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# Temperature responses of three *Fibrocapsa japonica* strains (Raphidophyceae) from different climate regions

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*The harmful bloom alga Fibrocapsa japonica has a worldwide distribution in temperate regions and is occasionally responsible for mass mortality of fish. Little is known about requirements for optimal growth and survival of this species, especially about temperature constraints that define natural distribution. Therefore, we studied thermal traits in three Fibrocapsa strains from different climate regions. All strains were eurythermal and viable between 4 and 32°C, explaining their presence in temperate regions. Some differences in temperature response among the strains were observed, not only for growth rate but also for biovolume and net production. The implication of the observed responses was evaluated by translating growth performance of strains in the laboratory to potential performance in the natural habitats. Only the Japanese strain seemed to be well adapted to its environment, while the New Zealand strain exhibited growth and survival over a much broader temperature range, despite the small temperature fluctuations in its habitat. Interestingly, the German Wadden Sea strain encounters lethal temperatures in winter and must have a resting stage, able to survive temperatures <4°C, to explain its occurrence in this region. However, in general, the responses of the three F. japonica strains in culture were in good agreement with the observed seasonal occurrence in the field.*

## INTRODUCTION

The unicellular golden brown flagellate *Fibrocapsa japonica* Toriumi and Takano is one of the harmful marine algal bloom (HAB) species of the class Raphidophyceae (Hara and Chihara, 1985) and has been held responsible for mass mortality events of economically important fish stocks and other marine wildlife (Iwasaki, 1971; Okaichi, 1972; Toriumi and Takano, 1973; Okaichi, 1989). *Fibrocapsa japonica* toxicity has been attributed to various causes. There is the possibility that *F. japonica* cells eject their characteristic mucocysts, causing mucus threads to clog fish gills. *Fibrocapsa japonica* also appears to be able to produce neurotoxins (Khan *et al.*, 1996b). In addition, it produces reactive oxygen species (ROS) (Oda *et al.*, 1997) responsible for gill-tissue injury, resulting in asphyxia (Ishimatsu *et al.*, 1996). *Fibrocapsa japonica* produces haemolytic compounds too, which could be involved in the ichthyotoxicity of the species (Marshall *et al.*, 2003; Fu *et al.*, 2004, in press). It is still unknown what effect or combination of effects kills the fish.

*Fibrocapsa japonica* has a global distribution (Fig. 1; Table I), being mainly present in coastal warm and cold temperate regions (Lüning, 1990). So far, the species has never been reported from the Arctic and Antarctic regions. The chronology of the first records (Table I) may suggest a recent range expansion possibly via discharge of ship ballast water or introduction with mariculture species (Hallegraeff, 1993; Scholin *et al.*, 1995; Hayes, 1998; Nehring, 1998). Alternatively, *F. japonica* may not have expanded its distribution, but the species could have been overlooked for quite some time (Billard, 1992), and first sightings could reflect the scientific efforts made rather than its first arrival on the investigated sites. It seems unlikely that *F. japonica* cells could have been detected in the preserved samples of phytoplankton records or that their could have been remains which could be detected in

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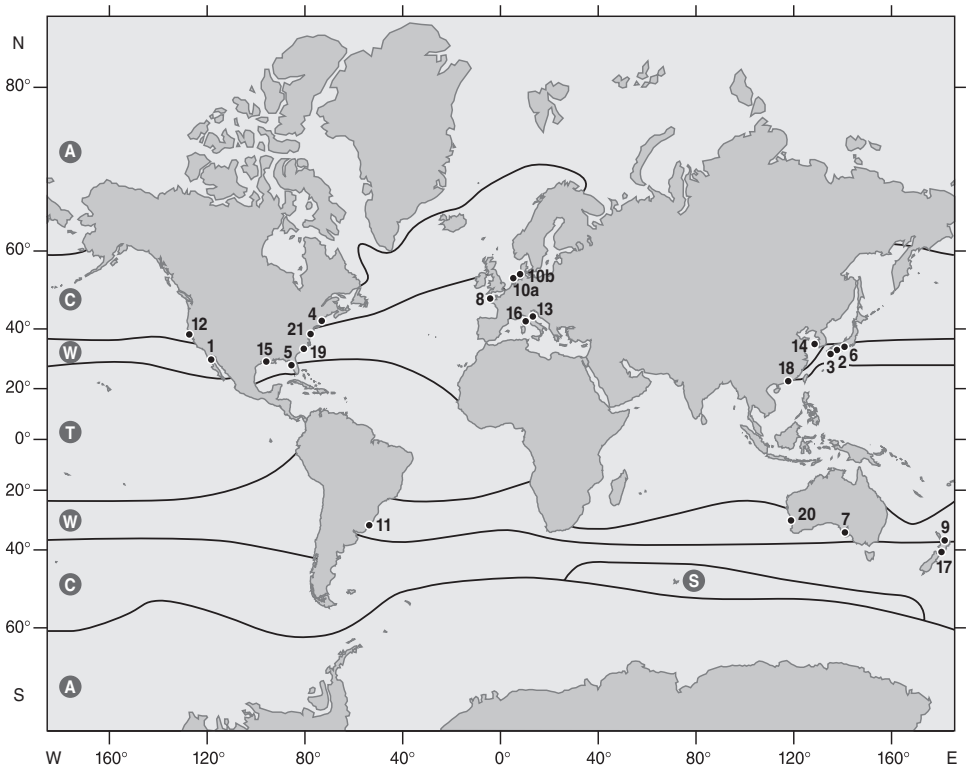


**Fig. 1.** Distribution of *Fibrocapsa japonica*: with observations since the 1970s for the North Pacific Ocean, since the 1980s for Australia and the North East Atlantic Ocean, since the 1990s at New Zealand, the North West Atlantic Ocean and the South East Atlantic Ocean and since the end of the 1990s in the Mediterranean Sea. The numbers correspond to data summarized in Table I. Seven groups of biogeographic regions are shown: **A**, Arctic, Antarctic region; **C**, cold temperate region Northern and Southern Hemisphere; **W**, warm temperate region Northern and Southern Hemisphere; **T**, tropical region; **S**, sub-Antarctic cold temperate island region (After Lüning, 1990).

fossil data. *Fibrocapsa japonica* lacks a cell wall, and therefore the cells are fragile and hard to preserve (Billard, 1992; Vrieling *et al.*, 1995; Rademaker *et al.*, 1998), although cysts may stay intact (De Boer *et al.*, in press).

A way to understand the natural distribution and bloom formation of *F. japonica* is to study its response to temperature, which is shown to be one of the most important factors to define the natural distribution of both terrestrial plants (Larcher and Wieser, 1995) and seaweeds (Lüning, 1990; Van den Hoek *et al.*, 1990). The temperature tolerance of a species is the result of both evolutionary imprints and recent adaptation (Breeman *et al.*, 2002). For seaweeds, it has been demonstrated that experimentally determined temperature limits for survival, growth and reproduction may set biogeographic boundaries (Van den Hoek, 1982; Breeman, 1988) and explain large-scale biogeographical distribution patterns (Cambridge *et al.*, 1990; Pakker *et al.*, 1995; Pakker and Breeman, 1996; Orfanidis and Breeman, 1999). By comparing these temperature limits with sea-surface isotherms at the boundaries of distribution (US Navy, 1981; Lüning, 1990), it is possible to determine which responses are likely to be responsible for delimiting the distribution range (Pakker, 1995). A study on growth responses to temperature of several diatom species isolated from various temperature environments suggests that temperature may also control the distributions of microalgae (Suzuki and Takahashi, 1995). In the HAB species *Gymnodinium catenatum* Graham, temperature was found to determine the geographical range of its different ecotypes (Hallegraeff and Fraga, 1998).

