AUTECOLOGY OF CTENOCLADUS (CHLOROPHYCEAE) IN SALINE ENVIRONMENTS

by

DEAN W. BLINN

B.A., Simpson College, Indianola, Iowa, 1964

M.A., University of Montana, Missoula, Montana, 1966

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Department of Botany

The University of British Columbia
Vancouver 8, Canada

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Abstract

Ctenocladus circinnatus Borzi, a member of the Ulotrichales, has a limited distribution being restricted to saline aquatic environments of specific physico-chemical composition. Most of the collections of this alga have been in North America, with a few scattered collections in Peru, Sicily and Siberia.

Confusion in the nomenclature of this taxon has led to the use of two generic names Ctenocladus and Lochmiopsis. Based on field observations, laboratory cultures and herbarium material it appears that Lochmiopsis and Ctenocladus are one with Ctenocladus a monotypic genus.

In order to study the ecology of Ctenocladus, seasonal physico-chemical parameters of seven saline habitats were investigated over a two year period in the dry interior zone of British Columbia. Three of the sites contained Ctenocladus while the remining four habitats were used for comparative purposes. Additional collections of Ctenocladus and water analyses were made from several saline habitats in California and Nevada. In addition, controlled laboratory regulation of environmental parameters (pH, temperature, light, osmotic potential and specific ions) considered to be important in the ecology of Ctenocladus were conducted and correlated with field observations.

The cation composition of the waters appears significant in the distribution of Ctenocladus. Waters composed predominately of Na^+ salts with Na^+:Mg^+ ratios greater than 1.5 were required for normal vegetative growth. Cultured material placed in the field in various investigated

saline habitats substantiated this as well. Akinete germination could only be induced with Na^{+} salt solutions, while other cations (K^{+}, Mg^{++}; Ca^{++}) were extremely toxic to these cells. Anion constituents do not appear to be significant in the distribution of Ctenocladus as collections were made in all of the major anion solutions in nature ($SO_4^{=}$, $CO_3^{=}$, Cl^{-}) and substitution of various anion salts to laboratory cultures showed no significant changes in growth and development.

Temperature may also be significant, particularly in shallow open waters of semi-permanent habitats where temperatures rise above 35°C during the summer. Corresponding laboratory experiments indicated that resting akinetes were extremely susceptible to these high temperatures.

Light intensity appears to influence the condition of akinetes with levels above 12,000 lux detrimental to viability. Salt encrustment of clones of akinetes and burial beneath the sediment and precipitated salt deposits may play an important role in survival of these resting stages.

Waters of saline habitats with Ctenocladus were above pH 9.0 during most of the year while other sites without the alga were generally below this value. Laboratory studies showed pH below 7.5 to be detrimental to akinetes indicating pH to be indirectily significant as it reflects the ionic constituents of the natural solutions.

Akinete production appears to have substituted for the resting zygote in maintaining the population in these extreme environments as shown in both field and laboratory experiments. Most cells are converted into akinetes at osmotic levels above 3000 mOsm and remain

in this condition for most of the year. Massive akinete germination occurs following spring dilution from runoff and when water temperatures are above 5°C. Zoosporangia production may not occur every year in shallow saline habitats when high salinities are achieved early in the season. Zoosporangia were only induced in laboratory cultures at osmotic potential levels below 1300 mOsm. Field observations substantiated this as well.

The total lack of genetic recombination in Ctenocladus populations may have restricted the organism to a very narrow ecological niche as witnessed both in the field and the laboratory.

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Autecology of Ctenocladus (Chlorophyceae) in Saline Environments

INTRODUCTION

Autecological investigations on Ctenocladus circinnatus Borzi, a representative of the Ulotrichales, were stimulated by the restricted distribution of this alga. Ctenocladus is limited to aquatic saline environments of specific physico-chemical composition.

Recorded collections of Ctenocladus on a world wide basis indicate it to be restricted to only a few inland or coastal saline aquatic habitats, as shown in Appendix Table I. Most of these collections have been in North America with scattered reports in Peru, Sicily and Siberia. Several North American collections have not been published. These include several localities in British Columbia and Southern Oregon.

Investigations of saline waters have been primarily restricted to permanent habitats, ignoring temporary and semi-permanent environments. In addition, regional surveys of inland saline waters (Livingstone 1963; Bayly and Williams 1966; Colinvaux 1968) include only spot water collections. Other studies are concerned with seasonal dynamics (Anderson 1958; Castenholz 1960; Cole, et al. 1967; Jones and Van Denburgh 1967) and are based only on a limited number of collections (i.e. spring and fall). Investigations of saline environments in the dry interior of British Columbia, where this study was conducted, are equally sparse. These include one general survey of the fauna in several of the saline habitats (Cameron 1953) and two investigations now in progress involved with the distribution of insects (G.G.E. Scudder, personal communication) and fungi (C.L. Anastasiou, personal communication).

Experimental studies on algal species occupying saline waters have been

limited due to the lack of isolates in culture. Most studies of this nature have been conducted on organisms collected and isolated from marine or brackish water habitats, (Droop 1958; Gibor 1956). Very little research therefore, has been conducted on organisms of inland saline habitats where Cl^- becomes secondary to divalent anions ($SO_4^=$ and $CO_3^=$).

Only one experimental study has been conducted on Ctenocladus (as Lochmiopsis siberica). This was by Ruinen (1933) who showed that active growth is possible in NaCl concentrations up to 1.5 mol. The resting stages tolerated a saturated salt solution. He also reported that the alga was polymorphic when subjected to different salt concentrations.

Pringsheim (1967) stressed the increasing need of field and laboratory phycologists to continue efforts to better understand individual species as well as the enviromental parameters influencing algal characteristics. Consequently, it was the purpose of this investigation to: 1) characterize the physico-chemical enviromnent of those habitats occupied by Ctenocladus; 2) measure the seasonal physico-chemical parameters in these saline habitats to reveal seasonal dynamics of the system and its influence on Ctenocladus: and 3) correlate these field measurements with experimental laboratory regulation of factors (i.e.; pH, temperature, osmotic potential, light and specific ions) thought to be significant in the ecology of Ctenocladus circinnatus.

Taxonomic Considerations

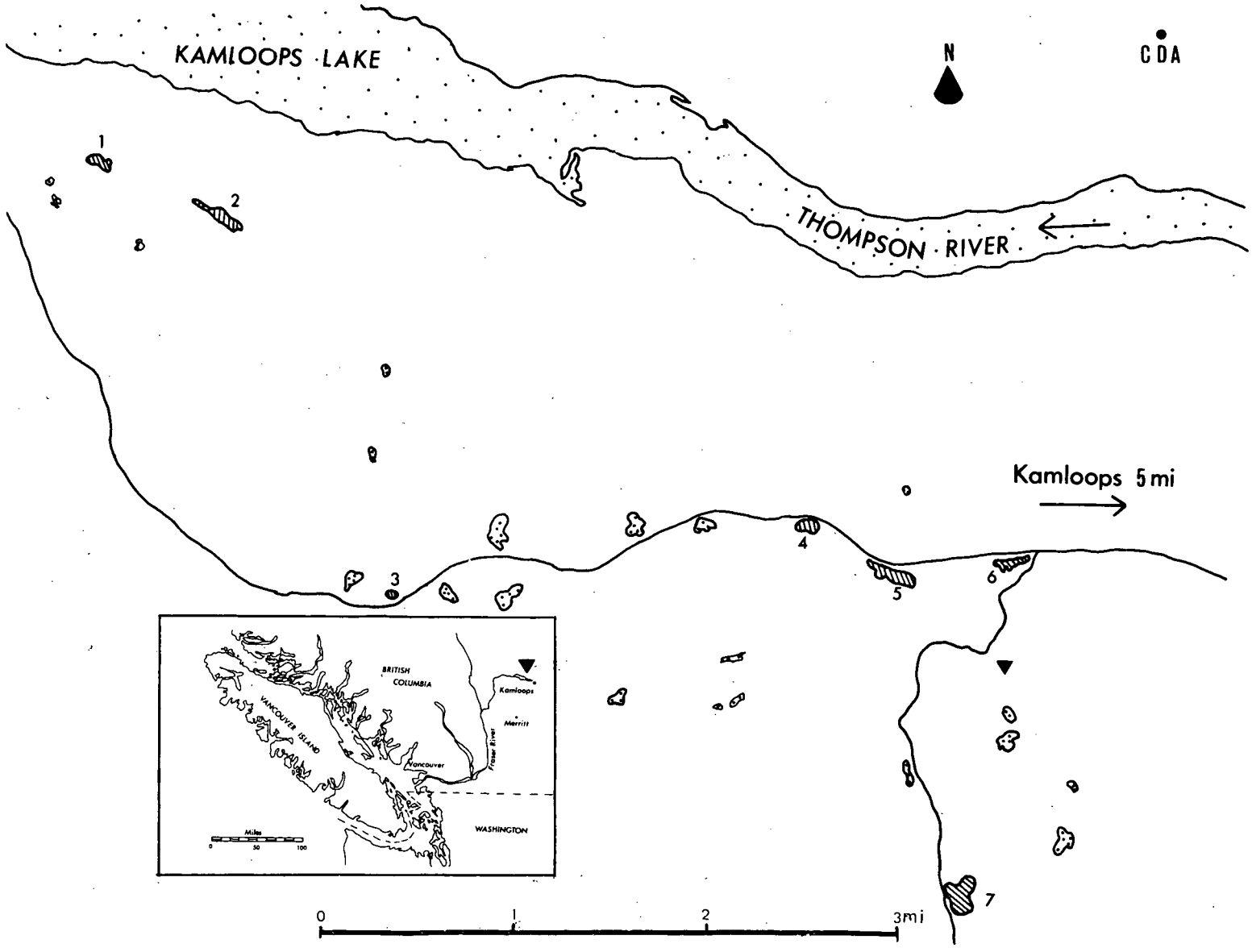
Confusion in the nomenclature of this alga for the last 50-60 years has led to the use, by various authors, of two generic names, Ctenocladus and Lochmiopsis. Some authors consider the two synonymous whereas others recognize separate taxa. The alga was originally collected as an epiphyte on Salicornia sp. and Ruppia sp. in a saline habitat in Sicily by Antonino Borzi (1883). He named the alga Ctenocladus circinnatus. Approximately 45 years later, Woronochin and Popova (1929) collected an alga from several inland saline lakes in Siberia. They named their alga, Lochmiopsis as it resembled Lochmium in possessing numerous akinetes and having intercalary zoosporangia. Woronochin and Popova (1929) added more confusion by describing two species, L. siberica and L. Printzii based on cell length to width dimensions and general size. Recently Printz (1964) created a third species L. Chodatii, separating it from the original two by the angle of branching. Smith (1950) and Bourrelly (1966) have combined Ctenocladus and Lochmiopsis and retained the original name, Ctenocladus. Throughout the remainder of this discussion the author will use the original name Ctenocladus, which is in accordance with the International Rules of Botanical Nomenclature (1964).

FIELD METHODS:

Selection of Saline Lakes and Ponds

Seven aquatic saline habitats were selected near Kamloops, British Columbia (approximately 280 miles NE of Vancouver, B.C.) for intensive limnological investigation (Fig. 1). Selection of these habitats was based on preliminary surveys conducted in the dry interior area around Kamloops and Merritt for the presence of Ctenocladus circinnatus. This region lies east of the Coastal Range rain shadow with an annual precipitation generally less than 30cm. The preliminary survey indicated that Ctenocladus occurred in only one shallow saline pond approximately 7.6 miles west of Kamloops on Highway 1. Additional investigations during the following year revealed the presence of Ctenocladus in two additional ponds located approximately 3.5 miles NW of the initial site where Ctenocladus was collected. Since the saline habitats throughout the study area appeared to be similar in appearance, size and flora, except for Ctenocladus in three of the habitats, convenience and accessibility were major factors involved in selecting four more sites for comparison. Only three of the saline habitats (Bowers Lake, Ironmask Lake and Wallender Lake) selected for this study were officially named, therefore names have been temporarily assigned to the remaining four saline habitats. Unofficial names for semi-permanent ponds are in quotes; official names of lakes are used without such designation. The following saline habitats, located within a 6 mile radius of the initial Ctenocladus site, were ultimately selected for intensive study.

Figure 1. Investigated saline habitats in Kamloops area. (1. "1st Salt Mine pond", 2. "2nd Salt Mine Pond", 3. "Cherry Creek Pond", 4. "Polygon Pond" 5. Ironmask Lake, 6. Bowers Lake, 7. Wallender Lake). Insert of southern British Columbia.


KAMLOOPS LAKE
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1. "Cherry Creek Pond" (50°39'N 120°32'W) 7.6 miles W of Kamloops; elevation 2150'; and semi-permanent basin in shaded depression with water only in small isolated pockets in late summer; maximum depth less than 1 meter; Ctenocladus present.

2. "1st Salt Mine Pond" (50°42'N 120°32'W) 7.2 miles WNW of Kamloops; elevation 1750'; semi-permanent basin in shaded depression; maximum depth less than 1 meter; Ctenocladus present.

3. "2nd Salt Mine Pond" (50°41.5'N 120°32'W) 7.2 miles WNW of Kamloops; elevation 1800'; semi-permanent basin in partially shaded depression; maximum depth less than 1 meter; Ctenocladus present.

4. "Polygon Pond" (50°40.5'N 120°32'W) 7.2 miles WNW of Kamloops; elevation 2300'; semi-permanent in open expanded basin; maximum depth less than 1 meter.

