations at J and L Environmental Services, Inc., include research interests in

- Prevention and treatment for bone loss and dementia in seniors and astronauts.
- Use of physical agents and natural products, with emphasis on the role transition metals play in the development of Alzhiemer's disease.
- Growing and harvesting algae for pharmaceutical and natural agents at a lower cost.

His education includes: CW Post College Greenvale Masters in Marine Science and Environmental Science, NY, New York University and Polytechnic University, Doctor of Philosophy in Environmental Health Science and Environmental Engineering Brooklyn NY. **Email: drjacobpaz@gmail.com**

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Chapter 1. What are algae and why are they important?

By David Arieti and Peter Winkler

OVERVIEW

This introductory chapter provides very basic information about biology, evolution, and classification of algae. Further chapters in this book will address these topics in more detail. At the end of this chapter there are lists of the benefits and detriments of algae. The lists are cross-referenced the chapter, or chapters that develop the topics in detail.

Scientifically speaking, the algae are a large and diverse collection of aquatic organisms that are grouped together because they share a variety of structural similarities as well as an ability to perform oxidative/oxygenic photosynthesis. In oxidative/oxygenic photosynthesis, free-living cyanobacteria (blue-green algae) and chloroplasts within algal cells use three inorganic ingredients, carbon dioxide (CO_2), sunlight, and water (H_2O), to produce high-energy, carbon-based molecules including carbohydrates, proteins, and lipids, cellular building block, and more. The process is called oxidative/oxygenic photosynthesis because in the reaction, two hydrogen atoms are removed from H_2O, liberating oxygen (O_2).

Yet to use the word "algae" in a non-scientific context, as a fisherman, a recreational boater, a landscaper or gardener, or a poet or pirate, is a common occurrence. Hence, in this book we strive to bring information to the discussions and conversations. We answer the questions posed, because the stakes are high. Global citizens, at all levels, in all sectors, make decisions everyday that intersect with the opportunities, and the warnings, of the "algal empire." The rise of the "algae industry" in recent decades has recruited many workers, managers, and researchers to meet the growth of the diverse businesses and agencies. A large proportion of scientists, and even biologists, have vague understandings of the organisms that are called "algae." As we see every day on Wall Street and in other critical setting, knowledge is power. This introduction, and the chapters that follow, bring practical understanding of algal subjects to the reader, and key information about in selected subject areas. Let us first consider how to define the "algae."

A prose poet, using the word "algae," in her 2014 book, *An Ocean Garden: The Secret Life of Seaweed*, author Josie Iselin writes to frame her subject—beautiful pictures of marine seaweeds, or kelps. Removed from their turbulent habitats, and isolated against white, or sometimes black backdrops, the specimens present an extraordinary display of diversity in form and color. Ms Iselin also provides interesting summaries of information of their "secret" lives underwater, as well as their not-so-secret lives in billion dollar industries. In describing her own attraction to her subject matter, she

turns to the work of Rachel Carson writing in her epic 1955 book, *The Edge of the Sea*. To expand on her own connection to art and science, Josie Iselin summarizes Rachel Carson's insight into the "secret" lives: "Yet it is astonishing how often the seaweeds are overlooked when describing life in the sea... seaweeds are ignored. Carson, however, saw the integral nature of all organisms living at the seashore. Her ecological view (rare at that time, when most scientists were nose down in the study of specific species) is as contemporary today as when she wrote it, and she celebrates seaweed as one of the great ecosystem engineers of our planet. It fixes carbon, generating the base of the foodchain, and creates habitat; it is fundamental not only to life in the sea but to all life on earth" (Iselin, 2014). The definition of the term algae is amply set by Rachel Carson writing in her earlier 1941 book, *Under The Sea-Wind,* and sets the stage for a growing scientific understanding:

> **Alga** (ăl'-gả; pl. algae (-jẽ). The algae belong to the first of the four major divisions of the plant Kingdom and are the simplest and probably the oldest plants. They do not have true roots, stems, or leaves, but usually consist of a simple leaflike frond. They range in size from microscopic spheres to giant seaweeds several hundred feet long.

Figure 1-1. *Sargassum muticum,* a type of kelp. Photograph by © W. Bay-Nouailhat. Long frond-like structure, several meters in length. With permission by Phycokey. http://cfb.unh.edu/phycokey/Choices/Fucophyceae/SARGASSUM/Sargassum_Image_page.htm #pic05 (viewed 5/7/22)

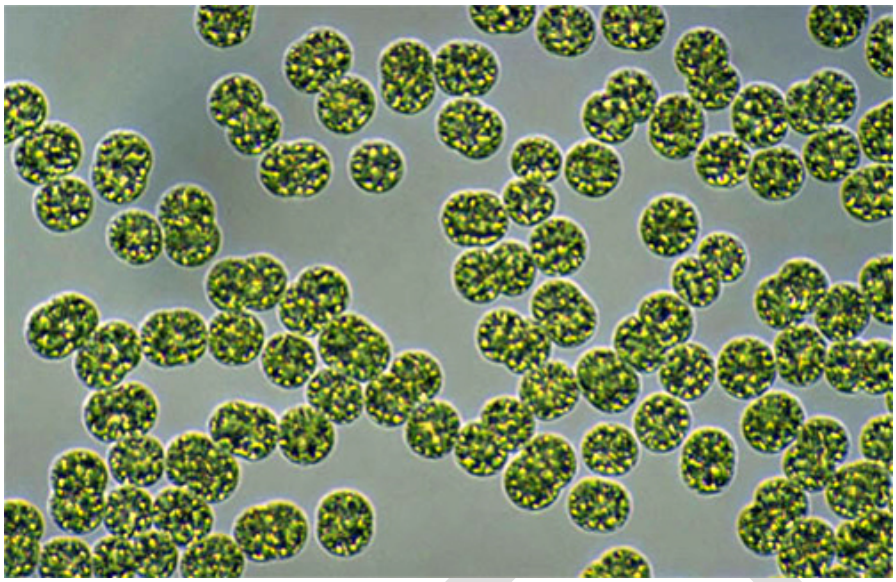


Figure 1-2. *Microcystis aeruginosa* from Natural History Museum, London, England. Tiny microscopic spheres. Many cells are dividing. With permission by Phycokey. http://cfb.unh.edu/phycokey/Choices/Cyanobacteria/cyano_unicells/unicell_of_microcystis/Microcystis_culture_Image_page.htm#pic03 (viewed 5/7/22

Considering that this definition is not as contemporary as it once was, nonetheless it sets the stage for a historical understanding of algal taxonomy (the science of biological classification that groups sets of organisms based on similarities and differences). Because scientific research continually adds to, revises, edits, and sometimes discards, all theoretical constructions change over time. To best contribute to any part of taxonomy, or science in general, knowledge of the history of the work adds to the understanding. The glossary at the end of the book provides a current definition of alga. The most definitive statement that we can make regarding the term algae, is that it is, at this time, not a formal taxonomic category, but a word of both biological science, or botany, and the vernacular, common use of the word. It's a word used to describe apparent plant life in water. That plant life may vary from a green scum, floating or covering submerged surfaces, to discoloration of open waters, to long fronds or strands trailing in the currents.

Moreover, the use of the term, as defined by Rachel Carson, and conditionally, but readily agreed upon by phycologists and botanists, does not include all plants growing in water. The oceanic niches of the kelp species, so beautifully displayed in Josie Iselin's picture book, are filled in freshwater systems by the so-called aquatic macrophytes. These are vascular plants with an evolutionary ancestry on dry land. In what can be understood to be one of the most cogent examples of evidence of an evolutionary process, land plants evolved back into aquatic niches (ecological roles), replacing the marine kelps in freshwaters in a type of evolutionary convergence of forms. Some species look much like the marine kelps as long trailing fronds, and have the added benefit and competitive advantage of recovering nutrients from the bottom sediments by their root systems. Also having flowers emerging

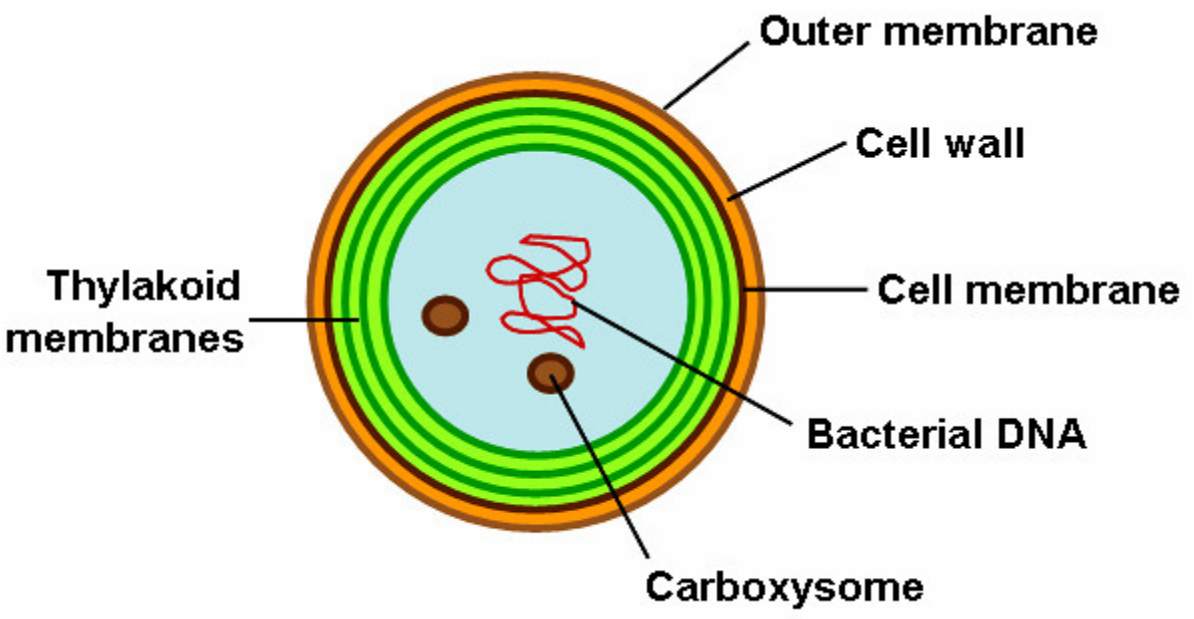
from the water, attracting pollinators, or underwater flowers secondarily adapted to the release of pollen to be carried by water currents. Sometimes science makes its most powerful statements by saying what something is not. Although people sometimes point at submerged aquatic macrophytes and say "algae!" These vascular, flowering plants are definitively not algae. So, not everything green, growing in water is an algal species. See Figure 1-3 to note the appearance of an aquatic macrophyte species.

Figure 1.3 *Potamogeton gramineus,* grass-leaved pond weed.
Submerged leaves and emerging flower stalks are evident, and few if any floating leaves. Photo by J.D. Madsen posted at aslo.org. With permission, by Phcokey.
http://cfb.unh.edu/phycokey/Choices/Anomalous_Items/Aquatic_macrophytes/submerged_leaves/POTAMOGETON/Potamogeton_Image_page.html#pic02 (viewed 5/7/22)

While defining what the algae are, and are not can seemingly be done with simplicity (an application of Occam's razor?), categorizing the myriad of forms and colors within the algae is not. Algal taxonomy is not a subject of simple explanations in this modern era of advanced tools of investigation—DNA sequencing/genomics, biochemical analysis/metabolomics, cell ultrastructure (internal construction) study with electron microscopes, and cytogenetics (the behavior of chromosomes relative to heredity and gene expression) all factor into determinations of taxonomic relationships at all levels— species, genus, and higher, such as family, order, class, etc... In the beginning, William Henry Harvey, an Irish botanist seeking to develop scientific understanding of seaweeds, proposed a classification system based on the colors that were consistent for typical forms. The year was 1839 and his research and explanations accord to him credit as the "father" of modern phycology. Like so many aspects of algal biology, his scientist history is much less known than that of his 19th century contemporaries—Carl von Linn (Linnaeus) for the method of functional biological taxonomy, Louis Pasteur for the germ theory of fermentation, Robert Koch for the germ theory of

disease, Gregor Mendel for the theory of heredity, Charles Darwin and Alfred Russel Wallace for the theory of evolution by means of natural selection, Ernst Haekel and ecosystem theory, Matteus Schleiden, Teodor Schwann, and Rudolf Virchow for cell theory, and Francis Crick, James Watson, Maurice Wilkins, and Rosalind Franklin for the theory of heredity through DNA (deoxyribonucleic acid). All theories with powerful and far-reaching ramifications and major resultant bodies of science. It seems that it is not an overstatement to say that algal-based biologicals will be great movers of progress for humanity during the 21st century.

The perspective of cell theory and the study of cells (**cytology**) serves to better understand the scope of algal diversity. How cells are structured, how they function, how they might differentiate in various multicellular constructions, and how they accomplish sexual reproduction answers riddles of taxonomy, at least partially in all cases. Other than viruses (acellular entities),all living things on Earth are composed of cells, of which there are two types: **prokaryotic and eukaryotic**. Prokaryotic cells (see Figure 1-4) differ from Eukaryotic cells (see Figure 1-5) in that they are much smaller, lack nuclei, and have no membranous organelles. Eukaryotic cells are diverse in form, but, with rare exceptions, all eukaryotic cells contain a nucleus, mitochondria, endoplasmic reticula, and Golgi bodies. A variety of other organelles, such as chloroplasts, peroxisomes, lysosomes, and vacuoles, are present in some taxonomic groups and absent in others. See Cell Structure and Function Outline Box below



***Synechocystis* diagram**

Figure 1-4, A schematic representation of a cyanobacterial cell (*Synechocystis*) with its photosynthetic membranes and particles. The light reactions happen at the thylakoid membranes, the carboxysome is

the site of carbon dioxide fixation. We owe the presence of oxygen in our atmosphere to these little critters. Photosynthetic prokaryotes like this gave rise to the chloroplasts in green algae and plant cells through endosymbiosis with a eukaryotic host cell.
This image has been released into the public domain on Wikipedia,
https://www.flickr.com/photos/blueridgekitties/4619195795 (viewed 5/9/22)

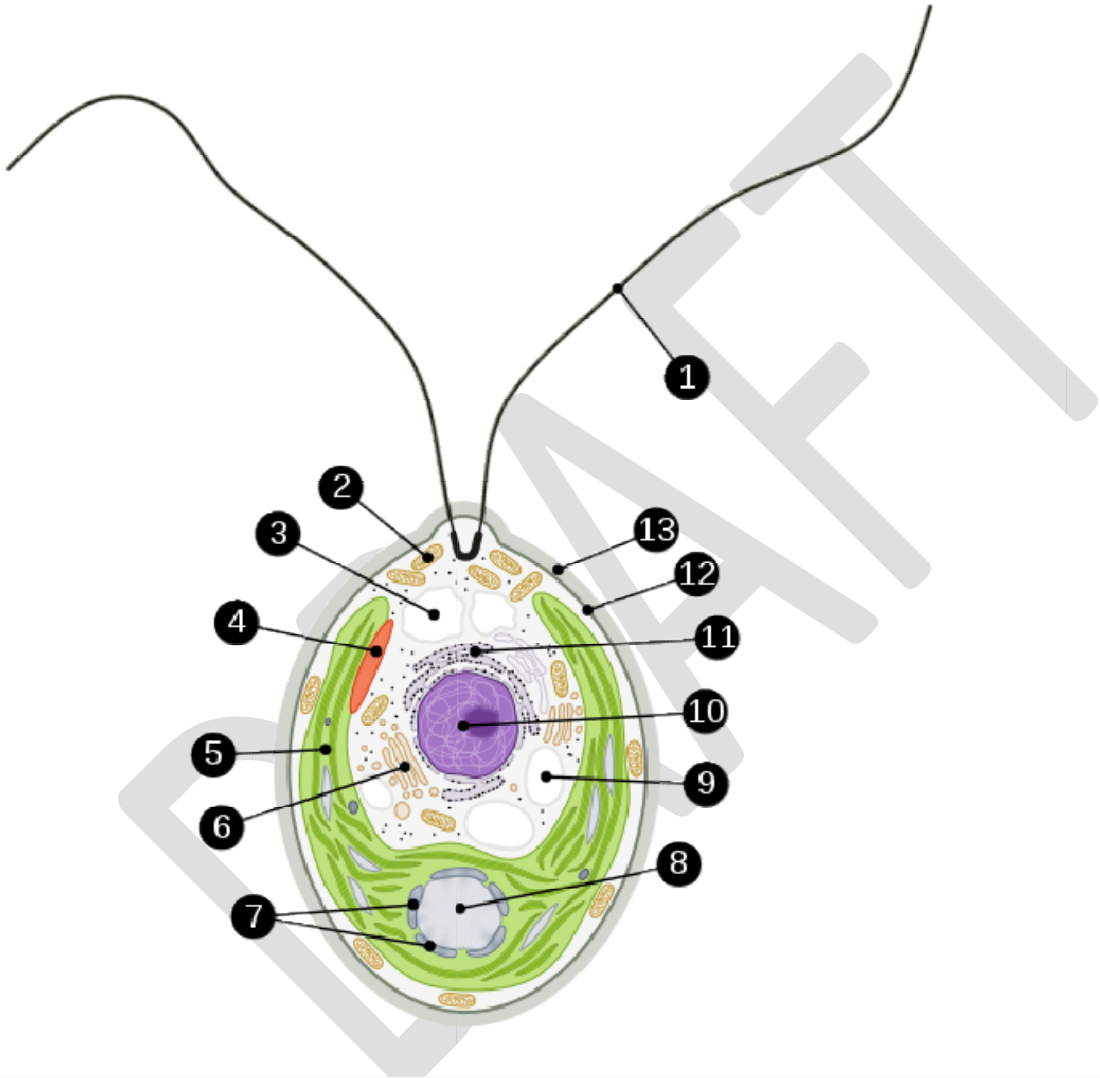


Figure 1-5. Scheme of the *Chlamydomonas reinhardtii* cell; schematically redrawn, based on this TEM micrograph: http://cellimagelibrary.org/images/CIL_37252

1) flagellum 2) mitochondrion 3) contractile vacuole 4) eyespot (stigma) 5) chloroplast 6) Golgi apparatus 7) starch granules 8) pyrenoid 9) vacuole 10) nucleus 11) endoplasmic reticulum 12) cell membrane 13) cell wall

Cell Structures and Functions Outline Box

A. Cell Structures and Functions Outline Box

Theory: All living organisms are composed of cells, and cell products, e.g. skin, hair, bone, teeth, wood, bark, mucilage, etc… and all cells arise from pre-existing cells.

Two main taxonomic (classifications) groups of cell types: Basis in morphology of the cells Morphology: Descriptions of organisms based on internal and external form, structures, and materials; cellular morphology describes cells.

1) Prokaryotic (Pro = before; karyon = nucleus): Simple cell types having no membrane bound organelles (cell structures), prokaryotes – bacteria
    a. Nucleoid region or genophore - location of DNA, cell instructions and heredity material.
    b. Cell membrane - diffusion, osmosis, and tonicity of cell.
    c. Cell wall including external sectreted adhering materials - protection of cell against physical and chemical shock.

2) Eukaryotic (Eu = true; karyon = nucleus): Contains membrane bound organelles which provide a higher level of organization and efficiency. Eukaryotes – protozoams and multicellular organisms.

    The most important organelle structures and functions are:
    a. Nucleus – containment of chromosomes, coordination of cell controls and all other cellular functions.

    b. Chloroplast – capture of light energy and production of food.

    c. Mitochondrion – production of usable energy from foods by aerobic respiration.

    d. Rough endoplasmic reticulum – A membrane system with ribosomes for protein synthesis; ribosomes occur unbound in prokaryotes.

    e. Smooth endoplasmic reticulum – The synthesis of fats, steroids, and starches.

    f. Golgi apparatus (or body) – Packaging, transport and secretion of cell products.

    g. Centrioles – Generation of cilia, flagella, mitotic astors and other microtubule structures.

    h. Other organelle structures tend to be elaborations or derivations of these.

    Study applicable diagrams, illustrations and tables for a complete consideration.

B. Reproduction of cells: Cell Division

1) Prokaryotes – binary fission

2) Eukaryotes – Mitosis and meiosis, governed by circadian rhythms, the cell cycle:

a. Binary fission (as in bacteria, blue-green algae):

b. Mitosis and meiosis in eukaryotes:
Mitosis – chromosomes are replicated and full sets of copies are evenly divided between daughter cells.

c. Meiosis – Chromosome number is reduced to a ½ size set in each of 4 daughter cells, this forms the sex cells (gametes) in animals, and forms spores in land plants, fungi, and protozoans, while in the algae life cycle either variation will occur depending on the taxonomic group.This difference in chromosome number is expressed as being a haploid or diploid number...

If the chromosome number = n
Diploid = 2n
Haploid = n

In mitosis: A 2n cell divides and 2 (2n) cells result, or a 1n cell divides and 2 (1n) cells result.
In meiosis: A 2n cell divides twice and 4 (n) cells result

3) A meiotic division is like two mitotic divisions, but with modifications:

First meiotic division: A reductional division, reduces chromosome # to haploid.

Second meiotic division: An equational division, replicated copies of chromosomes are separated, as in mitosis.

C. Three processes prevail in eukaryotic cell divisions:

1) Chromosome replication – occurs during the S (synthesis) phase of the cell life cycle.

2) Karyokinesis: Division of the nuclear contents and chromosomes.

3) Cytokinesis: Division of the cytoplasm and cell body.

D. Two cellular life cycle events confer sexual reproduction.
   1) Meiosis: Variable outcomes, gametes or spores.

   2) Fertilization; Typically gametes needed, but cellular fusions occur in some species. Syngamy, synkaryon complete 2n, or greater number.

Chloroplasts and mitochondria differ from other organelles in that they closely resemble bacteria. Like free-living bacteria, both organelles contain their own DNA, synthesize many of their own proteins, and reproduce by cell division within the host cell. Detailed morphological and genetic/genomic comparisons provide convincing evidence that mitochondria and chloroplasts descended from free-living bacteria. In the case of chloroplasts, that would be the cyanobaceria as the presumptive ancestor. Primeval symbiosis with so-called lateral transfer of functional cellular components reconstructed early eukaryotic cells and natural selection accounted for fitness, or effective survivability of variants.

All algal forms can be said to have converged in their evolutionary outcomes, being essentially all photosynthesizing cells. With exception of the cyanobacteria, or cyanophytes, which contain their photosynthetic pigments within their cell membranes (see Figure 1-4), all eukaryotic species of algae (with a very few exceptions), possess chloroplasts for photosynthesis. Carrying an organelle that produces food out of three available inorganic ingredients, many, but not all algae lost adaptations needed for directed movement and for finding, engulfing, and digesting prey. Instead, they evolved structures and lifestyles that optimized exposure of their chloroplasts to solar radiation. The fitness benefits of high chloroplast productivity and the reduced necessity for hunting resulted in considerable convergence of algal morphology and life histories. Some became completely planktonic (except during reproductive stages), with diminished motility as would be conferred by the flagellum or flagella (plural) . The resulting vulnerability to predation may have selected for production and sequestration of toxic secondary metabolic products, organic compounds to serve as repellants to herbivores., as in land plants, too. Other groups (e.g., dinoflagellates) retained their flagella, presumably because this helped them find or remain in sunny locations. Euglenoid algae also have this capacity. The necessity for CO_2 diffusion into the cell undoubtedly selected for more extensive perforation of cell walls, a problem to be reckoned with in hard walled forms, such as the diatoms. Multicellular species, such as the kelps, evolved attachment structures, elongation structures, and flattened shapes that maximized the proportion of cells that could access sunlight, and/or air bladders to stay close to the surface. An addition outcome for the kelp is a diversity of sizes and shapes in response to depths, currents, and turbulence in water. The algae as a group are delineated none the less, by their similarities: The ability to perform photosynthesis. in a wide variety of aquatic settings, seemingly only limited by the depth to which sunlight will penetrate. This convergence of evolutionary trends, along with the bright colors of photosynthetic pigments, explains the former, and continued, widespread assumption that algae are a group of closely related organisms.

Variations among forms as the raw material is a tenet of evolutionary theory. An important mechanism to generate variation among individuals in a population is sexual reproduction. The numerous algal species with varied life cycles and alternations of asexually and sexually reproducing stages were the laboratory for perfecting sexual reproduction with new combinations of parental lineages early in the history of eukaryotic cells. Importantly, sexual reproduction requires chromosomes, and chromosomes require a nucleus. not possessed by prokaryotic cells. The equal division of the replicated chromosomes during mitosis (cell division) and the reductional division accomplished by meiosis both require the formation of mitotic or meiotic spindles. That is further cell ultrastructure not possessed by prokaryotes, which cannot be said to have sexual reproduction. Without sexual reproduction, although they have a few mechanisms to recombine genetic material, prokaryotes evolve very slowly. The cyanobacteria of today resemble those of the earliest fossil records very closely. With both primevel symbiosis (endosymbiosis) and sexual reproduction with recombined parental lineages, the eukaryotic algae have diversified vastly into numerous niches in global aquatic ecosystems, and have ventured into intermittently wet locations to compete with terrestrial organisms for space.

The success of algae represents an excellent example of “combogenesis”, a process in which new kinds of entities form as a result of combination and integration of pre-existing, usually simpler entities. Often, the combination is synergistic; the new entities are far more successful than either of their simpler progenitors. This has led to increasingly complex organizations of matter and energy (Volk 2017). This pattern repeats itself in the construction of multicellular organisms, where different organs complement each other in structure and function producing inproved homeostasis (internal control). The same also holds true for ecosystems, where different species complement each other and contribute to increased system stability and productivity.

The diversity of life forms, species, if you will, all fueled by photosynthesis in great gambol through evoluionary and geologic past can be distilled in biological theory to one thing: **Sexual reproduction!** In the case the algae a topic covered in more than on chapter following in this book. An introductory explanation of the reproductive biology the algae must first consider the terms in the preceding Cell Structures and Functions Outline Box. The types of cell divisions and the numerical accounting for the chromosomes are key to understanding sexual reproduction. Asexual, or vegetative reproduction, part of essentially all algal life cycles, produces daughter cells or propagules (single- or multicellular fragments from the parent) from multicellular stages, offspring that do not arise from a combination of parental lineages. There is no use of sex cells (gametes) in asexual reproduction. Sexual reproduction always includes the fusion of gametes derived from differing lineages, be they near or far. The production of the gametes and the "fertilization" events have great variability among the algae. For example, Figure 1-6 illustrates the life cycle of the chlorophyte species *Acetabularia acetabulum.* The fusion of gametes and the resultant zygote are shown unlabeled at the bottom of the illustration.

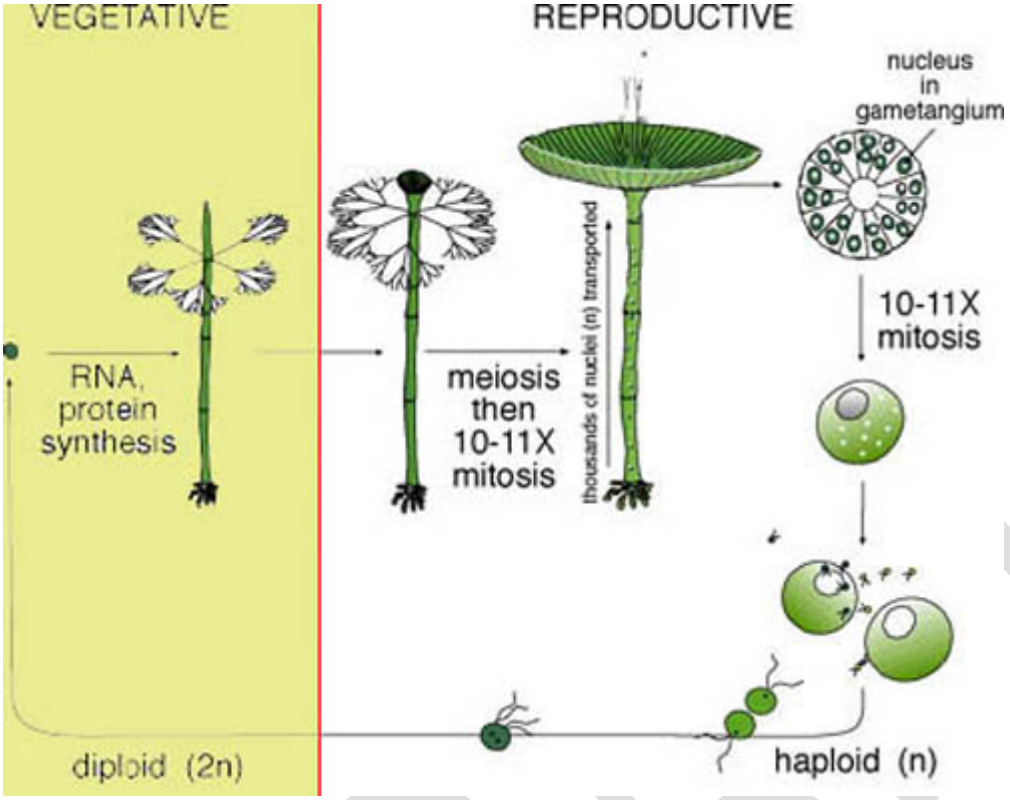


Figure 1-6. *Acetabularia acetabulum* life cycle (Henry et al. 2004)
With permission by Pycokey.
http://cfb.unh.edu/phycokey/Choices/Chlorophyceae/siphonous_greens/Dasycladales/ACETABULARIA/Acetabularia_Image_page.html#pic10 (viewed 5/11/22)

Our conventional view of reproduction, from the perspective of animal reproductive biology, is that of most, but not all, reproduction to be sexual. Many botanists marvel at the "love dances" of animal life. Mating rituals with epic rivalries lead to a ready observation of the interbreed-able gene pool, or population, and the unity of a species group. Also the process that conserves genetic fitness of that group by limiting the intrusions of untested genes or combinations of genes—reproductive isolation—non-interbreed-ability. Species, by definition, remain distinct from other species by the barriers to interbreeding. For algae, living their secret lives underwater, the barrier must be at least partially the incompatibility of gametes.

Categorizations of life cycles among the algae produces types that may vary from 3, to 5 or more as descriptions of gametes, zygotes, are variable. So too, are there variations in the roles of the mutlticellular phases of life cycles. A systematic view of algal life cycles is found in Chapter 2. Once the life cycle of an algal species is well known, the species can be said to be defined as a functional

biological unit. To be defined as a species is to exist at the absolute level of taxonomy, not to further debated in the absence of qualified new evidence.

Much of the field biology of the algae exists at level of genus, not including the so-called species epithet. So *Acetabularia acetabulum* collected in the field as *Acetabularia, or Acetabularia sp.,* Thus stating that it the precise species is not known at the time of identification. Identification to the genus level tends to have real functionality in algal field operations. Figure 1-7 shows a typical sampling from a classical "scum-covered pond."

Figure 1-7. Collected sample from a suburban pond. Note green coloration indicative of algal "blooming." Photo by David Arieti.

Microscopic examination reveals the distinctive liamentous form of *Spirogyra* with its characteristic helical chloroplast construction.

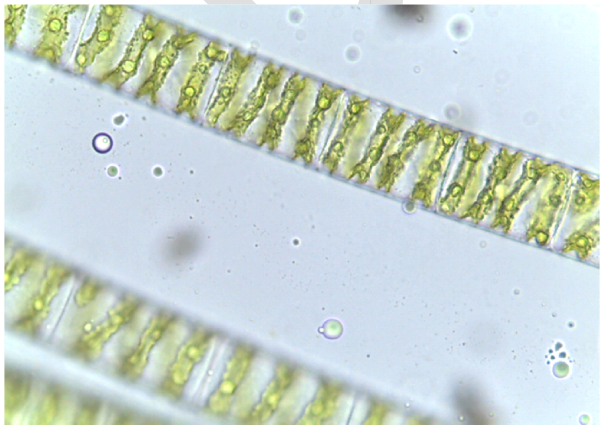


Figure 1-8. *Spyrogyra* sp. Photo by David Arieti

Michael D. Guiry of the AlgaeBase website and the Irish Seaweed Research Group, reports on the number af algal species (2012) that scientific estimates range from 30,000 to over 1,000,000. By his own conservative estimate there are 72,500 species of algae. His publication rightly raises the question, how is the concept species to be defined? The algae, with varied and elaborate life cycles challenge the standard definition of a species being a group of interbreeding individuals. Not an easy definition to apply to unicellar forms that multiply vegetatively by cell divisions, or to multicellular forms that generate offspring by asexual budding of propagules to give rise to genetically identical offspring, considered clones of the parent. The collection of algae to cultivate them for a myriad of purposes will yield strains and cultivars of known species, with defined genomes, or genotypes. The applications of genetic engineering to yield genetically modified organisms (GMOs), defined with great precision. The so-called biological definition of a species calls for all available evidence to be applied to the identification of a species. With such exhaustive demands, the use of the genus name alone will suffice for many functions of algology in the field.

The most recent taxonomic classification divides cellular life forms into three enormous "domains". All members of the domains Bacteria and Archaea have prokaryotic cells. Members of Domain Eukarya have eukaryotic cells. Cyanobacteria are in domain Bacteria. The Eukarya includes animals, plants, fungi, algae, and protists or protozoans, and many other, mostly microscopic groups. Recent classifications divide the Eukarya into six "super groups" . Reflecting their diverse origins, algal taxa are found in four of the six supergroups: Stromenopila, Alveolata, Excavata, and Archaeplastida. Further reading on the topics of the evolutionary origins and the taxonomy of the "algae," is found in Chapter 2,

Importance to diversification of life forms on Earth. It is difficult to overestimate the importance of free-living cyanobacteria and their endosymbiotic descendants (chloroplasts) that live within the cells of algae and land plants. Cyanobacteria/chloroplasts have produced and continue to produce all the oxygen and most of the monomeric building blocks of protein, carbohydrates, lipids, and nucleic acids. There was little or no oxygen in Earth's atmosphere for the first 2 billion years after the Earth formed. Over hundreds of millions of years, the cyanobacteria and the chloroplasts of algae liberated oxygen, which eventually accumulated in the atmosphere, rising as high as 25%. They also produced huge amounts of high-energy organic compounds, most of which have been used as food by heterotrophs.

