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THE IMPACT OF HARMFUL ALGAL BLOOMS ON FINFISH MORTALITY, PATHOLOGY AND TOXICOLOGY

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“Et toutes les eaux qui étaient dans la rivière s'étaient changées en sang. Et les poissons se trouvaient dans la rivière et moururent. Et la rivière empestait, et les Egyptiens ne pouvaient pas boire l'eau de la rivière.”

(Livre de l'Exode, 7, 20-24)

REPÈRES OCÉAN N° 10 - 1995

**The IMPACT of HARMFUL ALGAL BLOOMS on FINFISH
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***IMPACT des TOXINES ALGALES sur les POISSONS
MORTALITÉ, PATHOLOGIE, TOXICOLOGIE***

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Nantes, 18-22 octobre 1993

sous le titre

**“The impact of harmful algal blooms on finfish :
occurrence of fish kills, pathology, toxicological mechanisms,
ecological and economic impacts.
A review.”**

par

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TABLE DES MATIÈRES

SOMMAIRE EN FRANÇAIS	5
INTRODUCTION.....	9
CHAPTER I - OCCURRENCE OF FISH KILLS ASSOCIATED WITH "RED TIDES"	11
Europe	13
North america	14
Indo pacific region.....	14
Other countries.....	15
CHAPTER II - ALGAL SPECIES INVOLVED IN FISH KILLS.....	25
CHAPTER III - CAUSATIVE MECHANISMS OF FISH DEATH	35
O ₂ depletion	35
Mechanical damage to gills	36
Chemical injury from toxins.....	37
Miscellaneous negative factors.....	41
CHAPTER IV - TOXICOLOGY	43
CHAPTER V - INTEREST OF TOXIC ALGAL BLOOMS	51
General toxicology.....	51
Ecological and economic impacts	51
Human health hazard	53
CONCLUSION	54
BIBLIOGRAPHIE	55

SOMMAIRE

IMPACT DES TOXINES ALGALES SUR LES POISSONS : REVUE DES CAS DE MORTALITÉ, DES EFFETS PATHOLOGIQUES ET DES MÉCANISMES TOXICOLOGIQUES

Des blooms algaux se produisent, sous forme de “marées rouges ou brunes”, dans les divers milieux aquatiques du monde entier : large, littoral, lacs, rivières Ils se révèlent très souvent dangereux pour les organismes marins ou d’eau douce et, **en particulier, pour les poissons**. Leur diversité, leur fréquence, leur extension géographique et l’importance des dommages causés aux pêcheries et aux exploitations aquacoles où les pertes économiques sont élevées, justifient l’intérêt croissant que leur portent biologistes, halieutes, éleveurs et toxicologues.

La littérature scientifique, peu importante jusque dans les années soixante-dix – moins de 50 références – s’est considérablement enrichie au cours des récentes décennies, soit actuellement 300 références. Les publications et rapports concernent des observations aussi bien en milieu naturel que dans des élevages, et des expériences d’intoxications testées en laboratoire.

Ceci est une revue exhaustive des connaissances actuelles, tant sur les espèces algales responsables des accidents de mortalités massives de poissons que sur les biotoxines impliquées et leurs mécanismes d’action.

LES MORTALITÉS DE POISSONS ASSOCIÉES AUX BLOOMS ALGAUX

Si des données “anecdotiques” révèlent l’existence de blooms depuis des époques préhistoriques et historiques, leur nombre s’est accru de façon spectaculaire ces dernières décennies, ainsi qu’en témoignent les publications consacrées à leur description, soit 54 en Europe, 56 en Amérique et 36 dans l’Indo-Pacifique ... L’inventaire consiste en une présentation, continent par continent, dans les eaux intérieures et dans les eaux marines côtières, des espèces algales nocives, des poissons qui en sont victimes ainsi que des causes probables de cette létalité.

LES ESPÈCES ALGALES RESPONSABLES DES MORTALITÉS

Six classes d’algues unicellulaires sont impliquées dans les blooms mortels pour les poissons : Cyanophycées, Dinoflagellés, Chloromonades, Prymnesionomades, Silicoflagellés et Diatomées. L’ensemble représente une soixantaine d’espèces algales classées en trois catégories en fonction de leur mode d’action sur les poissons.

CAUSES DE MORTALITÉ

Trois types principaux de pathogénicité sont considérés.

Privation d’oxygène résultant de proliférations massives – plusieurs millions de cellules/litre – suivies de mortalités brutales des populations algales qui provoquent une forte

demande en oxygène (DBO) et conduisent à des conditions hypoxiques ou anoxiques responsables de l'asphyxie des poissons.

Lésions mécaniques des branchies

Divers Diatomées et Silicoflagellés provoquent des lésions cellulaires des lamelles secondaires des branchies. A une telle atteinte de l'intégrité de l'épithélium respiratoire s'ajoute une hypersécrétion de mucus, tous deux responsables d'une réduction des échanges gazeux et ioniques. Les troubles de respiration et d'osmorégulation ainsi induits se révèlent souvent mortels pour les poissons, en particulier les Salmonidés d'élevage.

Effets toxiques

De nombreuses algues produisent des toxines (biotoxines) dont les effets sont perceptibles par des observations histopathologiques des principaux organes-cibles que sont la branchie, le foie, le tractus digestif et le tégument.

TOXICOLOGIE

Plusieurs biotoxines algales ont été isolées (anatoxine, aplantoxine, microcystine, prymnesine, brevetoxine, saxitoxine...) et testées sur des organismes : souris et poissons tels que *Gambusia*, *Poecilia*, *Brachydanio*, *Fundulus*, *Oncorhynchus*..., ceci, en dépit de nombreuses difficultés liées à leur très grande diversité (peptides, polyéthers, alcaloïdes, acides gras...), très faible concentration, extrême variabilité en rapport avec diverses souches génétiques, ainsi qu'à la complexité des méthodes de purification et de séparation des métabolites, Les principales approches expérimentales relatives à des tests toxicologiques sont les suivantes :

- immersion dans des extraits naturels ou produits en culture,
- administration par voie orale,
- injection intrapéritonéale d'extraits toxiques,
- toxicité transmise le long de la chaîne trophique, les vecteurs d'intoxication étant des *Invertébrés consommateurs de phytoplancton*, tels que des Copépodes et les poissons cibles étant des larves (maquereaux ...) ou des adultes planctonophages (anchois, harengs ...).

Compte tenu du type de leurs effets toxiques, ces toxines ont été classées en plusieurs catégories :

- neurotoxines (STX, neoSTX, gonyautoxines, brevetoxines, anatoxine ...) ayant des effets sur les pompes ioniques (Na^+) des cellules nerveuses et des jonctions neuromusculaires.
- hépatotoxines (microcystine, prymnesine, nodularine ...)
- hémolysines
- cytotoxines
- chtyotoxines

Compte tenu de la variété des toxines et de la grande diversité des protocoles expérimentaux sur de nombreuses espèces cibles, une compréhension claire des mécanismes toxiques fait souvent défaut.

INTÉRÊT DE LA CONNAISSANCE DES BLOOMS ALGAUX

Trois thèmes d'intérêt, tant du point de vue fondamental qu'appliqué, se dégagent.

Toxicologie générale et pathologie des poissons

Nous manquons de connaissances en ce domaine. Plusieurs questions se posent quant à savoir si les poissons sont capables de détecter les souches toxiques et surtout quant au devenir (métabolisation, détoxification, excrétion, stockage ...) de ces toxines.

Impact écologique et économique

Les effets négatifs des blooms algaux s'exercent clairement sur les pêcheries marines (sardines, harengs, anchois...) et surtout sur les populations d'élevage (Salmonidés, sérioles, morues...) où les pertes peuvent être considérables. Les incidences sur les larves marines de poissons, donc sur leur recrutement, sont mal connues mais peuvent être graves. Des troubles s'exercent aux niveaux les plus élevés de la chaîne alimentaire, notamment chez les Oiseaux piscivores (cormorans, hérons, mouettes ...) et les Mammifères marins (dauphins, phoques, baleines...).

Risques pour la santé humaine

Divers cas de morbidité et même de mortalité ont été recensés chez des humains consommateurs de poissons toxiques.

CONCLUSION

Compte tenu de l'ampleur des phénomènes d'efflorescences algales et de leurs effets négatifs sur les poissons (populations sauvages et espèces d'intérêt aquacole), ainsi que sur les consommateurs de poissons que sont les Oiseaux et les Mammifères, et surtout l'espèce humaine, un **effort de recherche scientifique interdisciplinaire** paraît s'imposer, justifiant d'ailleurs une large coopération internationale.

INTRODUCTION

Algal blooms occur worldwide and, in some areas, are a common seasonal occurrence. Indeed, certain algal species undergo a rapid increase (their number may attain several million cells per litre) and form visible patches on the water surface (open sea, coastal areas, lakes, rivers...) referred to as "red tides" or "brown tides". These blooms may be noxious for aquatic species and especially marine and freshwater fish, either causing anoxia through the process of decay and clogging of fish gills, or producing specific toxins.

Intensified scientific and public awareness together with an increased concern for fishery and aquacultural industries are leading to a growing number of reports on harmful algal blooms in the world, and especially in European coastal waters (ICES, 1992), the US and Canada (WHITE, 1982a, 1984) as well as in Australian waters and the Indo-Pacific region (HALLEGRAEFF, 1993). Most phytoplankton blooms appear to be harmless events, but, under exceptional conditions, they may become so densely concentrated that they deplete oxygen or produce toxins noxious to fish. There is evidence of toxic blooms from prehistoric and historic times (presence of fossil cysts in bottom sediments : DALE *et al.*, 1993) but it appears that the incidence and diversity of blooms has been increasing in recent years due to anthropogenic and non-anthropogenic factors (DALE and NORDBERG, 1993). Harmful algal blooms (HABS) are of immense importance causing damage to ecosystems, fisheries, aquaculture and human health. Thus, bloom algae are becoming not only one of the major economic threats to fisheries and fish culture industries (WHITE, 1988) but are also a public health hazard.

This paper reviews the literature available on the occurrence of toxic algal bloom and fish kills (50 references up till 1975 and more than 250 from 1976 to 1993) and discusses their noxious effects on fish resulting in economic losses. Harmful effects associated with red tides have been reviewed in many scientific papers (TANGEN, 1977 ; SHUMWAY, 1990 ; HALLEGRAEFF, 1987, 1993 ; WHITE, 1982a, 1984 ; WALDICHUK, 1990 ; ICES, 1992) and are reported on the proceedings of many international workshops, especially over the past 20 years (GRANELLI *et al.*, 1990 ; FREMY, 1991 ; GORDON, 1991 ; HALLEGRAEFF, 1991 ; PARK and KIM, 1991 ; SMAYDA and SHIMIZU, 1993).

Three types of data are available in the ever expanding literature currently available : a) wild fish in natural habitats, b) cultured fish in fish farms, and c) experimental investigations on fish tested in laboratory conditions. Their total number is nearly 300.



Figure 1 - A fish kill of unknown origin of the shores of the Gulf of Lion, summer 1987,

(Photography from M. Coupeau)

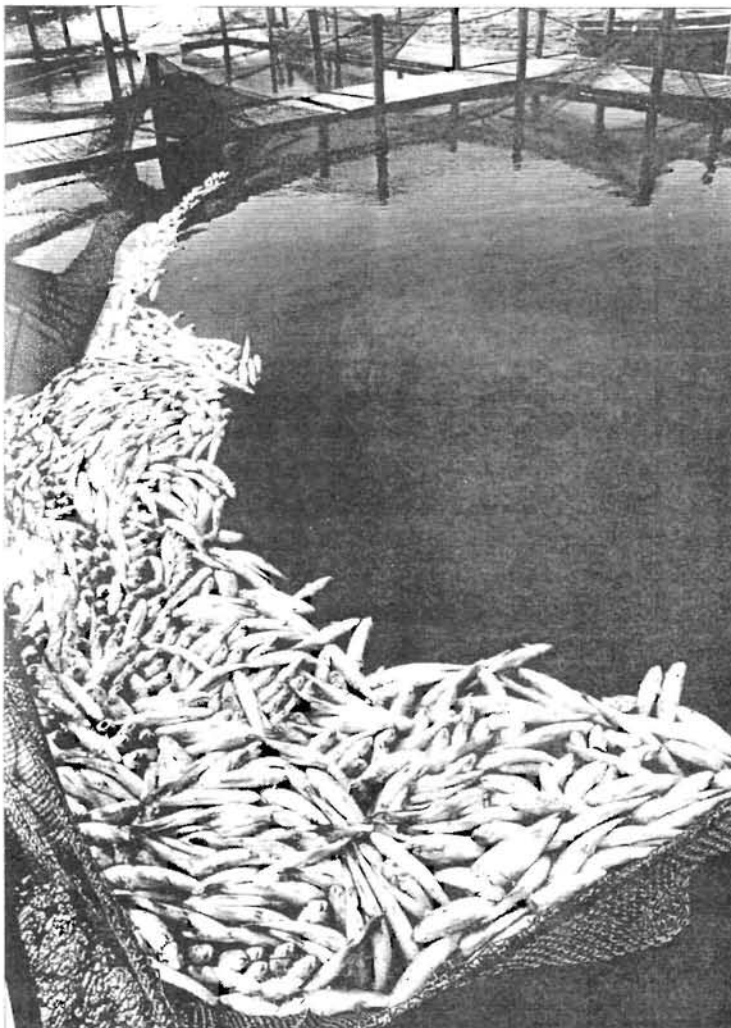


Figure 2 - Fish cage farm strucky by a sudden bloom of the toxic algae *Chrysochromulina polylepis*

(from C. Folke and N. Kautski *Ambio*, 1989, 18, p. 240)

CHAPTER I

OCCURRENCE OF FISH KILLS ASSOCIATED WITH "RED TIDES"

Discolored water and its association with fish mortality as well as mortality in other vertebrates (birds and mammals) have been noted since biblical times (i.e. the famous Egyptian plagues with rivers "turning to blood", Exodus). Red tides were also observed as early as 1770 during Captain Cook's voyage through the Coral Sea (HALLEGRAEFF, 1992), in 1844 along the West coast of Florida (GUNTER *et al.*, 1948), in 1878 in an Australian lake (PHILLIPS *et al.*, 1985) in 1891 in Sydney Harbor (HALLEGRAEFF, 1992)... There is however a limited number of records for over a long time period documenting a series of harmful blooms. Although they should be regarded as completely natural events, their worldwide increasing frequency in recent years has since been correlated with environmental changes of anthropogenic origin. Since the 1970's, records of the incidence of injury to wild and cultured fish owing to toxic phytoplankton have increased. It has also been demonstrated (HALLEGRAEFF, 1993) that not only one toxic algal bloom spreading in intensity, but also in geographic distribution (open sea as well as sea shores, estuaries, lakes and rivers) and in the number of algal species involved.

Although cold-blooded vertebrates have been previously considered to be relatively insensitive to algal toxins (PRAKASH *et al.*, 1971), a great number of more or less catastrophic mass mortalities of fish, coinciding with phytoplankton blooms, have been reported in various marine, brackish and freshwater bodies of many geographical areas. Diseased and dead fish include bottom-dwelling fish as well as pelagic fish, both wild species (Fig. 1) and cultured species (reviews in WHITE, 1984 ; HALLEGRAEFF, 1993 and Fig. 2). Wild fish populations have the freedom to swim away from noxious areas while caged fish tend to be extremely vulnerable to algal blooms. Indeed, some blooms are only detected as the result of their detrimental effects on caged fish.

Papers devoted to fish kills in natural habitats, as well as in cultural conditions, are numerous (149), reflecting a problem which occurs on a global scale (Tables I, II, III, and IV and Fig. 3).

