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GROWTH OF THE FILAMENTOUS GREEN ALGA *CTENOCLADUS CIRCINNATUS* (CHAETOPHORALES, CHLOROPHYCEAE) IN RELATION TO ENVIRONMENTAL SALINITY[1]

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ABSTRACT

Clones of the filamentous green alga Ctenocladus circinnatus *Borzi were isolated from algae collected at Abert Lake (Oregon) and Mono Lake (California). Stock cultures were exposed to varied salinities of natural lake water to examine the effects on growth rate, cell form, chlorophyll* a, *and water content. Growth rates were reduced in both clones with increased salinity over the range 25–100 g·L^{-1} and were almost completely inhibited at 150 g·L^{-1}. Chlorophyll* a *increased between salinities of 25 and 100 g·L^{-1}, reflecting slower growth, higher proportions of akinetes, and smaller cell sizes as salinity increased. Tissue water content remained essentially constant from 25 to 100 g·L^{-1} salinity. Shorter cell dimensions with increased salinity suggest that a lower surface-to-volume ratio may reduce the potential for passive loss of cell water. Prior acclimation of stock cultures to elevated salinity provided no enhancement of growth response at any salinity. The results indicate that environmental salinity can limit the productivity and distribution of* Ctenocladus *in nature.*

Key index words: algal physiology; Abert Lake; benthic algae; Chlorophyta; Ctenocladus circinnatus; *Mono Lake; salinity tolerance; periphyton*

The filamentous green alga *Ctenocladus circinnatus* Borzi is known primarily from western North America and Asia (Blinn and Stein 1970) and lives in saline inland waters and coastal brine pools. Vegetative cell growth in *Ctenocladus* occurs in the form of branching filaments that produce terminal thick-walled akinetes, often in chain-like rows. Formation of the dormant akinetes is induced by unfavorable conditions, and this stage is resistant to damage from freezing, desiccation, or saturated brines. Asexual reproduction occurs under favorable conditions by the release of motile zoospores, which settle on the substrate to form new filaments. Although isogamous sexual reproduction has been described (Smith 1950), Blinn (1970) concluded that akinete formation was the most important life history feature contributing to the persistence of *Ctenocladus. Ctenocladus* usually exhibits an attached epilithic growth habit, but germination of free-floating akinetes, zoospores, or detached vegetative cells often leads to radial growth of filaments that eventually form floating or loose benthic balls. These wash ashore and are often seen along the margins of salt lakes and ponds.

Previous experimental studies of environmental factors influencing *Ctenocladus* have either employed only akinete germination as a bioassay (Blinn 1971) or used solutions of NaCl rather than natural source waters to examine growth (Ruinen 1933). Our study extends these earlier investigations by constructing vegetative growth curves for different clones of *Ctenocladus* in varied salinities of natural waters from permanent, alkaline lakes.

Seasonal observations of *Ctenocladus* at Abert Lake (Oregon) and Mono Lake (California) suggested that substantial differences in the abundance of this alga existed between these lakes (Herbst 1988). At Abert Lake, floating balls and thick mats of *Ctenocladus* attached to rock surfaces cover a major portion of the shoreline and littoral lake bottom. At Mono Lake, only sparse tufts were present, often in shaded and protected crevices of calcareous tufa rock. At the time of these observations (spring–summer 1983), Mono Lake was more saline (83 g·L^{-1}) than Abert Lake (25 g·L^{-1}), although both are alkaline (pH = 10) sodium chlorocarbonate–type lakes. Earlier collections of attached *Ctenocladus* from Mono Lake (in 1968, D. W. Blinn, pers. commun.) indicate the alga was easier to locate and more abundant during this period of lower salinity (ca. 70 g·L^{-1}). These observations suggested the hypothesis that *Ctenocladus* productivity and distribution is limited by increasing environmental salinity. The objective of the research reported here is to test this hypothesis by comparing algal growth over a range of salinities using clones derived from Mono and Abert lakes.

MATERIALS AND METHODS

Tufts of *Ctenocladus* were collected from rock substrates at both Abert and Mono lakes in spring 1983. Clones were isolated as single filaments using sterile forceps to pull a free strand repeatedly over a sterile 1.5% agar surface to remove adhering algae.