The addition of massive amounts of food and oxygen fundamentally altered Earth's environment into one conducive to the evolution and adaptive radiation of millions of heterotrophic aerobic (oxygen-using) organisms. Had cyanobacteria not evolved oxidative, oxygenic photosynthesis, none of the oxygen-dependent life forms on Earth, including aerobic microorganisms, animals, and fungi would exist. Cyanobacteria and their endosymbiotic descendants "terraformed" the Earth.

Vital ecosystem services and commercial products provided by algae and Cyanobacteria.

1. Cyanobacteria and/or algae form the base of food webs in many of Earth's ecosystems. The energy-rich organic molecules they synthesize are assimilated, modified, and passed up through food chains. Photosynthetic organisms are essential for the survival of all the non-photosynthetic organisms at higher trophic levels. The crustaceans, fish, and other aquatic animals that depend on the products of photosynthesis provide a significant percent of the food eaten by humans each year.
2. When the sun is out, Cyanobacteria and algae liberate oxygen into the water and atmosphere. Oxygenated water is essential for many types of aquatic animals, including segmented worms, roundworms, crustaceans, insects, and fish .
3. Algae and free-living Cyanobacteria absorb gargantuan amounts of carbon dioxide every year . When land plants die, saprophytic bacteria and fungi process the dead tissues and, in most ecosystems, return nearly all the carbon to the atmosphere as carbon dioxide. This kind of decay also occurs for algae that live and die in lakes and streams. In contrast, when marine algae die, they drift to the bottom of the ocean, where decay is slowed by cold temperatures and burial under the continuous rain of dead organic matter and mineral sediments. If it weren't for fossil fuel extraction, these deposits would be permanently removed from Earth's carbon cycle.
4. Algae themselves have been used as food in many cultures and are excellent sources of essential minerals such as magnesium, iodine, iron. Red algae produce carrageenan, a polysaccharide used in many processed foods and in agar.
5. They provide a variety of medicines.
6. Other ecosystem services such as providing animal habitat for invertabrates and fish.

In mid-20th century U.S. there was little awareness that chloroplasts were descendants of Cyanobacteria. A popular bumper sticker at the time read, "Have you thanked a green plant today?" Based on our current understanding of the evolution of chloroplasts, the bumper sticker could now be amended to read, "Have you thanked free-living Cyanobacteria and their endo-symbiotic descendants today?"

Ecological, Environmental, and Medical Problems caused by algae and Cyanobacteria.

1. **Eutrophication:** Depending on the environment, pollutants such as nitrates, phosphates, or iron can cause algal blooms, a common initial step in eutrophication. The resulting anoxia, produced during decay of dead algae by aerobic bacteria, can cause mortality in oxygen-dependent organisms including fish and crustaceans. Excessive growth of algae and subsequent decay can foul the water with slime and offensive odors.
2. **Toxins:** Some algae release toxic secondary metabolites into the water, causing fish kills. Other compounds released by algae are allelopathic (kill competitor photosynthetic organisms) and can disrupt local aquatic communities. Some algal toxins have the potential to be weaponized.

3. **Corrosive effects:** Through corrosive effects, growth of algaecan accelerate erosion of concrete, such as the walls of reservoirs.
4. **Clogs filters.** Excessive algae can cause filters to clog.
5. **Aesthetic problems**. Algal growth on decks, awnings, sidewalks, etc. can often be black or have other distasteful colors.
6. **Chemical algacides**: can be expensive and may have unwanted environmental impacts.
7. **Human disease**. In rare instances, algae can cause human disease, e.g., Protothecosis (Lass-Florl and Mayr 2007).

Moreover, the algae have provided "drama" in recent years, if you can call illness debilitation, and death of people to be drama. As chronicled in the book *And The Waters Turned To Blood* (Barker,1997) the menace lurking underwater was not a huge man-eating shark, but something more stealthy and sinister. That something was the subject of research by a phycologist, Dr. JoAnne Burkholder, PhD., of the Microbial Ecology Lab at the University of North Carolina. Her process of the discovery of the dinoflagellate species, *Pfiesteria piscicida* was masterful study of an algal species, and also difficult and trying process with a number of agency and business establishments who were not receptive to the news of a menace in the waters of their economic and regulatory interests. Much like mayor in the movie *Jaws*, where the supposed shark menace should be eliminated without causing alarm among the seaside resorts. The dinoflagellate hazard, much less definitive than the shark, was a much harder sell for the microbial ecologist, Dr. Burkholder, taking years for her findings to shift the perceptions and practices of the stakeholders. *P. piscicida* continued its rampage in the estuaries of North Carolina as the "killer of fish," and conceivably as the killer of so much more. Secreting a deadly toxin, able to diffuse through water or air, functioning as a neurotoxin, rendering the "prey" immobile and next able to be consummed by the flesh-eating stages of the *P. piscicida* life cycle. Woe to the marine mammals, or the humans swimming or feeding from the waters inhabited by *P. piscicida*. Dr. Burkholder's isolation and description of the *P. piscicida* toxin gained the attention of agents from one of the national security agencies. They approached her at a scientific conference regarding the potential to weaponize the toxin! See Chapter 12 for a more detailed look at toxic algae.

While many other dinoflagellates are toxic, not all are. The horrors of *P. piscicida* are a stark contrast to some other dinoflagellates, such as *Noctiluca scintillans* ("sparkling night light"), although known to be toxic, it is better known for its night time bioluminescence (see Figure 1-9). A tourist spectacle in many tropical areas of the world. Bioluminescence revelers take kayak rides into calm bays to watch the water light up. Places like Bioluminescent Bay in Puerto Rico advertise their dinoflagellates and bay as a top rate ecotourism destination. The reasons not really known to science, perhaps algal of warning coloration to herbivores with eyes, photoluminescence by dinoflagellates must be a zenith of evolution. To gather light energy produced by one sun, to convert it and re-radiate it to combine with the lights of a million suns and the celestial bodies of

great favor for the revelers. Drama and splendor from a group of algae that perhaps once fed on dinosaurs that succumbed to the toxic exudates of billions of tiny cells.

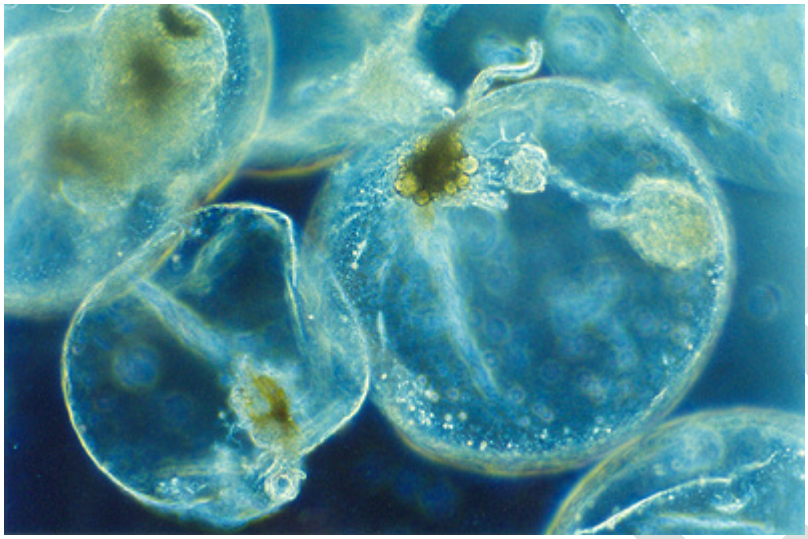


Figure 1-9. Noctiluca scintillans, a bioluminescent dinoflagellate.
Photo by Maria Antónia Sampayo, Instituto de Oceanografia, Faculdade Ciências de Universidade de Lisboa - http://planktonnet.awi.de (provided under a Creative Commons Attribution 3.0 License), CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=4611082 (viewed 5/12/22)

Aside from their historical importance to life on Earth, algae are valuable because they can be used to address some of our current environmental challenges. For example, algae can be a source of new medicinal compounds, they can be used to create alternative fuels, and they may be able to accelerate the rate at which carbon dioxide is permanently removed from the atmosphere.

Despite their immense diversity and ecological importance, the algae have received relatively little attention in comparison to the land plants, their terrestrial descendants. A search for English language, non-juvenile, non-fiction books published between 2010 and 2020 (WorldCat) identified 54,127 books containing the word “plant” in the title, but only 1,979 books with a title containing the word “algae”.

One book that delivers a timely and comprehensive exploration of algal potential, both realized and sought for is *Slime: How Algae Created Us, Plague Us, and Just Might Save Us* by Ruth Kassinger (2019). She covers a broad spectrum algal benefits and detriments by telling the stories of the businessmen, cultivators, and researchers working with algae hands-on. The book is a well documented examination of the people doing the hard and smart work of building partnerships with nature, and producing examples of sustainable industries and renewable products. Ms Kassinger is likely to recruit many brilliant young biologists to the field of Phycology. Through her

insights into human ingenuity and innovation there is much vision and accomplishment in partnership with the algal "empire."

This book surveys past, present, and possible future benefits of algae to environmental health and human well-being. We also review causative relationships of algae to certain medical conditions and environmental problems. We emphasize the importance of human culture partnership with nature. We include many images and diagrams to assist in field knowledge, to illustrate points discussed in the text, or to showcase the beauty and diversity of these organisms. The following chapters are intended to satisfy curiosity and set the direction for future study, a bright prospect, now knowing what algae are, and why they are important.

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Chapter 4. GROWING ALGAE FOR REDUCATION OF CARBON DIOXIDE, FOOD FOR ANIMALS AND HUMANS, AND FOR NATURAL PRODUCTS INCLUDING PHARMACEUTICAL AGENTS

by Dr.Jacob Paz, PhD

OBJECTIVES

The objectives of this chapter are:

- To examine the methods to grow algae: Sanitary landfills, the use of anaerobic digesters for biofuels, stabilization ponds, photobioreactors, and open changes.
- Algae growing for food production, natural agents, and for pharmaceuticals.
- Briefly discuss a cost/benefit analysis for methods i.e., pros and cons of biofuel costs.

What Are Microalgae

Microalgae "are small, unicellular, photosynthetic microorganisms using CO2, water, and light to synthesize organic compounds for nutrition. Macroalgae are typically called seaweeds. Macroalgae are multicellular, photosynthetic, plant-like organisms. They are often used in saltwater aquariums as a food source and a natural filter, removing nitrogenous wastes. ?"(no p.) [1] They can grow on three types of carbon media autotrophy, heterotrophy, and mixotrophy. In autotrophic growth, microalgae produce the needed organic matter and energy by using inorganic CO_2 as a carbon source and sunlight as an energy source. While heterotrophic microorganism use organic carbon sources for growth and metabolism. Whereas mixotrophic organisms can grow both on inorganic or organic carbon sources. [2-3]

Nutrient Requirements and other Factors for Growing Algae

Both nitrates and phosphates are important limiting nutrients needed for algae growth and

development in wastewater treatment and algae growing cultures. In particular, the levels of both nitrates and phosphate are extremely important for growth and development of algae. A 1998 report indicated that "Generally, if the soluble ortho-phosphorous (P) concentration is 0.002 mg/l or lower, phosphorus would be considered limiting. If the available nitrogen (N) concentration is less than about 0.015 mg/l, N would be considered to be limiting." [4]

Another approached using the ratio of N:P Algae as an indicator for the limiting nutrient. Generally, speaking Algae generally use nitrogen and phosphorus in typically constant proportions, on the order of 16 to 1 on an atomic basis (7.5 to 1 on a mg/l basis). It is, however, not a narrow range about 16:1; the

atomic ratio can range from about 5:1 to 25:1. [5] Micronutrient elements are need by algae in media for growth and development are: Fe, Mn, Cu, Zn Mo, V, B CI, Co, and S are needed at concentrations of 10^{-5} M or lower. Whereas macronutrients requirements are needed at a level of 10^{-2} to 10^{-4} M for C, H, O, N P, S, K, Mg Ca and Na. [6]

The factors of temperature and light also effect microalgae algae growth and productivity. Overall, microalgae tolerated a wide range of temperatures from 10 to 30 ^{o}C. While the optimum array temperature is a somewhat a smaller range of 15 to 25 ^{o}C respectively. On the whole, temperature increases usually associated with higher productivity and growth rate also increase the metabolic rate. Illumination is an equally important factor controlling the rate of microalgae photosynthesis. [7]. "Light irradiance varies between 33 µmol m^{-2} s^{-1} to 400 µmol m^{-2} s^{-1}. Maximum growth rate was found to be 1.73 $d^{-1,}$ for example, in *Selenastrum minutum* at 35 °C and 420 µmol m^{-2} s^{-1} irradiance. Minimum growth rate (0.10 d^{-1}) was reported for *Botryococcus braunii* KMITL 2 strain at temperature 25 °C, photoperiod 24:0 and 200 µmol m^{-2} s^{-1} irradiance." [8]

Algae and pH

The optimum pH range is around 8.2 for marine species, while freshwater strains prefer a pH around 7.0. Meanwhile, spirulina (*Arthrospir*a) prefers a pH of about 10. The pH of water is controlled by levels of CO_2, HCO_3^- , and CO_3^{-2}. Most lake and pond organisms desire pH levels of 6.5 to 9. The pH value is dependent upon the given time of the day, and it is subject to daily fluctuation due to inorganic alkalinity, photosynthesis, and respiration. If pH levels are not maintained within the desirable range, it could have a negative effect on the algae culture, especially its yield. [9]

Rationale for algae growing to capture carbon dioxide

- Algae have a high efficacy for absorbing CO_2 because of their fast-growing, short life cycles, and their ability to produce a large cell mass within a short period of time. [10]
- A 2013 publication reported that 1 kg of algal biomass can utilize up to 1.83 kg of $CO_{2.}$ per growth cycle. [11]
- Microalgae are single-celled, photosynthetic micro-organisms which have the ability to produce energy-rich oils. These algae can accumulate high oil levels in total dry biomass as observed in a small laboratory study of 30 micro-algae species in 2008. [11]. For instance, some algae can accumulate up to 50% of their dry mass stored as long chain hydrocarbons. [12]
- Currently, there is a global research effort aimed at increasing and modifying the accumulation of lipids, alcohols, hydrocarbons, polysaccharides, using bio-engineering or genetic engineering (GE) with microalgae. This is leading to improvements such as increased lipid and carbohydrate production. Because of the wide variety of algae species, it provides a wide range of options for finding genetic information that can be used to improve production

of algae biofuel. [13] An example is a 2014 discovery of the use of GE yeast for biofuel production, making yeasts more tolerant to the self-produced ethanol. [14]

Fossil-fuel powered electric plants and algae growth

Of the three fossil-fuels used in power plants, (coal, natural gas, and oil), coal-powered plants are the worst polluters. They present a potent environmental hazard to algae growth. Coal combustion releases large amounts of both sulfur dioxide (SO_2) and nitrogen oxides (NOx), into the air which react with water and oxygen to reduce the pH in aqueous effluents. Thus, it creates an unsuitable environment to grow algae for biofuel. [15] Therefore, coal combustion emissions require pretreatment to remove these contaminants before they can be used by algae to generate biofuel. Such pretreatment increases costs of operation and maintenance.

The amount of SO_2 in air emission is varied and depends upon coal type and sulfur content which may ranges from 0.2 to 2%. [16] In addition, reduction of SO_2 in the effluent depends upon the process which in turn can generate hydrogen sulfide. H_2S, gas is a flammable, smelly a t low levels, toxic gas that can inhibits algae growth. [17] Similarly, oil combustion generates electricity but produces CO_2, SO_2, and NO_x, levels of which also depend upon the sulfur and nitrogen content in the petroleum used. In contrast, burning natural gas, methane (CH_4) for energy, it emits the lowest amount of air pollution, for example produces only carbon dioxide and water vapor. [18]

Anaerobic Digester

Another method to reduce the level of CO_2 emissions from landfills, anaerobic digesters can convert organic waste into products that can be used for energy production with little or no CO2 emission. The use of an anaerobic digester (AD) is based on a natural process in which bacteria, in the absence of oxygen, degrade and break down organic materials into products which can be used for capturing GHG. It can use materials such as plant or animal matter, sludge from waste-water treatment, and animal manure to generate methane, carbon dioxide (CO_2), and sludge residue. AD output, or product, contains between 55% to 70% CH_4, and 30% to 45% CO_2. The CH_4 gas is captured and used for power generation. The CO_2, it appears, can be removed so that CO_2 from AD capture technology use is very limited or absent. When the methane (CH_4) is used as a fuel, the CO_2 produced can be scrubbed from the emissions.

Anaerobic digestion (AD) has been used in the past to use decaying organic matter as a fuel for heat. An archeological finding noted that heating baths by gases generated by AD is dated to the 10 century

BC in Assyria. In the 17th century, a Belgian named Jan Baptist VanHelmont first revealed that decaying organic matter emits a flammable gas. Later, a British chemist named Sir Humphry Davy in 1801 discovered that methane was present in cow manure. The first known plant to use anaerobic digesters was built in a leper colony in Bombay, India in 1859. [19]

Introduction to Sanitary Land Fill (SLF) Algae Growing and Rationale

The U.S. EPA defined "Sanitary landfill" as the following: "A municipal solid waste landfill is a discrete area of land or excavation that receives household waste. A ..."landfill" …may also receive other types of nonhazardous wastes, such as commercial solid waste, nonhazardous sludge, conditionally exempt small quantity generator waste, and industrial nonhazardous solid waste."[20] The rationale for SLF management is to reduce emissions of greenhouse gases that contribute to global climate change and thereby help to improve local air quality. The rationale for using sanitary landfills is to reduce CO_2 and CH_4. SLF is the third-largest source of human-related methane emissions in the United States accounting for about 15.1% of methane in the US inventory. In addition, SLF contributes to global greenhouse gas (GHG) emmissions. SLF gas composition is composed of roughly 50 percent methane (primary component of natural gas), and 50 percent carbon dioxide. [21]

Landfills leaching water rich in nutrients could pose a groundwater environmental hazard. (SLF) leachates contain four main components: nutrients (namely nitrogen), volatile organic compounds, heavy metals and toxic organic compounds. Further characteristics in leachate include the following:

- The pH in general ranges from 6.5 to 9.5,
- BOD ranges from 4000–13,000 mg/L
- Ammonia ranges between 500–1500 mg/L after a period of 3–8 years. [22]

Environmental Pollutants from SLF and Wastewater Treatment (WWT)

Hydrogen Sulfide (H_2S)

Hydrogen sulfide (H_2S) is an odorous gas even at very low levels of 0.0005 ppm. The average Sanitary landfill (SLF) emissions of H_2S ranges with an average concentration of between 2.0 ppm and 96.6 ppm. [23] The Hydrogen sulfide emissions to the atmosphere have been implicated as an indirect source which may contribute to global warming gas effects. In addition, H_2S in SLF effluents is varied and it has been reported that for one landfill site as quoted in the EPA document, "H_2S is generated at less than 1000 ppm and for the second landfill site in which construction waste, domestic waste, and industrial waste are mixed, the H_2S concentration reaches at least 4000 ppm and at most 30,000 ppm." [24] Hydrogen sulfide has become an environmental problem and concern in recent years.

Nitrogen Discharge EPA Limits

The US EPA treatment of wastewater and leachate from SLF treatment plants discharging to a nearby stream, river or wetland may have a total nitrogen limit of 3 mg/L, or an un-ionized ammonia (NH_3) limit of 0.2 mg/L. [25] Nitrogen discharge released untreated into a body of water can form nitrate (NO_3^-), a primary contaminant in drinking water and can cause a human health condition called methemoglobinemia. In addition, reduction of NO_3^- into NO_2^- by nitrate reducing bacteria in the human gastrointestinal tract forms nitrosamine, a known carcinogen which has been associated with the development of colorectal cancer. [26]

Elevated levels of nitrogen in effluents can cause nutrification, or **eutrophication**, which is defined as characterized by excessive plant and **algal growth** due to the increased availability of one or more nutrients needed for photosynthesis. Other factors that influence eutrophication include sunlight, carbon dioxide/bicarbonate (HCO_3^{-1}) and phosphate fertilizers. Most USA states have a phosphorus limit for effluents in the range of 0.5 to 1.5 mg/L. In the USA, states can use the EPA standard or may have a more stringent discharge standard. [27] In addition, discharge permits from EPA involve pH, as a discharge from Waste Water Treatment effluents with low pH have negative effects on aquatic life and enhance metal corrosion. The state of California (CA) for example has more stringent pH discharge permits than EPA Federal requirements. The CA pH limit is set for a pH range between 6.5 and 8. Effluents outside that range would need pretreatment to comply. This limits effluent discharge and increases treatment cost.

Biochemical Oxygen Demand (BOD)

Another important requirement set by the state of California involves an index of the degree of pollution in a body of water. Termed the Biological Oxygen Demand (BOD), it is a measure of the amount of oxygen required to remove waste organic matter from water by aerobic decomposer bacteria. In California, treated sewage effluents should be limited to a monthly average of a BOD of 45 mg/liter. (Moderately polluted rivers have BOD's ranging from 2-8mg /liter.) A higher BOD indicates a higher level of pollution. In contrast to CH_4 capture from SLF, at the present time, there is no CO_2 dual commercial plant which capture both CO_2 and CH_4, Furthermore, pretreatment may/can be required for odorous H_2S. [28-29]

Leachate in water which is allowed to percolate through SLF can be used for growing algae for animal feed, and as a component in wastewater treatment. Other benefits from algae cultivation include using algae as a protein source for the following examples are: in anti-inflammatories, for disease prevention,

for fatty acids in lipids and glycolipids, (important for cell membranes), and for polysaccharides (important for energy storage or structural elements in marine algae and terrestrial plants).

Methods of Growing Algae

Open Vs. Closed System, an Overview

There are two major systems to grow microalgae, open and closed. An open system involves a shallow pond, and a closed system involves a type of bioreactor, a photobioreactor. The selection process is complicated; it is not based solely upon a comparison of the costs of construction and operation costs but potential profits as well. The benefits and disadvantages of each method must also be considered before choosing one.

Closed photobioreactor (PBR) systems offer far more flexibility in controlling conditions affecting the algae's growth such as exposure to contaminants, and other conditions. It's easier to maintain a specific water temperature, pH level, or nutrient level when evaporation and dilution processes are controlled. With indoor growing environments for algae culture, closed reactors are likely the best choice to control humidity levels. However, closed systems are highly expensive, require a lot of maintenance and are much more limited in size than open ponds.

The majority of algae that are purposely cultivated fall into two categories:

- Microalgae (also referred to as phytoplankton, microphytes, which include planktonic algae)
- Macroalgae, commonly known as seaweed, also have many commercial and industrial uses, but due to their size and the specific requirements of the environment in which they need to grow, they do not lend themselves as readily to cultivation. [30]

Open and Closed Pond Algae Growing

Open pond systems are the most common system of algae cultivation, already used commercially in the United States to produce nutritional products and treat wastewater. Open pond systems use shallow ponds ranging from about 20 to 60 cm in depth. They range in area between one acre to several acres, in which the algae are exposed to natural solar radiation necessary to produce biomass. The main advantage of open ponds is lower construction.

Figure I. Illustrates Open and Close Systems for Algae Growth

The open pond has a lower power demand. The open pond is easier to clean compared with a closed photobioreactor. The disadvantages of open pond for algae growth are the following: daily and seasonal changes in temperatures and humidity, greater difficulty of maintenance, variations in light exposure and subsequently changes in algae yield. Light irradiation is the main problem which can affect the commercial production of algae.[31]

A closed pond with a transparent or translucent barrier effectively turns it into a greenhouse. The advantage of a closed system is that it can solve many of the problems associated with an open system. It allows more species to be grown. It also allows algae species that are being grown to stay dominant, and it extends the growing season. If the system is heated, the pond can produce year-round. Other advantages of closed systems for algae growth are: it allows more species to grow, and to remain dominant, and it can increase the CO_2 level in the media, thus increasing productivity. The disadvantages of closed pond systems are: requirements for temperature control and maintenance, periodic cleaning due to biofilm accumulation, need for external light sources and higher construction and operation costs. [32] A partial solution is to incorporate solar panels for lights and temperature control.

A closed system is where the algae growing system is completely closed. The most common closed system is the photobioreactor (PBR). Closed systems provide a controlled environment to culture algae with proper growth conditions to include nutrients, temperature, light and dark cycles. Closed systems growing algae usually produce significant higher production rates than open systems. PBR algae average

growing yields is about 25 g/m^2/d on ash-free dry weight. [79] In contrast to open ponds algae growing is about 0.05 g/L/m^2/d wet weight or 0.05 wt% ash-free dry weight. [80]

The advantages of closed algae system are: Low construction costs, reduced evaporation rates and lower water usage. The system provides protection against dust and bacterial contamination which results in a higher quality of algae products. Carbon Dioxide gas has been used in algae culture to stimulate growth. [31&33]

The question, which system to select, closed vs. open system, depends upon several factors:

- human food,
- cosmetics
- pharmaceuticals
- surface area for algae growth ponds
- location
- available funds for construction, operation, and maintenance.

The following table is summarizing the advantages and disadvantages of each system.

Growing Methods	Advantages	Disadvantages
Open pond	**Low construction and operation cost. Low energy requirements, easy to clean.**	**Low biomass productivity; poor mixing and light utilization; requires large area; risk of microbial contamination; limited algae species can grow; high rate of water loss due to evaporation; and poor mixing.**
Closed system Photobioreactor	**good control of system inputs i.e. temperature, light, pH, and nutrients. Smaller area is needed for algae growth. Can produce higher quality of algae products- medical and cosmetics,**	**Very difficult to clean, higher cost of operation t, and more technical/difficult to operate. High cost of construction, higher risk of imbalance throughout the system. Oxygen accumulation can reduce growth rate of algae**

human & animal food and drugs.	
Lower water requirement; Greater stability,	
more resistant to external adverse effects.	

Table I. Shows the Advantages and disadvantages of open and closed algae systems [34,)]

Algae growth using Waste Stabilization Ponds (WSP)

Waste stabilization ponds are shallow basins that use natural factors such as biodegradation, sunlight, temperature, sedimentation, predation, and adsorption to treat wastewater. The facultative stabilization pond for growing algae is usually designed to be aerobic throughout its depth and the facultative lagoon will be anaerobic at the bottom and aerobic at the top. The system is suitable to use in rural areas to treat waste for pig and cow manure. Furthermore, waste stabilization ponds have several advantages such as: high removal efficiency, simplicity, and low cost, which have been demonstrate by various researchers. [(35)] Photosynthetic algae, results in growth and endogenous respiration, gas exchange for oxygen, carbon dioxide, ammonia, ionic equilibrium processes, and pH. Solar radiation and wind is mixed in the upper layer of pond and provides oxygen for algae and other organisms. Solar radiation is the principal factor influencing algae growth and development. [(94). (36)] In addition, combinations of various WSPs have been used for of fish growing and floating microalgae in Indonesia, China, and Thailand. [(36)]

The first man-made treatment pond, named Mitchell Lake with 275 ha of surface area and 1.4 m of average depth, was accidentally built in San Antonio, Texas in 1901. At the present time North America waste water treatment plants are WSPs in the US, accounting for about 8000 facilities, which are also the first choice for nearly all remote areas. About 50% are WPS plants. [((37)] The advantages of using WSPs are as follows:

- Low energy requirements.
- Easy to operate and maintain and generally require only part-time employees.
- Low construction and operation cost.

- The use of ponds for wastewater treatment (WWT) in small communities meet the needs of low construction costs as well minimal operation and maintenance requirements. Recent combinations of WWTs with the combination of algae provide dual benefits of WWT and income form algae harvesting for GHG and animal feeding.
- The main disadvantages are:

- The need of large area needed for WWT in urban area.
- Hydrogen sulfide generating odors during algal blooms.
- Ponds provides breeding areas for mosquitoes and other insects if not properly maintained.
- Some effluents comprise algae and often require additional treatment or polishing to meet discharge standards. [38-39]

Operation of Stabilization Ponds

Stabilization ponds and facultative lagoons are mostly used in rural areas for domestic WWTs. The stabilization pond is designed to be aerobic throughout its depth and the facultative lagoon

will be anaerobic at the bottom and aerobic at the top. Stabilization ponds can also offer a secondary biological WWT to meet regulatory requirements for effluent discharge. Table II. Shows EPA permit discharge of secondary effluents**.**

Parameter	30-day average	7-day average
BOD_5	30 mg/L (or 25 mg/L $CBOD_5$)	45 mg/L (or 40 mg/L $CBOD_5$)
TSS	30 mg/L	45 mg/L
BOD_5 and TSS removal (concentration)	not less than 85%	--
pH	within the limits of 6.0–9.0*	

Table II. Displays EPA Permits of Secondary Effluents Requirements. [91]

BOD_5: Measure corresponds more specifically to the quantity of oxygen consumed. pH: Is measure the acidity or alkalinity of wastewater or the hydrogen concentration in Wastewater or water. Total Suspended Solids (TSS) are referring to small solid particles which remain in suspension in water as a colloid suspension or due to the motion of the water. This is used to measure water quality. [90]

Introduction to Aquaculture Algae Growing

A 2018 publication by the U.S. Geological Survey stated that in the U.S., there are two main types of aquaculture: marine and freshwater farming. Marine aquaculture (or mariculture) produces more species than fresh water including oysters, clams, mussels, shrimp, seaweeds, and fish such as salmon, black sea bass, sablefish, yellowtail, and pompano. Marine fish farming is typically done in net pens in the water or in tanks on land. U.S. freshwater aquaculture in ponds and lake produces fewer species such as catfish and trout. [42] Aquaculture is done for the production of:

- animal feed
- pharmaceuticals and cosmetics
- seafoods such as fish, shrimp, lobsters and crayfish.

Contribution of Aquaculture to the GHG

An interesting report that aquaculture systems is also emit GHG such as: CO_2 which is generated by heterotrophic bacteria mainly producing CO_2 during the consumption of organic matter including the decay of feed residues and fecal matter of fishes. The total N_2O CO_2-eqv assessed was 9.30×10^{10} g eqv CO_2, and it will grow to about 3.83×10^{11} g eqv CO_2 by 2030, assuming an annual growth rate of 7.1%. CH_4, formed by methanogenic bacteria producing methane gas by utilizing dissolved organic carbon under anaerobic conditions at the bottom of the pond. N_2O is made by nitrifying bacteria through autotrophic aerobic nitrification, and denitrifying bacteria produce nitrous oxide gas through anaerobic denitrification. Algal photosynthesis also releases NO_3^- and dissolved oxygen. [40-41] Fish fecal matter and food residues are the major sources for GHG in aquaculture. Other minor sources of CO_2 emission into the atmosphere are respiration of fish feeding on microorganisms and mineralization of organic matter. [40-42] Aquaculture is contributing to global warming GHGs.

