Chattonella and Fibrocapsa (Raphidophyceae)

Vrieling, E.G.; Koeman, R.P.T.; Nagasaki, K.; Ishida, Y.; Peperzak, L.; Gieskes, W.W.C.; Veenhuis, M.

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CHATTONELLA AND *FIBROCAPSA* (RAPHIDOPHYCEAE): FIRST OBSERVATION OF, POTENTIALLY HARMFUL, RED TIDE ORGANISMS IN DUTCH COASTAL WATERS

E.G. VRIELING[1,2], R.P.T. KOEMAN[3], K. NAGASAKI[4], Y. ISHIDA[5], L. PEPERZAK[6], W.W.C. GIESKES[1] and M. VEENHUIS[2]

[1]*Department of Marine Biology, University of Groningen, Biological Centre, P.O. Box 14, 9750 AA Haren, The Netherlands*
[2]*Laboratory of Electron Microscopy, University of Groningen, Biological Centre, P.O. Box 14, 9750 AA Haren, The Netherlands*
[3]*Koeman & Bijkerk B.V., Hydro-Ecological Research and Advice, P.O. Box 14, 9750 AA Haren, The Netherlands*
[4]*Red Tide Biology Section, Red Tide Research Division, Nansei National Fisheries Research Institute, Fisheries Agency of Japan, Onno-Cho, Saeki-gun, Hiroshima 739-04, Japan*
[5]*Laboratory of Microbiology, Department of Fisheries, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan*
[6]*National Institute of Coastal and Marine Management, Ministry of Transport and Public Works, P.O. Box 8039, 4330 EA Middelburg, The Netherlands*

ABSTRACT

Species of the potentially toxic and red-tide-forming marine-phytoplankton genera *Chattonella* and *Fibrocapsa* (Raphidophyceae) were observed for the first time in 1991 in samples taken in Dutch coastal waters; they were again recorded and enumerated in the following years. *Chattonella* spp. cell numbers varied with the season, with a maximum in May or June in the Dutch Wadden Sea. Cell numbers of *Chattonella* and *F. japonica* Toriumi et Takano were up to $6.0 \cdot 10^3$ cells·dm^{-3} in the Dutch Wadden Sea, except at one station in June 1993 when over 10^4 cells·dm^{-3} *Chattonella* were counted. In May 1993, a minor bloom (over $2.0 \cdot 10^5$ cells·dm^{-3}) was observed at a station in the southern central North Sea, 100 km northwest of the island of Terschelling. The potentially neurotoxic species *Chattonella marina* (Subrahmanyan) Hara et Chihara was identified and discriminated from morphologically related species within the class of Raphidophyceae by immunofluorescence. *F. japonica* could only be clearly identified in live samples; in fixed samples cell morphology was severely affected. The identification of this species was supported by the presence of mucocysts, structures that can be observed readily by optical and electron microscopy.

Key words: *Chattonella* spp., *Fibrocapsa japonica*, harmful phytoplankton, Raphidophyceae, red-tide organisms

1. INTRODUCTION

A variety of toxic marine phytoplankton species are observed each year in Dutch coastal waters (Kat, 1985; Leewis, 1985; Reid *et al.*, 1990). Since 1989 the potentially toxic dinoflagellates *Alexandrium* spp. and *Gyrodinium aureolum* Hulburt have been recorded during monitoring (Peperzak *et al.*, 1994). Representatives of the class Raphidophyceae were never observed during surveys before 1991, although species such as *Chattonella marina* (Subrahmanyan) Hara et Chihara, *C. antiqua* (Hada) Ono, *Fibrocapsa japonica* Toriumi et Takano, and *Heterosigma carterae* (Hulburt) Taylor (formerly *H. akashiwo* (Hada) Hada ex Hara et Chihara) are known as major red-tide organisms in coastal areas of Japan. There they are the cause of massive fish kills in cultures of yellowtails and sea breams (Iwasaki, 1971; Toriumi & Takano, 1973; Nakamura, 1983). Among the red-tide-causing species along the Chinese coast, *C. marina* has been identified as one of eight toxic species (Tseng *et al.*, 1993); *F. japonica* and *O. luteus* have a global distribution (Loeblich III & Fine, 1977; Smayda & Villareal, 1989; Billard, 1992; Taylor *et al.*, 1993). *F. japonica* has been identified in the German Bight (Elbrächter, pers. comm.), along the Belgian (Reid *et al.*, 1990) and near the French coast (Billard, 1992).

