Developments in Marine Biology, 3

TOXIC PHYTOPLANKTON BLOOMS IN THE SEA

(1)

DEVELOPMENTS IN MARINE BIOLOGY

1. **TOXIC DINOFLAGELLATE BLOOMS**
   edited by D.L. Taylor *and* H.H. Seliger 1979 (out of print)

2. **PHYTOFLAGELLATES**
   edited by E.R. Cox 1980 (out of print)

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TOXIC PHYTOPLANKTON BLOOMS IN THE SEA

Proceedings of the Fifth International Conference on
Toxic Marine Phytoplankton,
Newport, Rhode Island, U.S.A., 28 October - 1 November 1991

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ELSEVIER Amsterdam — London — New York — Tokyo 1993

ELSEVIER SCIENCE PUBLISHERS B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

ISBN: 0-444-89719-4

This book is printed on acid-free paper.

Printed in The Netherlands

PREFACE

The Fifth International Conference on Toxic Marine Phytoplankton held in Newport, Rhode Island, USA, from October 28-November 1, 1991, was convened by Dr. Ted Smayda of the Graduate School of Oceanography, University of Rhode Island. It brought together most of the active researchers in taxonomy, molecular biology, ecology, physiology, and toxicology, and public health officials concerned with the apparent global epidemic of harmful, toxic, noxious and nuisance phytoplankton events in the sea. In all, 325 scientists from 44 countries presented 217 oral contributions and poster papers. Their collective findings published in these Conference Proceedings will be helpful to public health officials and organizations; coastal management specialists; international, national, regional and state agencies concerned with the sea and its resources; aquaculturists; and also contribute towards resolution of the extent to which the current epidemic of undesirable blooms in the sea is linked to anthropogenic activity.

The Conference was dedicated to Professor Trygve Braarud (1903-1985), University of Oslo, Norway, in honor of his numerous distinguished and seminal publications on the taxonomy, life cycle, physiology and ecology of dinoflagellates and other bloom species. These are summarized in the Conference dedicatory paper.

A forum and round table discussion by invited participants, chaired by T. Smayda, with C.R. Tomas serving as *rapporteur*, was held on the problems of toxic species' definition and the degree to which these various definitions help, or hinder, the diverse scientific and applied needs for such definition by physiological ecologists; by public health officials, monitoring programs, and in methodological development; for aquacultural, vendor and consumer protection, and in public communication. We had intended to publish the round table discussions. However, the extraordinary number of papers presented at the Conference, and submitted for peer-reviewed publication in the Conference Proceedings, fully utilized the page limit set by the publisher. This volume is about twice the size of the previous Conference Proceedings! Regrettably, we are therefore unable to publish the round table discussions. We wish to thank the following for their participation in the toxic species' definition forum: D.M. Anderson, R. Boragine, A. Cembella, Y. Fukuyo, J.C. Gallagher, K. Kizer, S. Maestrini, Y. Oshima, E. Paasche, Y. Sako, C.A. Scholin, Y. Shimizu, F.J.R. Taylor, A. White, R. Williams, T. Wyatt and T. Yasumoto.

P. Lassus confirmed his offer and intention to host the 1993 meeting in France, originally presented and accepted by consensus at The Fourth International Conference on Toxic Marine Phytoplankton held in 1989 in Lund, Sweden. Professor T. Yasumoto's offer in Newport to host the 1995 conference in Japan was gratefully accepted by conference attendees.

On 2-3 November, immediately following the Conference in Newport, a Workshop jointly sponsored by the Intergovernmental Oceanographic Commission and the Scientific Committee on Oceanic Research was convened; participants were invited by the sponsors. The chairman was Dr. Robert Fournier, Secretary of SCOR. The IOC was represented by Dr. Thomas Osborn, Assistant Senior Secretary for Ocean Sciences in Relation to Living Resources, and Dr. Henrik Enevoldsen from the Harmful Algal Bloom sub-program. This Workshop was the third in a series designed to develop a program on Harmful Algal Events; the previous Workshops were held in Takamatsu, Japan (1987), and Paris, France (1991). The objective of the Workshop was to complete the broad outline of an international program to deal with the serious and growing problem of Harmful Algal Bloom events in the sea. This was achieved, and the report and recommendations of the Workshop subsequently prepared for presentation to the First Session of the Joint IOC-FAO Intergovernmental Panel on Harmful Algal Blooms convened in Paris in June, 1992.