ECONOMICAL VALUE OF GROWING ALGAE

Commercial Algae Cultivation Worldwide and US

Commercial algae growing can be tracked back to 1910 in Germany with the culturing of Chlorella for aquaculture purposes. Since about the 1970 there is a growing interest in algae growing for capturing GHGs and for the production of a wide range of products such as:

- astaxanthin (sunscreen)
- lutein
- beta carotene
- chlorophyll
- phycobiliprotein
- Polyunsaturated Fatty Acids (PUFAs)
- Pharmaceutical and cosmetic agents

- Animal and human food
- Nutraceuticals. [43-44]

There is an annual increase in related publications at a rate of 22% for the years 2009 to 2019. [45] Growing algae has a distinct advantage over corn and soy bean farming. Aquaculture of algae produces about 7-13-fold the amount for soy or corn. Algae feed contains 40 to 50% higher protein content. In addition, algae contain fatty acids, amino acids., pigments, and vitamins which are valuable in animal feed. [46]

By 2050 the world's population will reach about 9.1 to 9.8 billion. Several factors are associated with increases in food demand. They are the following: Global warming such as floods, drought, change in sea levels, rise in temperature of the oceans resulting in migrations and population growths. The question is, how we can increase food supply for growing world populations? It is a difficult task. [47] In underdeveloped countries and in especially in the sub-Sahara region there are concerns about erasing malnutrition, improving poverty, and achieving food security and environmental health Coupled with population growth, and demand for animal protein, is a supply problem due to over fishing and the depletion of fish stocks. Consequently, we are required to develop a new method for both animal and human food supply. [48]

Open and Close Systems R&D needs

An excellent 2013 article outlined and discussed the algae growing requirements needed to cultivated high value algae product in both open channel and bioreactor growing systems. These technologies were developed world wide for algae growing for animal feeding and biofuel. Key R&D issues are: R&D to develop strains of microalgae which has high content lipid and protein for animal feeding or biofuel. Including strains resistance fluctuation in growth conditions such as, pH, O_2, temperature and light, and strains that are tolerance, stable cultivation to invasion of weed algae, parasitic fungi, viruses, and bacteria. Additional requirements are: algal strains that can be able to be economically harvested and processed. Open channel algae growing are more susceptible to environmental changes such as temperature, light, pH changes and media contamination by invasion of foreign microorganisms. In contrast, bioreactors allow for where the environmental conditions are controlled, and invasive or pathogenic species are generally not a major issue. [49]

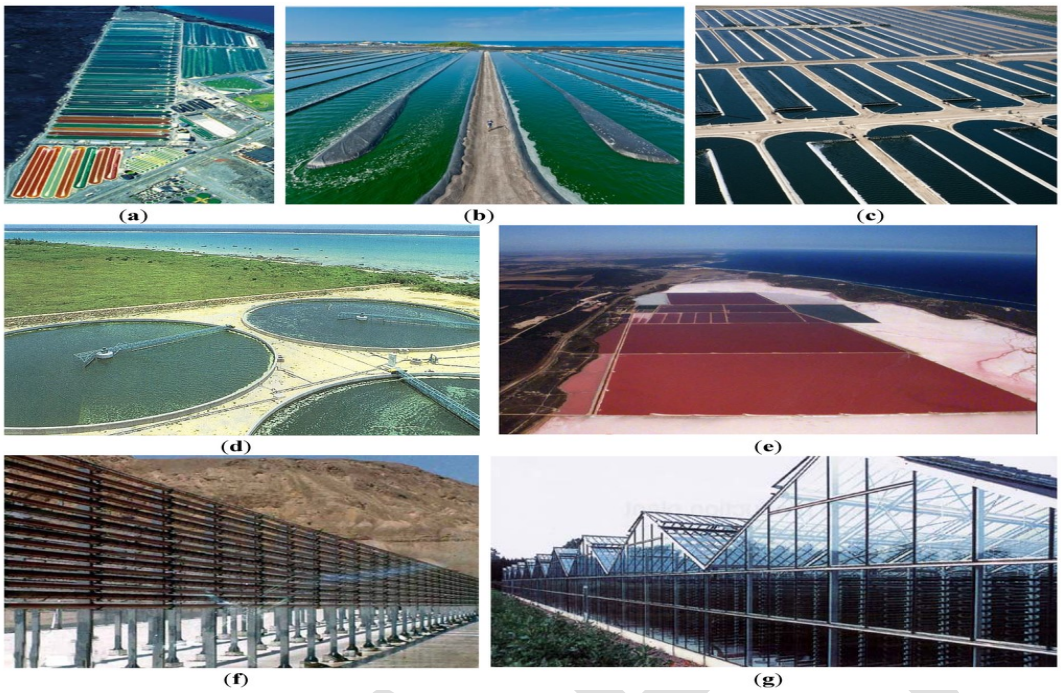

Figure II. Commercial microalgae production systems: ponds and photobioreactors. (a–c) Commercial production systems using paddle wheel mixed raceway pond systems. (a) Cyanotech Co., Hawaii, producing *Haematococcus pluvialis* (red ponds) and Spirulina; (b) Close-up of Cyanotech Spirulina ponds, ~0.3 ha, note paddlewheels are at far end; (c) Earthrise Nutritionals LLC, California, ~0.4 and 0.8 hectare ponds, note paddle wheels; (d) Chlorella Industries, Japan, circular ponds, each ~500 m^2, with central pivot mixing; (e) Betatene (Cognis), W. Australia, unmixed ponds, *Dunaliella* production ~500 hectares; (f) Algal Technologies, Israel, tubular photobioreactors for astaxanthin production *Haematococcus pluvialis*; (g) Production of *Chlorella* in tubular photobioreactors, inside greenhouse, in Germany (facility now owned by Roquette). [49]

Conditions noted that raceway pond algae growth is limited by water sorption of CO_2 from air of about 412 parts per million. Systems which used a 100% CO_2 bubble supply showed a substantial increase of up to 10 X higher compared to open channel. In Taiwan and Japan growers use a supplement of acetate (CH_3COOH) as an organic source of C to stimulate growing cycle of *Chlorella.* A 2020 laboratory study reported that *Nannochloropsis oceanica* can grow either on sodium-acetate or bicarbonate. The experimental data showed that: (i). This alga can grow on inorganic carbon media i.e., that they are strict autotrophic organisms. (ii). They also can also grow on organic media. (iii). The combination of sodium acetate and sodium bicarbonate significantly enhanced algal growth with higher acetate assimilation, when compared with single inorganic carbon sources. [50] Similar test results observed growing *Micractinium inermum* microalgae under comparable growing condition reported a 32.7-fold and 2.4-fold surge using dissolved inorganic carbon and dissolved oxygen levels parameters to assess the impact of autotrophic and heterotrophic cultures growth respectively. [51]

Algae Growing and Human Consumption

Algae growing can be used both for a protein source for human food and various important dietary supplements and pharmaceutical agents. The increase in population growth results in an increasing demand for human protein sources such as meat and dairy products. The question is could the conventional food supply meet the growing demands for protein needs for the next 20 to 30 years? Algae proteins, extracted from aquaculture grown algae, contain high percentages of proteins, varying from 40% to 60%. [52] Arguments for using micro algae for growing fish as protein are: They help fish develop faster, and can be economically and environmentally sustainable. Algae cells contains a mixture of crucial amino acids, including healthy triglycerides as fat, vitamins, and natural pigments. [53]

Additionally, algae growing for protein has several benefits over traditional high-protein crop use in terms of productivity and nutritional value. Seaweeds and microalgae have higher protein yield per unit area (2.5–7.5 tons/Ha/year and 4–15 tons/Ha/year, respectively) compared to terrestrial crops, such as soybean, pulse legumes, and wheat (0.6–1.2 tons/Ha/year, 1–2 tons/Ha/year, and 1.1 tons/Ha/year, respectively)." (p.2) [55] Three groups of marine algae have been shown to have high levels of protein content ranging from 24 to 376 grams dry weight per kg^{-1}. The protein content of brown macroalgae is generally lower which is frequently below 150 g kg^{-1} of dry matter (DM). Green macroalgae, and especially red macro algae, have a higher protein content on a DM basis. [55]

Macroalgae are broadly classified as brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) these algae, and are a various group of marine algae. They have quite variety of high levels of nutritional value of macroalgae is highly variable such as: Amino acid, Polysaccharides, lipids, phytochemicals a, and minerals. Variation of protein observe within these three groups, the lowest levels protein tests were brown algae ranges from 24-168; followed by green 32 – 362; and the highest protein concentration observed in red algae fluctuating from 64-376 (all test results reported as Values in g kg−1. [56]

Green seaweed macroalgae *Ulva* which also has a high protein content. This alga is frequently found in in brackish water (defined a water that is saltier than fresh water with a salinity of between 0.5-35 parts per thousand, but not as salty as sea water.) Ulva has a potential as an alternative source of proteins for animal food. It contains highly insoluble dietary fiber and soluble fiber and having higher protein content than other green seaweeds. *Ulva* has high levels of minerals, proteins and vitamins which is very appealing for study at a nutritional level. [57]

The global algae protein market using was estimated at $690.8 million in 2018 and it is expected to expand at a compounded annual growth rate (CAGR: defined as the annualized average rate of revenue growth between two given years, assuming growth takes place at an exponentially compounded rate of 6.6% during the forecast period and is expected to continue to grow at a rate) of over 6% CAGR from 2020 to 2026. This will require a shift in consumer preferences from animal-based meat and dairy products in the direction of plant-based and other alternative sources of protein. This is expected to

drive the demand for algae protein during the forecast period. Animal feed applications consumes about 30% of global algal production [58] By the year 2050 the projected algae production may account for 18% of protein sources across the more diverse world market according to the National Center for Biotechnology Information. [59] The [market value for plant protein production from algae is forecast to be 15.6 billion U.S. dollars in 2026. Compared to the 10.3 billion the market was estimated to be worth in 2020, this would constitute an increase of close to five billion U.S. dollars in 2024. [60]

Algal aquaculture growing is an excellence source of antioxidants, vitamins, A, B-2, B- 6, B-12, and good source for minerals such as of K, Fe, Mn, Cu, and I. [86] Other beneficial macromolecule supplements and compounds derived from algae cultivation are: biomolecules including astaxanthin, lutein, beta-carotene, chlorophyll, phycobiliprotein, Polyunsaturated Fatty Acids (PUFAs), beta-1,3-glucan, and other pharmaceutical agents. Examples of algal natural products and their uses are: (i). Astaxanthin is a natural pigment which can be used to enhance antioxidant defense mechanism, it also was found to provide protection against UV- sunlight irradiation. [55-56] (ii). Lutein, is a carotenoid pigment that plays a role in stimulating the immune system and prevents eye deceases such as cataracts. The 2018 estimated market value is $309 million and continues to grow. [56]

The Growing Algae Market Value for Biomolecules, Biofuel, Food, and Pharmacological Agents Brief Review

The World market for Algae Biofuels was estimated to be US$ 6.8 billion in the year 2020.It is projected to reach a revised size of U$11.4 billion by 2027, growing at the annual growth rate (CAGR) of 7.5% over the period 2020-2027. It represents a growth rate of 59%. The USA biofuel market will rise from a value of $1.8 billion in the year 2020, and by the year 2027 it is anticipated to reach $2.5 billion which is a 72% increase. China, is the world`s second largest economy and is forecast to reach a projected market size of US$ 2.5 billion by the year 2027 trailing a CAGR of 11.4% for the period 2020 t0 2027. [61]

Algae Biofuel Pro and Cons and a Commercial General- Overview Value

Advantages of Biofuel

Major biological sources of biofuels are algae cultivation and commercial value of biofuel:

- Algae provides additional income to farmer especially in underdeveloped countries for small scale agricultural producers and small and medium-sized enterprises.

- In Brazil, bagasse (residues of sugarcane) is burned and the heat is used for distillation processes and electricity generation.
- Biofuel extraction can produce glycerin which is a valuable commodity. [62]
- The most important factor is that use of algae can capture CO_2 from air and reduce GHE.
- Biofuels can be used for fuel substitute such as in motor vehicles and in airplanes. [63-64]
- In general, several publications reported that the combustion of biodiesel in diesel engines has been established as an alternative fuel to reduce major transportation pollutant emissions CO (Carbon Monoxide), PM (Particulate Matter), NO_x (Oxides of nitrogen), and VOCs (Volatile organic compounds) all of which contribute to smog formation and poor air quality. [65]
- Biodiesel from algae is an area of ongoing research. Algae could potentially produce 10 to 100 times more fuel per acre than other crops. [66]
- Open Channel Algae farms can produce about 14.5 metric tons of algae per acre (4000 M^2) per year which is considered the industry standard." [67]

Disadvantages to Use of Biofuel

However, the use of biofuel generated by algae has several major disadvantages that must be taken into evaluation of use of biofuel. [68]

- A major economical concern is the estimated cost of a barrel of algae-based biofuel. Using current technology, the cost of a barrel of biofuel ranges from $300 to $2600, compared with $40–$80 (not taking into account an inflation rate of 2009) for crude oil production. [69]
- Fertilizers with supplements of phosphate and nitrogen to stimulate algae growth could have a negative impact on the environment. For example, contamination of groundwater with nitrogen can cause eutrophication and thus increase operation costs. [70]
- Also, biofuels can reduce CO_2 emission rates. Nevertheless, we cannot ignore the generation of CO_2. A few studies noted that a blend of biofuels with diesel results in a slight increase in NOx of about 7% to 12%. [71]

Biofuels have a lower energy value compared to crude oil which has an energy value of 5,691,000 BTU (British thermal unit) per barrel, or 103,470 BTU per gal (gallon) Compared with biofuel: Use of gallon of finished motor gasoline (containing about 10% fuel ethanol by volume) equal to 120,286 BTU; 1 gal of gasoline has an energy value of 124,000 BTU. [72-73] On the other hand, the energy density of petroleum diesel is about 130,000 BTU per gal and that of biodiesel is about 118,000 BTU per gal. [74] In addition, the gross energy values of biodiesel fuel is ranges from 112,114 to 116,090 BTU per gal now, but will be increased in the future by process enhancements. [75]

One of a major important concern issue is that biofuels are not petroleum-based fuel therefore they will work differently in different engines which are developed for petroleum-based fuel. In particular it is more difficult to start biofuel in cold weather and why? Generally, it can be required to install heating units to protect the fuel tank and lines from freezing. This phenomenon is called "gelled fuel" it

happened in cold temperatures it causes diesel and biofuel to turn from a liquid into a gel-like substance. Biodiesel tends to thicken and "gel up" at low temperatures more readily than petroleum diesel. This phenomenon is a major concern, especially in states where temperatures drop below or near freezing point.] [89]

Others, disadvantages of biofuel are: It increase fuel viscosity, decrease the cetane number, and an increased melting point and viscosity; subsequently reduces lubricity**.** **[90-91]**
In 2012 review discussed and evaluated the concept of "investment return": They deliberated about the following method [How to evaluate the production of algal biofuels is to calculate the energy return on (energy) investment (EROI), which is defined as the amount of energy produced divided by the amount of energy required for that production which is similar to the net energy ratio. In brief, the EROI is the amount of energy produced divided by the amount of energy required for that the EROI which is the amount of energy produced divided by the amount of energy required for that production, and it has been used to characterize many resources].

For example, the EROI for production of conventional oil and gas, coal, wind energy, and corn, and ethanol has been estimated to be ~ 15, ~80, ~19, and ~1, respectively. The investigator concluded that "algae biofuel large scale production must be inexpensive and can compete with all current energy sources. " (p. 3.) (Emphasis added. [76]. A 2009 experiment testing the EROI of three plant species containing oil levels similar to algae species. Examples are the following: *Linum usitatissimum, Camelina sativa,* and *Brassica carinata* species *w*ere 1.46, 1.26, and 0.96 respectively. [77]

These three plant species have a similar oil content found in algal species used for biofuels, their EROI are far below that of conventional oil, gas, coal, wind energy, and corn ethanol. The estimated EROI for algal biofuels produced in either open ponds or photo-bioreactors range from 0.13 to 0.71. The recommended biofuel EROI should be > 3 for use as a fuel and a sustainable energy source. [78] Similar, the EROI for algae-based biofuel is 1.06:1, which is barely efficient compared with conventional energy sources. [79] In conclusion, EROI is not an absolute economy indicator but, it can help in understanding the regional national, and global energy markets.

A 2018 publication by The Northwest Pacific Laboratory reported one of the biggest limitations of growing algae is the initial high cost of construction; for example: Capital construction of 1000 acres of a PBR (Photobioreactor) costs millions which accounts for 78% of the total cost of start up. Other capital expenditures include: cleaning the PBR, supplying CO_2, algae inoculation, dewatering, and storage. [80] In contrast, growing algae in open channels has a lower capital cost compared with PBRs. [81] It is estimated that the minimum percentage of CO_2 emissions captured by an algae farm is about 10% to 30%. [82].

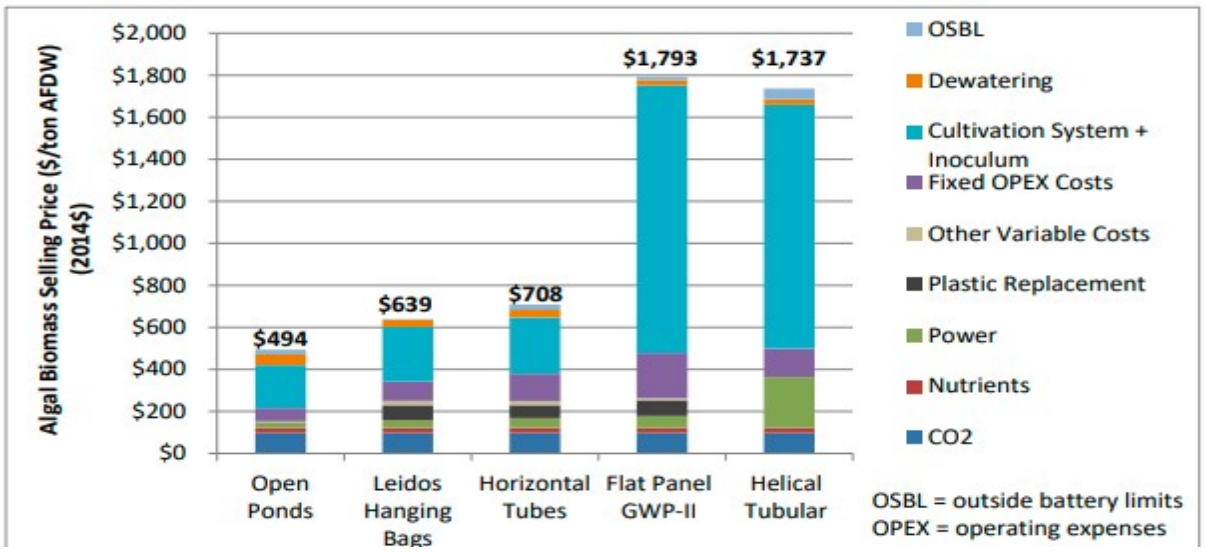

Figure IV. Display Minimum Biomass Selling Price (MBSP), MBSP results for each PBR scenario (as well as open pond reference case) broken out by major contributions [81]

It's estimated that algae growing is about 30 times more productive than soy (and 50 times more productive than corn), but requires only 1% as much fresh water supply. It also has a much higher protein content up to 70 percent, compared with about 10 percent in corn and 40 percent in soy. In addition, algae contain also fatty acids, amino acids, pigments, and vitamins which are valuable in animal feed.] [83] By the year 2050 the world's population will reach population number of about 9.8 billion and by 2100 it will reach 11.2 billion. Several factors are associated decline of food supply and the increases in food demand due are due to the GHG are [84]:

- Change in sea levels,
- Rising temperature of the oceans result in fish migrations and population growths. The question is how we can increase food supply for growing world populations is a difficult task.
- The dropped of ocean pH which lad to migration changes of fish and marine animals.
- Other GHG effects are floods, and forest fire
- Drought [85]

In underdeveloped countries and especially in the sub-Saharan region there are concerns about erasing malnutrition, improving poverty, and achieving food security and environmental health coupled with population growth and demand for animal protein is a problem due to overfishing and the depletion of fish stocks. Consequently, we are required to develop a new method for animal and human and food supply. [86]

In underdeveloped countries and in especially in the sub-Sahara region there are concerns about erasing malnutrition, improving poverty, and achieving food security and environmental health coupled with population growth and demand for animal protein is a problem due to over fishing and the depletion fish stocks. Consequently, we are required to develop a new method for animal and human and food supply. (86)

One of the most important therapeutical productions from algae (green, brown and diatoms) is monoclonal antibody which is defined as: Monoclonal antibodies (also called mAbs) are proteins made in cell cultures in laboratories that act like proteins called antibodies in our bodies. Antibodies are parts of the immune system, mABs in medicine use are used to treat a wide range of illnesses such as:

- Cancer, Breast Cancer, Colorectal Cancer, Lung Cancer and Ovarian Cancer
- Autoimmune Diseases
- Inflammatory Diseases
- Virus infection disease such as Covid -19, hepatitis C, and Ebola.
- Virus Viral Infectious Diseases such as: Viral Hepatitis C, Ebola virous, and Covid-19 and, hematological disorder. (87)

The global monoclonal antibody therapy market size is projected to grow from $178.50 billion in 2021 to $451.89 billion in 2028 at a CAGR of 14.1%(88). One main factor contributing to the mAb financial growth is the use of mAb to treat Covid-19 epidemics.

DISCUSSION

While growing algae for capturing CO_2 due to GHE has several serious limitations such as:

- It can only absorb 10% 30% of CO_2 from air.
- High cost of construction specifically PBR, maintenance, including operation and the large area needed for cultivation is a limited factor.
- Limitation use of biofuel in cold environment which can cause "**gel up" at low temperatures.**
- **At the present time the European Union (EU) has a regulation to use and define g**enetically modified organisms (GMOs), and genetically modified micro-organisms that can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination. The EU legislation on GMOs has been in place since the early 1990s. The EU introduced specific legislation on GMOs to protect its citizens' health and the environment while simultaneously creating a unified market for biotechnology. (89) **In contrast, to the EU, the US the Food and Drug Administration (FDA) does not have a similar regulation like the EU.**
    - On the other hand, growing algae for biofuel, cosmetic products, animal and human food, due to the GHG reductions is extremely important progress.

- Production of pharmaceutical agents such as monoclonal antibodies for treatment of various diseases i.e., antivirals, antimicrobials and cancer therapeutics.
- Additional R&D is needed to increase production of mAbs and for the development of new drugs from algae for treatment of illnesses, including the
- reduction of the high cost of mABs.

CONCLUSION

We cannot ignore the economic value generated by algae cropping for food, cosmetics, natural agents and pharmaceutical drugs. Business, industry, and scientific sectors will all redefine their futures in algal terms, be it of their own motivations, or that of their competitors. The solutions for both people and planet will be found closest to the source of essentially all things organic— **photosynthesis!**

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Chapter 14. Treatment and Control of Algae—The War on Algae

by Peter Winkler

As I now suspected, there were no fish down there. The pool was dead apart from some cavorting tiddlers, only six inches long. So much for the Hindu belief that rivers are sacred, the way to new life. Those cremated remains had not been consigned to a living river but rather to a degraded ecosystem where the predominant life forms are mats of algae.

(Jeremy Wade, *River Monsters,* 2011)

Modern society views the algal life spectrum as much more of a nuisance, than anything else. That is to say, a shallow view, not even a full appreciation of, and respect for, the risks and damages that algal forms may pose to social and ecological assets, much less for the numerous benefits of healthy and balanced algal communities. Algal imbalances in ecosystems of human interest are relegated to the concept of chemical treatment, the greatest possible convenience in dealing with the problem. Underlying root causes of algal imbalances, meaning noxious species in overabundance, have a great deal of scientific understanding at this point in time. There are also profound scientific tools of inquiry and investigation available to generate solutions to perceived problems with algal imbalances on a case-by-case basis. However, it shouldn't surprise anyone that the need to shift societal perceptions and priorities is, here again, too steep of a resistance to progress when chemical companies offer the quick and convenient, hence cosmetic only approach. This would be the backdrop to my work when I assumed the position of Customer Service Manager at Integrated Lakes Management in 1997, to take up warfare against the "river monster."

There was no formal mission statement at ILM, as I recall, but if there were, it would have been "promoting and providing sustainability in water resources." ILM was, at the time that I was there, and I believe it to be to this day, a very ethical small business. Such an ideal mission, combined with the strong ethics promoted by founder and owner, Jim Bland, set the venture apart from conventional landscapers and exterminator companies—All such entities operating commercial pesticide supply and application businesses, with their respective visions of business in the real world. Sadly, idealism and ethics on the part of conscientious operators don't represent a competitive advantage in the water resources management business, making success a hard-fought prospect, with repeat customers hard to keep, due to the esthetic issues with algae being the most intractable problem. Hence, one of our major metrics of business success was the amount of product (algaecide) delivered by application to ponds and lakes, and their outflows.

The use of pesticides in water resources is different than in other sectors, such as food production, habitation protection, and disease management. Pesticide usage in aquatic ecosystems, including algaecides, is almost entirely to serve an aesthetic sense of beauty in the landscape and the

convenience of clear waterways for boating sportspersons. The use of algaecides along with aquatic herbicides, is practiced as an art as much as a science by aquatics managers. Lake management professional associations are conduits for exchange of management knowledge in materials and methods. Advertising of products along with programs of development of methods of application of products continues to assure that chemicals are a cornerstone of IPM (integrated pest management) planning in aquatic algal management. Everyone who worked a full time position at ILM understood, scientifically of course, they were degreed and committed to scientific quality, that our industry was largely treating symptoms and not the root causes of aquatic ecology degradations. Meaning that the landscape that we were working to promote and sustain in terms of the perceived aesthetic, was being prodded, chemically speaking, into a downward spiral of degradations of ecosystem quality, including populations of fish, aquatic invertebrates, rooted aquatic macrophytes (including many beautiful flowering plants such as the lotus and water lily, and, curiously and poignantly, the planktonic aquatics including many seasonal native species of phytoplanktonic algae, the natural base of the open water food chains. The corporate business disconnect with sustainability was obvious to the scientific mind, and we were uncomfortable with it, and only infrequently discussed it, but a few of the discussions included Jim Bland. In the business model we operated on a contingency basis, and there was always a sense of urgency to deliver on behalf of the customers, who always liked to see crisis management in full swing. Boatloads of chemicals on-site, and subsequent clearing of algae-fouled water is a dramatic and tangible result, with a good sales and business outcome.

Discussions with customers about algaecide-based approaches to problems was pursued as an ethical mission of ILM, including the ownership. Risks of ecosystem damages and side-effects were routinely related to customers and sales prospects. Over the 2 and a half years that I was there, a number of customers were receptive to alternatives, and we did applications of barley and wheat straw bales, water aerator installations, alum precipitations, and bacterial applications on a couple of dozen ponds with mixed results (discussion of alternative treatments later in this chapter). Any scientific evaluation of the mixed results was difficult due to a lack of controls and to the fact that all of the managed ponds had histories and prior side-effects. The fact that alternative approaches, which were only conditionally proven to work, were purchased by customers is a great testament to a human need to feel natural and non-toxic in the relationship to the environment. This was a great ideal to promote and believe to be ethical, but ILM was a business that would ultimately have to meet the needs of customers; where the owner professed that he didn't need to give out pay raises because he could get people to work for free, since he could always have internships under the aegis of "help the environment." This is a very important point here, scientifically speaking. If you can't hire and retain the quality of people, who can apply discipline and insight to safety, and over the course of on-going study and observation, be able to discern and react to the early signs of degradations in aquatic ecosystems, disasters would be unavoidable.

After the great soaring flight of the spirit in compassion for all the lesser creatures of the water world, as experienced by aquatic ecologists of all sorts, the hard realities of customers phoning in complaints about the algae fouling their ponds assured the emphasis of chemicals in IPM evaluations (integrated pest management, a science based approach). Ass-chewings from Jim would wear down the any sense of ideals over time, and a need to protect your job by taking immediate action on any algal problem made the nozzle handle feel very reassuring in the hand. I was the tenth service manager hired at ILM in 11 years of its existence in the summer of 1997, when I joined the company. As the facts of working life at ILM, and Jim Bland's management style, both slowly grew into well formed images going into 1998, I was, none-the-less undaunted in my resolve to make my role successful. Indeed, my job at ILM was my favorite ever, at that time and even now as a memory. Because I did it all in some way or another. Even wearing the chemical engineering hat, having designed boat mounted, pumped, mixing and delivery systems exceeding anything that ILM had historically deployed. There was substantial pressure on employees, and management in particular to sell and deliver product with decisive outcomes.

We/ILM had the 200 gallon vat in the shop, which received the 50 lb. bags of copper sulfate and citric acid to provide for the bulk stream of algaecide product, an icon of corporate pride. Delivery of product, or active agent, to aquatic habitats was metered to provide a 1 ppm dose to nuisance algae. The target dose was a mean average, not spread evenly by way of application alone, but assisted by currents in its distribution to target plant life. A good application requires not only a dose calculation based on water habitat volume, but also as accurate an assessment of currents as possible. An ability to assess and respond, like a doctor modifying a treatment regimen for a patient, is essential to good results. My growth in abilities to assess in both the short and the long term was highly satisfying to me professionally. The diminishment of scientific council in sales and customer decisions was a growing disappointment and dissatisfaction for the long term prospects of the aquatic resources under our care.

With sustainability in mind it serves the practitioner well to thoroughly consider the safety and hazard features of the chemical controls. The most fundamental step in doing so is to read the algaecide product label information. Hazards to people and the environment, as well as safe usage and disposal, are detailed on product labels.

To be continued...

Chapter 12. Harmful Algal Blooms, Algal Toxins, and Human Health and Medical Implications

HARMFUL ALGAL BLOOMS (HABs) AND THEIR TOXINS [i]

Algae are one of the most important organisms on the planet Earth. Normally they are the best friends of ponds, lakes, oceans, ponds, rivers and places on land as is the case with lichens (a structure where algae and fungus live together such as on rocks and trees) all because they supply nutrients and oxygen to zooplankton (little animals such as copepods that eat algae known as phytoplankton: see Fig 12-1), amphibians, fish and other organisms. In other words, they are at the base of aquatic food chains. Put another way algae makes life on earth possible because of base nutrition and the production of oxygen.[ii]

What are algae? Algae are mainly a large and diverse group of eukaryotic and prokaryotic aquatic organisms where many photosynthesize but not all. The Eukaryotic algae include the flagellated forms such as dinoflagellates, certain green algae, the Diatoms, Euglenoid types, Seaweeds such the large Kelps, and other types. The prokaryotic algae formerly called Bluegreen algae are bacteria, so their new collective name is Cyanobacteria (Cyan is greenish blue). The cyanobacteria can appear cyan or other colors such as red, yellow, black, or even red. They also photosynthesize like plants do.

[iii]

Fig 12-1 Copepods are the most abundant animal on earth. The structures that look like fins are egg sacs from the females. They are the quintessential example of zooplankton. Photos by Andrei Savitsky.

In this chapter I discuss algal blooms ,give a brief description of the major groups of algae, their reproduction and a detailed discussion of diseases caused by their toxins in humans. Many algae are also involved in animal deaths. This chapter mainly deals with human toxicities.

Fig 12-2 Photo of an algal bloom. [iv]

What are harmful algal blooms (HABs) and why are they discussed in this book? Algal blooms are defined as rapid increases in a population of algae. There may be over one million cells per liter of water (1,000,000 or 10^6 expressed in scientific notation). Blooms can appear in lakes, rivers, and oceans. These blooms are often referred as "Red Tides" because of the colors of the algae but many blooms do not appear red in color.

The reason why I cover this topic in this book is because blooms may be caused by climate change, and they can cause lots of damage to aquatic and other organisms including humans. HABs cause millions of dollars every year. The damage includes many socioeconomic, ecosystem and human health issues.

Socioeconomic issues include loss of scallops, Alaskan shellfish, fish, commercial wild shellfish industry, losses to watermen such as fishers, seafood dealers and seafood restaurants and more aquaculture losses occur due to certain algae such as the diatom ***Chaetoceros*** which caused mortality in many salmon due to the algae lodging in their gills causing mucus production, suffocation and death. [v]

Ecosystem damage is caused by certain species that outcompete, overgrow which reduce light penetration to benthic (bottom) communities and may result in low oxygen (hypoxia).[vi]

Many species of algae produce toxins. Toxins are secondary metabolites. Primary metabolites are metabolites which are needed for reproduction and growth whereas secondary metabolites are those not involved in reproduction and growth such as antibiotics, pigments and toxins.

Toxins associated with algal blooms are Hepatotoxins (toxic to the liver), Neurotoxins (Nerve toxins), skin toxins and intestinal toxins. It should be pointed out that different species of algae can produce the same toxins**.**

Toxic algae diseases discussed in this chapter are the following:

Amnesiac shellfish poisoning
Paralytic shellfish poisoning
Diarrhoeic shellfish poisoning
Neurologic shellfish poisoning
Azaspiracid shellfish poisoning
Palytoxin poisoning
Microcystin poisoning
Palm Island Mystery
Pfiesteriosis
Ciguatera poisoning

Blooms occur when the following conditions are met: increases in phosphorous, nitrogen, other plant nutrients, and others which runoff from agriculture and industrial activities into the waters, temperature increases, pH changes and increases in solar energy (sunlight).[vii]

A little ecology about the three major groups that produce toxins while they bloom are included below because even though this is not a biology book per se we must give the reader some information regarding these organisms.

This chapter will cover the major groups of algae that result in HABs. The three major groups of algae are the following: The **Cyanobacteria** (bacteria like prokaryotes), the **diatoms** and the **dinoflagellates** both eukaryotic**).**

THE MAJOR BLOOM FORMING ALGAE GROUPS

CYANOBACTERIA

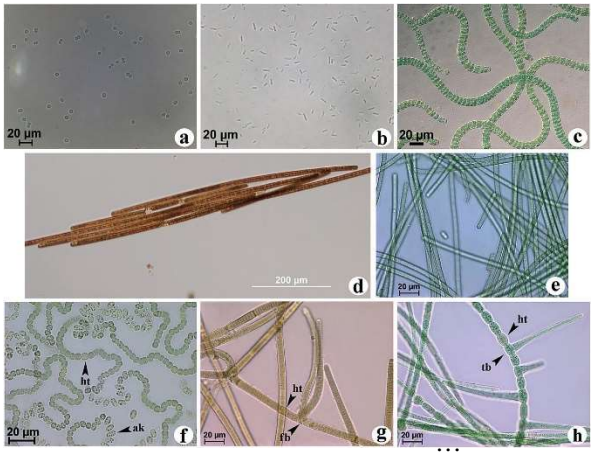


Fig 12-3 Photos of Cyanobacteria[viii]

Photos by Alberto A. Esteves-Ferreira, João Henrique Frota Cavalcanti, Marcelo Gomes Marçal Vieira Vaz, Luna V. Alvarenga, Adriano Nunes-Nesi and Wagner L. Araújo.

These are a group of prokaryotic photosynthetic organisms which fix nitrogen and live in a variety of ecosystems from soils, water and as symbionts with other organisms such as with lichens. One of their most prominent claims to fame is the fact that the chloroplasts of plants are cyanobacteria which contributed to the origin of plants.[ix]

Cyanobacteria could either be filamentous, single cell, or colonial living in a mucilaginous matrix. Many are involved with toxic algal blooms and cause algal diseases which are discussed in detail in this chapter. See Fig 12.3.

It should also be pointed out that warmer temperatures make good conditions for the cyanobacteria to multiply like crazy causing toxic algal blooms. Below is a photo of ***Microcystis aeruginosa***, the producer of the toxin, Microcystin, which is a very potent hepatotoxin (effects the liver) and might cause cancer.