To expand the very limited knowledge on the ecophysiology of *F. japonica*, we studied the temperature responses of three strains of this species. The strains originating from very different climate regions (German Wadden Sea, Western Europe; Seto Inland Sea, Japan; and Wellington Harbour, New Zealand). The effect of temperature was not only analysed on growth and survival but also on motility and morphology, because these are known to depend on abiotic factors in *F. japonica* and other raphidophytes (Khan *et al.*, 1998; Marshall and Hallegraeff, 1999). Furthermore, biovolume of the cell and net production rates were measured to test for temperature dependency. The observed temperature

*Table I: First sightings of Fibrocapsa japonica (the numbers correspond to Fig. 1)*

| Number | Date | Place | Reference |
|---|---|---|---|
| 1 | 10 July 1970 | Point Loma, San Diego, California, USA | Loeblich and Fine (1977) |
| 2 | August 1970 | Bingo Nada, Seto Inland Sea, Japan | Iwasaki (1971) |
| | 19 July 1978[a] | Tsuda Bay, Kagawa (Ken), Seto Inland Sea, Japan | NIES, Japan |
| 3 | 27 October 1972 | Atsumi Bay, Aichi-Ken, Japan | Toriumi and Takano (1973) |
| 4 | 1985 | Rhodes Islands, USA | Smayda and Villareal (1989) |
| 5 | May–June 1986 | Tampa Bay, Florida, USA | Tomas (1998) |
| 6 | May 1987 | Hasaki (Ibaraki-Ken), Japan | NIES, Japan |
| 7 | 7 January 1988 | Port Philip Bay, Hobsons Bay, Victoria, Australia | Bigelow, USA |
| 8 | 16 October 1991 | Luc-sur-mer, Calvados, Normandy, France | Billard (1992) |
| 9 | 23 October 1991 | West Coromandel Coast, New Zealand | Rhodes *et al.* (1993) |
| 10a | 1991 | Dutch coastal waters, The Netherlands | Vrieling *et al.* (1995) |
| 10b | 1992 | Wadden Sea, Germany | ICES-IOC HAEDAT |
| | 1995[a] | Büsum Harbour, Germany | Rademaker *et al.* (1998) |
| 11 | 4 May 1995 | Patos Lagoon, Brazil | Odebrecht and Abreu (1995) |
| 12 | November 1996 | Monterey Bay, California, USA | O'Halloran *et al.* (2002) |
| 13 | 1997 | Ancona, Adriatic Sea (Mediterranean Sea), Italy | ARPAM (2001) |
| 14 | 1997 | Kamak Bay, Korea | Lee *et al.* (2001) |
| 15 | 1998 | Corpus Christi, Texas, USA | C. R. Tomas, Wilmington, personal communication |
| 16 | August 1999 | Mare di Sabaudia, Thyrrenian Sea (Mediterranean Sea), Italy | Congestri *et al.* (2000) |
| 17 | 8 October 1999[a] | Wellington Harbour, New Zealand | Cawthron Institute, New Zealand |
| 18 | 1999 | Hong Kong, China | Wong (2002) |
| 19 | 21 May 2001 | Hilton Head Pont, South Carolina, USA | Lewitus *et al.* (2002) |
| 20 | May 2001 | Perth, Western Australia | J. Cosgrove, East Perth, personal communication |
| 21 | 2001 | Delaware Coastal Bay, Delaware, USA | E. B. Whereat, Lewes, personal communication |

The areas were defined by latitude and longitude and were limited by a landmass or by a difference in latitude or longitude of more than 3° from other sightings.
[a]Isolation date and place of the clonal strains used for this study.

responses of the strains were compared with temperature regimes at the collection sites in order to find possible explanations for the natural distribution, seasonal development and bloom formation of *F. japonica*.

## METHOD

### Algal cultures

Three clonal strains of *F. japonica*, originating from different climate regions (Fig. 1; Table I), were used for comparative studies. The German Wadden Sea strain, CCRuG-Cl3 (Culture Collection University of Groningen, the Netherlands), was originally established from a sample of a monospecific bloom taken from Büsum harbour in 1995 (U. Tillmann, Bremerhaven, personal communication), and the clonal culture CCRuG-Cl3 was established in 1997. The Japanese strain, CS-332 (CSIRO, Australia), was separated in 1993 from NIES-136 isolated in 1978 (National Institute of Environmental Studies, Japan). Finally, the New Zealand strain CAWR19 (Cawthron Institute, New Zealand) was chosen for the experiments.

All strains were cultured in autoclaved seawater enriched with f/2-Si nutrients (Guillard, 1975). Cultures were maintained under long-day (16:8 h L:D) conditions, at a salinity of 25 and a photon flux density of $35 \pm 5$ μmol photons $m^{-2}$ $s^{-1}$ (provided by fluorescent tubes; L36W/19 light, Osram, Germany and 20W/33RS, Philips, The Netherlands). The irradiance was measured with a Li-Cor quantum meter equipped with a cosine collector. Stock batch cultures were maintained in 1 L Erlenmeyer flasks at $18 \pm 1$°C. Before each experiment, cultures were precultured for at least two generations in exponential phase at 18°C in order to ensure that cells were in an active physiological state. No differences in pH and salinity were detected during the experiments.

### Morphology

Possible morphological changes in response to low temperatures were studied with an IMT-2 Inverted

Research Microscope (Olympus T041) by comparing control cultures at 18°C with cultures kept at 3–8°C for 9 days. An MX5 video camera (AIS Imput System) recorded images that were processed using the computer program Optimas 5.2. Short-term effects (within 30 min) of increasing temperature (≥32°C) were monitored using a Zeiss-Axioscope microscope with a phase contrast filter. Pictures were taken with a charged-couple device (CCD) camera (Princeton Instruments Inc.).

## Growth experiments

Growth experiments were carried out in waterbaths at the temperatures of 6, 9.5, 12.5, 16.5, 21, 24.5, 27.5, 29, 32 and 35 ± 1°C. Inoculated cultures (~1300 cells $mL^{-1}$) of exponentially growing cells pre-cultured at 18°C were gradually brought to the experimental temperatures (stepwise 4°C per 12 h) in order to avoid potential harmful effects of abrupt temperature shifts. At each temperature, the three strains were grown at least in triplicate. Before sampling, cultures were gently swirled; after sampling they were placed back randomly in the waterbaths. Every 2 days, 2 h after the end of the dark period, cell numbers and biovolume per cell were determined using a particle counter (Coulter Counter ZM equipped with a channelyzer 256 and a 100 μm aperture, Coulter Electronics Ltd, Luton, UK). When the cell density was too high for accurate measurements, samples were diluted in 0.2 μm filtered artificial seawater. Four replicate counts were made at each sampling time. Analysis took place within 10 min. Particle size was calibrated with latex particles 8.7 μm in diameter (Coulter Electronics Ltd, Luton, UK).

The particle-counter measurements were first checked with microscopic observations on cell density in the same samples ($n$ = 8). Cell numbers of living cells were counted in a Sedgwick-Rafter counting chamber. The particle-counter readings of biovolume per cell in an exponentially growing culture were compared with calculated biovolumes based on measurements of diameter and height ($n$ = 200 cells) using a microscope. Assuming *F. japonica* to be a prolate spheroid (Hillebrand *et al.*, 1999), biovolume was calculated as:

$$V = \frac{\pi}{6} d^2 h \quad (1)$$

where $d$ is the diameter and $h$ the height, with $h > d$. The volume distribution was not completely even, and to avoid bias through extreme data points, we chose the median biovolume per cell as the parameter. No differences in median biovolume per cell were detected between the microscope and particle-counter measurements.

Growth rates (μ) were calculated from a regression on at least four data points in the exponential phase of growth using the following equation:

$$N_t = N_0 e^{(\mu,t)} \quad (2)$$

where $N_t$ and $N_0$ are cell concentrations at time $t$ and 0 (in days) respectively.

Every day, before sampling, cultures were classified as non-motile, non-motile/motile and motile. Mucus production during the motile phase was recorded by observing the presence of mucus threads and aggregation of cells.

The net production of *F. japonica* was calculated as the product of cell volume and the specific net daily change in abundance (i.e. net cell volume per day: $\mu m^3\ d^{-1}$) (Weisse and Montagnes, 1998).

## Viability experiment

Viability (ability to recover from a temperature treatment) was determined over a broad range of temperatures: 2, 4, 6, 8, 10, 12, 17, 21, 24, 28, 30, 32 and 35 ± 1°C. Before the experiment was started, the pre-cultures kept at 18°C were grown to a density of at least 5000 cells $mL^{-1}$. Cultures were then brought directly to the experimental temperatures where they were incubated for up to 15 days, and every day the motility of the cells and aggregate formation were recorded by visual examination. Stirring of cultures was avoided, and after daily inspection they were placed back randomly at their fixed temperature. Viability experiments were done at least in triplicate. After 8 days of treatment at different temperatures, *F. japonica* cells of each culture were checked for viability by transferring samples 1:1 into a new medium and bringing them stepwise (<4°C per 12 h) to 18 ± 1°C. Viability was checked by observing the presence of swimming cells by visual inspection over the following month.

## Statistical analysis

Differences in growth rate, biovolume and net production among strains and temperatures were tested with ANOVA. Significant strain–temperature interactions were further analysed with single-factor ANOVA (i.e. effect of strain at each temperature and effect of temperature on each strain), followed by Scheffe's posthoc tests. Levene's test of homogeneity of variances was performed to check the assumptions of parametric statistics, and untransformed data were used in the final analysis when different scales of transformation did not achieve normalization (Underwood, 1997). All statistical tests and analyses were performed in accordance with Sokal and Rohlf (Sokal and Rohlf, 1995) using SPSS for Windows version 11.0 (2001).