The editors wish to thank the sponsors and co-sponsors for their support, without which this Conference would not have been possible, and which greatly facilitated the participation of many scientists who would otherwise not have been able to attend because of financial limitations. We are grateful for the advice and counsel of many individuals helpful in the organization of the Conference. Dr. Edna Graneli, Convener of the 1989 Conference in Lund, Sweden, was particularly helpful; and valuable insights provided by Dr. Donald Anderson. Special thanks and acknowledgement are extended to Blanche Coyne, Einar Hjorleifsson, Rhonda Kenny, Marilyn Nigrelli, and Jean Stutz for their tremendously effective assistance during the various stages of organizing and convening the Conference, and their help with the myriad number of tasks involved in the preparation of these Proceedings for publication. Rhonda Kenny's desktop publishing and word processing skills were of special importance. Dean Margaret Leinen, Graduate School of Oceanography, URI, kindly provided institutional support at a critical juncture in the preparation of the Conference Proceedings for publication.

The editing of the Proceedings proved to be particularly taxing, given the great number of papers and reviews to process; the need to photoscan a significant number of papers for reformatting, word processing and linguistic changes; transoceanic telecommunication inaccessibility causing significant delays in a number of instances, and some unexpected publishing and funding issues. We apologize for the modest delay in meeting our publication target date, but feel that this will not compromise the timeliness and relevance of the publications.

We are most grateful to our sponsors, Conference participants, advisors, supporting staff, and host institution for their support in helping us to facilitate, through the Conference and these Proceedings, communication of the advances to our knowledge made by our colleagues on undesirable phytoplankton organisms and their blooms in the sea, and presented herein.

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OCTOBER 28 - NOVEMBER 1, 1991
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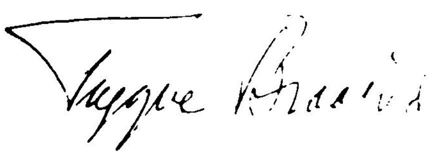

Toxic Phytoplankton Blooms in the Sea
T.J. Smayda and Y. Shimizu, editors

FLAGELLATES AND THEIR BLOOMS IN THE SEA: TRYGVE BRAARUD'S CONTRIBUTIONS TO OUR KNOWLEDGE

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ABSTRACT

This overview summarizes the contributions of Trygve Braarud (1903-1985), in whose honor the FIFTH INTERNATIONAL CONFERENCE ON TOXIC MARINE PHYTOPLANKTON is dedicated. His scientific contributions on flagellates and their blooms; his autecological and ecological phytoplankton studies, and his various pioneering contributions during the course of his scientific career are focused upon.

INTRODUCTION

The scientific career and legacy of Professor Trygve Braarud (1903-1985), in whose honor the FIFTH INTERNATIONAL CONFERENCE ON TOXIC MARINE PHYTOPLANKTON is dedicated, have been summarized in moving *in memoria* by his Norwegian colleagues, Professor Grethe Rytter Hasle [1] and Professor Eystein Paasche [2]. They have recorded Braarud's seminal, often pioneering contributions to our knowledge of marine phytoplankton at the organismal and ecological levels; his remarkable pedagogical skills; his inspirational direction of the first class institute which he founded, and his role in helping to advance marine science through membership on numerous national and international committees. Braarud's remarkable contributions, which have influenced the direction of past and contemporary phytoplankton research, transcended his focus on the phytoplankton. Mills [3], in his book on the early history of Biological Oceanography, recognizes Braarud as *"one of the pioneers of quantitative biological oceanography"*.

The philosophical cornerstone of Braarud's unique institute, which can be termed the "Oslo School" [2], and which expanded upon the approach of his mentor, Professor Haakon H. Gran, was that the organisms comprising the marine phytoplankton - their taxonomy, life cycles, physiology, and ecology - were worthy of study in of themselves, rather than as adjuncts to other marine problems. He believed that holistic, integrated approaches rooted in organismal biology and population ecology, and which combined field and experimental studies, were required to quantify regulation of phytoplankton dynamics in the sea, such as distribution, blooms, succession, toxic events, etc. [4]. This *raison d'etre* of the "Oslo School" clearly differs from the more prevalent approach to phytoplankton growth in the sea, which might be termed the *"whole community school"*. That approach is often governed by the legitimate need to elucidate the *collective* role of the phytoplankton as a subset of the major processes regulating, *inter alia*, the pathways and properties of oceanic biogeochemistry, productivity and trophodynamics. However, the pragmatic conceptual and reductionist approaches, and the application of gravimetric, mass balance and other whole community methodologies required in such phytoplankton studies are inappropriate for resolving such problems as the mechanisms of bloom species' selection, the processes regulating their bloom dynamics, and related topics of great interest to Braarud and relevant to our Conference. Braarud [5], for example, puzzled over why *Heterocapsa triquetra* and *Scrippsiella trochoidea*, despite many autecological features in common and a similar distributional pattern, exhibit pronounced differences in their bloom outbreaks. The "whole community school", in contrast, might formulate this problem generically, i.e., "Why a red tide?"; a different question!