Fig 12.4 *Microcystis aeruginosa*. Photo by David Arieti

The general mode of cyanobacterial reproduction is as follows:
The cyanobacteria grow when temperatures increase. Vegetative cells for long chains and float near the surface. When the bloom expands nutrients are depleted. When nutrients are depleted specialized cells called heterocysts are formed. These ***heterocysts*** can fix the nitrogen from the air into a type of nutrient. There are about 80 species out of the thousands that cause toxic blooms. Algal toxins can be classified as being hepatotoxins (liver toxins), neurotoxins (nerve toxins), gastrointestinal and skin toxins.

When temperatures fall in the autumn there is less energy for them, thus they form a type of cell called an akinete. The akinetes sink to the bottom and remain in the sediments for long time periods. When

environmental conditions are right the cells will grow. The cells produce gas vacuoles and float to the surface where they will photosynthesize, and the colony grows.[x]

DIATOMS

Diatoms are unique forms of algae that have capsules made from silicon called frustules (glass houses: see fig 12-5). They come in all shapes including round ones (pennate) and circular ones called (centric). They are all Eukaryotic. You might say that they resemble petri dishes used to grow bacteria. They can live virtually everywhere whether it be soils, streams, lakes, rivers and oceans. They account for 40% of photosynthesis on the planet thus are very important for those of us who like to breathe oxygen.

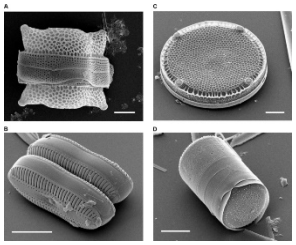


Fig 12-5 Examples of diatoms with their frustules[xi].These photos were made with the Scanning Electron Microscope (SEM). Photos by May Ann Tiffany, San Diego State University

Reproduction of diatoms is generally by asexual means. Some diatoms such as ***Pseudo-nitzschia*** sp form long chains. Two cells are produced each genetically identical but one is slightly smaller. Eventually this results in a reduction of population. When the diatom reaches about half its original size after many divisions it reproduces sexually where two parent cells align and produce gametes (sex cells). Gametes meet and fuse. The fused gametes form an auxospore. They protect the developing ***Pseudo-nitzschia*** cell until it is fully grown.[xii]

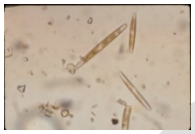


Fig 12-6 Typical diatoms. Photo by David Arieti

Fig 12-7 The Diatom , *Cymbella hantzschiana*.[xiii]

DINOFLAGELLATES

The dinoflagellates are a major eukaryotic algal group containing close to 2000 known species. These algae have characteristics unique to this group. They have two flagella, one is trailing like a sperm cell

and the other is transverse, coming from the side. Dinoflagellates also have a unique type of chromosome called dinokaryons in that they lack histones (protein structures which are in chromosomes) which are in most other known Eukaryotic organisms including protists.

Some species are known for their bluish glow called bioluminescence. It is thought that there may be a few hundred toxins produced by dinoflagellates. Dinoflagellates also produce substances which also have therapeutic effects such as being antiviral, antibacterial and antioxidant activity and their toxins although only around 80 species produce toxins. They are unique in that many species are both photosynthetic and heterotrophic (they eat). The main types of illnesses by dinoflagellates are respiratory paralysis and gastrointestinal problems such as diarrhetic poisoning.

Reproduction of the dinoflagellates is described as follows: Cysts of dinoflagellates lay dormant buried in sediment until environmental conditions are just right for them to germinate (grow). Swimming cells will then emerge from the germinating cysts. When abundant nutrients are present the cells will reproduce exponentially. One cell can divide many times. Within a week there will be many. When conditions are no longer right growth stops and gametes are formed.

Two gametes fuse and form a motile zygotic, the planozygote. The zygote's wall will thicken and it becomes a cyst which may stay for long periods of time on the sea floor germination and start the whole cycle again.

Fig 12-8 Two species of Dinoflagellates: *Peridinium gatunense* (left) and ***Ceratium hirundinella*-Photo by David Arieti**

Fig 12-9 Toxic algae bloom in Lake Erie Photo by Jesse Allen and Robert Simon[xiv]

ALGAL DISEASES

Amnesiac shellfish Poisoning

Domoic Acid (DA)

Amnesic Shellfish Poisoning is also known as Domoic Acid Poisoning because it is caused by domoic acid. It can be a life-threatening syndrome manifested by neurologic and gastrointestinal symptoms. After these initial symptoms, some patients may develop dementia. It was initially reported in Cardigan in Prince Edward Island in Canada, but now it is an ongoing problem in the state of Washington and Oregon as well as other parts of the world.

CASES / OUTBREAKS

EUROPE

Belgium - In 2000 and 2001, there were 151 and 154 samples respectively tested for ASP, but no toxic events were detected in shellfish (10).

Denmark - There were several investigations done in Denmark that showed a diatom species (***Pseudo-nitzchia. seriata***) was present in colder areas of Northern Hemisphere that produced domoic acid (DA) (10). Other species of ***Pseudo-nitzschia*** were tested for DA. In 1993, there was algae bloom caused by these types of diatoms. DA was detected but in low concentrations.

France - In May 2000, a bivalve (***Donax trunculus***) was tested with DA levels above the regulatory limit. The causative diatoms were the Pseudo-nitzschia types. There was one episode reported in April 2002, but the toxin level was low (10).

Ireland - In December 1999, high concentration of DA up to 3,000ug/g was found in the scallops hepatopancreas on every Irish coast. In 2000 and 2001, the ASP toxin were again detected above regulatory limits in scallops. In 2002, for the first time, ASA toxin was detected in mussels (10).

Italy - In 2002, DA was detected at levels above regulatory limit in the scallop (Pecten maximus) (10).

The Netherlands - In the Dutch Wadden Sea, diatoms of the species Pseudo-nitzschia were detected between November 1993 and July 1994 although there was no shellfish poisoning recorded (10).

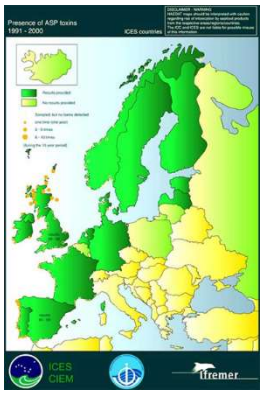
Norway - In 2000 and 2001, mussels and scallop were found to have DA but not above regulatory limits (10).

Portugal – In 1996, DA was detected in almost every species of bivalves around the Portuguese coast. In 1999, DA was detected in 960 samples (10). DA in shellfish are detected several times a year, mainly in the spring and autumn months. Levels recorded can be twice as high as the accepted limits and are not unusual. This DA was also detected in other shellfish such as oysters, furrow shells, razor clams, and sardines. However, in sardines, the DA was restricted in the gut and not on the muscle tissue.

Spain - The first detected DA was in mussels in October 1994. These mussels came from Galicia in northwestern Spain. From September to December 1995 and 1996, DA was detected in scallops. In 2002, there were toxin episodes in Galicia and Andalucía (10). Due to the presence of diatoms Pseudo-nitzchia, the production area was closed.

The United Kingdom of Great Britain and Northern Ireland - In July 1999, on the west coast of Scotland, a scallop harvesting area of 8,000 square miles was closed due to the presence of ASP toxin. In 2000 and 2001, ASP toxin were again detected above the accepted limit among the scallops, restricting fishing in the affected areas (10). In Northern Ireland, scallops were found to contain ASP toxin above regulatory limits. The United Kingdom Food Standards Agency banned scallop fishing in the affected areas.

Figure 12-10: Occurrence of ASP toxins in coastal waters of European ICES countries from 1991 to 2000



Source: Food and Agriculture Organization of the United Nation, 2004, Marine Biotoxin, Amnesic Shellfish Poisoning,

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NORTH AMERICA

Canada - Canada is where the first ASP was reported in 1987. During November and December 1987, there was an outbreak in the Cardigan Region of Eastern Prince Edward Island. There were 3 deaths and 108 cases of acute intoxication after eating blue mussels. The toxin was identified as DA and the diatoms as the ***Pseudo-nitzschia*** species. In the years after 1987, algae blooms happened but were less extensive. Since 1988, phytoplankton samples have been collected in 4 stations in the western Bay of Fundy. Usually, algae blooms occurred in late summer. The highest concentrations were recorded in 1988 and 1995 during late August and early September, leading to the closure of harvesting areas (10).

United States of America

Alaska - There was no problem with ASP even though toxic ***Pseudo-nitzschia*** species had been identified. In 1992, approximately 3,000 samples of commercially valuable shellfish and finfish were tested; the highest value was 11.1 mg/g 17 samples above the regulatory level (10).

West Coast - In October and November 1991, razor clams from the surfing zone on Pacific coast beaches in Washington and Oregon contained DA with levels as high as 154 mg / g, triggering a ban on clam harvesting In Washington state, there were 24 cases of reported illness with gastrointestinal symptoms and one with memory loss.. In the autumn of 1994 in Hood Canal in western Washington, there was bloom of ***Pseudo-nitzschia*** that lasted for 6 weeks (10). Shellfish tested for DA contained less than the regulatory limit. In Penn Cove, Washington, a bloom of the same species occurred, but the level of DA was less than the limit.

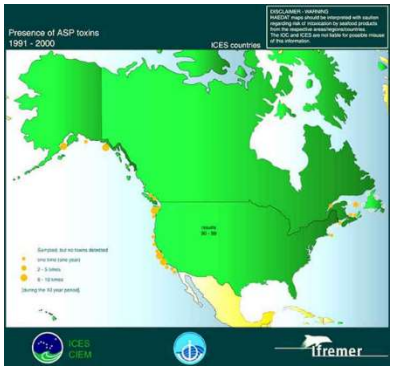
In the autumn of 1991, several species of ***Pseudo-nitzschia*** were detected in Monterey Bay, California but there was no ASP reported (10).

In May and June 1998, over 400 California sea lions (Zalophus californianus) died along the central California coast. The death was believed to be caused by DA produced by P. australis. In April and May 2002, many marine mammals and birds were dying along the California coast. About 70 dolphins, more than 200 sea lions, and 200 seabirds died (10). No human illness was reported.

East Coast - In January and February 1991, DA producing diatoms were isolated along the Nantucket coast in Massachusetts, but the levels were half of the regulatory limits (10). A multi year study in Louisiana showed the presence of ***Pseudo-nitzschia*** species in the water, but there were no reported cases of ASP.

Gulf of Mexico - Except for isolated cases, there is no direct evidence of ASP toxin in the shellfish in the Gulf of Mexico. Therefore, ASP is not considered a public health hazard in this area.

Figure 12-11: Occurrence of ASP toxins in coastal waters of North American ICES countries from 1991 to 2000



Source: Food and Agriculture Organization of the United Nation, 2004, Marine Biotoxin, Amnesic Shellfish Poisoning,
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http://www.ifremer.fr/envlit/documentation/dossiers/ciem/aindex.htm

CENTRAL AND SOUTH AMERICA

Argentina - In the winter of 2000, two massive mortality episodes of seabirds were reported in Mar de Plata (10). The dominant species was P. australis and the toxin were identified in mussels.

Chile - Since 1967, shellfish samples have detected DA levels exceeding the regulatory limit. Up until 2001, there had been no ASP intoxications recorded (10).

Mexico - 150 dead brown pelicans were found in Cabo San Lucas on the tip of Baja California Peninsula in January 1996 (10). These birds were fed with mackerel (*Scomber japonicus*) with DA from diatoms.

During January and February 1997, many marine organisms died in the Gulf of California. They were 766 common loons (***Gavia immer***) and 182 sea mammals (10). In their examination, they found DA and its isomers in some the stomach of some dolphins.

Asia

Japan - ASP toxin screening has been implemented in Japan since 1991. DA had not been detected except in August 1994 when there was a red tide in Hiroshima bay and DA was detected in a few diatoms of ***Pseudo-nitzshcia*** species (10).

OCEANA

Australia – A wide taxonomic survey was carried out on the potentially toxic diatom ***Pseudo-nitzschia.*** The dominant diatoms of this species were not toxic.. There was no P. australis identified. Along the coast of Tasmania and Victoria, cultured diatoms were of the non-toxic type.

In New Zealand during the summer of 1992 and 1993, DA was detected at low levels from the diatom samples in the Bay of Islands, the Hauraki Gulf, and Bay of Plenty. The highest level of DA was found in early 2003 (10). These were found in green mussels from Marlborough Sound, scallops from Tauranga Harbor and Whangaroa Bay.

CAUSATIVE ORGANISMS

The causative toxin domoic acid (DA) is produced by the diatom of ***Pseudo-nitzschia*** species and by a seweed , ***Chondria armata***. These species are widely distributed around the word and are: ***Pseudo-nitzchia multiseries, , P. australis, P. delicatissima, P. pungens, P. seriata, P. multiseriata, P. turgidula, and P. fraudulenta***. These diatoms are consumed by different shellfish, most notably by mussels and scallops which are eventually harvested for human consumption.

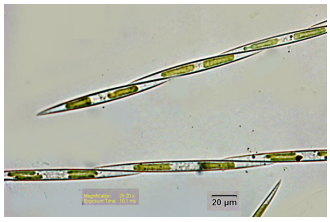


Fig 12-12- Pseudonitzchia fraudulenta[xv]

Fig 12-13-Chondria sp.[xvi]

MECHANISM OF ACTION

Domoic acid is an amino acid like glutamate, the body's most prominent excitatory neurotransmitter in the nervous system. It has been noted that the affinity of domoic acid to glutamate receptors (a-amino-5-methyl-3-hydroxyisoxazolone-4-proprionate - AMPA) in the brain is 100 times more than glutamate. The presence of domoic acid increases activation of these receptors causing increased brain activity that can precipitate seizures and damage the amygdala and hippocampus of the limbic system. Aside from these, activation of AMPA increases calcium into the brain cells. This can cause toxicity to the brain cell by signaling apoptosis. The hippocampus which plays a role in memory and is particularly at risk due to its abundance of glutamate receptors. This the reason why patients can develop amnesia after having the illness. (11)

Domoic acid is heat stable and will not be destroyed by cooking of freezing. As little as 1.9mg/g concentration of domoic acid can cause CNS toxicity in humans. This level is of concern since some muscles of the shellfish contain as much as 128 mg of domoic acid per 100-gram tissue. (11)

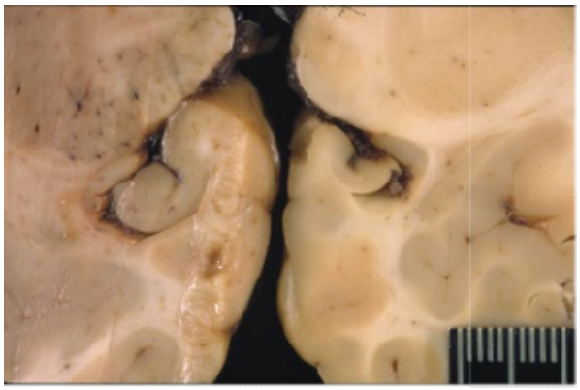
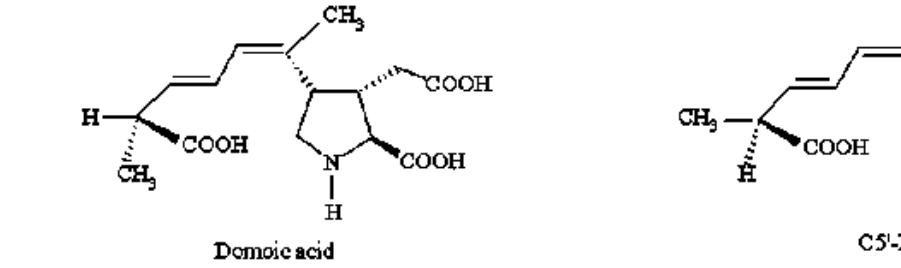
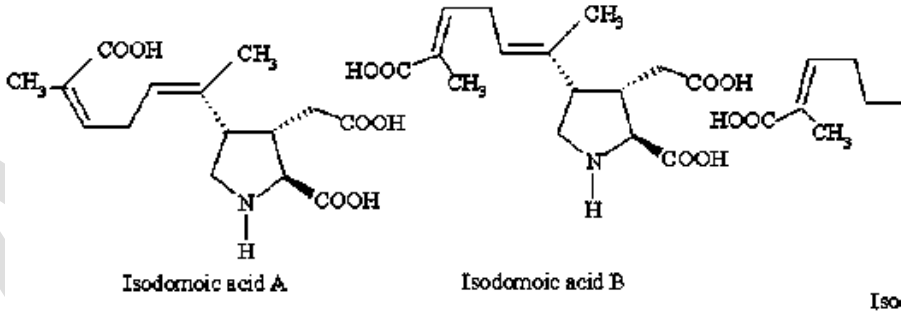
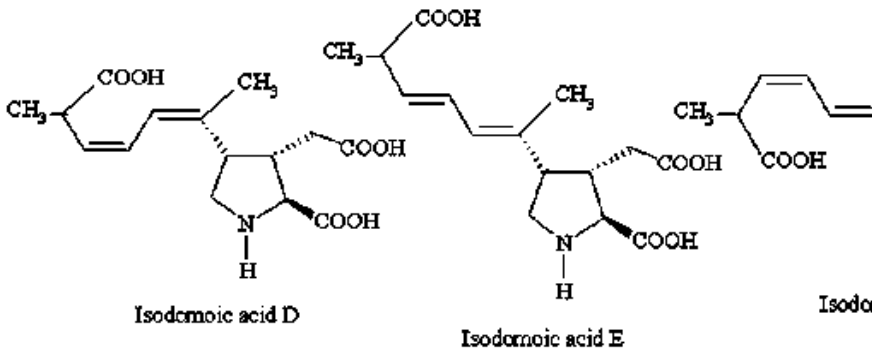
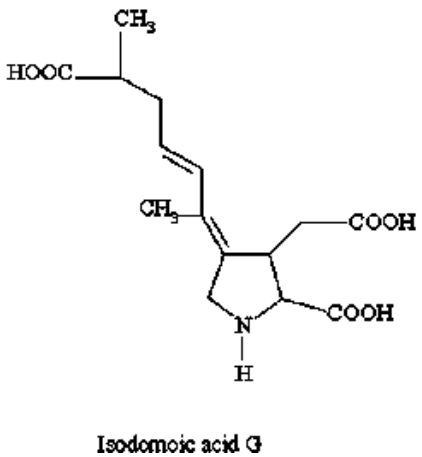


Fig 12-14 Hippocmpus of sea lions. Left:Normal California Sea Lion brain-
Right: California sea lion brain affected by domoic acid exposure.[xvii] Used with permission from the Marine Mammal Center.









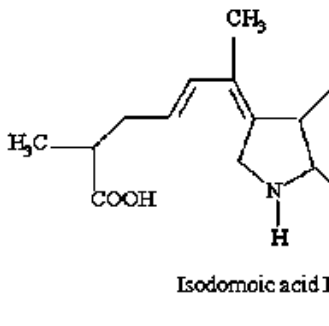


Figure 12-15: Chemical structures of domoic acid and its isomers[xviii]

Source: Food and Agriculture Organization of the United Nation, 2004, Marine Biotoxin, Amnesic Shellfish Poisoning,
xix

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CLINICAL MANIFESTATIONS

Patients who have taken seafood such as mussel, and clams contaminated with ASP may present with headache, abdominal pain, cramping, nausea, vomiting, and diarrhea which may start 30 minutes to 24 hours after intake. These symptoms are not that serious and can be managed readily by symptomatic treatment. Since domoic acid is like glutamic acid, central nervous system toxicity is a major concern. A patient with more toxicity can progress to memory loss, hyporeflexia, hemiparesis, ophthalmoplegia, agitation, seizure, coma, or shock over the next 48 hours. Permanent cognitive dysfunction can occur in older patients (older than 60) and in younger patient with pre-existing illness like diabetes, chronic renal disease, and stroke.

The frequencies of symptoms were reported as follows: vomiting (76%), abdominal cramps (50%), diarrhea (4%), severe headache (43%), and loss of memory (25%) (12).

There was a study done on 14 patients with severe neurologic complications (Teitlebaum et al., 1990): Neurophysiological testing was performed on these patients several months after the acute episodes.

12/14 developed severe antegrade memory deficits with relative preservation of cognitive functions.
11/14 had clinical and electromyographic evidence of pure motor or combined motor and sensory neuropathy.
4/14 showed decreased glucose metabolism in the medial temporal lobes by PET scan.

All 14 patients developed confusion and disorientation within 1.5 to 48 hours after consumption. Acute coma was associated with the slowest recovery while seizures became more frequent up to 2 months but ceased by 4 months.
For those 4 fatalities, their brain revealed necrosis and loss predominantly in the hippocampus and amygdala. (12)

DIAGNOSIS

Since hospitals and clinics do not have laboratory assay for ASP toxin, diagnosis is based on clinical manifestation and history. Presented with these arrays of common clinical manifestations, a practicing physician will find it hard to consider ASP. He has to think of many diseases and conditions with the same manifestation. The only probable clue pointing to poisoning is a history of intake of shellfish within minutes or to a few hours. More so if there is an outbreak of ASP.

Since the concern of this type of poisoning are the neurologic deficits, laboratory and ancillary procedures are geared toward the brain. A CT scan, EEG, or MRI can be requested to determine abnormalities in the brain, specifically in the hippocampus and **amygdala.**

For any suspected ASP, a sample of the food must be obtained, frozen, and sent to the Department of Health.
Currently, there are no private laboratories offering domoic acid testing.

- The amnesia in ASD can be differentiated from Alzheimer's disease due to its relative preservation of intellect and higher cortical function. The lack of confabulation with preserved frontal function will distinguish ASP from Korsakoff syndrome.

TREATMENT

There is no clear consensus for the treatment of ASP. The management is more symptomatic or supportive. Antipyretic for fever, antiemetic for nausea and vomiting, oral hydration fluid or intravenous fluid for diarrhea. Patients with seizures are given phenobarbital and diazepam. If patient is resistant to benzodiazepines, propofol or barbiturates can be given as adjunct therapy.
For amnesia, some treatments include cognitive therapy, neurofeedback, and nutritional supplements.

There is no antidote available for domoic acid toxicity.

There are animal studies that pyridoxine (vit B6) decreases the level of domoic acid.

Like any other poisoning, a case must not be considered isolated as this maybe
be followed by an outbreak. Report the case to the health authorities and proper follow up must be done to prevent spread. Every effort must be made to obtain the contaminated source for laboratory testing.

REGULATION AND MONITORING

EUROPE:

Member states in the European Union set 20 mg/g as the regulatory limit. Samples with more than this value are destroyed. (10)

Denmark - Shellfish monitoring was initiated in 1993. (10)

Ireland - A Biotoxin Monitoring Program started in 1984. Initially, it was done on DSP poisoning but now it includes domoic acid and azaspiracid poisoning. They have weekly shellfish testing using mouse bioassay and reports are sent to regulatory agencies, health officials, shellfish producers and processors by web-based communications (10).

NORTH AMERICA

Canada - Regulation started in 1988 with 20 mg/ kg of mussel as the regulatory limit. Harvesting areas are closed once the toxic level exceeds the regulatory limit.
Phytoplankton monitoring is also done at 4 stations in the Western Bay of Fundy
(10).

United States - There is a non-official guideline of 20 mg/ kg of bivalves. For cooked crabs, the guideline is set at 30 mg/kg. Closure is done once the level of 20 mg/kg is reached. Exported shellfish are accompanied by health certificates (10).

Central and South America

Argentina - Argentina has a national monitoring program for mussel toxicity. regional laboratories are in each coastal province and there is one fixed station in Mar de Plata (10).

Brazil - Had a pilot monitoring in for one year but a national monitoring program has not been initiated (10).

Chile - Chile has a national monitoring program for shellfish and phytoplankton (10).

Uruguay - Regularly monitors mussel toxicity and phytoplankton (10).

Oceana

Australia - Monitoring and regulation on mussels and algae were initiated in 1993.
(10)

New Zealand - Monitoring for shellfish started in 1993 and their guideline limit is also 20 mg of DA / kg of shellfish meat. The New Zealand Biotoxin Program monitors shellfish and phytoplankton using mouse assay (10).

PARALYTIC SHELLFISH POISONING (PSP)

Paralytic Shellfish Poisoning (PSP) is a food borne disease acquired by eating shellfish contaminated with diatoms, dinoflagellate, and toxic algae. These shellfish are clams, mussels, oysters, geoduck and scallops. In crabs, the toxin is found in the guts of crabs but not on the crab meat. If you do not want to suffer from PSP, remove the guts before cooking the crab and do not drink the broth.

There are many organisms that produce saxitoxin. The majority are either dinoflagellates or cyanobacteria

Table 12-1 List of the organisms that produce Saxitoxin

NAME OF TOXIN	GENUS	GROUP	TOXIN TYPE
SAXITOXIN (STX)[xx]	Alexandrium sp …[xxi]..	Dinophyceae	Neurotoxin
	Gymnodinium sp	Dinophyceae	
	Anabaena spp	Cyanophyceae	
	Cylindrospermiopsis[xxii] sp	Cyanophyceae	
	Lyngbya sp	Cyanophyceae	
	Planktothrix	Cyanophyceae	
	Pyrodinium bahamense	Dinophyceae	
	Aphanizomenon sp	Cyanophceae	
	Gessnerium monilatum.		

This is a photo of one of the many organisms that produce Saxitoxin

Fig 12-16
***Alexandrium ostenfeldi*. Photo by Nancy Lewis.**[xxiii]

Viewed 16 May 2020.

On June 5, 1990, in on the Island of Nantucket, Massachusetts, six fishermen developed an illness after eating blue mussels ,***Mytilus edulis*** - harvested in deep water (1). They manifested the following symptoms 1-2 hours after eating the shellfish: numbness of the mouth, tongue, throat, face with perioral edema and paresthesia of the extremities. These symptoms lasted for 14 hours. Low back pain was also noticed, which started 24 hours after the onset of neurologic symptoms and lasted for 3.3 days. Out of the six fishermen, four recovered right away but two were hospitalized, one of which lost consciousness but was discharged after 3 days.

OUTBREAKS OF PSP

There is an increasing distribution around the globe of paralytic shellfish poisoning (Hallegraeff,1993). In 1970, PSP was well known from temperate waters of Europe, North America, and Japan. Arriving in 1990, PSP was documented in South Africa, Australia, India, Thailand, Brunei Darussalam, Malaysia, Philippines and Papua New Guinea. (1)

Figure 12-17: Occurrence of PSP toxins in coastal waters of European ICES countries from 1991 to 2000[xxiv]

Source: Food and Agriculture Organization of the United Nation, 2004, Paralytic Shellfish Poisoning Reproduced with permission

Countries with PSP outbreaks:

Denmark - At the east coast of Jutland, PSP episodes in 1987, 1990, 1996, 1997 (1)

France - Atlantic coast in 1992, PSP toxin was found in mussels. In 1998, toxin was found on (in?) clams, oysters, mussels and their productions were closed for two months. In 2000, two in Britanny was closed (1)

Germany --- In 1972, mussels were monitored for toxins. In 1987, 3 cases of PSP poisoning were reported in Lower Saxonia. In March 1992, PSP producing algae has been isolated.

Ireland - In July 1992, PSP was noted in Cork Harbour and persisted for one week.. In 1999, A. tamarensis was detected and in 2002 PSP toxins were above the limit in mussels, oysters. (1)

Italy - HAB had been recurring in the Adriatic coast but there have been no reported cases of PSP. Monitoring station in Emilia Romagna in1994 to 1996 had greater than 80 ug / 100 gram of mussels' meat but no public health case was reported (1)

The Netherlands -- In the North Sea in 1989, PSP producing algae of Alexandrium sp. was identified but no PSP cases were found. (1)

Norway --- Among the Scandinavian countries, Norway has the earliest recorded cases of PSP. They have recorded PSP seven times (1901, 1939, 1959, 1979, 1991, 1992) with a total of 32 victims with 2 deaths. Surveillance conducted in 1994 led to closures of several fishing Communities. (1)

Portugal --- Along the north coast of Roca Cape, there were occurrence of PSP from 1996 to 1990. In 1992, there were cases of PSP along the south coast of Lisbon and coast of Algarve with a concentration of 100-500 ug / 100 gram of shellfish meat. In October 1994, PSP was reported among 9 patients (6 women and 3 men) after eating mollusks (Mytilus edulis) from the west coast of Portugal. (1)

Spain --- Among the European countries, Spain is the country most concerned with PSP because mussel aquaculture is an important industry in the Northern Atlantic coast of Galician Rias. In 1976, several countries in Europe including Germany, France, Switzerland, and Italy developed PSP after consuming mussels exported from Spain. There were a total of 120 people affected in Western Europe; however, there was no mortality. In 1993, this unfortunate episode lasted for an unusually longer period. They found out that the culprit was the Dinoflagellate (Alexandrium catenatum) that contaminated the mussels. In 1994,

there were outbreaks along the Atlantic shore of Spain. Beginning in 2000, because of the toxic events, the harvesting of bivalves has been prohibited in the area of Galicia. In 2002, production of scallops were closed due to the presence of G.catenatum. (1)

Sweden --- In Sweden, contamination of mussels usually occurs at the end of spring and beginning of summer. In 1985 and 1988, PSP toxin has been detected in mollusk meat. (1)

United Kingdom of Great Britain and Northern Ireland

The initial cases of PSP poisoning were reported in 1968, along the Northern coast of England. There were 78 people hospitalized but no mortality. From 1978 to 1980, sporadic toxic events were reported along the Northern coast, spreading toward Scotland. In August 2001, the United Kingdom Standard Agency prohibited scallop fishing along the sea adjacent to the Northern Ireland. (1)

Morocco - From October to November 1994, HAB was associated with PSP outbreaks. In November 1994, PSP toxin was showed 6000 ug eq/ 100 gram of mollusk meat. (1)

South Africa -- In 1969, six cases of PSP poisoning were reported and 17 cases in 1979. There were two fatalities in 1984. (1)

Tunisia --- In 1998, more than 700 tons of cultured sea bass and sea bream and several species of wild fish were found dead along the lagoons of Burger and Gar el Melh. (1)

Fig. 12-18: Occurrence of PSP in North America
Source: Food and Agriculture Organization of the United Nation, 2004, Paralytic Shellfish Poisoning
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Canada --- In 1993, 4 crewmen of from an expedition became sick and one died after consuming shellfish in the water of British Columbia. PSP has been documented as early as 1880 in St. Lawrence Estuary (Quebec) and the Bay of Fundy (between New Brunswick and Nova Scotia). Overall, from 1880 to 1995, there were 106 incidents reported with 538 cases and 32 deaths. In May 1999, two workers of Salmon farm in Herbert Inlet, British Columbia developed symptoms after consuming wild scallops. (1)

United States -- East Coast

In On the east coast, he first reported case of PSP was in the coast of Maine near the Canadian border in 1958. In 1972, there was HAB along the coast of Maine, New Hampshire, and Massachusetts cause PSP in 33 people,

however no one died – part of a sentence?. In 1979, mussel beds were closed Narragansett Bay, Rhode Island. In the following years, there were reports of PSP in small embayment in Connecticut and Long Island. (1)

From 1973 - 1987, the Massachusetts state health department reported 19 outbreaks. These were caused by the consumption of mussels, clams, oysters, scallops and cockles. In June 1990, the the same Health Department documented PSP among 6 fishermen in a fishing boat in the George Bank area off the Nantucket coast. The ate cooked blue mussels (Mytilus edulis) obtained in the deep water in by the island of Nantucket. Other areas in on the east coast with occasional PSP outbreaks? are in Long Island (New York), New Jersey, and Connecticut. Since January 2002, in Titusville, Florida 10 cases of PSP were reported. (2)

In June 26, 1990, Alaska Department of Health and Social Services (ADHSS) has documented that an Alaskan man died after eating shellfish (2). He took 25-30 steamed butter clams and 2 teaspoon of butter clam broth. He developed cardiorespiratory arrest two hours later and eventually died despite resuscitative measures. His stomach contained 370 ug/100 g of toxin (maximum safe level is 80 ug/ 100 g). The butter clam broth he drank contained 2650 ug /100 g. Two other companions had shared the butter clam broth. One manifested numbness of the face and hand followed by tingling sensations one hour later while the other one was asymptomatic.

West Coast:

In Alaska, PSP has occurred for centuries with the first reported case in 1799. From 1973 to 1994, there were 66 recorded outbreaks involving 146 individuals, 8 of them developed paralysis of the limbs, 8 required mechanical ventilation and 2 died. (1) Most of these outbreaks occurred in the island of Kodiak and southeastern Alaska between the month of May and June. Most of these cases were related to eating mussels.

In the strait of Juan de Fuca (international boundary between Canada and USA), PSP caused several deaths in 1942. In the Puget Sound region of Washington recurrent outbreak of Alexandrium was reported in the late 1970 (1). The Northern California and Oregon area have have these perennial problems of PSP outbreaks.