* Europa	54	* America	56
- Bulgaria	2	- Canada	18
- France	2	- USA	29
- Germany	1	- Others	9
- Italy	4		
- Netherlands	1	* Indo-Pacific	36
- Scandinavia	24	* South Africa	3
- Spain	1		
- Turkey	1		
- U.K.	16		
- Yougoslavia	1		
- Russia	1		

The first anecdotal records of fish kills associated with algal blooms were widespread. Several fish kills were been noted in the U.K. (Norfolk) in 1894, 1911, 1914, 1934, 1954, 1966 and 1967 (BOWLER, 1970 in HOLDWAY *et al.*, 1978), some of which were linked to the phytoplankton

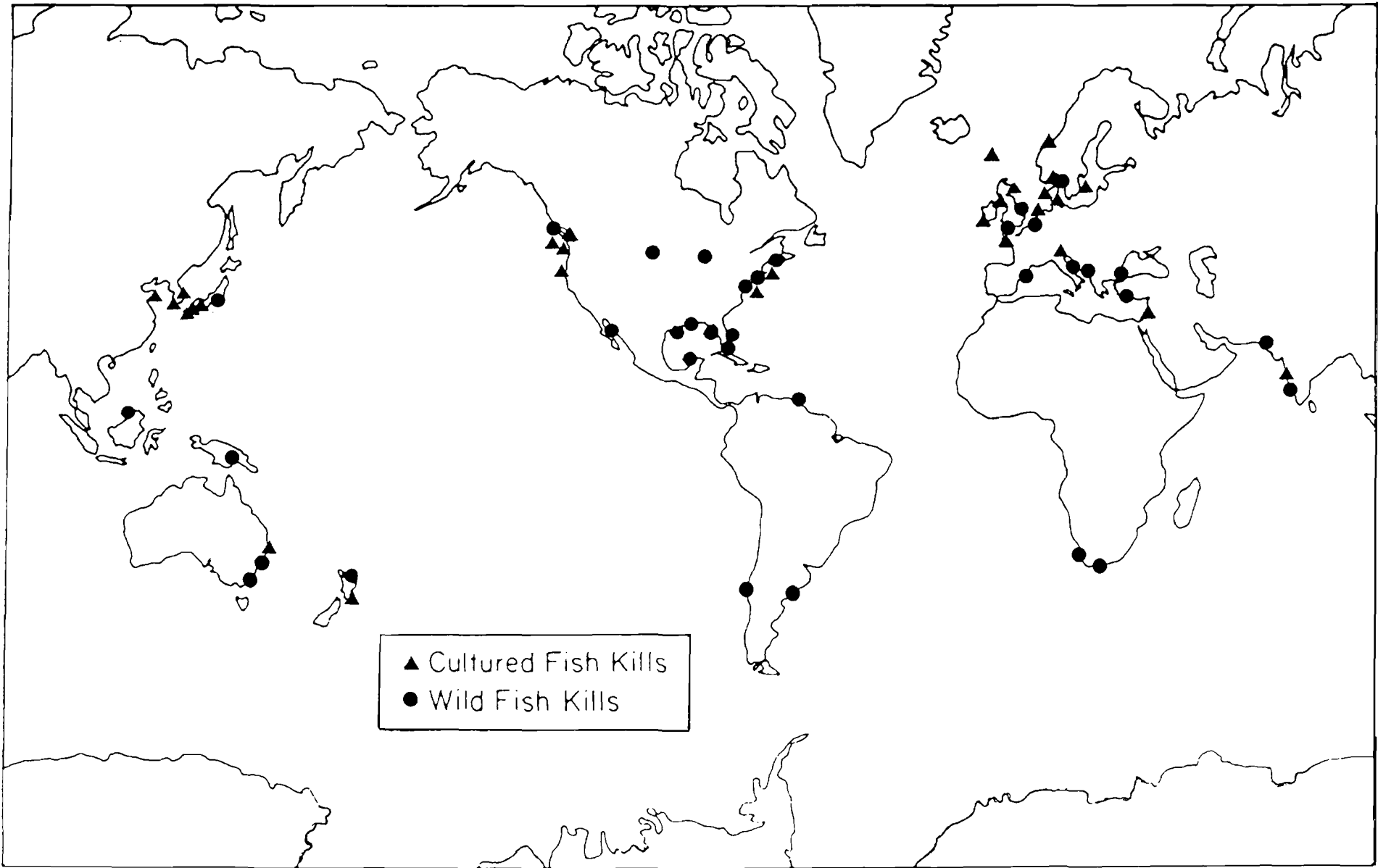


Figure 3 - Global world distribution of documented kills, both wild (●) and cultured (▲) fish (from Taylor, 1990, p. 539)

Prymnesium parvum, while in other cases, the phytoplanktonic blooms were only suspected to be related to mass fish mortality since a noxious alga could not be demonstrated (RAE *et al.*, 1965).

Numerous other reports varying in the degree of detailed scientific observation of fish kills have been published in Europe (Table I), North America (Table II), the Indo-Pacific region (Table III) and a number of countries (Table IV).

Some examples of fish mortality in various freshwater, brackish-water and marine ecosystems, are presented on the following pages :

EUROPE

• *Freshwater bodies*

Scientific data is easier to collect in the relatively limited area in which freshwater bodies tend to be confined as opposed to the open sea. Thus, widespread mortalities of pike (*Esox lucius*), perch (*Perca fluviatilis*), bream (*Abramis brama*), roach (*Rutilus rutilus*) and eel (*Anguilla anguilla*) in the Thurne River (Norfolk, U.K.) were soon linked to a *Prymnesium parvum* bloom (HOLDWAY *et al.* (1978).

• *Coastal marine areas*

The deaths of a number of benthic species (*Solea*, *Pleuronectes*, *Platichthys*) were observed in Southern Ireland (OTTWAY *et al.*, 1979) when a bloom of *Gyrodinium aureolum* developed during the summer of 1976. A few years later (1979), another *Gyrodinium* bloom occurred in the same area and was responsible for the deaths of *Myxocephalus* and *Nerophis* species (CROSS and SOUTHGATE, 1980). Another mass occurrence of *Gyrodinium* along the Southern coast of Norway in September 1987 was responsible for the death of rainbow trout, eel and cod kept in nets and cages (DAHL *et al.*, 1982). *Gyrodinium aureolum* blooms in Norwegian waters have been observed in 1966, 1974, 1976, 1977, 1979, 1981, 1982, 1983, 1984, 1985, 1988, 1989 and 1990. The majority of algal blooms which have caused fish mortality have amounted a total loss of at least 2,000 - 3,000 tons of Atlantic salmon and rainbow trout in Norwegian, Scottish and Irish fish farms. Thus, it has been the most important harmful algae for fish farming industry along the Norwegian coast until now (DAHL and TANGEN, 1993). Similar mortality in benthic fish species (*Solea*, *Arnoglossus*, *Gobius*, *Lithognathus*) took place in May 1969 along the coast of Romagna (Adriatic shores, Italy). It was associated with a *Peridinium depressum* bloom (PICCINETTI and MANFRIN, 1969). Deaths of sand eels (*Ammodytes sp.*), which occurred in May 1968 along the east coast of the U.K., were also associated with a *Gonyaulax tamarensis* bloom (ADAMS *et al.*, 1968).

One of the most spectacular and extensive blooms witnessed occurred along the coast of Sweden and Norway in May and June 1988, due to *Chrysochromulina polylepis*. More than 120 fish farms were affected and this algal catastrophe threatened the salmon and trout farming industry (SAUNDERS, 1988 ; ROSENBERG *et al.*, 1988). Wild fish (*Clupea*, *Labrus*, *Centrolabrus*, gobies....) also suffered from the bloom and were found dead (UNDERDAHL *et al.*, 1989) but garfish (*Belone belone*) were by far the worst affected with the highest number of recorded deaths on the Swedish coast (LEIVESTAD and SERIGSTAD, 1989 ; KLANG-JONASSON, 1988 in SKJOLDAL and DUNDAS, 1989). Several other phytoplankton blooms have been reported harmful to farmed fish (especially salmon) in U.K. waters owing to red tides of *Gyrodinium aureolum* in Scotland (AYRES *et al.*, 1982) and also owing to *Distephanus speculum* in Denmark (NIELSEN and AERTEBJERG, 1984) and Scotland (BRUNO *et al.*, 1989). *Gyrodinium aureolum* is probably the most serious phytoplankton threat to marine fish farming in north western European waters (PARKER, 1987). *Prymnesium parvum* remains a threat to salmon culture in Norway (JOHNSEN and LEIN, 1989).

Larval and juvenile fish can also suffer from phytoplankton blooms. POTTS and EDWARDS (1987) reported a dramatic reduction in the number of young fish off the coast of Plymouth following a bloom which adversely affected the vicinity. However, while mass mortalities of juvenile and adult fish have been frequently recorded in the literature, there are few field observations considering specifically the impact of toxic algae on the eggs and larval stages of fish (HEATH, 1992). This is due to difficulties in giving evidence and conspicuous proof of such phenomena. An almost complete absence of group 0 cod, whiting, saithe and pollack in some of the most seriously affected part of the Norwegian coast in May-June 1988, suggests that it might be damage to larval stages of these species by *Chrysochromulina polylepis* (LEIVESTAD and SERIGSTAD, 1989). The absence of capelin larvae in the Gulf of St Lawrence when cell densities of the dinoflagellate *Alexandrium excavatum* exceeded 13 cells/ml imply again the sensitivity of the larvae stages of certain fish species to algal blooms (ROBINEAU *et al.*, 1993).

NORTH AMERICA

• *Freshwater bodies*

Freshwater bodies in North Carolina have suffered from blue-green alga blooms (*Oscillatoria*, *Anacystis*, *Anabaena*) responsible for mortality of numerous fish species (sunfish, bass, carp, catfish, spot, silver perch, menhaden, flounder : Anonyme, 1990, 1991). A massive population of the common dinoflagellate *Ceratium hirundinella* also developed in a lake in Ontario during the summer 1976. The first fish to die were rainbow trout followed by white sucker then black bass (NICHOLLS *et al.*, 1980). Other fish mortalities (> 90 %) were observed in lakes of the North American midwest in 1971, 1972, 1975, 1976 (BARICA, 1975a, b, 1978) following blooms of blue-green algae (*Aphanizomenon flos-aquae*). Similar blooms again have been previously noted in a Wisconsin River and in the Iowa lakes (MAC KENTHUN *et al.*, 1945 ; PRESCOTT, 1948) with massive deaths of freshwater fish (*Cyprinus*, *Esox*, *Stizostedion*, *Pomoxis*, *Lepomis*, *Catostomus*, *Ameiurus* and *Anguilla*).

• *Coastal marine areas*

Fish kills associated with discolored water along Florida's coast were first reported as early as 1844 with an almost annual reoccurrence since. They are considered as a natural phenomenon following the rainy season on coastal waters of the Gulf of Mexico, with sporadic major outbreaks. STEIDINGER *et al.* (1973) listed those fish that are killed during *Gymnodinium* red tide outbreaks in the Gulf of Mexico (85 species including *Epinephelus*, *Gymnothorax*, *Haemulon*, *Diodon*, *Lutjanus*, *Mycteroperca*, *Scomberomorus*...). Equally, MILLS and Mc PHEE (1979) estimated that *Gonyaulax excavata* blooms might be responsible for the intoxication of winterflounder (*Pseudopleuronectes platessa*) larvae and consequently the decreasing year class size observed off the coast of Massachussets.

Death of adult herring (*Clupea harengus harengus*) occurred in the Bay of Fundy (Canada) in July 1979 during a bloom of *Gonyaulax excavata* (WHITE, 1980a, b). Phytoplankton blooms are a major problem in marine fish culture as in Canada. A bloom of *Heterosigma akashiwo* which occurred in Vancouver Island during the summer of 1990 was responsible for the mortality of juvenile chinook salmon (BLACK *et al.*, 1991). Phytoplankton bloom of *Chaetoceros convolutus*, *C. concavicornis* also induced losses of salmon (250,000 Atlantic and Pacific salmon) in Washington (RENSEL, 1992, 1993) and along the British Columbia coast (ALBRIGHT *et al.*, 1992). The two species *Chaetoceros* and *Heterosigma* are recognized in Pacific Canada as posing serious threats to Salmonids farmed in sea water (BRETT *et al.*, 1978 ; MARGOLIS and EVELYN, 1987).

INDO-PACIFIC REGION

- *Freshwater and marine coastal waters*

MACLEAN (1979) reviewed the occurrences of red tides in the IndoPacific region with special reference to the major causative agent, the Dinoflagellate *Pyrodinium bahamense* responsible for massive fish kills in Australia, New Guinea and Brunei. Widespread fish mortality was observed in Sydney Harbour as early as 1891 and in Port Philip Bay in 1984 (Anonyme, 1984). HALLEGRAEFF (1992) noted that, in the past two decades, there has been an apparent increase in the frequency, intensity and geographical distribution of harmful algal blooms in Australian coastal waters as well as in estuarine and freshwater ones.

In the inshore waters of New Zealand, fish mortalities were reported in the austral summer of 1983 and were associated with a phytoplankton bloom dominated by the diatom *Cerataulina pelagica* (CHANG and RAYAN, 1985). Later, in January 1989, a dense phytoplankton bloom (*Heterosigma akashiwo*) was associated with high mortality in cage-reared chinook salmon (CHANG *et al.*, 1990, 1993).

In Indonesia, mass mortality of demersal fish was first reported in Jakarta Bay and was suspected to be related to red tides of *Noctiluca scintillans* (ADNAN, 1987). Other fish kills were associated with *Pyrodinium bahamense* blooms (ADNAN, 1993). Abrupt set-backs to Indian fish populations are related by BHIMACHAR and GEORGE (1950) and coincide with a bloom of *Noctiluca*. Many other cases on the coast of India have also been recorded.

HASHIMOTO *et al.* (1968) reported mass mortality of fish (*Carassius*, *Cyprinus* ...) accompanying extensive blooms of *Peridinium polonicum* in a lake near Tokyo. Likewise, damage to cultured fish populations (yellowtail *Seriola quinqueradiata* and red sea bream *Pagrus major*) in the Seto Island Sea, due to *Heterosigma akashiwo* and *Gyrodinium nagasakiense* red tides, respectively were documented by OKAICHI (1989) and HONJO (1992). Mass mortalities of cultured yellowtail also occurred in 1970, 1974, 1977, 1978, 1979, 1984, 1985, 1986 and 1987 in the same area, associated in these years with *Chattonella* sp. red tides (OKAICHI, 1991).

OTHER COUNTRIES

A compilation of all red tide outbreaks in South Africa between 1959 and 1980 has been published by HORSTMAN (1981). *Gonyaulax catenella* was chiefly responsible for the mortality of large quantities of hagfish and suckerfish and, according to BROWN *et al.* (1979), a bloom occurring near Cape Town in 1976 resulted in a further mass mortality of numerous fish species i.e. *Lithognathus*, *Mugil*, *Diplodus*...

In South America, 200 tonnes of the plankton feeding sardine *Cetengraulis edenthus* died in 1982 off the coast of Venezuela (MIJARES *et al.*, 1985). *Ceratium* was the most abundant dinoflagellate at the time of incident. *Gonyaulax catenella* was also responsible for an outbreak of red tides in Southern Chile and Argentina when a high fish mortality was witnessed in summer 1991 and 1992 (BENAVIDES *et al.*, 1993).

TABLE I : FISH KILLS IN EUROPE

SITES	PERIODS	NOXIOUS ALGAE	IMPACT ON FISH SPECIES	CAUSATIVE MECHANISMS	AUTHORS
BULGARIA Varna lake	summer 1959	<i>Prymnesium parvum</i>	- fish kill (sp. ?)	?	VALKANOV, 1964
Black Sea coast	?	id.	id.	O ₂ depletion	MONCHEVA, 1993
FRANCE W Brittany (Douardenez)	April 1987	<i>Distephanus speculum</i>	- mortality of caged rainbow trout (15 tons)	O ₂ deficiency	HARACHE, 1987 ERARD-LE DENN and RICKAERT, 1990
GERMANY Fehmarn Isl.	September 1975	<i>Prymnesium parvum</i> (4.108 cells/l)	- mortality of pond carps (100 % of 4,000 carps) + eels (loss : 40,000 DM)	?	HICKEL, 1976
ITALY Pô delta (Rovigo)	June 1954	<i>Chromulina rosanoffii</i>	- mortality of eels, sea breams and grey mullets (<i>Mugil cephalus</i> in culture) (Valli di pesca)	O ₂ deficiency	D'ANCONA, 1951
Adriatic shores (Rimini)	May 1969	<i>Peridinium repressum</i>	- mortality of benthic fish (<i>Lithognathus</i> , <i>Callyonimus</i> , <i>Gobius</i> , <i>Arnoglossus</i> , <i>Solea</i>)	O ₂ deficiency	PICCINETTI and MANFRIN, 1969 ; FROGLIA, 1970
NETHERLANDS Bostshol lake (Utrecht)	fall 1990	<i>Prymnesium parvum</i>	- fish kill (sp. ?)	?	RIP et al., 1992
SCANDINAVIA - DENMARK NW Jutland	September 1973	<i>Oxyrrhis marina</i> (300,000 cells/ml)	- mortality of 200 kg eels (tanks in Horn So lake)	?	KRISTIANSEN, 1974
- NORWAY Oslo fjord	November 1976	<i>Gyrodinium aureolum</i> (20 million cells/l)	- mortality of farmed fish (salmon, rainbow trout, coalfish, sprat, cod, eel, black goby)	O ₂ deficiency	TANGEN, 1977 and 1979
Southern coast	Sept-Oct. 1981	(70.10 ⁶ cells/l)	- mortality of rainbow trout, eel and cod	?	DAHL et al., 1982
id.	1966, 1974, 1976, 1977, 1979, 1981, 1982, 1983, 1984, 1985, 1988, 1990, 1991	id.	id.		DAHL and TANGEN, 1993
- DENMARK Als Sound	May 1983	<i>Distephanus speculum</i>	- fish kill in farm	O ₂ deficiency	NIELSEN and AERTEBJERG, 1984

