Life Cycle Strategies of the Red Tide Causing Flagellates *Chattonella* (Raphidophyceae) in the Seto Inland Sea

ICHIRO IMAI*, SHIGERU ITAKURA* and KATSUHIKO ITOH†
*Nansei National Fisheries Research Institute, Ohno-cho, Hiroshima, 739-04 Japan
†National Research Institute of Aquaculture, Nansei-cho, Mie, 516-01 Japan

Chattonella antiqua (Hada) Ono and *Chattonella marina* (Subrahmanyan) Hara et Chihara are the most noxious red tide flagellates which cause serious damage to fish farming, especially to yellowtail culture, in Japanese coastal waters such as the Seto Inland Sea during summer. Cysts of *Chattonella* were identified from sediments of the Seto Inland Sea. These cysts overwinter in sea bottom and play an important role in initiating the summer red tides. Most of the cysts adhere to solid surfaces such as diatom frustules and sand grains, which may aid in keeping the cyst populations within seed beds for the red tides. Temperature is a principal factor affecting the physiology of cysts of *Chattonella*. No cysts germinate at 10 ° C. Optimum temperature range is between 20 ° and 25 ° C for germination. For maturation (acquisition of germinability) of the cysts, low storage temperature of 11 ° C or below for more than four months is essential, whereas no significant maturation is observed at 20° C or more. In freshly collected sediments, marked seasonality of germinability was confirmed in Suo-Nada. The cysts have germinability between spring and early summer. The cysts spend a period of spontaneous dormancy between autumn and the next spring, and they mature during the winter season. The life cycle of *Chattonella* is therefore well adpted to the seasonal temperature fluctuation in the Seto Inland Sea. And further, alternation between benthic and planktonic stage is presumably unconstrained by virtue of shallowness of the Seto Inland Sea. The life cycle of *Chattonella* can be regarded as superior strategies in conclusion for the occurrences of the red tides in temperate waters such as the Seto Inland Sea.

Introduction

The Seto Inland Sea (22,000 km^2) is the biggest enclosed coastal sea surrounded by Honshu, Shikoku, and Kyushu in Japan (Fig.1). The most part of the Seto Inland Sea is shallower than 50m in depth as shown in Fig.1. The Seto Inland Sea is the representative fish farming area, raising yellowtail, red sea bream, etc.

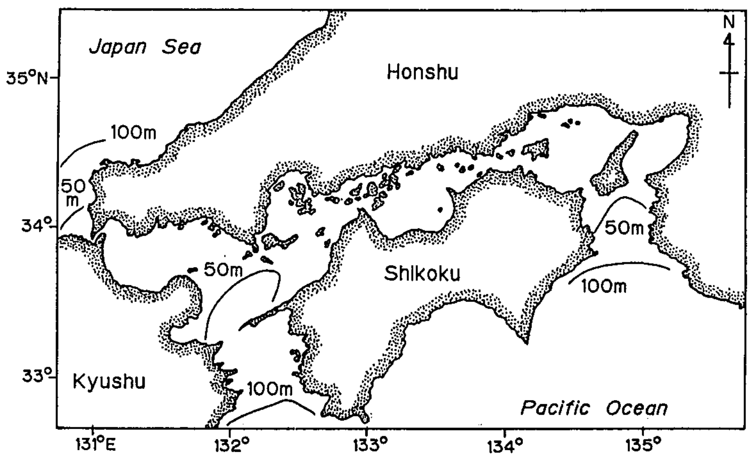

Fig.1. The Seto Inland Sea.

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In recent years, many red tide incidents have recurrently occurred in Japanese coastal waters, mainly in the Seto Inland Sea. Amongst the red tide causing organisms, Chattonella antiqua (Hada) Ono and Chattonella marina (Subrahmanyan) Hara et Chihara (Raphidophyceae) are known to be fish-killing flagellates which cause serious damage to fish farming (amounts of ¥ billion), especially to yellowtail cultures, during the summer season. These organisms have an overwintering benthic stage as cysts in their life cycle (Imai and Itoh, 1986, 1988). In the present paper, life cycle strategies of Chattonella are described dealing with the occurrences of the red tides in the Seto Inland Sea.