This PSP was followed by three more episodes in the Alaska Peninsula and Kodiak island in that month of June 1990. This outbreak had 13 cases among 21 individuals (62%) (1)

Epidemiologic study done showed the ff: (1)

- consumption of butter clam -- 7 / 13 – (54%)
  mussels ------ 6 / 13 -- (46%)
- number of shellfish consumed --- 3-30 (median 4 shellfish)
- onset of symptoms -- 0 to 2 hours (median 1 hour)
- duration of symptoms --- 1 to 24 hours (median 7.5 hours)
- sought medical care --- 7 persons / out of 13 (54%)
- mortality -- 1 /13 (8%)

- PSP toxin test done on 4 sites along the Alaskan Peninsula where the shellfish were harvested:
  - butter clams from Volcano Bay and King Cove -- 7750 ug / 100 g
  - mussels from Sand Point and Kodiak --- 1925-12,960 ug / g

From 1976 through 1989, there were 42 PSP outbreaks (accounting for 94 cases) were documented in Alaska. 31 of the 42 outbreaks occurred between May to and July. In 1942, due to several deaths from PSP, the Strait of Juan de Fuca was closed. In the 1970, the San Juan and Bellingham coasts were closed. PSP was also an annual problem along the coast of Northern California and Oregon. (1)

Argentina - The first HAB in this country was in 1980 in the Valdes Peninsula. Two members of a crew of a ship died after eating mussels. In 1991/1992 HAB was documented in the northeastern shore of Beagle Channel. This was followed in the summer of 1993, in the Buenos Aires shelf known as Rincon (1).

Brazil - Since Beginning in 1992, there have been reports of HAB with shellfish toxicity in the Tiera del Fuego almost every year. In 1998, G. catenatum algae were observed in off the coast of Santa Catarina State. (1)

Chile - As early as 1886, there were reports of PSP from eating mussels among the natives of Ushuaia. From October 1972 to January 1997, there were 329 PSP cases in the southern regions of Chile. 26 people died. PSP and DSP had severe public health and economic impact in Chile until 2001. In March 2002, there were 8 cases of poison and one death after consumption of shellfish in the southern Chile prohibiting the harvesting of shellfish in this area including the Ancud community (region?) (1).

Guatemala- There was an outbreak of PSP in 1987 with 187 cases and 26 deaths after the consumption of clam soup. 50% of the fatalities were children (1).

Mexico - In 1979, there were reports of PSP poisoning after eating local mussels. There were 20 cases with 3 fatalities. In November 1989, along the coast of Chiapas and Oaxaca, there were 99 cases of PSP poisoning with 3 fatalities. Between March 1993 and April 1994, shellfish harvesting was closed along the Gulf of California following increased density of dinoflagellate (A. catenella). (1)

Trinidad - The first PSP recorded in this area was in 1994. The PSP toxins were found in the meat extract of the mussel Perna viridis. (1)

Uruguay - In 1980, 60 cases of PSP poisoning were reported.They were not able to determine the the causative algae species. (1)

Venezuela - 171 cases of PSP were reported in 1979 and 9 cases were reported in 1981. (1)

China- The first PSP cases reported were 40 separate episodes involving 423 cases and 23 fatalities in the Zhejiang Province between 1967-1979. The next reported case was in Donghan (south of Fujian Province) with 136 cases and one fatality. From 1990 to 1992, there was a survey done in Guandong Province with the result of the presence of PSP toxins in 33 edible marine organisms (1)

Timor- Leste - There was a report of a man who died after ingestion of the crab Zosimus aeneus. (1)

Hongkong - There were three outbreaks of PSP in 1992. No details were available as to the number of cases and fatalities. (1)

India -- PSP was reported in 1981, with 98 cases and one fatality. (1)

Japan -- First reported PSP case was in 1992, in Hiroshima Bay. In March, 1997, in the island of Fukue, 20 people were poisoned after eating oysters. (1)

Malaysia -- In 1977, there were 201 cases of PSP reported after eating local calms. Most of these cases were confined in the west coast of Sabah in Borneo. In the Malayan Peninsula, the first reported PSP poisoning was in early 1991 when three people became ill after eating farmed mussels from Sebatu in the Strait of Malacca. In September 2001, there were six people who became ill after eating clams harvested from the coastal lagoon on Kelatan on the east coast of Malaysian Peninsula. One of them died. (1)

Philippines - PSP is a public concern in the Philippines. It causes economic loss in the fishery sector of the country, especially in shellfish industry. Between 1983 and 2005, there were reports of 2,161 cases and 123 fatalities in 27 coastal waters. The 1983 outbreak in the Central Philippines resulted in a 2.2 million loss with a dramatic decline in demands for fishery Products. During the 1988 outbreak in Manila Bay, it caused economic damage since the price of all seafood products dropped to 40% of the original price. (3)

There are reports dated Dec 27, 2016 from BFAR (Bureau of Fisheries and Aquatic Resources) of two deaths and 40 cases in the town of Mari pipi, Tacloban City, Philippines. (4)

Taiwan Province of China:
In January 1986, reports of two mortalities and 30 morbidities after eating Soletellina dipos. In February 1991, 8 cases of morbidity were reported after eating Soletellina dipos (1). The PSP in this area were found among purple clams, xanthid crabs and gastropods.

Thailand: In 1983, 62 cases of PSP were reported with one fatality in Thailand after the consumption of local mussels. A freshwater puffer poisoning case was reported in 1990 (1).

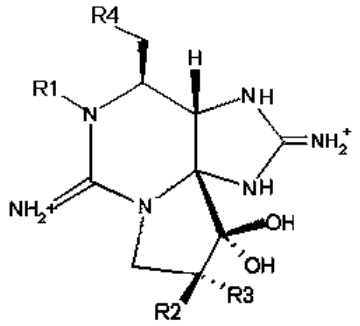
Australia: There were reports of high levels of PSP toxins in the Port of Philip Bay and Western Port Bay in Victoria from 1987 to 1997 (1).

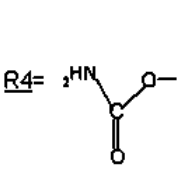
Nez Zealand: - In January 1983, there were more than 180 reports of respiratory irritation from air-borne toxins in sea spray. From January 1993 to July 1996, samples of shellfish taken around the coastal area was found to have toxic levels above regulatory limits (1).

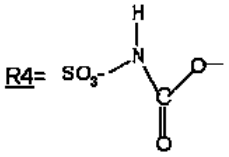
CAUSATIVE AGENTS

Diatoms, Toxic algae, Dinoflagellates (Alexandrium catenella, A. tamarensis, A. minutum, A. fraterculus, A.fundyense, A.cohorticula, , Pyrodinium bahamense, Gymnodinium catenatum) that produce a toxin called "Saxitoxin". This is a water soluble but heat stable substance unaffected by standard cooking or steaming.

Fig 12.19: Chemical structures of PSP toxins







R4= OH

R4=H

R_1	R_2	R_3	carbamate toxins	N-sulfo-carbamoyl toxins	decarbamoyl toxins	deoxy-decarbamoyl toxins
H	H	H	STX	GNTX5(B1)	dcSTX	doSTX
H	H	OSO_3^-	GNTX2	C1	dcGNTX2	
H	OSO_3^-	H	GNTX3	C2	dcGNTX3	
OH	H	H	neoSTX	GNTX6(B2)	dcneoSTX	doneoSTX
OH	H	OSO_3^-	GNTX1	C3	dcGNTX1	doGNTX1
OH	OSO_3^-	H	GNTX4	C4	dcGNTX4	

Source: Food and Agriculture Organization of the United Nations, 2004, Monse et al, 1988, Quilliam et all 2001, Paralytic Shellfish Poisoning
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MOLECULAR ACTION OF SAXITOXIN

Saxitoxin blocks the Na receptors on the external portion of the Na channels in the cell membrane of the nerves and muscles. As we know, the nerve impulse itself is the Na influx across the Na channels. Blocking the Na influx stops the flow of nerve impulse thereby paralyzing the muscles. What if it affects your respiratory muscles (diaphragm and intercostal muscles)? It may result to in death.
Natural reservoir --- shellfish, mussels, clams and other marine fish.

TRANSMISSION

These dinoflagellates and other types of phytoplankton are considered microscopic plants in watery environments both salty and fresh. They depend on ocean current for transport. The growth of the phytoplankton depends on the presence of carbon dioxide, sunlight and nutrients such as nitrate, phosphate, silicate, calcium. and a low concentration of iron. These factors came from human inputs into the sea such as untreated sewage, farming, and gardening products like fertilizers. Their growth are affected by water temperature, salinity, water depth, wind, and the type of predators. Once the right set of environmental factors are present, they grow explosively, a phenomenon known as "bloom" or "harmful algae bloom" (HAB). Rainfall, sunlight, and high temperatures increase the ocean current, and are favorable for the phytoplankton to spread out. These microscopic plants in enormous numbers produce biotoxin, specifically saxitoxin (which is one of the most toxic compounds known to man) which has deleterious effects on the marine

environment. During the HAB, fish, and shellfish consume the phytoplankton without apparent harm. Any marine mammals, humans, fish, or birds who that consume these shellfish and small fish acquire the toxin and may develop PSP.

CLINICAL PRESENTATION.

As early as 5 to 30 minutes after ingestion of contaminated shellfish, a person may develop tingling sensation around the mouth that eventually spreads to the face and neck. Numbness may follow. As the toxin spreads from the mouth to the throat, the patient may develop dysphagia, sense of constriction on the throat, or worse, aphasia. Within 2- 12 hours in severe cases, these symptoms leads to paresis and eventually paralysis. If it affects the respiratory muscles (diaphragm, intercostal muscles), the patient cannot breathe, leading to death. If the patient is fortunate enough to last for more than 12 hours without respiratory paralysis she starts to recover and improve. Usually she becomes symptoms free after few days.

Aside from the paralysis, the patient may experiences other symptoms, such as nausea and vomiting, headache, and dizziness. There is no loss of consciousness, however.

The highest mortality rate was reported in Guatemala in 1987 with a 14% fatality rate. Children among the fatalities comprised 50%. This is due to the higher vulnerability of children to the toxin and the poor emergency access and inadequate medical services in the area. The overall global mortality rate is 8.5-9.5% (Meyer in 1953.Ayers and Cullum in1978). (1)

DIAGNOSIS

Presented with a patient with complaints of a tingling sensation on the mouth with numbness would not point to PSP as these symptoms are common in other forms of oral poisoning. However, the history of intake of shellfish and the quickness of the appearance of symptoms right after intake will lead you to think of PSP. More so if these shellfish were taken from areas suspected with Red Tide.

Laboratory confirmation is the best basis of the diagnosis. The recommended diagnostic method is the "Mouse Bioassay". However, this diagnostic test cannot differentiate the PSP toxins from other tetrodotixin.. A "mouse unit" is the minimum amount of toxin that can cause the death of an 18-22 g. of white mouse in 15 minutes. The lethal amount for human is 5,000 - 20,000 mouse unit (equivalent to 1 to 4 mg.) depending upon the age and physical status of the patient.

Enzyme linked immunosorbent assay (ELISA) and Radioimmunoassay has been developed for saxitoxin and their results have good correlations with the result in mouse bioassay.

TREATMENT

In severe cases, just like with other poisonings, the first thing to do is to remove the obnoxious material in the gastrointestinal tract. This is done by gastric lavage that washes the stomach of the ingested toxin. Insert a gastric tube through the nose or the mouth up to the stomach, and pour in a saline solution. Let it stay for few minutes and aspirate the fluid. This is done several times. This remedy must be given as early as possible as the time element is important in poison treatment. Knowing that ingested materials

usually stay in the stomach for 3-4 hours, a patient with history of intake of shellfish within 4 hours has a greater chance of recovery as the toxin is still in the stomach not in the whole body. Other medicines that can be used are activated charcoal or diluted bicarbonate. These substances attract the toxin thereby preventing them from being absorbed in the stomach. Any respiratory difficulty must be treated with ventilator and blood gases level must be corrected. It has been reported that 75% of severely affected people die within 12 hours. Drugs used ordinarily for poisoning may have some beneficial effects like DL amphetamine (Benzedrine). Others like anticholinesterase agents and anti curare agents are non beneficial. There are some studies that show these drugs may be harmful to PSP. Metabolic acidosis must be corrected accordingly. Knowing that these this poison will circulate in the body and eventually end up in the kidney and may possibly cause kidney failure, hydration will dilute the toxin in the body and furthermore enhance the elimination of the toxin. Kidney function must be checked.

Areas affected by this Red tide have traditionally developed some local treatment. In the Philippines, a concoction of coconut milk and sugar is given. Studies shows that this combination has some detoxification property.

Less severe cases without respiratory failure may manifest headache, nausea, vomiting, and/or dizziness. These are treated symptomatically; analgesic for headaches and intravenous fluid for vomiting.

Most important in the treatment of PSP is the elimination of human contact with shellfish. Any incident must be reported to the authorities who then should conduct laboratory testing on sample shellfish. If proven true, (something missing) must initiate measures to contained the area and if necessary close the area for commercial fishing. Surveillance must follow. In the USA, 800 ug or more of PSP /kg by mouse assay is tantamount to closure of the area.

PREVENTION:

Depuration - a process of removing the contaminated shellfish from the area and transfer them to water free of algae and allow them to self-depurate (purify). However, this process is very tedious and costly as some shellfish (Crassostrea, Plactopectin, Spisula, and others) require several months to detoxify. In the clam Saxidomus gigantea, the elimination of toxin may take more than a year. They studied that the rate of elimination of toxin is dependent on what part of the shellfish is the toxin is stored. Shellfish that stored the toxin in their gastrointestinal tract are easily detoxified while those shellfish which stored toxin in their tissue will take time to detoxify.

Detoxification by instantaneous electric shock accelerated the toxin excretion in scallops. Acidification of the water to detoxify butter clams was not successful. Chlorination of water resulted to in the alteration of the flavor of the shellfish, thereby decreasing he market value. Ozonation has been tried with some success for shellfish recently contaminated by toxin and it prevents further accumulation of the toxin. However, this method is not feasible in those areas where the shellfish has been contaminated for a longer period of time.

Cooking the shellfish reduces the toxicity but does not totally (completely?) remove the toxin. This maybe effective for those shellfish with low levels of toxicity. Boiling the oysters for 10 minutes at 98 degree centigrade can reduce the toxicity to 68-81%, but not enough for extremely toxin shellfish. Pan frying is better than simple boiling, as the toxin stays in the soup which maybe drunk.

At present, large scale detoxification is not commercially feasible. Fishermen depend more on extensive monitoring programs with immediate containment procedures in the event of incidence. There must be regular inspections of seawater bodies, especially during the bloom seasons. The presence of this harmful algae like the dinoflagellates is a warning sign to test the level of toxin in the shellfish. In case of contamination, implement measures to prevent consumption of the shellfish. Any incidence should be reported to the proper authorities right away.

In Chile, since 1997, aside from constant monitoring, teaching strategies has have been applied, such as training workshops to fishermen and extensive dissemination of information about harmful algae blooms to the community including the teachers and the students.

Worldwide regulations: (1)

European Union set the limit for PSP using mouse assay at 80 ug of STX/ 100 grams of the meat of shellfish.
Morocco, Africa -- at 80 ug/ 100 grams of meat of shellfish
Canada -- 80 ug/ 100 g of meat of mollusks
-- 160ug/100 g of meat of soft-shell clams and mussels
---500 ug /100 g of meat of butter clams after removing the entire siphon
-- 300 ug/ 100 g of meat of butter clams after removing the distal portion of siphon
USA --- 80 ug / 100 g of tissue of bivalves
Argentina -- -400 MU / 100 g of mollusks
--160 ug/ g for snails. Argentina has a station in Mar del Plata that monitors mussel toxicity in each coastal province.
Brazil has started a monitoring initiative but does not have national monitoring program
Chile -- 80 ug / 100 g. of meat of mollusks
Guatemala -- 80 ug/ 100 g of meat of mollusks
Mexico --- 30 ug/ 100 g as regulator limit
Panama --- 400 MU /g of bivalves
Uruguay ---400 MU / g of mollusks
Venezuela -- 80 ug/ 100 g of mollusks
China, Hongkong Special Administrative Region -- 400 MU / 100 g of shellfish
Japan -- 400 MU / 100g of bivalves
Malaysia -- In 1990, the Malaysian Department of Fisheries has started the shellfish toxicity monitoring program that has greatly decreased the incidence of PSP. An additional monitoring facility was established in the east coast of Malaysian Peninsula after PSP cases were reported
Philippines -- has set a lower limit of 40 ug/100 g
Singapore---- 80 mg/ 100 g for bivalves
Republic of Korea --- 400 MU / 100 g. for bivalves
Australia --- 80 mg / 100 of shellfish meat
New Zealand --- 80 ug / 100 g of shellfish meat

DIARRHOEIC SHELLFISH POISONING (DSP) .

Diarrhoieic Shellfish Poisoning (DSP) is a food borne disease like Paralytic Shellfish Poisoning (PSP) but it causes gastrointestinal symptoms, not neurologic symptoms. It is caused by eating contaminated bivalves such as mussels, scallops, oysters, or clams. The toxins in DSP accumulate in the fatty tissue of the bivalves. Patients develop gastrointestinal symptoms such as nausea, vomiting, abdominal pain and diarrhea which start as early as 30 minutes to a few hours after ingestion and last for 3 days.

DSP was first reported in Netherlands in 1960. This was followed by outbreaks from Japan in the late 1970s. Similar incidents were reported in Europe, South America and Far East (5).

Figure 1: Occurrence of DSP toxins in coastal waters of European ICES countries from 1991 to 2000

The organisms responsible for DSP ***Dinophysis and Prorocentrum***.

Fig 12-20 ***Dinophysis acuminate***. Photo by Fjouenne.[xxv]

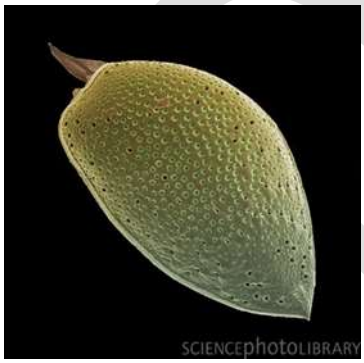

Fig 12-21 Prorocentrum sp. (Scanning electron micrograph).[xxvi]

Fig 12-22

Source: Food and Agriculture Organization of the United Nations, 2004, Diarrhoeic Shellfish Poisoning,[xxvii]
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OUTBREAKS OF DSP

EUROPE

Belgium - In February 2002, 430 cases of DSP were reported in Antwerp (5). The blue mussels that they ate came from Denmark. The remaining imported mussels were not sold.

Croatia - In 1994, toxin analysis of mussels (Mytilus galloprovincilis) from Central Adriatic Sea yielded DSP toxins. In the summer of 1995, DSP toxins were also found in mussels in Kastela Bay (5).

Denmark - There were reports of DSP poisoning involving 415 persons in the North Danish coasts. In 2001, these toxins were found in high concentrations in commercially fished mussels. In 2002, there were reports of people who became sick after eating Danish mussels (5).

France- From 1978 onwards, several areas in France (Normandy, South Brittany, West Brittany, Mediterranean coast) reported DSP poisoning. In 1984 and 1985, 10,000 cases and 2,000 cases, respectively, developed symptoms of DSP after eating mussels raised in France. In 2000, several areas on the Atlantic coast and Mediterranean coast were closed due to DSP toxins (5).

Germany - In 1987, DSP toxins were detected in mussels from the Wadden Sea. In 2000, two elderly women were reported to have DSP intoxication (5).

Ireland - The first DSP were recorded in 1980. A high level of DSP toxin was found in the Southwest coast of Roaring water, Dunmanus, Bantry, Kenmare and Dingle Bay in 1988. Aggressive detection of DSP toxins resulted in the closure of 30 shellfish harvesting areas in August 2000 (5).

Italy - On the northern and central Adriatic coast, there were reports of DSP from 1989 onwards. Monitoring of the Italian shellfish banks . began in 1989. Due to cases of DSP, the Emilia Romagna region was closed from August to December 2000 and closure of the Veneto region from October to December 2000 (5).

Netherlands- The first reported case of DSP was reported in 1960. The Warden Sea coasts have had several cases of DSP since 1961. In the summer of 2001, a bloom of D. acuminata occurred in the Dutch Wadden Sea contaminating the mussels (5).

Norway - There was an outbreak of DSP in the Oslo Fjord area in 1979. From October 1984 to October 1985, there were 300 to 400 cases of DSP in the southeast Norway (5).

Portugal – Since 1987, DPS toxins have been detected in Portugal but no human poisoning has been reported. In the summer of 2001, there was a DSP outbreak after eating razor clams harvested in Aveiro Lagoon. In 2002, due to algae bloom (D. acuminata) (5), the entire northwest coast producing mussels and other bivalves were closed.

Spain - The first confirmed DSP was in 1978. From April to December 1995, coastal areas in Galicia were closed due to high level of DSP and PSP toxins detected. In 2002, toxic events occurred in Galicia and Andalucia causing long closure periods and in Cataluna causing a short closure period (5).

Sweden - In 1971, mussel farming was started in Sweden. There were no toxic events 1983, when they observed DSP among the people who ate mussels. During the winter 1989 and 1990, harvesting mussels was stopped due to the high concentration of DSP toxin. It is common in Sweden to close the production areas of mussels during the period of September to March (5).

United Kingdom of Great Britain and Northern Ireland
First reported incidence of DSP in the United Kingdom was in 1997 when 49 patients developed symptoms of DSP after eating mussels in two London restaurants. Harvesting shellfish were banned in several parts of England in March 2002. In the West Coast of the Isle of Man, harvesting of queen scallops were banned. In Northern Ireland in 2001, positive results of . DSP toxins on oysters, mussels, cockle, and scallops, led to the United Kingdom Food Standard Agency banning fishing in the sea adjacent to Northern Ireland (5).

AFRICA

South Africa - During the autumn of 1991, DSP cases were reported in the west coast of South Africa and in the autumn 1992, DSP cases were reported in the south coasts (5). The causative organism was Dinophysis acuminata.

NORTH AMERICA

Figure 2: Occurrence of DSP toxins in coastal waters of North American ICES countries from 1991 to 2000

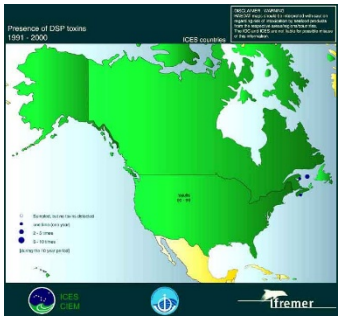

Fig. 12-23

Source: Food and Agriculture Organization of the United Nations, 2004, Diarrheoic Shellfish Poisoning, Reproduced with Permission
Canada- In 1989, DTX1 DSP toxin was isolated from mussel in the Prince Edward Island. In August 1990, 13 out of 17 persons in Eastern Nova Scotia developed gastroenteritis one to eight hours after eating boiled or steamed mussels. In October 1993, in Bonavista Bay, Newfoundland, several persons developed DSP after eating. mussels (5).

United States of America - Before 1980, there were sporadic cases of DSP in the New York and New Jersey areas. There were 4 reported cases of DSP like illnesses between 1983 and 1985 in Philadelphia and Long Island, New York after eating clams and mussels. In 1989, there was a high level of D.acuta that discolored the water in Long Island. No cases of DSP were reported (5).

In 2010, a pilot study was created by the Washington Department of Health and the FDA Gulf Coast Seafood Laboratory to monitor the presence of Dinophysis species and its associated toxins. They found out that the Dinophysis counts were above threshold, but the toxins (OA, DTX) were below the FDA guidance level (6).

The US Food and Drug Administration set the limit for PSP toxins at 80 micrograms per 100 grams of shellfish tissue.

The WHO says that 100,000 cells/mL is a moderate human health risk, but there are currently no standards for cell or toxin concentrations in the United States.

CENTRAL AND SOUTH AMERICA

Argentina - In 1999, 40 cases of DSP were reported in Patagonia (5).

Brazil - In the region of Florianapolis, several persons developed gastrointestinal distress and diarrhea after consuming mussels (5).

Chile - In 1970 and1971, there were sporadic blooms of Dinophysis causing gastrointestinal disorders. 120 people became ill after consuming mussels in January 1991. DSP in Chile caused severe public health and economic impacts until 2001, when fishing beds were closed from 44o SL southwards and nationwide monitoring was established (5).

Mexico -In 1993 and 1994, there were increased densities of dinoflagellate D. caudata in the Gulf of California (Punta arena, Playa Escondia, Amorales, and San Ingacio). Shellfish extracts were positive for DSP in samples from Bahia Conception in the Gulf of California. No cases of DSP were reported though (5).

Uruguay - In 1990, several persons developed diarrhea and gastrointestinal distress after consuming mussels. In January 1992, DSP was detected in shellfish along the coast of Uruguay. In the area of La Paloma, a partial ban was implemented on shellfish harvesting (5).

ASIA

China – DSP is widely distributed among the different types of shellfish along the coast of China. In 1996 and 1997, high levels of DSP toxins were found in 26 out of 89 samples. The highest level was found in Pema viridis from the region of Shenzen (5). No human poisoning was reported though.

Japan - DSP was first documented in Japan in June 1976 and 1977 when 164 persons suffered severe diarrhea and vomiting. Between 1986 and 1982, about 300 cases of DSP were reported (5)

India - There was a two-year study in India that showed DSP toxins were present in several shellfish samples (5). There DSP cases are known.

Philippines - While most of the shellfish poisoning in the Philippines is of the Paralytic type, there were some reports of DSP. In 1995, five species of Dinophysis had been detected to have DSP toxins but no human cases of poisoning happened (5).

OCEANA

Australia and New Zealand - A pipi (Donax delatoides) shellfish poisoning with 56 hospitalized cases was reported in New South Wales, Australia in December 1997. Another reported poisoning due to pipi shellfish was reported when an elderly woman became ill after consuming pipis from a local beach. Between the period of September 1994 and July 1996, there were outbreaks of human DSP involving 13 cases in the coastline of New Zealand (5). Samples collected on a weekly basis showed toxic levels above the regulatory limits.

CAUSATIVE ORGANISM

The causative organisms are marine dinoflagellates that belong to the genera Dinophysis and Prorocentrum spp. There are seven Dinophysis species which are: D.fortii ((in Japan), D.acuminata (in Europe), D. acuta, D. norvegica (in Scandinavia), D. mitra, D. rotunda and D. tipos. The Prorocentrum species are the following: Prorocentrum lima, P. concavum, and P. redfieldi. These algae, under favorable conditions, can grow in large numbers causing an algae bloom. These dinoflagellates produce the DPS toxins. The toxins can be divided into three groups depending on the chemical structures. The first group are the acid toxins. This group includes Okadaic acid (OA) and its derivatives named dinophysistoxin (DTXs). The second group are the neutral toxins which are polyether-lactones of the pectenotoxin group (PTXs). The third group is the sulphated polyether and its derivatives yessotoxins (YTXs)

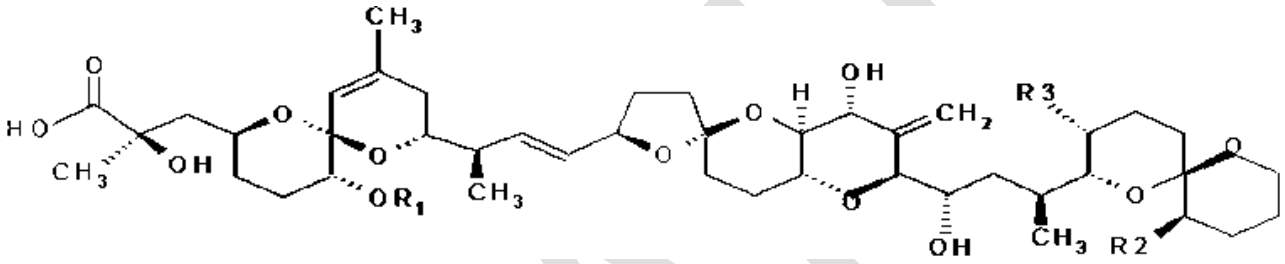

Fig 12-24

R1	R2	R3
okadaic acid (OA)	H	H CH_3
dinophysistoxin-1 (DTX1)	H	CH_3 CH_3
dinophysistoxin-2 (DTX2)	H	CH_3 H
dinophysistoxin-3 (DTX3)	acyl	CH_3 CH_3

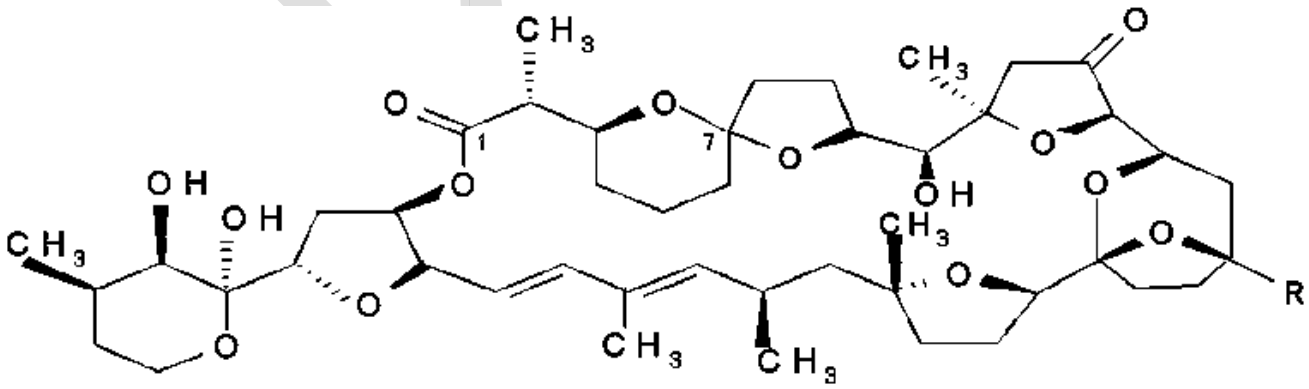

Fig 12-25

R	***C-7***

pectenotoxin-1 (PTX1) CH_2OH R
pectenotoxin-2 (PTX2) CH_3 R
pectenotoxin-3 (PTX3) CHO R
pectenotoxin-4 (PTX4) CH_2OH S
pectenotoxin-6 (PTX6) COOH R
pectenotoxin-7 (PTX7) COOH S

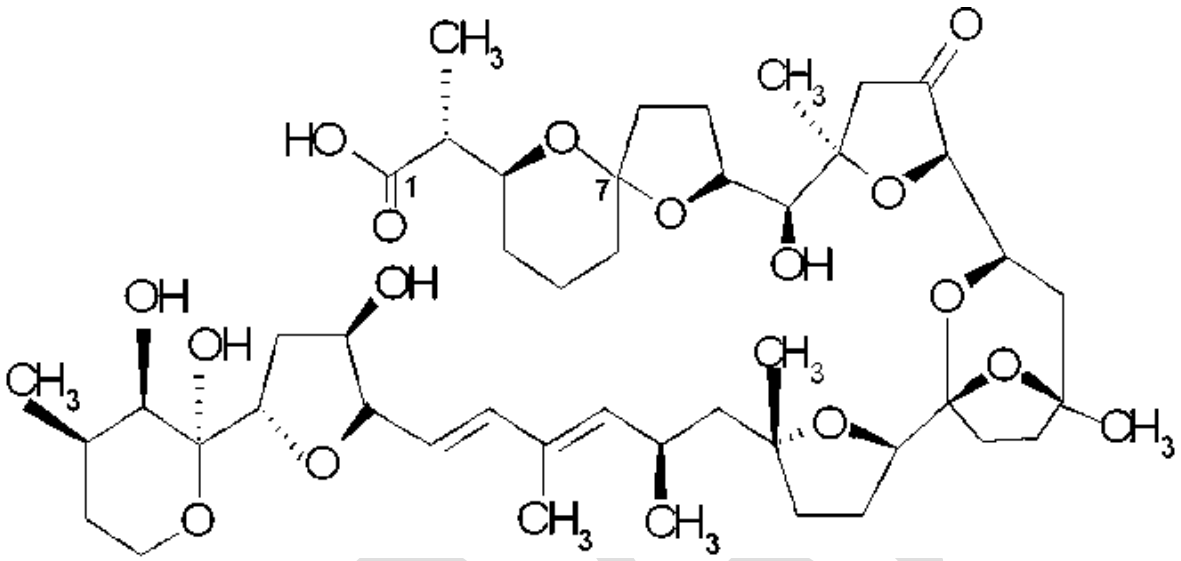

Fig 12-26

C-7

pectenotoxin-2 seco acid (PTX2SA) R

7-epi-PTX2SA S

Figure 3 Chemical structures of okadaic acid, dinophysistoxins, and pectenotoxins
Source: Food and Agriculture Organization of the United Nation, 2004, Yasumoto et al., 2001, Diarrhoeic Shellfish Poisoning,
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MOLECULAR MECHANISM OF ACTION

The minimal amount of DSP toxin that can induce disease in humans is 12 MU. One mouse unit (MU) is the amount of toxin that can kill one mouse with a body weight of approximately 20 grams in 24 hours.

One example of the DSP toxin is Okadaic acid (OA). OA being an acid toxin is lipophilic. It promotes phosphorylation that controls the sodium secretions by the intestinal cells. The presence of OA in the intestinal lumen promotes more sodium secretions of the intestinal mucosa. Since sodium is an osmotic electrolyte, it tags with it water. Therefore, sodium and water are copiously secreted by the intestinal mucosa in the presence of OA resulting in copious diarrhea. The

increased amount of fluid in the intestinal lumen will cause an increase in peristalsis causing the abdominal pain and nausea and vomiting.

CLINICAL PRESENTATIONS

Diarrheal Shellfish Poisoning is not as serious as Paralytic Shellfish Poisoning. This is a self-limited diarrheal disease without a known chronic sequela. Although nonfatal, the illness is characterized by abdominal pain, nausea, vomiting, and incapacitating diarrhea. The diarrhea was the most common symptoms (92%), followed by nausea (80%), and vomiting (79%). These symptoms start 30 minutes to 12 hours after ingestion of contaminated shellfish. Complete recovery is usually seen in 3 days, even in severe cases. There is no evidence of neurotoxicity and no fatal cases have ever been reported (Halstead 1988, Viviani 1992).