- FAROE ISLANDS	July 1984	<i>Gonyaulax excavata</i> (20,000 cells/l)	- fish kill in farmed salmon and rainbow trout (27 of 35 metric tons) and wild flounder	?	MORTENSEN, 1985 SIMONSEN et al., 1993
- DENMARK - NORWAY - SWEDEN	May-June 1988	<i>Chrysochromulina polylepis</i> (4-80 million cells/l)	- mortality of wild (<i>Labrus</i> , <i>Centrolabrus</i> , <i>Clupea</i> , <i>Gobies</i>) and farmed (salmon, cod) fish	?	UNDERDAHL et al., 1989 ROSENBERG et al., 1988 SAUNDERS, 1988
Skagerrak and Kategatt	id.	id.	- fish kill salmon and rainbow trout (480 metric tons)	toxicity	RAVN, 1991 LEIVESTAD and SERIGSTAD, 1989 KLANG-JONASSON, 1988 in SKJOLDAL and DUNDAS, 1989
id.	id.	id. (12.10 ⁶ cells/l)	- id. + mortality of wild fish (<i>Labrus</i> , <i>Centrolabrus</i> , <i>Raniceps</i>)	toxins	DAHL et al., 1989 GRANELI et al., 1989 and 1993 LINDAHL and DAHL, 1990
id.	April-May 1992	id.	- death of cultured fish (sp. ?)	toxicity	HANSEN et al., 1993
id.	July 1989	<i>Prymnesium parvum</i> (107-10 ⁸ cells/l)	id. (750 tons) (loss: 5 million US \$)	O ₂ deficiency	JOHNSON and LEIN, 1989 KAARTVEDT et al., 1991 GRANELI et al., 1993
id.	May-June 1991	<i>Chrysochromulina leadbeateri</i>	- mortality of salmon (550 tons) (loss: 5 million US \$)	toxicity	AUNE et al., 1992 HEIDAL and MOHUS, 1993
RUSSIA Amoursky Bay	October 1987	<i>Chattonella</i> sp.	- fish kill (sp. ?)	?	KONOVANOVA, 1993
SPAIN Lagoon of the Ebre delta	Nov. Dec. 1977	<i>Prymnesium parvum</i>	- fish kill eel, sea bass and grey mullet	?	COMIN and FERRER, 1978
U.K. Scotland Moray Firth	September 1963	<i>Ceratium</i> sp. (?) <i>Peridinium foliaceus</i> (?)	- mortality of cod, haddock, whiting and conger eels	?	RAE et al., 1965
Lincolnshire River	May 1962, 1963 and 1964	<i>Prymnesium parvum</i> (1,6.10 ⁶ cell/ml)	- fish kill (sp. ?)	ulcerative liver	FARROW, 1969
Eastern Scotland	May 1968	<i>Protogonyaulax tamarensis</i>	fish kill <i>Ammodytes</i> sp.	?	ADAMS et al., 1968 WOOD, 1968
River Thurne (Norfolk)	August-Sept. 1969	id. (800,000 cells/l)	- mortality of <i>Anguilla</i> , <i>Esox</i> , <i>Perca</i> , <i>Rutilus</i> , <i>Abramis</i>	?	HOLDWAY et al., 1978
Southern Ireland (Hickling Broad Lake)	1969-1970	<i>Prymnesium parvum</i>	- fish kill (sp. ?)	?	MOSS and LEAH, 1982

Plymouth	August 1978	<i>Gyrodinium aureolum</i> (5 million cells/l)	- fish kill (sp. ?)	O ₂ deficiency	BOALCH, 1979
Ireland	July-August 1978	<i>Gyrodinium aureolum</i>	- mortality of <i>Solea</i> , <i>Pleuronectes</i> , <i>Platichthys</i> and <i>Ammodytes</i> sp.	toxicity ?	OTTWAY et al., 1979
Ireland	August 1978	<i>Gyrodinium aureolum</i>	- mortality of caged rainbow trout	?	PARKER in CROSS and SOUTHGATE, 1980 PARKER et al., 1982
Scotland (Firth of Clyde)	Sept. 1980	id. (2-10 ⁷ cells/l)	- fish kill of 3,000 pond reared salmon	gill and gut lesions	AYRES et al., 1982 JONES et al., 1982
Plymouth	August 1986	id.	- mortality of larvae, postlarvae and juvenile fish (sp. ?)	?	POTTS and EDWARDS, 1987
Scotland Loch Torriden Shetlands Isl.	June-July 1988	<i>Chaetoceros wighami</i> <i>Distephanus speculum</i>	- fish kill of 550,000 farmed salmon (post smolt) with gill lesions	mechanical injury by Diatom frustules	BRUNO et al., 1989
W. Scotland	summer 1979 and 1982	Flagellate X (<i>Chattonella</i> or <i>Olisthodiscus</i>)	- fish kill of farmed fish (sp. ?)	?	AYRES et al., 1982
id.	May and July 1989	id.	- fish kill of farmed salmon (20-30 T) (loss : 90,000 £)	?	JOHNSON, 1988
TURKEY Bay of Izmir	1955	<i>Alexandrium minutum</i> (6-10 ⁶ cells/l)	- mortality of mullet, sardine, anchovy and gobiids	?	KORAY, 1992
YUGOSLAVIA Kastela Bay	September 1980	<i>Gonyaulax polyedra</i>	- fish kills <i>Sardinella</i> <i>aurita</i>	gill impairment (?)	MARASOVIC and VUKADIN, 1982

TABLE II : FISH KILLS IN NORTH AFRICA

SITES	PERIODS	NOXIOUS ALGAE	IMPACT ON FISH SPECIES	CAUSATIVE MECHANISMS	AUTHORS
CANADA					
- South Western Manitoba (eutrophic lakes and land-locked ponds)	1971, 1975, 1976	<i>Aphanizomenon flos aquae</i>	- fish kill (sp. ?)	O ₂ depletion	BARICA, 1975 and 1978
- Vancouver Harbour	summer 1976	<i>Olisthodiscus luteus</i>	- mortality of juvenile salmon	?	HARRISON et al., 1983
- Strait of Georgia (B.C.)					
- Heart Lake (Ontario)	August 1976	<i>Ceratium hirundinella</i>	- mortality of rainbow trout white sucker (<i>Catostomus</i>) and black bass	O ₂ depletion	NICHOLLS et al., 1980
Bay of Fundy (N.B.)	July 1976	<i>Gonyaulax excavata</i>	- kill of <i>Clupea harengus</i> harengus (25 tons)	toxic contamination through Cladoceran or Pteropod food chain	WHITE, 1977, 1980 a,b
Gulf of St Lawrence	1988-1989	id.	- mortality of <i>Mallotus villosus</i> larvae	toxicity	ROBINEAU et al., 1993
British Columbia	August 1977	<i>Chaetoceros convolutus</i>	- mortality of reared sockeye salmon	mechanical damage in gill	BRETT et al., 1978
id.	July 1988	<i>Alexandrium fundyense</i>	- mortality of <i>Scomber scombrus</i>	PSP toxins (STX) accumulated from food (contaminated herring)	HAYA et al., 1990
Vancouver Island	?	<i>Chaetoceros convolutus</i>	- mortality of lingcod <i>Ophiodon elongatus</i>	gill injury by siliceous spines	BELL, 1961
British Columbia	September 1986 and 1989	<i>Heterosigma akashiwo</i>	- mortality of reared Pacific salmon 1,500 tons (loss : 8.5 million \$)	?	Anonymous, 1989 BLACK, 1991 TAYLOR and HAIGH, 1993
id.	spring and fall 1988	<i>Chaetoceros convolutus</i> <i>C. concavicornis</i>	- mortality of reared salmon	mechanical damage in gill	ALBRIGHT et al., 1992 and 1993
Vancouver Island	July August 1990	id. (200,000-793,000 cells/ml)	- mortality of farmed <i>Oncorhynchus tshawitscha</i>	ichthyotoxicity	BLACK et al., 1991
USA					
Narragansett Bay (Rhode Island)	Nov. 1898	<i>Gymnodinium</i> (?)	- mortality of eel, menhaden, tautog and flatfish	?	NIXON, 1989
Yahara River (Wisconsin)	1946	<i>Aphanizomenon flos-aquae</i> (4.10 ⁵ cells/ml)	- mortality of <i>Cyprinus</i> , <i>Esox</i> , <i>Stizostedion</i> , <i>Pomoxis</i> , <i>Lepomis</i> , <i>Catostomus</i> , <i>Ameirus</i> ...	O ₂ depletion + toxins	MACKENTHUN et al., 1945

Lake Winnisquam (New Hampshire)	1966	id.	- mortality of 6 tons of fish (sp. ?)	?	SAWYER et al., 1968
Iowa Lakes	1948	<i>Aphanizomenon</i> sp. <i>Microcystis</i> sp. <i>Anabaena</i> sp.	- mortality of <i>Perca</i> , <i>Lepomis</i> <i>Ameiurus</i> , <i>Cyprinus</i> , <i>Pomoxis</i> , <i>Pimephales</i> ...	O ₂ depletion + H ₂ S + hydroxylamine	PRESCOTT, 1948
West Florida coast	from Nov. 1946 to August 1947	<i>Gymnodinium brevis</i>	- mortality of 60,000 bottom fish : mullet, snake-eel (<i>Ophichthus</i>), blue angelfish (<i>Angelichthys</i>) and short-nosed batfish (<i>Ogcocephalus</i>) + grunts groupers, yellowtail	toxicity	GUNTER et al., 1948 BEIN, 1954
id.	May and August 1951	id.	id.		BEIN, 1954
Offatt Bayou lagoon (Gulf of Mexico)	summer 1949	<i>Gonyaulax</i> sp.	- mass mortality of fish (sp. ?)	anaerobic conditions	CONNELL and CROSS, 1950
id.	September 1955	<i>Gonyaulax monilata</i>	id.	?	GATES and WILSON, 1960
South Florida and Texas	id.	<i>Gymnodinium brevis</i> (500 cells/ml)	id. (sp. ?)	?	WILSON and RAY, 1956
Florida Gulf coast	1973-1974	id.	- pathology <i>Mugil cephalus</i> and <i>Haemulon aurolineatum</i>	?	QUICK and HENDRICKS, 1975
Mission Bay, California	July 1961	<i>Gymnodinium flavum</i>	- fish kill (sp. ?)	toxicity	LACKEY and CLENDENNING, 1963
Sarasota Bay (West Florida)	July 1971	id. (150,000 cells/l)	- mortality of catfish, eel and others (85 species including <i>Epinephelus</i> , <i>Mycteroperca</i> <i>Gymnothorax</i> , <i>Lutjanus</i> , <i>Scomberomorus</i> , <i>Diodon</i> ... (2,367 tons) (=155,763 \$ loss)	toxicity	STEIDINGER and INGLE, 1972 STEIDINGER et al., 1973
Dickinson Bayou estuary (Texas)	1971-1972	?	- fish kill : over 4 million fish (sp ?)	O ₂ deficiency	KNUDSON and BELAIRE, 1975
Tampa Bay (Florida)	October 1973 May 1974	<i>Gymnodinium breve</i>	- mortality of <i>Haemulon</i> <i>aurolineatum</i> and <i>Mugil cephalus</i>	?	FORRESTER et al., 1977
New York Bay	summer 1976	<i>Ceratium tripos</i>	- mortality of bottom fishes	O ₂ depletion and H ₂ S	MAHONEY and STEIMLE, 1979
Coast of Maine	July 1976	<i>Gonyaulax excavata</i> (<i>tamarensis</i>)	- kill of <i>Clupea harengus</i> <i>harengus</i>	toxic contamination through Pteropod food chain	WHITE, 1977
off South-west Florida	from September to December 1976 fall 1977	<i>Gymnodinium breve</i> (2.10 ⁴ to 6.10 ⁴ cells/l)	- mortality of mullets and other fish (?)	?	ROBERTS, 1979
Galveston Bay (Texas)	June and July 1984	<i>Gymnodinium splendens</i>	- mortality of <i>Micropogonias</i> , <i>Mugil cephalus</i> , <i>Polydactylus</i> and <i>Menticirrhus</i>	O ₂ deficiency and H ₂ S	HARPER and GUILLEN, 1989
Texas coastal waters	Oct. Nov. 1986	<i>Ptychodiscus brevis</i> (10,000 - 47,680 cells/ml)	- mortality of larvae <i>Sciaenops ocellatus</i>	toxicity	RILEY et al., 1989

Washington	1987	<i>Chaetoceros convolutus</i>	- mortality of 250,000 Atlantic and Pacific salmon (loss: over 0.5 million \$)	?	RENSEL et al., 1989, HOPNER et al., 1990
Puget Sound	?	<i>Chaetoceros concavicornis</i>	- mortality of farmed salmon	mucus production	RENSEL, 1992
Port Townsend Bay (Washington)	summer 1986 and 1987	?	- mortality of farmed <i>Salmo salar</i> , <i>Oncorhynchus tshawtscha</i> , and <i>O. mikyss</i>	toxicopathic liver disease	KENT, 1990
freshwater bodies and Pamlico River estuary (North Carolina)	1989 and 1990	<i>Oscillatoria erythraea</i> , <i>Anacystis</i> , <i>Anabaena</i>	- mortality of sunfish, bass, carp, catfish, spot, silver perch, menhaden, flounder	O ₂ deficiency	Anonyme 1990 and 1991
Pamlico River estuary (North Carolina)	May-Dec. 1991 and Feb. 1992	"phantom" Dinoflagellate (35,000 cells/ml)	- mortality of 1 million Atlantic menhaden <i>Brevoortia tyrannus</i> + flounder, eel, mullet and others	?	BURKHOLDER et al., 1992
Florida	1992	<i>Pfiesteria piscimorte</i>	- mortality in marine nonspecific fish aquarium	?	LANDSBERG et al., 1993

TABLE III : FISH KILLS IN INDOPACIFIC REGION

SITES	PERIODS	NOXIOUS ALGAE	IMPACT ON FISH SPECIES	CAUSATIVE MECHANISMS	AUTHORS
AUSTRALIA Sydney Harbour Port Phillip Bay	1890 1950	<i>Scripsiella trochoidea</i> (= <i>Glenodinium rubrum</i>)	- fish kill (sp ?)	?	HALLEGRAEFF, 1992
Port Phillip Bay	January February 1984	?	- mortality of flathead, flounder and spikey globefish	?	Anonyme, 1984
id.	?	<i>Gymnodinium sp.</i>	- mortality of farmed fish (sp ?)	?	WOODS, 1964 in HALLEGRAEFF, 1992
CHINA ?	July-October 1989	?	- fish kill (sp. ?)	?	YUZAQ et al., 1993
Coast of Fujian	June 1990	<i>Cochlidinium sp.</i>	- mortality of wild (<i>Mobula</i> , <i>Muraenesox</i> , <i>Lateolabrax</i>) and cultured fish <i>Epinephelus akaara</i> , <i>sparus</i> <i>macrocephalus</i> : losses 600,000 yuan)	asphyxiation ?	QI et al., 1993
HONG KONG Tolo Harbour	May 1988	<i>Gonyaulax polygramma</i>	- fish kill in farms (sp. ?) (35 tons)	O ₂ deficiency	WU, 1988 LAM and YIP, 1990
id.	1980	<i>Prorocentrum minimum</i>	- fish kill (sp. ?)	?	HO and HODGKISS, 1993
INDIA West coast	October 1948	<i>Noctiluca miliaris</i>	- mortality of <i>Arius</i> , <i>Synagris</i> , <i>Caranx</i> and <i>Cynoglossus</i>	?	BHIMACHAR and GEORGE, 1950
Karnataka state	December 1989	<i>Gymnodinium nagasakiense</i> (4.10 ⁸ cells/l)	- mortality of farmed <i>Tilapia</i> , <i>Chanos</i> and <i>Etrophus</i> (5 tons) (4.10 ⁸ cells/l)	?	KARUNASAGAR, 1993
West coast	?	<i>Gymnodinium mikimotoi</i> (2.410 ⁶ cells/l)	- mortality of farmed <i>Tilapia</i> , <i>Etrophus</i> and <i>Chanos</i>	toxicity	KARUNASAGAR and KARUNASAGAR 1993
INDONESIA Djakarta	July 1986	<i>Noctiluca scintillans</i>	- mortality of demersal fish (sp. ?)	NH ₃	ADNAN, 1987
JAPAN Sagami Lake (Tokyo)	September 1962	<i>Peridinium polonicum</i> (= <i>Glenodinium sp.</i>)	- mortality of <i>Carassius</i> <i>auratus</i> , <i>Cyprinus carpio</i> and <i>Zacco platypus</i>	toxicity	HASHIMOTO et al., 1968

Seto Island Sea	1965	<i>Gymnodinium nagasakiense</i>	- mortality of farmed <i>Seriola quinqueradiata</i>	?	DOI et al., 1981 OKAICHI, 1989
id.	?	<i>Heterosigma akashiwo</i>	- mortality of <i>Seriola quinqueradiata</i> and <i>Pagrus major</i>	?	HONJO, 1992
Seto Island Sea + Osaka Bay	1970, 1972, 1977 1978 and 1987	<i>Gymnodinium sanguineum</i> <i>Heterosigma akashiwo</i> <i>Chattonella antiqua</i>	- mortality of juveniles yellowtail jack (loss : 7.1 billion yen)	?	MURAKAWA, 1987 OKAICHI, 1989 and 1991
KOREA Chinhae Bay	since 1989	<i>Cochlodinium polykrikoides</i> <i>Gymnodinium mikomotoi</i>	- mortality in fish farms	toxicity	PARK, 1991
South Sea	August 1992	<i>Gyrodinium</i> (sp. ?)	- mortality in farmed yellowtail and bastard halibut	toxicity	KIM et al., 1993
NEW ZEALAND East coast	December 1982 January 1983	<i>Cerataulina pelagica</i> (+ <i>Prymnesium calathiferum</i>)	- mortality of <i>Chrysophrys auratus</i>	O ₂ deficiency mucus production	TAYLOR et al., 1985
Big Glory Bay	id.	id. (8.5 10 ⁶ cells/l)	lethality of 600 tons salmon (loss : 17 million \$)		CHANG et al., 1993
Stuart Island	January 1989	<i>Heterosigma akashiwo</i> (5.9 - 10 ⁶ - 4.5 10 ⁷ cells/l)	- mortality of farmed chinook salmon (>800 tons) (loss : 12 million \$)	gill pathology	BOULSTEAD et al., 1989, in MACKENZIE, 1991 CHANG et al., 1990 MACKENZIE, 1991
PAKISTAN Gwador Bay	November 1987	<i>Prorocentrum minimum</i> (8-45 - 10 ⁶ cells/l)	- mortality of <i>Congresox</i> , <i>Pomadasy</i> , <i>Terapon</i>	?	RABBANI et al., 1990
PAPUA NEW GUINEA Morobe District	November 1971 and 1992	<i>Pyrodinium bahamense</i> (3.5 million cells/l)	- mortality of juveniles <i>Gerres</i> sp.	toxicity	MACLEAN, 1975

