Effects of temperature, salinity and irradiance on the growth of the toxic dinoflagellate Gymnodinium catenatum (Dinophyceae) isolated from Hiroshima Bay, Japan

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ABSTRACT: Temperature and salinity ranges in which Gymnodinium catenatum (Hiroshima Bay strain) showed specific growth rates higher than 0.2/day were approximately 20–30°C and 20–32. The specific growth rate (µ) expressed as a polynomial equation as functions of temperature (T; °C) and salinity (S) were 

\[
\mu = (-6.84 \times 10^{-4}T^2 + 0.0354T - 0.213) \times (-1.03 \times 10^{-3}S^2 + 0.0579S - 0.548)/0.31;
\]

the maximum growth rate (0.31/day) was obtained at 25°C and 30. From a comparison with field data recording temperature, salinity and light intensity, this species may be expected to bloom from summer to autumn in Hiroshima Bay.

KEY WORDS: growth; Gymnodinium catenatum; irradiance, salinity, temperature.

INTRODUCTION

The chain-forming dinoflagellate Gymnodinium catenatum is known as a paralytic toxin producer among Gymnodinoid members. This species was first reported in the Gulf of California. The first bloom associated with paralytic shellfish poisoning (PSP) was reported in Spain in 1977, and the first incidence associated with human fatalities was reported from the Gulf of California in 1979. To date, the appearance of this toxic species has been reported from the coastal waters of Australia and Portugal. The increasing number of reports has been attributed to coastal eutrophication, increasing scientific awareness, transportation by ship ballast water and currents, and global warming.

In Japan, although this organism was first reported in Hiroshima Bay c. 35 years ago by Hada, there has not been any progress in the research of this species, with the exception of descriptions of several local PSP incidents. This is because the major PSP problems have been associated with the toxification of scallops by Alexandrium sp. in the northern part of Japan. However, the spread of G. catenatum to the southern part of Japan is obvious from several recent reports.

Despite its wide geographic distribution and serious impact on shellfish resources, data on the physiology of G. catenatum, which are required to help understand toxic events, are limited. Published experimental studies on this species mainly consider morphology and life history, cyst formation and germination, comparison of toxin profiles and changes in toxin composition with environmental conditions. Of these publications, only that of Blackburn et al. describes the species’ growth response characteristics to temperature and salinity. Experimental studies are necessary for future modeling work to clarify the population dynamics of G. catenatum in terms of species competition in natural assemblages.

In the present study, experiments were designed to elucidate the effect of temperature, salinity and irradiance on the growth of G. catenatum isolated from Hiroshima Bay, Japan, and the season and depth at which this species might occur in this bay is discussed.
MATERIALS AND METHODS

Strain and culture conditions

Gymnodinium catenatum was isolated from Hiroshima Bay in December 1997. Temperature and salinity at the site of sample collection were approximately 14°C and 31, respectively. The strain was washed during the log growth phase with sterile seawater by pipetting and the cells were then propagated using axenic methods. This procedure was repeated until almost no bacteria-like fluorescence was measured using a fluorometer (model 10-100R; Turner Designs, Mountain View, CA, USA). This method has been used frequently for this purpose as it has the advantage of not risking contamination by opening the cap to count the number of cells. The cap was, however, opened under axenic conditions (on a clean bench) once at the middle of the log-growth phase to check microscopically whether the cells had deformed as a result of non-optimal growth conditions.

To obtain a stable fluorescence value, samples were kept in a dark place for 10 min before measurement, and measurements were carried out in the laboratory in dim light. The test tubes were shaken gently but thoroughly to ensure that any chain-forming cells had dispersed and were distributed homogeneously because this species produces mucilage. To determine the amount of shaking required to achieve this, preliminary experiments were performed to determine the shaking intensity required. Measurements were conducted in triplicate. Data showing an obviously different trend from the other two were excluded from the calculation.