# RESULTS

## Cell morphologies at different temperatures

Microscopic observations showed no morphological differences among the three *F. japonica* strains grown at the control temperature (18°C). All strains had mainly prolate spheroid cells (Fig. 2a) with two unequal flagellae emerging from the anterior end of the cell: one trailing and one anterior flagellum. These flagellae are not visible in Fig. 2a because of their rapid motion, but the nucleus, chloroplasts and the mucocysts were clearly visible in the cell. The many rod-shaped mucocysts were located in the posterior part of the cell. At 3°C all cells of *F. japonica* lysed, and at 8°C most of the cells were spherical, with shorter flagellae than usually seen in vital cultures at the control temperature (18°C). At low temperatures (<8°C) as well as high temperatures (≥32°C), fast degradation of flagellae was observed. Different morphologies were also seen when a culture was exposed to increasing temperatures. When temperature increased, the motility of the cells stopped, the flagellae contracted and the apical part crumbled off (Fig. 2b and c), while a thickened remnant remained (Fig. 2d), which was lost later. During the degradation of the flagellae, the shape of the cells became spherical (Fig. 2b and c). In the course of the experiment, the plasmalemma of the cell became less smooth (Fig. 2d). Shortly before cell lysis, the cell distended (Fig. 2e). Before complete cell lysis, the mucocysts ejected threads with a length of a few hundred micrometers (Fig. 2f–h).

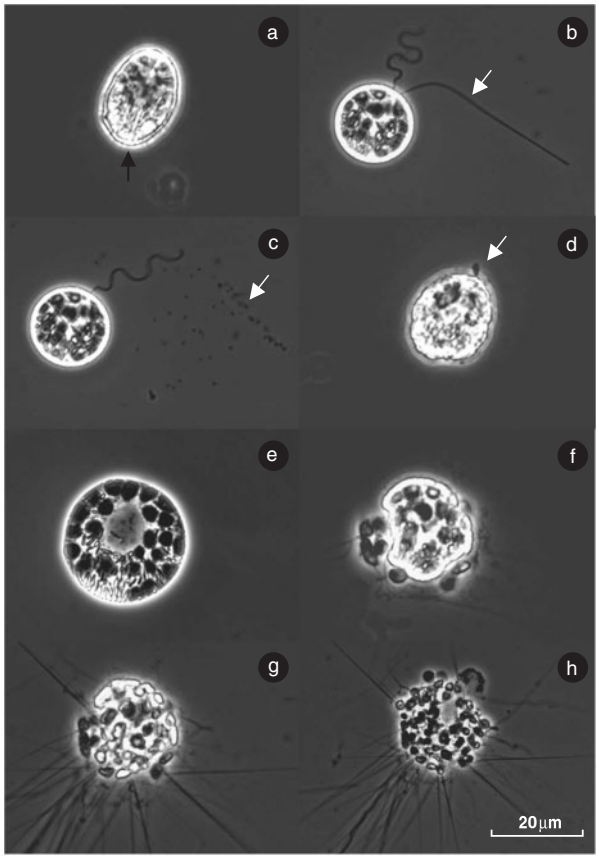


**Fig. 2.** Change in cell morphology of *Fibrocapsa japonica* caused by increasing temperature. All pictures, except **a**, **d** and **e**, are from the same *F. japonica* cell. White arrows point to the flagellum, and black arrows point to mucocysts. Cells are positioned with the anterior end of the cell top right and the posterior end down left.

## Growth experiments

Analysis of variance on growth rate ($\mu_{max}$) showed a significant strain–temperature interaction ($F_{14,91}$ = 21.075, $P$ < 0.001). Single-factor ANOVAs showed significant effects of temperature in each strain, and at each temperature significant differences among strains were detected (Table II). The strains from the German Wadden Sea and Japan did not grow at temperatures ≤9.5°C and ≥32°C (Fig. 3). No growth was observed in the New Zealand strain at ≤6°C and ≥35°C. Therefore, the New Zealand strain had a broader temperature range for growth (9.5–32°C) than the German Wadden Sea and the Japanese strains (12.5–29°C). In all strains, the temperature sustaining maximal growth rates was much closer to the upper than to the lower temperature limit for growth (Fig. 3).

Optimum temperatures for growth were between 21 and 24.5°C in all strains (Table II: bold letters). High growth rates were also observed at 29°C in the New Zealand strain. Compared to the two other strains, the German Wadden Sea strain consistently showed the highest growth rates between 12.5 and 29°C (Fig. 3), and values were significantly higher than in one or both other strains (Table II: posthoc strain). In the optimum temperature range of 21–24.5°C, the Japanese strain grew faster than the New Zealand strain (Fig. 3; Table II). At suboptimal (12.5°C) and supraoptimal (29°C) temperatures, no differences in the growth rates for the German Wadden Sea and the New Zealand strain were observed, but the growth rate of the Japanese strain was only half the values of the other two strains (Fig. 3; Table II).

The New Zealand strain could potentially grow over the whole annual range of field temperatures, albeit at suboptimal growth rates (Fig. 3). No growth is expected at low ambient winter temperatures in the German Wadden Sea strain and the Japanese strain, but temperatures would support optimal growth in summer (German Wadden Sea strain) or in autumn and spring (Japanese strain).

## Biovolume

During exponential growth, the biovolume of *F. japonica* cells did not change (data not shown). The analysis of

*Table II: Fibrocapsa japonica: Summary of significant differences in maximum growth rate, biovolume and net production among temperatures and strains (single-factor ANOVAs with Scheffe's posthoc tests)*

| Temperature (°C) | Maximum growth rate ($d^{-1}$) | | | | | Biovolume ($\mu m^3$) | | | | | Net production ($\mu m^3\ d^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | German Wadden Sea | Japan | New Zealand | *P* value | Posthoc strain | German Wadden Sea | Japan | New Zealand | *P* value | Posthoc strain | German Wadden Sea | Japan | New Zealand | *P* value | Posthoc strain |
| 9.5 | | | e | <0.001[a] | (NZ > WS = J) | | | a | <0.001[a] | (NZ > WS = J) | | | **ab** | <0.001[a] | (NZ > WS = J) |
| 12.5 | d | c | d | <0.001 | WS = NZ > J | a | a | b | <0.001 | J > NZ > WS | bc | c | **a** | <0.001 | NZ = WS > J |
| 16.5 | bc | b | c | <0.001 | WS > NZ = J | b | b | c | <0.001 | J > NZ > WS | **a** | b | **a** | 0.044 | WS = J = NZ |
| 21 | **ab** | **a** | **ab** | <0.001 | WS = J > NZ | c | c | d | <0.001[a] | J > NZ > WS | **ab** | **a** | **a** | <0.001 | J > WS = NZ |
| 24.5 | **a** | **a** | **a** | <0.001 | WS > J > NZ | **d** | **d** | **f** | <0.001 | J > WS > NZ | **ab** | b | b | <0.001 | WS = J > NZ |
| 27.5 | bc | b | bc | 0.035 | WS > J; NZ = WS = J | **cd** | **d** | **f** | 0.001 | J > WS = NZ | c | c | b | 0.089 | WS = NZ = J |
| 29 | c | b | **abc** | <0.001 | WS = NZ > J | **d** | **d** | **f** | <0.001 | J > WS > NZ | c | c | b | 0.002 | WS = NZ > J |
| 32 | | | e | <0.001[a] | (NZ > WS = J) | | | e | <0.001[a] | (NZ > WS = J) | | | c | <0.001[a] | (NZ > WS = J) |
| *P* value | <0.001 | <0.001 | <0.001 | | | <0.001 | <0.001[a] | <0.001[a] | | | <0.001 | <0.001 | <0.001 | | |

Rows show comparisons among strains at each temperature (posthoc strain: highest to lowest value). Columns show comparisons among temperatures in each strain; significant differences in posthoc test indicated with different letters (a, highest value). WS, German Wadden Sea; J, Japan; NZ, New Zealand. Temperature ranges with highest maximum growth rate and net production and with lowest biovolume are indicated in bold type.

[a]Variances heterogeneous; posthoc results between brackets: no growth in German Wadden Sea and Japanese strains.