DIAGNOSIS

Medical history is particularly important when considering DSP as the diagnosis. Since patients manifest the usual gastrointestinal symptoms of abdominal pain, nausea, vomiting, and copious diarrhea, a physician must consider many gastrointestinal infectious conditions. The only clue to DSP is a history of ingesting shellfish. More so if the shellfish came from areas known to have outbreaks of DSP. Remember, there are no neurologic manifestations. These manifestations will differentiate this type of shellfish poisoning from the Paralytic type (PSP).

Confirmation of the diagnosis might be difficult. One way of confirming the diagnosis of DSP is the mouse bioassay (commonly used in Japan). This is done by injecting the toxic extracts into the abdominal cavity of the mouse and observe the mouse for 24 hours. A DPS toxin level more than 50 MU are considered toxic to human consumption. Other countries consider a 15 MU level toxic to humans.

MANAGEMENT AND TREATMENT

Treatment is symptomatic and supportive with regards to diarrhea and accompanying fluid and electrolyte losses. In general, hospitalization is not necessary; fluid and electrolytes can usually be replaced orally. Patient must be on a "nothing by mouth" except for the fluid and electrolyte replacements for several hours until their diarrhea subsides. Abdominal pain, nausea, and vomiting are usually not treated unless they are severe enough to cause other symptoms. Other diarrhetic illnesses associated with shellfish consumption, such as bacterial or viral contamination, should be ruled out (Aune & Yndstad 1993).

Just like any other shellfish poisoning, the presence of one case must not be considered lightly. This might be an initial case of an impending outbreak. Any suspected case of DSP must be reported right away to the appropriate public health authorities. These case/s must be monitored properly. Necessary precautions must be implemented to prevent the spread.

PREVENTION

Depuration is a purification process wherein harvested shellfish are placed in a land-based plants containing clean estuarine to purge or expel the gastrointestinal content enhancing the separation of the contaminant from the shellfish. This is an effective way of removing the toxin from the shellfish but not effective in removing other contaminants like norovirus and hepatitis A.

In Japan, depuration decreased the DSP toxin from 4.4 to 2.5 MU in one week and then to 0.5 MU by the next week.

The main strategy to prevent DSP is effective monitoring of mussels with respect to the DSP toxin so that the contaminated shellfish will not be available for consumption. Frequent monitoring of the seawater around aquaculture facilities or shellfish farms for the presence of phytoplankton strains known to produce toxins. This must be done routinely regardless of an outbreak or not. Then data on the occurrences, types, and concentration of algae species must be collected. These will serve as the basis for determining what type of toxin maybe expected for a particular period. Some countries monitor only the two most common algae species, while other countries have a long list of species to monitor. Once the algae exceed certain concentrations, the harvesting area is closed. In Italy, closures are done if the toxin is detected only in shellfish.

REGULATIONS AND MONITORING

Europe- In 1996, EU-National Conference Laboratories Meeting on Analytic Method and Toxicity (5) Criteria agreed that mouse assay (MU) is the preferred method for the detection of DSP toxins. Tolerable levels are 80-160 mg of OA per kg of shellfish meat. This is equivalent to 20-40 Mu/kg of whole shellfish meat.

In March 2002, the European Commission laid out the following rules (5)

1. Maximum levels of OA, DTXs and PTXs together in edible tissue of mollusks, echinoderms, tunicates, and marine gastropods shall be 60 mg equivalent/ kg meat.
2. The maximum level of YTXs in edible tissue of mollusks, echinoderms, tunicates, and marine gastropods shall be 1 mg YTX equivalent/kg.
3. The mouse and rat bioassay are the preferred methods of analysis for the toxin. In the event of discrepancies between these two methods, the mouse bioassay must prevail.

Ireland - In 1984, the Biotoxin Monitoring Programme was initiated (5). This was based on screening samples for the presence of DSP toxins. Recently, they included other toxins for monitoring like azaspiracids and the monitoring is done now on the weekly basis. A website information system is being developed to have easy access to this information.

Turkey - Regulation and detection is based on mouse assay.

North America

Canada - In 1995, Hallegreaff et. al reported that in Canada, monitoring for Dinophysis and Prorocentrum spp. is done and when the DSP toxin exceeds the tolerable level, closure of harvesting areas is implemented (5).

United States - There is no monitoring of DSP in the United States simply because there is no confirmed DSP. The FDA (Food and Drug Administration) is the primary agency on seafood safety and marine biotoxin. The National Marine Fishery Service of the National Oceanic and Atmospheric Administration has several programs geared on fishing and wildlife. Internationally, it is the FDA that sets up the memoranda of understanding with other countries to regulate imported seafood products (5) Central and South America –

Argentina - Argentina has a national monitoring program for mussel toxicity and its fixed station is in Mar del Plata.

Brazil - Brazil had one pilot monitoring initiative for one year but does not have a national monitoring program (5).

Chile - The Chile government has two monitoring agencies. The National Health Service is responsible for monitoring toxicity using bioassay at 40 stations on monthly basis. and the Fisheries Research Institute which is university based. The Chile Minister of Health through its regional Health Services is responsible for assessment and the closure of harvesting areas. The National Fish Service (NFS) is the agency for seafood export. It has a memorandum of agreement with the US and Europe to permit shellfish export. Before 2001, PSP and DSP severely affected the public health and economy of Chile (5).

Uruguay - Uruguay has national monitoring programme for mussels and toxic phytoplankton. Their analysis is based on mouse assay (5).
Venezuela - Regulation is based on the mouse assay (5).

Asia

China - China has no regulatory program for DSP toxin and no regulatory program for algae biotoxins. One major program on red tide was funded that includes regular monitoring in two areas. This monitoring is biweekly for shellfish and plankton (5).
Japan - Japan has the Perfectural Fisheries Experimental Station in major shellfish areas that periodically collect plankton samples and carries out cell count of Dinophysis species. Shellfish are also collected and assayed. Maximum level of DSP toxin for human consumption is set at 5 MU/100 gram of whole meat detected by mouse assay. Japan has a well-defined network for easy dissemination of information connecting government agencies, fisheries cooperatives, fishermen, mass medias and the general public (5).
The Republic of Korea- The Republic of Korea has the National Fisheries Research and Development Institute (NFRDI) that assesses plankton samples in key areas biweekly. It has over 200 stations. They test these samples for PSP, DSP, and ASP. They set up a tolerance limit for DSP at 5 MU / 100 grams (5).
Thailand - Regulation is based on mouse assay (5).
Oceania:
Australia - Their recommended limit for DSP is 16-20 ug OAeq/100 grams of shellfish meat (5).
New Zealand - The New Zealand government has the Biotoxin Monitoring Programme that assesses phytoplankton and performs regular shellfish testing (5).

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4. NEUROLOGIC SHELLFISH POISONING (NSP)

Neurotoxin shellfish poisoning is caused by ingesting shellfish contaminated with brevetoxin which are produced by unarmored dinoflagellates. Cases of NSP are usually associated with shellfish harvested red tide blooms. This type of poisoning may not be as serious as the PSP or DSP, but some cases required

hospitalization. There are no reported fatalities so far for NSP. Patients suffering from NSP develop gastrointestinal, respiratory, dermatological, and neurological symptoms.

This poisoning is caused by polyether "brevetoxin" produced by unarmored dinoflagellates. This is tasteless, odorless, heat and acid stabile, lipid-soluble, cyclic polyether neurotoxin. This poison is toxic to humans, birds, marine mammals, and fish, but not to shellfish (oysters, clams, mussels).

CASES and OUTBREAKS
North America

Figure 12-22. Occurrence of NSP toxins in coastal waters of North American ICES countries from 1991 to 2000

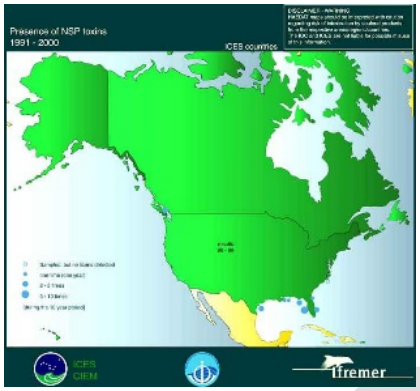

Fig 12-27

Source: Food and Agriculture Organization of the United Nation, 2004, Neurologic Shellfish Poisoning
http://www.fao.org/3/y5486e/y5486e0o.htm#bm24
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France - In October 1991, F. japonica was reported in the Channels coast of Normandy (8)

Germany - ***H. akashimo*** was detected in the German Wadden Sea beginning in the summer of July1995. F. japonica was observed near Sylt in summer of 1997. In March to May of 1998 2000, and 2001, an extensive algae bloom of ***Chattonella*** sp. occurred killing fish (8).

Netherland - ***F. japonica, Chattonella antiqua and Chattonella marina*** were reported in 1991 to 1993 in the Wadden Sea, North Sea and/or Delta area south of Rhine. In summer of 1997, F. japonia was found along the Dutch coast from Noordwijk to Borkum (8).

The Russian Federation - There were reports of fish deaths in the Amurskii Bay caused by ***Chattonella sp*** (8).

The United Kingdom of Great Britain and Northern Ireland - There were reports of red tide of H. akashimo from England and Bermuda that caused fish deaths (8)

South Africa - In the coastal resort of Hermanus in Walker Bay, episodic respiratory problems were seen on residents in the summer of 1995 and 1996 (8). This was due to the bloom of toxin Dinoflagellate species ***Gymnodinium.***

Canada - Report of red tide due to ***H. akashiwo*** has caused mortality of cultured fish in Canada (Van Apeldoom, et al., 2001) (8)

United States of America:

East Coast - From 1987 to 1988, there were reports of bottlenose dolphin deaths (***T. truncatus)*** attributed to Brevetoxin along the mid Atlantic coast. In November 1987 till early 1988, there was an algae bloom due to G. breve in North Carolina coast resulting in 48 cases of NSP in humans (8). Red tide due to ***H. akashimo*** have caused mortality on cultured fish.

Florida and Gulf of Mexico -

As early as 1946, there were already reports of bottlenose dolphin mortalities in Southwest Florida due to an etiologic agent which later on was identified as brevetoxin. In 1987 and 1988, there were reports of bottlenose dolphin deaths associated with brevetoxin (8).

On June 16, 1996, three patients developed symptoms of NSP according to the Sarasota County Health Department. They had eaten clams (Chione cancellate) and whelks (unidentified or unknown? species) harvested from areas closed for harvesting clams. The clams were cooked until they opened. From March to April 1996, there was a significant red tide dinoflagellate bloom in the South coast of Florida resulting in the deaths of at least 149 manatees (8).

West coast - In California, brevotoxin was identified as the cause of summer mortality in common murres (Fleming and Baden, 1999). There were reports of mortality of cultured fish by ***H. akashimo*** (Van Apeldoorn et al, 2001) (8)

Mexico - In Gulf of Mexico, along the coasts of Veracruz and Tamaulipas, G. breve developed huge blooms almost every year in the autumn killing fish. Beginning in 1994, this algae bloom increased in permanence, resulting to in fish kills (8). Residents were affected by exposure to sea sprays or immersion in seawater.

Hongkong - In March 1987, Hong Kong Special Administrative Region reported the first harmful bloom of raphidophytes in Yim Tim Tsai. In 1991, a bloom of ***Chattonella*** marina occurred, killing fish and posing a serious threat to finfish cultures (8).

Japan - In 1972, a red tide of F. japonica was reported from coastal areas of Ehime Perfecture causing heavy mortalities of caged young yellowtail. This red tide was later reported in Atsumi Bay, the Seto Inland Sea and Harima Nada (8).

Republic of Korea - Red tide due to H. akashiwo was reported in embayments, killing cultured fish (8).

Australia - In Boston Bay, Southern Australia, an algae bloom of Chattonella marina occurred, affecting the tuna industry. High levels of a breve-like toxin were found the liver of these farmed bluefin tuna. Another finding is the epithelial swelling of the tuna gills with copious mucus. In January 1994, mussels in the Gippsland coast of Victoria were reported to have high level of NSP toxin (8).

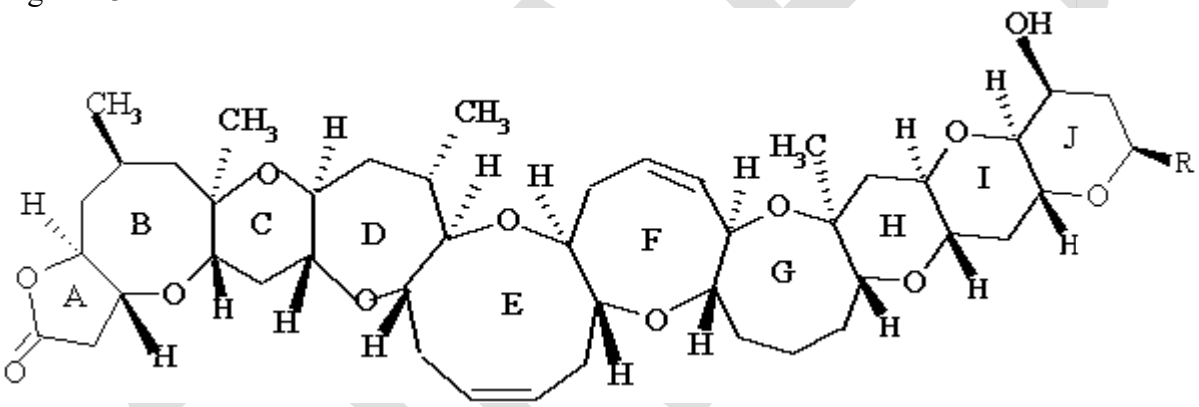
New Zealand - There were 186 cases of NSP recorded (Van Appeldoorn et al, 2001). From September 1994 to July 1996, about 0.2% of weekly samples of shellfish along the coastline of New Zealand showed NSP toxin beyond regulator limits (8).

From mid February to April 1998, there was a severe toxic outbreak in Wellington Harbor that decimated almost all marine life (including seaweeds) (8). This outbreak killed eels, flounders, pelagic fish, and other marine invertebrates. 87 people developed a respiratory illness ranging from dry cough, severe sore throat, runny nose, and skin irritation. The people affected were beach goers, swimmers, and windsurfers. The algae bloom was dominated by ***Gymnodinium*** sp.

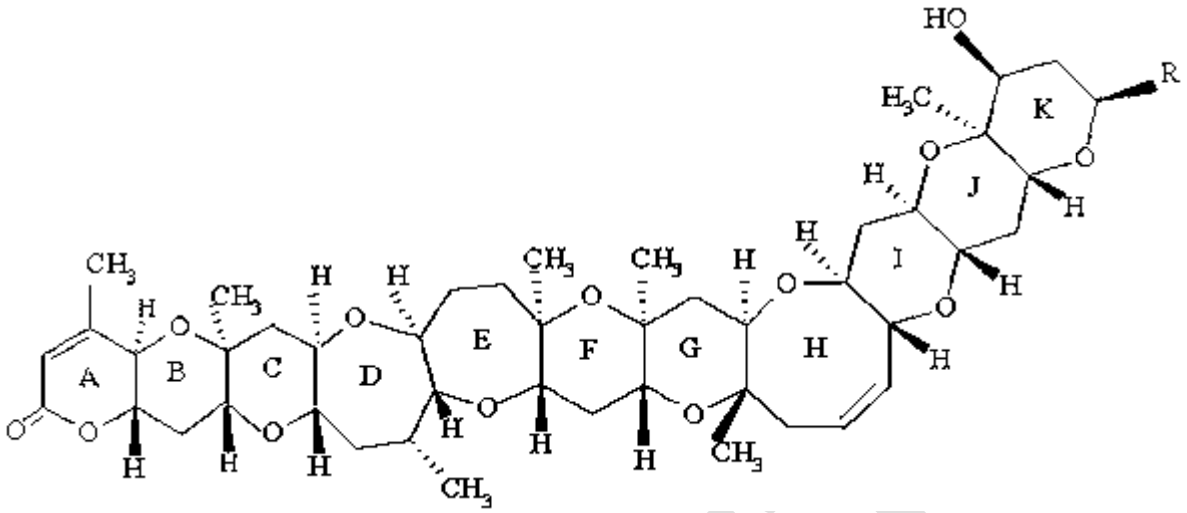
CHEMICAL STRUCTURE OF BREVETOXIN:

Brevetoxin is a heat and acid stable, lipid soluble substance produced by marine dinoflagellates. There are four analogues of brevetoxin isolated from contaminated shellfish: BTX- B1 analyzed from cockles (Ishida et al, 1995), and the BTX- B2, BTX-B3, BTX - B4 analyzed from mussels (Morohashi et al, 1995).

Fig 12-23

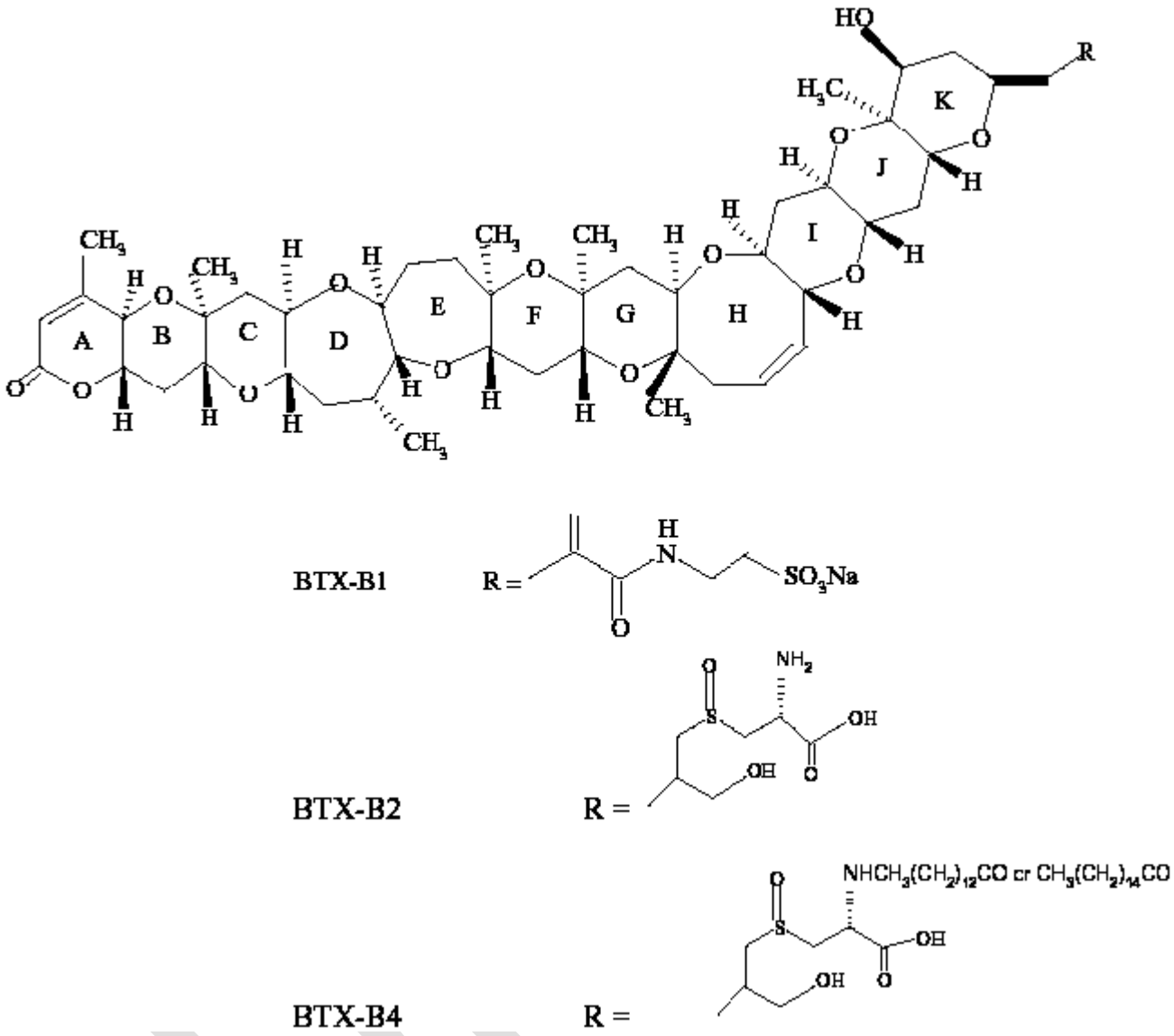


Type 1 (A) brevetoxins: PbTx-1, R = $CH_2C(=CH_2)CHO$
PbTx-7, R=$CH_2C(=CH_2)CH_2OH$
PbTx-10, R=$CH_2CH(CH_3)CH_2OH$



Type 2 (B) brevetoxins: PbTx-2 R = $CH_2C(=CH_2)CHO$
oxidized PbTx-2 R=$CH_2C(=CH_2)COOH$
PbTx-3 R=$CH_2C(=CH_2)CH2OH$
PbTx-8 R=CH_2COCH_2Cl
PbTx-9 R=$CH_2CH(CH_3)CH2OH$
PbTx-5 the K-ring acetate of PbTx-2
PbTx-6 the H-ring epoxide of PbTx-2
Fig 12-18

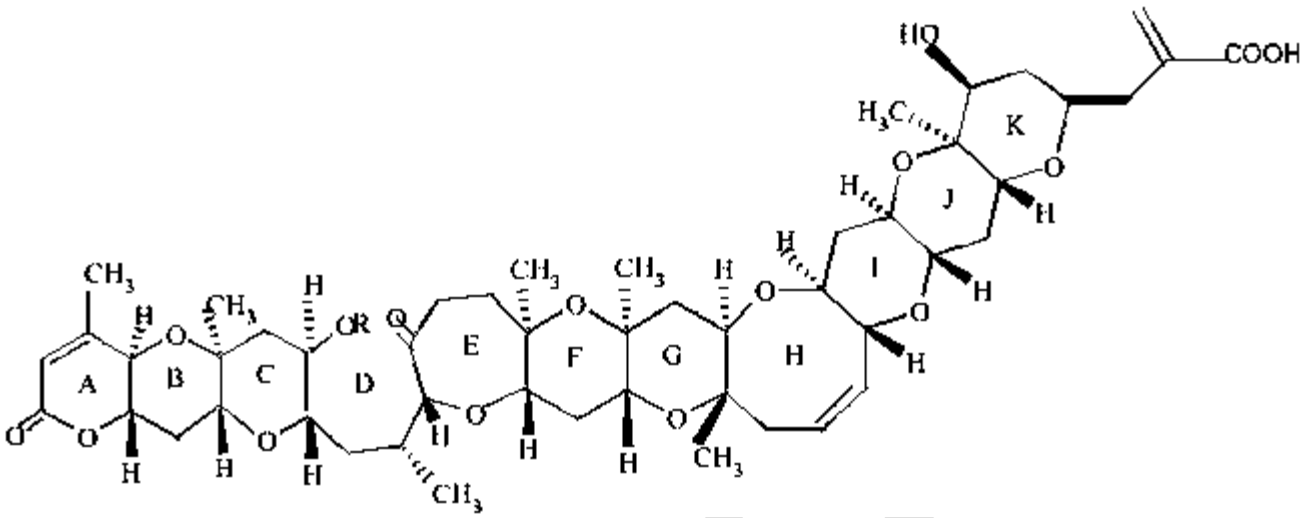
Source: Yasumoto et al., 2001
Fig. 12-28: Chemical structures of type A and B brevetoxins (Hua et al., 1996)



Source: Yasumoto et al., 2001

Figure Fig 12-19: Chemical structures of brevetoxin analogues BTX-B1, -B2 and -B4 isolated from contaminated shellfish

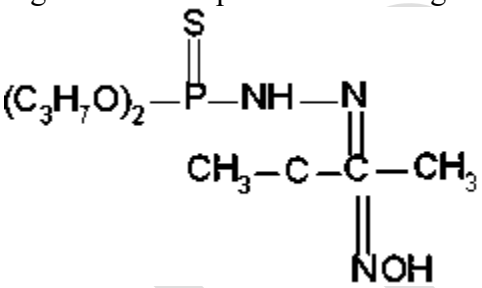
Fig 12-29



Source:Yasumoto et al., 2001
Fig.12-30`: Chemical structure of brevetoxin analogue BTX-B3 isolated from contaminated shellfish

In addition to brevetoxins, some phosphorus containing ichthyotoxic compounds resembling anticholinesterases, have also been isolated from G. breve. One example is an acyclic phosphorus compound with an oximino group in addition to a thiophosphate moiety, namely O,O-dipropyl(E)-2-(1-methyl-2-oxopropylidene)phosphorohydrazidothioate-(E)oxime (Van Apeldoorn et al., 2001).

Fig. 12-31: Phosphorus containing ichthyotoxic toxin isolated from G. breve.



Phosphorus containing ichthyotoxic toxin isolated from G. breve
Source: Food and Agriculture Organization of the United Nation, 2004, Neurologic Shellfish Poisoning, http://www.fao.org/3/y5486e/y5486e0o.htm#bm24

SOURCE ORGANISMS:
Dinoflagellates ***Gymnodinium breve*** aka syn. ***Ptychodiscus breve)***
Algae species belonging to class Raphidophyceae like ***Chattonella antiqua***
Algae species Fibrocapsa japonica
Algae species ***Heterosigma akashiwo***

SHELLFISH CONTAINING NSP TOXINS

Most common shellfish that contain brevetoxin are the oysters, clams, mussels, and welks. These shellfish are not susceptible to the toxin, but fish, birds and other mammals are.

Some copepods (***Temora tubinata, Labidocera aestiva, Acartia tonsa***) have been traced to have brevetoxin; however, in experiments when these copepods were combined with juvenile fish, the fish were not killed.

Brevetoxin were also found in some tuna in Australia, in menhaden and mullet from the coast of Florida, and Muir birds from the coast of California.

PREDISPOSING CONDITION FOR GROWTH

G. breve blooms in the west coast of Florida from summer to winter, more frequently in autumn. They initially appear on summer when the winds are weakest and continue into the autumn when the winds are strong. With these strong winds, these algae are transported to the shore. The growth of these algae are further enhance from additional human made sources.

In the great flood of Mississippi in 1993, huge amounts of agricultural nutrients from Midwest farms were poured into the Gulf of Mexico, creating a so called "dead zone" of water with low oxygen leading to the growth of this Dinoflagellates. It was documented in 1987 and 1988 that wind coming from Gulf of Mexico carried G. breve to the east coast of Florida and north to North Carolina (8).

C. marina belonging to raphidophytes produces brevetoxin in coastal areas rich in organic materials.

H. akashiwo blooms, which are found in the Pacific and Atlantic coasts, requires metals such as iron and manganese, and nitrogen, phosphorous and vitamin B12 for growth. These are supplied by water with low oxygen content plus the wind induced turbulence of water sediments.

The growth of ***Chattonella antiqua*** are supported by nitrate, ammonium and, to a lesser extent, urea. Iron and vitamin B12 promote growth. Maximum/terminal growth is 25 degree centigrade, at salinities between 25 and 41%, and under light intensities above 0.04 ly min

PATHOGENESIS

Brevetoxin opens voltage gated sodium ion channels in the cell membrane. This will enhance the influx of sodium ion altering the cell membrane potential, making it less negative. This will result in slowing of flow of impulse or action potential. Moreover, because these sodium channels are opened, there are persistent activations and repetitive firings.
Neuro-excitation from continuous nerve membrane depolarization leads to spontaneous firing. In most cases, aside from depolarizing the nerves, the muscles are also depolarized. (9) Since the toxin is aerosolized, once inhaled it can cause bronchospasms leading to different respiratory symptoms. Not only that, but brevetoxin can cause the release of the neurotransmitter ACH from the autonomic nerve endings. This will cause contractions of smooth muscles in organs, specifically the lungs, stomach, and

the uterus. In the lungs in particular, contractions of the smooth muscles of the trachea and bronchi cause further bronchial constriction and bronchial spasms, resulting to in breathing difficulty.

Also, there is an increased histamine release which causes the capillaries to dilate and increases permeability. This will allows fluid in the plasma and cells to squeeze between the endothelium of the capillary walls to migrate to interstitial space. This leads to the symptoms of an allergic reaction that an exposed person experiences, such as conjunctival irritation, rhinorrhea, and a non-productive cough.

Since histamine causes vascular dilation and an increase in permeability, it will decrease the intravascular volume, leading to lower fluid volume and low blood pressure.

Histamine is one of the substances that can stimulate hydrochloric acid secretions by the gastric mucosa.

CLINCAL SYMPTOMS / TREATMENT

Oral exposure: Eating raw or cooked contaminated shellfish may cause a toxic syndrome similar to PSP and ciguatera poisoning, but to a lesser degree. The latency period from the intake of shellfish to the appearance of symptoms is usually 30 minutes to three hours and these symptoms last for three to four days The gastrointestinal symptoms are diarrhea, abdominal pain, nausea, and vomiting. In severe cases, the patient may develop hypotension due to increase vascular permeability, arrhythmias, sweating, chills, numbness, and paresthesia of the lips, face, and extremities. Respiratory symptoms such as breathing difficulty follows. If no immediate treatment is initiated, this may lead to paralysis, seizures, or coma. So far, no mortality has been reported from NSP (Cembella, et al 1995).

Treatment of NSP is more symptomatic and supportive as the patients recover spontaneously.
An antispasmodic is given for abdominal pain. If vomiting is severe, an injectable anti-emetic drug can be administered. Particular attention must be given to diarrhea. If the patient shows signs and symptoms of dehydration, fluid administration is a must and electrolyte imbalance, if any, must be corrected. The neurologic manifestations, such as paresthesias, numbness, or weakness are usually self limiting. Respiratory symptoms from oral exposure are milder compared to those from aerosol exposure.

Aerosol exposure - Inhalation of aerosolized surf or its red tide causes respiratory distress and eye and nasal allergy symptoms.

Respiratory symptoms include rhinorrhea, non-productive cough, and difficulty breathing due to bronchoconstrictions. Allergic reactions including conjunctival irritation, copious catarrhal exudates, mucus secretions, and skin rashes are present. Asthmatics and the elderly with chronic pulmonary disease whose respiratory status are already compromised, are particularly susceptible. Brevetoxin - 3 has been implicated as the primary toxin responsible for respiratory symptoms in humans.

Treatments for respiratory symptoms are usually supportive. In normal individuals, respiratory symptoms are usually temporary. The individual should leave the beach and enter an air-conditioned room, and the symptoms will subside.

Dermal exposure - Since the G. breve organism has no outer shell covering like other dinoflagellates, it is easily broken open in the surf releasing the toxin. Direct contact with swimmers causes eye irritation,

mucous membrane irritations, and skin irritations. These are usually temporary and will subside after leaving the beach area and entering an air-conditioned room.

REGULATIONS AND MONITORING IN SOME COUNTRIES

Europe:

Denmark - Monitoring program for selected species exists. For Gymnodinium sp., anything above 5.10 to the 5th power cells/ liter causes the fish harvesting areas to close (8).

Italy - Harvesting areas for fishery products are closed once there is both the presence of algae in water and toxin in mussels (8).

United States of America - A level of 80 mg of PbT-2 in 100 grams of shellfish tissue analyzed by mouse bioassay in shellfish would necessitate regulator action by FDA (8).

Florida and the Gulf of Mexico - Since mid-1970, the Florida Department of Natural Resources has enacted a control program. In 1984, the G. breve concentration exceeded 5,000 cells/liter leading to the closure of shellfish beds. The closure would last from few weeks to six months. This measure above prevents NSP from eating shellfish, but not respiratory irritation associated with exposure to aerosolized red tide toxins. Unlike Texas, Florida does not close beaches for recreational and occupational activities, even during an active near shore algae bloom (8).

Central and South America

Argentina - This country has national monitoring program of mussel toxicity. and The fixed monitoring station is in Mar de Plata (8).

Brazil – Has a pilot monitoring initiative but does not have a national monitoring program (8).

Uruguay - Has national monitoring program on mussel toxicity and toxic phytoplankton (8).

Oceana:

New Zealand - With the advent of the NSP in 1993, New Zealand created a management strategy of weekly sampling of commercial and non-commercial shellfish harvesting areas, which monitors for phytoplankton (8).

5. AZASPIRACID SHELLFISH POISONING (AZA)

Azaspiracid was identified in 1995, when eight people in the Netherlands became sick after consuming mussels (***Mytilus edulis***) that originated from Killary, Ireland (13). The symptoms were similar to diarrheic shellfish poisoning but investigators found that the outbreak was not caused by DSP toxins (dinophysistoxin and okadaic acid). Furthermore, samples collected were different from the known

samples that cause DSP. The causative toxin was later identified as azaspiracid. Since then, azaspiracid has been isolated in shellfish from countries all around the world.

CASES / OUTBREAKS

EUROPE

Ireland: The first recorded case of Azaspiracid Shellfish Poisoning (AZP) that led to the identification of azaspiracid as the causative toxin occurred in Ireland in 1995. Eight people became ill after consumption of mussels (Mytilus edulis) from Killary Harbor, Ireland. They presented with gastrointestinal symptoms similar to DSP, but it was noted that their okadaic acid and dinophysistoxin levels were low enough to not cause symptoms. They later found out that the toxin was azaspirtoxin. In the following years, several ASP incidents were reported in the Arranmore Island region of Donegal, Northwest Ireland, among other countries. They also noted that it was not only mussels that carries the toxin but other bivalves like oysters.

