

In: Sterr, H., Hofstede, J. & Plag, H.-P. (Eds.), Proceedings of the International Coastal Congress, ICC-Kiel '92 : interdisciplinary discussion of coastal research and coastal management issues and problems. Lang, Frankfurt/M.: 454-467, 1993

Mechanisms for recurrent nuisance algal blooms in coastal zones: resting cyst formation as life-strategy of dinoflagellates

Stefan Nehring

Institut für Meereskunde an der Universität Kiel,
Düsternbrooker Weg 20, D-2300 Kiel 1,
Federal Republic of Germany

ABSTRACT: The increasing occurrence of nuisance blooms and their negative ecological and economic impact has led to intensified monitoring activities. However, mechanisms of bloom formation are poorly understood. Among the most frequent producers of nuisance blooms, dinoflagellate species with a benthic cyst stage in their life cycle are prominent. The role of those resting cysts in bloom initiation, species dispersal, genetic recombination, survival of environmental stresses and as vectors of toxicity is discussed. Distribution studies of cysts in recent sediments of the coastal North Sea are presented together with a comprehensive list of cyst-forming, planktonic dinoflagellate species. Furthermore the collection, preparation and identification of resting cysts is described.

INTRODUCTION

Phytoplankton blooms are temporary phenomena and occur primarily in coastal zones, in landlocked areas, fjords and lakes. The increasing pollution of coastal waters by urban and agricultural sources, especially with nutrients, has helped to cause a worldwide increase in nuisance phytoplankton blooms over the past 20 years. Among the negative effects of such blooms there are esthetic problems of water quality (discoloration, scum-forming with all consequences on the water-based tourist industry) as well as the massive kills of fish and other marine animals, induced by oxygen deficiency or algae toxin production. Phytoplankton toxins may directly cause allergic problems to man and may directly involve the death of e.g. fish (Burkholder et al. 1992) as well as domestic animals (Nehring 1991). They may be concentrated in the food chain e.g. by mussels with fatal results for the consumers (man, marine mammals, birds). Toxins of dinoflagellate are known to cause Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurological Shellfish Poisoning (NSP) and Ciguatera fish poisoning which may be a hammer for a variety of aquaculture branches. At present more and more sea areas were closed down temporarily for sale of shellfish. All of these events were often associated with occurrence of toxicity in phytoplankton species that had been thought to be harmless. For ecological and economic reasons monitoring activities were intensified but the mechanism of exceptional phytoplankton bloom formation is still poorly understood.

Among causative organisms of nuisance blooms, dinoflagellate species, which often include a dormant cyst stage in their life cycle, play an important role. At present aspects of the timing, location, and duration of dinoflagellate blooms have been linked to the encystment/excystment of resting cyst-forming species (Anderson & Morel 1979, Anderson et al. 1983). Moreover there is some evi-

dence that factors such as cyst resuspension and current transport but also cyst transport in ship ballast water may repeatedly infect areas with toxic species (Anderson 1989, Hallegraeff & Bolch 1991). Highly toxic resting cysts, containing up to 10 times the toxin of vegetative cells, may present a source of poison to organisms well after the motile species have disappeared from the water column (Dale et al. 1978, Oshima et al. 1982).

The occurrence and distribution of dinoflagellate resting cysts is subject of increasing interest because cysts represent stable populations assuring geographical maintenance in contrast to the transient bloom from which they may be derived. The fact that resting cysts can be collected and enumerated during non-bloom periods, offers a potential tool for the prediction of future toxic blooms. The purpose of this paper is to provide an introduction to modern dinoflagellate resting cyst studies.

RESTING CYSTS IN THE LIFE CYCLE OF DINOFLAGELLATES

Many plankton groups include a non motile dormant egg or cyst stage in their life history (e.g. copepods, cladocerans, tintinnids, diatoms, and dinoflagellates). Fossilized dinoflagellate resting cysts as an important group of microfossils ('hystrichospheres') are known from sedimentary deposits of 230 million years ago (Triassic) and are extensively studied by geologists for biostratigraphy (e.g. in oil exploration). More than 100 years ago, living dinoflagellate resting cysts were first observed in plankton samples of the North Sea (Hensen 1887, Stein 1883) and were occasional mentioned in the phycological literature mainly about freshwater during the first half of the twentieth century (e.g. Braarud 1945, Diwald 1938, Klebs 1912, Zederbauer 1904). Detailed investigations were, however, not carried out until the 1960s as the cyst-teca relationships of modern dinoflagellates were recognized (Evitt & Davidson 1964, Wall & Dale 1966).

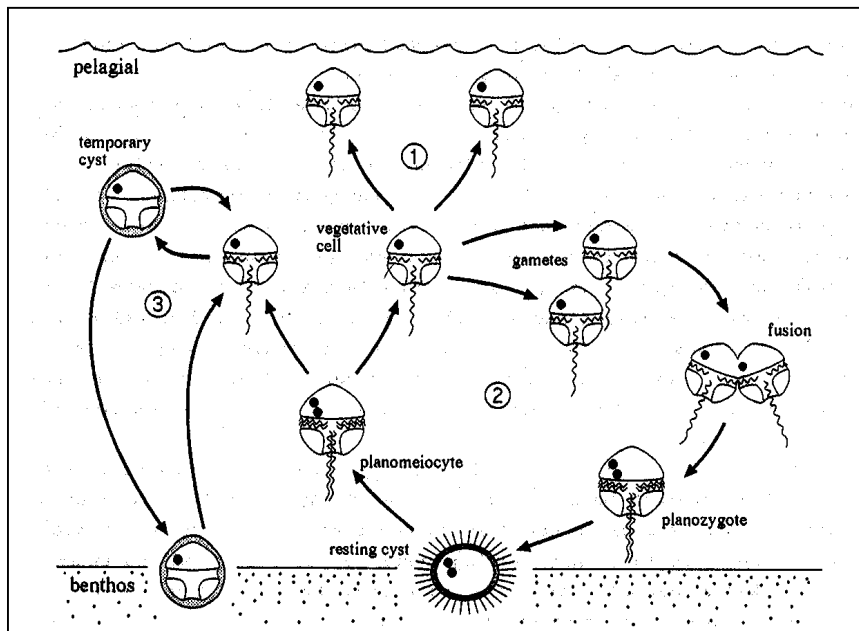


Fig. 1. Schematic diagram of basic dinoflagellate life cycle: 1) Asexual phase with motile, planktonic vegetative cell dividing by binary fission. 2) Sexual forming of non-motile resting cysts. 3) Asexual forming of non-motile temporary cysts. (o: cell nucleus).