5. Ironmask Lake (50°40'N 120°27'W) 3.6 miles W of Kamloops elevation 2350'; semi-permanent in open expanded basin; maximum depth less than 1 meter.

6. Bowers Lake (50°40.2'N 120°26'W) 3.0 miles W of Kamloops; elevation 2350'; permanent in open expanded basin; maximum depth greater than 1 meter.

7. Wallender Lake (50°38'N 120°26.5'W) 4.6 miles WSW of Kamloops; elevation 2750'; permanent in open expanded basin; maximum depth greater than 1 meter.

In addition to the seven lakes and ponds in the Kamloops area, Lyons Lake, located approximately 40 miles north of Kamloops, was investigated during June and July 1968 for the presence of C. cincinnatus.

In early June 1968 various saline lakes and ponds were examined in arid regions (annual precipitation less than 51cm.) in Nevada and California. This survey was designed to locate new collection sites for Ctenocladus and to compare these habitats chemically to the saline habitats investigated

in British Columbia. Ctenocladus occurred at only three sites (Fig. 36-38). For comparative purposes, additional saline ponds located within the immediate vicinity of these three habitats were also selected for study. Again, most of these habitats, with the exception of Mono Lake, are not officially named, therfore names have been assigned.

1. Mono Lake (119°00'W 38°00'N) approximately 1.5 miles ENE of Lee Vining, California; permanent; maximum depth 51.5 meters; Ctenocladus present.

2. "Little Mono Lake Pond" (119°00'W 38°01'N) isolated pond approximately 600' NW of Mono Lake; permanent; maximum depth approximately 1.5 meters.

3. "Hazen Pond" (119°00'W 39°50'N) 5.0 miles E of Hazen, Nevada; semi-permanent open basin; maximum depth about 1.0 meter; Ctenocladus present.

4. "2nd Hazen Pond" (119°00W 39°50'N) 1.0 mile W of "Hazen Pond" semi-permanent open basin; maximum depth less than 1.0 meter.

5. "Stateline Pond" (122°00'W 42°00'N) 8 miles W of Tulelake, California; permanent open basin; maximum depth about 1.0 meter; Ctenocladus present.

6. "2nd Stateline Pond" (122°00'W 42°00'N) 0.5 mile E of "Stateline Pond"; permanent open basin; maximum depth approximately 1 meter.

Sampling, Physico-Chemical Analyses:

Measurements of selected physico-chemical factors were made at various times of the year to determine if seasonal conditions (salinity, temperature, ionic constituents, etc.) were significant in limiting Ctenocladus to a particular habitat. Therefore, all of the detected saline habitats in the Kamloops area, with the exception of the two "Salt Mine Ponds",

were investigated at 3 week intervals from 19 May 1967 to 23 September 1967. Samples were also taken through the ice in January. During the following year these same five ponds were investigated monthly from March to August. The two "Salt Mine Ponds" were sampled once a month from 18 May 1968 to 29 August 1968.

During each collection on Bowers Lake and Wallender Lake, surface and bottom water samples were taken at a station in the center of the lake along with one sample near the margin. Bottom samples were obtained ca. 5-10 cm above the sediment with a 1-liter polyethylene bottle clamped to a calibrated pole. Surface samples were obtained ca. 0-10 cm. Marginal water samples were collected from Ironmask Lake, "Polygon Pond" and the two "Salt Mine Ponds" in duplicate. In "Cherry Creek Pond", distinct crystal beds were scattered throughout the pond, therefore surface and bottom samples were taken at one station over the crystal bed and one station over the non-crystal zone. Eighteen 1-liter samples were normally collected for chemical analysis from the seven habitats during each visit. Water samples for chemical analysis were filtered through silk bolting cloth (No 20 mesh) into 1-liter polyethylene bottles. These samples were frozen within 5 hr after collection and transported back to the laboratory for analyses.

A detailed description of procedures used in measuring the physico-chemical factors in this study is included in Appendix Table II. Values for NO_2^-, $-NO_3^-$, NH_4^+, $PO_4^{\equiv}$, Cl^- and $SO_4^{=}$ were obtained in the laboratory within 72 hr with Hach Chemicals (Hach Chemical Co., Ames, Iowa). Major cations (Mg^{++}, Ca^{++}, Na^+, K^+) were analyzed on a Perkin-Elmer atomic absorption spectrophotometer from frozen and Millipore filtered samples

stored at -15°C. Analysis of major cations and anions for the 11 August 1967 collections as well as the salt crystals taken from the crystal bed in "Cherry Creek Pond" on 1 July 1967, were conducted by Wood Laboratory, Vancouver. Total dissolved solids and osmotic potential values were also obtained from the frozen samples.

Temperature, pH, depth visibility, CO_2 and O_2 were determined *in situ*. Surface measurements were made at, 0-5 cm whereas bottom readings were taken *ca.* 5-10 cm above the sediment. Values for the dissolved gases were obtained by the Winkler method with Hach Chemicals and YSI model 54 (Yellow Springs Instrument Co.) oxygen probe. Temperature readings were made with mercury thermometers and a YSI model 54 thermister. Depth visibility reading were obtained from a standard 20 cm Secchi Disk. Values for HCO_3^- and $CO_3^=$ were obtained by potentiometric titration procedures from separate 250 ml unfrozen samples within 6 hr of collection.

A spectrographic analysis of trace elements was conducted by Coast Eldridge, Vancouver, for surface samples collected 18 May 1968 from all the Kamloops ponds and lakes except Ironmask Lake. Monthly water level recordings were obtained from a calibrated stake positioned in the ponds.

Climatological data for the Kamloops area was recorded by the Canada Department of Agriculture at a weather station in the area (50°43'N 120°26'W) shown in Fig 1 (See C.D.A.). Average values for precipitation and temperature are based on a 15 yr and 14 yr recording period respectively.

Soil samples for preparation of culture media were collected five times from November 1966, to April 1968. Submerged marginal sediment samples were obtained from Bowers Lake, Wallender Lake, Ironmask Lake, and "Polygon Pond". Sediment samples from both the crystal and non-crystal

zones were collected from "Cherry Creek Pond". All of these samples were air-dried and stored in plastic bags.

Duplicate 1-liter samples for physico-chemical analyses were collected in polyethylene bottles from each of the six selected lakes and ponds in the California and Nevada study areas. Similar analyses for major cations to those from the Kamloops area conducted on these samples.

Biological:

During each visit to the Kamloops study area, algal collections were made with plankton net hauls (#20 mesh), dipping nets (for bottom sediment samples) or were collected as epiphytes from various substrates along the margin and within the pond. Algal material was normally preserved in either Lugol's IKI Solution (Ruttner 1966) or formalin-acetic acid-alcohol (FAA) plus 5% $CuSO_4$ (Smith 1950). Algal nomenclature follows that of Geitler (1932) for Cyanophyceae, Smith (1950) Patrick and Reimer (1966) for Bacillariophyceae, Huber-Pestalozzi (1941) for Chrysophyceae, and Prescott (1961) for Chlorophyceae and remaining groups. A representative sample of each collection remains in the University of British Columbia phycological collection. Marginal and aquatic vascular plants from each habitat were collected and pressed with identifications made using Muenscher (1964), and Hitchcock et al. (1964). Voucher specimens are deposited in the University of British Columbia herbarium. Zooplankton and insect larvae were collected from each pond with plankton net hauls (#20 mesh) and preserved in 70% alcohol. Live chironomid larvae from the Wallender Lake collection on 19 May 1968, were transported back to the laboratory for predation experiments.

Akinetes (resting cells) for laboratory experiments were collected during late summer and stored in the following conditions: 1) frozen at -15°C; 2) maintained in filtered concentrated pond water collected during late summer; 3) collected and stored as dried material.

Three hundred vegetative cells from "Cherry Creek Pond" were randomly selected and measured monthly from 26 March 1968 to 29 August 1968. One hundred cells were selected from three different collections to account for the total. Measurements were made with a light microscope with an ocular micrometer at a magnification of 100x.

A Hiller-borer was used in collection 50 cm sediment cores from "Cherry Creek Pond", Ironmask Lake and Bowers Lake. Sediment cores were taken next to the margin and toward the center of each habitat. Water mounts were prepared in the laboratory at 10 cm intervals along each 50 cm core and examined for resting stages of Ctenocladus as well as for other algal resting stages.

Collections of Ctenocladus were taken at the three locations previously described in California and Nevada. Material was examined within 4 hr after collection for reproductive structures and general cytological condition. Both preserved and live collections were brought back to the laboratory. Cultures, as mentioned above were established from Ctenocladus collected at the three sites and maintained for further investigation.

LABORATORY MATERIALS AND METHODS

A. Media

The chemically undefined media used to culture Ctenocladus included two sea-water based media (Appendix Table III), Provasoli ES enrichment (Provasoli, 1968) and Chihara Marine Medium (M. Chihara personal communication). In addition, a saline soil water (SSW) medium as described below was used either in the biphasic stage (biphasic SSW) or as filtered extract (SSW extract). Other media used in this study included standard biphasic soil water with garden loam (Starr 1964), Beijerinck Medium (Stein 1958), Bold Basal Media (Nichols and Bold 1965), Erd-Schreiber (Starr 1964), modified von Stosch (vonStosch 1965) and ASP (Provasoli et al. 1957).

Biphasic SSW was prepared by adding ca. 20ml distilled water to ca. 0.5g air dried sediment. This was steamed at 95°C for 1 hr on three consecutive days. The pH of the extract ranged from 8.4 - 8.7. Major cations of the filtered (#1 Whatman paper) extracts were measured on an atomic absorption spectrophotometer.

Attempts were made to develop a chemically defined mineral medium in order to obtain a more critical idea of the influence of individual ions on various stages in the life history of Ctenocladus. Ionic concentrations for waters occupied by Ctenocladus in previous studies (Mason 1967; Rawson and Moore 1944; Wetzel 1964; Cole, et al. 1967) as well as values from this study were systematically analyzed for a potential culture solution. A basic defined mineral solution (BSM) was prepared as indicated in Appendix Table XXl through variations of each constituent.

B. General Culture Conditions

Unless otherwise indicated for specific experiments, general culturing conditions with optimum development of Ctenocladus was 19-20°C at 4066-4280 lux on 8-hr dark/16-hr light photoperiod. Culture units were equipped with overhead banks of F 48T 12 Cool White Sylvania fluorescent lamps. Diurnal fluctuations in the culture chambers averaged ±2°C. Stock culture chambers were maintained on similar light cycles at a lower light intensity of ca. 2140 lux at 10°C or left in the resting stage as akinetes. Optimum pH for culture solutions ranged from ca. 8.5 - 9.5.

C. Experimental Methods

1. Germination Studies

Throughout this investigation procedures for germination studies were conducted under similar conditions unless otherwise indicated for specific experiments. A solution of concentrated saline pond water was filtered through a sterile .22u Millipore filter, placed at 10°C and utilized as the storage medium for akinetes. Akinetes collected during midsummer 1968 were isolated into this solution. When investigating the influence of various environmental factors on akinete germination ca. 250-350 akinetes were selected from this solution, rinsed through three sterile water baths and inoculated into duplicate 20-ml test tubes or 60mm Petri dishes. Cultures with media were placed at respective temperature settings 24 hr prior to inoculation to allow for temperature adjustment. In germination studies with variations of pH and specific ions, akinetes were treated with a potassium telluride (.002 g/l) and caffeine (.001 g/l) preparation for 5-6 days to obtain near bacteria-free conditions. Length of time for

optimum germination percentages for each factor considered was established experimentaly prior to inoculation. Generally, cultures remained under optimum conditions for 10 days with one factor varied. SSW extract was normally used as the culture medium for various studies unless otherwise indicated. Germination percentages, based on 200 akinetes, were recorded from each inoculation and compared to a controlled condition in SSW. In addition, the number of cells per germination tube was generally noted. Each individual experiment was conducted at least twice unless otherwise stated.

2. Akinete Tolerance Experiments

The ability of akinetes to germinate after subjected to various levels of temperature, pH and light intensity for a designated period of time was also investigated in the laboratory. General procedures included four replicates of 60mm Petri dish cultures, each inoculated with 250-350 akinetes and placed at determined conditions as indicated in each section. Cultures remained under described conditions for a period as indicated for each factor considered. After this period two cultures were removed from each setting and placed at optimum conditions for the factor being investigated leaving two cultures at each initial condition for controls. Germination percentages were measured after a given period at the new and the initial condition.

D. Individual Factors Studied

1. Temperature

The effect of temperature on akinete germination was investigated.

Temperatures utilized in the experiment ranged from -15°C to +35°C as indicated in results in Appendix Table XXIII. Cultures were maintained at optimum conditions at the various temperature settings for a period of 14-16 days. Cultures were transferred once into temperature-adjusted SSW extract after 6 days.

The time required for germination and the time required to reach the 2-cell stage were determined at temperatures ranging from 0-31°C as indicated in the results (Appendix Table XXII). Triplicate cultures of filtered SSW extract were inoculated with akinetes and placed at the designated temperature levels. Cultures were examined ca. every 4-8 hr. This experiment was conducted at least three times for all settings.

Temperature influence on zoosporangia formation was also established in the laboratory. Duplicated 20-ml test tubes of biphasic SSW were inoculated with Ctenocladus akinetes. Temperatures utilized in this experiment ranged from 0-31°C at ca. 4°C intervals as indicated in results in Appendix Table XXII. Cultures were maintained for ca. 60 days with transfers every 12-14 days.

