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# COMPETITION BETWEEN THE PLANKTONIC ALGAE *SCENEDESMUS* STRAIN 170 AND *GOLENKINIA* SP IN UNI AND BIALGAL CULTURE[1]

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## ABSTRACT

*Scenedesmus* strain 170 and *Golenkinia* sp. were grown in uni and bialgal culture under different nutrient, light intensity and temperature regimes to determine competitive interactions between the two organisms. Total salt concentration (mg • $l^{-1}$) in the inorganic media used, ranged from 17 (dilute) to 170 (concentrated). The two algae were found to have similar growth rates in both concentrated and dilute media. *Golenkinia* sp. always favored higher light intensity (8000 lux) whereas, *Scenedesmus* strain 170 favored different light intensities, depending on the medium used. *Scenedesmus* strain 170 had a strong preference for lower temperatures (22° vs. 28°C) than *Golenkinia* sp.

Intrinsic differences in growth rates, determined in unialgal culture, held true in bialgal culture and accounted for most of the observed competitive interactions. However, distinct competition between the two algae for nutrients was observed in dilute but not concentrated media. In dilute medium, at 22°C and 8000 lux, *Scenedesmus* strain 170 outcompeted *Golenkinia* sp for nitrate. At 28°C and 8000 lux, *Golenkinia* sp outcompeted *Scenedesmus* strain 170 for phosphate. Application of the results to nature is discussed.

## INTRODUCTION

Phycologists have historically grown algae in culture media with an overabundance of nutrients, especially nitrogen and phosphorus (1). Some of these culture media (e.g. Bristol's medium) have 2 to 3 orders of magnitude more nutrients than are found in natural waters (1). Probably as a consequence of using concentrated media, phycologists have also historically worked with cell concentrations greater than those in nature. For example, many scientists use cell concentrations greater than $10^6$ cells • $ml^{-1}$ in their experiments, several orders of magnitude larger than typically found in nature ($10^3$-$10^5$ cells • $ml^{-1}$).

Laboratory findings using concentrated media and high cell numbers are questionable with rspect to their application to nature. Recent studies have shown that certain observed laboratory phenomena, using concentrated media, did not necessarily occur in nature (2, 3, 4). As a result, there has been a trend towards the use of more dilute media that more closely mimics natural waters (5). Chu (6) attempted to use dilute media but was forced to increase nutrient concentrations when he continued to use high cell numbers. Others lowered cell levels, but failed to obtain good growth because the cultures quickly became nutrient limited (7). More recently, the use of dilute media in combination with lower cell numbers, was more successful when used in combination with daily nutrient replenishment because the possibility of nutrient limitation was reduced (8, 2, 5).

The use of multialgal cultures in studying algal competition, especially its' application to nature, has not been developed to its' fullest (9). Laboratory experimentation utilizing multialgal cultures will show intrinsic differences in growth rates (9) and nutrient uptake patterns (10) between its' members as well as allelopathic interactions (11). It has been documented that algal growth patterns delineated in laboratory multialgal experiments also occur in the field, suggesting that multialgal cultures be incorporated in studies pertaining to algal seasonal successional patterns (9, 12).

In this study growth and competition between two common green algal phytoplankters, *Scenedesmus* strain 170 and *Golenkinia* sp were observed. The objectives of the study were to: (1) compare the growth of *Scenedesmus* strain 170 and *Golenkinia* sp in concentrated (25% Bristol's medium) and dilute (Harter's Dilute medium) media under different nutrient, temperature and light intensity regimes, (2) use bialgal cultures to study competition between the two organisms and determine the conditions that favor each species and (3) determine which medium (dilute or concentrated) was more appropriate to use in algal competition studies.

## MATERIALS AND METHODS

A xenic culture of *Scenedesmus* strain 170 was obtained from E.M. Swale (13). An axenic culture was established and stock cultures were kept in dim light (1000 lux using cool-white fluorescent bulbs), on modified Bristol's agar slants and liquid culture. An axenic culture of *Golenkinia* sp was obtained from L. Harter (9) and maintained in 25% Bristol's liquid medium at dim light. *Golenkinia* sp required vitamin $B_{12}$ for growth (9) which was added to the media (See Table 1). Cells were pretreated for experimentation as follows. Substocks were made by transferring 0.5 to 1.0 ml of liquid stock into 150 ml Harter's Dilute medium (9) and allowed to grow for 2 to 3.5 weeks at 1000 to 3000 lux. Inocula were prepared from the 2 to 3.5 week old substocks by transferring 1/3 volume of substock to 2/3 volume of fresh medium and diluting twice with equal volumes on succeeding days. Cells were allowed to grow for 4 to 5 days before they were used for experimentation.