Braarud's publications reveal a long-term interest in flagellates (dinoflagellates, coccolithophorids, euglenids) and their blooms, an interest which spanned the problems of species' definition, life cycles, physiology, ecology and harmful bloom events. Many of his insights, approaches and conclusions are highly relevant to, and even anticipated, contemporary issues associated with Harmful Algal Bloom species' dynamics in the sea. Some of these are sketched in this dedicatory note offered in honor of his contributions, and as guidelines to unresolved scientific issues.

Those of us who studied and/or worked with Braarud know that he was given to understatement; he frequently presented his opinions as *"tentative conclusions"*. This trait was not out of hesitancy; rather, it reflected his profound understanding and experience that in the changing polymixtures of species making up phytoplankton communities, complex physical-chemical-biological interactions regulated their recruitment, growth, succession and blooms, and that measurements of habitat variables, interactive effects and *in situ* processes are inevitably incomplete, inadequate or inappropriate. Braarud, however, was not deterred by the enormous problems associated with quantifying ecological processes. In an invited paper presented at the First International Oceanographic Conference (1959), Braarud [5] challenged Alfred C. Redfield's (of Redfield Ratio fame) conclusion that marine experimentation was inadequate for the intended ecological extrapolations. Braarud argued that his own experiments, designed to explain *in situ* behavior, led him to conclude that a *"combination of field work and laboratory experimentation is essential to progress"*, and pointed out the need not to confuse experimentation for *"orientation purposes"* with that needed for ecological assessments, which require analyses of factor interactions.

Taxonomic and Life History Studies

Braarud possessed a keen eye for morphological detail, honed by vast experience from microscopic examination of field phytoplankton samples. Among new taxa described by him, are the red water species *Gymnodinium galatheanum* from Walvis Bay [6], and the remarkable coccolithophorid from the Gulf of Maine [7], renamed *Braarudosphaera bigelowi*, a relict from the Cretaceous Period where its four massive blooms at 30 million year intervals were accompanied by the evolutionary appearances of marine superpredators 10-18 m long [8]. Braarud [7] also emended Lebour's description of *Alexandrium tamarense* based on material from the Gulf of Maine, beginning his continuous interest in this toxic species.

Braarud's interests in taxonomic and speciation issues were utilitarian; he considered such knowledge to be fundamental to his primary interest in the phytoplankton: their ecology. He stated [9]: *"For the study of the ecology and physiology of this important group* [= dinoflagellates], *a knowledge of its taxonomy, the demarcation of the taxonomical units, is most essential"*. To achieve this, he stated two approaches were needed: the use of electron microscopy [10] and clonal culture experiments [9]. Braarud's [10] enthusiasm for electron microscopy is evident in his statement that not only would it *"initiate a new era for the study of taxonomy, systematics and phylogeny"*, but would also *"establish a sound basis for ecological surveys"*. And in the early 1950's, he and his "Oslo School" colleagues introduced and applied transmission electron microscopy to coccolithophorid systematics [10] and rejuvenated and revolutionized such research. This successful use of electron microscopy was then applied to diatom systematics, with distinction, by his colleague, Grethe Hasle. One of her early studies [11] has recently facilitated taxonomic resolution of the newly discovered, unexpected diatomaceous production of toxic domoic acid by certain *Nitzschia* species, and splendidly demonstrates the validity of Braarud's viewpoint that taxonomic research is relevant to ecological problems. Braarud considered dinoflagellates to be large enough to be studied adequately by light microscopy, except for the smallest species. In his only published effort [12] on the electron microscopy of a dinoflagellate, *Prorocentrum balticum*, his ecological interests prevailed. Observing the unexpected presence of numerous fine, thecal spines, he concluded that these morphological features increased the nutrient absorption area and might *"partly"* explain the wide distribution and growth of this species in nutrient-poor waters.