Red-tide phenomena are caused by the motile biflagellated stage in the life history of dinoflagellates whereby the dominant reproductive mode is asexual fission (Fig. 1). Often towards the end of a bloom some species are also capable of sexual reproduction, forming gametes that fuse into a swimming planozygote which, in most cases, transform into a resting stage. These cysts are morphologically distinctive and differ from the motile planktonic stage. The cyst wall in most species is composed of organic matter (sporopollenin) but species of the genera *Scrippsiella* and *Ensiculifera* produce calcareous cyst walls (Fig.2). Organic cyst walls are extremely resistant to natural decay and good fossilizable. The calcareous cysts and the cellulose wall of motile stages are less resistant, and they would probably not persist into the fossil record. To date, among the approximately 2000 extant dinoflagellate species more than seventy species of marine and more than twenty species of freshwater planktonic recent dinoflagellates produce a resting cyst as part of their sexual life cycle (Tab. 1). Of these cyst-forming species, more than 20 have been known to cause red-tides and several species of *Alexandrium*, *Gonyaulax*, *Gymnodinium* and *Pyrodinium* are toxic.

Induction of sexuality in laboratory cultures is most often accomplished by sudden or gradual nitrogen depletion (Anderson et al. 1984), iron stress (Doucette et al. 1989), phosphorus limitation (Anderson & Lindquist 1985) or an unfavourable temperature was also required (Anderson et al. 1985a). Sexual phenomena such as cellular fusion was, however, often mistakenly interpreted as cell division and together with the fact that only one in up to several hundred motile cells may produce cysts probably accounts for the paucity of cyst records in plankton studies.

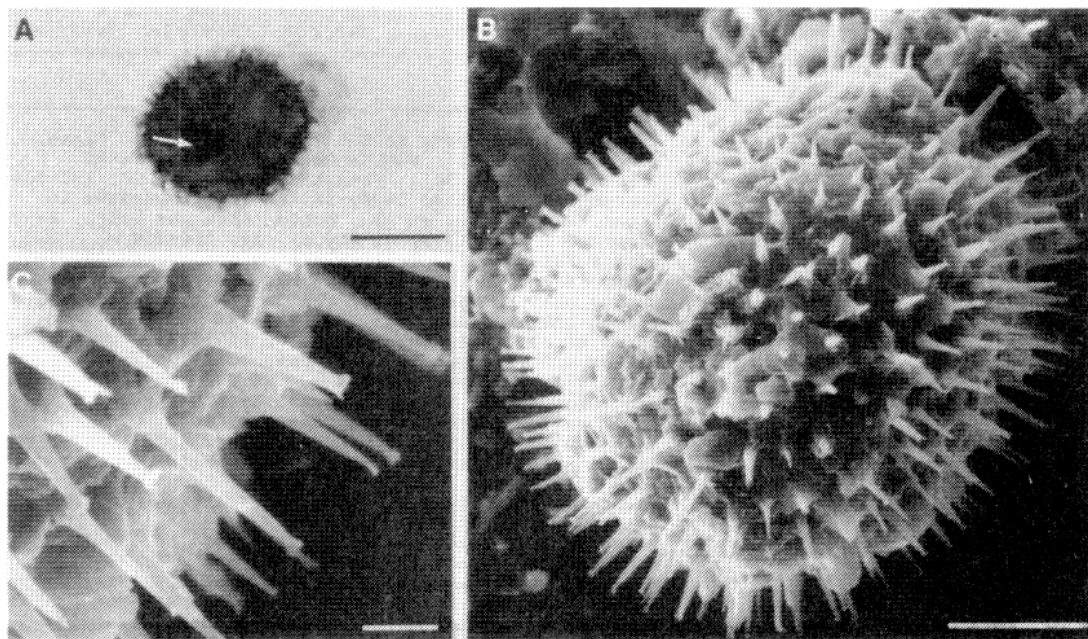


Fig. 2. *Scrippsiella trochoidea*, the most common resting cyst in North Sea sediments. (A) Living resting cyst showing red body (arrow). (B) SEM, cyst with numerous calcareous spines. (C) SEM, detail of ornament. Scale bars: 20 μm in (A), 10 μm in (B), 2 μm in (C).

In contrast to the vegetative cells, cysts have a negative buoyancy and accumulate on the sea bottom (Anderson et al. 1985b). A newly formed cyst generally has a mandatory resting period during which it cannot germinate, even when growth conditions are optimal (Anderson 1980). The duration of dormancy varies significantly between species. It can last few days but also several months. When the

dormancy stage is completed, germination of the cyst depends on external factors. Temperature is often cited as the major environmental factor regulating germination (Anderson 1980), but also light and oxygen as well as more subtle factors may be important (Anderson et al. 1987, Burkholder et al. 1992). In deep and relatively invariant bottom waters an endogenous circannual rhythm in cysts can control germination (Anderson & Keafer 1987).

RESTING CYSTS AS A STRATEGY IN SPECIES SURVIVAL

The formation of resting cysts has a variety of potential functions in the overall ecology of dinoflagellates:

- A)** The sexual reproduction of dinoflagellates is resulting in a genetic exchange which may lead to increased variation important for species survival. In this way, Anderson (1984) noted that cyst-forming dinoflagellates may maintain a viable, dormant seed population in the sediments year after year optimizing the growth and proliferation of motile cells as well.
- B)** This thick-walled resting stage may enable forms to survive unfavorable conditions in their environment and can be indicated as an overwintering stage. The cysts may lie dormant in bottom sediments for many years even at anoxic conditions (Anderson et al. 1987, Dale 1983).
- C)** Some resting cysts are themselves toxic and resuspended cyst may be then a direct source of shellfish toxicity (Dale et al. 1978). In Japan, Oshima et al. (1982) also found that the toxicity of natural cysts of *Alexandrium* spp. was as much as 5 to 10 times that of vegetative cells germinated from the same cysts.
- D)** Cysts can act as seed populations, inoculating the water column with vegetative cells to initiate blooms (Steidinger 1975).
- E)** Sexuality and cyst formation may appear to dictate the dynamics of bloom decline (Anderson et al. 1983, Burkholder et al. 1992).

Anderson & Morel 1979 showed an *in situ* demonstration of the initiation of a dinoflagellate bloom via the excystment process. In a shallow restricted embayment cysts of *Alexandrium tamarense* were found in sediments only within the bloom area. In this stable system with reduced circulation, cyst germination initiated recurrent blooms in the overlying water.