Morphology of cysts

It had long been unsuccessful to identify the morphology of cysts of Chattonella despite many efforts devoted by a number of workers. In 1986, cysts of Chattonella were found for the first time from surface sediments of Suo-Nada, western Seto Inland Sea (Imai and Itoh, 1986). Cysts of C.antiqua and C.marina are shown in Fig.2. The living cysts of Chattonella are yellow-greenish to brownish in color, and provided with several spots of dark brown or black materials. The cysts are mostly hemispherical 25-35μm in diameter and 15-25μm in height, and are much smaller than common vegetative cells. The living cysts show auto-fluorescence of chloroplasts when observed under blue-light excitation (Imai and Itoh, 1988). No distinguishable differences were noticed in morphology between the cysts of C.antiqua and C.marina, as in case of cysts of the toxic dinoflagellate Alexandrium tamarense and A.catenella (Fukuyo et al., 1982).

Most of the cysts were found to adhere to solid surfaces such as diatom frustules, sand grains, etc (Imai and Itoh, 1988). Adhesion to any substrata presumably offers the following ecological advantages to the cysts: enlargement of apparent cyst size will protect the cysts from predation; increase in apparent specific gravity of the cysts with heavier particles increase their weight which will in turn keep these cysts within seed bed areas for the red tides.

Cyst formation of Chattonella marina and C.antiqua were investigated in culture under laboratory conditions (Imai, 1989, 1990). The cysts of both species produced in culture displayed morphological characteristics quite similar to those natural cysts observed in sediments of the Seto Inland Sea. The combination of factors such as

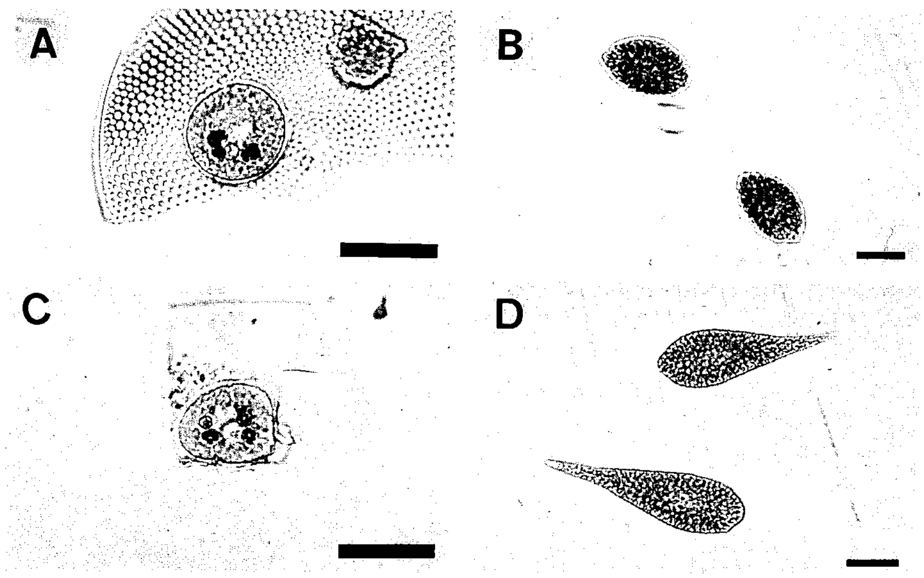

Fig.2. Cysts and vegetative cells of Chattonella antiqua and C.marina. (Scale bar=30μm)
A: Cyst of C.marina adhered to a fragment of diatom frustule.
B: Cultured vegetative cells after the germination from the cyst shown in A.
C: Cyst of C.antiqua adhered to solid surface.
D: Cultured vegetative cells after the germination from the cyst shown in C.

nutrient depletion (nitrogen limitation), adhesion to solid surfaces (glass beads), and low light intensities of 1,000 lux or below (or darkness) was essential for the cyst formation (Imai, 1989, 1990).

Distribution of cysts

Distribution of cysts of Chattonella in sediments were investigated by the direct count technique (Imai and Itoh, 1988; Imai, 1990) in Suo- and Harima-Nada (Fig.3). Densities of cysts in sediment samples ranged from 0 to 490•cm^{-3} (average 91) in Suo-Nada in June 1987, and from 0 to 723•cm^{-3} (average 265) in Harima-Nada in April 1988. Highly accumulated areas were found in both Suo- and Harima-Nada. In case of the toxic dinoflagellate Alexandrium excavatum, White and Lewis (1982) reported that rich deposit of the cysts found offshore in the southwestern Bay of Fundy is consistent with, and can be explained by, hydrographic and sedimentary processes within the Bay. Dense seed bed formation of Chattonella is also probably affected by hydrographic and sedimentary processes.