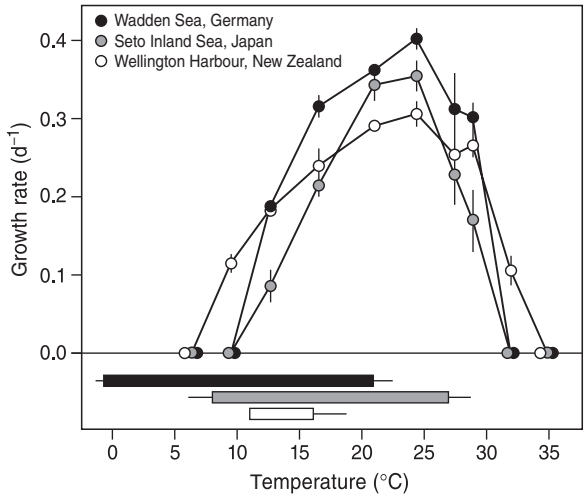


**Fig. 3.** Growth rates (d$^{-1}$) at different temperatures for three strains of *Fibrocapsa japonica*. Data represent means and standard deviations ($n$ = 3). Bars show the annual temperature range in the different habitats; thin lines indicate extreme temperatures recorded in the field (Yoshimatsu and Ono, 1986; FTZ-CAU, 1995, 1997b; Chang, 2000).

variance on biovolume showed a significant strain–temperature interaction ($F_{14,91}$ = 1029.340, $P$ < 0.001). Single-factor ANOVAs showed significant effects of temperature in each strain, and at each temperature significant differences among strains were detected (Table II). The biovolume of cells of all strains decreased with increasing temperatures up to 27.5°C (Fig. 4). In all strains, cells were smallest between 24.5 and 29°C (Table II: bold letters).

Between 12.5 and 21°C, the German Wadden Sea strain consistently had the smallest cells, while the biggest cells were observed in the Japanese strain (Fig. 4; Table II: posthoc strain). The highest biovolume of ~7800 μm$^3$ was observed in the New Zealand strain at 9.5°C, a temperature too low to support growth in the other strains. Cells of the three strains were different from each other in biovolume at each temperature, except at 27.5°C for the German Wadden Sea and the New Zealand strain. The New Zealand strain, the only one able to grow at 32°C, had a significantly higher biovolume per cell at this temperature than at 29°C.

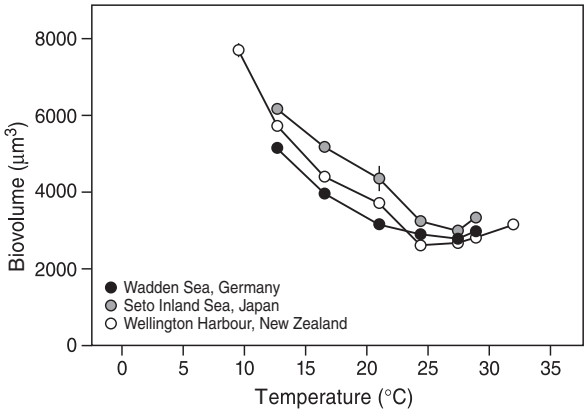


**Fig. 4.** Cell biovolume (μm$^3$) at different temperatures for three strains of *Fibrocapsa japonica*. Data represent means and standard deviations ($n$ = 3).

## Net production

Analysis of variance on net production showed a significant strain–temperature interaction ($F_{14,91}$ = 37.697, $P$ < 0.001). Single-factor ANOVAs showed significant effects of temperature in each strain, and at most temperature significant differences among strains were detected (Table II). As a result of differences among the strains in their temperature response with regard to cell volumes (Fig. 4), the relationship between temperature and net production (Fig. 5) differed considerably from that of growth rates based on cell numbers (Fig. 3; Table II). Optimum temperatures shifted downward, and the temperature range supporting maximal net production became wider in the New Zealand and German Wadden Sea strains (Fig. 5; Table II, bold letters). The highest maximal net production of 1480 μm$^3$ d$^{-1}$ was reached by the Japanese strain at 21°C. The temperature range of the maximal net production was 9.5–21°C for the New Zealand strain and 16.5–24.5°C for the German Wadden Sea strain. The New Zealand and German Wadden Sea strains showed relatively high net production at temperatures close to the lower limit for growth (e.g. German Wadden Sea strain at 12.5°C = 960 μm$^3$ d$^{-1}$; New Zealand strain at 9.5°C = 880 μm$^3$ d$^{-1}$).

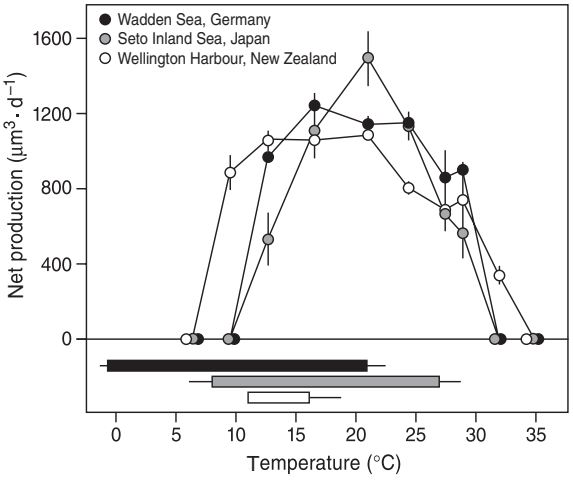


**Fig. 5.** Calculated net production (i.e. the product of cell volume and specific net daily change in abundance; μm$^3$ d$^{-1}$) at different temperatures for three strains of *Fibrocapsa japonica*. Data represent means and standard deviation ($n$ = 3). Bars show the annual temperature range in the different habitats; thin lines indicate extreme temperatures recorded in the field (Yoshimatsu and Ono, 1986; FTZ-CAU, 1995, 1997b; Chang, 2000).

Because of the shifts in optimum temperatures, significant differences among isolates were observed over the temperature range (Table II: posthoc strains). At 21°C, a near-optimum temperature for all strains, the German Wadden Sea and the New Zealand strain had the same net production, but values were lower than in the Japanese strain. At low (e.g. 12.5°C) as well as high (e.g. 29°C) temperatures, the opposite pattern was found; values were lower in the strain from Japan than in the other two strains (Table II).

The New Zealand strain did not grow at maximum rates at the ambient temperatures (Fig. 3) but did have maximal net production over the whole field temperature range (Fig. 5). The German Wadden Sea strain and the Japanese strain had comparable responses for growth and production. For the Japanese strain this means vegetative growth in almost the whole range of local temperatures, although growth and net production rates would be suboptimal at the highest and lowest ambient temperatures. The German Wadden Sea strain would be capable of maximal net production at the higher ambient temperatures, but vegetative growth would not be possible at the lower ambient temperatures.

## Cell behaviour

The non-motile state of cells did not merely depend on the absence of flagellae because microscopic observations (Fig. 2) showed that there were sometimes normal flagellae in non-motile cells.

The influence of temperature on cell behaviour observed in two experiments (growth and viability) is combined in Fig. 6. All strains were consistently non-motile at 2, 4 and 35°C, and the Japanese strain also at 32°C. At 6–10°C, at least part of the cells became non-motile in all strains in the course of the experiments. Part of the cells of the Japanese strain became also non-motile at 12°C. All cells of the New Zealand and German Wadden Sea strains at 12–30°C were motile, which was not seen for the cells of the Japanese strain.

Only at higher temperatures mucocyst ejected threads (Fig. 2g–h) and aggregation of cells by mucus was seen at the culture surface, especially at the wall of the flask. The cells of the German Wadden Sea strain showed persistent aggregation of cells by mucus from the first week onwards at temperatures between 24 and 30°C (Fig. 6). In the Japanese strain, aggregation of cells was seen between 21 and 30°C, starting the second week. Aggregation of cells was observed to a lesser extent in the New Zealand strains. The cells of the New Zealand strain showed only intermittent aggregation by mucus at 28 and 30°C. Comparison of the presence of mucus and therefore cell aggregation with growth phase showed the following results. German Wadden Sea strain: 21°C, stationary growth phase; 24–30°C, mid exponential growth phase. Japanese strain: 17 and 28–30°C, end of the exponential growth phase; 20–24°C, stationary growth phase. New Zealand strain, occasional presence of aggregates at the end of the exponential growth phase. The ejection of mucus threads and therefore cell aggregation by mucus does not seem to be related to cell density.