A posthumous publication by Paulsen [13] in 1949, edited by Grøntved, suggested that dinoflagellates similar in appearance, but morphologically distinguishable, should be regarded as separate *species*, if they are geographically isolated, but as *formae* of a given species, if they co-occur in a given region and intergrade morphologically. He accepted the general belief of that time that marine dinoflagellates reproduce only asexually. Paulsen's speciation concept and problematic exclusion of sexual reproduction capability greatly interested Braarud [9, 14], given his considerable field experience with species' form variations and earlier culture studies on morphological variability. In 1944, Braarud [15] carried out culture studies on two clones of *Alexandrium tamarense* which he isolated from Oslofjord that February in *"an attempt towards understanding its specific characters"*. This study followed up his previous emendation of this taxon based on the Gulf of Maine material [7]. Although he accepted that *"a certain variability in form is always to be expected"*, the clear, persistent differences in the habitus of these two clones despite their cultivation in the same media and at similar growth conditions surprised him, and he subdivided *A. tamarense* into three varieties: var. *typica* (= Lebour's original description), var. *excavata* and var. *globosa*. He was more impressed, however, with the indication that within the same population of a species different genotypes exist, an insight which he returned to in his physiological experiments.

Braarud's subsequent (1951) morphological studies [9] on two clones of *Scrippsiella trochoidea* to evaluate whether phenotypic drift occurred [it did not] towards the closely related, if not identical taxon, *Peridinium* [sic] *faroënse*, reinforced his impressions based on his *A. tamarense* results. He endorsed [9] Paulsen's view that the *"use of a collective species would seem to be natural, gathering the various genotypically different, smaller units in one larger"*, at least until other research approaches were developed. This call for newer approaches reflected Braarud's view that morphometric analyses alone, whether on cultured or natural populations, would not resolve Paulsen's speciation issue. This conclusion was supported by a prodigious effort on *Dinophysis* [14] that his student, Ingrid Solum, undertook at Braarud's initiative. [Although not a co-author of this paper, Braarud prepared and interpreted the results for publication.] Pointing out that everyone who has worked on *Dinophysis* recognizes that it presents a *"serious obstacle for identification to species"* [9], and wishing to test the validity of Paulsen's [13] partially revised taxonomy of this genus, Braarud had Solum make thousands of biometric measurements on seven *Dinophysis* species collected during different years and seasons at six localities along the Norwegian coast. Applying Paulsen's own criteria, i.e., that two taxa within the same region linked by morphological transitional forms should be considered the same species, they invalidated some of Paulsen's taxonomic revisions of *Dinophysis*. The conceptually more meaningful result of this study, however, was the demonstration that a variable correlation occurred between environmental conditions and morphology and, hence, taxonomic status.

Collectively, Braarud's efforts to assess Paulsen's concept dealt with the still unresolved questions of: *"how is variation in size brought about when the cells are subjected to the same environmental conditions"* [22], and are observed morphological variations environmentally induced, or does the environment select for genotypes having the observed morphologies [13]? A related, contemporary analogue of these problem formulations is the major, unresolved issue as to how toxigenesis is regulated *in situ*.

Braarud also made observations on the cell division process, its diel periodicity, on phototaxis, encystment, and aberrant cell formation during his various experimental studies on *Alexandrium tamarense, Heterocapsa triquetra, Mesoporus perforata, Prorocentrum micans, Protoceratium reticulatum* and *Scrippsiella trochoidea* [9, 15, 22-24]. He provided one of the first descriptions of the life cycle and cyst formation in a dinoflagellate, *Heterocapsa triquetra* [23]. He established cultures of *H. triquetra* from cysts kept in a refrigerator for eight months; observed excystment stages in hanging drop cultures, and carried out [unsuccessful] pairing experiments with four clones in seeking information on zygote formation. He also described, from natural populations, the annual reproductive cycle, including aplanospore formation, of the positively buoyant *Halosphaera viridis* [25].