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TABLE 1

*The composition of 25% Modified Bristol's Medium and Harter's Dilute Medium. An organic buffer is added to dilute medium.*

| NUTRIENT | CONCENTRATION ($mg \cdot l^{-1}$) | |
|---|---|---|
| | 25% Modified Bristol's | Harter's Dilute |
| $NaNO_3$ | 62.5 | 2.0 |
| $K_2HPO_4$ | 18.75 | 0.03 |
| $KH_2PO_4$ | 4.3 | — |
| $MgSO_4 \cdot 7H_2O$ | 18.75 | 1.0 |
| $NaSiO_3 \cdot 9H_2O$ | 10.0 | 10.0 |
| TRIS | — | 40.0 |
| Vitamin $B_{12}$ | 0.003 | 0.003 |
| $CaCl_2 \cdot 2H_2O$ | 6.3 | 2.7 |
| NaCl | 6.2 | — |
| $FeCl_3 \cdot 6H_2O$ | 1.25 | 0.1 |
| $MnCl_2 \cdot 4H_2O$ | 0.75 | 0.3 |
| $CoCl_2 \cdot 6H_2O$ | 0.005 | 0.02 |
| $CuSO_4$ | 0.0025 | 0.01 |
| $ZnSO_4 \cdot 7H_2O$ | 0.010 | 0.04 |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.005 | 0.02 |
| $Na_2EDTA \cdot 2H_2O$ | 1.725 | 0.69 |

The designations cells • $ml^{-1}$ and cell doublings • $day^{-1}$ refer to the number of cells per ml and the number of cell divisions per day respectively.

In all experiments 10 ml of the test medium were inoculated on day 1 with $3.5 \times 10^4$ cells • $ml^{-1}$ of each test organism (i.e., bialgal cultures contained a total of $7.0 \times 10^4$ cells • $ml^{-1}$). On the next four successive days, at the beginning of the light period, 5 ml of each culture were removed aseptically and replaced with 5 ml of fresh medium. This 1:1 transfer provided a daily replenishment of nutrients and maintained cell concentrations below $10^5$ cells $\cdot$ $ml^{-1}$. Cultures were analyzed for cell densities on day 5 using haemacytometers and the mean number of cell doublings • $day^{-1}$ were calculated using the $\log_2$ technique.

Stationary cultures were used for all experimentation. Cultures were grown on a 16 hour dark : 8 hour light cycle at either 4000 lux (low light intensity) or 8000 lux (high light intensity), using cool-white fluorescent illumination. Experiments were carried out at 22 ± 1 °C (low temperature) or 28 ± 2 °C (high temperature). At the end of an experiment each culture was tested for bacterial contamination by inoculating a nutrient broth/yeast extract mixture with 0.5 ml of the culture. This mixture was allowed to incubate for two weeks at 36 °C.

Modified 25% Bristol's medium (referred to as 25% Bristol's medium) and Harter's Dilute medium (9) were routinely used throughout the study (Table 1). Media were prepared fresh from stock solutions, autoclaved and allowed to equillibrate with the atmosphere for one day before use. For several experiments the amounts of nitrogen (as nitrate) or phosphorus (as phosphate) were increased by adding more stock solution.

Competition was investigated by comparing the growth of each organism in bialgal culture. Growth was compared in two ways: 1) The proportion of the total population for each species was directly compared. These comparisons indicated competition that may be a result of intrinsic growth rates and/or an actual effect of one alga on the other. 2) The final cell concentrations of each organism in bialgal culture were compared to growth in controls (unialgal culture). This comparison eliminated intrinsic differences in growth rates between the organisms, however, showed effects from one member of the bialgal culture on the other.

Differences in growth rates greater than 0.3 doublings • $day^{-1}$ were considered to be significant, since they resulted in at least a two-fold difference in final cell concentration over a five day period. Thus, in bialgal cultures where the final cell concentration of one alga was greater than 2 x the other, competition was said to occur.