Specific growth rate (per day) was calculated using a least square regression on log-transformed in vivo fluorescence data during the exponential growth phase, which was determined using the following formula:

\[ \mu = \frac{1}{\Delta t} \ln \frac{N_t}{N_0} \]  

(1)

where \( N_0 \) and \( N_t \) are the initial and final in vivo fluorescence values of the exponential period (relative unit); and \( \Delta t \) is the period of exponential growth phase (day).

Curvilinear fitting was applied to create growth parameters as functions of temperature and salinity, using the statistical program package STATVIEW (SAS Institute Inc., Cary, NC, USA).

Light intensity experiments

Incubation of G. catenatum was carried out at 15°C, 30 and different light photon flux densities (PFD) of 10, 20, 30, 50, 75, 100, 200, 300 and 500 \( \mu \text{mol/m}^2 \text{per s} \) (measured with a QSL2100; Biospherical Instruments Inc., San Diego, CA, USA) were provided by cool-white fluorescent lamps using a 12 h : 12 h light/dark cycle. As described earlier, growth rate was calculated from the increase in fluorescence during the exponential growth phase. The incubation experiment was conducted in triplicate.

Equation 2 was fitted to the PFD and specific growth rate data. This equation is that of modific-
tion, in terms of including the compensation PFD, of the rectangular hyperbola by Lederman and Tett, which they originally proposed for the photosynthesis–irradiance curve for cases in which no photoinhibition occurs.

\[ \mu = \mu_m \frac{E - E_0}{(K_E - E_0) + (E - E_0)} \]  

(2)

where \( \mu \) is the specific growth rate (per day); \( \mu_m \) is the maximum specific growth rate (per day); \( E \) is the PFD (\( \mu \)mol/m\(^2\) per s); \( E_0 \) is the compensation PFD (\( \mu \)mol/m\(^2\) per s); and \( K_E \) is the PFD at \( \mu_m/2 \) (half-saturation light intensity).

**Field observations**

Field observations were carried out seasonally (11–14 January, 10–14 May, 2–6 August, and 4–8 November) in 1999 at 17 stations in Hiroshima Bay (Fig. 1). At all stations, temperature, salinity and light intensity were observed using a conductivity–temperature–depth system (SBE-9/11 plus; Sea Bird Electronics Inc., city, Seattle, WA, USA) with a spherical quantum meter (QSP-200L; Biospherical Instruments Inc.) at every 1 m depth. Data were averaged arithmetically for all stations in each season. The temperature and salinity data were then summarized in a temperature–salinity diagram, and the underwater light intensity data were plotted according to depth profiles. These field data were compared with the experimental data obtained in the present study to discuss the probable season and depth at which *G. catenatum* might form blooms.

**RESULTS**

**Effect of temperature and salinity on growth rate**

Individual growth curves of *G. catenatum* at different temperatures and salinities are shown in Fig. 2 and summarized in Fig. 3 as growth isoclines. At temperatures below 10°C, growth was not observed at salinities of 10–20. At salinities below 10, they did not grow even at 15°C, and neither at 15, 12.5°C nor at 20, 10°C. Moderate growth rates of approximately more than 0.2/day were obtained in the ranges of 20–30°C and 20–35. The maximum growth rate of 0.31/day was obtained at 25°C and 30.

Differences in chain length were observed along with differences in growth rate when cells were checked once during the course of cultivation. While they were usually long chains (sometimes 32 cells) at the optimum temperature and salinity conditions, they formed single, sometimes round-shaped, cells at non-optimum conditions of 10 and 15 at 7.5°C, 10 at 30°C, and 10 at 12.5°C.

From the results obtained in these experiments, the growth rate (\( \mu \)) was expressed using a polynomial equation as functions of temperature (\( T; ^\circ C \)) and salinity (\( S \)).

\[ \mu = (-6.84 \times 10^{-4} T^2 + 0.0354 T - 0.213) \]

\[ \times (-1.03 \times 10^{-3} S^2 + 0.0579 S - 0.548) / 0.31 \]  

(3)

The first and second terms of the right-hand side of the equation describe a convex parabola with the apex of the maximum growth rate (0.31) at 25°C and 30°C, respectively. This equation describes the observed values well (\( r=0.746, P<0.0001 \)).