In certain coastal and estuarine environments sediments contained only few toxic cysts but transport of suspended cysts from offshore seed beds by either periodic upwelling, storms or onshore subsurface currents to a localized site may also contribute to direct shellfish intoxication or bloom formation (Anderson & Wall 1978, Cembella et al. 1988, Dale et al. 1978, Seliger et al. 1979, Steidinger 1975, Yentsch & Mague 1979).

There is some evidence that besides to current transport human intervention, in the form of ship de-ballasting or the transfer of shellfish stocks may also be a factor to infect uncontaminated areas with toxic cyst-forming species (Anderson 1984, Hallegraeff & Bolch 1991).

The resting cyst forming dinoflagellate *Gymnodinium catenatum* was previously known only from southern Californian water. Hallegraeff et al. (1988) described the spreading of this toxic species in shellfish rearing areas in Spain, Japan and Tasmania. In European coastal waters a lining infection of *G. catenatum* from the south (Spain) to the north (France) is taken place (Wyatt 1992). A remarkable Indo-Pacific spreading of the toxic resting cyst forming *Pyrodinium bahamense* var. *compressum*

Tab. 1.: Known recent planktonic and benthic (+) dinoflagellates producing a resting cyst (*= toxic; ?= observation of cyst forming has not since been verified).

MARINE SPECIES	REFERENCE
<i>Alexandrium affine</i>	Fukuyo & Inoue 1990, in Red Tide Organisms in Japan, eds. Fukuyo et al., Uchida Rokakuho Tokyo, 84-85
<i>catenella*</i>	Yoshimatsu 1981, Bull. Plankton Soc. Japan 28, 131-139
<i>cohorticula*</i>	Fukuyo & Pholpunthin 1990, in Red Tide Organisms in Japan, eds. Fukuyo et al., Uchida Rokakuho Tokyo, 88-89
<i>excavatum*</i>	Anderson & Wall 1978, J. Phycol. 14, 224-234
<i>hiranoi</i>	Kita & Fukuyo 1988, Bull. Plankton Soc. Japan 35, 1-7
<i>leei</i>	Fukuyo & Pholpunthin 1990, in Red Tide Organisms in Japan, eds. Fukuyo et al., Uchida Rokakuho Tokyo, 92-93
<i>lusitanicum</i>	Blanco 1989, Scient. Mar. 53, 785-796
<i>minutum*</i>	Bolch et al. 1991, Phycologia 30, 215-219
<i>monilatium</i>	Walker & Steidinger 1979, J. Phycol. 15, 312-315
<i>ostenfeldii*</i>	Dale 1977, Sarsia 63, 29-34
<i>pseudogonyaulax</i>	Montresor et al. 1991, in Abstracts 5th Intern. Conf. on toxic Marine Phytoplankton, Newport, 86
<i>tamarense*</i>	Anderson & Wall 1978, J. Phycol. 14, 224-234
<i>Amphidinium carterae*</i>	Cao Vien 1967, Comptes Rendus Acad. Paris Ser. D 264, 1006-1008
<i>Cachonina ? niei</i>	v. Stosch 1969, Helgol. wiss. Meeresunters. 19, 558-568
<i>Coolia monotis</i> ⁺	Faust 1992, J. Phycol. 28, 94-104
<i>Diplopelta parva</i>	Matsuoka 1988, Rev. Palaeobot. Palynol. 56, 95-112
<i>Diplopsalis lebourae</i>	Matsuoka 1988, Rev. Palaeobot. Palynol. 56, 95-112
<i>lenticula</i>	Matsuoka 1988, Rev. Palaeobot. Palynol. 56, 95-112
<i>Diplopsalopsis orbicularis</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>Ensiculifera carinata</i>	Matsuoka et al. 1990, Bull. Plankton Soc. Japan 37, 127-143
<i>Fragilidium ? subglobosum</i>	v. Stosch 1969, Helgol. wiss. Meeresunters. 19, 569-577
<i>Gonyaulax digitalis*</i>	Wall & Dale 1967, Rev. Palaeobot. Palynol. 2, 349-354
<i>polyedra*</i>	Nordli 1951, Nytt Mag. Naturvid. 88, 207-212
<i>scrippsae</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>spinifera</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>verior</i>	Matsuoka et al. 1988, Jap. J. Phycol. 36, 311-320
<i>Gotoius abei</i>	Matsuoka 1988, Rev. Palaeobot. Palynol. 56, 95-122
<i>Gymnodinium ? breve*</i>	Walker 1982, Trans. Am. Microsc. Soc. 101, 287-293
<i>catenatum*</i>	Anderson et al. 1988, J. Phycol. 24, 255-262
<i>Gyrodinium instriatum</i>	Matsuoka 1985, Rev. Palaeobot. Palynol. 44, 217-231
<i>resplendens</i>	Dale 1983, in Survival strategies of the Algae, ed. G.A. Fryxell, Cambridge Univ. Press, 69-136
<i>uncatenum</i>	Tyler et al. 1982, Mar. Ecol. Prog. Ser. 7, 163-178
<i>Heterocapsa ? triquetra</i>	Braarud & Pappas 1951, Lebour. Vid. Akad. Avh. I. M. N. Kgl. 2, 1-23
<i>Katodinium fungiforme</i>	Spero & Moree 1981, J. Phycol. 17, 43-51
<i>Perdinium dalei</i>	Lewis 1991, Bot. Mar. 34, 91-106
<i>Pheopolykrikos hartmanni</i>	Matsuoka & Fukuyo 1986, J. Plankton Res. 8, 811-818
<i>Polykrikos kofoidii</i>	Morey-Gains & Ruse 1980, Phycologia 19, 230-232
<i>schwartzii</i>	Reid 1978, New Phytol. 80, 219-229
<i>Prorocentrum lima</i> ⁺ *	Faust 1991, in Abstracts 5th Internat. Conf. Toxic Marine Phytoplankton Newport, 41
<i>? pyrenoideum</i>	Bursa 1959, Canad. J. Bot. 37, 1-31
<i>Protoceratium reticulatum</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>Protoperidinium ? achromaticum</i>	Popovsky & Pfiester 1990, Dinophyceae, Fischer, 272pp