## RESULTS

### *Growth Rates*

Effect of medium:

No considerable difference in growth rates for either *Scenedesmus* strain 170 or *Golenkinia* sp. (measured as doublings • $day^{-1}$) were found between 25% Bristol's and Harter's Dilute

TABLE 2

*The mean cell doublings per day for* Scenedesmus *strain 170 and* Golenkinia *sp. in unialgal and bialgal culture at various ight intensity/temperature regimes. Cultures were grown under a 16L:8D cycle and transferred 1:1 daily with fresh medium for five days. (n = 3 or greater).*

| TEMPERATURE LIGHT CONDITION* | SCENEDESMUS STRAIN 170 | | SCENEDESMUS STRAIN 170 WITH GOLENKINIA | | GOLENKINIA SP. | | GOLENKINIA WITH SCEMEDESMUS STRAIN 170 | |
|---|---|---|---|---|---|---|---|---|
| | 25% B | HD | 25% B | HD | 25% B | HD | 25% B | HD |
| HIGH TEMPERATURE HIGH LIGHT | 1.32 | 1.09 | 1.28 | 0.87 | 1.47 | 1.28 | 1.55 | 1.15 |
| HIGH TEMPERATURE LOW LIGHT | 0.86 | 0.96 | 0.64 | 0.88 | 0.98 | 0.85 | 1.05 | 0.79 |
| LOW TEMPERATURE HIGH LIGHT | 1.49 | 1.36 | 1.57 | 1.38 | 1.14 | 1.31 | 1.12 | 0.76 |
| LOW TEMPERATURE LOW LIGHT | 1.16 | 1.29 | 1.27 | 1.12 | 0.73 | 0.99 | 0.85 | 0.76 |

*High temperature = 28 °C, Low temperature = 22 °C; High light = 8000 Lux, Low light = 4000 Lux; 25% Bristol's medium = 25%B, Harter's Dilute medium = HD

TABLE 3

*The mean percentage of* Scenedesmus *strain 170 and* Golenkinia *sp. in bialgal culture. Comparisons are shown for 25% Bristol's and Harter's Dilute medium at various environmental conditions. Cultures were grown on a 16L:8D cycle and transferred 1:1 daily with fresh nutrients.*

| MEDIUM | 25% BRISTOL'S MEDIUM | | HARTER'S DILUTE MEDIUM | |
|---|---|---|---|---|
| EXPERIMENTAL CONDITION* | % SCENEDESMUS STRAIN 170 | % GOLENKINIA | % SCENEDESMUS STRAIN 170 | % GOLENKINIA |
| HIGH TEMPERATURE HIGH LIGHT | 32 | 68 | 26 | 74 |
| HIGH TEMPERATURE LOW LIGHT | 25 | 75 | 68 | 32 |
| LOW TEMPERATURE HIGH LIGHT | 77 | 23 | 81 | 19 |
| LOW TEMPERATURE LOW LIGHT | 76 | 24 | 65 | 35 |

*High Temperature = 28 °C, Low Temperature = 22 °C; High Light = 8000 Lux, Low Light = 4000 Lux

TABLE 4

*The mean percentage of the control cultures (i.e., unialgal cultures of* Scenedesmus *strain 170 or* Golenkinia *sp.) represented by each species in bialgal culture. For example, a 50% value means the organism greq half as much in bialgal culture as it did in the control vessel (unialgal culture). Values are shown for both 25% Bristol's and Harter's Dilute media at various environmental conditions. Cultures were grown on a 16L:8D cycle and transferred daily 1:1 with fresh nutrients.*

| MEDIUM | 25% BRISTOL'S MEDIUM | | HARTER'S DILUTE MEDIUM | |
|---|---|---|---|---|
| EXPERIMENTAL CONDITION* | % SCENEDESMUS STRAIN 170 | % GOLENKINIA SP. | % SCENEDESMUS STRAIN 170 | % GOLENKINIA SP. |
| HIGH TEMPERATURE HIGH LIGHT | 91 | 129 | 26 | 60 |
| HIGH TEMPERATURE LOW LIGHT | 56 | 125 | 95 | 50 |
| LOW TEMPERATURE HIGH LIGHT | 142 | 97 | 85 | 34 |
| LOW TEMPERATURE LOW LIGHT | 134 | 147 | 108 | 77 |