TABLE IV : FISH KILLS IN OTHER AREAS

SITES	PERIODS	NOXIOUS ALGAE	IMPACT ON FISH SPECIES	CAUSATIVE MECHANISMS	AUTHORS
SOUTH AFRICA Cape Town	March, April 1962	<i>Gonyaulax polygramma</i>	- mortality of <i>Chrysoblephus</i> , <i>Coracinus</i> , <i>Chlorisochismus</i> , <i>Gymnobatrachus</i> and juveniles <i>Tachysurus</i>	O ₂ deficiency	GRINDLEY and TAYLOR, 1964
West and South coast	February 1967	<i>Noctiluca miliaris</i>	- mortality of mackerel and shark	NH ₃	HORSTMAN, 1981
id.	December 1967	<i>Gonyaulax catenella</i> (4 - 10 ⁶ cells/l)	- mortality of hagfish and suckerfish	PSP toxins	id.
Cape Town	August September, 1976	<i>Gymnodinium</i> sp.	- mortality of <i>Lutjanus</i> , <i>Mugil</i> , <i>Sarpa</i> , <i>Diplodus</i> , <i>Hepsetia</i> , <i>Pomatomus</i>	clogging of gill	BROWN et al., 1979
SOUTHERN AMERICA CHILE	October 1972	<i>Gonyaulax catenella</i> (600 cells/ml)	- fish kill (sp. ?)	?	AVARIA, 1979
SOUTH CHILE	spring 1988 winter 1991	<i>Chaetoceros convolutus</i>	- fish kill Atlantic salmon and sea trout in farms	?	CLEMENT AND LEMBEYE, 1993
	June 1991	id.	id.	?	LEMBEYE, 1991
	1989, 1990, 1991	<i>Leptocylindricus</i> <i>minimus</i> (26,000 cells/ml)	- mortality (low) of Atlantic salmon	?	CLEMENT AND LEMBEYE, 1993
CHILE-ARGENTINA	spring-summer 1991 and 1992	<i>Alexandrium catenella</i>	- mortality of fish (sp.?)	?	BENAVIDES et al., 1993
VENEZUELA central coast	March 1982	<i>Noctiluca scintillans</i> <i>Ceratium furca</i>	- mortality of sardine <i>Centropomus edentulatus</i> (200 tons)	O ₂ deficiency	MIJARES et al., 1985 LA BARBERA-SANCHEZ et al. 1991
PALESTINE	fall 1945	<i>Prymnesium parvum</i> (500,000 - 800,000 cells/l)	- mortality of carp, tilapia and <i>Gambusia</i> (brackish water)	toxicity	REICH and ASCHNER, 1947
ISRAEL	?	id.	- mortality of carp, tilapia and mullet in polyculture	?	OHAYON and SMITH, 1975

CHAPTER II

ALGAL SPECIES INVOLVED IN FISH KILLS

Several “algal” classes are involved in harmful blooms :

- Cyanophyceae (genus *Microcystis*, *Anabaena*, *Aphanizomenon* ...)
- Dinoflagellates (genus : *Amphidinium*, *Gambierdiscus*, *Gymnodinium*, *Gyrodinium*, *Noctiluca*, *Peridinium*, *Pyrodinium*, *Ptychodiscus*, *Protogonyaulax* ...)
- Chloromonads (genus *Chattonella*, *Heterosigma* ...)
- Prymnesiomonads (genus *Chrysochromulina*, *Prymnesium*...)
- Silicoflagellates (genus *Distephanus* ...)
- Diatoms (genus : *Chaetoceros*, *Nitzschia* ...)

The identity of the cited organisms is questionable in many cases and some of the generic and species names have recently been revised as the result of further studies causing a great deal of confusion (SHIMIZU, 1987). The most obvious examples of the adoption of new names include a change in name from *Gonyaulax* sp. to *Protogonyaulax* sp. and from *Gyrodinium breve* to *Ptychodiscus brevis*. Another problem is the possibility of strain variations in toxin productivity because toxic and non-toxic strain even coexist sometimes. For example, *Pseudonitzschia pungens* from the Gulf of St Lawrence appears as two morphotypes - forma multiseriata, a domoic acid producer and forma *pungens*, a non toxic organism (BATES and DOUGLAS, 1993). 300 species of marine phytoplankton can at times occur in “red tides” while only 40 species have the capacity to produce potent toxins that can find their way through fish to humans (HALLEGRAEFF, 1993). There are still problems of specific identity to be resolved. Thus, the old “morphological species” concept (morphospecies) seems becoming inadequate and replacement for a “biological species” concept is recommended by TAYLOR (1993), taking into account inductors of probable toxicity according to genetic variability within each species and regarding selective inductors by environmental conditions.

A list of the phytoplankton species suspected to cause harm in European countries was presented by ICES (1992) and different types of harmful algal blooms have been recognized and classified into three major categories (HALLEGRAEFF, 1993) :

- Species which cause harmless discoloration of water but can grow so dense that O₂ depletion can cause fish kills (*Noctiluca*, *Gonyaulax* ...)
- Species harmful to fish by damaging or clogging their gills (*Chaetoceros*, *Heterosigma*...)
- Species which produce potent toxins that can find their way through the food chain (*Gambierdiscus*, *Gymnodinium*, *Microcystis*, *Pyrodinium* ...).

Different species are now listed with respect to their geographical occurrence along with those authors reported how they participate in harmful blooms.

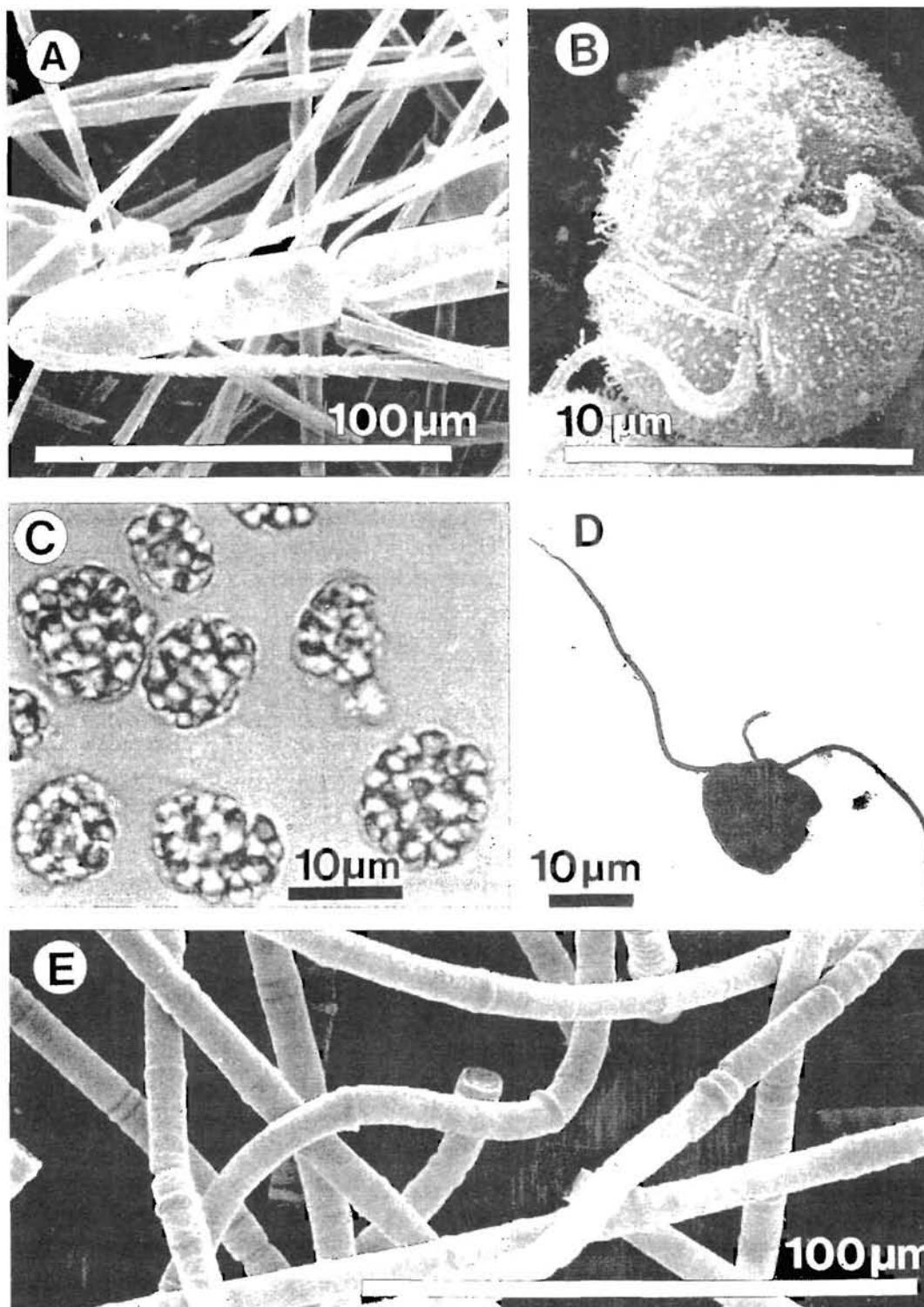


Figure 4 - Algal species which can kill cage-reared fish.

- (A) SEM. Diatom *Chaetoceros concavicornis* from British Columbia.
- (B) SEM. Dinoflagellate *Gymnodinium galatheanum* from Norway (closely related to *Gyrodinium aureolum*).
- (C) LM. Raphidophyte *Heterosigma akashiwo* from New Zealand. (Lugol preserved sample).
- (D) TEM. Prymnesiophyte *Prymnesium parvum* from Australia.
- (E) SEM. Hepatotoxic filamentous cyanobacterium *Nodularia spumigena* from Australia.

(In : Hallegraeff, 1993, *Phycologia*, 32, p. 88)

I - CYANOBACTERIA (ex *Cyanophyceae* or blue-green algae or Cyanophytes)

- 1/ *Anabaena circinalis* HALLEGRAEFF, 1992 - Australia
ANONYME, 1990 and 1991 - USA
- 2/ *Aphanizomenon flos-aquae* MACKENTHUN *et al.*, 1945 - USA
PRESCOTT, 1948 - USA
SAWYER *et al.*, 1968 - USA
JACKIM and GENTILE, 1972 - U.K.
BARICA, 1975a, b, 1978 - Canada
SHILO, 1972 -
PHILLIPS *et al.*, 1985 - U.K.
CODD and POON, 1989 - U.K.
RABERGH *et al.*, 1991 - Norway
BEVERIDGE *et al.*, 1993 - U.K.
- 3/ *Microcystis aeruginosa* SHILO, 1972 -
PHILLIPS *et al.*, 1985 - U.K.
CODD and POON, 1989 - U.K.
RABERGH *et al.*, 1991 - Norway
BEVERIDGE *et al.*, 1993 - U.K.
- 4) *Nodularia spumigena* (Fig. 4) KONONEN *et al.*, 1993 - Finland
- II - DINOFLAGELLATES (= *Dinophyceae*)**
- 5/ *Alexandrium excavatum* ROBINEAU *et al.*, 1991 a, b, 1993 - Canada
- 6/ *A. fundyense* HAYA *et al.*, 1990 - Canada
ICES, 1992 -
- 7/ *A. minutum* KORAY, 1992 - Turkey
- 8/ *A. monilatum* (see *Gonyaulax*)
- 9/ *A. tamarense* ICES, 1992 -
SIMONSEN *et al.*, 1993 - Faroe Islands
- 10/ *Amphidinium carterae* THURBERG and SASNER, 1973 - USA
NAKAJIMA *et al.*, 1981 - Japan
YASUMOTO, 1990 - Japan
ICES, 1992 -
- 11/ *A. klesii* NAKAJIMA *et al.*, 1981 - Japan
- 12/ *Ceratium furca* MIJARES *et al.*, 1985 - Venezuela -
LA BARBERA-SANCHEZ *et al.*, 1991 - id.
- 13/ *C. hirundinella* NICHOLLS *et al.*, 1980 - Canada
- 14/ *C. tripos* MAYONEY and STEIMLE, 1979 - USA
NIELSEN and AERTEBJERG, 1984 - Denmark
- 15/ *Cochlodinium sp.* QI *et al.*, 1993 - China
- 16/ *Cochlodinium helix* HALLEGRAEFF, 1992 - Australia

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- 17/ *C. polykrikoides* PARK and KIM, 1991 - Korea
- 18/ *Cosniodona pseudogonyaulax* TERA0 *et al.*, 1989 - Japan
- 19/ *Gambierdiscus toxicus* too many references ...
- 20/ *Gonyaulax sp.* WHITE, 1982 a,b - Canada
- 21/ *G. catenella* CONNELL and CROSS, 1950 - USA
 McLAUGHLIN and DOWN, 1969 - USA
 BATES *et al.*, 1978 - Canada
 AVARIA, 1979 -Chile
 HORSTMAN, 1981 - South Africa
 YAZDANDOUST, 1985 - USA
 BENAVIDES *et al.*, 1993 - Chile, Argentina
- 22/ *G. excavata* (see *Protogonyaulax*)
- 23/ *G. monilata* (ex *Alexandrium monilatum*) GATES and WILSON, 1960 - USA
 ALDRICH *et al.*, 1967 - USA
 SIEVERS, 1969 - USA
 WHITE, 1988 - USA
 RILEY *et al.*, 1989 - USA
- 24/ *G. polyedra* OGURI *et al.*, 1975 - USA
 MARASOVIC and VUKADIN, 1982 -
 Yougoslavia
- 25/ *G. polygramma* GRINDLEY and TAYLOR, 1964 - South Africa
 WU, 1988 - Hong-Kong
 LAM and YIP, 1990 - Hong Kong
- 26/ *Gymnodinium sp.* WOOD, 1964 in HALLEGRAEFF, 1992 -
 Australia
 BROWN *et al.*, 1979 - South Africa
 DOI *et al.*, 1981 - Japan
 SHIMADA *et al.*, 1982 - Japan
- 27/ *G. breve* (see *Ptychodiscus*)
- 28/ *G. galatheanum* HALLEGRAEFF, 1992 - Australia
 NIELSEN, 1993 - Norway
- 29/ *G. mikimotoi* OKAICHI, 1989 - Japan
 PARK and KIM, 1991 - Korea
 HALLEGRAEFF, 1992 - Australia
 KARUNASAGAR and KARUNASAGAR, 1993 -
 India
- 30/ *G. nagasakiense* PARTENSKY *et al.*, 1987 and 1989 - Brittany
 OKAICHI, 1989 - Japan
 PARTENSKY, 1989

- 31/ *G. splendens* KARUNASAGAR and KARUNASAGAR, 1993 - India
ARZUL *et al.*, 1993 - France
HARPER and GUILLEN, 1989 - USA
- 32/ *Gyrodinium sp.* KIM *et al.*, 1993 - Korea
- 33/ *G. veneficum* ABBOTT and BALLANTINE, 1957 - U.K.
BALLANTINE and ABBOTT, 1957 - id.
- 34/ *G. aureolum* (= *Gymnodinium aureolum*) (Fig. 4) TANGEN, 1977 and 1979 - Norway
BOALCH, 1979 - U.K.
OTTWAY *et al.*, 1979 - Ireland
CROSS and SOUTHGATE, 1980 - Ireland
AYRES *et al.*, 1982 - U.K.
DAHL *et al.*, 1982 - Norway
JONES *et al.*, 1982 - U.K.
ROBERTS *et al.*, 1983 U.K.
NIELSEN and AERTEBJERG, 1984 - Denmark
PARTENSKY et SOURNIA, 1986 - NE Atlantic
PARKER *et al.*, 1988 - U.K.
WHITE, 1988 - Canada
PARTENSKY, 1989 - France
POPPE, in TAYLOR, 1990 - U.K.
YASUMOTO, 1990 - Japan
GENTIEN and ARZUL, 1991 - North Atlantic
JACKSON *et al.*, 1991 - U.K.
DAHL and TANGEN, 1993 - Norway
TANGEN et DAHL, 1993 - Norway
- 35/ *Ichtyodinium chabelardi* MENESES and RE, 1992 - Portugal
- 36/ *Noctiluca scintillans* (= *N. miliaris*) BHIMACHAR and GEORGE, 1950 - India
OKAICHI and NISHIO, 1976 - Japan
HORSTMAN, 1981 - South Africa
SUBRAMANIAN, 1985 - India
ADNAN, 1987 - Indonesia
LA BARBERA-SANCHEZ *et al.*, 1991 - Venezuela
JACKSON *et al.*, 1991 - U.K.
ICES, 1992 -
CHEN and GU, 1993 - China
- 37/ *Oxyrrhis marina* KRISTIANSEN, 1974 - Denmark
- 38/ *Peridinium depressum* (= *Protoperidinium d.*) PICCINETTI and MANFRIN, 1969 - Italy
FROGLIA, 1970 - Italy
- 39/ *P. foliaceum* (= *Glenodinium f.*) RAE *et al.*, 1965 - U.K.
HASHIMOTO *et al.*, 1968 - Japan