2. Light

Akinetes were subjected to various light intensities to determine the effect on germination percentages. Light intensity settings ranged from 0-12,305 lux as indicated in results in Appendix Table XXV. Cultures in the dark were wrapped several times with aluminum foil.
Values for each light setting were read from a Gossen #1.67-873 light meter through 15-ml of SSW extract and the bottom of the glass 60mm Petri dish to compensate for the removal of light by the solution and the dish cover.

All cultures were maintained between 9-12.5°C on optimum light/dark cycles. Since cultures at higher light intensities were located near the fluorescent lamps, temperature reading were taken daily with a Yellow Springs Instrument model 425 C Telethermometer. Temperatures in the culture media never fluctuated more than 3.5°C during the experiment.

Encrusted fragments of akinetes collected along the margin of "Cherry Creek Pond" during late summer were exposed to a high light intensity in the laboratory. These dry encrusted fragments were placed in 20-ml of SSW extract in Petri dishes and exposed to a light intensity of 11,770 lux for 10 days. At the end of this period, encrusted fragments were immersed in 20-ml of SSW extract. Two were exposed to 4280 lux light and two to 11,770 lux. After 10 days, akinete germination was measured.

3. Osmotic Potential

Water collected from several saline habitats from May through July with osmotic potential values ranged from low (690 mOsm) to high (3000 mOsm) as indicated in results (Appendix Table XXIX). Inoculated cultures of akinetes were placed at optimum conditions for 12 days.

Saline pond water collected from "1st Salt Mine Pond" on 29 August 1968 was diluted with distilled water to determine the response of akinete germination and the effect of cell dimensions. A total of 10 volumetric dilutions were made providing osmotic potential values ranging from 10 mOsm to 3000 mOsm as indicated in results in Appendix Table XXX. After 10 days at these conditions, germination percentages were recorded for two cultures at each dilution. The remaining cultures were transferred into the respective solutions. After another 10 days, cell dimensions

for 200 cells were measured from cultures with the following osmotic potential values: 375 mOsm, 1050 mOsm, 1510 mOsm, 2540 mOsm or ca. the range of seasonal field conditions.

The osmotic potential of solutions was regulated with "polyethylene glycol 1000". Four different levels of osmotic potential (32 mOsm, 72 mOsm, 176 mOsm and 375 mOsm) were established by the addition of various concentrations of this high molecular weight compound to distilled water. Tris buffer (1.0 g/l) was also added to each culture as well as to distilled water. Preliminary studies indicated levels of polyethylene glycol 1000 up to 150 g/l did not retard akinete germination. Water from the "1st Salt Mine Pond" collected on 29 August 1968 was also diluted to provide osmotic potential values of 112 mOsm and 360 mOsm for control purposes.

In order to study effects of osmotic potential on zoosporangia formation, water from "Cherry Creek Pond" collected on 29 August 1968 was diluted with distilled water to give 8 different osmotic potential reading ranging from 225 mOsm to 1625 mOsm (Appendix Table XXXI). Inoculated cultures were transferred at the end of 12 and 24 days during the 32 day incubation period.

4. Hydrogen-ion Concentration

The culture medium used in this experiment to demonstrate the effects of pH on akinete germination was the defined mineral medium (BSM) developed specifically for cultivation of Ctenocladus. A 1:3 dilution with distilled water was made to eliminate precipitation at lower hydrogen-ion concentrations. Various pH values ranging from 6.0 to 12.0 as indicated in Appendix Table XXVII were obtained with .145 N HCL for values below

9.0 and .1N NaOH for values above this level. Cultures were maintained under optimum conditions for 8-10 days. Fluctuations for cultures between pH 7-8.5 never exceeded $\pm$0.1 of a unit while those between pH 9-11 ranged from 0.3 to 0.5 units. Settings at pH 6.0 fluctuated from pH 6.0 to 6.3, while those above pH 11.0 ranged from 0.4 to 0.8 units. The pH of distilled water plus Tris buffer (0.5 g/l) was adjusted to ea. pH 7.5 ($\pm$0.2) with .145N HCl. Triplicate tubes each with 20ml of the adjusted solution as well as an unadjusted Tris (0.5 g/l) solution at pH 9.0 were inoculated with akinetes. Cultures were maintained under optimal conditions for six days.

5. Specific Ion Influence

Chihara Seawater Medium (Appendix Table III) was prepared and adjusted to different Na:Mg ratios by the addition of Mg^{++} salts. The medium was diluted by four parts distilled water to one part Chihara Seawater Mix. Previous investigations indicated dilutions of this medium of up to 1:5 allowed normal development of vegetative cells and production of zoosporangia. Tris buffer (0.2 g/l) was also added to this diluted seawater preparation. Major cations for the basic solution were measured on an atomic absorption spectrophotometer. The Mg^{++} levels were manipulated by the addition of known concentrations of Na:Mg at ratios of 1:1, 1:2, 3:1. Cultures remained at optimum conditions for ca. 30 days with cultures transferred every 7-8 days. After this period, cultures were examined for general cell shape, appearance, type of branching and reproductive structures. Cell dimensions of 200 cells were also measured from each culture.

Saline soil water extract was prepared from Bowers Lake sediment as previously described (p12). The extract was filtered through a .45μ Millipore filter and a dilution of one part distilled water: one part extract was prepared. Tris buffer (1 g/l) was also added to this preparation. Six different concentrations of Na_2SO_4 were added to separate aliquots of soil extract preparation to obtain the Na:Mg ratios indicated in results in Appendix Table XXXIII. The pH of the preparation was adjusted to 8.7 ($\pm$0.2) with .1N KOH. Cultures remained at optimum conditions for ca. 40 days with transfers every 8-10 days.

A series of cultures was prepared where the Cl^- and $SO_4^=$ salts for each of the four major cations were added to distilled water at concentrations ranging from .05 g/l to 6 g/l (Appendix Table XXXIV). Tris buffer (0.5 g/l) was also added to each preparation. Inoculated cultures were maintained at optimal conditions for 6 days at pH 8.6 ($\pm$0.2).

6. Response of Ctenocladus in Other Saline Water

Water was collected from Ironmask Lake, Wallender Lake, Bowers Lake, and "Polygon Pond" during the spring (April 1968) and late summer (August 1967) and treated in the following ways: 1) unfiltered and unsteamed; 2) filtered through #1 Whatman filter paper and steamed at 80-100°F for 30-40 minutes on two consecutive days; 3) filtered, steamed and diluted one part saline pond water:two parts distilled water. Approximately one month after each seasonal collection, 20-ml of each of the three types saline water was added to duplicate 20-ml test tubes and each inoculated with vegetative filaments of Ctenocladus. Another similar set was inoculated with akinetes. Temperatures used for the two seasonal collections

were approximated to similar field conditions at the time of collection. Spring water was maintained at 10°C and summer water at 20°C. The vegetative material was transferred every 14-15 days over a 60 day period.

7. Chelator in Seawater

A portion of seawater collected from the southwest coast of Vancouver Island (near Sooke) was treated with EDTA-Na_2 (.004 g/l) and another portion was left untreated. Four test tubes with 20 ml of each solution were inoculated with akinetes and placed at optimal conditions. Cultures were examined after 6 and 12 days for germination percentages and reproductive structures.

E. Experimental transplants:

Ctenocladus was introduced on glass microslides into those lakes and ponds where it was not previously collected; Wallender Lake, Bowers Lake, Ironmask Lake and "Polygon Pond". Similar transplants were made into "Cherry Creek Pond" for control purposes. The plants were grown for three weeks on etched glass microslides (hydrogen fluoride) which were used for better zoospore attachment. The plants were started from zoospores produced by plants growing in biphasic SSW cultures. These cultures were maintained at 20°C at 4066 lux (16 light/8 dark cycle). Six slides with young Ctenocladus plants were placed at approximately 45° angles in clear plastic transfer chambers in each pond. The boxes were ca. 8x15cm in which 0.6 cm holes were randomly spaced to allow circulation of water (Fig. 2). The boxes were fastened securely to a stake ca. 10cm below the water surface at one location in each habitat except in the control

pond where transplants were made over the crystal bed and in the non-crystal zone. Transfer boxes were placed in the five habitats on three different occasions during the period of 28 March 1968 to June 1968. Transplants were made on 28 March 1968, 20 April 1968 and 19 May 1968. Four of the six microslides from the March transplant were collected and replaced with four new slides on 20 April 1968. The two slides remaining from the March transplant along with two slides from the April transplant were collected and replaced with four new inoculated slides on 19 May 1968. On 16 June 1968, all six of the slides were collected and the experiment terminated. The above collecting procedure allowed for both one and two month incubation periods in each habitat.

After each collection, the slides were examined within 3 hr for general cell shape and appearance as well as for reproductive structures. Material was transported back to the laboratory in biphasic SSW containers for further study.

Due to heavy grazing pressures of chironomid larvae in Wallender Lake, two transfer chambers were placed in this lake during the period of 19 May 1968 to 16 June 1968. One of these chambers was slightly modified by covering the opening with #20 mesh silk bolting cloth.

F. Biological Factors:

1. Biological Antagonism

Isolates of several algal species occurring in two of the investigated Kamloops lakes were inoculated into cultures of Ctenocladus. The two species selected were Cladophora fracta (Dillw.) Kütz. from Wallender Lake and Rhizoclonium hieroglyphicum (C.A.Ag.) Kütz. from Bowers Lake.

Inoculations of each were made into duplicate 20-ml biphasic SSW containing young vegetative filaments of Ctenocladus. Duplicate cultures of each isolation were placed at 10°C, 15°C and 20°C. Cultures were maintained under optimum conditions for ca. 60 days through two transfers.

2. Predation

The feeding capabilities of Cricotopus sp. larvae identified by A.L. Hamilton (personal communication) were investigated in the laboratory. Inoculations of Ctenocladus on etched glass slides (See transplant experiment) with five-six Cricotopus larvae were introduced into five sterile 250-ml containers of biphasic SSW. These cultures were maintained at 15°C or similar to field temperature conditions at the time of collection. Transfers were made only once after a period of 8 days. In addition to examination of droppings, larvae were sacrificed every 1-2 days to examine gut contents.

G. Herbarium Material

Herbarium sheets designated as either Ctenocladus circinnatus or Lochmiopsis sibirica were examined from 13 major herbaria. A listing of the herbaria and specimens is given in Appendix Table XXXVI. Vegetative cell size, type of branching and presence of akinetes were generally noted when possible for each specimen. Permanent slides from each collection were also prepared for later reference.

FIELD RESULTS - KAMLOOPS AREA

A. Description of Study Area in Kamloops Region

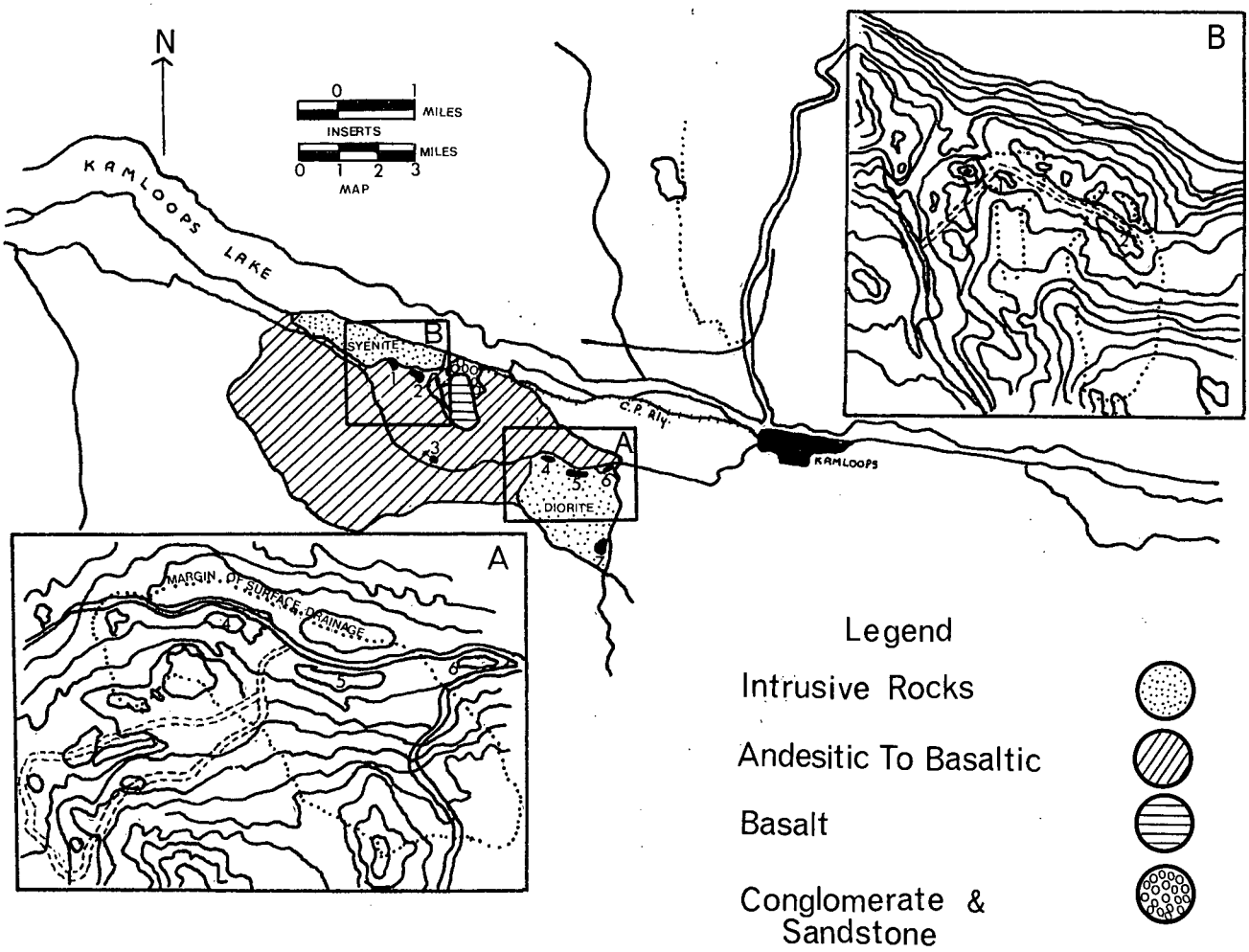
The seven lakes and ponds investigated in the Kamloops area are situated within the ponderosa pine-bunch grass biogeoclimatic zone of British Columbia (Krajina 1965). The lower slopes of the hills and valleys generally are covered with sagebrush.