Experimental and Physiological Studies

Braarud's first published paper on marine phytoplankton (1931), co-authored with Føyn [16], experimentally evaluated Schreiber's bioassay method to measure nutrients. The stimulus for this study was a special ICES *"meeting of experts"* convened in Oslo (1928) to test, develop and recommend standard methods for the measurement of the major phytoplankton nutrients [3]. However, Braarud and Føyn's experiments clearly went beyond this objective; they also examined the uptake of inorganic and organic nitrogen and the secretion of dissolved organic matter in bacteria-free cultures of a marine *Chlamydomonas* sp. and *Carteria* sp. Their interest in the secretion of dissolved organic matter reflected the controversial hypothesis August Pütter developed in a series of papers between 1908-1925 [see 17]; namely, the magnitude of phytoplankton production in the sea and the availability of other particulate and detrital matter were inadequate to nourish aquatic animals. Therefore, their uptake of dissolved organic matter (= parenteral feeding) was needed. Although the two microalgae secreted about 30% of their photosynthate, in characteristic fashion Braarud was cautious in his extrapolation to *in situ* processes, noting that their experimental green algal species are insignificant in natural communities and *"erst wenn ähnliche Kulturversuch mit Diatoméen gemacht worden sind, wird man sehen können, welche Bedeutung es für den Stoffwechsel in Meere hat"*.

In September 1931, the Danish zoophysiologist and 1920 Nobel laureate in physiology and médicine, August Krogh, published [17] his definitive refutation of Pütter's hypothesis, presented at a special ICES meeting convened in Copenhagen that March. I am uncertain to what extent this common interest in Pütter's hypothesis was a factor, but in 1935, after completing two major studies, that on the Gulf of Maine [7] and his doctoral dissertation on Arctic phytoplankton [18], Braarud began a 4-1/2 month fellowship in Krogh's laboratory. Although Krogh was awarded the Nobel prize for his research on the mechanisms of vertebrate gas exchange, his subsequent studies on limnetic phytoplankton and zooplankton [see 17] clearly facilitated Braarud's Fellowship objectives [19] to develop experimental culturing techniques to provide data on *"the physiological background for ecological observations and ecological classification"*. His pioneering experiments on seven winter-spring diatom species, the dominant flora available for isolation into culture from the Kattegat during his January-April visit, introduced quantitative autecological experimental techniques into phytoplankton research.

Continuance of his experimental studies upon his return to Oslo had to await completion of culturing facilities. In late 1938, he initiated experiments on diatom growth rates, form variation and life history [20]. However, World War II disrupted these and other planned studies, and the major results were not published until 1945 [20]. Of special interest is Braarud's report [20] that during this period he established numerous bloom species into culture, including: *Emiliania huxleyi, Phaeocystis pouchetii, Alexandrium tamarense, Heterocapsa triquetra, Scrippsiella trochoidea, Prorocentrum micans and Halosphaera viridis.* This success in cultivating marine flagellates was a notable pioneering achievement. Previously, Allen may have been the first to cultivate a dinoflagellate, *Heterocapsa triquetra* [21], and in 1935 Barker [21] published his remarkable physiological experiments on *Heterocapsa triquetra* and *Prorocentrum micans,* and reported that he established several other dinoflagellates into culture, including a *Dinophysis* sp.

Having established flagellates into culture, Braarud rarely experimented on diatoms thereafter. This appears to reflect his view [5] that diatoms were difficult experimental organisms, whereas *"The species . . . most suitable for culture work and studies on the influence of environmental conditions on their occurrence are those which reproduce by binary fission, without any complications in the form of sexual reproduction or cyst formation."* Dinoflagellates, he believed, satisfied this requirement; even the cyst-formers, since *"there are no difficulties in keeping the cultures in active growth without cyst formation"*.

Dinoflagellate experiments initiated during the war years (1941-1942) evaluated the effects of salinity, inorganic phosphate and various sewage dilutions on growth rate in *Heterocapsa triquetra* [23], and the effects of salinity and sewage on *Prorocentrum micans* [24]. Between 1943-1945, he apparently studied the effect of salinity and temperature on growth in *Scrippsiella trochoidea* [22; see 5, 26], followed by minor observations on growth in *Mesoporus perforata* and *Protoceratium reticulatum* during his 1944 experiments [15].

In 1951, Braarud published his classical paper on Salinity as an Ecological Factor in Marine Phytoplankton [26]. The problem formulation was simple: to establish the role of salinity in regulating species' distributions, but the results brought him back to Paulsen [13] and the issue of species' definition. His experiments with single clones of two dinoflagellates, a coccolithophorid and a cryptomonad showed the surprising euryhaline nature of all four taxa, accompanied by interspecific differences in their salinity optima. He then grew *Scrippsiella trochoidea* over the same salinity gradient in three parallel experiments at different irradiance and temperature levels, and also compared the salinity responses of two clones of *Prorocentrum micans.* He wished to establish whether even a single experiment with one clone of a given taxon was representative for that clone, and whether the response of one clone of a given taxon was characteristic of that taxon generally.