*High Temperature = 28 °C, Low Temperature = 22 °C; High Light = 8000 Lux, Low Light = 4000 Lux

media for all temperature/light conditions (Table 2). The greatest different in growth rates between the media were at high light/high temperature for *Scenedesmus* strain 170 (Δ 0.23 doublings • day$^{-1}$) and at low light/low temperature for *Golenkinia* sp. (Δ 0.26 doublings • day$^{-1}$). All other growth rate comparisons between Harter's Dilute and 25% Bristol's media had a difference of less than 0.19 doublings • day$^{-1}$.

Both *Scenedesmus* strain 170 and *Golenkinia* sp. were found to have a similar range in growth rates. The range of growth rates for *Scenedesmus* strain 170 was 0.86 to 1.49 doublings • day$^{-1}$ while *Golenkinia* sp. varied from 0.73 to 1.47 (Table 2). Growth rate comparisons between the organisms for the two media showed major differences. Except at high light intensity in the Harter's Dilute medium *Scenedesmus* strain 170 had a considerably higher growth rate than *Golenkinia* sp. (0.3 doublings • day$^{-1}$) in both media at low temperature (Table 2). Although not as significant, *Golenkinia* sp. seemed to favor 25% Bristol's medium at high temperature.

Effect of Light and Temperature:

Light intensity had a large effect on the growth rates of both organisms (Table 2). For *Golenkinia* sp., lower light intensity (4000 lux) caused a reduction from between 0.32 to 0.49 doublings • day$^{-1}$. The same low light intensity caused a reduction in the doublings • day$^{-1}$ rate of greater than 0.33 for *Scenedesmus* strain 170 only in 25% Bristol's medium. However, in Harter's Dilute medium there was no difference in the growth rates between high and low light intensity (i.e. less than Δ 0.13 doublings • day$^{-1}$).

Temperature was found to have a significant effect on the growth rates of both organisms (Table 2). *Scenedesmus* strain 170 showed a strong preference for the lower temperature (22 °C) for each experimental condition. For example, at low light levels in Harter's Dilute and 25% Bristol's media there were, respectively, 0.35 and 0.3 more doublings • day$^{-1}$ at 22 °C. *Golenkinia* sp. favored the higher temperature (28 °C) when grown in 25% Bristol's medium, however, had no temperature preferences when tested in Harter's Dilute medium.

*Competition Between Scenedesmus strain 170 and Golenkinia sp. in Bialgal Culture*

There are considerable differences between the final cell concentrations for *Scenedesmus* strain 170 and *Golenkinia* sp.

when grown in bialgal culture (Table 3). In general, after five days, *Scenedesmus* strain 170 outnumbered *Golenkinia* sp. 3:1 for every experimental condition associated with low temperature. At low temperature in Harter's Dilute medium at 8000 lux, the concentration of *Scenedesmus* strain 170 was four times *Golenkinia* sp. At high temperature, the situation was reversed where *Golenkinia* sp. had significantly greater cell concentrations at every experimental condition except low light intensity in Harter's Dilute medium.

The differences in final cell concentrations of the two algae in bialgal culture were reflective of the differences in growth rates between the two algae (compare Table 2 and 3). For example, in Harter's Dilute medium at low temperature/high light intensity, *Scenedesmus* strain 170 had 0.62 doublings • day$^{-1}$ more than *Golenkinia* sp. As a consequence, after a five day period, the population of *Scenedesmus* strain 170 was over 4 times that of *Golenkinia* sp. The question of whether the competition advantages of *Scenedesmus* strain 170 and *Golenkinia* sp. at low and high temperature respectively, were merely a reflection of intrinsic differences between the organism's growth rates or the result of a direct inhibition of one alga on ther other was investigated. This was determined by comparing the growth of each organism in bialgal culture to that in the controls i.e., growth of each alga separately (Table 4). If one alga grew 2 or 3 times more in the controls than in bialgal culture it would be reflected by a value of 50% or 33%, respectively in Table 4.