High density areas of Chattonella cysts do not always coincide with those of vegetative cells during the summer red tide season (Imai et al., 1986; Imai, 1990). Accumulation mechanism of cysts is probably different from that of vegetative cells (Imai et al., 1986).

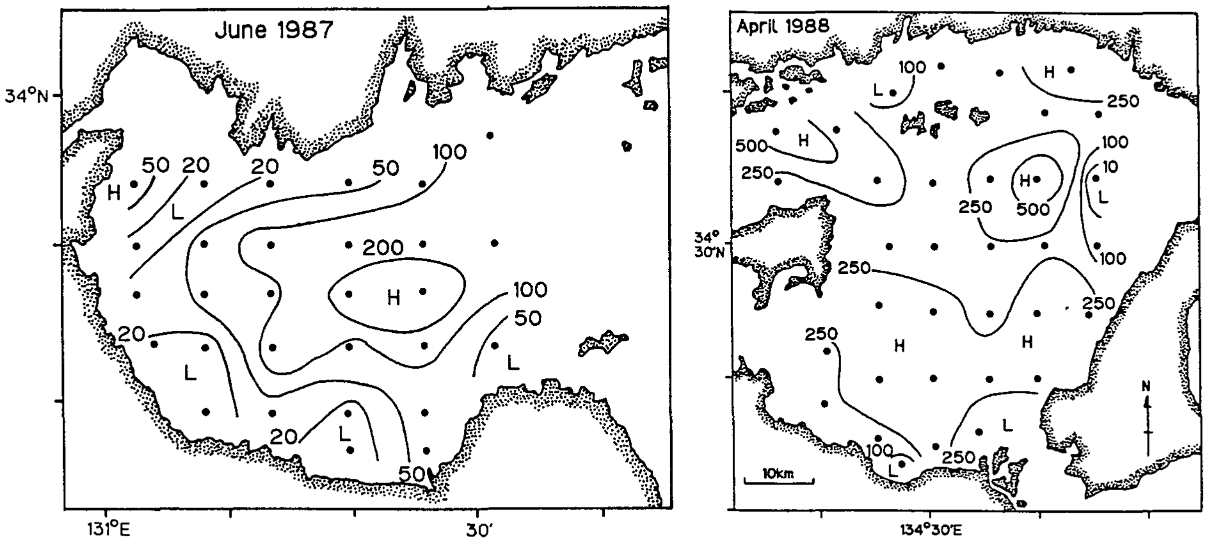

Fig.3. Distribution of cysts of Chattonella in Suo-Nada in June 1987 (left), and in Harima-Nada in April 1988 (right). Numerals indicate the number of cysts in cubic centimeter wet sediment. Surface sediments (3cm depth) were used for enumeration. From Imai (1990)

Physiology and ecology of cysts

In the Seto Inland Sea, water temperature fluctuates seasonally. Temperatures of around 10°C or below are usual in winter, and those of 25°C or higher in summer. From laboratory experiments using sediment samples collected from the Seto Inland Sea, it was confirmed that temperature is a principal factor affecting the physiology of cysts of Chattonella (Imai et al., 1984, 1989; Imai and Itoh, 1987). Fig.4 shows effects of incubation temperature on germination of matured cysts and of storage temperature on maturation (acquisition of germinability) of cysts in sediments. Germination of cysts is not found at 10°C. Optimum temperature range is between 20 and 25°C for germination.

Maturation and dormancy of cysts of Chattonella are significantly affected by temperature. For maturation of cysts, low storage temperatures of 11°C or below is essential (Fig.4). No significant maturation is observed at storage temperature of 20°C or more.

In sediments freshly collected from Suo-Nada, marked seasonality of germinability