<i>americanum</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>avellana</i>	Lewis et al. 1984, J. Micropalaeontol. 3, 25-43
<i>brochii</i>	Blanco 1989, Scient. Mar. 53, 797-812
<i>claudicans</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>compressum</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>conicoides</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>conicum</i>	Kobayashi & Matsuoka 1984, Jap. J. Phycol. 32, 251-256
<i>denticulatum</i>	Harland 1982, Palaeontology 25, 369-397
<i>divaricatum</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>cf divergens</i>	Dale 1983, in Survival strategies of the Algae, ed. G.A. Fryxell, Cambridge Univ. Press, 69-136
<i>excentricum</i>	Lewis et al. 1984, J. Micropalaeontol. 3, 25-34
? <i>expansum</i>	Hallegraeff & Bolch 1992, J. Plankton Res 14, 1067-1084
? <i>granii</i>	Meunier 1910, Campagne Arctique de 1907, Buleus, 343pp
? <i>hangoei</i>	Lewis et al. 1984, J. Micropalaeontol. 3, 25-34
<i>latissimum</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>leonis</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>minutum</i>	Fukuyo et al. 1977, Bull. Plankton Soc. Japan 24, 11-18
<i>nudum</i>	Harland 1983, Palaeontology 26, 321-387
<i>oblongum</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>pentagonum</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>punctulatum</i>	Wall & Dale 1968, Micropaleontology 14, 265-304
<i>subinermis</i>	Bolch & Hallegraeff 1990, Bot. Mar. 33, 173-192
<i>thorianum</i>	Lewis et al. 1984, J. Micropalaeontol. 3, 25-43
<i>thulesense</i>	Dodge 1985, Atlas of Dinoflagellates, Farrand Press London, 119pp.
<i>Pyrodinium bahamense</i> var. <i>bahamense</i>	Wall & Dale 1969, J. Phycol 5, 140-149
var. <i>compressum</i> *	Matsuoka 1990, in Red Tide Organisms in Japan, eds. Fukuyo et al., Uchida Rokakuho Tokyo, 112-113
<i>Pyrophacus horologium</i>	Wall & Dale 1971, J. Phycol 7, 221-235
<i>steinii</i> var. <i>steinii</i>	Matsuoka 1990, in Red Tide Organisms in Japan, eds. Fukuyo et al., Uchida Rokakuho Tokyo, 116-117
var. <i>vancampoae</i>	Wall & Dale 1971, J. Phycol. 7, 221-235
<i>Scrippsiella crystallina</i>	Lewis 1991, Bot. Mar. 34, 91-106
<i>lachrymosa</i>	Lewis 1991, Bot. Mar. 34, 91-106
<i>precaria</i>	Montresor & Zingone 1988, Phycologia 27, 387-394
<i>rotunda</i>	Lewis 1991, Bot. Mar. 34, 91-106
<i>sweeniae</i>	Wall & Dale 1968, J. Paleontol. 42, 1395-1408
<i>trifida</i>	Lewis 1991, Bot. Mar. 34, 91-106
<i>trochoidea</i>	Anderson et al. 1985, Limnol. Oceanogr. 30, 1000-1009
<i>Zygabikodinium lenticulatum</i>	Matsuoka 1988, Rev. Palaeobot. Palynol. 56,95-122

FRESHWATER SPECIES

REFERENCE

<i>Amphidinium</i> ? <i>cryophilum</i>	Wedemayer et al. 1982, J. Phycol. 18, 13-17
<i>Ceratium carolinianum</i>	Wall & Evitt 1975, Micropaleontology 21, 18-31
<i>cornutum</i>	v. Stosch 1972, Bull. Soc. Bot. Fr., Mem. 53, 201-212
<i>furcoides</i>	Hickel 1988, Hydrobiologia 161, 41-48
<i>hirundinella</i>	Chapman et al. 1982, J. Phycol. 18, 121-129
<i>rhomvoides</i>	Hickel 1988, Hydrobiologia 161, 49-54
<i>Cryptocodinium cohnii</i>	Tuttle & Loeblich III 1975, J. Phycol 11 (Suppl.), 15
<i>Cystodinium</i> ? <i>bataviense</i>	Pfiester & Lynch 1980, Phycologia 19, 178-183
? <i>cornifax</i>	Schilling 1891, Z. wiss. Mikroskop 8, 314
<i>Glenodinium</i> ? <i>emarginatum</i>	Klebs 1912, Verh. Naturh.-Med. Ver. Heid.N.F.11, 369-451
<i>Gloeodinium montanum</i>	Kelley & Pfiester 1989, J. Phycol 25, 241-247

<i>Gymnodinium</i> ? <i>chiastosporum</i>	Cridland 1958, <i>New Phytol</i> 57, 285-287
<i>dodgei</i>	Sarma & Shyam 1974, <i>Br. phycol. J.</i> 9, 21-29
<i>fungiforme</i>	Biecheler 1952, <i>Bull. Biol. France Belg. Suppl.</i> 36, 1-149
? <i>fuscum</i>	Bourelly 1970, <i>Les algues d'eau douce III</i> , N. Boubee Paris, 512pp
<i>helveticum</i> forma <i>achroum</i>	Skuja 1948, <i>Symb. Bot. Upsal.</i> 9(3), 1-399
? <i>impatiens</i>	Skuja 1964, <i>Nova Acta Reg. Soc. Sci. Upsal.</i> 18(3), 1-645
<i>paradoxum</i>	v. Stosch 1972, <i>Bull. Soc. Bot. Fr., Mem.</i> 53, 201-212
<i>pseudopalustre</i>	v. Stosch 1973, <i>Br. phycol. J.</i> 8, 105-13
<i>Peridinium</i> <i>aciculiferum</i>	Popovsky & Pfiester 1990, <i>Dinophyceae</i> , Fischer, 272pp
<i>cinctum</i> forma <i>ovoplanum</i>	Pfiester 1975, <i>J. Phycol.</i> 11, 259-265
forma <i>westii</i>	Eren 1969, <i>Verh. Internat. Verein. Limnol.</i> 17, 1013-1016
<i>cunningtonii</i>	Sako et al. 1984, <i>Bull. Japan. Soc. Sci. Fish.</i> 50,743-750
<i>gatunense</i>	Pfiester 1977, <i>J. Phycol.</i> 13, 92-95
<i>inconspicuum</i>	Pfiester et al. 1984, <i>Am. J. Bot.</i> 71, 1121-1127
<i>limbatum</i>	Wall & Dale 1968, <i>Micropaleontology</i> 14, 265-304
<i>lubiniensiforme</i>	Dilwald 1938, <i>Flora (Jena)</i> 131, 174-192
<i>penardii</i>	Sako et al. 1987, <i>Bull. Jpan. Soc. Sci. Fish.</i> 53,473-478
<i>volzii</i>	Pfiester & Skvarla 1979, <i>Phycologia</i> 18, 13-18
<i>willei</i>	Pfiester 1976, <i>J. Phycol.</i> 12, 234-238
<i>wisconsinense</i>	Evitt & Wall 1968, <i>Stanford Univ. Publ. Geol. Sci.</i> 12 (2), 1-15
<i>Wolozynskia</i> <i>apiculata</i>	v. Stosch 1973, <i>Br. phycol. J.</i> 8, 105-134
? <i>cestocoetes</i>	Thompson 1950, <i>Lloydia</i> 13, 277-299
? <i>coronata</i>	Wolozynska 1917, <i>Bull. Acad. Sci. Cracovic (B)</i> 1917, 114-122
? <i>reticulata</i>	Thompson 1950, <i>Lloydia</i> 13, 277-299
? <i>tenuissima</i>	Wolozynska 1917, <i>Bull. Acad. Sci. Cracovic (B)</i> 1917, 114-122
<i>tylota</i>	Bibby & Dodge 1972, <i>Br. phycol. J.</i> 7, 85-100

between 1972 and 1984 has been documented (MacLean 1989). For Japanese waters between 1978 and 1982 *Alexandrium tamarense* and *Gymnodinium catenatum* spread into eight new areas, accompanied by shellfish poisoning (after Smayda 1990).