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Single filaments cleaned of such contaminants were cut out of the agar in small blocks and dropped into filtered source lake water (Whatman GF/A) to establish a series of stock clone cultures from each lake. Stock cultures derived from a single clone from each lake were used as the source inoculum for all experiments. These were maintained in filtered source water adjusted to a specific gravity of 1.030 at 20° C (ca. 40 $g \cdot L^{-1}$) prior to inoculation into treatment salinities. Separate subcultures of these clones were also transferred into lake water adjusted to a specific gravity of 1.050 (ca. 65 $g \cdot L^{-1}$) and were acclimated at this higher salinity for 35 days prior to use in experimental cultures.

Treatment salinities of 25, 50, 75, 100, and 150 $g \cdot L^{-1}$ were prepared from the waters of both lakes by evaporation under vacuum at low temperatures (30–40° C in a rotary evaporator) or by dilution with distilled water. The salinity range selected covers the natural range of variability found in Mono and Abert lakes over the past 50–100 years (Herbst 1988). Major ion chemistry of the two lakes is quite similar, both being of the sodium chlorocarbonate type, and ion proportions remain constant over the range of salinities examined. Final salinity was adjusted to the specific gravity corresponding to the desired total dissolved solids concentration. All culture media were filtered through GF/A filters to remove potential contaminating algae and were enriched with sterile stock solutions prior to partitioning into replicate culture flasks. Enrichment consisted of primary nutrients (1.76 mM $NaNO_3$, 0.28 mM Na_2HPO_4), trace elements (chelated 2:1 with ethylenediaminetetraacetate; in μM: 1.8 Fe, 7.3 Mn, 0.8 Zn, 0.3 Cu, 2.0 Mo, 0.2 Co), and vitamins (in $\mu g \cdot L^{-1}$: 250 thiamine, 5 biotin, 2.5 cyanocobalamine).

Tufts of algae from stock clonal cultures were homogenized into a uniform suspension for inoculation by repeatedly drawing into and expelling algae from a 10-mL syringe. This well-mixed suspension of intact filament cells and akinetes was then immediately inoculated as 1.0-mL aliquots into three 50-mL replicates of each experimental culture treatment. Culture flasks were placed at random over a white background sheet beneath a bank of 40-W cool-white fluorescent tubes. The light field varied from 60 to 90 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ at the culture level. Flasks were periodically rearranged to ensure that light availability was uniform (although illumination levels were probably saturated). Light was maintained on a 14:10 h LD photoperiodic cycle, and temperature was held at 14° C.

Growth yields of *Ctenocladus* were determined over a 6-month period. Harvesting consisted of vacuum-filtering flask contents through a pre-tared Whatman GF/A filter. The filters were then rinsed with 150 mM ammonium formate (an isotonic rinse that volatilizes completely) to remove any adhering salts and weighed after drying 48–72 h at 55° C. Small subsamples of algae were taken from each flask prior to harvest to determine the relative frequency of vegetative cells and akinetes. These subsamples were homogenized, dispersed on a slide, and 250–500 cells were counted in random fields of view (at 400×), noting cell type and general morphological condition.

At the final harvest, rinsed algal filters were held on vacuum until they appeared free of excess water and then were gently blotted on absorbant tissue paper to remove adhering water. The weight difference between the fresh-harvested filter and the dried filter weight (corrected for the wet weight of the filter disk) was taken to represent the total water remaining in algal cells. Whole filters (dry) were extracted in 10-mL volumes of alkaline ($MgCO_3$ added) 90% acetone in a ground-glass homogenizer, stored 24 h at 4° C in darkness, and centrifuged. The optical density of the supernatant was read at 750 (turbidity correction) and 663 nm.

To examine whether chlorosis observed in Abert Lake *Ctenocladus* at low salinity was related to nutrient limitation, the final set of three replicate flasks at 25 $g \cdot L^{-1}$ Abert Lake water were re-enriched with either NO_3, PO_4, or both, complete with trace elements and vitamins. Chlorophyll *a* levels in these cultures were then compared at the final harvest.