The area is comprised of thick accumulations of Tertiary volcanic rocks composed mainly of basalt and basalt breccia (Cockfield 1961). Several localized deposits occur within this sheet which include the Ironmask Batholith and a syenite intrusive bordering Kamloops Lake to the south within the vicinity of the two "Salt Mine Ponds" (Fig 3). The Ironmask Batholith is composed mainly of diorites rich in hornblende of which the main constituent is magnesium-rich amphibole (G. Rouse and W.H. Mathews personal communication).

Climatic conditions place the area in the dry belt with an average annual precipitation ca. 24.3 cm, with the highest monthly average (2.99 cm) received in June (Canada Department of Agriculture). Minimum average monthly precipitation is received during March and April with values at 0.71 and 0.91 cm respectively (C.D.A.). Average annual winter snowfall is ca. 73.4 cm, with most of this falling in December and January (C.D.A.).

Average annual temperature for the area is ca. 9°C with average monthly high is July (21.5°C) and August (20.0°C) (C.D.A.). Average monthly lows occur in December (-1.5°C) and January (-5.0°C). Temperatures first rise above freezing during March (3.5°C) and normally fall below the freezing point in December (C.D.A.).

Figure 3. Saline Deposits in the Vicinity of Kamloops, British Columbia Illustrating the Geology of the Drainage Basins for Saline Habitats. (1 ="1st Salt Mine Pond", 2 = "2nd Salt Mine Pond", 3 = "Cherry Creek Pond", 4 ="Polygon Pond", 5 = Ironmask Lake, 6 = Bowers Lake, 7 = Wallender Lake) After J.M. Cummings, 1940.


N
0 1
MILES
INSERTS
MILES
0 1 2 3
MAP
KAMLOOPS LAKE
SYENITE
B
A
C.P. RLY.
KAMLOOPS
DIORITE
MARGIN OF SURFACE DRAINAGE
Legend
Intrusive Rocks
Andesitic To Basaltic
Basalt
Conglomerate & Sandstone

B. Seasonal Changes in the Habitats

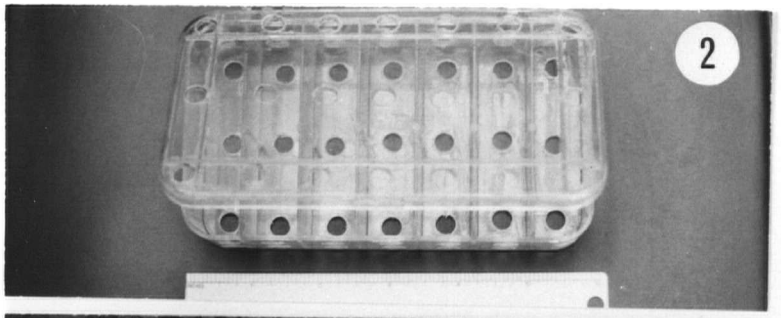
Seasonal changes in the appearance of the closed saline habitats in the Kamloops area are extreme as would be expected with evaporation rates exremely high and precipitation very low throughout the summer. Many of the shallow undrained basins and depressions are temporary, with water present during only spring and early summer. Others may be classified as semi-permanent, characterized as having only isolated pockets of concentrated water in the late summer. Formation of a thin, transparent encrusted salt sheet over the surface of these isolated water pockets minimizes evaporation, consequently total drying is infrequent. Total drying for any given year depends on climatic conditions i.e. winter snowfall, spring and early summer precipitation and summer temperatures.

Seasonal changes for each habitat investigated in this study are illustrated in figures 4 - 20. A characteristic white efflorescent ring of dried salt appears along the margin of all habitats throughout the summer months as water levels drop (Fig 5,13,16,18). Major losses of water occur in July and August as would be expected when temperatures are at their highest (Appendix Table IV). Wallender Lake (Fig 17,18) and Bowers Lake (Fig 15,16) remain permanent throughout the year. The remaining five habitats are semi-permanent with isolated pockets of water throughout the undrained basin (Fig 6,11,14). "Polygon Pond" (Fig 9-11) is unique and similar to patterned ground in the Artic (Washburn 1956) in that it is characterized by polygonal raised ridges of sediment apparent during the late summer. Cumming (1940) reports that permanent crystal beds ca. 1.2 m beneath the soft black ooze in "1st Salt Mine Pond" and Ironmask Lake. Cores taken indicated crystal beds up to 10 m occur

Figure 2. Experimental field transfer chamber (8 x 15 cm).

Figure 4-8. Seasonal Condition of "Cherry Creek Pond".

4. Spring condition (April) at maximum depth ca. 45 cm.
5. Early summer condition (May) with open water zones (crystal beds) and Ruppia zones.
6. Mid-summer condition (July) with water only remaining in the crystal pockets (depressions).
7. Late summer condition (September) with no standing water.
8. Winter condition (January) with visible crystal zones as spherical semi-frozen regions.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Figures 9-11 Seasonal condition of "Polygon Pond".

9. Spring condition at maximum depth ca. 40 cm (Note polygonal raised ridges not visible).

10. Late summer condition (September) with visible polygonal raised ridges.

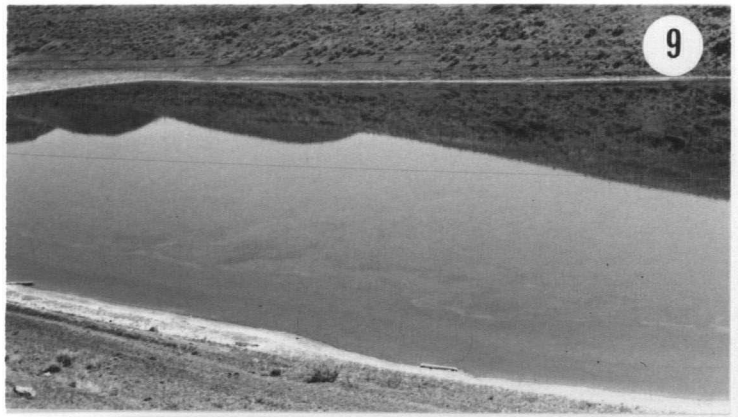
11. Open pockets of concentrated water in the late summer (September).

Figures 12-14. Seasonal condition of Ironmask Lake.

12. Spring condition (April) at maximum depth ca. 42 cm.

13. Late summer condition (August) with only scattered pockets of concentrated water remaining.

14. Scattered regions of open concentrated water pockets in the late summer (September).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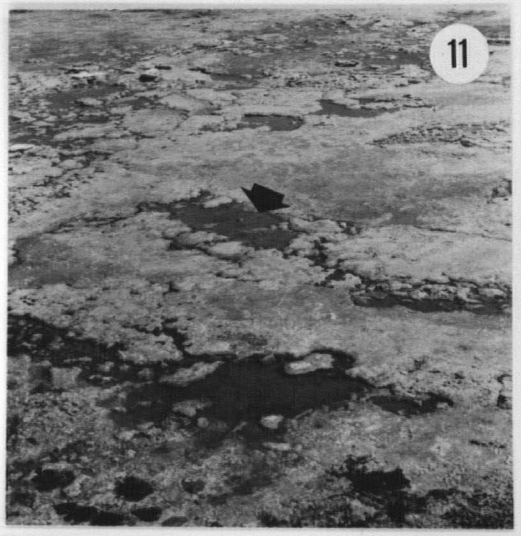
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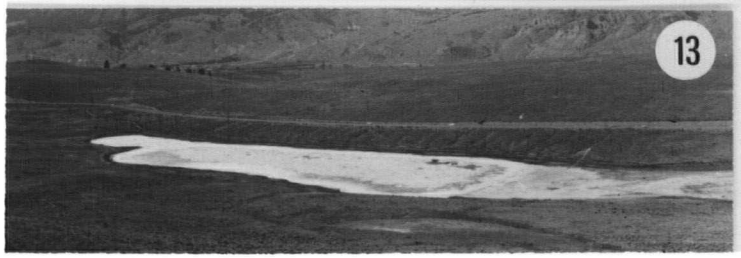
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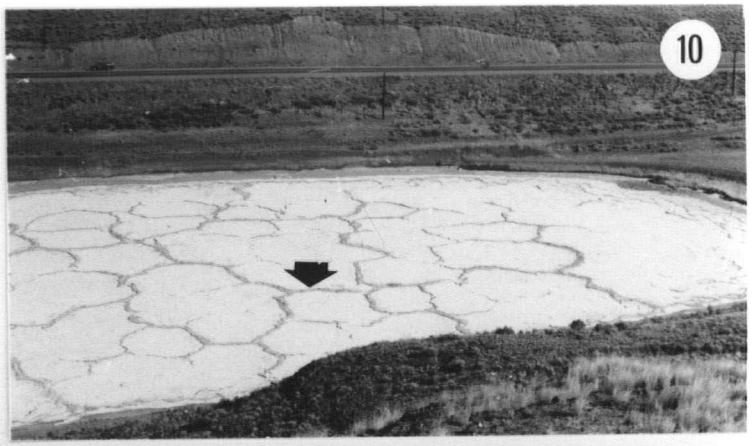
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14

Figures 15-16. Seasonal condition of Bowers Lake.

15. Spring condition (April) at maximum depth *ca.* 140 cm .

16. Late summer condition (September) with characteristic efflorescent white marginal ring of salt.

Figures 17-18. Seasonal condition of Wallender Lake.

17. Spring condition (April) at maximum depth *ca.* 150 cm .

18. Late summer condition (September).

Figure 19. "1st Salt Mine Pond" during May .

Figure 20. "2nd Salt Mine Pond" during May.


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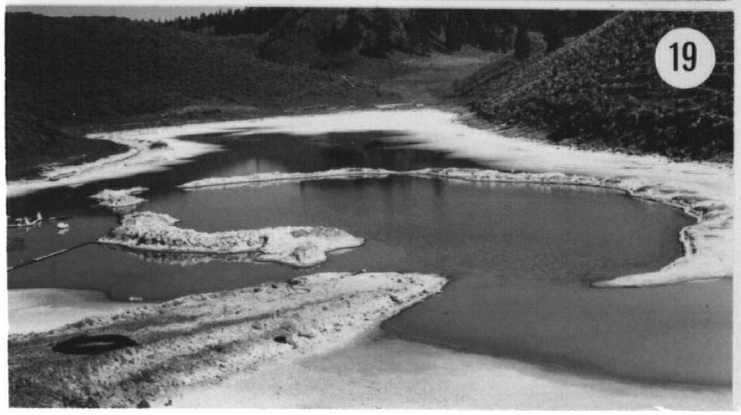
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20

in the "1st Salt Mine Pond" (Report of Minister of Mines for British Columbia, 1930).

All habitats are characterized by a narrow band (up to 3 m) of Salicornia rubra A. Nels. around the margins. The boundaries for these primary colonists of saline environments vary from year to year depending upon the annual fluctuations in the water level. The dominant aquatic macrophyte in "Cherry Creek Pond" and Wallender Lake is Ruppia maritima L., whereas the alga Chara canescens Desv. & Lois and scattered patches of Potomogeton spp. are dominants in Bowers Lake. Small isolated remnant patches of Ruppia were observed in "Polygon Pond" and Ironmask Lake. These two habitats along with the two "Salt Mine Ponds" were too concentrated to support abundant growth of macrophytes.

"Cherry Creek Pond" is unique in that there are four isolated pockets of crystal deposits throughout the year where Ruppia does not occur (Fig 5,6), whereas throughout the rest of the pond the plant is quite abundant. These crystal zones are sometimes evident during the winter months when the water freezes leaving clearly visible semi-frozen pockets of slush (Fig 8).

During the late summer there is usually a brilliant reddish-pink color cast along the margins and isolated patches due to high concentrations of sulfur bacteria which prevail in these habitats. They are particularly noticeable around masses of decomposing Cladophora, Rhizoclonium and Ctenocladus in Wallender Lake, Bowers Lake and "Cherry Creek Pond" respectively.