Because of the absence of a rigid cell wall, the

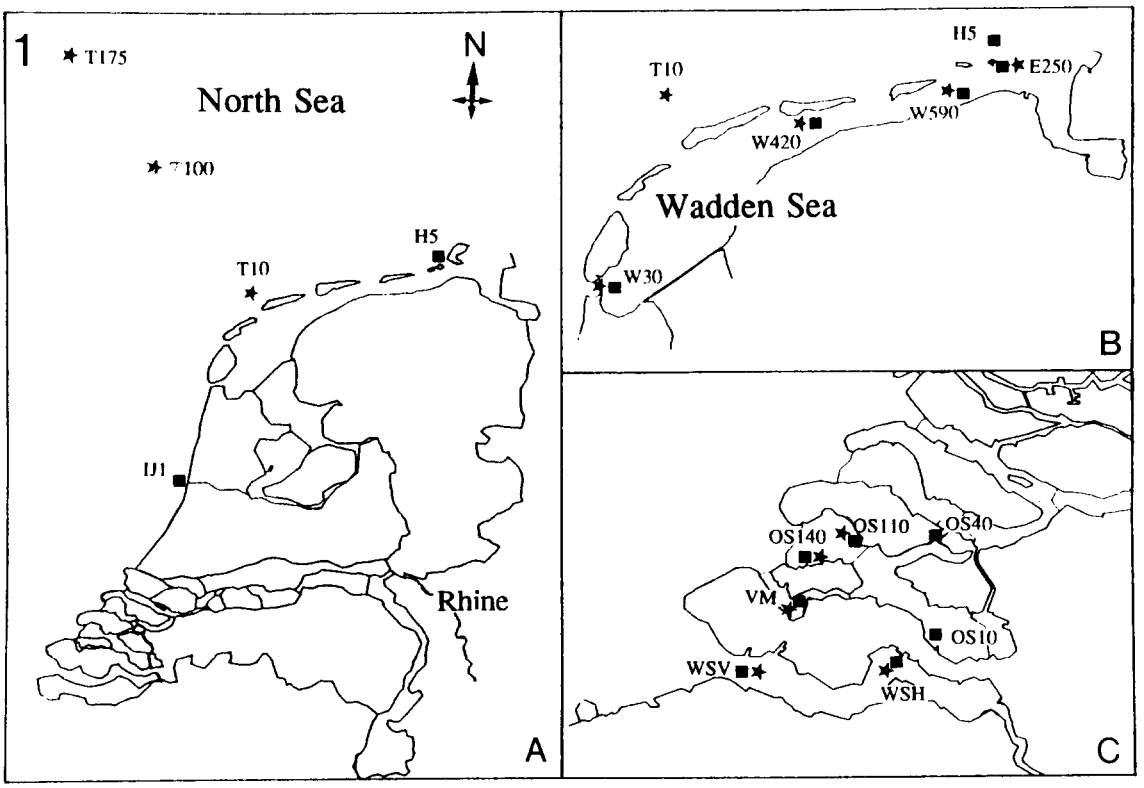

Fig. 1. Stations along the Dutch coast at which the raphidophyceans (★) *Chattonella* (*C. marina* and *C. antiqua*) and (■) *Fibrocapsa japonica* were observed. Enlargements are of the Dutch Wadden Sea (B) and the Delta area south of the Rhine estuary (C).

shape and size of Raphidophyceae tend to change rapidly with changes in environmental conditions (Hiroishi *et al.*, 1988; Nagasaki *et al.*, 1989); identification is further affected by sampling, isolation, and fixation (Billard, 1992). For example, *H. carterae* (formerly *H. akashiwo*) has been misidentified as the sand-dwelling species *Olisthodiscus luteus* Carter in reports describing this last species for North American coastal waters (Taylor *et al.*, 1993) although they can be distinguished easily by light microscopy after careful examination (see illustrations in Larsen & Moestrup, 1989 and Fukuyo *et al.*, 1990).

To study toxic algal blooms in a biological and ecological context, one should be able to identify species, inducing red tides, even when they co-occur with morphologically indistinguishable and closely related non-toxic species. Immunochemical approaches to the identification of toxic phytoplankton species have therefore been designed (Hiroishi *et al.*, 1988; Nagasaki *et al.*, 1989; Anderson *et al.*, 1990; Sako *et al.*, 1993; Bates *et al.*, 1993; Vrieling *et al.*, 1993a, b, 1994). Monoclonal antibodies directed against cell surface antigens of *Chattonella* species have already allowed the distinction between *C. antiqua* and *C. marina* and grouping of serologically related cell types (Hiroishi *et al.*, 1988; Nagasaki *et al.*, 1989; Uchida *et al.*, 1989).

The massive fish kills in Japanese coastal areas due to exposure to *C. antiqua* or *C. marina* were initially thought to be caused by anoxia (Matsusato & Kobayashi, 1974; Ishimatsu *et al.*, 1990), but histological studies revealed severe damages of fish gills induced by biotoxins (Matsusato & Kobayashi, 1974; Doi *et al.*, 1981; Endo *et al.*, 1985; Toyoshima *et al.*, 1985). The major effect of neurotoxins produced by *C. marina* is a decrease of the heart rate, presumably resulting in anoxia following reduced blood circulation in the gills (Endo *et al.*, 1992). The mortality of zooplankton species such as the copepod *Acartia tonsa* (Tomas, 1981) and tintinnids (Verity & Stoecker, 1982) is also linked to Raphidophyceae.

In this contribution the presence of two different genera of Raphidophyceae in Dutch coastal waters is described; it is suggested that they may become a threat to fish and benthic life.

2. MATERIALS AND METHODS

Samples were taken in 1991, 1992, and 1993, between April and September at two-week intervals

for the Dutch biomonitoring programme by the National Institute of Coastal and Marine Management (NICMM). Fig. 1 shows the location of the stations in the Dutch Wadden Sea (E250, W30, W420, and W590), North Sea (T10, T100, T175, H5, and IJ1), and the Delta area south of the Rhine estuary (VM, WSH, WSV, OS10, OS40, OS110, and OS140) where Raphidophyceans were identified. Samples were taken from the surface and the thermocline (T100 and T175) by filling Niskin bottles mounted on a rosette sampler. One series of subsamples of 1 dm^3 (brown flasks) were fixed directly with 0.4% (v/v) Lugol's iodine and stored at 4°C for later examination, others were investigated within two days without fixation to obtain isolates and confirm identification of species. In live samples, motile phytoplankton cells were concentrated by hanging a bright light (intensity approximately 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) above the brown sampling flasks for 1 h resulting in a photoactive reaction towards the light of the light-inhibited motile cells. Phytoplankton concentration was determined in both concentrated Lugol-fixed and in the photo-active concentrated live samples by microscopical counting using an Olympus IMT2 inverted microscope.