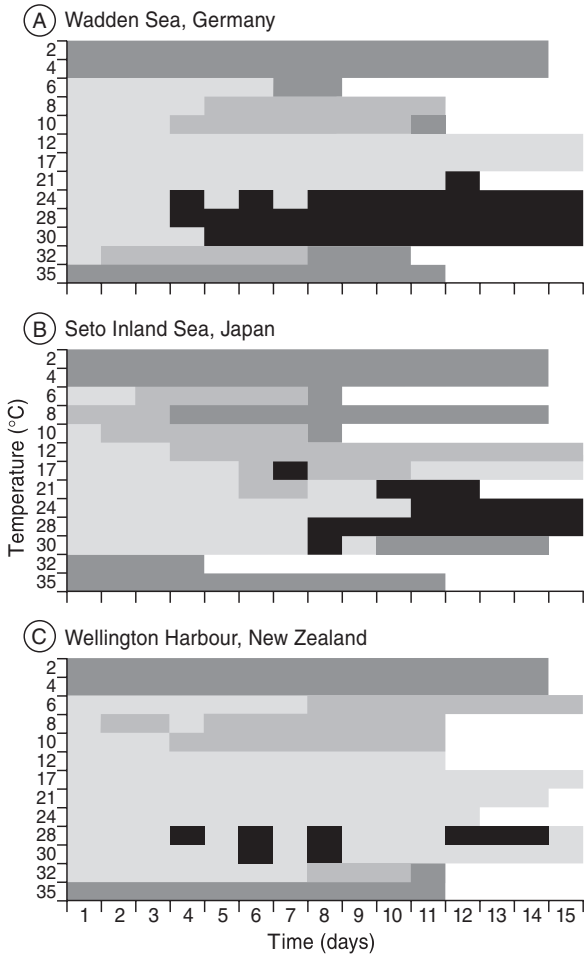


**Fig. 6.** Observations in cultures on three strains of *Fibrocapsa japonica* at different temperatures. Light grey, motile (swimming) cells; middle grey, both motile cells and non-motile cells; dark grey, only non-motile cells; black, motile cells and aggregation of cells by mucus; white, experiment terminated.

## Viability experiment

All cultures recovered at 18°C (Table III) after treatment at temperatures between 6 and 30°C. At 4°C all strains showed a similar decrease in viability. Only the New Zealand strain survived at 2°C (one replicate). After incubation at high temperatures (≥32°C), no recovery was observed, although the New Zealand strain initially showed growth.

*Table III: Viability of the three Fibrocapsa japonica strains after temperature treatment, as percentage recovery*

| Strain origin | Temperature (°C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10–30 | 32 | 35 |
| Wadden Sea, Germany | 0% (14) | 33.3% (12) | 100% (7) | 100% (8) | 100% | 0% (4) | 0% (3) |
| Seto Inland Sea, Japan | 0% (14) | 36.4% (11) | 100% (7) | 100% (8) | 100% | 0% (4) | 0% (3) |
| Wellington Harbour, New Zealand | 5.9% (17) | 33.3% (12) | 100% (7) | 100% (8) | 100% | 0% (4) | 0% (3) |

Number of samples in brackets (10–30°C: $n \geq 5$).

# DISCUSSION

## Growth performance in laboratory cultures

The *F. japonica* strains studied (from the German Wadden Sea, Japan and New Zealand) had their maximal growth rates between 21 and 24.5°C, could grow over a broad range of temperatures (9.5–12.5 to 29-32°C) and may, therefore, be considered eurythermal. Despite their rather similar temperature growth response, some small differences between strains were observed (Fig. 3). Small differences in temperature response were also observed for cell biovolume (Fig. 4), net production (Fig. 5) and temperature tolerance (Fig. 6).

### *Growth rate*

In our experiments, the German Wadden Sea strain, which had the highest maximal growth rate of the strains studied, had exactly the same maximal growth rate ($\mu$, 0.4 $d^{-1}$) as reported by Khan *et al.* (Khan *et al.*, 1996a) under comparable culture conditions (25°C, 35 µmol photons $m^{-2}$ $s^{-1}$) for a Dutch Wadden Sea strain collected and isolated in 1992 (reported as cell divisions per day).

Like for most microorganisms, the optimum temperature for growth for *F. japonica* strains was much closer to the upper than to the lower temperature limit. Therefore, the maximal growth rate can be considered as the result of activating and inhibitory effects of temperature and it is questionable whether the optimum growth temperature reflects the temperature that is optimal for the organisms under field conditions. It has been suggested that one should consider a 'physiological optimum', which would be some degrees below the growth optimum (Aragno, 1981).

### *Biovolume*

Temperature affected the biovolume of *F. japonica* cells, which decreased with increasing temperature up to 24.5°C. Enlargement of cells at low temperatures seems to be a general mechanism and has also been found in *Emiliania huxleyi* (Lohman) Hay and Mohler (Van Rijssel and Gieskes, 2002), some diatoms (Montagnes and Franklin, 2001) and ciliates (Weisse and Montagnes, 1998). For each *Fibrocapsa* strain, the minimal biovolumes per cell were not found at the optimal temperatures for growth but at higher temperatures, indicating that the minimum biovolume per cell is not strictly coupled to maximal growth rate. The different biovolumes per cell between strains at all temperatures again illustrate strain-specific responses.

### *Net production*

Because of the differences in cell biovolume related to temperature, growth was also expressed as net production: the product of biovolume and growth rate (i.e. net cell volume per day, $\mu m^3$ $d^{-1}$) on the assumption that biovolume is directly coupled to biomass. This assumption seems reasonable since *F. japonica* cells are in the size range where N and C are constant per unit volume (Verity *et al.*, 1992). Cell volume seems to increase because of the rate of cell division that is low compared to biomass production. Net production is a more appropriate measure than growth rate when considering the amount of biomass produced, which is available for transfer within the food web (Weisse and Montagnes, 1998). The temperature ranges for maximal net production were shifted downward, compared to those for maximal growth (Figs 3 and 5; Table II). For all strains, cellular metabolism seemed already impaired above the temperatures for maximal production, while growth rates were still high. Thus, the net production optimum temperature, combining both growth rate and biovolume changes, corresponds well with the physiological optimum proposed by Aragno (Aragno, 1981). The maximal net production of the New Zealand and German Wadden Sea strains seemed to be better adapted to colder temperatures than in the Japanese strain. This was also observed for the motility of the cells.

### *Cell behaviour and viability*

*Fibrocapsa japonica* cells were motile when growing at half $\mu_{max}$ or higher, which is in agreement with a study on a

Dutch Wadden Sea strain (Khan *et al.*, 1998). In general, there was a shift from a pelagic stage (motile cells) to a benthic stage (non-motile cells) with decreasing temperatures. Unfortunately, with visual examination of cell behaviour, we could not distinguish cysts from non-motile vegetative cells. Cells showed lysis <4°C or after a short period >32°C. These lower and upper temperatures will set the lethal limits in the field.

## Mucus production

In this study, aggregation of cells by mucus production does not seem to be triggered by growth phase or cell density but seems to depend on temperature. Aggregation of cells by mucus was observed at temperatures just at or above maximal net production temperatures. For *Chattonella* aff. *verruculosa*, it is suggested that a mixture of algae and mucus may simply have caused clogging of the fish gills and that suffocation subsequently killed the fish (Backe-Hansen *et al.*, 2001). In April–May 1998, both wild fish and fish in pens were killed during a bloom in parts of the Skagerrak and the North Sea, where along the southwest coast of Norway the temperature at 1 m rose from 8 to 13°C during May (Backe-Hansen *et al.*, 2001).

## Growth performance in the natural habitat

The temperature supporting maximal growth rates of an alga in the laboratory may be considerably different from the temperatures at which the alga predominates in the field. This phenomenon indicates that temperature alone does not determine the dominance of algal populations in the natural environment (Smayda, 1969; Durbin, 1974; Eppley, 1977). However, comparison of experimentally determined temperature responses with ambient field temperatures may indicate how well adapted each strain is to the local conditions and could provide an explanation for the periodicity of *F. japonica* blooms observed in the field. We used the temperature response of growth rate since growth rate is based on cell numbers and represents population size. Cell numbers are more relevant for studies on population growth than is biomass expressed as net production (Weisse and Montagnes, 1998).