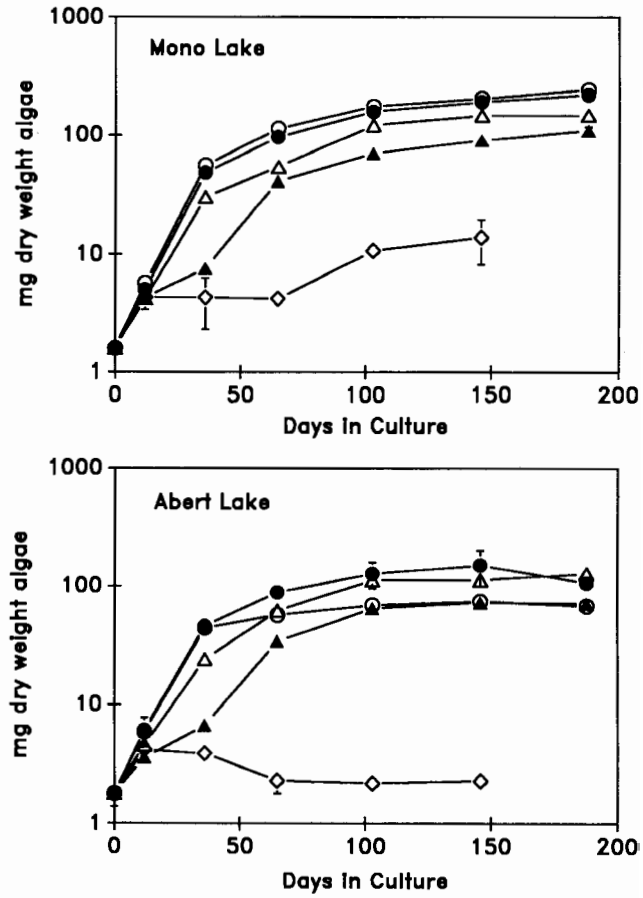

FIG. 1. Growth of Mono Lake and Abert Lake *Ctenocladus* clones in relation to salinity of Mono Lake and Abert Lake water, respectively. Salinities in $g \cdot L^{-1}$: 25 (○), 50 (●), 75 (△), 100 (▲), 150 (◇). n = 3 for each data point; bars indicate ± 1 SD (unless smaller than data point symbol).

The influence of lake source, salinity, and acclimation on final biomass yield was examined in cultures after 5 months of growth. Replicates from stock and acclimated cultures derived from both lakes and grown at salinities of 50, 100, and 150 $g \cdot L^{-1}$ were compared using analysis of variance.

RESULTS

Increasing salinity of lake source culture water reduced vegetative growth of *Ctenocladus* clones from both Mono and Abert lakes (Fig. 1). Growth occurred up to 100 $g \cdot L^{-1}$, comparable to the results of Ruinen (1933), and was almost completely inhibited at 150 $g \cdot L^{-1}$. Exponential growth rates (occurring over the initial growth period of about 1 month) decreased over the range 25–75 $g \cdot L^{-1}$, with algal growth rates declining from 1.2–1.5 $mg \cdot d^{-1}$ at 25 and 50 $g \cdot L^{-1}$ to about half this rate, 0.6–0.8 $mg \cdot d^{-1}$ at 75 $g \cdot L^{-1}$, with no exponential growth apparent at 100 $g \cdot L^{-1}$ (Table 1). Maximum yield also decreased in increasing salinities, with the exception of the Abert clone at 25 $g \cdot L^{-1}$. At this low salinity, despite initially high growth rates, the growth period of this clone was abbreviated, and the cultures

TABLE 1. *Growth characteristics, chlorophyll* a, *and water content of* Ctenocladus *clones at different salinities. Exponential growth calculated over early culture period (0–36 d) for cultures showing linear increase in log log plots (Figs. 1, 2). Sample n = 3.*