From light-concentrated field samples, cultures of *C. marina* and *F. japonica* were established. Single cells were selected and transferred into F/2 enriched seawater (Guillard & Ryther, 1962) and cultured at 16°C under a 12/12 h light and dark regime (light intensity approximately 75 $\mu E \cdot m^{-2} \cdot s^{-1}$. After preliminary growth, the species were cloned whereafter unialgal batch cultures were obtained.

For species identification of *C. marina*, several samples containing *Chattonella* (as determined from microscopical observations) and cultures were treated with an enhanced immunofluorescence assay (Vrieling *et al.*, 1993a), with the exception that Lugol-fixed samples were treated as described by Anderson *et al.* (1990). Briefly, cultured cells were fixed *in situ* for 1 h at room temperature (RT) using freshly prepared paraformaldehyde (final concentration 2% (w/v)). Fixed cells were washed three times with incubation buffer (phosphate buffered saline (PBS) with 0.8% (w/v) bovine serum albumine), before they were incubated with the species-specific and cell surface antigen-directed monoclonal antibodies AT-86 against *C. antiqua* and MR-18 against *C. marina* (Hiroishi *et al.*, 1988; Nagasaki *et al.*, 1989; Uchida *et al.*, 1989). Both monoclonals were diluted 1:5 in incubation buffer. After incubation for 1.5 h at RT, cells were washed three time with incubation buffer, incubated for 1 h at RT with sheep anti-mouse FITC conjugated antibodies. After the final incubation, cells were washed three times with PBS and examined for fluorescence. For enhanced labelling, biotinylated sheep anti-mouse antibodies were used as secondary antibodies followed by a third incubation with FITC conjugated streptavidin. Secondary antibodies and streptavidin were obtained from Amersham and diluted 1:100 in incubation buffer before use. Non-specific labelling of the secondary antibody and background autofluorescence was examined by either omitting the primary antibody or using normal serum. Labelled samples were screened for FITC-fluorescence with a Zeiss Axioscope epifluorescence microscope and a Leica Cambridge confocal laser scanning microscope. The latter was equipped with an Argon-ion laser (excitation wavelength 488 nm); emitted fluorescence was separated using a 525-550 nm (green fluorescence) and a longpass 650 nm (red fluorescence) filter in the detection channels.

3. RESULTS

3.1. MORPHOLOGY

Microscopical observations of unfixed and Lugol-fixed samples revealed the presence of *Chattonella* spp. and *F. japonica* from 1991 to 1993 at the stations shown in Fig. 1. Only once another Raphidophycean, probably *O. luteus*, was observed. The cells of *C. marina* are characteristic: obovoid with a mucilage like layer (Figs 2A and 2B). The cells measure 30-45 μm in length and 20-30 μm in width. Ellipsoid chloroplasts can easily be recognized by their radial arrangement (Figs 2A and B). The cells possess two flagella that are equal in length, both emerging from the anterior end (the arrow in Fig. 2A indicates one of the flagella). In a small number of samples, a larger, slightly flattened and slender tail-bearing species was observed, which resembled *C. antiqua* (Figs 2C and D).

The cells of *F. japonica* measure up to 20-30 μm in length and 15-20 μm in width. Numerous yellowish-brown discoid chloroplasts and mucocysts (ejectile organelles similar to trichocysts of dinoflagellates), the latter generally located at the posterior site of the cell (white arrows Fig. 2E), can be recognized by optical microscopy (Figs 2E and F). The two flagella, one of which is longer, emerge from the anterior end of the cell (arrows Fig. 2F).

3.2. DISTRIBUTION

The total cell numbers of *Chattonella* spp. varied considerably between the years 1991, 1992, 1993 and for the stations where they were observed (Fig. 1). In 1991, the species were observed for the first time and the cell concentration could only be estimated for a small number of all samples. As has been observed later again in 1993, the cell number was almost always below 10^3 cells·dm^{-3}. During the 1991-1993 period, the cell concentration of *Chattonella* spp. exceeded $2.0 \cdot 10^3$ cells·dm^{-3} at only two occasions: once at station W590 (Fig. 1B) in April 1992 (just over $6.0 \cdot 10^3$ cells·dm^{-3}) and once at station E250 (Fig. 1B) in June 1993 (over 10^4 cells·dm^{-3}). In 1993, *Chattonella* spp. were observed for the first time in the