Although his results supported such conclusions, he challenged whether they were representative, or even conclusive. *Prorocentrum micans,* he noted, was a *"good species"*, showing little morphological variation in Nature, and therefore *"may behave differently from a more variable species"*. As for *Scrippsiella trochoidea*, cultured for more than five years at varying temperature, salinity and nutrient conditions, it showed no trace of the *faroënse*-type of the species [see also 22]. He also pointed out that if the well known morphological variation in *Scrippsiella trochoidea* [and dinoflagellates, generally] was due only to phenotypic variation, the appearance of the *faroënse*-type in some of the cultures would be expected. This evidence, added to his previous field and experimental experience, led him to conclude that *"it seems probable that in the marine dinoflagellates, the species may often be collective species, consisting of morphologically different genotypes"*. But the corollary of this was the more significant: *"If clones of the same 'species' exhibit morphological differences, there might be reason to suspect that they also differ in their physiological characters."*

The theoretical and practical consequences of this possibility were, and remain, enormous. Ecologists would not only have to contend with the problems inherent in sampling, measuring, experimenting on, and modeling the polymixtures of species making up phytoplankton communities, but any given species may be a physiological polymixture, further complicating such analyses and extrapolation of experimental results. Braarud's focus on this problem of clonal physiological variation was the *tour de force* of his experimental studies.

In seeking to resolve this issue, he supplemented his own studies by having others of the "Oslo School" initiate autecological experiments. Nordli [27] overcame the difficult [see also 21] technical problems of establishing the sensitive ceratian species into culture, and carried out experiments (1950-1956) on the "red tide" forms *Ceratium tripos, C. fusus, C. furca* and *C. lineatum*. The growth of three clones of the coccolithophorid, *Emiliania huxleyi*, a cause of episodic water-discoloring blooms in the Oslofjord termed "*Coccolithus* [sic] *huxleyi* Summers" [28], was examined by Mjaaland [29].

In 1961, Braarud summarized [5] the results of the autecological studies carried out by him, by his "Oslo School" associates, and related studies in a paper entitled: Cultivation of Marine Organisms as a Means of Understanding Environmental Influences on Populations. He showed that the evidence for physiological clones was divergent, and depended on whether one considered responses to salinity or temperature. His results raised the unresolved issue of whether clonal differences expressed in response to one habitat variable characterizes responses to all habitat variables. Discrepancies were found between *in vitro* and *in situ* responses. Braarud cautioned that deductions from regional distribution and seasonal occurrence patterns for a given species might *"blur the ecological picture"* for that species, and may be just as serious a problem as faulty experimental procedures in contributing to extrapolation discrepancies. He also stressed that the *"tolerance of. . . species to extreme conditions should be studied with special care"*, such as the *"Extreme mass occurrences of certain species, as for instance in red tides, [which] represent an interesting and spectacular example of societies presumably resulting from hitherto obscure conditions favoring growth in certain species [which] are normally members of societies of a more balanced composition"*.

Ecological and Bloom Studies

Braarud's ecological insights derived from his considerable field experience with phytoplankton in the Arctic [18], the Gulf of Maine [7], at numerous locations along the Norwegian Coast and its fjord systems [see 30], and the North Sea [31]. The diverse ways in which hydrography regulates phytoplankton processes were a major focus of Braarud, as was his interest in nutrient effects. His classical 1931 paper [32], co-authored with Klem, first revealed his interest in hydrographic regulation and provided the definitive field evidence and interpretation that initiation of the spring bloom resulted from a dynamic interplay between vertical circulation, stability of the water column and light penetration, rather than being solely a light or nutrient-regulated event. This understanding, embellished upon in the Gulf of Maine study [7], has been termed *"the last great discovery in phytoplankton ecology"* [see 2].