Clone source	Salinity ($g \cdot L^{-1}$)	Exponential growth rate ($mg \cdot d^{-1}$)	Maximum mean yield (mg dry wt)	Chlorophyll content [SE] (μg chlorophyll $a \cdot mg^{-1}$)	% water content [SE]
Mono Lake	25	1.50	244.2	0.46 [0.05]	88.7 [0.5]
	50	1.29	220.5	0.55 [0.03]	89.1 [0.5]
	75	0.79	147.4	1.33 [0.22]	88.4 [0.4]
	100	[a]	109.7	4.35 [0.01]	88.0 [0.7]
	150	[a]	[a]	[a]	[a]
Abert Lake	25	1.18	74.4	0.44[b] [0.05]	90.6 [0.9]
	50	1.23	150.8	1.44 [0.04]	85.8 [0.7]
	75	0.62	127.5	2.10 [0.04]	84.7 [0.5]
	100	[a]	72.7	3.71 [0.64]	85.8 [0.3]
	150	[a]	[a]	[a]	[a]

[a] Growth unmeasurable.

[b] Shows chlorophyll at penultimate harvest. Selective enrichment of this treatment before the final harvest did not alter yields but changed chlorophyll as follows: complete, 1.89; N, 1.70; P, 0.50 (same units as above).

became increasingly chlorotic with age. Final yield was only equivalent to that found at 100 $g \cdot L^{-1}$, the highest salinity permitting growth.

Prior acclimation of algae at higher salinity (65 $g \cdot L^{-1}$) than that in stock cultures (40 g L^{-1}) produced no significant differences in yield (Table 2). With or without acclimation, yields at 100 $g \cdot L^{-1}$ were about half those found at 50 $g \cdot L^{-1}$, and essentially no growth occurred at 150 $g \cdot L^{-1}$. Nonetheless, differences in growth between the population sources do suggest that genetic variation for growth response to salinity exists. The Mono clone consistently showed greater growth than the Abert clone at comparable salinities in native lake waters (Tables 1, 2).

Both absolute and relative amounts of chlorophyll *a* increased in clones from either population as the culture medium salinity increased from 25 to 100 $g \cdot L^{-1}$ (Table 1). Observations of cell form suggest that chlorophyll accumulated in small, slow-growing cells at higher salinity and diminished in elongate, fast-growing cells at dilute salinity. For the Abert Lake clone at 25 $g \cdot L^{-1}$, however, less chlorophyll was formed as compared to 50 $g \cdot L^{-1}$, despite the fact that growth rates were higher and yield greater at 50 $g \cdot L^{-1}$ (Fig. 1, Table 1). Nutrient enrichment was equivalent for all salinities initially and far in excess of ambient levels of dissolved nitrogen in lake waters prior to enrichment. Supplemental enrichment of the final set of flasks containing chlorotic Abert algae at 25 $g \cdot L^{-1}$, with either nitrate or complete enrichment, resulted in substantial increases in chlorophyll *a*, but addition of phosphate had no effect (Table 1).

TABLE 2. Ctenocladus *growth yields (21 weeks in culture) with and without acclimation to elevated salinity and analysis of variance for source, salinity, and acclimation effects and interactions. Algal inoculum was derived from a stock medium (40 $g \cdot L^{-1}$) or an acclimation medium (65 $g \cdot L^{-1}$) of native late water. Mean and [SE] for n = 3.*

Source	Salinity ($g \cdot L^{-1}$)	Stock	Acclimated
Mono Lake	50	191.8 [4.3]	188.1 [3.3]
	100	90.5 [4.7]	106.1 [16.1]
	150	13.8 [5.6]	15.1 [0.6]
Abert Lake	50	150.8 [50.2]	104.1 [3.0]
	100	72.7 [3.3]	68.6 [2.2]
	150	2.3 [0.2]	2.6 [0.6]

ANOVA table

	df	*F* ratio	*P*
Main effects			
A) Lake	1	42.10	0.000
B) Salinity	2	273.05	0.000
C) Acclimation	1	1.41	0.246
Interactions			
AB	2	8.08	0.002
AC	1	4.07	0.054
BC	2	3.35	0.051