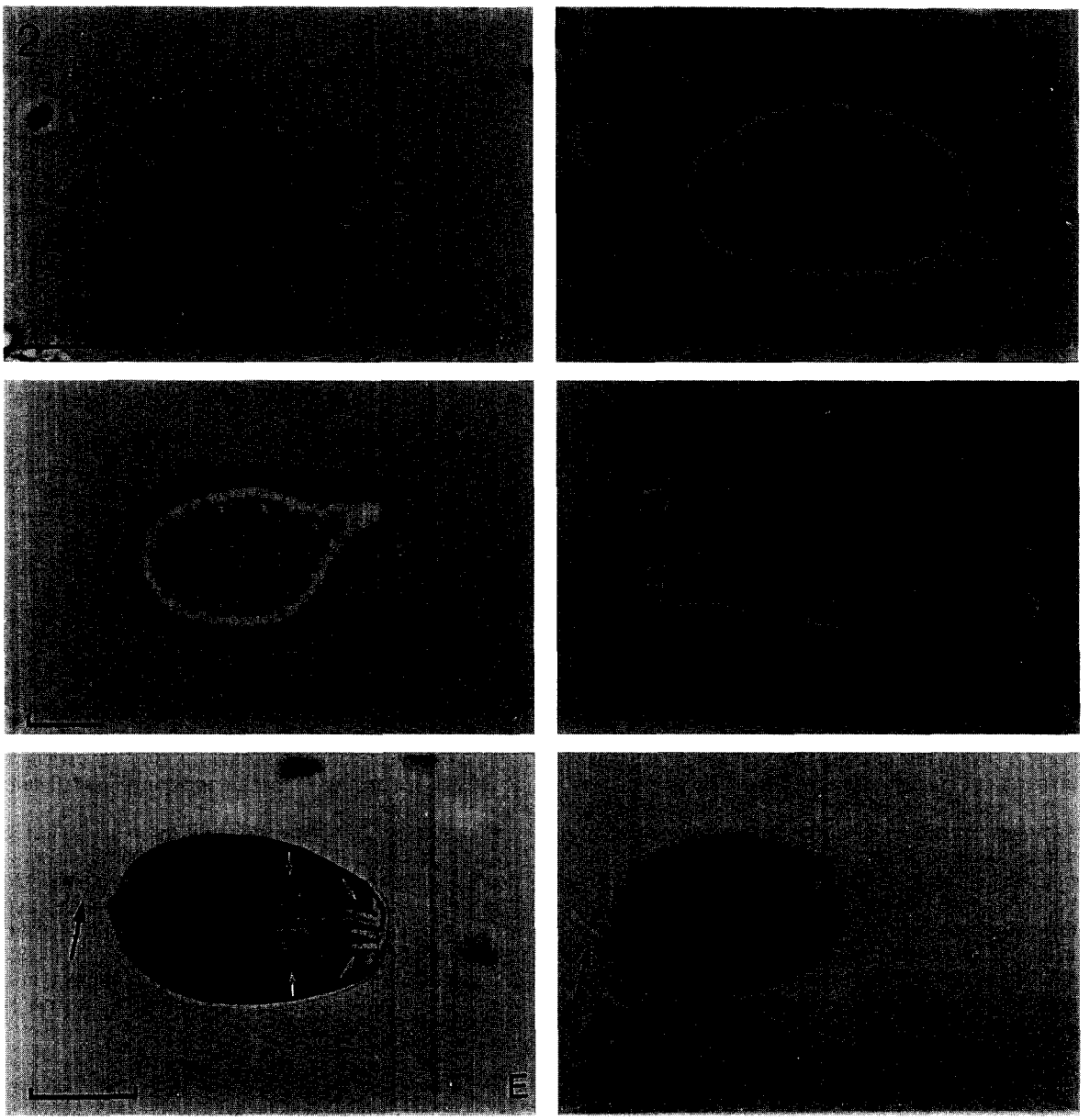

Fig. 2. Micrographs of live and Lugol fixed *Chattonella marina* (A & B), *C. antiqua* (C & D), and *Fibrocapsa japonica* (E & F). The black arrows indicate the position of flagella, whereas the white arrows indicate the position of the mucocysts. Note: cells of B and D were fixed by Lugol. Bar length is 15 μm.

central North Sea, a minor bloom density 100 km northwest of the island of Terschelling (T100, Fig. 1A), with concentrations over $2.0.10^5$ cells·dm^{-3} in May. The bloom seemed to be restricted to this location; at other stations on this Terschelling transect the species was not observed during that time. In the months before the bloom, cell densities increased from about 500 cells·dm^{-3} in March to 2000 cells·dm^{-3} in April, while after the bloom cell numbers never exceeded 200 cells·dm^{-3}. Remarkably, *Chrysochromulina* spp. co-dominated the *Chattonella* bloom at a density between 10^5 and 10^6 cells·dm^{-3}, whereas near the coast (Fig. 1A, at station T10) the characteristic spring bloom of *Phaeocystis* spp. dominated the plankton. In the Delta area south of the Rhine estuary (Fig. 1C), *Chattonella* spp. have occasionally been

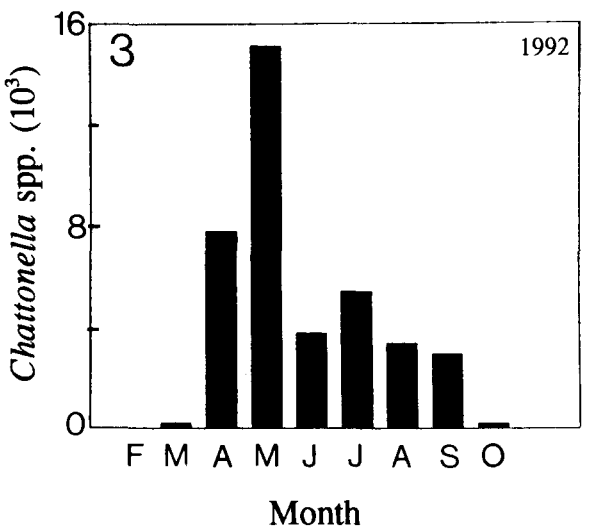

Fig. 3. Variation of total cell numbers of *Chattonella* spp. (*C. marina* and *C. antiqua*) recorded for the stations W30, W420, W590, and E250 (see Fig. 1B) in the Dutch Wadden Sea between February and October 1992.

recorded at cell numbers of about 10^3 cells·dm^{-3}, but normally smaller numbers were observed.

In contrast to 1991 and 1993, *Chattonella* spp. were observed on almost every sampling date and sample stations in 1992. Cell densities of *Chattonella* spp. in the Wadden Sea (Fig. 1B) during the sampling period in 1992 are presented in Fig. 3. Cell numbers varied considerably from late spring to early autumn; the highest cell numbers were recorded in May, ranging from about $2.0 \cdot 10^3$ cells·dm^{-3} at station W420 to more than $6.0 \cdot 10^3$ cells·dm^{-3} at station W590. The contribution of these species to the whole phytoplankton crop was minor, below 0.01% of total cell numbers; they could not be noticed during blooms of *Phaeocystis* spp. or *Skeletonema costatum* (Greville) Cleve. An increase and a subsequent gradual decrease in monthly cell numbers of *Chattonella* spp. were observed between March and October 1992 (Fig. 3).