- 40/ *Pfiesteria piscimorte* BURKHOLDER *et al.*, 1993 - USA
LANDSBERG *et al.*, 1993 - USA
GLASGOW and BURKHOLDER, 1993 - USA
- 41/ *Prorocentrum concavum* NAKAJIMA *et al.*, 1981 - Japan
- 42/ *P. minimum* RABBANI *et al.*, 1990 - Pakistan
JACKSON *et al.*, 1991 - U.K.
HO and HODGKISS, 1993 - Hong Kong
- 43/ *Protogonyaulax tamarensis* (= *Gonyaulax excavata*) ADAMS *et al.*, 1968 - U.K.
WOOD, 1968 - U.K.
WHITE, 1977, 1980 a,b, 1981 a et b, 1982 a,b,
1984,
1988 - Canada
BATES *et al.*, 1978 - Canada
MILLS and KLEIN-MACPHEE, 1979 - USA
MORTENSEN, 1985 - Faroe Islands
FUKYO and ISHIMARU, 1986 - Japan
OGATA *et al.*, 1987 - Japan
HALLEGRAEFF, 1987 - Australia
SHIMIZU, 1988 -
GOSSELIN *et al.*, 1989 - Canada
WHITE *et al.*, 1989 - Canada
SHUMWAY, 1990 -
TANGEN and DAHL, 1993 - Norway
- 44/ *Ptychodiscus brevis* (ex *Gymnodinium breve*) GUNTER, 1948 - USA
BEIN, 1954 - USA
WILSON and RAY, 1956 - USA
RAY and WILSON, 1957 - USA
STARR, 1958 - USA
LACKEY and CLEDENNING, 1963 - USA
SIEVERS, 1969 - USA
MARTIN et CHATTERJEE, 1970 - USA
STEIDINGER and INGLE, 1972 - USA
WILSON *et al.*, 1975 - USA
SPIEGELSTEIN *et al.*, 1973 - Israël
STEIDINGER *et al.*, 1973 - Israël
FORRESTER *et al.*, 1977 - USA
RISK *et al.*, 1979 - USA
ROBERTS, 1979 - USA
BADEN and MENDE, 1982 - USA
CHOU and SHIMIZU, 1982 - USA
SHIMIZU *et al.*, 1986 - USA
SHIER, 1988 -
WHITE, 1988 - Canada
MAYONEY, 1989 - USA
RILEY *et al.*, 1989 - USA
EDWARDS *et al.*, 1990 - USA
ANDERSON and WHITE, 1992 - USA

- 45/ *Pyrodinium bahamense* MAC LEAN, 1975 and 1979 - Papua-New Guinea
 MACLEAN and WHITE, 1985 SE Asia
 SHIMIZU, 1988 -
 WHITE, 1988 - SE Asia
 MACLEAN, 1989 - SE Asia
 OSHIMA, 1989 - Borneo
 ADNAN, 1993 - Indonesia
 GONZALES, 1993 - Philipines
- 46/ *Scripsiella trochoidea* (= *Glenodinium rubrum*) HALLEGRAEFF, 1992 - Australia
- 47/ a new "phantom" dinoflagellate (?) BURKHOLDER *et al.*, 1992 - USA
- III - CHLOROMONADS (*Raphidophyceae* sp.)**
- 48/ *Chattonella* sp. ISHIMATSU *et al.*, 1991 - Japan
 KONOVALOVA, 1993 - Russia
- 49/ *C. antiqua* SHIMADA *et al.*, 1983 - Japan
 OKAICHI, 1989, 1991 - Japan
- 50/ *C. marina* (= *Hornella marina*) ENDO *et al.*, 1985, 1992 - Japan
 SAKAI *et al.*, 1986 - Japan
 WHITE, 1988 - Japan
 MACLEAN, 1989 - IndoPacific
 MURATA *et al.*, 1989 - Japan
 SAGIR *et al.*, 1993 - Japan
- 51/ *Heterosigma* sp. LEMBEYE, 1991 - Chile
- 52/ *H. akashiwo* (= *Olisthodiscus luteus*) (Fig. 4) HARRISSON *et al.*, 1983 - Canada
 MARGOLIS and EVELYN, 1987 - Canada
 WHITE, 1988 -
 ANONYME, 1989 - Canada
 CHANG *et al.*, 1990 - New Zealand
 BLACK, 1991 - Canada
 MACKENZIE, 1991 - New-Zealand
 HONJO, 1992, 1993 - Japan
 CHANG *et al.*, 1993 - New Zealand
 TAYLOR et HAIGH, 1993 - Canada
 YANG, 1993 - Canada
- 53/ Flagellate "X" (?) ICES, 1992 -

IV - PRYMNESIOMONADS (= *Prymnesiophyceae* = *Chrysophyceae*)

- 54/ *Chrysochromulina leadbeteri* AUNE *et al.*, 1992 - Norway
ICES, 1992 -
HEIDAL and MOHUS, 1993 - Norway
- 55/ *C. polylepis* LEIVESTAD and SERIGSTAD, 1988 - Norway,
Sweden
ROSENBERG *et al.*, 1988 - Denmark
SAUNDERS, 1988 - Norway
DAHL *et al.*, 1989 - Denmark, Norway,
Sweden
GRANELLI *et al.*, 1989 - Scandinavia
SKJOLDAL and DUNDAS, 1989 - Norway,
Sweden
UNDERDAHL *et al.*, 1989 - Norway
EVARDESEN *et al.*, 1990 - Norway
RAVN, 1991 - Denmark
GRANELI *et al.*, 1993 - Norway
HANSEN *et al.*, 1993 - Scandinavia
LINDAHL and DAHL, 1990 - Norway, Sweden
MELDAHL *et al.*, 1993 - Norway
TANGEN and DAHL, 1993 - Norway
- 56/ *Phaeocystis pouchetii* SAVAGE, 1931 - U.K.
CHANG, 1983 - New Zealand
JACKSON *et al.*, 1991 - U.K.
WEISSE *et al.*, 1994 -
- 57/ *Prymnesium parvum* (Fig. 4) REICH et ASCHNER, 1947 - Israël
REICH et PARNAS, 1962 - Israël
PARNAS, 1963 - Israël
VALKANOV, 1964 - Bulgarie
PARNAS et ABBOTT, 1965 - USA
ULITZUR et SHILO, 1966 - Israël
PASTER, 1968 - Israël
FARROW, 1969 - U.K.
SHILO, 1972 -
OHAYON and SMITH, 1975 - Israël
HICKEL, 1976 - Germany
COMIN et FERRER, 1978 - Spain
HOLDWAY *et al.*, 1978 - U.K.
MOSS and LEAH, 1982 - U.K.
SHIER, 1988 -
WHITE, 1988 - Europe
JOHNSEN et LEIN, 1989 - Norway
KAARTVEDT *et al.*, 1991 - Norway
HALLEGRAEFF, 1992 - Australia
RIP *et al.*, 1992 - Netherlands
ICES, 1992 -

GRANELI *et al.*, 1993 - Norway
 IGARASHI *et al.*, 1993 - Japan
 MELDAHL *et al.*, 1993 - Norway
 MONCHEVA, 1993 - Bulgaria
 TERAO *et al.*, 1993 - Japan

V - SILICOFLAGELLATES

58/ *Distephanus speculum* (= *Dictyocha fibula*) NIELSEN et AERTEBJERG, 1984 - Denmark
 BRUNO *et al.*, 1989 - U.K.

VI - DIATOMS (*Bacillophyceae*)

- 59/ *Cerataulina pelagica* TAYLOR *et al.*, 1985 - New Zealand
- 60/ *Chaetoceros* sp. BRETT *et al.*, 1978 - Canada
 JOHNSON, 1988 - U.K.
 ICES, 1992 -
- 61/ *C. concavicornis* (Fig. 4) BRETT *et al.*, 1978 - Canada
 RENSEL, 1992, 1993 - USA
 ALBRIGHT *et al.*, 1992, 1993 - Canada
 YANG and ALBRIGHT, 1992, 1993 a,b
- 62/ *C. convolutus* BELL, 1961 - Canada
 ALBRIGHT *et al.*, 1992, 1993 - Canada
 CLEMENT and LEMBEYE, 1993 - Chile
 MARGOLIS and EVELYN, 1987 - Canada
 RENSEL *et al.*, 1989 - USA
 RENSEL, 1992, 1993 - USA
- 63/ *Chaetoceros wighami* BRUNO *et al.*, 1989 - U.K.
- 64/ *Nitzschia pseudoserrata*
 (= *Pseudonitzschia australis*) ANDERSON and WHITE, 1992 - USA
 FRITZ *et al.*, 1992 - USA
 WORK *et al.*, 1993 - USA
- 65/ *N. pungens* BATES and DOUGLAS, 1993 - Canada

CHAPTER III

CAUSATIVE MECHANISMS OF FISH DEATH

Deaths of wild and cultured fish can be divided into three categories according to three negative effects (Fig. 5), attributed in some cases to the **deoxygenation** of the water when blooms decay, while in others, there is clear evidence of the involvement of biotoxins. There is either a **direct** action of the algae on the fish itself, especially on sensitive organs such as the gills, liver, and tissue of the nervous system or **indirect** death of those predators which have consumed herbivores by feeding on blooms.

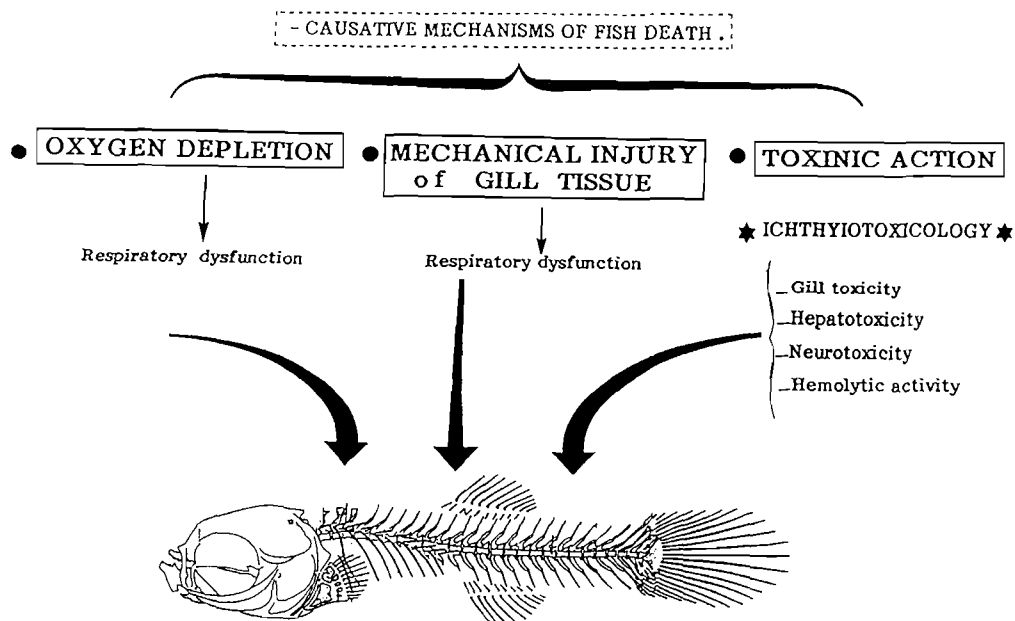


Figure 5 - Three types of pathogenicity in fish kills

O₂ DEPLETION

There are **non-specific** fish kills following intense blooms of algae which the cells die and decay resulting in a high biological oxygen demand (BOD). Anoxic or hypoxic conditions result in high fish mortality, as has been observed in many areas (Tables I, II, III and IV).

A lowering of the concentration of dissolved oxygen in water occurs mainly in the early morning when respiration rates of large quantities of algae are highest and decaying processes are especially rapid (SMAYDA, 1979). The products of bacterial decomposition (H₂S) can contribute an additional stress factor to fish, often resulting in asphyxiation. Schools of fish under stress can be observed gulping for air at the water surface before death through suffocation, as was witnessed when *Ceratium* blooms developed in a Canadian lake (NICHOLLS *et al.*, 1980) and Danish and Swedish waters (NIELSEN and AERTJEBERG, 1984). Other fish kills due to O₂ deficiency have been described in the marine coastal waters of Italy (PICCINETTI and MANFRIN, 1969 ; FROGLIA, 1970), Scandinavia (TANGEN, 1977 and 1979), the United States (KNUDSON and BELARE, 1975 ; HARPER and GUILLEN, 1979), South Africa (GRINDLEY and TAYLOR, 1964) and Australia (HALLEGRAEFF, 1992).

MECHANICAL DAMAGE TO GILLS

Some algal species harm fish by clogging up their gills (i.e mucous producing algal cells) or damaging their gill tissues leading in both cases to asphyxiation and eventually to death (Table V). *Gonyaulax polygramma* is one of such harmful algae species which has proven to be lethal to cultured fish in Hong-Kong (LAM and YIP, 1990) by producing a thick mucous layer which reduced the efficiency of oxygen uptake and leads to anoxia. The effects of this particular event were further demonstrated to result solely from mechanical damage to the fish gills rather than any toxins. *Gyrodinium aureolum* and *Heterosigma akashiwo* have also been observed to contribute towards mucus secretion in the gills of fish causing interferences with oxygen exchange and affecting osmoregulation. Other harmful diatoms such as *Chaetoceros concavicornis* and *C. convolutus* first observed in the gill tissue of lingcod *Ophiodion elangatus* (BELL, 1961) have led to the mortality of fish in Canada (YANG and ALBRIGHT, 1992) and the United States (RENSEL, 1992) when barbed spines of the skeleton of diatoms penetrated and damaged the respiratory epithelium of rainbow trout. Histological examination of gills showed hyperplasia, hypertrophy, fusions of secondary lamellae with severe oedema and localized hemorrhaging. In addition, excessive amounts of mucus can inhibit the ability of the gills to diffuse oxygen resulting invariably in mortality within 72 hours exposure while similar mortalities of Salmonids have been reported in British Columbia by BRETT *et al.* (1978). Treatment of salmon with a mucolytic agent (L-cysteine ethylester) can greatly decrease the production of mucus thereby retaining the viability of fish stocks (YANG and ALBRIGHT, 1993a). ALBRIGHT *et al.* (1993) however have noted high mortality rates in penned salmon at concentrations of *Chaetoceros sp.* which have been not previously been reported as lethal (less than 5 cells/ml). On closer examination, they observed an increase in vibriosis-related mortalities (BDK, *Vibrio anguillarum*) and concluded that the hypoxic condition induced by phytoplankton causes a cascade of events disrupting the normal functioning of the metabolic system of fish and particularly their immunodefensive system. Thus, concentration of harmful *Chaetoceros*, as low as approximately 0.40 cell/ml may increase the mortality rates of chinook salmon because phytoplankton-pathogen interaction. BRUNO *et al.* (1989) showed that a Silicoflagellate *Distephanus speculum* caused extensive necrosis and sloughing of the gill lamellae in *Salmo salar*. Clogging and abrasion of these delicate structures by the frustules resulted in mortality of farmed Atlantic Salmon.

TABLE V : MECHANICAL DAMAGES TO FISH INDUCED BY PHYTOPLANKTON

FISH SPECIES	GEOGRAPHICAL ARE	HARMFUL ALGAE	TARGET-ORGANS	PATHOLOGICAL OBSERVATIONS	AUTHORS
Rainbow trout <i>Onchorynchus mykiss</i> and other Salmonidae	CANADA	<i>Chaetoceros concavicornis</i> (Diatoma)	gill	hyperplasia, hypertrophy and fusion of secondary lamellae oedema and hemorrhaging	BELL <i>et al.</i> , 1974 BRETT <i>et al.</i> , 1978 YANG and ALBRIGHT, 1992 ALBRIGHT <i>et al.</i> , 1993
Atlantic Salmon <i>Salmo salar</i>	SCOTLAND	<i>Distephanus speculum</i> (Silicoflagellate)	gill	necrosis and sloughing of the lamellae	BRUNO <i>et al.</i> , 1989
Chinook Salmon <i>Oncorhynchus tshawitscha</i>	NEW-ZEALAND	<i>Heterosigma akashiwo</i> (Raphidophyceae)	gill	oedema and degenerative change of the epithelium	CHANG <i>et al.</i> , 1990
id.	USA	<i>Chaetoceros concavicornis</i>	gill	mucous production ---> suffocation	RENSEL, 1992

CHEMICAL INJURY FROM TOXINS (Table VI)

Algae which produce toxins include a number of species belonging to at least three classes : Chrysophyceae, Dinophyceae and Cyanophyceae. However, the ability to synthesize toxins does not appear to be a strictly phylogenetic specialization. Only a few species of dinoflagellates are known to have a confirmed toxicity and some twenty out of a total number of 1500 species of algae are known to produce **potent toxins** (BADEN, 1983 ; HALLEGRAEFF, 1987). Intoxication of fish is often associated with sudden lysis of algal blooms resulting in the release of algal endotoxins into the surrounding water. To site an example, in German ponds, the release of haemolytic exotoxin from *Prymnesium parvum* caused the death of almost an entire 4,000 carp population (HICKEL *et al.*, 1976). The toxins produced by *Prymnesium parvum* were also witnessed to spread throughout the fjord system of South-western Norway killing salmon and rainbow trout (750 tons) as reported by KAARTVEDT *et al.* (1991). *Heterosigma akashiwo* on the other hand produce an ichthyotoxic compound (BLACK *et al.*, 1991) as does the dinoflagellate *Ceratium* which produces a neurotoxin of great ichthyotoxic potential responsible off the coast of Venezuela for 200 tonnes of dead plankton feeding sardine (*Cetengraulis*) (MIJARES *et al.*, 1985). Many other chemical injuries in fish due to toxins from algal blooms have been described. Death of freshwater fish in Wisconsin River (MACKENTHUN *et al.*, 1945) was attributed primarily to the depletion of oxygen and secondarily to toxic substances released into the water by the decomposition of the algae *Aphanizomenon flos-aquae*, a blue-green algae still responsible for fish kills in the eutrophic lakes of the North American mid-west (BARICA, 1975, 1978). H₂S (8.5 ppm) and hydroxylamine (a poisonous protein), were produced when dense masses of blue green algae (*Aphanizomenon*, *Microcystis*, *Anabaena*) decayed resulting in freshwater fish kills (PRESCOTT, 1948). Histopathological findings on fish are related to two major target-organs : the gill and the liver

- The gills

Some dinoflagellate species can damage fish gills, not only purely by physical contact, but also through chemical poisoning :

- Toxins produced by *Gymnodinium* are responsible for damage to the gill epithelium of the Japanese yellowtail *Seriola quinqueradiata* (DOI *et al.*, 1981 ; SHIMADA *et al.*, 1982). Secondary lamellae become impaired and swollen with modifications of mucous cells and chloride cells, which reduce the exchange of gases and interfere with osmoregulation resulting in the death of the fish.