The proportion of akinetes versus vegetative cells changed at the different culture salinities (Fig. 2). Zoosporangia were infrequently observed and so were not included in counts of cell type. Experimental cultures consisted almost entirely of vegetative cell filaments during early growth phase, except at 150 $g \cdot L^{-1}$, where the proportion of akinetes remained essentially the same as in the inoculum (30–50%). This indicates that virtually all akinetes from the inoculum (at either stock or acclimation salinity) germinated at 25–100 $g \cdot L^{-1}$, while those at 150 $g \cdot L^{-1}$ remained dormant (consistent with the results of Blinn 1971). Vegetative cells were swollen, empty (no chloroplast visible), and apparently dead. As reported by Blinn and Stein (1970), we also observed a decrease in the length of filament cells with increasing salinity. As growth rates declined with age in culture, akinetes began to form. This process also appeared to be accelerated by increasing salinity. Higher proportions of akinetes formed in the Abert clone (terminating filament growth) may have accounted for lower growth rates and yields compared to the Mono clone. In lower salinity cultures (25 and 50 $g \cdot L^{-1}$) of the Mono clone, some *Ctenocladus* occurred as an unusual cell type. Though not having the heavy walls of akinetes, these cells were similar in shape (spherical) and size (slightly smaller) and had dense dark-green contents (possibly unger-

minated zoospores or incipient akinetes?). Due to difficulty in distinguishing these cell differences, proportions are not reported for these treatments beyond the time that this other cell form appeared.

Water accounted for 85–90% of the wet weight of *Ctenocladus* (Table 1). Over the 25–100-g·L^{-1} salinity range, water content was unchanged in the Mono clone (ca. 88–89%) but decreased from 90% at 25 g·L^{-1} to about 85% at each of the higher salinities in the Abert clone. This may be associated with the relative proportions of cell types rather than salinity effects per se, because the Abert clone consisted entirely of vegetative cells at 25 g·L^{-1} and 14–30% akinetes at 50–100 g·L^{-1} (Fig. 2). The thick-walled, dense cell contents of akinetes probably contain less water than the large, vacuolated spaces of vegetative cells.

Acclimated algae showed no significant growth enhancement compared to stock cultures of algae from either lake (Table 2). The main effects of the treatments were highly significant differences in yield due to salinity and lake source. A significant lake source × salinity interaction suggests the Mono clone may be more sensitive to salinity increase over the range 50–100 g·L^{-1}.

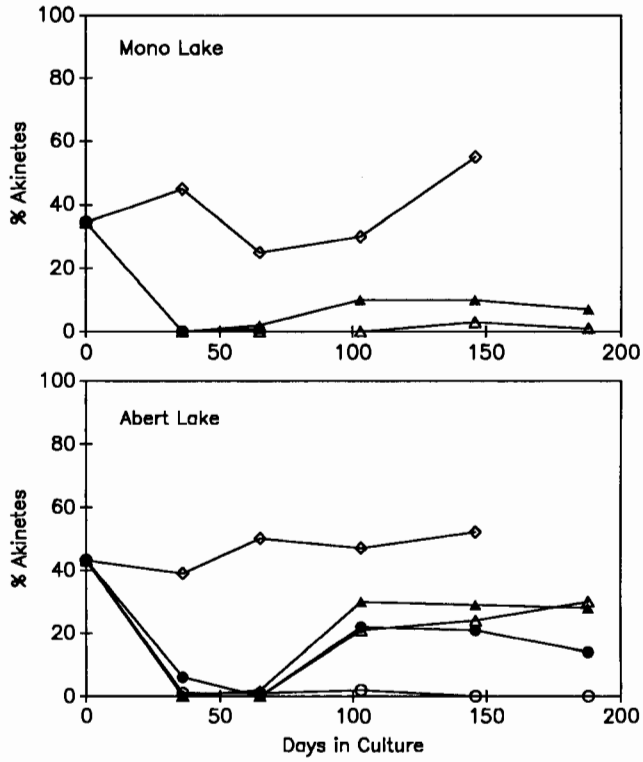

FIG. 2. Changing percentage of *Ctenocladus* cells that are dormant akinetes (versus vegetative filament cells) over the time course of culture growth for the Mono Lake clone (upper panel) and Abert Lake clone (lower panel). Unreplicated counts of 250–500 cells from subsamples of each treatment. Symbols for salinity treatments as in Figure 1.