Besides the formation of resting cysts another strategic mechanism, i.e. the asexual formation of temporary cysts may be of importance in dinoflagellate life cycle (Fig. 1). Hypertrophic semi-enclosed, brackish basins situated behind the coast line appear to be very favorable sites for the mass development of dinoflagellates, as could be demonstrated for recurrent red tides of *Glenodinium foliaceum* in some of these ponds at the German North Sea coast (Hesse et al. in press). During and in the aftermath of these blooms, the sediment is covered by a carpet of *G. foliaceum* temporary cysts (Fig. 5A), the formation of which may be correlated due to changing environmental conditions. Temporary cysts, however, have no mandatory resting period and the protoplast of the *G. foliaceum* cyst can be divided in up to 8 daughter cells per cyst which may re-seed a second red tide. There is some evidence that the temporary cyst of *G. foliaceum* is also used as an overwintering stage. Culture experiments showed that temporary cysts of this species, which were stored in the dark at 4 °C for 6 months, germinated immediately after transfer in broad daylight at room temperature (Nehring unpubl.). The mechanism of temporary cyst encystment/excystment may play an important role in the long term persistence of red tides in confined areas, but the occurrence of such cysts in natural waters has rarely been recorded.

Common phytoplankton monitoring activities only show the actual stock of vegetative cells. However, the life cycle of plankton organisms are undoubtedly important in many aspects of nuisance bloom formation and should be considered also in monitoring systems.

DISTRIBUTIONS OF RESTING CYSTS

Accumulations of dinoflagellate resting cysts have been observed in a variety of marine ecosystems, including offshore trenches and depressions, fjords, estuaries and shallow coastal embayments (e.g. Cembella et al. 1988, Dale 1976, Dale et al. 1978, White & Lewis 1982). The comparative distribution of cysts showed a general increase in cyst diversity and abundance from the inshore areas to offshore seas and is positively correlated with muddy sediments. Hydrodynamic conditions can produce high local cyst accumulations ("hot spot" for bloom initiation) e.g. convergence zones have been associated with high deposition of cysts which were found at the downstream periphery of the fronts (Cembella et al. 1988, Garcon et al. 1986, Tyler et al. 1982).

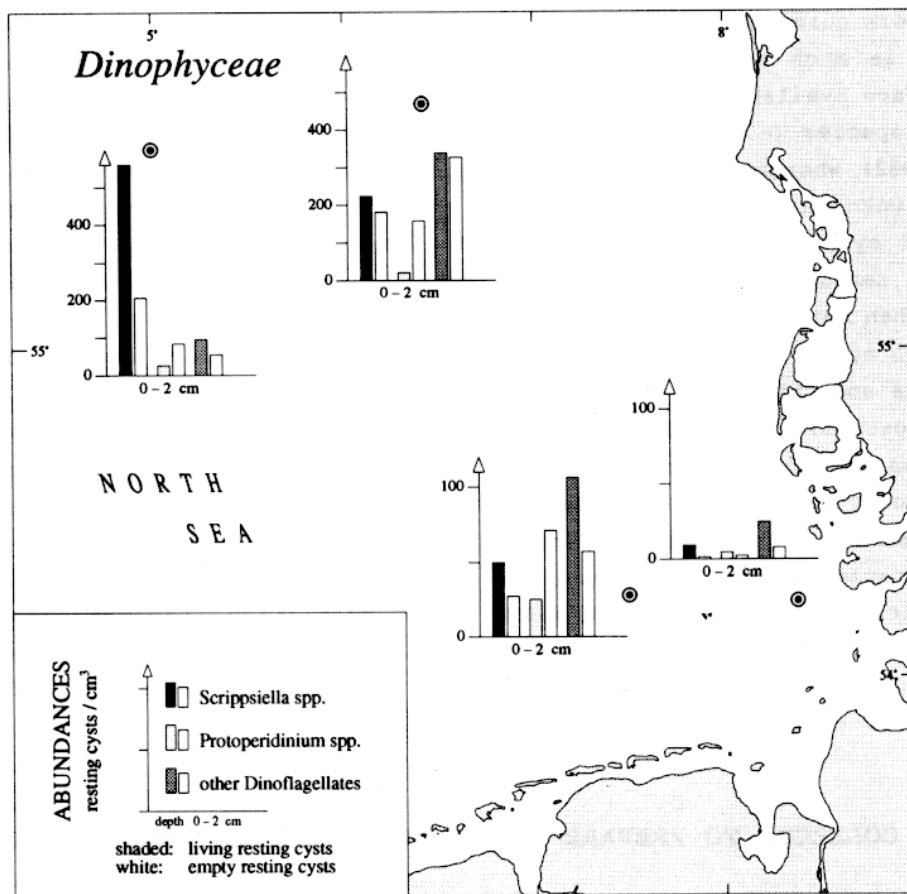


Fig. 3. Abundance of living and empty dinoflagellate resting cysts (*Scrippsiella*: 3 species; *Protoperidinium*: 7 species; other dinoflagellates: 10 species) in the topmost 2-cm of sandy mud North Sea sediments, collected beginning of October 1991.

In sandy mud sediments of the North Sea the top 2-cm of offshore stations have concentrations of 583-682 living cysts/cm³. Empty cysts constituted 34-53 % of the total cyst abundance. The inshore stations have only concentrations of 39-180 living cysts/cm³. The fraction of empty cysts was 25-56 % of the total cyst count (Fig. 3). The dominance of *Scrippsiella* spp. calcareous cysts is remarkable, because Dale (1986) suggested that calcareous cysts are relatively more important in tropical and oceanic rather than temperate regions.



Fig. 4. View of a modified Meischner & Rumohr (1974) gravity corer after collecting an undisturbed sediment core.