In 1999, they tested 1,800 samples and 5% tested positive for azaspiracid. (13)

Norway - Azaspiracid has been identified in mussels. (13)

Portugal - There were some reports of toxicity similar to AZP from cockles (***Cerastoderma edule***). (13)

United Kingdom - Azaspiracid has been found in mussels. (13)

Figure 1: Occurrence of AZP toxins in coastal waters of European ICES countries from 1991 to 2000

Fig 12-32

Source: Food and Agriculture Organization of the United Nation, 2004, Marine Biotoxin, Azaspiracid Paralytic Shellfish Poisoning,
Reproduced with permission

CAUSATIVE ORGANISM

Azaspiracids are produced by the small dinoflagellates ***Azadinium spinosum*** and ***Protoperidinium crassipes***. These dinoflagellates are eaten by bivalves such as oysters, mussels, and scallops,also by marine crabs.

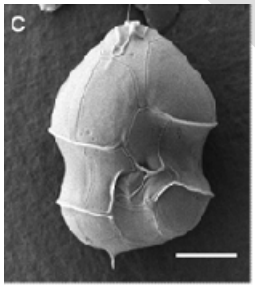


Fig 12-33- Azadadinium sp (SEM) [xxviii]

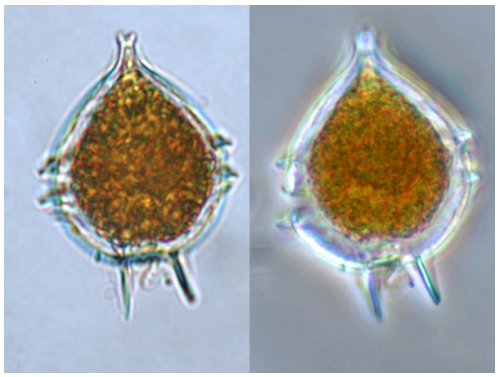


Fig 12-34 Protoperidinium steinii. Photo by Rajashree Gouda[xxix]

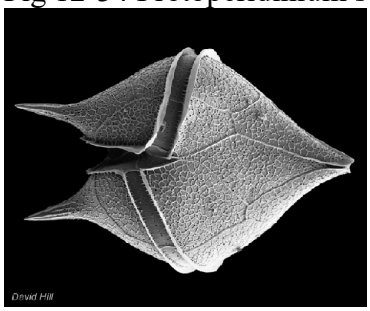


Fig 12-35- Protoperidinium sp. (SEM) Photo by David Hill[xxx]

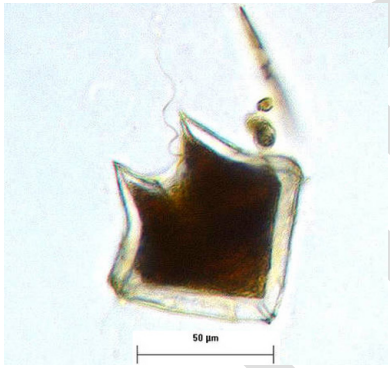


Fig 12-36- ***Protoperidinium sp.***[xxxi]***(LM light micrograph))***

Mechanism of Action: There is no data for mechanism of action for azaspiracid; however, research has determined that the lethal dose of AZA is 150 ug/kg. Injection of this lethal dose to the mice causes the swelling of the liver due to fatty accumulation and vacuole formation in the hepatocytes. The stomach became swollen leading to erosion and bleeding. It causes pyknosis in the pancreas leading to fragmentation of the nucleus and cell apoptosis. Dead lymphocyte debris were present in the thymus and spleen. No histological changes were reported in the heart, lung, and kidney. In another study, they injected acetone extract from contaminated mussels to the mice intraperitoneally causing "neurotoxin-like" symptoms such as respiratory difficulties, sluggishness, spasm, progressive paralysis, and death within 20-90 days. These pathologic changes are considered different from those changes induced by toxins of other shellfish poisoning.

CHEMICAL STRUCTURE

Azaspiracid is a colorless, amorphous substance with no UV absorption maxima above 210 nm. Azaspiracid (AZA) has four analogues, AZA 2 to AZA 5. AZA 4 and AZA 5 are oxidized metabolites of AZA 3. So, AZA, AZA 2, and AZA 3 are likely the genuine substance that causes Azaspiracid Shellfish Poisoning. This compound is considered stable because its toxicity remains unchanged even if heated at 5oC for 150 minutes in 1.0 N acetic acid/ methanol. Figure 2 is the chemical structure of AZA.

: Chemical structures of azaspiracids [xxxii]

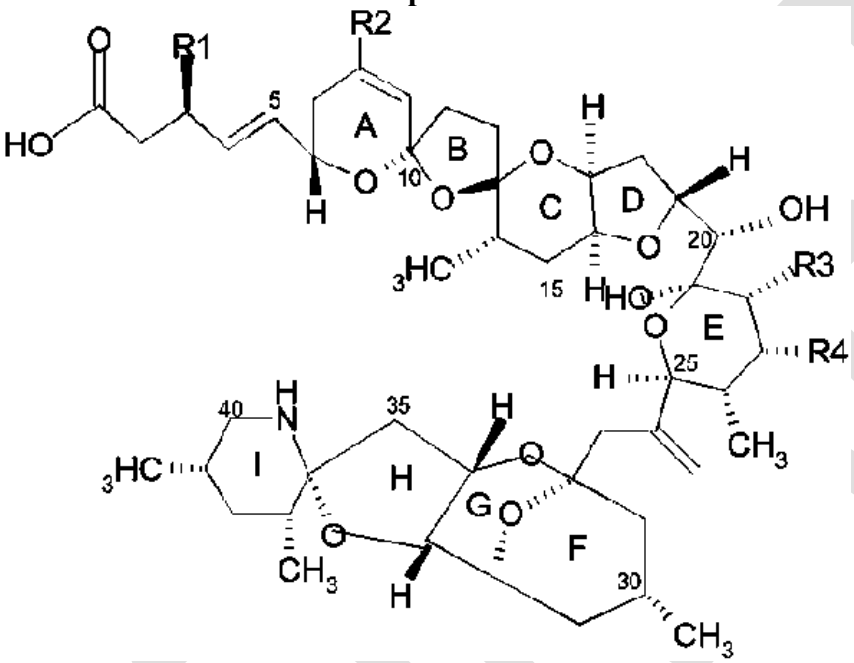


Fig 12-37
Source: Food and Agriculture Organization of the United Nation, 2004, Marine Biotoxin, Azaspiracid Paralytic Shellfish Poisoning, **http://www.fao.org/docrep/007/y5486e/y5486e0p.htm#bm25**
Reproduced with permission

Clinical Manifestation: Symptoms of AZP are similar to those from diarrheic shellfish poisoning such as nausea, vomiting, stomach cramps, and diarrhea. Investigators initially thought that these were DSP cases, but the toxins responsible for DSP were very low, so it was thought that these cases were of a different entity. These symptoms usually appear within hours of ingestion and persisted for 2-3 days, after which there was a full recovery.

As to the long-term effects, studies showed AZA can cause damage to epithelial cells of the intestinal tract explaining the diarrhetic symptoms in humans. Because of these finding, it is possible that AZA may lead to chronic gastrointestinal conditions such as Crohn's disease, ulcerative colitis, or even cancer of the gastrointestinal tract, but it remains to be established.

For AZP management, for persons suffering from generalized manifestations, the treatment is symptomatic such as antipyretic for fever and antispasmodic for abdominal pain. Since patients may develop diarrhea and vomiting, make sure that their electrolytes are balanced by giving intravenous fluid if necessary. No mortality has been reported to date.

REGULATION AND PREVENTION:

In 2001, the EU Commission for Consumer Health and Protection sponsored a workshop and established a regulatory limit of 160 ug AZA/ kg whole shellfish flesh. Since this regulatory limit was established, there were only five reported AZP events between 1995 and 2000, a period of over seven years. This proved that a regulatory mechanism is very important in prevention of diseases. (14)

Depuration has been suggested as another way to prevent AZP. This is a procedure by which marine or freshwater animals are placed into a clear water environment for a specified period of time to purge them of biological contaminants and impurities. This preventive method was mentioned as a way of freeing shellfish from the toxin AZA. Some researchers opined that this might be a slow process considering that the toxin is located in the digestive tract and other tissues of mussel.

PALYTOXIN POISONING

Introduction:

Palytoxin is a very potent and dangerous marine toxin that can be acquired by inhalation. ingestion, and skin exposure. This toxin has been sourced from some marine corals in the aquarium at home. This toxin can be found also in certain marine bacteria and some fish and seafoods in tropical countries. Here are some reported palytoxin poisoning.

A case of a a man together with his wife and daughter went to the hospital due to shortness of breath, worsening non productive cough. These were followed by fever, chills and myalgia. These symptoms started after the man cleaned an exotic corrals in the aquarium. His wife was in the basement while his daughter was upstairs. All the three persons in the family had the same symptoms with degree of severity commensurate to the distance from the aquarium. This cause of their symptoms were later sourced from soft marine corals belonging to the Zoantharian (zoanthids) genera known as Palythoa.[xxxiii]

In May 2017 in Adelaide, Australia, a family member cleaned the saltwater aquarium inside the house by removing and scrubbing unwanted growth in the rocks and corals. Unknowingly, the aerosolized palytoxin was released. All the family members in the house developed severe shortness of breath ending up in the hospital. [34]

Between 2000 and 2004, The US National Poison Date System reported 171 + phone calls from people who have inhaled or came in contact with the palytoxin. 80% of these cases were call from the residences leading them to surmise that the cause was from the home aquarium. [34]

In August 2019, a family of five from Shropshire, England was poisoned and hospitalized after cleaning a tropical aquarium. [33]

In 2018 and 2019, two poisonings involving the handling of corals were reported in Quebec.[33]
In 2017, seven family members and their dog were treated for breathing difficulties after their saltwater aquarium was cleaned [33]

Reports of the marine bacteria, fish, and seafood as the source of the poisoning were reported. One was in
in 2005, when 200 beach going tourists developed respiratory symptoms ending up in the hospital instead
in the beach. 10 % of the patients required intensive care. [xxxiv]

In the Philippines, one person died after eating a crab species ***Demania reynaudii*** containing palytoxin. [xxxv]

Causative Organisms:

Palytoxin is produced by species of Palythoa and Zoanthus soft coral. These are the corals used in the house aquarium. People underestimate the health risk of these corals because there are only few reported cases

Palytoxin can be found also in species of the phytoplankton genus Ostreopsis dinoflagellates and other bacteria. Palytoxin can be found in sponges, mussels, starfish, cnidaria and fish and crabs by the process called biomagnification. The thing is that this toxin does not kill the fish, crustaceans, worms and other marine inhabitants. Humans acquired the poisoning by ingesting these marine inhabitants. It is noteworthy to know that palytoxin destroy the stony hard corals but not the soft corals so it is not advised to put them together in the aquarium.

Toxicology

Palytoxin is a huge molecule weighing around 2,680 daltons and has a backbone of 115 carbon atoms long.
A 1989 C&EN story considered palytoxin the Mount Everest of chemistry for the monumental effort required to understand and synthesize the complex molecule.[34] Palytoxin is heat labile so boiling water cannot remove the toxin.

Here is the chemical structure of palytoxin.[xxxvi]

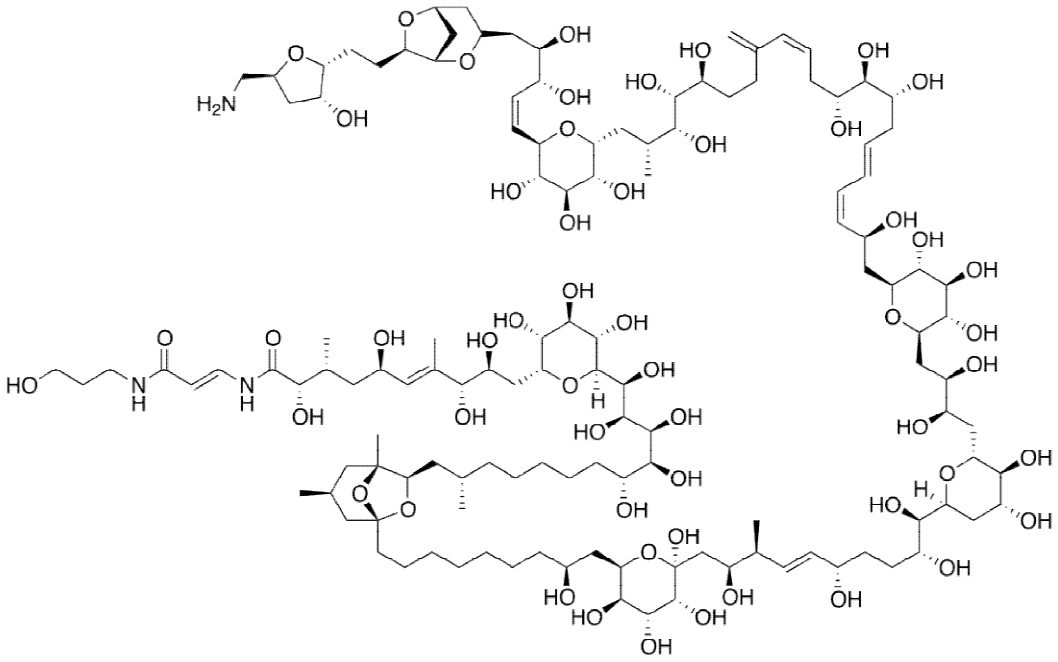


Fig 12-38

Mechanism of action:

Palytoxin binds with the Na+/K+-ATPase, a trans membrane protein enzyme found in cell membrane of every vertebral cells. This enzyme is necessary to start Na+/K+ pump. This pump is very important in maintaining homeostasis of the cells. It pump Na+ out of the cell and pumps K+ ion into the cell to maintain the concentration gradient between the intracellular fluid and extracellular fluid. This is necessary for viability of all cells. Normally, there are more Na+ outside the cell and there are more K+ inside the cell. When a stimulation happens, these Na+ channels open and there is sudden rush of Na+ into the cells. To counteract this event, the K+ rush out of the cell to go to the extracellular fluid. Eventually this Na+ inside the cell (as a result of rushing in) must be brought back outside the cell and the K+ outside the cell (as a result of rushing out) has to go back into the cell. This is accomplished by Na+/K+ pumps. Disruption of the pump would cause free diffusion of Na+ and K+ in and out of the cell eventually leading to loss of the normal electrolyte gradients between the inside and outside of the cell. In RBC, this will result to hemolysis of blood cells while in the heart and muscle cells, it cause continuous contraction of the cells.[xxxvii]

https://en.wikipedia.org/wiki/Palytoxin#:~:text=Palytoxin%20is%20a%20polyhydroxylated%20and,over%201021%20alternative%20stereoisomers.

Palytoxin can also affect the eyes. It causes corneal edema, corneal ulcer leading to blurring of vision. The posterior surface of the cornea of the eye is lined by simple squamous epithelium with Na-K Pump keeping sodium and water out of the cornea to make the cornea clear. Disruption of this cellular pump cause more water
to remain in the cornea because it cannot be pump out. This hydrated cornea becomes edematous and later develop bulla and ulcers leading to blurring of vision

Clinical Manifestations:

Palytoxin is acquired by ingestion, inhalation, skin exposure (through skin wound) and ocular exposure. Among these routes, the skin and respiratory routes pose grave problems for human health. The thing is that the palytoxin does not kill these marine inhabitants and but harmful to human. Upon ingestion, the toxin accumulates in the bloodstream and spread to the different organs of the body causing the systemic symptoms. These symptoms appear suddenly within minutes to hours after exposure.

The respiratory symptoms are cough, sore throat, runny nose shortness of breath with fever and in severe cases,
accumulation of fluid in the lungs (hydrothorax) that may result to respiratory failure. The 200 patient-tourists (see introduction above) in Italy presented with fever, cough, sore throat and dyspnea. Ten percent of these patients were brought to intensive care unit.

In another reported case in Virginia, a patient developed respiratory symptoms after cleaning a zoanthid coral with boiling water. Physical examination revealed a febrile, tachypneic and tachycardic patient.

Those patients who acquired the palytoxin from ocular exposure, develop conjunctivitis, photophobia, blurring of vision, and worse corneal ulceration.

Those who acquired the toxin from skin exposure develop rashes, itchiness, numbness, and dermatitis

Those patients who acquired palytoxin by ingestion, developed abdominal cramps. nausea, vomiting, diarrhea. There might be some oral symptoms such as distortion of taste called dysgeusia, or paresthesia of the perioral area. Once the toxin enters the bloodstream, patient may develop systemic symptoms affecting the nervous system such as dizziness, speech disturbance, tremors, and numbness of the extremities. The toxin can cause damage to the cardiac muscles manifested as irregular slow or fast heart rate, low or high blood pressure.
Severe cases can develop multi organ failure such as renal failure, heart failure and respiratory failure.

Diagnosis

Diagnosing Palytoxin Poisoning is mainly based on clinical presentations and history of exposure. There is no confirmatory test nor a laboratory marker for this type poisoning. Other types of poisoning may present similar manifestations making it hard to diagnose this condition. Presented with this typical symptoms of poisoning, the Palytoxin poisoning is considered more of a differential diagnosis than a primary diagnosis. The only item that will pinpoint to Palytoxin poisoning is the exposure to aquarium. Public and even medical professional have little awareness even though it is a serious condition.

A medical professional can request for Chest X-ray to check for lung conditions specially pumonary edema and congestion, EKG to check the status of the heart. Laboratory examinations like CBC is important because cases of Palytoxin poisoing usually cause Leukocytosis (increased numbers of WBC). Arterial blood gasses determination (ABG) can be requested to check for hypoxemia. For severe cases, the major organs of the body must be checked like requesting for liver function tests to check the liver status, kidney function tests to check for the renal status.

Treatment:

No definite treatment nor antidote for Palytoxin poisoning is available. Generally, the treatment is supportive and symptomatic. Giving antipyretic for fever, anti-tussive medications for cough, bronchodilators for dyspnea, antidiarrheal and anti-emetic for gastrointestinal symptoms and anti-arrhythmia for heart involvement. Oxygen inhalation for hypoxemia and IVF administration for hypotension and electrolyte imbalance.

Serious cases however must be hospitalized. There was one case in Danville, PA who was hospitalized due to fever and dyspnea [35]His condition deteriorated and he was transferred to ICU. In this case, the blood oxygen level spiraled down and he was connected to mechanical ventilators. In serious gastrointestinal symptoms with diarrhea and vomiting, electrolyte imbalance is the usual complications. The electrolyte level in the blood must be monitored and corrected right away for any imbalances. If the palytoxin was taken within 3-4 hours, gastric lavage maybe done to bring out the toxin from the stomach. Those patients with cardiac involvement develop arrhythmias. Such patients must be put to CCU and hook to cardiac monitor. Treating the arrhythmias is a must to prevent heart failure.

Those patient with ocular symptoms are usually given artificial tears and corticosteroids

In dermal exposure, antihistamine maybe given. Topical corticosteroids can be given too.

There is no antidote for Palytoxin poisoning at this time. Palytoxin however, being a very potent vasoconstrictor can be treated with vasodilators as an antidote. Examples of these vasodilators are papaverine, isosorbide dinitrate. These drugs must be injected immediately upon exposure.

Preventions

There are no official evidence base guidelines from CDC for Palytoxin Poisoning as quantitative data are still inadequate and further research are still to be done. It is worthwhile though, to give some advice to people handling or maintaining aquarium. These corals indeed add color and beauty to your aquarium, but they also bring naturally occurring toxin.

When cleaning aquarium, one must wear long gloves, eye goggles, and face mask to prevent exposure to aerosolized palytoxin. Better soak the rocks and corals with 10% bleach solution for thirty minutes before cleaning. This will neutralize the remaining palytoxin. If possible clean the rocks and corals outdoors to disperse the aerosolized palytoxin. If this is not possible, you can clean them indoor with open windows or run an exhaust fan. Do not use pressurized stream water in cleaning because this will aerosolize the palytoxin from the rocks and corals.

As far as eating contaminated fish and crustaceans, this is extremely rare but fatal. Probably this is another reason there is no official CDC guidelines. There are no regulations and toxicity determination on palytoxin in shellfish. There is no way for us consumers to know if the fish. crabs we buy from the market have palytoxin The fact is that the toxin is thermostable and cannot be remove by boiling and they remain stable in aqueous solution for longer period. The good thing is that its toxicity is lost in acidic or alkaline solution. Having said that, probably it will be better to mix the fish, crabs, shellfish to acidic solution like vinegar before cooking

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MICROCYSTIN POISONING (MCYST)

Introduction:

When water bodies are stagnant, warm, rich in phosphorus and nitrogen from sources such as agricultural land and runoff from sewage and septic tanks, the surface water appears in different colors raging from red to brown. They are also called “pond scum" These colors are due to the rapid multiplication of algae called "Algae Blooms". These algae may appear in surface waters or at the bottom of the water. They emit a foul odor described as rotten plant odor. A particular alga that

commonly causes this bloom is the blue green algae called Microcystis, a cyanobacterium. They are called as such because they share common characteristics with bacteria. This algae bloom can occur anytime of the year but more commonly during late summer or early fall. These blue green algae produce several toxins called cyanotoxins and the most common among them is microcystin which will be discussed in this section.

If you happen to wade on this stagnant pond with blue green algae bloom, you may develop skin rashes or respiratory symptoms. Worse if you happened to drink water contaminated with microcystin, you may develop renal or liver damage

Microcystin producing algae blooms are a worldwide problem especially in Australia, Brazil, China, South Africa, United States and Europe countries. The Hartbeespoort Dam in South Africa is believed to be the most contaminated site in the world with cyanobacteria[xxxviii].

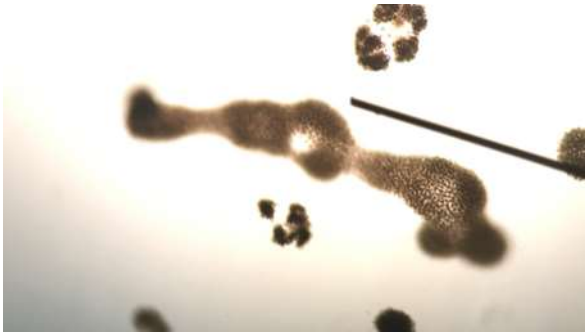


Fig 12-39 Microcystis ***auroginosa*** Photo by David Arieti

Toxicology

Microcystin is a toxin produced by the freshwater cyanobacteria of Microcystis species but is also produced by other species which include . ***Anabaena, Hapalosiphon, Nostoc, Planktothrix, and Phormidium.***

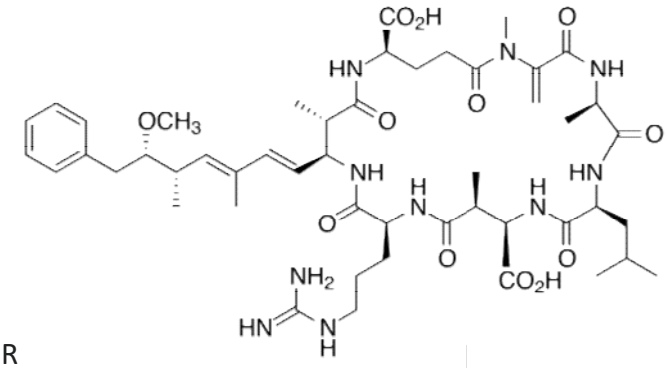
. This toxin is lethal and is one of most studied toxins by scientists, biologists and ecologists.

Microcystin is relatively stable and resists hydrolysis, oxidation and other common chemical reactions. Ordinary cooking will not eliminate the toxins. it breaks down in at ph. extremes. There are some bacteria that produce proteases (enzymes that breakdown proteins) and these enzymes break down microcystin. These types of bacteria however are seldom found in the water bodies.

Microcystin LR has several non-proteinogenic amino acids which are covalently bonded and inhibit protein phosphatase PP1 and PP2A. We know that these phosphatase enzymes are necessary for cellular phosphorylation. This biological process is an important part in mitosis, signal transduction, cell growth, protein synthesis, activation or deactivation of some cellular enzymes. Failure of this biological

process can lead to abnormal proliferation, differentiation of cells, cancer or apoptosis (cell death). Although microcystin causes gastrointestinal symptoms, neurologic symptoms, the main effect of the toxin is in liver cells. The liver cells enlarge, bleeds and become congested, eventually leading to liver cell necrosis. It is interesting to note that microcystin is a potent tumor promoter in rats. It acts as tumor initiator. In China, there were reports of liver cancer from ingesting microcystin contaminated drinking water.

In February 1996, in Caruaru, Pernambuco state in Brazil, 116 (89%) patient out of 131 undergoing dialysis developed neurologic and hap ototoxic symptoms after undergoing dialysis. 100 patients developed acute liver failure and 76. them died. The microcystin originated from the a lake they used as the source of water due to severe drought. This lake was found out later to have blue green algae bloom.



xxxix

Chemical structure of Microcystin LR[xl]

Fig 12-40

Symptoms:

The microcystin can be acquired through direct skin contact as you wade intentionally or accidentally in a pond with blue-green algae. It can be acquired by inhalation of airborne droplets containing the toxin in boating or waterskiing . and acquired accidental by swallowing water from this pond. Animals that are fond of wading or swimming on the pond are vulnerable because they usually lick their body to dry off after leaving the water.[xli] Symptoms of Microcystin poisoning may appear several hours to days after exposure but the usual incubation period is within the first week after exposure.

Those individuals who had skin contact with the toxin, present erythema on the skin, with blisters, hives, rashes, irritations and other allergic like manifestations. They are more frequently located on the lips and under the swimsuits.

Those individuals who acquired the toxin by inhalation would present respiratory symptoms, runny nose and eye, dyspnea, sore throat, cough, other asthma-like symptoms and allergic-like symptoms.

Those individuals who accidentally drink water with the toxin would develop nausea, vomiting, diarrhea, fever, headache. Ingestion of greater amounts of toxin can cause liver damage called acute hepatic necrosis which will eventually lead to hepatic failure which is usually manifested as pain in the right upper quadrant, jaundice, elevated liver enzymes levels and neurologic symptoms such as disorientation.

Wildlife, livestock and even pets are more vulnerable than humans as they swim. wade and drink water in the ponds and lakes. Symptoms of microcystin poisoning in the animals includes vomiting, anorexia, diarrhea, excessive salivation and difficulty of breathing. In severe cases, they may develop seizures or death.

Diagnosis:

Presented with sudden onset of gastrointestinal symptoms such as nausea, vomiting and diarrhea with neurologic symptoms of weakness, headache, and disorientation followed by symptoms of acute liver failure, one has to consider microcystin poisoning. This is further supported if the patient has a history of exposure to stagnant water bodies or has accidentally drank water from those water bodies.

Water samples can be tested by microscopy for the presence of blue green algae. It can be analyzed for the presence of microcystin by the ELIZA test or liquid chromatography- triple mass spectrometry. These examinations are offered in California Animal Health and Food Safety Laboratories in Davis, California.

(http://cahfs.ucdavis.edu) and Auburn University CyanoPros,

(http://www.cyanopros.com).

If there are elevations of these of liver enzymes (alkaline phosphatase, ALT, AST) in blood levels then it will further boost your diagnosis of microcystin poisoning.

Other tests to determine microcystin are kidney function tests (blood urea nitrogen level, creatinine lever), serum electrolytes and chest X-rays if respiratory symptoms are more pronounced.

Treatment:

There isn't an antidote for microcystin poisoning. The treatment is symptomatic and supportive. For gastrointestinal symptoms, the patient must be put to NPO (nothing per orem –nothing by mouth)) and if necessary, give the patient fluid and electrolytes intravenously. If the intake is within 3-4 hour before, gastric lavage is utmost importance to remove the toxin from the stomach.

Those patients with more severe poisoning develop liver damage manifested as jaundice, fever, confusion or disorientation. They are monitored in the hospital. Treatment is geared to prevent hepatic encephalopathy, which is basically hepatic failure. The damaged liver cannot breakdown the toxin in the body which eventually accumulates to affect the central nervous system causing confusion and disorientation. In worse case scenarios there may be a coma.

The goal of treatment is to eliminate the toxins from the body. They are first given lactulose. It act as laxative to draw the toxin to the large intestine and defecated out. They also soften the stools. Antibiotics such as neomycin is given to eliminate bacteria that create the toxin from the digested food thereby decreasing the toxin in the body. Low protein diet is recommended to decrease the production of ammonia.

For those patients with a history of inhaled aerosolized toxin, movement of the patient to non-contaminated areas is recommended. Bronchodilators may be given for patients with difficulty breathing.

If you come in contact with water contaminated with microcystin, remove your clothing, jewelry and other accessories and wash with fresh water and soap for 10-15 minutes as soon as possible. Antihistamines or steroid cream may be applied for skin rashes, irritation, and itchiness.

For eye exposure, wash your eyes with normal saline for 15 minutes. Remove contact lenses, if symptoms persists after washing, consult an ophthalmologist.

Prevention:

For the public especially children and pregnant women, avoid wading, playing or swimming in the lake, pond and other water bodies that appear with different colors because they are likely to have algae blooms. Refrain from drinking or swallowing any recreational water especially those coming from lakes or streams.

For livestock and other animals, they should be prevented from staying close to water bodies with algae blooms. When they get out of water after wading or swimming, they lick their body to dry off, thereby acquiring the toxin. If there is no other water area for drinking, these animals should drink on the shore of the lake only. Placing barriers such as logs, floating plastic to keep the algae bloom from reac ***Demania reynaudii***

hing the shore were proven to be ineffective.

From the government side, several water treatment techniques have been used. Among them, are chlorination and ozonation. Chlorine and ozone are oxidants that kill cyanobacterium cells. Some environmentalists are skeptical of these techniques because as the cells are killed by the chemicals,

they release the mycotoxin in the water. Unless another process is added to remove these toxin, these techniques are not recommended.

Another chemical compound they used is copper sulfate (CuSO4) or any copper based compound. It prevents the formation of cyanobacteria algae blooms. The formula of the mixture of copper and water is based on gallons of water or acres of water bodies. This technique is different from ozonation or chlorination because copper sulfate prevents the formation of cyanobacteria.

Of particular attention is addition of chemicals that bind and remove phosphorous from the eutrophic water. This type of water is rich in chemicals which serve as nutrients for the algae. These waters are usually run off from chemical factories, agricultural land using pesticides and fertilizers and from commercial establishments. Phosphorous is one of the nutrients for algae formation. Among the chemicals that bind with phosphorous are aluminum sulfate, ferric chloride and some particles in clay. Another chemical that binds and removes phosphorous from water bodies is Lanthanum. This is a naturally occurring earth element that is commercially used to increase the water qualities in ponds, lake, reservoirs. This compound has been patented in USA since 2010.[xlii]

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6. PALM ISLAND MYSTERY DISEASE (CYN)

In November 1979, an outbreak of a "hepatitis-like illness" (associated with dehydration and bloody diarrhea) was reported in Palm Island, northern Queensland, Australia. These involved 148 people (10 adults and 138 children) of Aboriginal and Torres Strait Islander descent. Investigators found that all the patients drank water from the same source, the Solomon Dam. Residents who had other sources of water were not affected. A few days before the incident, the Solomon Dam was treated with copper

sulfate to control the algae bloom. Copper sulfate at 1ppm (1 ug/ ml) breaks the cyanobacteria and releases its toxic components into the water (16).

Investigators received samples of water from the dam, cultured the organism, and the results were administered to mice. The mice later developed tissue injury on their gastrointestinal organs, kidneys, and liver. It was determined that the causative algae was ***Cylindrospermopsis***. They later identified the cyanobacteria as ***Cylindrospermopsis rackborskii*** and the toxic element produced as cylindrospermopsin raciborskii. Eventually, the identified toxin produced by this cyanobacterium was identified as cylindrospermopsin (CYN).

Fig. 11-7: *Cylindrospermopsis raciborskii* (microscopic picture, upper left corner) can form toxic cyanobacterial blooms (big picture).

Source: https://natoxaq.ku.dk/toxin-of-the-week/cylindrospermopsins/

(Still waiting for permission to reproduce, otherwise I will delete the picture)

Fig 12-41 Palm Island from Wallaby Point, Queensland , Australia[xliii]

The affected people manifested anorexia, malaise, headache, and vomiting with initial constipation followed by bloody diarrhea at varying degrees of dehydration. Laboratory results showed elevated liver enzymes indicating liver damage. Most of these patients needed intravenous fluid while those with a severe condition developed hypovolemic/acidotic shock. The stools and food were examined, eliminating common infectious organisms and toxins as possible causes of the outbreak. All patients eventually recovered after treatment. There was no mortality.

Causative organism: Cylindrospermopsis rackborskii which produces a toxin called cylindrospermopsin.

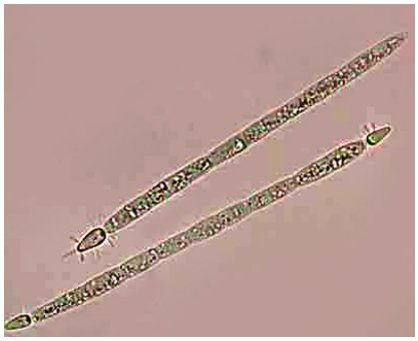
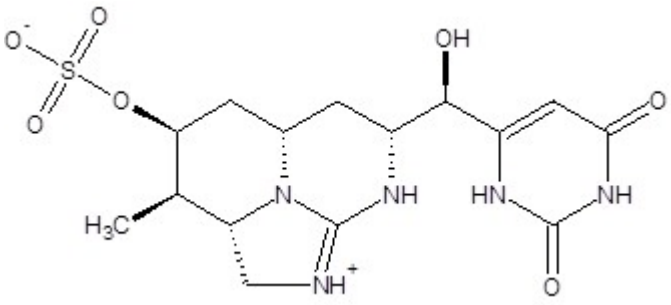


Fig 12- 42 Cylindrospermopsis sp.[xliv]

Figure 12:43 Here is the chemical structure of Cylindrospermopsin (CYN.[xlv])



Source: https://natoxaq.ku.dk/toxin-of-the-week/cylindrospermopsins/

Cylindrospermopsin is a potent inhibitor of protein synthesis produced by cyanobacteria, most commonly by ***C. rackborskii***. This toxin can cause cell death with the liver as the main target organ (15). It has an uracil moiety attached to guanidino moiety suggesting that it may have a carcinogenic effect. CYN is not easily degraded in water because of its high water solubility and stability to a wide range of heat, light, and pH.