### *Competition for Nitrogen and Phosphorus in Bialgal Culture*

Competition between *Scenedesmus* strain 170 and *Golenkinia* sp., when grown together in Harter's Dilute medium, was further investigated by varying the concentrations of nitrogen and phosphorus (Table 5). At 28 °C, reduced growth rates for *Scenedesmus* strain 170 in bialgal culture were not changed by increasing the levels of nitrogen. However, by increasing the concentration of phosphorus three fold, *Scenedesmus* strain 170 grew the same in bialgal culture as in the controls (Table 5). On the other hand, reduced growth rates noted for *Golenkinia* sp. at low temperature (in bialgal culture) were not changed by adding phosphorus. The addition of nitrogen did stimulate equal growth rates for *Golenkinia* sp. in bialgal culture as in the control (Table 5).

## DISCUSSION

The use of dilute media, with inorganic salt levels resembling nature, to grow microalgae, has only recently been successful (14, 5). It was determined that growth rates for microalgae using dilute media, in combination with low cell numbers and daily transfer, were similar to rates obtained using concentrated media (14). In this study, both *Scenedesmus* 170 and *Golenkinia* sp. were found to have similar growth rates in 25% Bristol's and Harter's Dilute media, supporting the use of dilute media in algal research. In addition, several cases of competition for nutrients were observed in Harter's Dilute media that were not observed in 25% Bristol's medium. This added difference leads us to conclude that future studies on microalgae, especially those that will compare laboratory and field results, should use dilute media.

As expected, higher light intensities yielded a considerable increase in growth rates. The average growth rates for *Golenkinia* sp at high and low light levels were 1.30 and 0.89 doublings • day$^{-1}$, respectively. *Scenedesmus* strain 170 had a similar increase (0.91 to 1.21 doublings • day$^{-1}$) in response to increased light intensities for experiments in 25% Bristol's medium. In Harter's Dilute medium, however, *Scenedesmus* strain 170 grew the same at both high and low light levels. This was unexpected and suggested that perhaps growth maxima for *Scenedesmus* strain 170 in Harter's Dilute medium exists over a broad range of light intensities.

Greater cell doubling rates for *Scenedesmus* strain 170 and *Golenkinia* sp have been recorded (4, 9), but are probably a result of higher light intensities. Moss (15) found maximal growth rates between 0.4 and 0.8 doublings • day$^{-1}$ for several green microalgae. The growth rates measured by Moss who used low light intensities (2000-4000 lux), are comparable to those found in this study at similar light intensity.

Chlorophycean algae are known to grow best at temperatures between 20 °C and 30 °C (16). In temperate lakes, such a temperature range usually spans from late spring to mid-summer. In addition, late spring is a critical period in the seasonal succession of algae since diatom populations decline and are usually replaced by green algae. In succeeding summer months, several different green algal populations may oscillate, depending on slight temperature shifts. Thus, temperature optima are a major consideration in algal successional patterns.

TABLE 5

*The effect of nitrogen and phosphorus concentration on the mean doublings · day$^{-1}$ of* Scenedesmus *strain 170 and* Golenkinia *sp. in control and (unialgal culture) and bialgal cultures. Values for* Scenedesmus *strain 170 at high temperature/high light, and for* Golenkinia *sp. at low temperature/high light are shown. All cultures were grown in Harter's Dilute medium on a 16L:8D cycle and transferred daily 1:1 with fresh nutrients.*

| | High Temperature/High Light | | Low Temperature/High Light | |
|---|---|---|---|---|
| mg · l$^{-1}$ | SCENEDESMUS STRAIN 170 (CONTROL) | SCENEDESMUS STRAIN 170 WITH GOLENKINIA SP. (BIALGAL CULTURE) | GOLENKINIA SP. (CONTROL) | GOLENKINIA SP. WITH SCENEDESMUS STRAIN 170 BIALGAL CULTURE |
| $NO_3$ | | | | |
| 1.5 | 1.00 | 0.70 | 1.31 | 0.76 |
| 4.4 | 1.20 | 0.47 | 1.44 | 1.40 |
| 18 | 1.20 | 0.50 | 1.34 | 1.32 |
| $PO_4$ | | | | |
| 0.1 | 1.00 | 0.70 | 1.31 | 0.76 |
| 0.7 | 1.18 | 1.14 | 1.23 | 0.95 |
| 2.8 | 1.16 | 1.04 | 1.32 | 0.95 |

*High Temperature = 28 °C, Low Temperature = 22 °C; High Light = 8000 Lux

In this study a slight temperature shift (6 °C) was found to have a significant effect on the growth of *Scenedesmus* strain 170 and *Golenkinia* sp. As previously found (4), *Scenedesmus* strain 170 grew well at 22 °C while *Golenkinia* had a distinct preference for the higher temperatures (9). Since growth at all temperatures were not examined, further study may show that the actual temperature optima for each taxa may be slightly different. Nonetheless, *Scenedesmus* strain 170 may, at least partially, have a competitive advantage in late spring or early summer and give way to *Golenkinia* sp. during mid and late summer.