Most of cyst distribution studies have been conducted only on qualitative status, presence or absence of a cyst species. These studies are valuable in describing the general geographic cyst distribution and can even indicate areas where nuisance blooms may be an unrecognized problem. Quantitative cyst mapping is much more time-consuming. At present only a few quantitative studies are available, mostly concerning abundances of potential toxic *Alexandrium* species (e.g. Anderson & Keafer 1985, Cembella et al. 1988, White & Lewis 1982) whereas the cyst distribution of non-toxic red-tide species is largely unknown (Nehring submitt.). In studies about the vertical distributions of cysts, the bulk was found to occur in the topmost two to three sediment centimeter (Anderson et al. 1982, Tyler et al. 1982, White & Lewis 1982). When buried in deeper sediment layers by sedimentation or bioturbation (Anderson et al. 1982), cysts may contribute little to bloom initiation, due to anoxia and other inhibitory micro-environmental factors (Anderson et al. 1987). However, cysts are able to survive for years in anoxic sediments (Dale 1983) and erosion or bioturbation bring buried cysts back to the surface. Consequently an unexpected number of cysts are present for bloom initiation.

The need for occasional dinoflagellate cyst mapping surveys is obvious, but quantification should be interpreted with caution. High cyst concentration may indicate potential seedbeds for bloom initiation, however, favorable conditions for germination are a prerequisite. Investigations of the abiotic factors of the sea area as well as studies of physiological ecology of cysts are necessary.

HOW TO COLLECT, TO PREPARE AND IDENTIFY DINOFLAGELLATE RESTING CYSTS

To document the presence of dinoflagellate cysts in sediments, a core sampler is used. Bottom samplers such as dredges or grab buckets (e.g. van Veen grab), which often lose the light fluffy material at

the sediment surface, are less suitable. Best results with respect to an undisturbed sediment surface will be received by a box corer or a gravity corer (e.g. Meischner & Rumohr 1974) (Fig. 4). Sediment cores (10 cm long, 2.6 cm diameter) as subsamples can be obtained from the corer and then be stored in the dark at 4 °C until further examination.

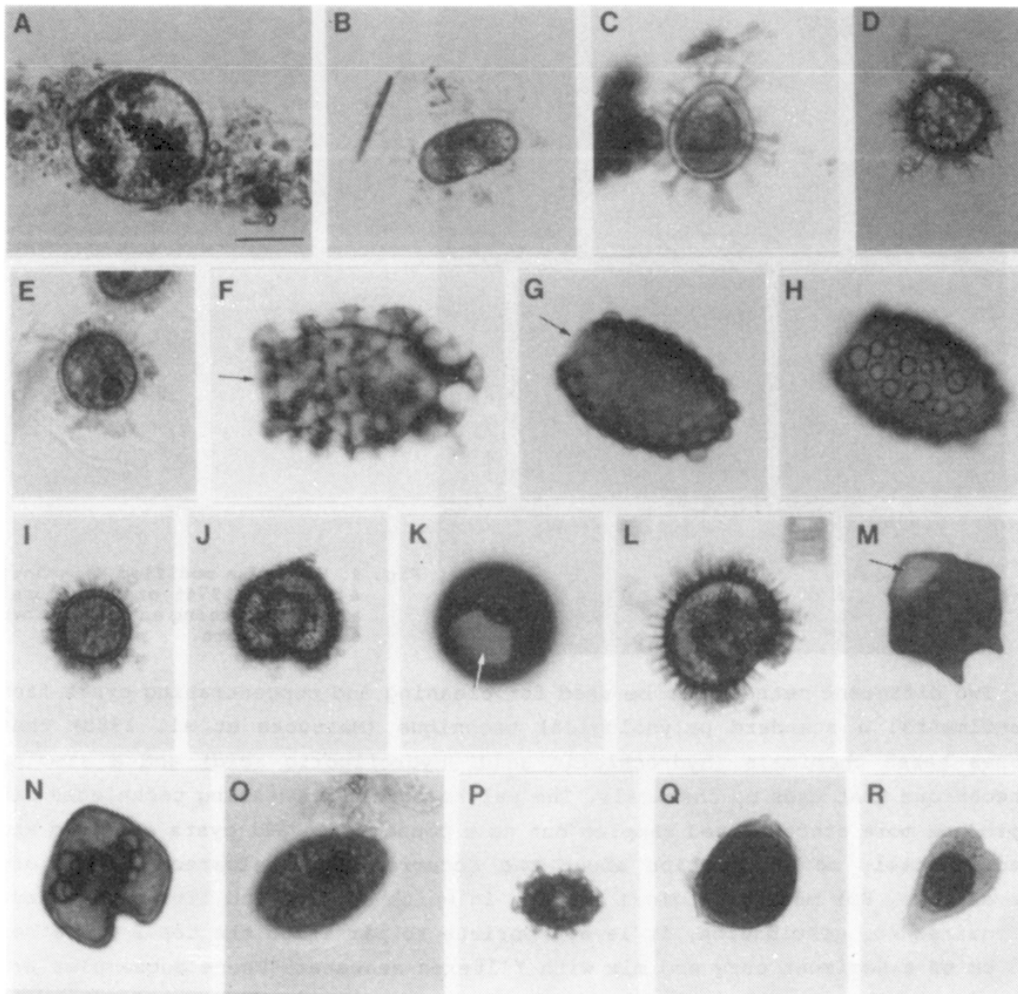


Fig. 5. Cysts of North Sea plankton organisms. **Dinoflagellate temporary cyst:** (A) *Glenodinium foliaceum*. **Dinoflagellate resting cyst:** (B) *Alexandrium* cf. *excavatum*. (C) *Gonyaulax digitalis*. (D) *Gonyaulax polyedra*. (E) *Peridinium dalei*. (F) Empty cyst of *Polykrikos kofoidii*, archeopyle (arrow). (G,H) Empty cyst of *Polykrikos* sp., archeopyle (arrow). (I) *Protoceratium reticulatum*. (J) *Protoperidinium claudicans*. (K) Empty cyst of *Protoperidinium conicoides*, archeopyle (arrow). (L) *Protoperidinium conicum*. (M) Empty cyst of *Protoperidinium leonis*, archeopyle (arrow). (N) *Protoperidinium oblongum*. (O) *Scrippsiella lachrymosa*. (P) *Scrippsiella trifida*. (Q) *Zygabikodinium lenticulatum*. **Tintinnid cyst:** (R) Heart-shaped cyst of ?*Favella* sp. Scale bar: 20 μm in (A,D,E,K,O), 40 μm in (B,C,F-J,L-N,P-R).