- Severe alteration of the gill tissue was also found in juvenile cod (*Gadus morhua*) exposed to *Gymnodinium galatheanum*, where extensive separation of the respiratory epithelium from the underlying pillar cells resulted (NIELSEN, 1993). An increase in plasma osmolarity, in the juvenile cod suggested further that the cellular-damage to gills results from oedema which in turn is responsible for respiratory and osmoregulatory impairment often proving lethal to fish. *Chaetoceros concavicornis* was responsible for severe blood hypoxia in cultured *Salmo salar*. Decreased Po₂ and elevated PCO₂ in exposed fish are further evidence of the severe impediment of gas exchange at the gills, which is reflected by histological studies showing profuse mucus production from gill tissues with large areas of edema in secondary lamellae (RENSEL, 1993).

- Pathological modifications can be observed in the gills of *Seriola quinqueradiata* when exposed to neurotoxins from *Chattonella marina*. They include hypertrophy and oedema of the epithelium of secondary lamellae and a decrease in the number of mucous cells (ENDO *et al.*, 1985 ; MURATA *et al.*, 1989) owing to a degeneration of the mucous cell membrane as revealed by TEM preparations (SHIMADA *et al.*, 1983). Such alterations lead to impaired osmoregulation and gas exchange followed by death. Neurotoxins produced by the same dinoflagellate contributed to similar harmful effects in red sea bream (*Pagrus major*) (ENDO *et al.*, 1992). Gill enzyme activity involving carbonic anhydrase was reduced in the gills of both fish species when exposed to *Chattonella* red tide according to SAKAI *et al.* (1986).

• *Gyrodinium aureolum* was also responsible for gill damage to rainbow trout under laboratory conditions (ROBERTS *et al.*, 1983) and in Salmonid farms where they are farmed off the south-west coast of Ireland (PARKER *et al.*, 1982). In both cases, changes included necrotic degeneration of the secondary lamellae along the epithelium. *Salmo salar* suffered from the same dinoflagellate *G. aureolum* in Scottish farms when gill lesions developed which comprised a severe necrosis and sloughing of the gill epithelium coupled with oedema (JONES *et al.*, 1982). It was suggested that ichthyotoxins produced by the dinoflagellates are severely necrotic to the delicate mucoid epithelial tissues of not only the gills but also of the intestine. Another cytotoxic material, prymnesin, is excreted from *Prymnesium parvum* and demonstrates a high ichthyotoxic activity by inducing an abnormal permeability of the gill (YARIV and HELSTRIN, 1961).

• The toxin produced by *Chrysochromulina polylepis* destroys epithelial tissues of fish, particularly of the gills, leaving fish unable to maintain an ionic balance. Thus, osmotic stress from damaged membranes killed both wild and farmed fish in Kattegatt-Skagerrak in 1988 (HORSTMANN and JOCHEM, 1988 in SKJOLDAL and DUNDAS, 1989). Finally, histological examination of a sample of gill tissue from rainbow trout affected by a *Distephanus speculum* bloom in France (April 1987) revealed oedema and significant hyperplasia (ERARD-LE DENN and RYCKAERT, 1990). In addition, there was a degeneration of the liver with occurrence of nuclear pyknosis.

- The liver

Severe toxicological damage to the liver of cultured Salmonidae (*Salmo salar*, *Oncorhynchus mykiss*, *O. tshawytscha*) was observed in Washington farms (KENT, 1990). Histological damages characteristic of the disease were a vacuolation and necrosis of hepatocytes with an infiltration of melanomacrophage into the liver parenchyma. The author hypothesized that the causative agent may be a phytotoxin originating from an algal bloom. Similar hepatic damage was observed in Atlantic salmon associated with a bloom of *Chattonella* in Scotland and *Gyrodinium aureolum* in Norway (TETT, 1980 and POPPE, pers. comm., both in TAYLOR, 1990). Ichthyointoxication of *Mugil cephalus* and *Haemulon aurolineatum* by *Gymnodinium breve* resulted in hepatic vascular endothelial hemosiderosis which was one of 16 other diseases involving anemia (QUICK and HENDERSON, 1975).

Blue green algae such as *Microcystis aeruginosa* are also toxic to fish liver. Following an intraperitoneal injection to rainbow trout of *Microcystis* culture extracts containing the peptide microcystin, fish displayed a focus of massive necrosis within the liver, a general loss of architecture and subsequently an overall degeneration of hepatic tissue (PHILLIPS *et al.*, 1985), giving evidence of inextendingly certain blue-green algal toxins. Using the same cyanobacteria and the same toxin (microcystin), when intraperitoneally injected to *Cyprinus carpio* showed dissociation of the normal parenchymal structure and swollen, pycnotic hepatocytes confirming the earlier findings of liver damage to fish (RABERGH *et al.*, 1991). Under the electron microscope, swollen mitochondria, vacuolization of cytoplasm, dissociation of hepatocytes and vesicularisation of RER, further gives proof of the toxic potential of this blue green algae. Toxin induced hepatic megalocytosis and a necrosis of hepatocytes observed in juvenile striped bass *Morone saxatilis* were used as biomarkers of harmful phytotoxins at the cellular level (GROFF *et al.*, 1992). The livers of affected fish were macroscopically enlarged and pale. Using light microscopy, hepatocytes appeared megalocytic and multinucleated, with a prominent karyomegaly and nuclear polymorphism. There was also an increase in the number of inflammatory cells (lymphocytes, monocytes and macrophages) within the liver parenchyma and areas of fibroplasia around the bile duct. Moribund fish have also demonstrated moderate gill epithelial hyperplasia and mild renal necrosis. At the ultrastructural level, hepatocytes displayed invaginations of the nuclear envelope, a proliferation of ER, cytoplasmic inclusions which contained abundant profiles of swollen endoplasmic reticulum and mitochondria, a depletion of stored glycogen, lipoprotein particles within ER and Golgi vesicles, and numerous autophagic vacuoles, lysosomes and residual bodies

which suggested an alteration of biliary epithelial cells. Hepatotoxic agents under consideration included algal phytotoxins but the etiologic agent was not determined.

- Other organs

Intestinal changes including of nuclear pyknosis of the epithelium combined with excess mucus production and resulting in a complete sloughing of the gut epithelium were described by JONES *et al.* (1982) in *Salmo salar* suffering from a *Gyrodinium aureolum* bloom. Other intestinal damage to fish from an algal bloom was described by CHANG *et al.* (1990) : Mild intestinal alterations were observed (oedema and epithelial cell hypertrophy) in chinook salmon during a bloom of *Heterosigma akashiwo*. White sucker (*Catostomus sp.*) suffered from granulomatous enteritis. WOLKE and TRAINOR (1971) speculate that silicon dioxide from the numerous phagocytised diatoms in the submucosal tissue of the foregut is responsible for the granulomatous reaction. Toxin excreted by the dinoflagellate *Pfesteria piscimorte* is known to create skin lesions and hemorrhaging in fish (GLASGOW and BURKHOLDER, 1993).

MISCELLANEOUS NEGATIVE FACTORS

Increase in viscosity of water by a *Gyrodinium* bloom should be harmful to the young fish stages as observed off Plymouth by POTTS and EDWARDS (1987). These authors estimated that the impact of blooms on nursery grounds could be considerable.

On the other hand, anoxic conditions may also result from a mucilage production by the Diatoms as *Cerataulina* (TAYLOR *et al.*, 1985) or Dinoflagellates as *Gyrodinium* (BROWN *et al.*, 1979). In addition, toxicity of *Noctiluca* should be due to vacuolar ammonia as observed in Jakarta Bay by ADNAN (1987).

CHAPTER IV

TOXICOLOGY

The production of so-called “red-tide” toxins was for a long time something of a mystery. Difficulties arise from different quarters.

- The wide variety of existing toxins
- The type of toxic molecules involved is diverse, ranging from such simple molecules as ammonia to the more complicated polypeptides, polysaccharides, or polyethers.
- Dinoflagellates have been shown to be a rich source of highly bioactive compounds. Unfortunately, only a very limited number of species are successfully cultured and even their studies are limited owing to a high variety of potential living conditions which can influence the production of toxic metabolites (SHILO, 1972). In addition, the toxicity of many clones are remarkably different from each other (up to one hundred times) (OGATA *et al.*, 1987). For example, the exotoxic production of *Gyrodinium aureolum* is highly variable and depends either on clone selection or an unknown stimulus (GENTIEN and ARZUL, 1991).
- The available quantity of toxin is generally low acting as a handicap in the structural analysis of the purified constituent molecules. Moreover, the molecular basis for poisoning or resistance to toxins is still not understood since metabolic effects need further analysis.
- Since fish deaths occur with such rapidity and with such devastating effects, accumulation of toxins in fish is unlikely, (except for ciguatera toxins which accumulate in muscle and liver tissue and become increasingly concentrated through the food chain). High levels of PSP toxins of *Pyrodinium bahamense var. compressa* have been found in fish viscera, whereas the toxins, which are highly lethal to fish do not appear to accumulate in fish muscle tissue (MACLEAN and WHITE, 1985). However, high levels of toxins (neOSTX, STX, GTX₁₋₆) were observed in contaminated (*Pyrodinium bahamense var. compressum*) Borneo planktivorous fish *Sardinella sp.* Dietary preference for *Pyrodinium bahamense* may be the reason for the high toxin content (total toxicity = 301 Mouse-Unit) in *Sardinella* (OSHIMA, 1989).

Scientific aspects of the problem include identifying the toxins and specific effects on fish. Initially chemical observations were undertaken on diseased or moribund fish in the field during an algal bloom. QUICK and HENDERSON (1975) noted hemolytic signs (cyanosis, normo-blastosis, increased hematocrit in fish affected by red tide toxins from *Ptychodiscus brevis* (*ex Gymnodinium breve*). A number of biological tests and bioassays have been developed in laboratory conditions using protocols either of intoxication by algae or purified extracts. There are however often of limited value because of their fragmentary character.

Toxicity was demonstrated when target fish (*Gambusia*, *Brachydanio*, *Oncorhynchus*, *Fundulus* ...) were killed, either by immersion in extracts from natural or cultured algae and direct inhalation (passage across respiratory membranes) or by intraperitoneal injection (i. p.) of toxic extracts. Toxicity can be also transferred (“vectorial intoxication”) through feeding by ingestion of toxins in fish considered as primary vectors (herbivorous and detrital feeders) or secondary vectors (higher carnivores).

- *Direct inhalation and immersion in extracts from natural or cultured algae*

A significant toxic effect of *Gymnodinium galatheanum* was found in juvenile cod *Gadus morhua* exposed to 115.10^6 cells/l. They demonstrated severe histopathological changes in the gill tissue and died within 2 days (NIELSEN, 1993). Toxicity of *Ptychodiscus brevis* to six species of fish (*Mugil*, *Fundulus*, *Mollienisia*, *Cyprinodon*, *Membras* ...) clearly indicated (RAY and WILSON, 1957) that this dinoflagellate produced a fish-killing substance (time of death varied from a few minutes to two hours when contaminated with an unialgal culture containing 4-8 million cells/l). In addition, *Cyprinodon variegatus* was used as the reference animal because of its sensitivity to the toxins produced by both *Gonyaulax monilata* and *Ptychodiscus brevis* : 100 % mortality was observed over a 48 h test period when fish were immersed in a concentration 1.2 million and 9.9 million cells/l respectively (SIEVERS, 1969). *Prymnesium parvum*, which produces and excretes a toxin killing fish, was used as a model for illustrating toxicogenicity (SHILO, 1972). Thus, the toxic effect of *Prymnesium parvum* was shown when *Gambusia affinis* died 24 hours after an immersion in the phytoflagellates (REICH and ASCHNER, 1947). The mosquitofish *Gambusia affinis* is still very susceptible to *Gonyaulax polyedra* (OGURI *et al.*, 1975) as well as *Gymnodinium breve* toxins because low doses of purified toxins (0.05 μ g) are lethal to fish (SPIEGELSTEIN *et al.*, 1973). Killfish *Fundulus heteroclitus* also died when placed in an aquarium containing supernatant from centrifuged *Amphidinium carteri* cultures (THURBERG and SASNER, 1973). *Pyrodinium bahamense*, at a density 5.10^6 cells/l, provoked the death of juvenile mullet *Valamugil seheli* within less than 50 min (MACLEAN, 1979). In the other hand, LD 50 determination for *Brachydanio rerio* immersed in *Ptychodiscus brevis* extracts was 4-6 ng/ml (310 ng/ml for 60 min death) according to RISK *et al.* (1979).

- *Oral administration*

WHITE (1981b) demonstrated the lethal effects to winterflounder, Atlantic salmon and cod of Atlantic herring toxins produced by *Gonyaulax excavata* by an administration of toxin extracts (LD 50 = 400 - 750 μ g STX / kg body weight). Similarly, fish larvae were proven to be highly vulnerable to the toxins. Winterflounder larvae raised in water with 200 - 250 cells/ml of *Gonyaulax excavata* exhibited symptoms of paralysis followed by high mortality rates after three days of feeding (MILLS and KLEIN-MACPHEE, 1979). Mortality of *Mallotus villosus* and *Clupea harengus harengus* larvae was strongly correlated with the percentage of larvae that ingested cells at a concentration of 1,500 cells/ml (GOSSELIN *et al.*, 1989). Symptoms of larvae following ingestion of toxic cells include erratic swimming, remaining at the bottom of the aquarium and paralysis followed by heart failure. Capelin larvae, which feed on *Alexandrium excavatum*, do not discriminate toxic from non-toxic cells. In addition, no evidence was found of a vertical migration to avoid the dinoflagellate. Consequently, overlap, in the field, of a large fraction of larval fish populations with that of *Alexandrium* during bloom periods (peak concentration at 5,000 cells/ml) could threaten the survival of *Mallotus villosus* larvae.

- *Intraperitoneal injection (i.p.) of toxic extracts*

A purified form of the toxin prymnesin, a glycolipid produced by *Prymnesium parvum*, exhibited lethal effects to fish : LD 50 for intraperitoneally injected *Gambusia affinis* was 1.8 ± 0.4 μ g / 300 mg of body weight (PASTER, 1968). LD 100 for *Fundulus heteroclitus* and *Cyprinodon variegatus* was 0.5 mg/kg (quite different from the 8 mg/kg for mice) with i.p. extracts of *Aphanizomenon flos-aquae* (GENTILE and MALONEY, 1969). Injection of 0.2 ml of extracts of lyophilized *Amphidinium carteri* in culture was responsible for the deaths of killfish (*Fundulus heteroclitus*) (THURBERG and SASNER, 1973). The lethal dose of injected *Aphanizomenon* extracts to killfish was as low as 29 μ g/kg and much greater for mice (JACKIM and GENTILE, 1968). PSP toxins, when injected intravenously to the Japanese eel *Anguilla japonica*, caused cardioventilatory

perturbations (fall in blood pressure, ventricular arrest) probably causing death by suffocation (CHAN AND LIU, 1991).