Braarud's exceptional understanding of the role of hydrographic and vertical physical structure, in combination with organismal biology, as factors regulating species' population dynamics is evident in his 1958, co-authored study [33] on Dramsfjord. Dramsfjord's complex, seasonally variable, multi-layer circulation system is partly driven by nutrient-enriched freshwater inputs; there is a sill at 10 m and an anoxic layer beginning at 65 m. Monthly phytoplankton surveys revealed the extremely complicated and time-varying vertical distributional and occurrence patterns of several dinoflagellate species. Almost everyone else would probably have considered the dinoflagellate data as too "noisy" to warrant serious analysis. But Braarud, who later cited [14] the view that *"it is not taxonomy, but Nature that is chaotic, and it is of Nature that we must try to give a picture"*, was not deterred. I personally remember his half-day tutorial with me during which he went through his analyses, clearly delighted in having unraveled a vexing phytoplankton patchiness problem and being able to teach about it. He explained the seemingly irregular horizontal and vertical dinoflagellate patterns as the reasonable result of *"a combined effect of local growth in the upper layers, vertical phototactic migrations, horizontal transport by the laminated current and counter-current systems and, after onset of the floods, outward transport of populations being caught in outflowing freshwater and brackish layers"*, with seasonal variations in the above process. The general importance of such an entrainment and transport phenomenon to dinoflagellate dynamics *in situ* was rediscovered and confirmed 20 years later in an excellent paper by Tyler and Seliger [34] on Chesapeake Bay [and subsequently by others] having the benefit of much better data sets. The elegant conceptual and analytical approaches, rooted in autecological and population biology, used by Braarud in his Dramsfjord analysis should be required reading (pp. 43-54 in [33]) for everyone interested in phytoplankton ecology and harmful blooms.

Braarud's other principal ecological focus, on the diverse roles nutrients play in regulating phytoplankton responses [5], was stimulated by his long-term studies in Oslofjord and its nutrient-enriched inner region [35, see also 30]. This interest was already evident in the experimental studies in the early 1940's to evaluate the effects of nutrient and sewage on growth of *Heterocapsa triquetra* [23] and *Prorocentrum micans* [24]. His extensive Oslofjord studies published in 1945 [35], and dedicated to Professor H.H. Gran on his 75th birthday, are highly relevant to contemporary interests [see 36] in how, and if, nutrients regulate harmful algal bloom events. Braarud viewed nutrient enrichment from sewage discharge as a *"fertilizing experiment in sea water on a large scale"*. As to his discovery of a similar species composition in the polluted and unpolluted Oslofjord regions, it *"indicates how relatively unimportant the pollution factor is for the qualitative composition of the phytoplankton"*; he found it *"remarkable that dinoflagellates play such an important part in the plankton of the polluted region"*, unlike in freshwater systems; and noted that mass occurrences of *"green flagellates"* in enriched waters contrasted with their reduced abundance in more pristine waters. In contrast, diatoms did not show a clear relationship to nutrient enrichment; their more evident response was a species' succesional pattern *"more varied than is usually found"*.

Braarud also thought that there was a linkage between toxic blooms and nutrient enrichment, and recommended [4]: *"Further research on toxic dinoflagellates is urgently needed in view of the danger of mussel poisoning in eutrophized localities."* He concluded that nutrient enrichment may primarily increase the abundance of certain autochthonous coastal flagellate species, rather than select for newly recruited bloom species, an issue relevant to contemporary concerns over whether some [and which] harmful bloom events result from newly introduced species, or are the efflorescense of rarer, autochthonous species cryptic in the hidden flora. He cautioned that phytoplankton responses in nutrient-enriched waters may differ from those in "normal" waters in many obscure ways, partly because of the complex *"physico-chemical nature of sewage"*, and that *"various species may react differently to pollution according to their physiological nature"*. He then came to an unexpected notion [35]: that the geographic distribution of marine phytoplankton *"seems mainly to be controlled by other factors of habitat than the concentration of nutrient salts (apart from the general dependency of phytoplankton growth upon a certain supply of nutrient salts)"*.

In 1962, Braarud returned to this issue [37] in his analysis of the regional and seasonal distribution and occurrence patterns of 14 different taxa; each taxon selected for its representation of the different combinations of organismal, autecological needs and habitat requirements characterizing the phytoplankton. He noted: *"complexity of the autecological reactions as well as of the environmental conditions causes such a diversity of distribution that hardly two species behave in the same way"*. Although Braarud focused on regional distribution patterns and the *"theoretical distribution factors"*, obviously those factors which regulate the spatial distribution of species can also influence their temporal occurrences. Hence, Braarud's analysis of the diverse distributional requirements is highly relevant to interest in the mechanisms which drive succession, influence seasonal bloom patterns, select for harmful species, etc. Among the 14 distributional (occurrence)