Two different methods can be used for cleaning and concentrating cysts from sediments: a standard palynological technique (Matsuoka et al. 1989) that uses harsh chemicals (hydrochloric acid, hydrofluoric acid) and a sieving technique that uses no chemicals. The palynological processing techniques can produce more concentrated samples but as a consequence all cysts are dead and additionally no information about the occurrence of calcareous cysts are available. For most biological studies in which species and living cysts are required for germination, it is appropriate to partition the top 2 or

rather 3 cm of a sediment core and mix with filtered seawater. These subsamples are sonicated for one minute in an ultrasonic cleaning bath, in order to separate the cysts from organic and inorganic aggregates. Pass the suspension through a 150 μm gauze and accumulate on 20 μm gauze. The residue on the 20 μm gauze has to be rewashed and filled up with filtered seawater. Parts of this preparation may be counted on common slides using a light microscope or on Utermöhl slides using an inverted microscope.

Unknown cysts and individual cysts may be used for germination experiments to identify the species by identification of the motile thecate cell stage. To do this, pick cysts out from Utermöhl slides using a micropipette and wash twice in filtered seawater. Then cysts should be placed in small sterile incubation chambers and filled up with filtered seawater of the sample location or incubated in F/2-nutrient solution (medium). The cysts can be kept at room temperature ($\approx 18^\circ\text{C}$) and examined regularly for germination.

Most resting cysts are spherical, ellipsoid or polygonal with or without spine-like ornamentations and range from 20 to 80 μm in diameter. The shape but also wall structure and color, paratabulation and the type of archeopyle (exit opening for germination) are important features used in cyst identification. The archeopyle is very useful in classifying the genus but the opening is not visible until excystment. Resting cysts are generally less conservative in morphology than their corresponding motile stages and therefore may be easier to identify (Fig. 5) (The asexual formed temporary cyst has no characteristics useful for identification; under non-suitable environmental condition motile cells cutting off the flagella and sometimes they shed their theca and cell membrane to transform into round ball-like cells (Fig. 5A)).

A first guide to resting cyst identification has been prepared by Matsuoka et al. (1989) but not all known recent cysts are considered. At present, palaeontologists and biologists often use different names for the life history stages of the same dinoflagellates. Palynologists use a cyst-based classification while biologists have developed a dinoflagellate classification system based on motile stages which is commonly used in biological cyst investigations. Unfortunately no comprehensive guide to recent resting cyst identification is available at the moment. The literature list given in this paper (Tab. 1) should provide a first introduction to cyst descriptions and taxonomy.

CONCLUSION

Many factors have been put forward to account for the development of nuisance blooms in coastal zones. Usually hydrological factors as temperature or salinity stratification and adequate nutrient and trace metal availability are held responsible for the phenomenon. The most frequent causative organisms for nuisance blooms are dinoflagellates. The role of the complex life-strategy of these forms in initiating bloom formation has not been considered sufficiently in the past. A partial explanation for the timing, persistence and recurrence of nuisance blooms may be achieved when studies of hydrodynamic, chemical and biological factors of the water column and the quantity, spatial and temporal distribution of resting cysts in the sediment are taken into account. It is suggested that the synthesis of sexuality, resting cyst formation and toxin production may be a very successful combination to balance short- and long-time variations in the ecosystem. That is why cyst-forming species may be of increasing importance in a changing world.

ACKNOWLEDGEMENTS: I thank Dr K.-J. Hesse for valuable discussions and comments on the manuscript and W. Huckriede for the drafting of diagrams. Part of this study is supported by the Federal Environmental

Agency, Environmental Research Plan of the Minister for the Environment, Nature Conservation and Nuclear Safety of the Federal Republic of Germany (Grant 108 02 085/1), and by the State of Schleswig-Holstein. This is publication no.41 of the project Ecosystem Research Wadden Sea.

LITERATURE CITED

- Anderson, D.M. 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. *J. Phycol.* 16: 166-172.
- Anderson, D.M. 1984. Shellfish toxicity and dormant cysts in toxic dinoflagellate blooms. In, *Seafood Toxins*, Ragelis, E.P. (ed.). Amer. Chem. Soc. Symposium Series No. 262, Wash. D.C.: 125-138.
- Anderson, D.M. 1989. Cysts as factors in *Pyrodinium bahamense* ecology. In, *Biology, epidemiology and management of Pyrodinium red tides*, Hallegraeff, G.M. & Maclean, J.L. (eds.). ICLARM Conference Proc. 21: 81-88.
- Anderson, D.M. & Wall, D. 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 14: 224-234.
- Anderson, D.M. & Morel, F.M.M. 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. *Estuar. Coast. Shelf Sci.* 8: 279-293.
- Anderson, D.M. & Keafer, B.A. 1985. Dinoflagellate cyst dynamics in coastal and estuarine waters. In, *Toxic Dinoflagellates*, Anderson, D.M., White, A.W. & Boaden, D.G. (eds.). Elsevier, Inc.: 219-224.
- Anderson, D.M. & Lindquist, N.L. 1985. Time-course measurements of phosphorus depletion and cyst formation in the dinoflagellate *Gonyaulax tamarensis* Lebour. *J. exp. mar. Biol. Ecol.* 86: 1-13.
- Anderson, D.M. & Keafer, B.A. 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. *Nature (Lond.)* 325: 616-617.
- Anderson, D.M., Aubrey, D.G., Tyler, M.A. & Coats, D.W. 1982. Vertical and horizontal distributions of dinoflagellate cysts in sediments. *Limnol. Oceanogr.* 27: 757-765.
- Anderson, D.M., Chisholm, S.W. & Watras, C.J. 1983. Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis*. *Mar. Biol.* 76: 179-189.
- Anderson, D.M., Kulis, D.M. & Binder, B.J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield batch cultures. *J. Phycol.* 20: 418-425.
- Anderson, D.M., Coats, D.W. & Tyler, M.A. 1985a. Encystment of the dinoflagellate *Gyrodinium uncatenum*: temperature and nutrient effects. *J. Phycol.* 21: 200-206.
- Anderson, D.M., Lively, J.J., Reardon, E.M. & Price, C.A. 1985b. Sinking characteristics of dinoflagellate cysts. *Limnol. Oceanogr.* 30: 1000-1009.
- Anderson, D.M., Taylor, C.D. & Armbrust, E.V. 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.* 32: 340-351.
- Braarud, T. 1945. Morphological observations on marine dinoflagellate cultures (*Porella perforata*, *Goniaulax tamarensis*, *Protoceratium reticulatum*). *Avh. norske Viensk. Akad. Oslo I. Mat.-Naturv. Klasse* 1944 (11): 18pp.
- Burkholder, J.M., Noga, E.J., Hobbs, C.H. & Glasgow Jr., H.B. 1992. New phantom dinoflagellate is the causative agent of major estuarine fish kills. *Nature (Lond.)* 358: 407-410.
- Cembella, A.D., Turgeon, J., Therriault, J.-C. & Beland, P. 1988. Spatial distribution of *Protogonyaulax tamarensis* resting cysts in nearshore sediments along the north coast of the Lower St. Lawrence estuary. *J. Shellfish Res.* 7: 597-609.
- Dale, B. 1976. Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. *Rev. Palaeobot. Palynol.* 22: 39-60.