- *Toxicity transmitted by vectoral intoxication*

Because herbivorous zooplankton are known to be capable of ingesting and accumulating potent neurotoxins (ROBINEAU *et al.*, 1991 a and b), toxins can disseminate through marine food webs starting often with resistant invertebrate vectors. Herbivorous marine zooplankton (i.e copepod *Acartia clausi*) fed cultures of *Gonyaulax excavata* were observed to retain toxins (PSP) ; 21 µg STX were sufficient to kill herring within a few minutes. Herring and sand lance known to feed on herbivorous zooplankton were also observed to be killed by intoxicated zooplankton (WHITE, 1977, 1981 a). In experimental conditions, the mortality rates of red sea bream *Pagrus major* fed on *Gonyaulax excavata* was about three times that of starved controls. Despite the low toxin contact of the dinoflagellates, a first feeding larva would need to eat only 6-11 algal cells to acquire a lethal dose (WHITE *et al.*, 1989). In contrast, first feeding Japanese anchovy *Engraulis japonica* larvae fed poorly on *Gonyaulax excavata* showed no difference in mortality between treated individuals and controls. In the same fashion, *Gonyaulax catenella* toxins may be transmitted through the food chain by crab (*Cancer anthonyi*) larvae fed on *G. catenella* and in turn fed to goby (*Lythrypnus dalli*). The goby exhibited a loss of balance with death following between 24-60 hours later (YAZDANDOUST, 1985). Exposure of mackerel larvae (*Scomber scombrus*) to neurotoxins produced by *G. excavata* could equally have lethal effects in natural ecosystems as shown in laboratory conditions when larvae were directly fed toxic cells (ROBINEAU *et al.*, 1991 a et b). A relationship between daily mortality and the number of toxic cells ingested was uncovered. Vectorial intoxications of mackerel postlarvae fed on natural zooplankton (adult copepods able to prey on *G. excavata*) was again carried out with the result of heavy mortalities suffered by the postlarvae. In addition, flesh toxicity has been induced experimentally in *Lutjanus bohar* by feeding them ciguatoxic fish flesh. It was found that only after the consumption of toxic material did the toxicity wear off 30 months (HELFRICH and BANNER, 1973). Liver extracts of mackerel *Scomber scombrus* caught in the Bay of Fundy during a bloom of *Alexandrium fudyense* were lethal to mice (HAYA *et al.*, 1990). Significant concentrations of toxins in the fish liver (up to one month after the disappearance of the bloom) indicated that mackerel could accumulate toxins from their food and that PSP toxins may be transferred further through the food web (to harbor seals for example).

However, one question can be raised : do the phytoplankton-feeding fish modify their feeding behaviour when presented with toxic strains (such as cyanobacteria which constitute an important part of the diet of a number of tropical Cichlids and Cyprinids, owing to the high levels of protein provided) ? BEVERIDGE *et al.* (1993) showed that Tilapia (*Oreochromis niloticus*) and silver-carp (*Hypophthalmichthys molitrix*) are able to differentiate between toxic and non-toxic cyanobacterial strains of *Microcystis aeruginosa* (a producer of microcystin). Grazing on toxic algae was depressed and almost completely suppressed in comparison to grazing rates on non-toxic strains. Such discrimination seems to be on the basis of the levels of microcystin (< 250 ng/l) a seemingly accurate means of assessing palatability of fish. In a similar way, the staghorn sculpin *Leptocottus armatus*, which forage extensively on the siphon tips of the butterclam (*Saxidomus sp.*), developed a significant aversion to siphon from toxic butterclams (those retaining a PST toxin - STX) but showed no hesitation in feeding on non-toxic butterclams (KVITEK, 1993).

Another point to consider is how fish cope with ingested toxins ? there is a shortage of adequate responses ...

Classification of toxins

Phytoplankton toxins (endotoxins, exotoxins) have been classified into different categories according to their organic or cellular targets (CARMICHAEL, 1988 ; SHIER, 1988 ; SHIMIZU, 1988 ; HOKAWA and SMITH, 1990 ; YASUMATO, 1990) and include the neurotoxins, hepatotoxins, hemolysins, cytotoxins and ichthyotoxins (Fig. 6).

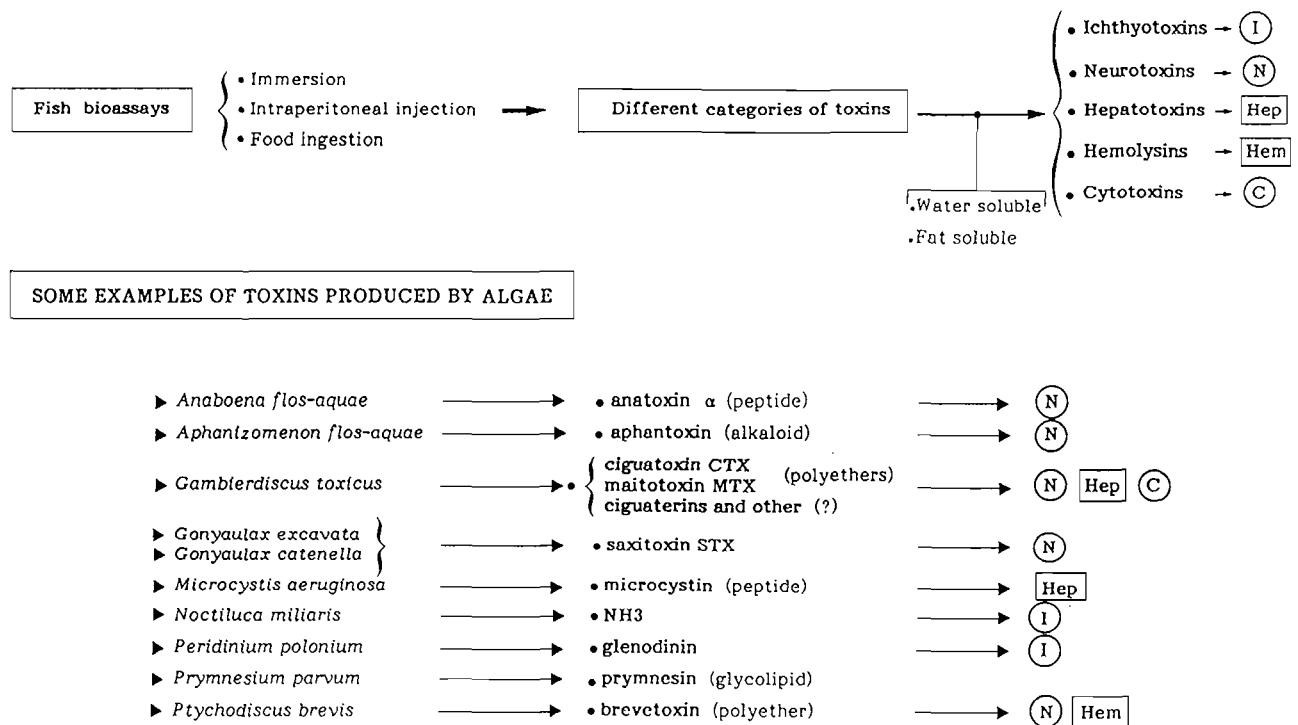


Figure 6 - Toxicology

• Neurotoxins

Fish appear to be as sensitive as most warm blooded animals to paralytic shellfish toxins PSP (WHITE, 1981 b). Thus, a complex of toxins produced by *Gonyaulax excavata* (STX, neoSTX, 5 gonyautoxins) was responsible for physiological disturbances in fish (salmon, cod). The fish were observed to be swimming in an irregular, jerky manner followed by a loss of equilibrium and arrhythmic breathing and finally death (WHITE, 1981 b, 1984). Symptoms of neurotoxic poisoning in various fish (*Morone*, *Brevoortia*, *Paralichthys*...) exposed to a new "phantom" dinoflagellate induced sudden sporadic movements, disorientation, lethargy and apparent suffocation (BURKHOLDER *et al.*, 1992).

Ion channels in plasma membranes are the primary targets for algal toxins. The physiological effects of toxins arise from a direct action on ion channels. Gonyautoxins exert their influence by altering or disrupting the membrane properties of excitable cell types in ways that enhance or inhibit the inward flow of Na⁺ ions (BADEN, 1983). Toxins which depolarize membranes are classified as occluders (STX), activators (brevetoxins) or stabilizers of Na⁺ channels (STRICHARTZ and CASTLE, 1990). Thus, a brevetoxin produced by *Ptychodiscus brevis* appears to be an extremely powerful ichthyotoxin (lethal to guppies at concentration of 10⁻⁸ g/ml : CHOU and SHIMIZU, 1982), altering the membrane properties of excitable cells. Radioactive tagging of toxins shows a binding to brain synaptosomes of *Tilapia* and it is believed that the neurotoxin binding site is associated with the Na⁺ channel (EDWARDS *et al.*, 1990). This toxin which was known to depolarize excitable

membranes (STEIDINGER *et al.*, 1973) was shown not to be an acetylcholinesterase inhibitor when applied to the brain of *Cyprinodon variegatus* (MARTIN et CHATTIERJEE, 1970). Ciguatoxin CTX from *Gambierdiscus toxicus* showed an anti-cholinesterase activity (LI, 1965). A toxin from *Prymnesium parvum* acts as a non-depolarizing blocking agent on the post-synaptic membrane of the endoplate of frogs (PARNAS and ABBOTT, 1965), blocking neuromuscular transmission. Several lipid soluble neurotoxins, according to BADEN and MENDE (1982), are highly toxic when injected (i.p.) or orally administered to mice or fish (bioassays on *Gambusia affinis*). Neurotoxic STX produced by *Gonyaulax excavata* was responsible for the deaths of Atlantic herring and capelin larvae, by direct ingestion as its concentration increased passing through the food chain, beginning with vectorial zooplankton (GOSSELIN *et al.*, 1989).

Cyanobacterial toxins such as aphantoxin (alkaloids produced by *Aphanizomenon flos-aquae* and anatoxins (peptide) produced by *Anabaena flos-aquae*, were classified as neurotoxins (SKULBERG *et al.*, 1984), when observed to act as postsynaptic blocking agents (CODD and POON, 1989). Anatoxin α from *Anabaena flos-aquae* was even considered as a fast death factor (FDF) or a very fast death factor (VFDF) by COLLINS (1978). *Aphanizomenon* toxin blocks calcium dependent action potentials ; when injected into fish, the toxins initially cause a darkening of pigmentation followed by a loss of coordination and death ensues within several minutes (JACKIM and GENTILE, 1972). Neurotoxins produced by *Chattonella marina* contribute to cardiac disorder (a large decrease in the heart rate through depolarization of the vagal nerve), followed by the death of *Pagrus major* (ENDO *et al.*, 1992). Causative substances killing yellowtails exposed to *Chattonella antiqua* blooms were uncovered as to be highly unsaturated fatty acids C16 : 4 and C18 : 4 (OKAICHI, 1989) which severely damage the gill lamellae and cause death by suffocation (OKAICHI, 1991). In addition, the physiological respiratory disorders were intensified by a modification of the normal acid-base status, a decrease in blood pH (metabolic acidosis) and PaO₂ (ISHIMATSU *et al.*, 1991).

- *Hepatotoxins*

Microcystin, a peptide toxin produced by a Cyanobacterium (*Microcystis aeruginosa*), showed affinity to hepatic parenchymal cells and induced remarkably rapid hepatic injury to mice (fast death factor or FDF = SHILO, 1972). It also caused liver damage again when administered intraperitoneally to rainbow trout (PHILLIPS *et al.*, 1985). Liver extracts of Atlantic salmon dying after intoxication with *Chrysochromulina* still displayed toxic effects to mouse hepatocytes which were totally destroyed owing to a strong powerful disintegrating effect on the cell membranes (AUNE *et al.*, 1992). Necroses in the liver was also induced by prymnesin (oral route or i.p. injection) in *Oryzias latipes* where hepatocytes revealed a swelling of mitochondria and cytoplasmic condensation (TERAO *et al.*, 1993). The *Nodularia spumigena* toxin, nodularin, displayed extreme toxic effects again to liver tissues when tested with a mouse bioassay (KONONEN *et al.*, 1993).

- *Hemolysins*

An extract from a culture of *Chrysochromulina polylepis* caused 100 % lysis in cod erythrocytes. Hemolysis of erythrocytes, (a useful model providing a direct measurement of membrane failure) was maximal at a concentration of 2.2×10^6 cells/ml (EDWARDSSEN *et al.*, 1990). Hemolytic sensitivity of the mullet to *Gymnodinium breve* toxins was about 300 times that of rabbit red blood cells ; the toxin involved in this case seemed to induce a further degradation of the hemoglobin following the lytic phase.

- *Cytotoxins*

Cytotoxic activity appears to often be directed towards the biological membranes, especially the plasmic membrane of erythrocytes, the gill epithelium ..., the final result often being a lysis of cells making up affected tissues (SHILO, 1972). Cytotoxicity of crude extracts of *Gyrodinium aureolum* were also tested on human cells (KB strain). However, owing to the very low abundance of toxin within algal cells from Brittany, characterization of the toxic compounds has not yet been possible (PARTENSKY, 1989). It is believed that this species is probably not sufficiently toxic on its own to account for the fish mortality observed but that the additional production of mucilages by the alga is thought to play a greater role in fish kills by limiting the dispersion of toxic molecules and creating an anoxic zone around fish gills. The activities of marine biotoxins at the cellular level were reviewed by MARTIN and PADILLA (1973) and a summary of the biological characteristics of algal action presented by SASNER (1973).

- *Ichthyotoxins*

The potential ichthyotoxicity of different dinoflagellates was examined by BATES *et al.* (1978) using fish (*Gambusia affinis*) bioassays. *Gonyaulax catenella* demonstrated a low level of toxicity (STX) whereas *Ptychodiscus brevis* displayed a potent ichthyotoxicity (hundreds of times more toxic than *G. catenella*) and was thought to be a FDF (fast death factor) when tested on *Poecilia reticulata* and *Catostomus commersoni* (GENTILE and MALONEY, 1969). The ichthyotoxic unit (ITU) is defined as the minimal amount of toxin per ml that can kill all the fish in an experimental set up. Lipid soluble toxins (one of them isolated from unialgal culture-molecular formula $C_{41}H_{59}NO_{10}$ - by CHOU and SHIMIZU, 1982) produced by this dinoflagellate induced damage to fish : clinical signs of diseases consist of violent twisting movements while swimming, pectoral fin paralysis, loss of equilibrium, convulsions and finally death (LD50, 24 h : 2-6 ng/ml : BADEN, 1983). Brevetoxin from *Ptychodiscus brevis* was estimated to be the most potent ichthyotoxin to guppies *Poecilia reticulata* (SHIMIZU *et al.*, 1986). *Gymnodinium nagasakiense* was shown to produce toxic lipid compounds containing polyunsaturated fatty acids (PUFA) (YASUMOTO *et al.*, 1990 in ARZUL *et al.*, 1993), especially 18 : 5 w 3 octadeca pentaenoic acid (ARZUL *et al.*, 1993). *Prymnesium parvum* also releases several soluble toxins such as ichthyotoxin (named prymnesin, a polyether), hemolysins and cytotoxin (PARNAS, 1963). Ichthyotoxic activity was confirmed by the mortality of *Gambusia affinis* (REICH and ASCHNER, 1947) and *Oryzias latipes* (IGARASHI *et al.*, 1993). Its negative effects confirmed by further studies indicating that the toxins exert their influence on the gills (SHILO et ROSENBERG, 1960). In addition, the *Prymnesium* toxin inhibits synaptosomal phosphatase activity which could be involved in increased levels of intracellular Ca^{2+} (MELDAHL *et al.*, 1993). The ichthyotoxicity of *Noctiluca miliaris* extracts on killfish was found to be closely related to ammoniacal contents of the dinoflagellate (OKAICHI et NISHIO, 1976). Ammonia (NH₃) intoxication (0.15 ng/l) could be a contributory factor to the mortalities suffered by cultured fish in Hong-Kong waters (LAM and YIP, 1990) as well as clogging of the gills by a mucous layer produced by *Gonyaulax polygramma*. Glenodinin produced by *Peridinium polonium* was also found to be a powerful toxin to *Oryzias latipes* (HASHIMOTO *et al.*, 1968). Hybrid striped bass was also sensitive to ichthyotoxins produced by the dinoflagellate *Pfisteria piscimorte* when reared in aquarium, they showed sudden distress (desorientation, skin loss, cutaneous hemorrhaging, suffocation) then death (BURKHOLDER *et al.*, 1993). Medaka (*Oryzias latipes*), when given prymnesin from *Prymnesium parvum* by either orally or by i.p. injection, died with injuries to the gills and liver (TERAO *et al.*, 1993). The toxic mode of action of *Heterosigma akashiwo* seems to be due to the formation of superoxide radicals, hydrogen peroxide and hydroxyl radicals. Indeed, juvenile sockeye salmon can be protected against such toxic effects by adding suitable amounts of superoxide dismutase (SOD) in the culture immediately before the addition of experimental fish (YANG *et al.*, 1993). *Chrysochromulina polylepis* toxins appear as a group of compounds rather than a single chemical.

They are lipophilic, resemble digalactosyl monoacyl glycerolipids produced by *Amphidinium carteri* and are similar to hemolysin isolated from *Prymnesium parvum* (LEIVESTAD and SERIGSTAD, 1989 ; SKJOLDAL and DUNDAS, 1989). These toxins operate at the cellular level on cell membranes, making them more permeable and thereby disturbing the ionic balance which results in the death of fish through osmoregulatory failure following toxic action on gill tissue (DAHL *et al.*, 1989). Toxin extracts disturb the ion fluxes in biological membranes by causing an increase in the intracellular calcium concentration in synaptosomes (MELDAHL *et al.*, 1993).

- *The case of ciguatera toxins*

Ciguatera is now a well documented tropical and subtropical fish poisoning. Ciguateric toxins (CTX, MTX, ciguaterins ...) produced by the benthic dinoflagellate *Gambierdiscus toxicus* and other related dinoflagellates were regarded, for a long time, to be harmless to fish despite the accumulation of high concentrations of these toxins (especially CTX) in muscle tissues and especially the liver. Their concentration however is always known to be potentially harmful to mammals and especially humans despite no obvious harmful effects on those fish that consumed ciguateric toxins directly (HELFRICH and BANNER, 1963).