The use of bialgal cultures indicated distinct competition between the two green algae. By comparing the proportions of each species in the bialgal cultures after a five day period, it was found that *Scenedesmus* strain 170 outcompeted *Golenkinia* sp. at 22 °C under all conditions tested. When the temperature was increased to 28 °C, the situation was reversed, with *Golenkinia* outcompeting *Scenedesmus* strain 170 (Table 3). The question arose whether the competitive advantages of *Scenedesmus* strain 170 and *Golenkinia* sp. at low and high temperatures respectively, were merely a reflection of differences between intrinsic growth rates or the result of a direct effect of one alga on the other (i.e., release of allelopathic substances or differences in nutrient uptake strategies). To test this difference, the growth of each organism in bialgal culture was compared to growth in a control vessel, i.e., unialgal culture (Table 4). It was found that under most conditions the growth rates of each organism in bialgal culture were similar to their controls, indicating that differences in intrinsic growth rates of the organisms accounted for observed competition. In two instances, however, growth in bialgal culture was considerably less than in the controls. In Harter's Dilute medium at high temperatures and high light intensity, *Scenedesmus* strain 170 grew 4 times more in the control than in bialgal culture, and at low temperature and high light intensity, *Golenkinia* sp. grew much less in bialgal culture than in the controls. In preliminary results, the growth of *Scenedesmus* strain 170 in media made from *Golenkinia* sp. culture filtrate (and vice versa for *Golenkinia* sp.) was similar to the control. Thus it is believed that the reduced growth rates found in bialgal cultures were not the result of allelopathic substances.

The role individual nutrients (nitrogen and phosphorus) played in the direct competition between the two algae in Harter's Dilute medium was tested (Table 5). It was found that by raising the nitrogen concentration, the growth rate of *Golenkinia* sp. in bialgal culture was no longer lower than in the controls. Similar results were not obtained when phosphorus levels were raised. This suggested that at low temperature, *Scenedesmus* strain 170 outcompeted *Golenkinia* sp. in Harter's Dilute medium by removing nitrate more effectively (Table 5). For *Scenedesmus* strain 170 in Harter's Dilute medium at high temperature, the situation was reversed where it competed more successfully with *Golenkinia* when the phosphorus concentration was increased. This suggested that phosphorus was limiting to *Scenedesmus* strain 170 in bialgal culture in Harter's Dilute medium at high temperature.

Similar competition as found at high light intensities were not observed under low light conditions. This may have been a result of the time duration of the experiment. Perhaps, at low light levels, where reduced growth rates occured, more than five days were needed to observe similar results.

In summary, competition between *Scenedesmus* strain 170 and *Golenkinia* sp. was found to be dependent on nutrient levels in dilute media, light intensity and temperature. The use of bialgal cultures predicted the environmental conditions necessary for dominance of each species. On the basis of this research, *Scenedesmus* strain 170 would be expected to outcompete *Golenkinia* sp. in late spring and early summer when temperatures are in the low 20's especially if nitrogen levels are minimal. As water temperatures increase (mid to late summer), *Golenkinia* sp would be favored over *Scenedesmus* strain 170, especially under phosphorus limitation. The next phase of this research will test the application of the above hypothesis by growing the organisms in bialgal culture in nature.

In the laboratory experiments, even though one species would dominate after five days, both taxa coexisted. The question remains whether total exclusion of one alga would occur given a longer culture time. Total exclusion of one species would be in accordance with the competitive exclusion principle (18). However, because of seasonal as well as daily fluctuations, coexistance is often found in nature (17, 9). Thus, one species may be found to comprise a large portion of the phytoplankton community but yet, not completely exclude the other species. A similar situation may be found between *Scenedesmus* strain 170 and *Golenkinia* sp.

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