### *German Wadden Sea strain*

A fairly broad temperature range from 21°C in August to −0.7°C in December exists in the cold temperate region of the harbour of Büsum, Wadden Sea, Germany (Fig. 7a). Optimal growth of this strain would occur in July and August, which corresponds with the period when this species was recorded in the field (FTZ-CAU, 1997a; Rademaker *et al.*, 1998). The maximal growth rate found in the laboratory is not reached, although net maximal production can be reached in the field. If the ejection of mucocysts is coupled to harmful effects on fish, this could be expected at temperature ≥21°C, an unusual phenomenon in this area. Two periods allowing suboptimal growth in the field are expected to occur in late spring (May and June) and early autumn (September

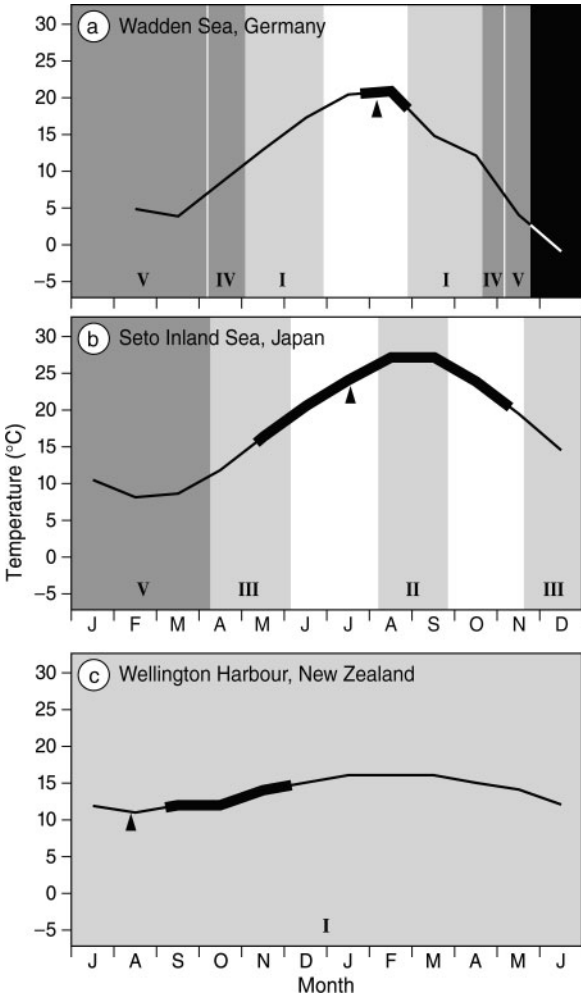


**Fig. 7.** Annual water temperature variation in habitats where the *Fibrocapsa japonica* strains were collected: **a**, Wadden Sea, Germany (FTZ-CAU, 1995); **b**, Seto Inland Sea, Japan (Yoshimatsu and Ono, 1986); **c**, Wellington Harbour, New Zealand (US Navy, 1981). Bold lines represent periods when *F. japonica* was recorded in the field: Büsum, Wadden Sea, Germany, 1997 (FTZ-CAU, 1997a, b; Rademaker *et al.*, 1998); Harima Nada, Seto Inland Sea, Japan, 1985 (Yoshimatsu and Ono, 1986); Leigh, New Zealand, 1992 (Rhodes *et al.*, 1993). Arrows indicate date of collection of each strain (German Wadden Sea strain, U. Tillmann, Bremerhaven, personal communication; Japanese strain, NIES; New Zealand strain, Cawthron Institute). White: period of optimal growth (≥80% of $\mu_{max}$); light grey: period of suboptimal growth (>20% and <80% of $\mu_{max}$) with motile cells (**I**), motile cells and aggregation of cells by mucus (**II**) and motile and non-motile cells (**III**); dark grey: period of no growth (<20% of $\mu_{max}$) with motile and non-motile cells (**IV**) and non-motile cells (**V**); black: lethal temperatures, lysed cells; based on experiments shown in Figs 3 and 6 and Table III.

and October). Pelagic motile *F. japonica* cells that do not grow are expected in April and in late October/early November. Between November and April, a period with non-motile benthic but viable cells is expected; however, temperatures (<4°C) that are lethal for vegetative cells can occur in December.

The German Wadden Sea strain is the only strain in this study which needs cyst formation in the field to survive lethal winter temperatures. Similar to other Raphidophyceae (Imai *et al.*, 1998; Tomas, 1998), low temperatures (≤12°C) are necessary for inducing viable *F. japonica* cysts (Yoshimatsu, 1987; De Boer *et al.*, in press). This means that between the end of October and the beginning of May, low temperatures could be inducing viable cysts. Above 9.5°C, the German Wadden Sea strain starts growing and therefore spring (mid April) would be the time for germination of cysts. Cyst formation and maturation is not likely to have taken place in our short-term experiments. Therefore, it is possible that cysts, as opposed to vegetative cells, can survive temperatures <4°C and reinoculate the water column after severe winters. Another explanation for reoccurring *F. japonica* at the Wadden Sea coast is yearly reinoculation by anthropogenic means. Seeding via natural currents with *F. japonica* cells from southern regions with temperatures >4°C seems less likely because the residual current in the North Sea takes a year to move water masses of the Channel and the southern North Sea to the German Bight (Kautsky, 1988). Moreover, *F. japonica* is not expected to flow easily with the water masses since benthic stages are induced at low temperatures (Yoshimatsu, 1987; De Boer *et al.*, in press). Nehring (Nehring, 1998) suggested that the recent but permanent colonization of the North Sea (and German Bight) by immigrated species such as *F. japonica* may be a biological signal of subtle changes in the climate, especially relatively mild winters. However, blooms of *F. japonica* were seen in the Wadden Sea after the cold winters of 1995/1996 and 1996/1997 (Rademaker *et al.*, 1998), so other hypotheses such as continuous introduction, but more likely the survival of cysts, seem a more plausible explanation for the reappearance of *F. japonica* in the southern North Sea region.

### *Japanese strain*

The southern Harima Nada, Seto Inland Sea of Japan (Fig. 7b), has a temperature range from 8°C in February to 27°C in August/September, with occasional extremes of 6.3°C in winter and 28.7°C in summer (Yoshimatsu and Ono, 1986). Our results indicate that the Japanese strain would be well adapted to the temperature variations in its own habitat. In southern Harima Nada, *F. japonica* was reported from mid-May to mid-November in 1985 (Yoshimatsu and Ono, 1986). Temperatures sustaining maximal growth rate as well as the maximal net production are met in the field.

Optimal growth of the Japanese strain is possible in two periods from June to July and from October to mid-November, interrupted by a period of suboptimal growth with mucus production due to high temperatures in mid-summer. These two expected periods of optimal growth agree with field observations. Between 1973 and 1985, two periods of red tides of *F. japonica* were repeatedly reported in southern Harima Nada (Yoshimatsu and Ono, 1986). The expected period of suboptimal growth with possible mucus production at temperatures >26°C is supported by the field report that the rise of water temperature >26°C was accompanied by a decrease in *F. japonica* cell density (Yoshimatsu and Ono, 1986).

Interestingly, harmful effects of *F. japonica* red tides accompanied by mass mortality of fish and other animals were also observed in the Seto Inland Sea in August 1970 (Iwasaki, 1971) and July and August 1972 (Okaichi, 1972; Toriumi and Takano, 1973). The water temperature was in the range from 22 to 27°C on these occasions. If the ejection of mucocysts at water temperature ≥21°C is coupled to *F. japonica* toxicity (21–24.5°C, stationary phase and >24.5°C exponential phase), this could imply the risk of yearly harmful effects of *F. japonica* in the Seto Inland Sea.

The periods of mid-April to the end of May and of the end of November to the end of December would support suboptimal growth with both pelagic and benthic stages, similar to the German Wadden Sea. The Japanese strain, however, would never meet a lethal limit in the field. The Harima Nada is the only region where *F. japonica* cysts have, so far, been found in the sediment (Yoshimatsu, 1987). At this location, cyst formation at temperatures ≤12°C (Yoshimatsu, 1987) would be possible in midwinter.

### *New Zealand strain*

Extremely small seasonal temperature variation, between 11°C in August and 16°C in January to March, characterizes the cold temperate region between the northern and southern Island of New Zealand (Fig. 7c).

Because data on *F. japonica* seasonality are lacking for Wellington harbour, data from Leigh, the most nearby place with *F. japonica* observations and with a similar temperature profile, were used (Rhodes *et al.*, 1993). At Leigh, *F. japonica* was observed from late August 1992 to early December 1992. The New Zealand strain was more cold tolerant in the laboratory experiments than both other *F. japonica* strains, a trait that is not expected to be of any use in this area. The New Zealand strain grows suboptimally but has a maximal net production

throughout the year, and a period of optimal growth (>18.8°C) could occasionally occur in this region (Chang, 2000). The presence of mucus threads induced by high temperatures (≥28°C) is not expected to occur in the field. In addition, induction of cysts by low temperatures is not expected. This explains why this strain could be collected in the field during the period with the lowest temperature.

## CONCLUSIONS

*Fibrocapsa japonica* is a typical temperate region species that cannot survive in polar regions. All strains are eurythermal, but there is some variation in the temperature response of growth within the species. The similarity of the temperature responses of the German Wadden Sea strain and the Japanese strain is remarkable, although they are from different climate regions. The Japanese strain is well adapted to the ambient temperature regime. In contrast, the German Wadden Sea strain will meet its lethal winter limit (for survival of vegetative cells) in the field. However, it would be able to survive by cyst formation. Alternatively, regional populations may be the result of repeated introductions from elsewhere.