C. Comparative Seasonal Physical and Chemical Changes in Habitats

1. Major Ions

The dominant cation and anion constituents differ markedly in the investigated saline lakes and ponds. Chemical results for June 1968 of major ions for all the lakes and ponds are compared to seawater and expressed as meq.% of the major cations and anions (Hutchinson 1957) for comparison (Table 1). Also, values for June and August 1967 and March, May and August 1968 expressed in meq.% and meq./l are shown in Appendix Tables V - IX. The dominant salt in "Cherry Creek Pond" and "1st Salt Mine Pond" is Na_2SO_4, whereas in the "2nd Salt Mine Pond", $CO_3^=$ replaces the $SO_4^=$ as dominant. Cation proportions in "Cherry Creek Pond" are quite similar to those of seawater with Cl^- and $SO_4^=$ proportions reversed (Table 5). Bowers Lake is definitely a $MgSO_4$ lake with Mg^{++} and Na^+ cations sharing the dominant role with $SO_4^=$ in Wallender Lake, Ironmask Lake and "Polygon Pond".

The monovalent:divalent (M:D) cation ratios also differ in each habitat (Appendix Table X). Similar seasonal M:D ratios occurred in all habitats except "Polygon Pond", where spring ratios were higher (1.2) than those in the late summer (0.49). Extremely high M:D cation ratios were found in the "1st Salt Mine Pond" (36.0) and "2nd Salt Mine Pond" (75.0), whereas seasonal ratios in "Cherry Creek Pond" remained between 5.3-8.8. All other habitats investigated had a M:D cation ratio of 1.2 or less with the lowest occurring in Bowers Lake (0.47) (Appendix Table X).

Seasonal percentage composition of K^+ and Ca^{++} remain low and fairly constant in all habitats with the highest percentage of each occurring in Bowers Lake (Appendix Table V - IX). Extremely high levels of each ion are achieved after evaporation in the concentrated solutions

Table 1. Chemical Data for Major Ions in June 1968 Compared with Normal Seawater (Harvey 1963) Expressed in meq. (per cent)

	K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	$CO_3^=$
Seawater	1.7	77.3	17.7	3.3	90.0	9.3	0.7
"Cherry Creek Pond"							
Crystal zone							
Surface	.6	82.5	16.2	.7	1.6	95.0	3.4
Bottom	.8	82.8	15.6	.8	1.4	96.0	2.6
Ruppia zone	1.0	78.4	20.0	.6	1.8	94.9	3.3
"1st Salt Mine"	.39	98.3	1.3	.01	3.2	28.8	68.0
"2nd Salt Mine"	.34	96.9	2.7	.06	.6	83.0	16.4
"Polygon Pond"	1.1	43.9	53.5	1.5	.9	98.7	.4
Ironmask Lake	1.1	48.8	49.3	.8	1.9	96.2	1.9
Bowers Lake							
Surface	2.8	37.1	56.2	3.9	1.3	97.4	1.3
Bottom	2.6	39.8	54.5	3.1	1.3	97.5	1.2
Wallender Lake							
Surface	1.2	52.0	43.9	2.9	3.7	93.6	2.7
Bottom	1.1	53.3	43.8	1.8	4.1	93.3	2.6

remaining in the late summer.

Highest Mg^{++} levels occur in "Polygon Pond" (3083 meq./l) and Ironmask Lake (1266 meq./l) in late summer, with highest Na^{+} levels found in "1st Salt Mine Pond" (4269 meq./l) and "2nd Salt Mine Pond" (4434 meq./l). Percentages of Na^{+} with respect to the other cations in these two ponds and in "Cherry Creek Pond" are extremely high ranging from 82.0% to 98.3% with percentages of others relatively low (Appendix Table V - IX).

Both vertical and horizontal differences in percentage composition of ions were noted in this study. Generally, the bottom levels have the greater concentration of ions. This is particularly noticeable in Wallender Lake where the bottom stagnant layer is formed in the late summer (Appendix Table IX). It also is evident on a smaller scale in "Cherry Creek Pond" (Appendix Table IX) and in addition, the percentages of Mg^{++} and Na^{+} differ between the crystal and Ruppia zone (Fig 5), with higher percentages of Na^{+} found in the crystal zone. Sodium:magnesium ratios (5:1) in the crystal zone are higher than in the Ruppia zone (3.5:1.0). The crystalline salt deposits in "Cherry Creek Pond" covering the bottom sediment in the crystal zone were analyzed with Na_2SO_4 being the major component.

As the season progresses, the percentages of Na^{+} and Mg^{+} in solution change with Mg^{++} increasing as the waters concentrate (Appendix Table V - IX). For example, waters in "Polygon Pond" in the spring are considered to be predominantly Na_2SO_4 with Na^{+} and Mg^{++} percentages of 52.3% and 42.6% respectively. However by midsummer the dominant Na^{+} becomes the secondary to Mg^{++} with Mg^{+} and Na^{+} percentages at 65.7% and 32.3% respectively. Similar patterns are apparent in all ponds,

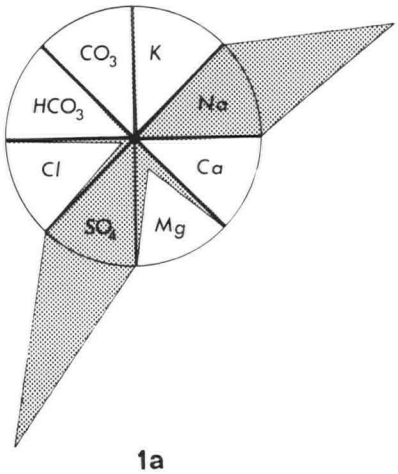
however they are more noticeable in "Polygon Pond" and Ironmask Lake due to greater concentration of ions (Appendix Table V - IX).

Proportional seasonal changes for "Polygon Pond", Ironmask Lake, Wallender Lake, Bowers Lake and "Cherry Creek Pond" are graphically illustrated in Fig 21 a & b.

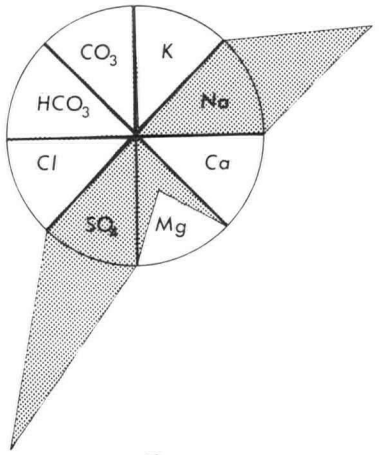
The relative anionic proportions of both Cl^- and $SO_4^=$ remained fairly constant throughout the year. One exception was "1st Salt Mine Pond" (Appendix Table V - IX) where percentages of $SO_4^=$ increased from 28.8% to 45.4%. Highest absolute values for Cl^- and $SO_4^=$ were achieved in "Polygon Pond", with Cl^- at 184 meq./l and $SO_4^=$ at 4125 meq./l (Appendix Table V - IX). Wallender Lake and the "1st Salt Mine Pond" had the highest relative proportions of Cl^- reaching as high as 5% (Appendix Table V - IX).

Seasonal changes in the $HCO_3^-/CO_3^=$ forms of combined CO_2 were evident in "Cherry Creek Pond", Wallender Lake and Bowers Lake. In the spring, HCO_3^- was the principle component; however as the seasons progressed, $CO_3^=$ became the dominant (Appendix Table V - IX). Both forms were generally similar in Ironmask Lake throughout the year. In "Polygon Pond", HCO_3^- was the seasonal dominant of the combined CO_2 fraction, whereas in the Salt Mine Ponds $CO_3^=$ was the principal component as reflected in the pH (Appendix Table V - IX). Vertical differences of HCO_3^- and $CO_3^=$ were apparent in Bowers Lake, Wallender Lake and "Cherry Creek Pond" with values at the bottom considerably higher for each component than at the surface by the latter part of the summer. Extreme vertical differences in the water column existed in Wallender Lake at the end of the summer when $CO_3^=$ (17.4 meq./l) was the major component at the surface with this form replaced by HCO_3^- (22.6 meq./l)

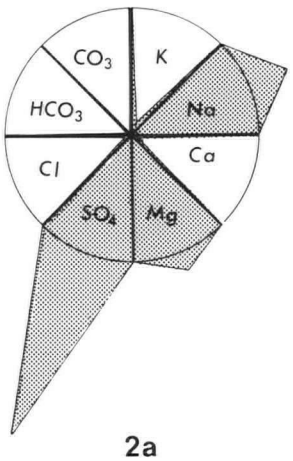
Figure 21a. Seasonal ionic diagrams for semi-permanent habitats ("Cherry Creek Pond" 1a & 1b, "Polygon Pond" 2a & 2b and Ironmask Lake 3a & 3b) in which the areas of the segments are proportional to the equivalent concentration of the major anions and cations. (a = spring values b. = late summer values).



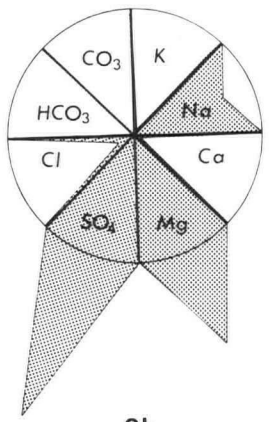
1a



1b



2a



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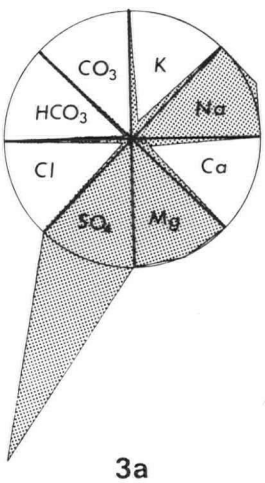
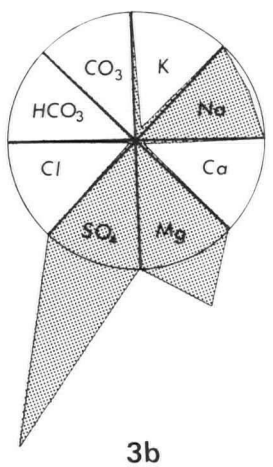
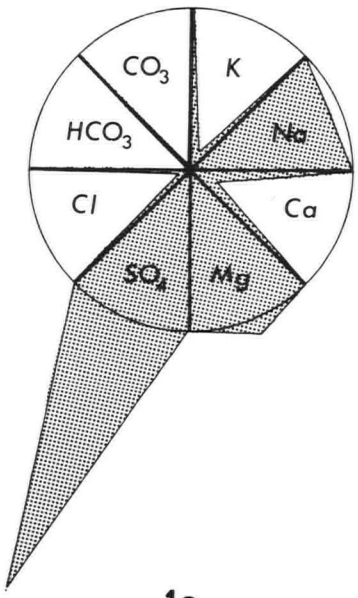


3a

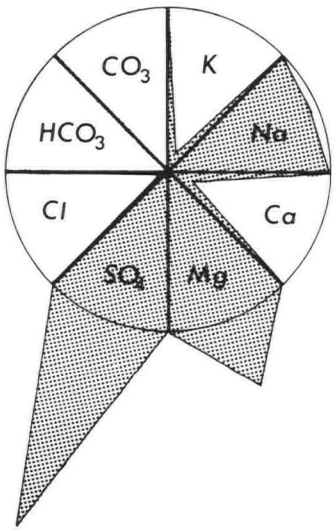


3b

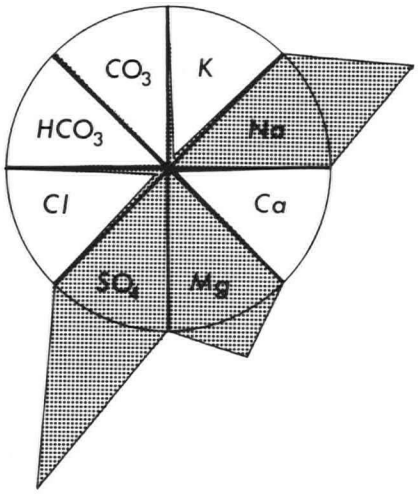
Figure 21b Seasonal ionic diagrams for permanent lakes (Bowers Lake 1a & 1b, Wallender Lake 2a & 2b) in which the areas of the segments are proportional to the equivalent concentration of the major anions and cations. (a = spring values, b = late summer values).


CO_3
K
HCO_3
Na
Cl
Ca
SO_4
Mg

1a


CO_3
K
HCO_3
Na
Cl
Ca
SO_4
Mg

1b


CO_3
K
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Cl
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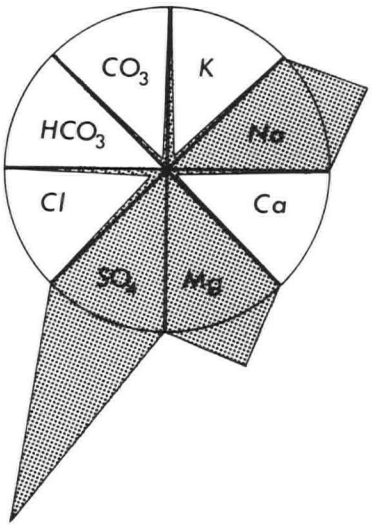
SO_4
Mg

2a


CO_3
K
HCO_3
Na
Cl
Ca
SO_4
Mg

2b

at the bottom (Appendix Table IX).

2. Nitrogen and Phosphorous

Nitrogen (NO_2^--NO_3^-) values expressed in mg/l as indicated in Appendix Table XI, were normally higher in spring and early summer, decreasing in late summer to trace amounts until after winter turnover (Fig 22). Highest levels in the spring were present in "Cherry Creek Pond" at .18 mg/l with increased concentration of $NO_2^-NO_3^-$ by midsummer in "Polygon Pond" and Ironmask Lake (Fig 22). Marked seasonal vertical differences were not detected in any of the habitats except in the early spring in "Cherry Creek Pond" and the late summer in Wallender Lake (Fig 22).