- Dale, B. 1983. Dinoflagellate resting cysts: "benthic plankton". In, Survival Strategies of the Algae, Fryxell, G.A. (ed.). Cambridge Univ. Press: 69-136.
- Dale, B. 1986. Life cycle strategies of oceanic dinoflagellates. Unesco Technical Papers in Marine Science 49: 65-72.
- Dale, B., Yentsch, C.M. & Hurst, J.W. 1978. Toxicity in resting cysts of the red-tide dinoflagellate *Gonyaulax excavata* from deeper water coastal sediments. Science 201: 1223-1224.
- Diwald, K. 1938. Die ungeschlechtliche und geschlechtliche Fortpflanzung von *Gleodinium lubiniense* spec. nov. Flora, Jena 131: 174-192.
- Doucette, G.J., Cembella, A.D. & Boyer, G.L. 1989. Cyst formation in the red tide dinoflagellate *Alexandrium tamarense* (Dinophyceae): effects of iron stress. J. Phycol. 25: 721-731.
- Evitt, W.R. & Davidson, S.E. 1964. Dinoflagellate studies. I. Dinoflagellate cysts and thecae. Stanford Univ. Publ. Geol. Sci. 10 (1): 12pp.
- Garcon, V.C., Stolzenbach, K.D. & Anderson, D.M. 1986. Tidal flushing of an estuarine embayment subject to recurrent dinoflagellate blooms. Estuaries 9: 179-187.
- Hallegraeff, G.M. & Bolch, C.J. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. Mar. Pollut. Bull. 22: 27-30.
- Hallegraeff, G.M., Steffensen, D.A. & Wetherbee, R. 1988. Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. J. Plankton Res. 10: 533-541.
- Hensen, V. 1887. Ueber die Bestimmung des Plankton's oder des im Meere treibenden Materials an Pflanzen und Thieren; nebst Anhang. Bericht der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere, in Kiel 1882-1886, 5: 1-108.
- Hesse, K.-J., Hentschke, U. & Brockmann, U. in press. A synoptic study of nutrient and phytoplankton characteristics in the German Wadden Sea with respect to coastal eutrophication. Proc. 25th Europ. Mar. Biol. Symp.
- Klebs, G. 1912. Über Flagellaten- und Algen-ähnliche Peridineen. Verh. Naturh.-Med. Ver. Heidelberg N.F. 11: 369-451.
- MacLean, J.L. 1989. Indo-Pacific red tide occurrences, 1985-1988. Mar. Pollut. Bull. 20: 304-310.
- Matsuoka, K., Fukuyo, Y. & Anderson, D.M. 1989. Methods for modern dinoflagellate cyst studies. In, Red Tides: Biology, Environmental Science, and Toxicology, T. Okaichi, D.M. Anderson & T. Nemoto (eds.). Elsevier, Inc.: 461-479.
- Meischner, D. & Rumohr, J. 1974. A light-weight, high-momentum gravity corer for subaqueous sediments. Senckenbergiana marit. 6: 105-117.
- Nehring, S. 1991. Lethal effects of a *Nodularia spumigena* bloom at the North Sea coast. Red Tide Newsletter 4 (2&3): 8-9.
- Nehring, S. submitt. Dinoflagellate resting cysts as factors in phytoplankton ecology of the North Sea. Helgol. Meeresunters.
- Oshima, Y., Singh, H.T., Fukuyo, Y. & Yasumoto, T. 1982. Identification and toxicity of the resting cysts of *Protogonyaulax* found in Ofunato Bay. Bull. Jap. Soc. Sci. Fish. 48: 1303-1305.
- Seliger, H.H., Tyler, M.A. & McKinley, K.R. 1979. Phytoplankton distributions and red tides resulting from frontal circulation patterns. In, Toxic Dinoflagellate Blooms, Taylor, D.L. & Seliger, H.H. (eds.). Proc. Sec. Int. Conf. on Toxic Dinoflagellate Blooms. Elsevier, North Holland: 239-248.
- Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In, Toxic Marine Phytoplankton, Graneli, E., Sundström, B., Edler, L. & Anderson, D.M. (eds.). Elsevier, Inc.: 29-40.
- Steidinger, K.A. 1975. Basic factors influencing red tides. In, Proc. First Intern. Conf. Toxic Dinoflagellate Blooms, V.R. LoCicero (ed.). Mass. Sci. Technol. Found.: 153-162.

- Stein, F. 1883. Der Organismus der Infusionstiere. III. Abteilung. Die Naturgeschichte der Flagellaten oder Geisselinfusorien. 2. Hälfte. Der Organismus der arthrodelen Flagellaten. Verlag W. Engelmann Leipzig: 30pp.
- Tyler, M.A., Coats, D.W. & Anderson, D.M. 1982. Encystment in a dynamic environment: deposition of dinoflagellate cysts by a frontal convergence. *Mar. Ecol. Prog. Ser.* 7: 163-178.
- Wall, D. & Dale, B. 1966. "Living fossils" in western Atlantic plankton. *Nature* 211: 1025-1026.
- White, A.W. & Lewis, M. 1982. Resting cysts of the toxic red tide dinoflagellate *Gonyaulax excavata* in Bay of Fundy sediments. *Can. J. Fish. Aquat. Sci.* 39: 1185-1194.
- Wyatt, T. 1992. *Gymnodinium catenatum* in Europe. *Harmful Algae News* 2, Suppl. to *IMS Newsletter* 63: 4-5.
- Yentsch, C.M. & Mague, F.C. 1979. Motile cells and cysts: two probable mechanisms of intoxication of shellfish in New England waters. In, *Toxic Dinoflagellate Blooms*, Taylor, D.L. & Seliger, H.H. (eds.). Elsevier, Inc.: 127-130.
- Zederbauer, E. 1904. Geschlechtliche und ungeschlechtliche Fortpflanzung von *Ceratium hirundinella*. *Ber. deutsch. Bot. Ges.* 22: 1-8.