CLINICAL MANIFESTATIONS: The mode of transmission for most of the documented cases of Palm Island Mystery Disease is drinking water. The main manifestations of the patient involved is in the gastrointestinal tract. These symptoms are abdominal pain, nausea and vomiting, bloody diarrhea, and headache. Another mode of transmission is by direct contact. The patient may develop skin or eye irritation. If inhaled, it may cause a sore throat, dry cough, or worse atypical pneumonia.

TREATMENT: As used in any other type of poisoning, if the case is of mild to moderate severity, the management is symptomatic or supportive. For gastrointestinal symptoms, fluids and electrolytes balance is very particularly important. If needed, the patient should have intravenous therapy.

For severe cases, aside from the supportive management, the patient must be monitored for the possibility of liver toxicity since the main target of the toxin is the liver. Have a liver enzyme determination especially for a patient with persistent symptoms. Some patients may developed atypical pneumonia and/or other respiratory symptoms. The respiratory functions must be well monitored in these cases.

PFIESTERIOSIS IS CAUSED BY THE DINOFLAGELLATE, PFIESTERIA PISCIDIDA

7. PFIESTERIOSIS / PFIESTERIA POISONING (Pf)

(Pfiesteriosis is the general name for any or all symptoms a human may experience after exposure to ***Pfiesteria piscicida(Pp)***. Pfiesteria poisoning is a general name for any or all manifestations of fish infected with ***Pfiesteria piscicida.***)

In 1988, researchers at the College of Veterinary Medicine at North Carolina State University were startled to find a significant number of dead fish in their aquaria. It was later discovered that the appearance of dead fish occurred simultaneously with an increased number of a specific microalga. The microalga was later identified as a freshwater dinoflagellate named ***Pfiesteria piscicida*** in honor of the late Lois Pfiester, who had done extensive research on this organism. This organism is mainly found in the Albermarle - Pamlico Estuarine System which is the second largest estuary in the United States. Sometime in summer of 1997, this toxic dinoflagellate that causes illness in fish was the hottest topic in

local media in the area. They coined this microalga as "Cell from Hell" or "Fish Killer". At present the common habitat of the ***Pp*** are the areas from the Gulf of Mexico to the Atlantic estuarine waters, including Florida, North Carolina, Maryland, and Delaware. There were some reports of the presence of ***Pf (Pp?)*** in the Mediterranean Sea. (21)

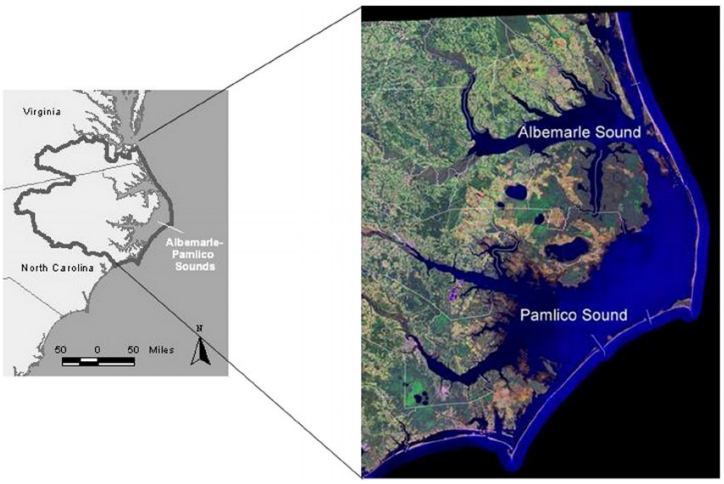


Fig 12-44

The location of the Albemarle-Pamlico Sound Estuary System relative to the State of North Carolina and the southeastern United States

Causative Organism.

The causative organisms are dinoflagellate species of genus ***Pfiesteria (Pp)***. Although many dinoflagellates act similar to ***Pfiesteria***, there are only two species that have toxin producing capabilities. These are ***Pfiesteria piscicida*** and ***Pfiesteria shumway***. The latter was named after renowned scientist Sandra Shumway, who also did extensive research on these toxic dinoflagellates.

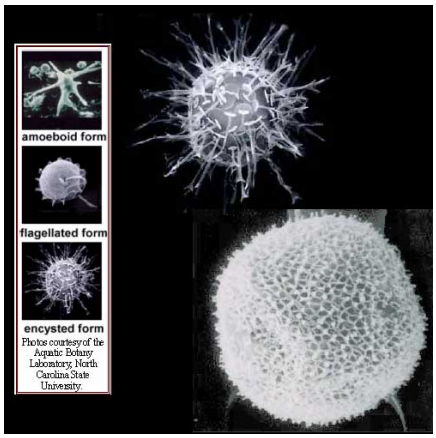


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Figure 12-45. Scanning electron microscopy showing toxic zoospore ***Pfiesteria*** [xlvi]

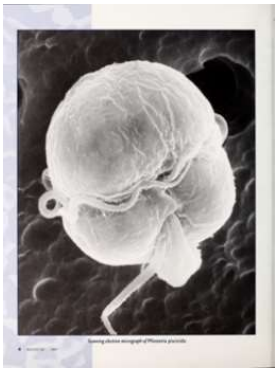


Fig 12-46 Pfiesteria piscicda. Photo by US Sea Grant College program[xlvii]

Pfiesteria piscicida has been found to be active at a temperature range of 9-33 degree Centigrade although most of the growth is at 18 degrees Centigrade. It is also active in brackish water of estuaries with a salinity of 2-20, parts per thousand (ppt) (note: fresh water is less than one and marine water is 35ppt on the salinity scale). (19)

Pfiesteria piscicida has a remarkable life cycle. It was the first toxic dinoflagellate to attack fish prey. Unlike other toxic dinoflagellates that are plant-like organism with pigment that may color water, the ***Pp*** are translucent unless they consumed pigmented fish prey. It is difficult to monitor its presence as it has more that 20 forms or stages in the water column or benthic sediments. This is also the first dinoflagellate to have dormant stages in a form of a cyst that can last for years. When the environment is conducive to their growth, they transform to a motile form that swarm the water and produce toxins. (19)

Pp has versatile nutrition. Its food ranges from bacteria and other algae to mammalian tissue, live or dead. They consume dissolved organic substances found in poorly treated sewage and animal waste. At the initial stage, ***Pfiesteria piscicida*** may be harmless to the fish but further contact will cause these dinoflagellates to multiply and become lethal. This will support the assumption that the growth of ***Pp*** is stimulated by the presence of the fish tissue itself, its secretions, and excretions. Furthermore, they can survive even without organic substances because they have a large food vacuole called kleptocholoplast that stores the chloroplasts they acquired from other algae. These serve as their nutritional supplement. (19)

Several ***Pfiesteria piscicida*** outbreaks in North Carolina happened in estuaries with a high concentration of anthropogenic origin such as animal waste, poorly treated human sewage, cropland, and lawn fertilizer runoff with a large phosphorous content.

Toxin of *Pfiesteria piscicida*

The lethal toxin produced by t ***Pp*** has not been identified yet. There were several toxicity studies done and all of them were not universally conclusive. As of now, what is definite is that the compound

responsible for epidermal damage to the fish is a fat-soluble compound. Another compound obtained from a dinoflagellate was a water-soluble compound with neurotoxin-like properties. Collaborative efforts between NOAA (National Oceanic and Atmospheric Administration) scientists and researchers in North Carolina State University and University of Miami are trying to characterize and identify the toxin.

Mechanism of Action:

Although the mechanism of action has not been established yet because the toxin has not been identified, several studies were done on the effect ***Pp*** toxin on fish and humans. Data shows that the ***Pp*** toxin lowers the fish white blood cells to 40-60% of the normal, compromising its immunity and lowering their resistance making them susceptible to other opportunistic pathogens. The toxin also destroys the osmoregulatory system located in the epidermis of the fish making them vulnerable to the high salinity of the estuary water. The toxin of ***Pp*** was observed to impair the reproductive system of some fish. Commercial fish such as striped bass (***Morone saxatilus***) and killifish (***Fundulus heteroclitus***) do not hatch after exposure to toxin of ***Pp***. (19)

Upon contact with toxic ***Pp*** or even indirectly with only the toxin, the fish became inactive, moving wayward and sluggishly. In acute exposure, the fish develop focal or diffused skin lesions ranging from ulcerations and hemorrhages leading mostly to death. These skin lesions appear within 2 - 12 hours upon exposure. (19)

There is no significant research as to the effects of ***Pp*** toxin in humans, but studies done in rats suggest that the toxin appears to target the N-methyl-p-aspartate (NMDA) receptors. As we know, the NMDA receptor is particularly important in the body. It controls the synaptic activity and memory functions. The toxin inhibits the NMDA receptors in the brain leading to cognitive and memory impairment. The intensity and duration of exposure to produce the symptoms is still unknown, but there is a dose-response relationship as more neurocognitive impairments were seen in people who have had longer exposures to water with fish killers.

Clinical Manifestations:

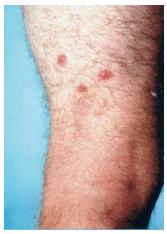
It is surprising to know that the potential effect of ***Pp*** toxin was first noticed in a laboratory setting when marine scientists developed symptoms while studying the effects of the toxin in fish health. One of them was hospitalized due to severe symptoms. Similar sickness was reported among fishermen in Maryland. They observed that those fishermen who had repeated contact with estuary water with fish kill developed the illness. (18)

The usual modes of acquiring the disease are through prolonged skin water contact or inhaled aerosols where fish were diseased or dying and where actively toxic ***Pfiesteria*** populations were present. Unlike poisoning associated with other marine biotoxin; ***Pp*** associated syndrome is not acquired by oral route.

The medical complaints of those exposed to the ***Pp*** toxin are respiratory irritation, eye irritation, gastrointestinal symptoms such as stomach cramps, nausea and vomiting, fatigue, and headache. These symptoms usually last for 2 weeks depending upon the time span of contact. Other individuals manifest neurologic symptoms such as altered mental status, confusion, disorientation, mood and personality changes, dyskinesia, and ataxia. Some individuals' manifest amnesia with impairment of

antegrade memory, less concentration, and impaired dexterity. In some individuals, lumbar tap was performed to assess the affection in the brain. They found elevated protein and immunoglobulins in the cerebrospinal fluid. These symptoms disappear 2-3 weeks after exposure. (18)

Skin irritation is manifested in the form of a burning sensation, itchiness, red bumps, or sores erythematous papules usually in the extremities and trunk. If symptoms persist, individuals may experience numbness and a tingling sensation of extremities. These lesions are suggestive of allergic reaction, inflammatory, or toxic process. (18)



Erythematous papules on legs of person exposed to

waterways where ***Pfiesteria*** species were present. (Photograph courtesy

Dr. Ritchie Shoemaker.) (21)

How about oral route of ***Pp*** toxin? At this time, it appears that ***Pp*** does not adversely affect people who ate seafood originating from areas with know toxic ***Pp***. Was this because of the high acidity of our gastric environment that will neutralize the toxicity? Studies show that it is not because of the inherent human protector in the stomach but with the characteristics of the toxin. The toxin has been observed to be labile and unstable in water. Studies were done when the water taken from estuaries with dead fish were filtered to remove the ***Pp*** strain, the remaining toxin in the water will kill fish only within 8 hours. Beyond this time the fish survive. Some consider the water safe for human use after 24 hours. As a precautionary measure, in Maryland, US their policy was to reopen the areas after three days without fish deaths.

More research needs to be done on the impact of ***Pp*** toxins to human health. Potential effects on humans during ***Pp*** outbreaks cannot be ruled out until more studies are done on the toxin in the fish tissue. The greatest obstacle standing in the way of more research on the ***Pp*** toxin to humans is the lack of an assay for toxin identification.

Diagnosis

Having the symptoms as mentioned above are not enough to have a diagnosis of ***Pfiesteria*** associated syndrome. These symptoms are not reliable to consider this syndrome as these lesions can be caused by fungal infections. A more reliable basis is the history of prolonged exposure to water known to have the ***Pf*** toxin. Although neurocognitive tests are nonspecific, positive results on these tests will support the diagnosis of ***Pfiesteria*** associated syndrome. At present, neuroscientists are working in

collaboration with the CDC to determine basic testing batteries for persons inclined to develop the ***Pp*** associated syndrome.

Regulation and Monitoring

Since ***Pp*** proliferate in an environment with high concentrations of anthropogenic origin such as animal waste, poorly treated human sewage, cropland, and lawn fertilizers, preventative guidelines are geared towards minimizing these factors. (20)

1. Local, state, and federal governments should enact regulations to lower and hopefully end water pollution, especially in estuarine systems.

2. Hog farms should be placed under strict regulations.

3. Companies whose chemical waste is leaking should be forced to meet high standards of containment for the waste.

4. Conduct further research. At this point, many details of ***Pfiesteria piscicida***'s way of life, origins, and effects remain unknown. Federal agencies have allocated $750,000 for ***Pfiesteria piscicida*** research at North Carolina State University. This is a substantial step in the right direction, but until ***Pfiesteria piscicida*** is fully understood, research efforts should continue.

5. Educate the public. Spread the word about ***Pfiesteria piscicida***.

6. Inform farmers and industrial companies about their effects and impacts on the watershed. Teaching people about the potentially hazardous consequences involved with their actions may help to terminate unsafe behavior.

7. Alert the public regularly and frequently about water conditions and warn them about the causes and symptoms of Pfiesteriosis (***Pfiesteria***?).

8. Protect wetlands and marshes which act as buffers from pollutants to rivers and streams. Create and enforce regulations to preserve these fragile and valuable ecosystems.

Regarding the prevention of ***Pp*** associated syndrome in the laboratory setting, there are no federal guidelines or regulations at this time. Federal agencies have recommended standard laboratory practices when handling toxic algae, which includes the use of laboratory coats, gloves, and other personal protective equipments. Universities engaged in ***Pp*** research have constructed a laboratory with BSL 3 (Biological Safety Level 3) protocols, described below.

BLS 3

A BSL-3 laboratory typically includes work on microbes that are either indigenous or exotic (non-native?) that can cause serious or potentially lethal disease through inhalation. Examples of microbes worked with in a BSL-3 includes yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

The microbes are so serious that the work is often strictly controlled and registered with the appropriate government agencies. Laboratory personnel are also under medical surveillance and may receive immunizations for the microbes that they work with.

Common requirements in a BSL-3 laboratory include:

- Standard personal protective equipment must be worn, and respirators might be required.
- Solid-front wraparound gowns, scrub suits or coveralls are often required.
- All work with microbes must be performed within an appropriate BSC.
- Accessible hands-free sink and eyewash stations are available near the exit.
- Sustained directional airflow to draw air into the laboratory from clean areas towards potentially contaminated areas (Exhaust air cannot be re-circulated).
- A self closing set of locking doors with access away from general building corridors.
- Access to a BSL-3 laboratory is restricted and controlled at all times.

CIGUATERA FOOD POISONING

Ciguatera Food Poisoning is the most commonly reported poisoning from seafoods. This is an illness caused by eating reef fish that contain toxin (ciguatoxin) produced by marine microalgae called "Gambier discus toxicus " . Symptoms include nausea, vomiting, diarrhea and neurologic symptoms such as tingling of the fingers and toes, dizziness and weakness. There are more that 400 species of fish, including edible fish such as sea brass, snapper, perch that are contaminated with ciguatoxin. These fish are typically inhabitants of coral reef that consumed the marine microalgae.

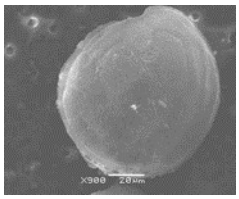


Fig 12-47 Gembierdiscus toxicus[xlviii.]

Incidence:

Ciguatera poisoning have been reported way back in the 1511 by Peter Martyr de Anghera in West Indies. It was also reported in 1601 by Harmansen in the islands of Indian Oceans. By 1606, there were reports of Ciguatera Poisoning in several archipelagos of the Pacific Ocean. The word " cigua" was referring to a certain univalve mollusk (Turbo pica) in Cuba which was believed to contain the toxin. In 1787, Antonio Parra in Cuba transferred the word to describe the intoxication caused by eating the mollusk.[xlix]

In the old days, the incidence of Ciguatera Food Poisoning were limited to coastal areas, islands communities of aboriginal people but now because of increase universal food consumption , commercialism and increase travel, this poisoning has been present worldwide.

Ciguatera Food Poisoning affects all age groups with male and female in equal numbers. It is interesting to note that ciguatoxin was detected in semen that can be transferred to female after intercourse. It was also detected in breast milk, so it is possible to transfer the toxin from the nursing mother to the babies.[l]

Toxicology/Pathogenesis

Ciguatera toxinis odorless, tasteless compound undetected by simple means, It is heat stable. A self-closing set of locking doors with access away from general building corridors.

e, lipid soluble.. It is composed of 13-14 rings fused by ether linkages into a ladder structure. It cannot be destroyed by cooking and exposure to mild acidic and alkaline condition

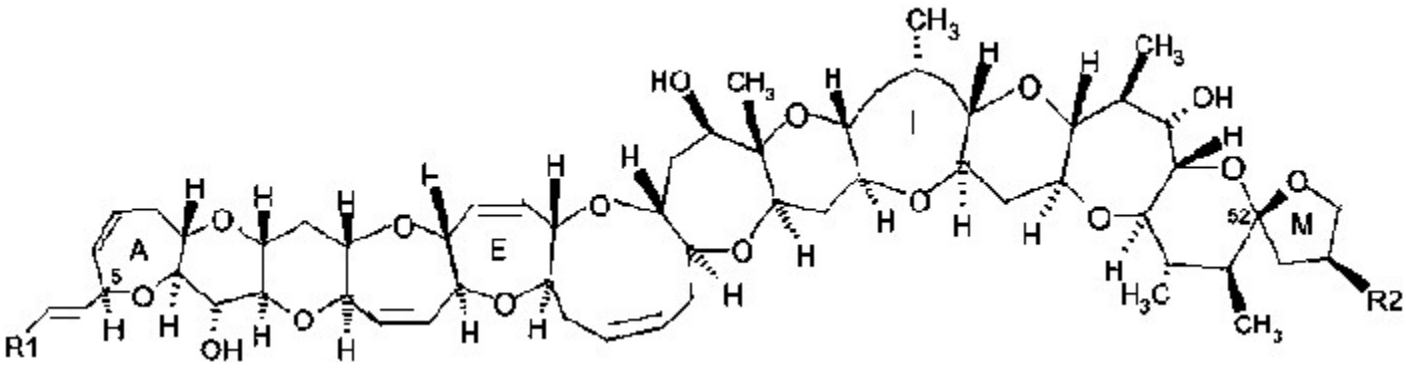
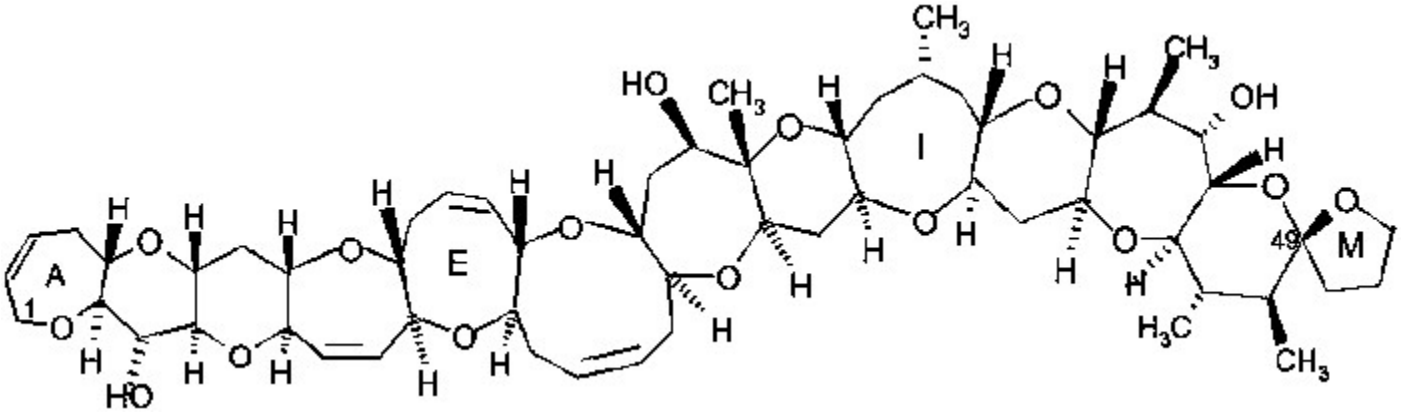


Fig 12-45



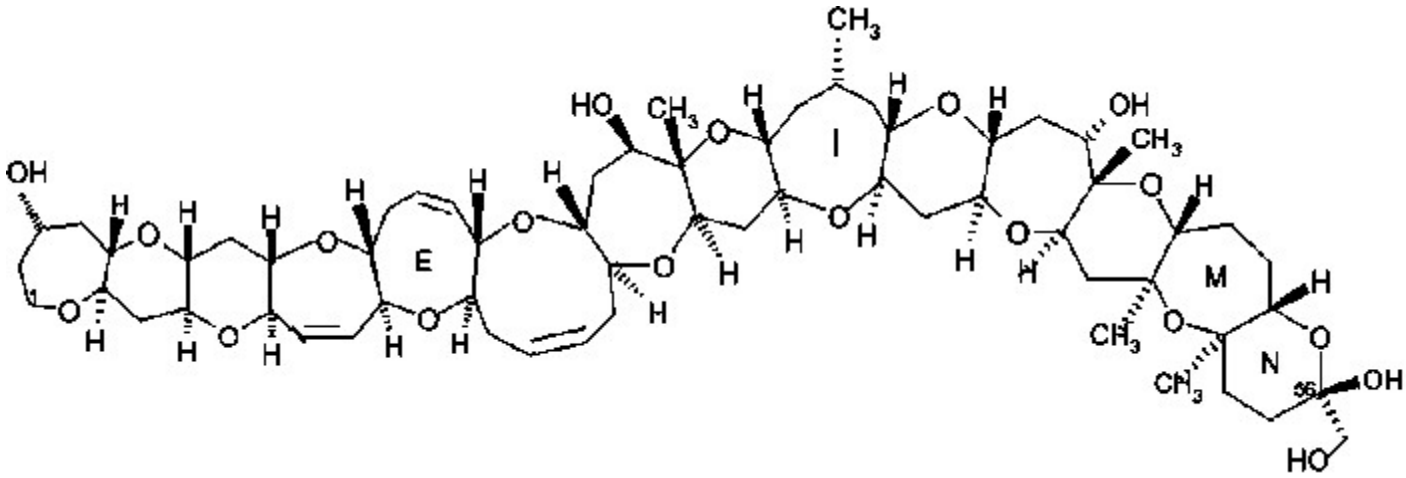


Fig12.48

Structure of Pacific (P) and Caribbean (C) ciguatoxins (CTXs) [li]

Source: http://www.fao.org/3/y5486e/y5486e0q.htm#bm26

Ciguatoxin are produced by marine microalgae called **Gambierdiscus toxicus.** These microalgae actually produce two toxin: maitotoxin which is water soluble and ciguatoxin which is lipid soluble. The maitotoxin has no role in Ciguatera Food Poisoning. The hervivorous fish eat the microalgea that contain the ciguatoxin and eventually eaten by the carnivorous fish. The ciguatoxin has been found in the liver, muscle, skin and bones of the large carnivorous fish which are eventually eaten by humans.

Here are some of the fish that may contain ciguatoxin: moray eel (**Muraenidee**), snapper such as red bass (**Lutianidae**), groupers (Serranidae), mackerel (**Scombridae)**, jacks **(carrangidae**), barracudas (**Sphyraenidae).** [.51]

In the continental US, grouper, red snapper, jack, barracudas have been implicated to cause CFP. From 1954 to 1992, the barracudas has been associated with ciguatera poisoning in Florida.

The ciguatoxin affects the excitability of the cell membranes of the nerve and muscle. It specifically binds to voltage gated sodium channels in the cell membrane causing influx of Na even at resting membrane potential. This cause imbalances among the electrolytes inside and outside the cell. This will result to increase Calcium entry into the cell that will cause increase muscle cell contraction. This will result to significant slowing of nerve conduction and prolongation of absolute refractory period. In the heart it causes increase cardiac muscle contraction (increase inotropic effects). In the intestinal mucosa, the increase Calcium entry into the smooth muscle causes more fluid secretions from the digestive glands leading to diarrhea.

Symptoms

The symptoms of Ciguatera poisoning start as early as 30 minutes after consumptions of the contaminated fish for severe symptoms. For mild form, the symptoms may appear 24-48 hours after consumption. The initial symptoms are numbness of the lips, tongue followed by tingling sensation of the hand and feet. Gastrointestinal symptoms follows which are nausea , vomiting, diarrhea and abdominal cramps. Neurologic symptoms may ensue such as generalized weakness, restlessness,

dizziness, blurring of vision and even coma. Cardiac symptoms may appear in the form of hypotension and bradycardia. Gastro intestinal symptoms last for days but neurologic symptoms lasts for several days. Severe cases can develop hypotension, bradycardia, respiratory difficulties but death is uncommon. The reason why death is uncommon is that the toxin in the fish rarely reach a level lethal to humans. The fish are vulnerable to high level of toxin. Researchers found out that ciguatoxin can be stored in the adipose tissue. Any condition that cause increase lipid metabolism such as in time of stress, exercises, weight loss may result to the toxin re-entering the blood stream and subsequently appearance of the symptoms and signs. This is the reason why the symptoms may last for weeks and months in some patients.

Treatment:

At present, there is no antidote for Ciguatoxin. Fortunately, most of the cases are mild and managed symptomatically such as hydration for diarrhea and vomiting,

paracetamol (acetaminophen) or nifedipine for headache, gabapentine for neuropathic pain. For severe poisoning, a gastric lavage can be administered if the contaminated fish was taken within 3-4 hours before. This means that food eaten is still in the stomach that can be brought out by lavage. Food eaten usually stay in the stomach for 3-4 hours. Severe gastrointestinal symptoms can be managed by continuous intravenous fluid infusion with monitoring of the electrolytes and kidney functions. Patient rarely develop respiratory failure and coma that require intubation and assisted ventilation. For those patients with more pronounced neurologic symptoms such as coma, Mannitol is given as infusion. Mannitol being an osmotic substance will attract fluid as it pass through the brain decreasing the cerebral and neuronal edema that cause the neurologic symptoms. Aside from that, Mannitol attracts free radicals brought about by the ciguatoxin eventually decreasing the action of the toxin to the voltage gated sodium channels. Note that Mannitol serve also as osmotic diuretic that may decrease the total fluid volume so it is recommended that patient must be hydrated first before administering Mannitol.

In some areas in New Caledonia, Western Pacific, inhabitants use traditional herbal medicine to treat ciguatera poisoning. These are extracts from Argusia argentea leaves or Davalliea sp. but there is no scientific evidence of their efficacy[lii]

Prevention:

It is hard to prevent Ciguatera poisoning because of the physical and chemical characteristics of the toxin. It is odorless and tasteless so it is hard to detect contaminated fish. It is heat stable so it cannot be eliminated by boiling, frying, salting, marinating and even freezing. It is interesting to note that most of the fish causing the Ciguateria Poisoning are those fish caught by sport fishing, not from commercial fishing. It is advised not to consume bigger fish because the bigger *the fish is, the greater is the toxin content. This toxin can remain in these fish throughout their* life span. It is also advised not to eat the visceral parts of the fish such as liver, head because these parts contain more toxin that the muscles of the fish.

Prevention is more on community outreach and education of residents especially in endemic areas. They should be made aware of the kinds of fish that harbor the toxin. Here are some examples of those fish:

-moray eel

-barracuda

-grouper

-kingfish

-jacks

-snapper

-surgeon fish

-parrot fish

-wrasses

-hogfish

-narrow barred Spanish mackerel

-coral trout

-flowery cod

red emperor

Any case of CFP, whether it is confirmed or suspected must be reported to authorities. In Florida, there is a law that requires medical practitioners such as physician, chiropractor, naturopathy practitioners and veterinarians to reports any incidence of CFP. Confirmed cases are recorded while suspected cases are reviewed to gather more data as well as obtain fish samples for analysis by FDA. When a report is done, immediate warning are issued to the residents as to what fish to avoid and areas not to fish.

Government agencies maintain a website for providing information to residents about the CFP. In Florida for example, there is such thing as Waterborne Disease Surveillance Program that serve as coordinating network among the medical practitioners, Florida Poison Information Center and other government agencies to review data, and give recommendations to the affected residents.

The Florida Poison Information Center- Miami has a 24 hour hotline (888-232-8635) where anyone can report CFP.[52]

TYPES AND ACTIVITIES OF TOXINS ASSOCIATED WITH CYANOBACTERIA

A. HEPATOTOXINS [liii] (Toxic to the liver)

TABLE 12-1MICROCYSTINS

ACTIVITY	GENERA THAT PRODUCE THESE TOXINS
Hepatotoxic	*Microcystis*
Protein phosphatase inhibition	*Anabaena*
Membrane integrity	*Nostoc*
Conductance disruption	*Planktothrix*
Tumor promotors (Cancer)	*Anabaenopsis*
	Hapalosiphon

TABLE 12-2 NODULARIN

ACTIVITY	GENERA THAT PRODUCE THIS TOXIN
Same as with microcystins	

TABLE 12-3 CYLINDROSPERMOPSIN

ACTIVITY	GENERA THAT PRODUCE THE TOXIN
Necrotic injury to liver	Cylindrospermopsis
Spleen	Aphanizomenon
Kidneys	Anabaena
Lungs	Raphidiopsis
Intestines	Umezakia
Protein synthesis inhibition	
Genotoxic	

B. NEUROTOXINS

TABLE 12-4 ANATOXIN-A

ACTIVITY	GENERA THAT PRODUCE THESE TOXINS
Postsynaptic , depolarizing neuromuscular blockers	*Apanizomenon*
	Anabaena
	Raphidiopsis
	Oscillatoria
	Planktothrix
	Cylindrospermum

TABLE 12-5 ANATOXIN –a(S)

ACTIVITY	GENERA THAT PRODUCE THESE TOXINS
Acetylcholinesterase inhibitor	*Anabaena*

TABLE 12-6 SAXITOXINS

ACTIVITY	GENERA THAT PRODUCE THESE TOXINS
Sodium channel blockers	Aphanizomenon
	Anabaena
	Planktothrix
	Cylindrospermopsis
	Lyngbya

A. DERMATOTOXINS

TABLE 12-7 LYGBYATOXIN-A AND APLYSIATOXINS

ACTIVITY	GENERA THAT PRODUCE THE TOXIN
Inflammatory agents	Lyngbya
Protein kinase C activators	Schizotrix
	Oscillatoria

ENDOTOXINS

TABLE 12-8 Lipopolysaccharides

ACTIVITY	GENERA THAT PRODUCE THE TOXIN
Inflammatory agents	Many cyanobacteria
Gastrointestinal irritants	

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[i] Jonsson,P.R. et al 2009. Formation of harmful algal blooms cannot be explained by allelopathic interactions. Proc Natl Acad Sci U S A. 2009 Jul 7; 106(27): 11177–11182. doi: 10.1073/pnas.0900964106 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2708709/

[ii] Kinkaid, C 2014. Toxic Algae: How to Treat and prevent Harmful Algal Blooms in Ponds, Lakes Rivers and Reservoirs.P14. Solardyne.com

[iii] https://commons.wikimedia.org/wiki/File:%D0%92%D0%B5%D1%81%D0%BB%D0%BE%D0%BD%D0%BE%D0%B3%D0%B8%D0%B5_%D1%80%D0%B0%D0%BA%D0%BE%D0%BE%D0%B1%D1%80%D0%B0%D0%B7%D0%BD%D1%8B%D0%B5_%D1%80%D0%B0%D0%B7%D0%BD%D1%8B%D1%85_%D0%B2%D0%B8%D0%B4%D0%BE%D0%B2.jpg Write in Wikipedia in English and the photo comes in the Wikipedia article.in English. Viewed 23 April 2022.

[iv] https://commons.wikimedia.org/wiki/File:Red_tide.jpg Viewed 18 May 2021.

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[xix] Saxitoxin (STX) has also been found in Bullfrog (***Rana catesbeiana***)plasma

[xx]

[xxi] There are many species of *Alexandrium* which also produce STX. They are *A.tamerense,A. fundyense, A.catenella*. Species of *Gymnodinium* include *G. catenatum.*[xxi]

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Below is a list of symptoms associated with the cyanobacteria.

- **\Liver damage**
- **Death**
- [liii] Saxitoxin (STX) has also been found in Bullfrog (***Rana catesbeiana***)plasma.
- [liii] There are many species of *Alexandrium* which also produce STX. They are *A.tamerense,A. fundyense, A.catenella*. Species of *Gymnodinium* include *G. catenatum.*[liii]
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