Ammonium ion concentrations were generally lower in "Cherry Creek Pond" and the two salt mines, than in the other lakes and ponds investigated (Appendix Table XI). Values as high as 49.25 mg/l were measured in "Polygon Pond" during June. Seasonal vertical differences in NH_4^+ concentrations existed with bottom measurements higher than those at the surface (Fig 23). This was particularly evident in the two permanent lakes (Bowers and Wallender) when surface measurements of NH_4^+ were .2 mg/l and 12.0 mg/l and bottom measurements at .75 mg.l and 26.0 mg.l respectively.

Phosphate concentrated as the seasons progressed to extreme levels as high as 177.3 mg/l recorded in "Polygon Pond" in the late summer (Fig 24). Phosphate levels were also higher in bottom samples than those taken from the surface, particularly in the stagnant bottom layer in Wallender Lake in late August, where levels were six times as high as at the surface (Appendix Table XII). In all of the habitats invest-

Figure 22. Seasonal values for NO_2 - NO_3 measured in mg/l for Kamloops' habitats during 1967 and 1968.
(Surface ——————; Bottom ----------)

"Cherry Ck. Pond"

"Polygon"

Ironmask

Wallender

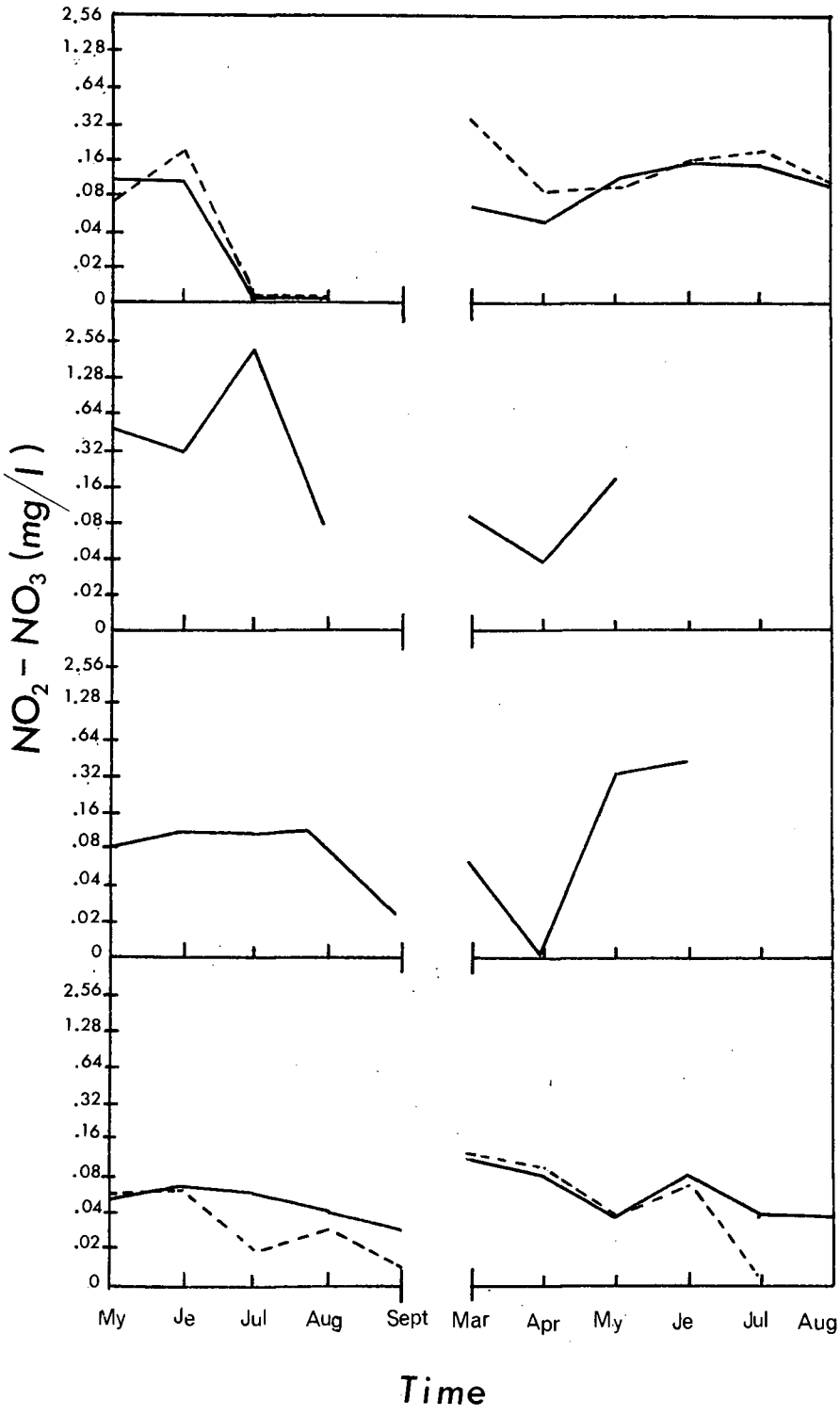


Figure 23. Seasonal surface and bottom ammonia values measured in Kamloops' habitats during 1968.
(Surface _____ ; Bottom ----------)

"Cherry Ck. Pond"

"Polygon"

Bowers

Wallender

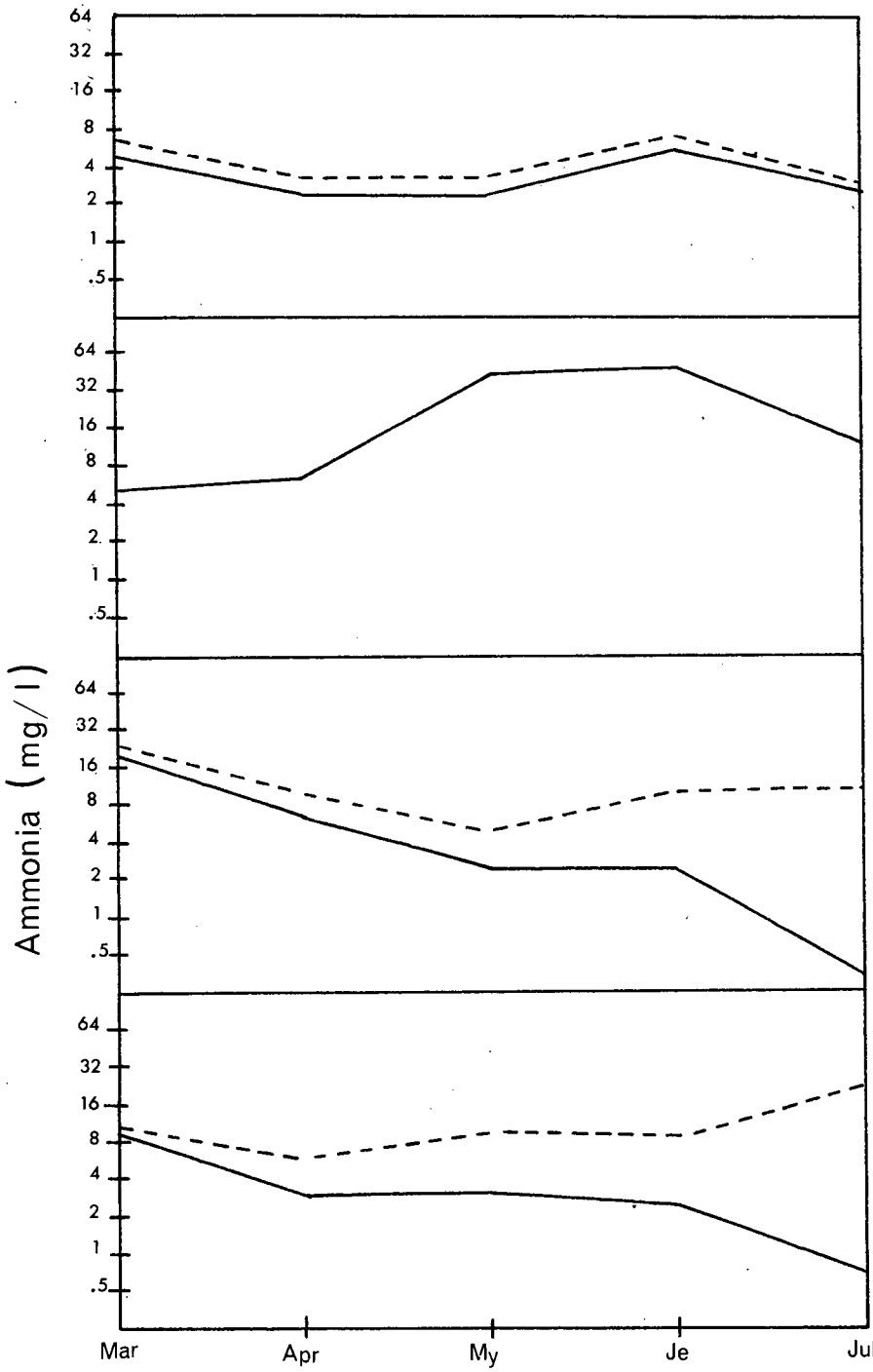
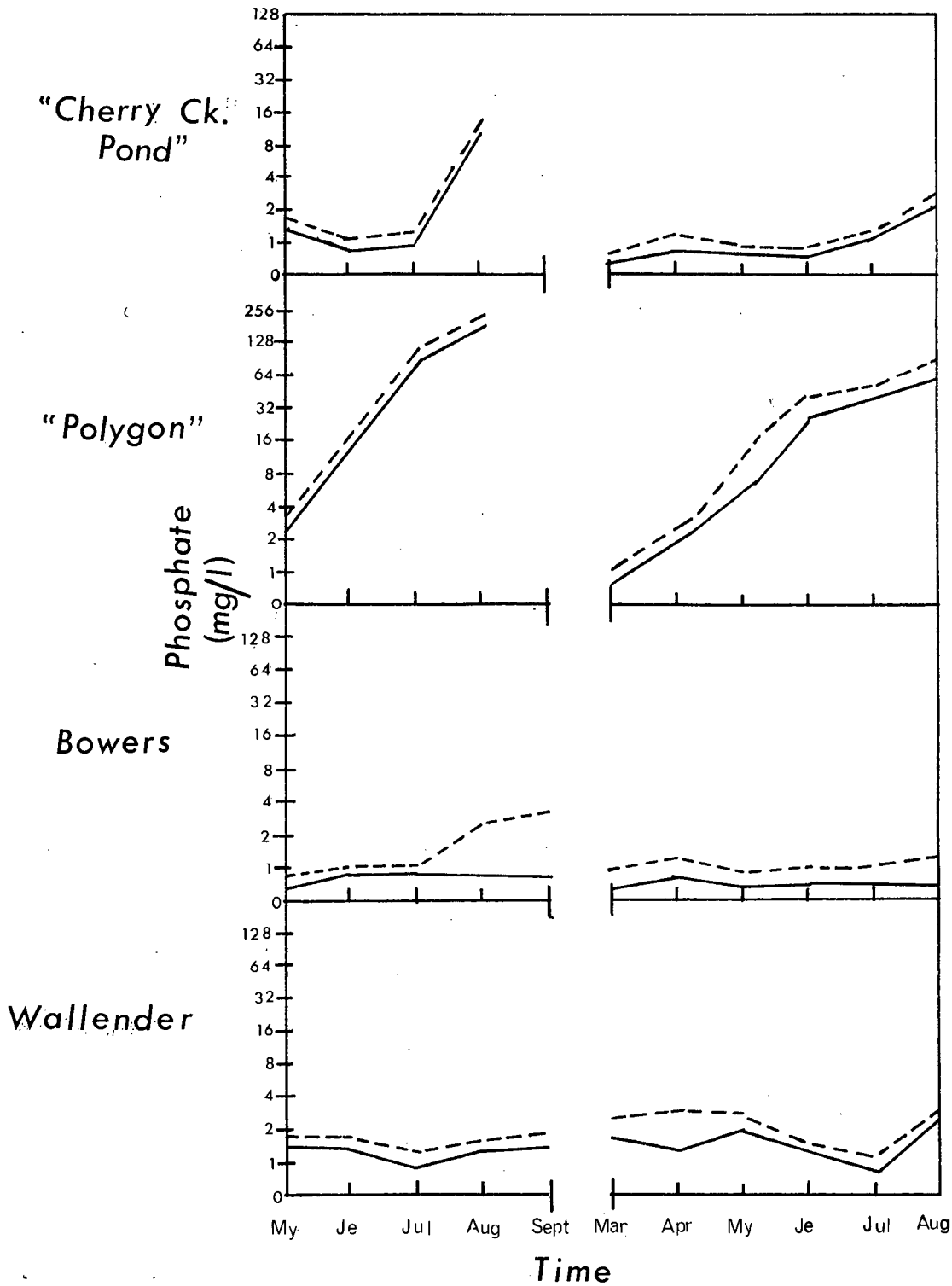


Figure 24. Seasonal values for phosphorus for Kamloops' habitats during 1967 and 1968.
(Ortho-phosphate ————————; Total phosphate ---------)


"Cherry Ck. Pond"
"Polygon"
Bowers
Wallender
Phosphate (mg/l)
128
64
32
16
8
4
2
1
0
256
My
Je
Jul
Aug
Sept
Mar
Apr
My
Je
Jul
Aug
Time

igated, except Bowers Lake and "Polygon Pond" most of the phosphate was as ortho-$PO_4^{\equiv}$ throughout the year (Fig 24). In these two habitats the meta-$PO_4^{\equiv}$ fraction made up a large percentage, consequently producing very low seasonal ortho-$PO_4^{\equiv}$ values.