DISCUSSION

The inhibition of growth by increased salinity shown in our experiments agrees with the hypothesis that productivity of *Ctenocladus circinnatus* in nature depends on salinity. Growth rates of clones from Mono Lake and Abert Lake declined progressively from 25 to 100 g·L^{-1} and ceased completely at 150 g·L^{-1}. The threshold limit for both vegetative growth and akinete germination was between 100 and 150 g·L^{-1}. Although low salinity limitations were not examined, yield and chlorophyll *a* content were reduced at 25 g·L^{-1} in the Abert Lake clone, possibly due to depletion of nitrate in batch cultures or reduced capacity for nitrogen uptake. When nitrogen supplement was provided, chlorophyll *a* levels increased in this treatment.

Although abundance of *Ctenocladus* at Mono Lake may have been reduced by recent increases in the salinity of this lake, the superior growth of the Mono clone compared to that from Abert Lake suggests the possibility that selection for salt tolerance has produced ecotypic variants. However, only limited growth of *Ctenocladus* occurred under hypersaline conditions (150 g·L^{-1}), with or without acclimation to higher salinity.

Blinn (1971) showed that akinete germination in *Ctenocladus* occurred with greatest success over a range from ca. 40 to 1700 mOsM (milliosmolar) and was almost completely inhibited above 2500 mOsM (equivalent to slightly more than 100 g·L^{-1} of either Mono or Abert Lake water). Furthermore, formation of zoosporangia was not observed above 1400 mOsM, with only akinete production occurring at about 1600–1800 mOsM. These studies were conducted with algae and experimental solutions originating from ephemeral pond water containing mainly Na-$MgSO_4$ salts at alkaline pH. Although these are natural waters, they represent a limited chemical class in the varied waters of inland saline habitats. Nonetheless, the results are consistent with the salinity levels at which vegetative growth was inhibited in our study. Similarly, Ruinen (1933), using only NaCl solutions, reported active growth of *Ctenocladus* (as *Lochmiopsis sibirica*) up to a concentration of 1.5 M NaCl (nearly 90 g·L^{-1} total dissolved solids).

Studies of cultures of mixed benthic algae from both Mono and Abert lakes (Herbst and Bradley 1989) have shown broad salinity tolerance over the range 50–150 g·L^{-1}. Initial growth rates and the organic matter and pigment content of algae were reduced, however, as culture salinities were increased over this range. The growth limitations observed in these mixed cultures may be due partly to the poor growth of *Ctenocladus* as exhibited in our experiments (Table 1). Though algae may survive or tolerate broad ranges of salinity, the rate of growth and amount of carbon fixed appear to be salinity-dependent.

Field studies also suggest that a lower salinity range is optimal for the growth of *Ctenocladus*. Wetzel

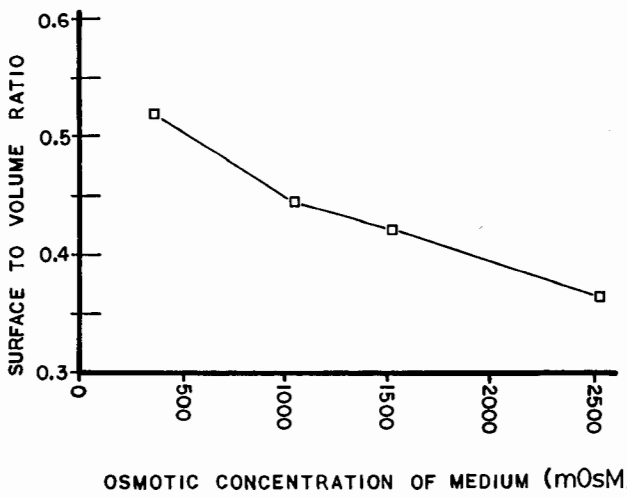

FIG. 3. Relationship of surface-to-volume ratio of *Ctenocladus* filament cells grown at different osmotic concentrations. Data used for these calculations based on mean length and width measurements given in Blinn and Stein (1970).