Concentrations of *F. japonica* were always below 10^4 cells·dm^{-3} at the stations were they have been observed (Fig. 1) and this species was only identified properly in live samples. Unfortunately, no live samples of the Delta area (Fig. 1C) were taken during 1993, so additional data about the presence of this species in that area in that year are missing. The estimates of cell numbers by the 'photoactive concentration method' applied are rough. Nevertheless, a good indication of the range of the amounts of flagellates can be obtained. Due to fixation, cells of *F. japonica* formed aggregates in which individual cells were hardly recognized, resulting in unsatisfactory identification and enumeration precision of this species in fixed samples.

3.3. IMMUNOFLUORESCENCE

From unfixed field samples, *C. marina* was successfully isolated and cultured for further identification. By both the indirect and enhanced immunofluorescence microscopy that we used, cultured cells reacted only with the monoclonal antibody MR-18, while the monoclonal specific to *C. antiqua* (AT-86) showed no reaction. A number of Lugol-fixed samples of each station, containing *Chattonella* spp., that were subjected to the enhanced IF-assay showed only a positive labelling with the MR-18 antibody directed to cell-surface antigens of *C. marina* (Table 1). Although intensities were quite low, fluorescence of enhanced labelled cells was clear with respect to controls. Occasionally, weak labelling was observed in samples labelled with the AT-86 antibodies, suggesting the presence of *C. antiqua*. Phase-contrast images of these cells, however, revealed obovoid cells and not the tail-bearing cell type. In almost all field samples treated with both antibodies, small heterotrophic naked dinoflagellates were present with an outspoken green autofluorescence of the cell wall.

4. DISCUSSION

Red-tide organisms of the class Raphidophyceae were not observed before 1991 in the Wadden Sea, the open North Sea, and the Delta area. Based on the morphology of Raphidophyceae, described by Hara & Chihara (1982, 1985) and summarized in Fukuyo *et al.* (1990), the following three species were identified in the samples: *Chattonella marina* (Subrahmanyan) Hara et Chihara, *C. antiqua* (Hada) Ono and *Fibrocapsa japonica* Toriumi et Takano (Fig. 2). The identification of *Olisthodiscus luteus* Carter remains uncertain, because this species was observed only once. Another member of the class, the apparently cosmopolitan *Heterosigma carterae* (Hulburt) Taylor (synonym *H. akashiwo*), which can easily be misidentified as *O. luteus* (Taylor *et al.*, 1993), has not been observed yet in samples of the monitoring programme.

The distinction between raphidophytes, and especially between the potentially neurotoxic species *C. marina* and *C. antiqua*, is difficult. However, in *F. japonica* the presence of numerous mucocysts at the posterior site of the cell is very clear (Figs 2E and 2F). Previous reports of Hiroishi *et al.* (1988), Nagasaki *et al.* (1989), and Billard (1992) mention that sampling and fixation affect cell morphology, so cells of different species may appear to be identical by optical microscopic examination. Cell size measurements revealed that the cells of *Chattonella* spp. in our cultures and in fixed field samples from Dutch coastal waters were somewhat smaller than those observed in Japan. The cells identified as *C. antiqua* had the morphology described by Ono & Takano (1980), but may have been transformed into an obovoid cell type

due to the absence of a rigid cell wall (Hiroishi *et al.*, 1988; Nagasaki *et al.*, 1989) and become almost identical to *C. marina*. According to the immunofluorescence-assay (Table 1), however, no, or occasionally very weak, labelling was observed for obovoid cells using the AT-86 monoclonal antibody.

Use of the species-specific monoclonal antibody MR-18 against cell surface antigens of *C. marina* (Hiroishi *et al.*, 1988; Uchida *et al.*, 1989) supported the microscopical identification of this species. The fluorescence of the cell surface of Lugol-fixed samples was only evident after the enhanced labelling described by Vrieling *et al.* (1993a), and even so at lower fluorescence intensities. Low fluorescence intensities must be expected, as has been concluded in previous studies of samples preserved with Lugol (Anderson *et al.*, 1990; Bates *et al.*, 1993; Vrieling *et al.*, 1994); enhanced labelling improves the fluorescence intensity significantly as a result of an increase in the amount of fluorescent molecules per antibody (Vrieling *et al.*, 1993a). The decrease of the antigen binding capacity of the monoclonal AT-86 due to Lugol fixation may have caused the failure of identifying *C. antiqua* in field samples immunochemically. Yellowish-green fluorescence of cell walls of small heterotrophic dinoflagellates in the field samples cannot be ascribed to cross-reaction with the antibodies; it is an autofluorescent phenomenon (Tsuji & Yanagati, 1981; Shapiro *et al.*, 1989; Vrieling *et al.*, 1994).

Raphidophycean cell numbers were not very abundant in samples of Dutch coastal waters, in contrast with high counts in the shallow seas in south east Asia (Imai, 1990; Nakamura & Umemori, 1991; Tseng *et al.*, 1993), Narragansett Bay (Smayda & Villareal, 1989), and the coastal waters of New Zealand (Chang *et al.*, 1990). However, when environmental conditions are optimal a few cells may initiate a bloom. Remarkably, at station T100 in the southern central North Sea (Fig. 1), a minor bloom, which was typically restricted to this area, was recorded for *C. marina* with concentrations over 10^5 cells·dm^{-3} in May 1993. Later that year, in June, high cell numbers of over 10^4 cells·dm^{-3} were recorded for the Dutch Wadden Sea at station ED250 (Fig. 1).