Results from DAVIN *et al.* (1986 and 1988) soon questioned, for the first time, the notion that fish are not harmed by ciguateric toxins. Indeed, distinct behavioral abnormalities (including skin color variations, inactivity, loss of equilibrium, erratic swimming, jerky feeding movements and loss of orientation) were displayed by blueheads *Thalassoma bifasciatum* after consumption of *G. toxicus*, one of the major vectors by which ciguatera toxins (CTX, MTX, ciguaterins) enter the food chain. Piscivorous fish such as largemouth bass *Micropterus salmoides* showed behavioral abnormalities following the consumption of either a ciguatoxic great barracuda *Sphyrnaena barracuda* or freeze-dried *G. toxicus* (DAVIN *et al.*, 1988).

Effects of barracuda ciguateric toxins on Teleost chromatophores was investigated by GALARZA and TOSTESON (1992). Ciguatoxins caused the pigment granules to aggregate in the centres of the melanophore cells (either in *in vitro* isolated scales or in i.p. inoculated damselfish *Stegastes planifrons*), resulting in altered coloration of the fish.

In experimental conditions, recent findings indicate that some fish may suffer from contamination of ciguateric toxins either through food consumption or through intraperitoneal injection of toxin extracts. Thus, intraperitoneal injections of CTX produced significant histopathological changes in the guts and gills of two Pomacentrids *Pomacentrus wardi* and *Chromis nitida* (CAPRA *et al.*, 1988).

On the other hand, Pomacentrid fishes (*Chromis chromis*) had a smaller and paler livers than control fish when fed on a diet containing *G. toxicus* (DURAND-CLEMENT *et al.*, 1987). In addition, modifications were observed in the liver of cabrilla sea bass *Serranus cabrilla* fed *G. toxicus* (AMADE *et al.*, 1990). Hepatic disturbances described at an ultrastructural level involved an increase in lipid reserves, degeneration of hepatocytes, perisinusoidal fibrosis and lysis of the sinusoidal envelope (GONZALEZ, 1990 and 1992). Some of these morphological injuries have also been recognized in the liver of mice (TERAO *et al.*, 1991). Thus, it became apparent that fish are not immune to ciguatera toxins and can be adversely affected by a diet containing ciguatoxic material. However, although pronounced disorders may be experimentally induced after ingestion of ciguatoxic food, proof of similar negative effects in the natural food chains of coral reef ecosystems is needed (GONZALEZ, 1992). Studies of this kind are few and, before any final conclusions can be made, it is important to consider that the effects of harmful algae can be modified by a number of factors including, for example, the health of the fish.

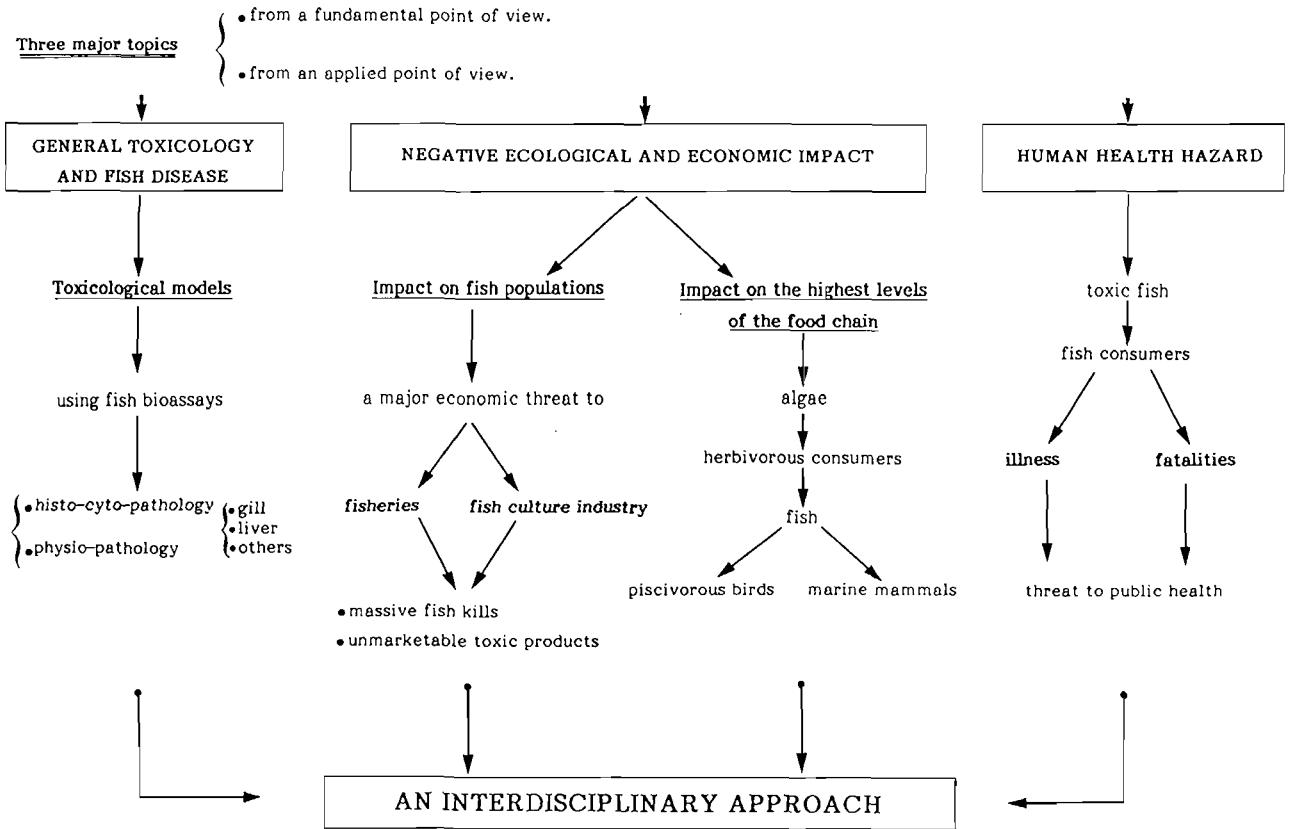


Figure 7 - Interest in harmful algal blooms

CHAPTER V

INTEREST OF TOXIC ALGAL BLOOMS

There is growing concern on three major topics, both from a fundamental and applied point of view (Fig. 7) :

GENERAL TOXICOLOGY

Toxicological models using fish bioassays have been developed and are still in progress, especially through the use of histocytological (liver, gill and other tissue) and physiological findings (see chapter III and IV). It is still necessary however to clarify the mechanisms leading to fish poisoning (i.e ability to accumulate toxins in organs) and especially to elucidate the transfer and detoxification mechanisms within the bodies of fish.

ECOLOGICAL AND ECONOMIC IMPACTS

The impact of algal blooms on fish populations and fisheries as well as on piscivorous birds and mammals has been well documented. Attention has been focused on blooms of toxic algae especially dinoflagellates (*Protogonyaulax*, *Gymnodinium*, *Pyrodinium*, *Ptychodiscus*) which present problems with respect to optimal utilization of marine and freshwater resources due to the economic losses they are responsible for following mass mortality of wild and cultured fish and also the threat they pose to public health (SHUMWAY, 1990 and 1992).

- *Impact on fisheries*

Although the impact of algal blooms on fisheries is difficult to evaluate both in terms of the monetary losses and when attempting to estimate the number of dead fish, it is clear, as described in chapter I, that such blooms often have devastating effects on fish populations.

Harmful phytoplankton have caused considerable damage by depleting fish stocks, restricting harvests with the result of great losses to the fishing industry and as a while the economic impact of fishery mortality as a direct result of algal blooms. It is now an important threat to coastal fisheries and proliferation of *Alexandrium excavatum* and other phytoplankton species is expected to increase particularly along the NW Atlantic coast (ROBINEAU *et al.*, 1991a and b).

The avoidance of contaminated areas by fish has been noted in some areas : scarcity of herring off the East coast of England during a *Phaeocystis* bloom (SAVAGE, 1931) in the Bay of Fundy (Canada) following a bloom of *Gonyaulax tamarensis* (WHITE, 1984) and also a reduction of catches along the Western coast of India during a *Noctiluca miliaris* bloom (SUBRAMIAN, 1985).

On the other hand, the widespread occurrence of a net-clogging "slime" due to the mucilage producing *Phaeocystis pouchetii* in the English Channel prevents fishermen from successfully using their nets (BOALCH, 1984). Catches in Tasman Bay (New Zealand) were highly reduced in the spring 1981 through similar events (CHANG, 1983). Acrylic acid and dimethylsulfide (DMS) production by *Phaeocystis* blooms may further act as a barrier to the migration of fish (WEISSE *et al.*, 1994).

High mortality rates of sardine eggs was attributed to the dinoflagellate *Ichthyodinium chabelardi* off the coast of Portugal (MENESES and RE, 1992). Fish larvae are highly sensitive to toxins and spatiotemporal expansion of phytoplankton blooms could jeopardize recruitment by

dramatically reducing survival of fish larvae by direct or vectorial poisoning as shown by ROBINEAU *et al.* (1991 b). Anchovy (*Anchoa mitchii*) recruitment was affected along the New-York coasts in this manner (SHIMA and COWEN, 1989). Mortality of fish resulted from a reduction in the visual detection of prey by larval fish and leaving them often exposed to predators and thereby leading to changes in both growth and mortality rates (CASTRO and COWEN, 1989). In addition, GOSSELIN *et al.* (1989) concluded that proliferation of the toxic dinoflagellate *Protogonyaulax tamarensis* in coastal waters, as observed in the St Lawrence Estuary, could jeopardize the early recruitment of *Mallotus villosus* and *Clupea harengus* in Canadian fisheries. Therefore, a spatio-temporal coincidence of the emergence of fish larvae and the proliferation of toxic dinoflagellates could lead to massive fish mortality and significantly impair recruitment of fish stocks.

- *Impact on fish culture*

The economic impact of harmful blooms has become increasingly severe at a time when aquaculture of fish is promoted extensively all along the coasts of Europe (PARKER, 1987), North America (WHITE, 1982), and Japan (FUKYO et ISHIMARU, 1986). Fish mortalities in Norway, Ireland and Scotland have shown that red tides of *Gyrodinium aureolum* are a serious obstacle to the development of Salmonid and cod farming in the sea (JONES *et al.*, 1982). The losses are, by far, the easiest to evaluate when considering only netted and caged fish of which commercial value is well established (Table 1).

- *Ecological impact on the highest levels of the food chain*

There are a number of pathways through which fish population may be negatively affected by dense phytoplankton blooms. The principal routes through which toxins reach fish and are transmitted to human throughout the food chain were summarized by WHITE (1984 and Fig. 8).

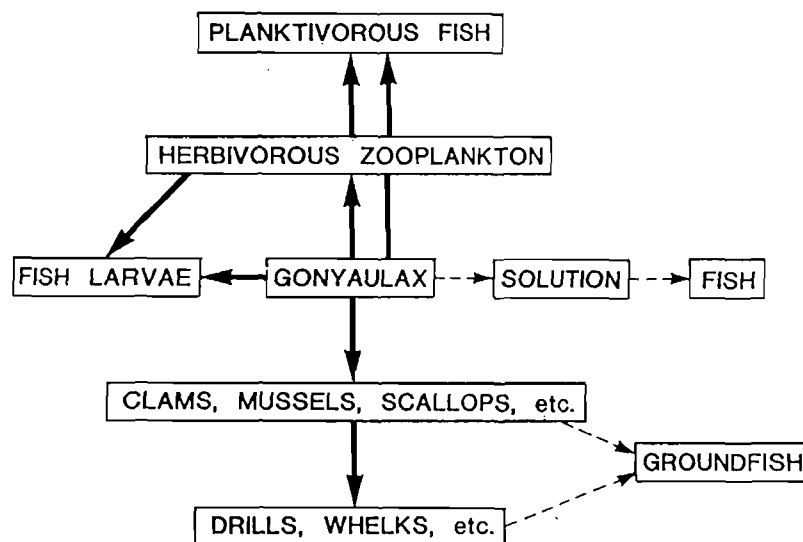


Figure 8 - Some routes through which paralytic shellfish toxins reach fish. Solid arrows show known routes. Broken arrows show possible routes (In White A.W., 1984, p. 177)

Thus, toxins can be transmitted from the algae to herbivorous consumers (Molluscs, Crustacea or phytophageous fish) and further to carnivorous fish, piscivorous birds and mammals. For example, a bloom of the diatom *Pseudonitzschia australis* occurring in California, during the month of September 1991, coincided with an episode of fish mortality rates in sea birds (*Pelicanus*, *Phalacrocorax*). High levels of domoic acid (DA), the ASP toxins, were found in the stomach

contents of affected birds and in the visceral contents of local anchovies (*Engraulis mordax*), the principal food source of sea birds (FRITZ *et al.*, 1992). Thus, herbivorous finfish can act as vectors of the DA toxin which is subsequently carried up the food chain from diatom producers to anchovies and finally to sea birds. Several thousand ducks were estimated to have died from a red tide episode *Gymnodinium breve* on the west coast of Florida (FORRESTER *et al.*, 1977). Exceptionally high mortalities in shag and other sea bird populations (puffins, gulls, guillemots) along the Northumberland coast of the U.K. resulted by consumption of PSP toxins concentrated in the fish they prey upon, particularly sandeels *Ammodytes* sp. (COULSON *et al.*, 1968). Along the coast of northern Florida brown pelicans and cormorants were paralyzed by domoic acid after eating anchovies that had consumed toxic diatoms *Pseudonitzschia australis* (ANDERSON and WHITE, 1992). A similar episode of mortality in brown pelicans and cormorants in Santa Cruz, California in September 1991 was attributed to the neurotoxin domoic acid produced by *Pseudonitzschia australis*. Diatoms and high levels of DA were found in the stomach contents of affected birds and in the viscera of anchovies (*Engraulis mordax*), the principal food source of sea birds (WORK *et al.*, 1993). It appears however that anchovies were not affected by the toxins. On the other hand, the death of hundreds of bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of the US (between New Jersey and Florida) in 1987-1988 have been associated with a transfer of brevetoxin through the food chain involving menhaden and spanish mackerel (*Scomberomorus regalis*) as well as other fish (GERACI, 1989 in MAYONEY, 1989). Dolphin livers tested positive when analysed for brevetoxin and it was speculated by ANDERSON and WHITE (1992) that toxin from *Gymnodinium breve* may have additionally been responsible for a weakening the immune system thereby rendering dolphins as well as other mammals susceptible to infection or other secondary ailments. The death of humpback whales (*Megaptera novaeangliae*) in Cape Cod originated from fatal poisoning through consumption of mackerel (*Scomber scombrus*) containing the neurotoxin STX produced by dinoflagellates (GERACI *et al.*, 1989 ; HAYA *et al.*, 1990 ; TAYLOR, 1990 ; ANDERSON and WHITE, 1992). It remains an enigma how and why Atlantic mackerel are able to survive with substantial levels of toxins in their gut. In addition, a *Chrysochromulina* bloom which occurred in 1988 along the Danish coasts might be involved in the death of harbor seals *Phoca vitulina* (SIMMONDS, 1991).

HUMAN HEALTH HAZARD

Accumulation of toxins in fish muscle tissue and viscera render the fish unsafe for human consumption. Thus, 270 ppm domoic acid was found in samples of viscera from frozen anchovies caught near Los Angeles in April 1991 (WEKELL *et al.*, 1993). The most famous algal toxin threatening human health through the consumption of fish is ciguatera. However, other algal toxins can be equally harmful to humans. The first illnesses (191) and fatalities (4) in humans, associated with the consumption of a planktonivorous finfish *Sardinella* sp. and *Selarodes leptolepis* (both suspected to be contaminated with PSP - *Pyrodinium bahamense var compressa*), occurred in 1983 in Indonesia when it was likely that fish were consumed whole (ADNAN, 1984, in MAYONEY, 1989 ; ADNAN, 1993 ; MACLEAN and WHITE, 1985). Inhabitants of Kaliningrad (URSS) avoid eating fish (and especially fish livers), especially burbot captured in lagoons because of the high incidence of cyanobacterial blooms. However, despite these precautions, over 1000 people fell ill between 1920-1930 along the Baltic coast (CODD and POON, 1989). *Pyrodinium* likewise has been responsible for more than 1000 human illnesses and 60 fatalities resulting from the consumption of contaminated shellfish as well as planktonivorous fish (sardines and anchovies) in the Pacific Ocean. A *Pyrodinium* bloom in 1987 along the Pacific coast of Guatemala resulted in the hospitalization of 187 people, 26 of which were fatal cases (HALLEGRAEFF, 1993). The involvement of those fish consumed by humans as vectors of algal toxins poses serious problem. One of which is the difficulty in surveying fish which have been exposed to algal blooms since fish can easily migrate far from bloom areas (OSHIMA, 1989).

CONCLUSION

The effects of harmful algal blooms on finfish may be given preferential treatments in future programs of research. Knowledge of the variation of biotoxin levels among individual fish caught in the same area is important for ecological and physiological purposes and is necessary for developing statistically managing and testing fish products. Some monitoring programs are presently being developed (SHUMWAY, 1990) but many questions remain to be answered and require further investigation especially through a interdisciplinary approach, because, as asserted by WHITE (1980 p. 4) : “many facets of the general problem of algal toxin remain to be explored”.

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