(1964) conducted quantitative studies of benthic and planktonic primary productivity in Borax Lake, California, and showed that benthic periphyton, consisting almost entirely of *Ctenocladus,* constituted about 70% of the annual total. During these studies, the salinity of this shallow alkaline lake was between 30 and 60 $g \cdot L^{-1}$, optimal conditions for *Ctenocladus* growth as defined by the present study. In algal floral surveys of the saline lakes of Saskatchewan, Hammer et al. (1983) found *Ctenocladus* in lakes over a broad range of salinities, from 20 to 200 $g \cdot L^{-1}$. However, most of the hypersaline lakes sampled showed wide seasonal and annual variations in total dissolved solids. Because *Ctenocladus* may persist at high salinity as akinetes, its presence in lakes of fluctuating salinity may indicate only that active vegetative growth occurs during the season of low salinity, and akinetes form when salt concentrations become prohibitive to growth. Blinn (1971) reported just such a phenology in ephemeral ponds of British Columbia and further noted the disappearance of *Ctenocladus* from Little Manitou Lake over a period of years when salinity increased from about 100 to more than 200 $g \cdot L^{-1}$. The epilithic mats of *Ctenocladus* common at Abert Lake under the low salinity conditions during our studies had largely disappeared by 1990, associated with falling lake levels from prolonged drought. By October 1992, salinity was well in excess of 200 $g \cdot L^{-1}$, and only akinetes of *Ctenocladus* were present in sediments. However, when introduced into media at salinities of 25–75 $g \cdot L^{-1}$, akinetes germinated and normal *Ctenocladus* arose.

Environmental factors other than salinity have also been reported to affect *Ctenocladus.* Akinete germination was found to be optimal under alkaline pH, at temperatures of about 10–13° C, and moderate irradiance (Blinn 1971). Blinn (1970) also found that Na/Mg ratios of less than 1.3 completely suppressed germination, and vegetative filament cells became longer and zoosporangia formed with an increasing proportion of sodium. Moreover, other monovalent cations could not replace sodium in producing these critical developmental processes. Although these results demonstrate dependence of growth on sodium, anionic proportions should also be examined as potential controlling influences.

The finding that the percent water content of *Ctenocladus* remained constant over the salinity range 25–100 $g \cdot L^{-1}$ indicates that water balance is regulated in this alga. Other halophilic chlorophycean algae (such as *Dunaliella*) accumulate organic solutes such as glycerol to replace water lost to osmotic desiccation in hypersaline solutions (Hellebust 1976). In cultures of *Ctenocladus,* Blinn and Stein (1970) found that filament cells became shorter and wider as osmotic concentrations were increased. Assuming a cylindrical cross-section for these cells, the convertion of mean length and width data (from Blinn and Stein 1970) to surface-to-volume ratios shows that the ratio decreases as osmotic concentration increases (Fig. 3). This may represent a possible adaptive growth response to salinity stress by a passive mechanism that would minimize the osmotic loss of intracellular water through a change in cell morphology.

Benthic algae of saline lakes constitute an important food resource to brine flies (Ephydridae), often the most abundant benthic invertebrates in these ecosystems (Collins 1980, Herbst 1988, 1990). The alkali fly, *Ephydra hians,* dominates the benthos of both Abert and Mono lakes, and though *Ctenocladus* has been shown to be a relatively poor-quality food (Bradley and Herbst 1994), it is a common part of the natural diet (Herbst 1986). Development and reproduction of this insect are limited by decreasing quantity or quality of algal food available (Herbst 1992). The results reported here suggest that increased salinity may limit *E. hians* growth and production by reducing the availability of an algal food resource. Other species of benthic algae such as diatoms (Kociolek and Herbst 1992) and cyanobacteria should also be cultured at varied salinities to further our understanding of benthic production and community structure in saline lake ecosystems.

The productivity of *Ctenocladus* has been shown here to depend on salinity, but interactions with other physical and ecological factors no doubt also affect its distribution. Grazing by fly larvae, rock substrate texture, wave shear forces, and the dynamics of competition with other epilithic algae are some of the influences that might profitably be examined and manipulated in field studies using introduced substrates and enclosures.

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