The 'photoactive concentration method' applied to enumerate *F. japonica* is not reproducible, but it gives a good impression of the amount of motile cells in the samples responding to light after light inhibition. Because this species could be identified clearly only in live samples and after isolation, the actual abundance at the stations and its seasonal variation must remain uncertain. In fixed samples, cells could not be counted properly due to aggregation, probably as a consequence of the stickiness of extruded mucocysts. Aggregation was noticed when cultured cells were fixed for optical and electron microscopical examination.

C. marina and *F. japonica* sampled in European waters have until now not been demonstrated to be toxic. Recent analytical studies revealed that biotoxins produced by *C. marina*, *Gymnodinium* spp., and *Cochlodinium* spp. (Onoue & Nozawa, 1989; Onoue

TABLE 1

Results of the IF-assay, using monoclonals MR-18 (*C. marina*) and AT-86 (*C. antiqua*), performed on samples containing *Chattonella* cells. IF-reactivity is expressed as (+), (±), and (-) for positive, weak and no labeling respectively.

station	*date* (m/d/y)	*location*	*latitude*	*longitude*	*Chattonella spp.* (cells·dm^{-3})	*IF-reactivity* MR-18	AT-86
W030	05/11/92	Wadden Sea	52°59'01"	04°45'01"	> 4000	+	-
	06/26/92				> 2000	+	-
	07/28/92				> 1800	+	-
W420	05/13/92	Wadden Sea	53°24'00"	05°43'17"	> 2000	+	-
	07/13/92				> 2000	+	-
	28/09/93				> 1300	+	-
W590	04/13/92	Wadden Sea	53°27'13"	06°30'52"	> 6000	+	-
	08/24/92				> 1000	+	-
	08/10/92				> 1000	+	-
	06/29/93				<10000	+	-
E250	05/13/92	Ems-Dollard	53°33'37"	06°39'42"	> 3000	+	-
	05/26/92				> 4600	+	-
	09/23/92				> 2000	+	-
	06/15/93				> 4800	+	±
T100	03/31/93	Central North Sea	54°08'58"	04°20'31"	< 500	+	-
	04/15/93				> 2000	+	-
	05/05/93				>250000	+	-
	05/13/93				< 200	+	-
T175	08/23/92	Central North Sea	54°43'09"	03°41'30"	> 1000	-	±

et al., 1990) are closely related to the brevetoxins of *Gymnodinium breve* (Syn. *Ptychodiscus brevis*, see Baden & Mende, 1982). Cultures should be started of more isolates from Dutch coastal waters to determine toxin content, production, and composition, as has been done in Japanese strains (Onoue & Nozawa, 1989; Onoue *et al.*, 1990).

As has been mentioned already, species of the class Raphidophyceae were never observed before 1991 because they were simply not present or could not be recognized properly (as a result of morphological changes following fixation or too low cell numbers). Harmful events caused by raphidophycean toxicity have not recorded in the Netherlands yet, but an outbreak cannot be excluded because the species discussed here can potentially be present each year. Except in summer, the temperature of Dutch coastal waters (<18°C) is well below the temperature at which Raphidophyceae occur as red tides in Japanese coastal waters, where about 16°C is the minimum (Imai, 1990). During warm periods the same conditions prevail in the Dutch Wadden Sea and the estuary south of the river Rhine. Strains adapted to the cooler environment of the North Sea may also be present. As to the salinity tolerance, Iwasaki (1971) showed that optimal growth occurred at salinities varying from S=11 to 20, which is in the same range as measured in the Dutch Wadden Sea and the estuary south of the river Rhine. The small bloom of *Chattonella* in May 1993 in the south of the central North Sea at a higher salinity (S=25 to 28) was therefore not expected.

Even cysts of Raphidophyceae may be present in Dutch coastal waters. Investigations of morphology, physiology, and ecology of cysts of *Chattonella* spp. and *F. japonica* have revealed that encystment takes place frequently whenever environmental conditions are unfavourable for 'normal' growth (Yoshimatsu, 1987; Imai, 1990; Nakamura & Umemori, 1991; Yamaguchi & Imai, 1994). Encystment-stimulating factors such as nutrient depletion, the presence of solid surfaces for cyst adhesion and low light intensities occasionally occur in Dutch coastal waters. *C. marina* and *C. antiqua* have a diplontic life cycle in which smaller pre-encystment cells are observed besides cysts (Yamaguchi & Imai, 1994). These cells and cysts are not known from Dutch coastal waters, maybe for lack of an adequate sampling scheme.

We suggest continuation of the combined immunochemical and microscopical biomonitoring approach presented here. First, it allows the collection of well-defined isolates of the different Raphidophyceae to investigate environmental conditions inducing toxicity in the laboratory and the phylogenetic relationship with Raphidophyceans observed elsewhere. Second, bloom development can be followed from the earliest stage; thus chemical, physical, and hydrographical factors causing the bloom can be revealed. Events of toxic marine phytoplankton occurrence often take us by surprise, because the period before the outbreak of the bloom has not been sampled. We are now able to detect the presence of cells at very low numbers in mixed phytoplankton populations by the immunofluorescence-assay. In addition, the presence of cysts of *Chattonella* spp. and *F. japonica* should be studied to identify and register survival stages in Dutch coastal waters.