**Effect of light on growth rate**

Cell growth of *G. catenatum* was observed at irradiance values of 20\( \mu \)mol/m\(^2\) per s and above. *Gymnodinium catenatum* did not grow at an irradiance value of 10\( \mu \)mol/m\(^2\) per s, at which cell density remained constant for 16 days. From the exponential growth phase, a hyperbolic equation was obtained as follows (Fig. 4):

\[ \mu = 0.13 (E - 10.0) / (E - 3.2) (r = 0.994) \]  

(4)
Effect of physical factors on the growth of G. catenatum

Fig. 2  Growth curves of Gymnodinium catenatum grown at various temperature and salinity conditions. Each symbol represents the average of triplicate data. pH 8.0 and 180 μmol/m² per s (cool-white fluorescent lamps, 12 h:12 h light/dark cycle).
From equation 4, the compensation PFD \( (E_c) \) was \( 10.0 \mu\text{mol/m}^2\text{ per s} \). The half-saturation PFD \( (K_E) \) and the maximum growth rate \( (\mu_m) \) were \( 16.8 \mu\text{mol/m}^2\text{ per s} \) and \( 0.13/\text{day} \), respectively. The \( \mu_m \) was low compared to that obtained at optimum temperature and salinity conditions \( (0.31) \), which was caused by the low experimental temperature set for this experiments \( (15^\circ\text{C}) \). However, this low value showed a good coincidence with that from temperature and salinity conditions of \( 15^\circ\text{C} \) and \( 30 \) in Fig. 3.

**Results from field observations**

The temperature and salinity data collected seasonally in 1999 from Hiroshima Bay are summarized as a temperature–salinity diagram in Fig. 5. The temperature and salinity ranges obtained from the four observations were approximately \( 12–28^\circ\text{C} \) and approximately \( 24.5–34.5 \), respectively, demonstrating that the temperature and salinity ranges set in the present series of experiments covers these ranges. Seasons were divided into two groups at temperature \( 20^\circ\text{C} \): winter and spring, and summer and autumn. Salinity tended to be low in the surface in summer due to stratification of the water column. Temperature and salinity ranges at which \( G.\text{catenatum} \) can grow at rates higher than \( 0.2/\text{day} \) \( (\text{approximately } 20–30^\circ\text{C} \text{ and } 20–32; \text{cf. Fig. 3}) \) were superimposed onto the temperature–salinity diagram. Most data points obtained during summer and autumn fell in this range.

The average light intensity just below the sea surface varied from approximately \( 800 \mu\text{mol/m}^2\text{ per s} \) in autumn and winter to approximately \( 2000 \mu\text{mol/m}^2\text{ per s} \) in summer in 1999 (Fig. 6). The
underwater light intensity decreased exponentially with depth, and the depth corresponding to the $E_0$ of $G. \text{catenatum}$ (10 µmol/m² per s) corresponded approximately to a depth of 26 m (range 19–28 m) in spring, 20 m (10–25 m) in summer, 17 m (9–23 m) in autumn, and 18 m (12–23 m) in winter. Although these observations were carried out between the hours 09.00 and 15.00 (not including data from early morning and late evening), in addition to the difference in the sampling time the underwater light intensity at any depths varied two- to threefold depending on the differences in the sampling locations (water quality) and the day’s weather (sunny or cloudy, no rainy days during the observations). Therefore, the seasonal change in the surface irradiance and the depth corresponding to the 10µmol/m² per s were not statistically significant.