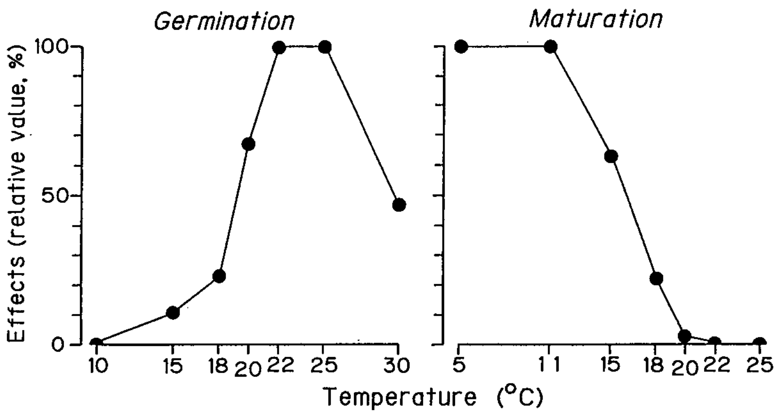

Fig.4. Effects of incubation temperature on germination of cysts, and of storage temperature on maturation of cysts in sediments. The ordinates show the percentage of maximum value obtained in experiments.

was confirmed in the cysts of Chattonella (Imai and Itoh, 1987). It was weak from autumn to early winter, then strengthened gradually up to a high level, which was maintained between spring and early summer, and again decreased rapidly during summer.

The annual life cycle of Chattonella (Fig.5), including vegetative and cyst phases, was summarized as follows: (1) vegetative cells in early summer originated from the germination of cysts in sediments; (2) they form cysts during the summer season; (3) the cysts spend a period of spontaneous dormancy until next spring; (4) the duration of post dormancy, an enforced dormancy due to low temperatures, follows until early summer (Imai and Itoh, 1987). The cyst populations, which remained in sediments and missed a chance of germination during summer, could be carried over to the next year through the ability of secondary dormancy (Imai et al., 1989). The life cycle of Chattonella is therefore well adapted to the temperature regime in temperate seas such as the Seto Inland Sea. And further, alternation of the life cycle between benthic and planktonic stage is presumably unconstrained by virtue of shallowness of the Seto Inland Sea (Fig.1)

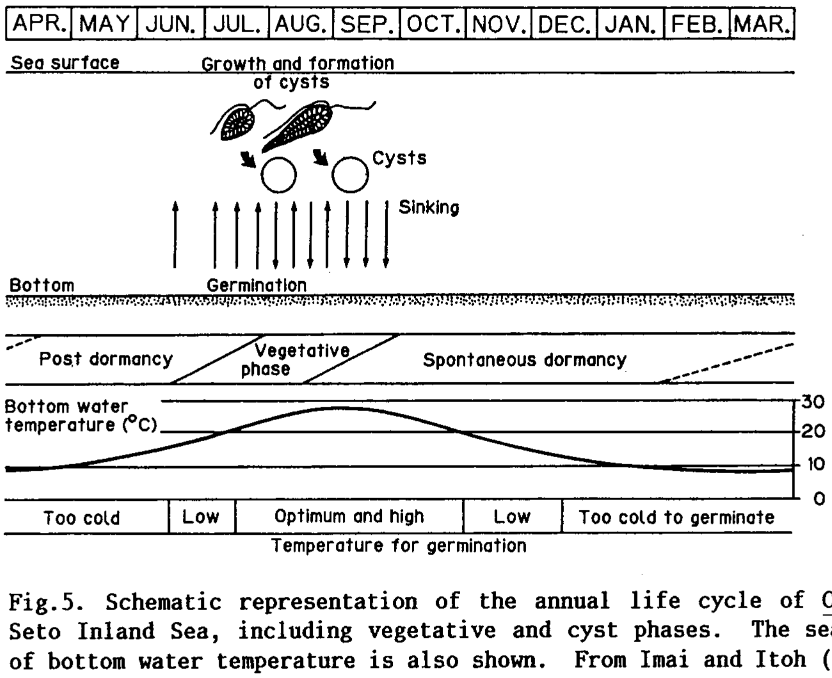

Fig.5. Schematic representation of the annual life cycle of Chattonella in the Seto Inland Sea, including vegetative and cyst phases. The seasonal fluctuation of bottom water temperature is also shown. From Imai and Itoh (1987)

Cysts and red tide

It is well known that in dinoflagellates resting cysts play an important role in initiating the blooms (Wall, 1971; Steidinger, 1975; Anderson and Wall, 1978; Dale, 1983; Sako et al., 1984). Possible functions of dinoflagellate cysts were well reviewed by Wall (1971, 1975). First, the cysts settle to bottom sediments to overwinter and thereby ensure persistent existence of the species in the same area. Second, the cysts need mandatory period for maturation, and so they act as timing device that control the time of repopulation. Third, since the cysts are resistan and durable to damage and predation, they aid in dispersal of the species. Hallegraeff et al. (1990) reported that microalgal species including toxic ones could be introduced from country to country through survival of cysts and spores in ship's ballast water. Since Chattonella marina and C.antiqua form cysts and overwintering of vegetative cells is impossible (Yamochi, 1984) in the Seto Inland Sea, the cysts of Chattonella presumably function as in the case of dinoflagellate cysts.