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5. REFERENCES

Anderson, D.M., D.M. Kulis & E.M. Cosper, 1990. Immunofluorescent detection of the brown tide organism, *Aureococcus anophagefferens*. In: E.M. Cosper, V.M. Bricelj & E.J. Carpenter. Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Springer Verlag, Berlin: 213-228.

Baden, D.G. & T.J. Mende, 1982. Toxicity of two toxins from the Florida red tide marine dinoflagellate, *Ptychodiscus brevis*.—Toxicon **20:** 457-461.

Bates, S.S., C. Léger, B.A. Keafer & D.M. Anderson, 1993. Discrimination between domoic-acid-producing and non-toxic forms of the diatom *Pseudonitzschia pungens* using immunofluorescence.—Mar. Ecol. Prog. Ser. **100:** 185-195.

Billard, C., 1992. *Fibrocapsa japonica* (Raphidophyceae), algue planctonique nouvelle pour les côtes de France.—Cryptogamie Algol. **13:** 225-231.

Chang, F.H., C. Anderson & N.C. Boustead, 1990. First record of a *Heterosigma* (Raphidophyceae) with associated mortality of cage reared salmon in Big Glory Bay, New Zealand.—N. Z. J. mar. Freshwat. Res. **24:** 461-469.

Doi, A., O. Hatase, M. Shimida, T.H. Murakami & T. Okaichi, 1981. Ultrastructural changes in gill epithelia of a yellowtail, *Seriola quinqueradiata*, exposed to sea bloom.—Cell Struct. Func. **6:** 375-383.

Endo, M., T. Sakai & A. Kuroki, 1985. Histological and histochemical changes in gills of the yellowtail *Seriola quinqueradiata* exposed to the Raphidophycean flagellate *Chattonella marina*.—Mar. Biol. **87:** 193-197.

Endo, M., Y. Onoue & A. Kuroki, 1992. Neurotoxin-induced cardiac disorder and its role in the death of fish exposed to *Chattonella marina*.—Mar. Biol. **112:** 371-376.

Fukuyo, Y., H. Takano, M. Chihara & K. Matsuoka, 1990. Red tide organisms in Japan. An Illustrated taxonomic guide. Uchida Rokakuho, Tokyo: 1-430.

Guillard, R.R.L. & J.H. Ryther, 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve).—Gran. Can. J. Microbiol. **8:** 229-239.

Hara, Y. & M. Chihara, 1982. Ultrastructure and taxonomy of *Chattonella* (Class Raphidophyceae) in Japan.—Jap. J. Phycol. **30:** 47-56.

——, 1985. Ultrastructure and taxonomy of *Fibrocapsa*

japonica (Class Raphidophyceae).—Arch. Protistenk. **130:** 133-141.

Hiroishi, S., A. Uchida, K. Nagasaki & Y. Ishida, 1988. A new method for identification of inter- and intra-species of the red tide algae *Chattonella antiqua* and *Chattonella marina* (Raphidophyceae) by means of monoclonal antibodies.—J. Phycol. **24:** 442-444.

Imai, I., 1990. Physiology, morphology, and ecology of cysts of *Chattonella* (Raphidophyceae), causative flagellates of noxious red tides in the inland sea of Japan.— Nansei Bull. Nat. Fish. Res. Inst. Japan **23:** 62-166 (in Japanese).

Ishimatsu, A., H. Maruta, T. Tsuchiyama & M. Ozaki, 1990. Respiratory, ionoregulatory and cardiovascular responses of the yellowtail *Seriola quinqueradiata* to exposure to the red tide plankton *Chattonella*.—Nippon Suisan Gakk. **56:** 189-199.

Iwasaki, H., 1971. Studies on the red tide flagellates. VI. On *Eutreptiella* spp. and *Exuviaella* spp., appeared in Bingo-Nada, the Seto Inland Sea, in 1970.—J. Oceanogr. Soc. Japan **27:** 152-157.

Kat, M., 1985. *Dinophysis acuminata* blooms, the distinct cause of mussel poisoning. In: D.M. Anderson, A.W. White & D.G. Baden. Toxic dinoflagellates. Elsevier, New York: 95-100.

Larsen, J. & Ø. Moestrup, 1989. Guide to toxic and potentially toxic marine algae. The Fish Inspection Service, Ministry of Fisheries, Copenhagen, Denmark: 1-61.

Leewis, R.J., 1985. Phytoplankton off the Dutch coast. A baseline study on the temporal and spatial distribution of species in 1974 and 1975. Ph.D. Thesis, University of Nijmegen, The Netherlands. Van Gorcum Press, Assen: 1-147.

Loeblich, A.R. III & K. Fine, 1977. Marine Chloromonads: more widely distributed in neritic environments than previously thought.—Proc. Biol. Soc. Wash. **90:** 388-399.

Matsusato, T. & H. Kobayashi, 1974. Studies on death of fish caused by red tide.—Bull. Nansei reg. Fish Res. Lab. **7:** 43-67 (In Japanese).

Nagasaki, K., A. Uchida, S. Hiroishi & Y. Ishida, 1989. Identification of red tide algae *Chattonella marina* and *Chattonella antiqua* by means of monoclonal antibodies. In: S. Miyachi, I. Karube & Y. Ishida. Current topics in marine biotechnology. Fuji Technology Press Ltd., Tokyo: 161-164.

Nakamura, Y., 1983. Growth characteristics of *Chattonella antiqua* (Raphidophyceae). Part I: Effects of temperature, salinity, light intensity, and pH on growth.—J. Oceanogr. Soc. Japan **39:** 110-114.

Nakamura, Y. & T. Umemori, 1991. Encystment of the red tide flagellate *Chattonella antiqua* (Raphidophyceae): Cyst yield in batch cultures and cyst flux in the field.—Mar. Ecol. Prog. Ser. **78:** 273-284.