**DISCUSSION**

The temperature range in which $G. \text{catenatum}$ (Hiroshima Bay strain) was able to grow was similar to that reported for the strain from Vigo, Spain (Table 1). Bravo and Anderson have reported that the growing temperature range of the Spanish strain was 22–28°C, and that the maximum growth rate of 0.37/day (0.53 divisions/day in the original paper) was observed at 24°C.15 However, the Tasmanian strain appears to grow better at lower temperature ranges. Blooms in Tasmanian waters occur between December and June when water temperature ranges from 12°C to 18°C and salinity ranges from 28 to 34,27 which is in keeping with data from the laboratory experiments of Blackburn et al.14 Conversely, the temperature range of 14–19°C reported by Fraga et al.29 for waters where this species appears in Spanish coastal waters is not consistent with the results of Bravo and Anderson.15 In subtropical regions, such as Palau, Venezuela and The Philippines, the growing temperature range is 23–29°C.29 These differences in the growing temperature range or optima along with salinity–growth characteristics indicate the importance of investigating conditions for individual strains.

Bravo and Anderson have observed germination of $G. \text{catenatum}$ cysts at temperatures ranging between 17°C and 29°C; the rate of success for germination was 75% at 25°C.15 These results for the conditions of cyst germination show good accordance with those found for the growth characteristics of vegetative cells in the present study. However, with the Tasmanian strain, up to 100% of germination was observed at 18°C,14 which is almost the lower temperature limit of germination for the Spanish strain. It is said that no mandatory dormancy period is needed for the germination of $G. \text{catenatum}$ cysts and this would be an important ecologic characteristic of this species.15 For

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**Table 1** Comparison of growing temperature and salinity ranges of various strains of Gymnodinium catenatum, and their optima (parentheses)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hiroshima Bay, Japan</td>
<td>20–30 (25)</td>
<td>20–32 (30)</td>
<td>Present study</td>
</tr>
<tr>
<td>Tasmania, Australia</td>
<td>14.5–20</td>
<td>23–34</td>
<td>Blackburn <em>et al.</em> (1989)²⁴</td>
</tr>
<tr>
<td>Vigo, Spain</td>
<td>22–28 (24)</td>
<td>–</td>
<td>Bravo and Anderson (1994)²⁵</td>
</tr>
</tbody>
</table>
example, compared to G. catenatum, A. tamarense needs 1 month at 22°C for cysts to mature. This suggests that whereas G. catenatum has a low growth rate (0.31/day in the present study), its bloom could last longer by forming cysts that can germinate faster, hence, recovering from any sub-optimal conditions they might encounter.

Figure 5 suggests that the temperature and salinity conditions from summer to autumn in Hiroshima Bay would be appropriate for the blooming of G. catenatum. In summer and autumn, the depths corresponding to the E0 of G. catenatum (10 µmol/m² per s) were equated to 17–20 m in summer and autumn. Even though the summer temperature (25°C) increased the value of E0 as a result of increased respiration, it would be, at most, three times after considering the general law of Q10. Conversely, the irradiance level at the surface in summer (average of 2000 µmol/m² per s, and up to 4900 µmol/m² per s at noon) is supposedly too strong for most phytoplankton species to perform normal photosynthesis, resulting in photoinhibition. Thus, the subsurface layer appears to provide the most suitable conditions for G. catenatum to reside. Data from Ogata et al., who have examined the relationship between light intensity and toxicity of the Senzaki Bay strain, have shown 0.2 divisions/day (=0.14/day in specific growth rate) even at light intensities below 1000 lux (approximately 14 µmol/m² per s) at 20°C. Below the depth of thermocline (5–8 m in summer), they can also exploit nutrients regenerated from the decomposition of organic matter. This strategy is quite similar to a genetically close species, Gymnodinium mikimotoi, which always forms blooms at the subsurface layer.

In Hiroshima Bay, blooms of G. catenatum so dense that they would damage the oyster industry have, fortunately, not yet occurred. However, the temperature, salinity and light fields during summer and autumn in Hiroshima Bay may provide suitable conditions for an outbreak of G. catenatum as discussed earlier. To understand the dynamics of this species, further physiological and ecologic studies are necessary, including both inorganic (some of which have been reported by Flynn et al. for the Spanish strain) and organic nutrient uptake, competition with other phytoplankton, and grazing by organisms of higher trophic levels, as well as those of many other abiotic factors.

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