3. Osmotic Potential, Total Dissolved Solids and Specific Conductivity

Extreme summer drying conditions produced a characteristic drop in the water level of all habitats. Seasonal values for total dissolved solids (TDS), osmotic potential and specific conductivity for all habitats are shown in Appendix Tables XIII - XV.

Extremely high values for TDS (Fig 25) were measured in isolated water pockets in the temporary habitats (565 g/l) in late summer. Since these habitats occupy shallow basins, salinity levels increase rapidly with extremely high salinity values occurring by early summer when water levels are relatively low as illustrated in Fig 25 for "Cherry Creek Pond" and "Polygon Pond". Conversely the two permanent lakes (Bowers and Wallender) are the least concentrated with less obvious monthly increases in salinity (Fig 25.). The two "Salt Mine Ponds" when first visited in May already had TDS values 140 g/l with values more than doubling (315.4 g/l) in "2nd Salt Mine Pond" by the end of August.

Vertical salinity differences in the water column are evident in spring and late summer as indicated in Fig 25-26. Early spring salinity differences recorded in March 1968 were apparent in all habitats except Ironmask Lake. In the crystal zone of "Cherry Creek Pond" (Fig 5-7) the bottom total dissolved solid readings was ca. three times greater than the surface. Corresponding osmotic potential differences at the surface (220 mOsm) and bottom (705 mOsm) were also recorded (Appendix

Figure 25. Seasonal total dissolved solids for Kamloops' habitats during 1967 and 1968.
(Surface ————————; Bottom ----------)

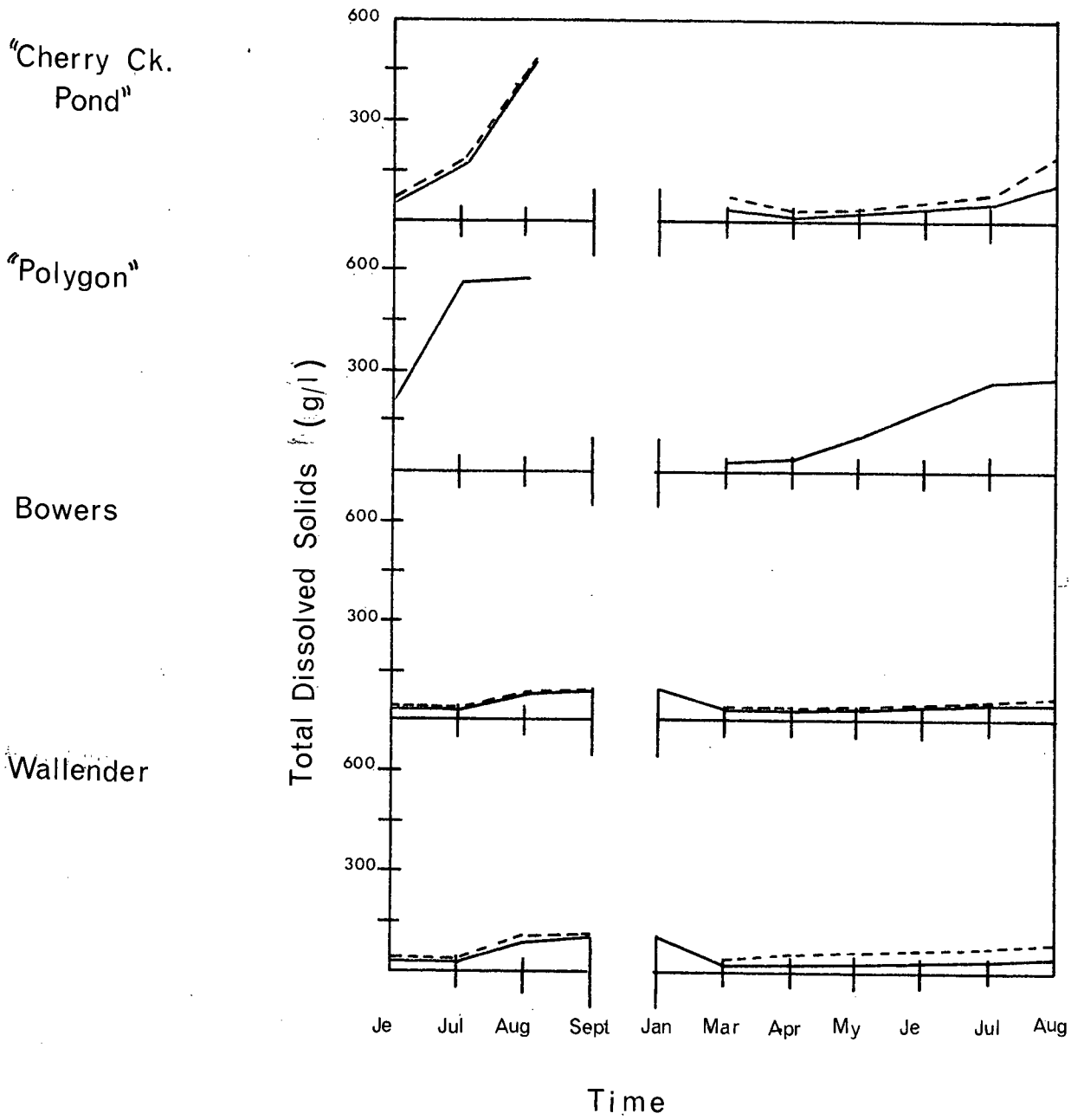

"Cherry Ck. Pond"
"Polygon"
Bowers
Wallender
600
300
Total Dissolved Solids (g/l)
Je
Jul
Aug
Sept
Jan
Mar
Apr
My
Je
Jul
Aug
Time

Figure 26. Seasonal specific conductivity values for Kamloops' habitats during 1967 and 1968.
(Surface ——————— ; Bottom ----------)

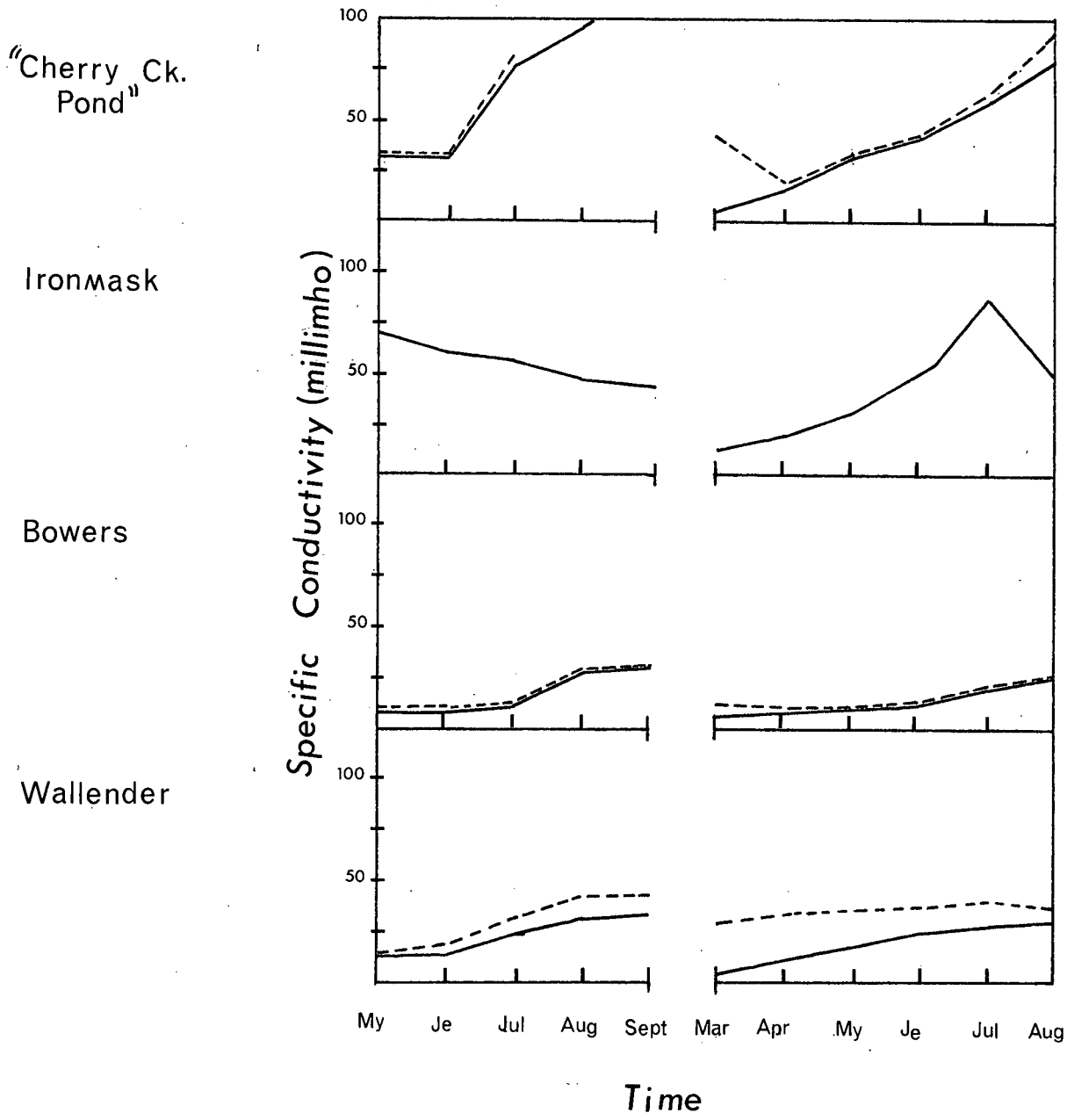

"Cherry Ck. Pond"
Ironmask
Bowers
Wallender
100
50
Specific Conductivity (millimho)
My
Je
Jul
Aug
Sept
Mar
Apr
My
Je
Jul
Aug
Time

Table XIV).

Simiar vertical differences in salinity levels were recorded for "Cherry Creek Pond" and Wallender Lake in the late summer (Fig 25-26). In all of the semi-permanent habitats, specific conductivity values would reach a summer peak and beyond this, conductivity values of the saturated solutions would fluctuate characteristically dropping to lower levels (Fig 26). A similar phenomenon occurred with total dissolved solids (Fig 25).

4. Dissolved Gases

Dissolved oxygen in all habitats gradually diminished to extremely low levels (<1 mg/l) by the end of the summer (Appendix Table XVI). Highest concentrations of dissolved oxygen (12.0 mg/l) were measured in "Cherry Creek Pond" during spring with marked differences between the open crystal zone and the Ruppia zone during the optimum growing season of Ruppia in May and June (Appendix Table XVI). Vertical oxygen differences in the water column were also noted, particularly in Wallender Lake and Bowers Lake with levels from trace amounts up to 1 mg/l at bottom levels in the latter part of the season.

Free CO_2 was normally absent in the waters, except in cases where the pH dropped below 8.3.

Hydrogen sulphide was present at the mud-water interface in all lakes and ponds investigated. Measurements varied from trace amounts to 6 mg/l in all habitats except in Wallender Lake where values >10 mg/l were recorded.

5. pH

Values for pH were high in all habitats with noticeably lower values (generally below pH 9.0) in the Mg^{++} habitats as would be expected (Fig 27). The seasonal range of pH for the permanent habitats was less than in the semi-permanent basins as shown in Appendix Table XVII. There was a noticeable trend of decreasing pH throughout the summer with increasing salinity in these semi-permanent habitats (Fig 27), whereas this was less noticeable in the permanent lakes (Wallender and Bowers). Both horizontal and vertical differences in pH were noted during certain times of the year. Horizontal differences approaching 1 unit were recorded in the early summer between the Ruppia and open water zones in both Wallender and "Cherry Creek Pond" during the active growing season of this macrophyte. (Appendix Table XVII). Vertical differences in pH were measured only in Wallender Lake where vertical differences of 1.5 units existed during late summer stagnation even though maximum depth was only 105 cm (Appendix Table XVII).

6. Depth Visibility and Temperature

The standard 20 cm Secchi Disk was visible to the bottom in all open habitats throughout the year except where there was extensive growth of Ruppia in "Cherry Creek Pond" and Wallender Lake, which made visibility of the disk impossible immediately below the surface.