Ono, C. & H. Takano, 1980. *Chattonella antiqua* (Hada) comb. nov., and its occurrence on the Japanese coast.—Bull. Tokai Reg. Fish. Res. Lab. **102:** 93-100.

Onoue, Y. & K. Nozawa, 1989. Separation of toxins from harmful red tides occurring along coast of Kagoshima prefecture. In: T. Okaichi, D.M. Anderson & T. Nemoto. Red tides, biology, environmental science, and toxicology. Elsevier Science Publ., New York: 371-374.

Onoue, Y., M.S. Haq & K. Nozawa, 1990. Separation of neurotoxins from *Chattonella marina*.—Nippon Suisan Gakk. **56:** 695.

Peperzak, L., J.F. Lawrence & P. Hagel, 1994. *Mytilus edulis* as a biomonitor for the potentially toxic dinoflagellates *Alexandrium*, *Dinophysis*, and *Gyrodinium aureolum* in the Central North Sea. In: P. Lassus, G. Arzul, E. Erard, P. Gentien & C. Marcaillou. Harmful marine algal blooms. Lavoisier Sci. Publ., Paris: in press.

Reid, P.C., C. Lancelot, W.W.C. Gieskes, E. Hagmeier & G. Weichart, 1990, Phytoplankton of the North Sea and its dynamics: A review.—Neth. J. Sea Res. **26:** 295-331.

Sako, Y., M. Adachi & Y. Ishida, 1993. Preparation and characterization of monoclonal antibodies to *Alexandrium* species. In: T.J. Smayda & Y. Shimizu. Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam: 87-93.

Shapiro, L.P., E.M. Haugen & E.J. Carpenter, 1989. Occurrence and abundance of green-fluorescing dinoflagellates in surface waters of the Northwest Atlantic and Northeast Pacific Oceans.—J. Phycol. **25:** 189-191.

Smayda, T. & T. Villareal, 1989. The 1985 'brown-tide' and the open phytoplankton niche in Narragansett Bay during summer. In: E.M. Cosper, V.M. Bricelj & E.J. Carpenter. Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Coastal Estuar. Studies 35. Springer-Verlag, Berlin: 165-187.

Taylor, F.J.R., R. Haigh & T.F. Sutherland, 1993. Phytoplankton ecology of Sechelt Inlet, a fjord system on the British Columbia coast. II. Potentially harmful species.—Mar. Ecol. Prog. Ser. **103:** 151-164.

Tomas, C.R., 1981. The influence of grazing by two *Acartia* species on *Olisthodiscus luteus* Carter.—Mar. Biol. **72:** 79-87.

Toriumi, S. & H. Takano, 1973. *Fibrocapsa*, a new genus in Chloromonadophyceae from Atsumi Bay, Japan.—Bull. Tokai Regional Fish. Res. Lab. **76:** 25-35.

Toyoshima, T., H.S. Ozaki, M. Shimada, T. Okaichi & T.H. Murakami, 1985. Ultrastructural alterations on chloride cells of the yellowtail *Seriola quinqueradiata* following exposure to the red tide species *Chattonella marina*.—Mar. Biol. **88:** 101-108.

Tseng, C.K., M.J. Zhou & J.Z. Zou, 1993. Toxic phytoplankton studies in China. In: T.J. Smayda & Y. Shimizu. Toxic phytoplankton blooms of the sea. Elsevier, Amsterdam: 347-352.

Tsuji, T. & T. Yanagati, 1981. Improved fluorescent microscopy for measuring the standing stock of phytoplankton including fragile components.—Mar. Biol. **64:** 207-211.

Uchida, A., K. Nagasaki, S. Hirioshi & Y. Ishida, 1989. The application of monoclonal antibodies to and identification of *Chattonella marina* and *Chattonella antiqua*.—Nippon Suisan Gakk. **55:** 721-725.

Verity, P.G. & D. Stoecker, 1982. Effects of *Olisthodiscus luteus* on the growth and abundance of tintinnids.—Mar. Biol. **72:** 79-87.

Vrieling, E.G., A. Draaijer, W.J.M. van Zeijl, L. Peperzak, W.W.C. Gieskes & M. Veenhuis, 1993a. The effect of labelling intensity, estimated by real time confocal laser scanning microscopy, on flow cytometric appearance and identification of immunochemically labelled marine dinoflagellates.—J. Phycol. **29:** 180-188.

Vrieling, E.G., W.W.C. Gieskes, F. Colijn, J.W. Hofstraat, L. Peperzak & M. Veenhuis, 1993b. Immunochemical identification of toxic marine algae: first results with *Prorocentrum micans* as a model organism. In: T.J.

Smayda & Y. Shimizu. Toxic phytoplankton blooms of the sea. Elsevier, Amsterdam: 925-931.

Vrieling, E.G., L. Peperzak, W.W.C. Gieskes & M. Veenhuis, 1994. Detection of the ichthyotoxic dinoflagellate *Gyrodinium* (cf.) *aureolum* and morphologically related *Gymnodinium* species using monoclonal antibodies: a specific immunological tool.—Mar. Ecol. Prog. Ser. **103:** 165-174.

Yamaguchi, M. & I. Imai, 1994. A microfluorometric analysis of nuclear DNA at different stages in the life history of *Chattonella antiqua* and *Chattonella marina* (Raphidophyceae).—Phycologia **33:** 163-170.

Yoshimatsu, S., 1987. The cysts of *Fibrocapsa japonica* (Raphidophyceae) found in bottom sediment in Harima-Nada, Eastern Inland Sea of Japan.—Bull. Plankton Soc.Japan **34:** 25-31.

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