In Suo-Nada, germinability of cysts in sediments and occurrence of vegetative cells during summer were investigated together with environmental factors such as surface and bottom water temperatures (Imai, 1990). From the results of field observations, it was suggested that the cysts started to germinate from coastal shallow area where bottom temperature reached the optimum level (ca. 20°C) for germination in early June. Since bottom temperature rises gradually from coastal shallow area to deeper area, the germination of cysts presumably continue for rather long period. Long period germination is considered to be superior seeding strategy for Chattonella red tides, because Chattonella can take more chances for population development as a whole during the summer season.

Sequential biological phases of Chattonella including cyst phase and the factors influencing the red tide development are schematically represented in Fig.6. Cyst distribution is affected by tidal flow during cyst forming period, and winter wind may also affect cyst distribution in areas such as Suo- and Harima-Nada (Itoh and Imai, 1988). Bottom temperature has great effects on the germination of cysts as mentioned before. Resuspension of cysts caused by turbulence (due to bioturbation, tidal flow in bottom layer, etc.) may encourage germination of more cysts. Vegetative populations after the germination of cysts are affected by factors such as irradiation, nutrient level, competitors, predators, etc. Vegetative populations grow in surface water, and thereafter experience nutrient depletion. This may act as a trigger inducing cyst formation (Imai, 1989). Newly formed cysts are supplied to sea bottom. Cyst populations remained in sediments during summer which missed a chance of germination are induced into secondary dormancy and carried over to the next year together with new cysts (Imai et al., 1989).

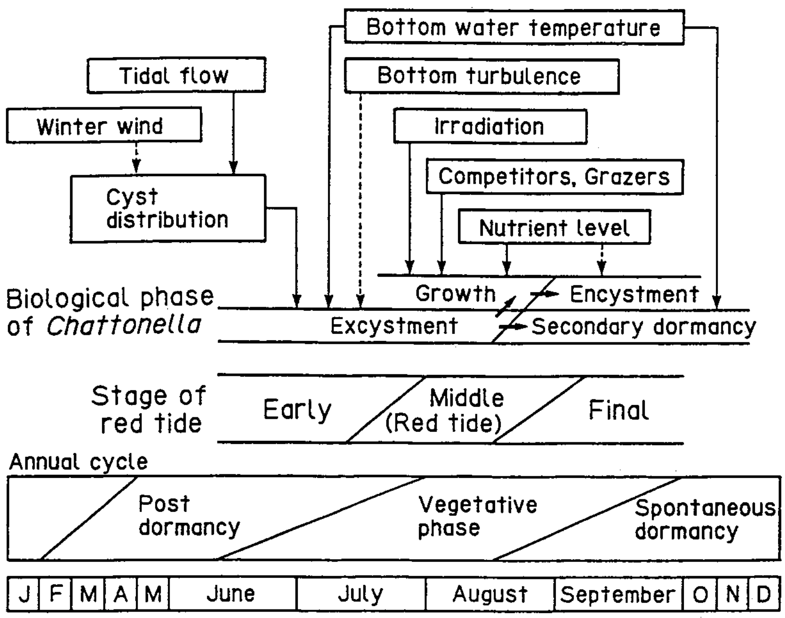

Fig.6. A schematic representation of sequential biological phase of Chattonella and the factors affecting the red tide development. From Imai (1990)

Since there is no feasible technique to exterminate Chattonella specifically at present, prediction of the red tide occurrences is essential to minimize damage of fish farming. For occurrence of Chattonella red tides, the cysts play a key role in seeding in the early stage of the red tides. It is therefore suggested that the monitoring of the rise of bottom temperature at shallow station located in dense seed bed from spring to summer is useful in predicting the appearance of vegetative populations. The vegetative growth is influenced by environmental factors, especially irradiation time in the early stage of the red tide (Imai, 1990). Meteorological conditions generally give much effects on changes in coastal environments. The efforts to utilize long-range and/or middle-range weather forecast may be hopeful trial in future for predicting the occurrences of Chattonella red tides in the Seto Inland Sea.

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