The New Zealand strain seems to be more cold adapted than the other strains (although it still can grow at 32°C), because this strain had higher growth rates at lower temperatures and a broader temperature range down to 9.5°C for maximal net production. The broad temperature range for growth of the New Zealand strain, compared to the temperatures in its habitat, indicates evolutionary imprints that enable this strain to grow at conditions never met in the field. The New Zealand strain may have been introduced from another area with a much broader annual temperature range. A reduction of the temperature range for growth is much more likely to occur than a broadening of the range because it may be the result of single mutations (i.e. making an essential protein more heat or cold sensitive) rather than gene modification (Aragno, 1981; Lüning, 1990).

The physiological profiles of the *F. japonica* strains studied here indicate differences mainly between the New Zealand strain and the two other strains, from the German Wadden Sea and Japan. On the basis of internal transcribed spacer (ITS) sequences (Kooistra *et al.*, 2001), the Japan and the New Zealand strains are expected to be more closely related to each other than to the German Wadden Sea strain. These minor intraspecific differences in ITS sequences do not correspond with the ecophysiological data presented here. Temperature responses, however, are not necessarily reflected by ITS differences that are caused by random mutations without selective pressure on temperature behaviour. A comparison between the German Wadden Sea strain and the Dutch Wadden Sea strain (Khan *et al.*, 1996a, 1998) is interesting because they showed identical physiological responses to temperature, suggesting not much physiological differences between strains from the same area. Comparable physiological responses were also observed in the German Wadden Sea strain and the Japanese strain. Because the water mass of the North Sea is not in direct contact with the North Pacific Ocean water mass, it is tempting to speculate on the exchange of populations by anthropogenic means, e.g. through exchange of ballast water, as the cause of *Fibrocapsa* sightings in European waters that started a decade ago (Billard, 1992; Vrieling *et al.*, 1995). Close inspection of the range of maximal net production of the German Wadden Sea and Japanese strains, however, suggests that the former is slightly more cold adapted. It remains uncertain whether the magnitude of this difference is big enough to presume the existence of an endemic European population that went unnoticed before HAB species caused problems elsewhere.

When comparing our results with the worldwide distribution of *F. japonica*, it seems that temperature may also be a controlling factor for this microalgal species. So far, *F. japonica* has not been observed north of the Wadden Sea area. Both the German Wadden Sea strain and the Dutch Wadden Sea strain (Khan *et al.*, 1996a, 1998) are representatives of biogeographic boundary areas because low winter temperatures would not permit the survival of vegetative cells in the field. An increase in seawater temperature due to global warming could possibly allow *F. japonica* to spread to new areas, causing a potential threat for economically important fish stocks north of the Wadden Sea.

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## REFERENCES

Aragno, M. (1981) Responses of microorganisms to temperature. In Lange, D. L., Nobel, P. S., Osmond, C. B. and Ziegler, H. (eds), *Physiological Plant Ecology*. Springer Verlag, Berlin, pp. 339–369.

ARPAM (Agenzia Regionale per la Preotexione Ambiantale delle Marche) (2001) Un anno di monitoraggio biologico marino. http://www.regione.marche.it/mareancona/eu1997(1).htm

Backe-Hansen, P., Dahl, E. and Danielssen, D. S. (2001) On a bloom of *Chattonella* in the North Sea/Skagerrak in April–May 1998. In Hallegraeff, G. M., Blackburn, S. I., Bolch, C. J. and *et al.* (eds), *Harmful Algal Blooms 2000*. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 78–81.

Billard, C. (1992) *Fibrocapsa japonica* (Raphidophyceae), planktonic marine alga reported for the first time in France. *Cryptogam. Algol.*, **13**, 225–231.

Breeman, A. M. (1988) Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phenological evidence. *Helgol. Meeresunters.*, **42**, 199–241.

Breeman, A. M., Oh, Y. S., Hwang, M. S. *et al.* (2002) Evolution of temperature responses in the *Cladophora vagabunda* complex and the *C. albida/sericea* complex (Chlorophyta). *Eur. J. Phycol.*, **37**, 45–58.

Cambridge, M. L., Breeman, A. M. and Van den Hoek, C. (1990) Temperature limits at the distribution boundaries of four tropical to temperate species of *Cladophora* (Cladophorales: Chlorophyta) in the North Atlantic Ocean. *Aquat. Bot.*, **38**, 135–151.

Chang, H. F. (2000) New blooms and old. *Water Atmos.*, **8**, 26–27.

Congestri, R., Albertano, P., Ravizza, P. *et al.* (2000) On blooms of *Fibrocapsa* along the middle Thyrrenian Sea (Mediterranean Sea), Italy, in spring–summer 1999. ASLO Abstract, Copenhagen.

De Boer, M. K., Van Rijssel, M. and Vrieling, E. G. (in press) Morphology of *Fibrocapsa japonica* cysts formed under laboratory conditions. In Steidinger, K. A., Landsberg, J. H., Tomas, C. R. and Vargo, G. A. (eds) *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, USA.

Durbin, E. G. (1974) Studies on the autecology of the marine diatom *Thalassiosira nordenskiöldii* Cleve. 1. The influence of day length, light intensity, and temperature on growth. *J. Phycol.*, **10**, 220–225.

Eppley, R. W. (1977) The growth and culture of diatoms. In Werner, D. (ed.), *The Biology of Diatoms*. University of California Press, Berkeley, pp. 24–64.

FTZ-CAU (Research and Technology Centre Westcoast (FTZ) at Büsum, Christian-Albrechts-Univerisity in Kiel) (1995) Bakterioplankton. http://www.uni-kiel.de/ftzwest/ag1/bakt-e.htm

FTZ-CAU (Research and Technology Centre Westcoast (FTZ) at Büsum, Christian-Albrechts-Univerisity in Kiel) (1997a) *Fibrocapsa japonica*: distributions and concentrations. http://www.uni-kiel.de/ftzwest/ag1/fib-vork-e.htm

FTZ-CAU (Research and Technology Centre Westcoast (FTZ) at Büsum, Christian-Albrechts-Univerisity in Kiel) (1997b) Büsum-Pier. http://www.uni-kiel.de/ftzwest/ag1/buemo-e.htm

Fu, M., Koulman, A., Van Rijssel, M. *et al.* (2004) Chemical characterisation of three haemolytic compounds from the microalgal species *Fibrocapsa japonica* (Raphidophyceae). *Toxicon*, **43**, 355–363.

Fu, M., Van Rijssel, M. and Liebezeit, G. (in press) Hemolytic compounds from *Fibrocapsa japonica* (Raphidophyceae). In Steidinger, K. A., Landsberg, J. H., Tomas, C. R. and Vargo, G. A. (eds), *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, USA.

Guillard, R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. and Chanley, M. H. (eds), *Culture of Marine Invertebrate Animals*. Plenum Press, New York, pp. 108–132.

Hallegraeff, G. M. (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia*, **32**, 79–99.

Hallegraeff, G. M. and Fraga, S. (1998) Bloom dynamics of the toxic dinoflagellate *Gymnodinium catenatum*, with emphasis on Tasmanian and Spanish coastal waters. In Anderson, D. M., Cembella, A. D, and Hallegraeff, G. M. (ed.), *Physiological Ecology of Harmful Algal Blooms*. Vol. G 41. Springer-Verlag, Berlin, pp. 59–80.

Hara, Y. and Chihara, M. (1985) Ultrastructure and taxonomy of *Fibrocapsa japonica* (Class Raphidophyceae). *Arch. Protistenkd.*, **130**, 133–141.

Hayes, K. R. (1998) Ecological risk assessment for ballast water introductions: a suggested approach. *ICES J. Mar. Sci.*, **55**, 201–212.

Hillebrand, H., Dürselen, C. D., Kirschtel, D. *et al.* (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Imai, I., Yamaguchi, M. and Watanabe, M. (1998) Ecophysiology, life cycle, and bloom dynamics of *Chattonella* in the Seto Inland Sea, Japan. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms*. Vol. G 41. Springer-Verlag, Berlin, pp. 95–112.

Ishimatsu, A., Sameshima, M., Tamura, A. *et al.* (1996) Histological analysis of the mechanisms of *Chattonella*-induced hypoxemia in Yellowtail. *Fish. Sci.*, **62**, 50–58.

Iwasaki, H. (1971) Studies on the red tide flagellates. VI. On *Eutreptiella* sp. and *Exuviaella* sp. appeared in Bingo-Nada, the Seto Inland Sea, in 1970. *J. Oceanogr. Soc. Jpn.*, **27**, 152–157.

Kautsky, H. (1988) Radioactive substances. In Salomons, W., Bayne, B. L., Duursma, E. K. and Förstner, U. (eds), *Pollution of the North Sea. An Assessment*. Springer-Verlag, Berlin, pp. 390–399.

Khan, S., Arakawa, O. and Onoue, Y. (1996a) Growth characteristics of a neurotoxin-producing chloromonad *Fibrocapsa japonica* (Raphidophyceae). *J. World Aquac. Soc.*, **27**, 247–253.

Khan, S., Arakawa, O. and Onoue, Y. (1996b) Neurotoxin production by a chloromonad *Fibrocapsa japonica* (Raphidophyceae). *J. World Aquac. Soc.*, **27**, 254–263.

Khan, S., Haque, M. M., Arakawa, O. *et al.* (1998) Influence of environmental factors on the morphology of red-tide producing phytoflagellate *Fibrocapsa japonica*. *J. Aquac. Trop.*, **13**, 119–132.

Kooistra, W. H. C. F., De Boer, M. K., Vrieling, E. G. *et al.* (2001) Variation along ITS markers across strains of *Fibrocapsa japonica* (Raphidophyceae) suggests hybridisation events and recent range expansion. *J. Sea Res.*, **46**, 213–222.

Larcher, W. and Wieser, J. (eds) (1995) *Physiological Plant Ecology*. Springer-Verlag, Berlin.

Lee, J. B., Lee, M. H., Lee, J. A. *et al.* (2001) Seasonal dynamics of dinoflagellates and raphidophytes and distribution of their resting cysts in Kamak Bay, Korea. In Hallegraeff, G. M., Blackburn, S. I., Bolch, C. J. and Lewis, R. J. (eds), *Harmful Algal Blooms 2000*. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 116–119.

Lewitus, A. J., Wilde, S. B., Kempton, J. W. *et al.* (2002) Harmful algal blooms in South Carolina brackish ponds associated with housing and golf courses. In *XHAB 2002 Book of Abstracts*. Florida Fish and Wildlife Conservation Commission, Florida, p. 170.

Loeblich, A. R. I. and Fine, K. (1977) Marine chloromonads: more widely distributed in neritic environments than previously thought. *Proc. Biol. Soc. Wash.*, **90**, 388–399.

Lüning, K. (ed.) (1990) *Seaweeds. Their Environment, Biogeography and Ecophysiology*. Wiley, New York.

Marshall, J. A. and Hallegraeff, G. M. (1999) Comparative ecophysiology of the harmful alga *Chattonella marina* (Raphidophyceae) from South Australian and Japanese waters. *J. Plankton Res.*, **21**, 1809–1822.

Marshall, J. A., Nichols, P. D., Hamilton, B. *et al.* (2003) Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis*

*polycanthus*) the synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae*, **2**, 273–281.

Montagnes, D. J. S. and Franklin, D. J. (2001) Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: reconsidering some paradigms. *Limnol. Oceanogr.* **46**, 2008–2018.

Nehring, S. (1998) Establishment of thermophilic phytoplankton species in the North Sea: biological indicators of climatic changes? *ICES J. Mar. Sci.*, **55**, 818–823.

O'Halloran, C., Silver, M. and Marin, R. (2002) Brevetoxin and brevetoxin-producing species in Monterey Bay, California. In *XHAB 2002 Book of Abstracts*. Florida Fish and Wildlife Conservation Commission, Florida, p. 218.

Oda, T., Nakamura, A., Shikayama, M. *et al.* (1997) Generation of reactive oxygen species by raphidophycean phytoplankton. *Biosci. Biotechnol. Biochem.*, **61**, 1658–1662.

Odebrecht, C. and Abreu, P. C. (1995) Raphidophycean in Southern Brazil. *Harmful Algae News*, **12/13**, 4.

Okaichi, T. (1972) Occurrence of red-tides related to neritic water pollution. In Anonymous (ed.), *The Cause of Red-tide in Neritic Waters*. Japan. Ass. Protec. Fish. Resour., Tokyo, pp. 58–76.

Okaichi, T. (1989) Red-tide problems in the Seto Inland Sea, Japan. In Okaichi, T., Anderson, D. M. and Nemoto, T. (eds), *Red Tides: Biology, Environmental Science and Toxicology*. Elsevier, New York, pp. 137–142.

Orfanidis, S. and Breeman, A. M. (1999) Geographic variation in thermal traits in *Digenea simplex* and *Champia parvula* (Rhodophyta) in relation to present and glacial temperature regimes. *J. Phycol.*, **35**, 919–930.

Pakker, H. (1995) Some like it hot. Thermal traits and biogeography of tropical to warm-temperate Atlantic seaweeds. PhD Thesis. University of Groningen.

Pakker, H. and Breeman, A. M. (1996) Temperature responses to tropical to warm-temperate Atlantic seaweeds. II. Evidence for ecotypic differentiation in amphi-Atlantic tropical-Mediterranean species. *Eur. J. Phycol.*, **31**, 133–141.

Pakker, H., Breeman, A. M., Prud'homme van Reine, W. F. *et al.* (1995) A comparative study of temperature responses of Caribbean seaweeds from different biogeographic groups. *J. Phycol.*, **31**, 499–507.

Rademaker, M., Reckermann, M., Tillmann, U. *et al.* (1998) *Fibrocapsa japonica* and *Heterosigma akashiwo*: new observations. *Harmful Algae News*, **17**, 8–10.

Rhodes, L. L., Haywood, A. J., Ballantine, W. J. *et al.* (1993) Algal blooms and climate anomalies in north-east New Zealand, August–December 1992. *N. Z. J. Mar. Freshw. Res.*, **27**, 419–430.

Scholin, C. A., Hallegraeff, G. M. and Anderson, D. M. (1995) Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia*, **34**, 472–485.

Smayda, T. J. (1969) Experimental observations on the influence of temperature, light and salinity on cell division of the marine diatom *Detonula confervacea* (Cleve) Gran. *J. Phycol.*, **5**, 150–157.

Smayda, T. J. and Villareal, T. (1989) The 1985 "brown tide" and the open phytoplankton niche in Narragansett Bay during summer. In Cosper, E. M., Bricelj, V. M. and Carpenter, E. J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 165–187.

Sokal, R. R. and Rohlf, F. J. (eds), (1995) *Biometry*. 3rd edn. Freeman, San Francisco.

Suzuki, Y. and Takahashi, M. (1995) Growth responses of several diatom species isolated from various environments to temperature. *J. Phycol.*, **31**, 880–888.

Tomas, C. R. (1998) Blooms of potentially harmful Raphidophycean flagellates in Florida coastal waters. In Reguera, B., Blanco, J., Fernández, M. L. and Wyatt, T. (eds), *Harmful Algae*. Xunta de Galiacia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostella, Spain pp. 101–103.

Toriumi, S. and Takano, H. (1973) *Fibrocapsa*, a new genus in Chloromonadophyceae from Atsumi Bay, Japan. *Bull. Tokai Reg. Fish. Res. Lab.*, **76**, 25–35.

Underwood, A. J. (ed.) (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.

US Navy (ed.) (1981) Marine climatic atlas of the World. Vol. IX. *World-wide Means and Standard Deviations*. U.S. Government Printing Office, Washington DC, 19 pp., 9 maps, 1 microfiche.

Van den Hoek, C. (1982) The distribution of benthic marine algae in relation to temperature regulation of their life histories. *Biol. J. Linn. Soc.*, **18**, 81–144.

Van den Hoek, C., Breeman, A. M. and Stam, W. T. (1990) The geographic distribution of seaweeds species in relation to temperature: present and past. In Beukema, J. J., Wolff, W. J. and Brouns, J. J. W. M. (eds), *Expected Effects of Climatic Change on Marine Coastal Ecosystems*. Kluwer Academic Publishers, Dordrecht, pp. 55–67.

Van Rijssel, M. and Gieskes, W. W. C. (2002) Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of *Emiliania huxleyi* (Prymnesiophyceae). *J. Sea Res.*, **48**, 17–27.

Verity, P. G., Robertson, C. Y., Tronzo, C. R. *et al.* (1992) Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.*, **37**, 1443–1446.

Vrieling, E. G., Koeman, R. P. T., Nagasaki, K. *et al.* (1995) *Chattonella* and *Fibrocapsa* (Raphidophyceae): first observation of, potentially harmful, red tide organisms in Dutch coastal waters. *Neth. J. Sea Res.*, **33**, 183–191.

Weisse, T. and Montagnes, D. J. S. (1998) Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora). *Aquat. Microb. Ecol.*, **15**, 285–291.

Wong, M. W. (2002) Phytoplankton monitoring in Honk Kong: review and way forward. In *XHAB 2002 Book of Abstracts*. Florida Fish and Wildlife Conservation Commission, Florida, p. 306.

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.

Yoshimatsu, S. and Ono, C. (1986) The seasonal appearance of red tide organisms and flagellates in the Southern Harima-Nada, Inland Sea of Seto. *Bull. Akashiwo Res. Inst. Kagawa Prefect.*, **2**, 1–42.