Temperature in the ponds and lakes demonstrated a typical seasonal pattern (Fig 28), with extremely high summer temperatures and low winter temperature conditions existing in the semi-permanent habitats. Winter measurements were recorded as low as $-4.5^{o}C$ during January with

Figure 27. Seasonal surface and bottom pH values for Kamloops' habitats during 1967 and 1968.
(Surface ——————— ; Bottom ----------)

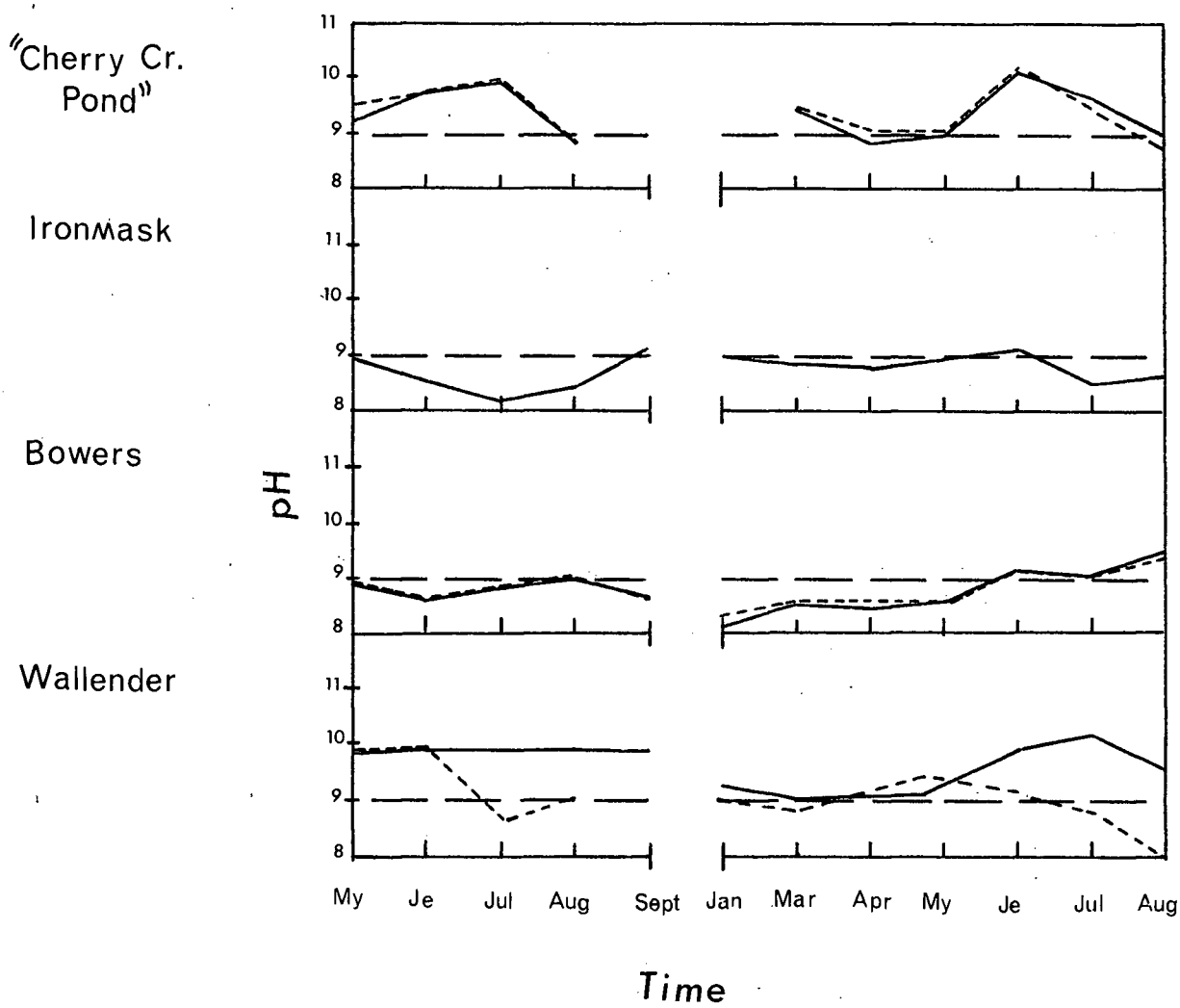
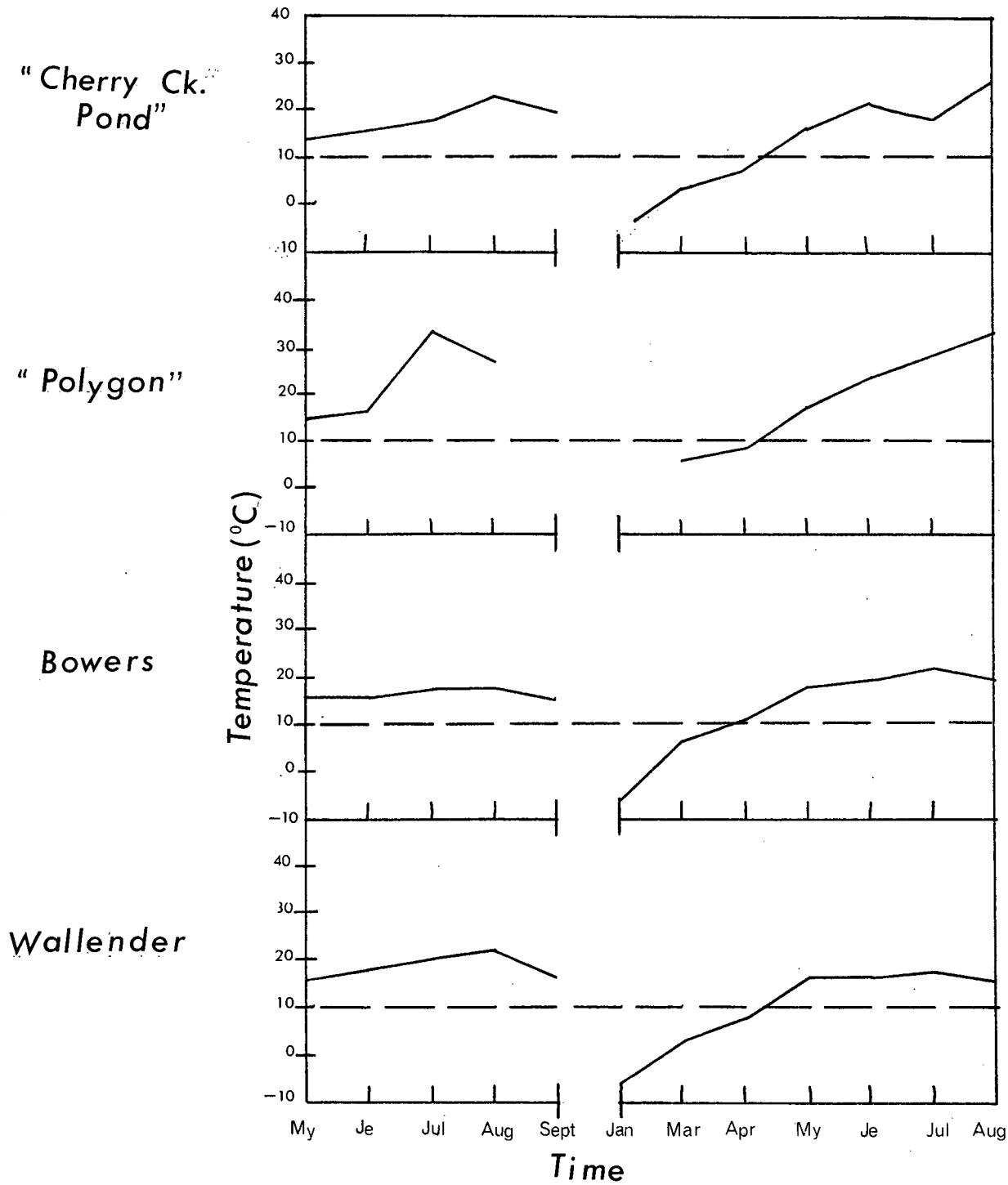

"Cherry Cr. Pond"
Ironmask
Bowers
Wallender
pH
11
10
9
8
My
Je
Jul
Aug
Sept
Jan
Mar
Apr
My
Je
Jul
Aug
Time

Figure 28. Seasonal Surface Temperature for Kamloop's Habitats During 1967 and 1968.


"Cherry Ck. Pond"
"Polygon"
Bowers
Wallender
Temperature (°C)
40
30
20
10
0
-10
My
Je
Jul
Aug
Sept
Jan
Mar
Apr
My
Je
Jul
Aug
Time

mid-summer readings up to 37.5°C in July (Fig 29). Summer peak temperatures in the two permanent lakes (Bowers and Wallender) were considerably lower (Fig 28). Afternoon temperatures in "Cherry Creek Pond", "1st Salt Mine Pond" and "2nd Salt Mine Pond", never measured above 28°C whereas those with open unshaded basins, such as Ironmask Lake and "Polygon Pond" ranged from 26 - 37°C during mid-summer in any one day (Fig 28).

The significance of microclimatic temperature conditions became evident when waters within small wooden enclosures (1.5m x 1.5m, from previous mining operations on the "Salt Mine Ponds"), indicated significant temperature differences depending upon the length of time exposed to direct sunlight during the day. In the two "Salt Mine Ponds" temperatures for waters that were shaded for the majority of the day particularly during the afternoon, ranged from 16-20°C whereas areas exposed to direct afternoon sunlight ranged from 25-28°C in any one day. Temperatures of solutions beneath the encrusted salt, as well as temperatures within the salt crystals were also normally 3-6°C lower than water exposed to the direct sunlight.

Vertical temperature differences in the water column were recorded in Bowers Lake and "Cherry Creek Pond", when density stratification occurred March 1968 (Appendix Table XVIII). Surface water temperatures in "Cherry Creek Pond" were recorded at 4.5°C with bottom temperatures at 11.5°C. Similarly, surface temperatures in Bowers Lake were 7.5°C with bottom temperatures at 12.0°C.

7. Trace Elements:

Table 19 in the appendix gives a comparative picture of the trace elements present in the surface waters of Bowers lake, Wallender Lake, "Cherry Creek Pond", "1st Salt Mine Pond", "2nd Salt Mine Pond" and "Polygon Pond". Certain elements (i.e. Al, B, Fe, Si and Ti) show relatively high concentrations in these saline habitats with very high values found in the semi-permanent habitats. Strontium values are relatively high (32-50 mg/1) in Wallender Lake, Bowers Lake and "Polygon Pond" with relatively lower levels measured in "Cherry Creek Pond" and the two "Salt Mine Ponds" (<20 mg/1)

BIOLOGICAL

Seasonal Condition of Ctenocladus

Early in the spring (mid-March) akinete germination (Fig 29) is initiated by reduced salinity levels and rising temperatures. Germination tubes are formed in several directions (Fig 30), producing cells with typical laminate chloroplasts. Rapid vegetative development (Fig 31) follows during the limited growing season, which lasts from mid-March to mid-June depending upon seasonal conditions. Zoosporangia may be present as early as mid-April on two to many-celled filaments. Released zoospores attach to various substrates (logs, rocks, rubber tires, macrophytic vegetation, etc.). By early summer (June) as the waters evaporate and salinity levels rise, some terminal cells form akinetes (Fig 32).

At this time large spherical masses, or "balls" (Fig 33) of Ctenocladus (from 15-25mm) achieved from initial akinete germination

Figures 29-35. Stages in the life history of Ctenocladus in the field and seasonal condition.

29. Germination of Ctenocladus akinetes with germination tubes at 2-3 cell stage (note chains of germinating akinetes) x300.

30. Akinete germination with several germination tubes arising in several different directions x1200.

31. Vegetative filaments of Ctenocladus illustrating general appearance with acute branching x300.

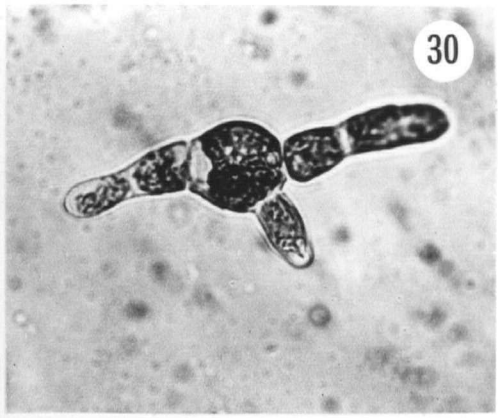
32. Formation of terminal akinetes (A) illustrating reduced cell size at terminal end of filament x1200.

33. Spherical colonies of Ctenocladus (10-25 cm) prior to salt encrustment. Ctenocladus at this stage is primarily vegetative.

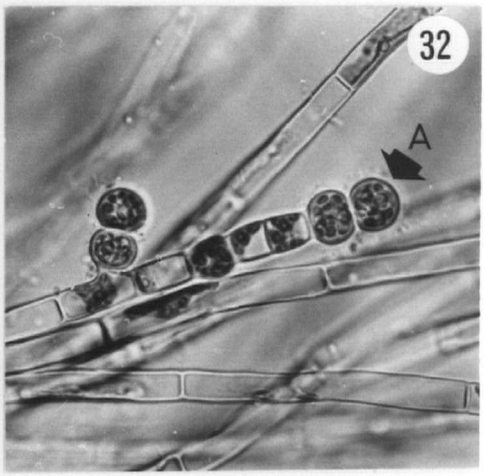
34. Encrusted spherical colonies of Ctenocladus (akinetes) deposited along the margin of "Cherry Creek Pond" in the late summer.

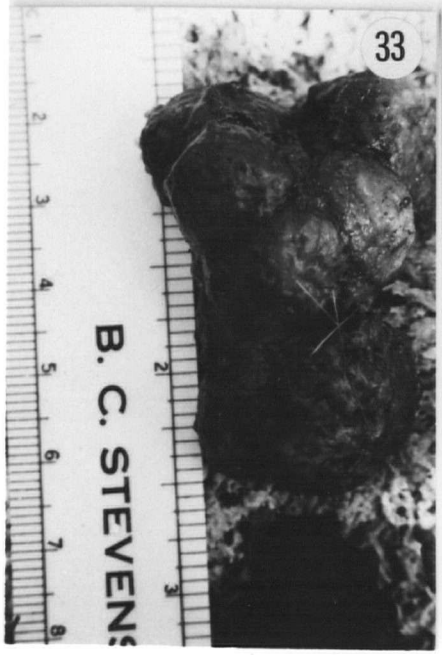
35. Encrusted spherical colonies (10-25 cm) illustrating salt encrustment. Ctenocladus at this stage is entirely akinetes.

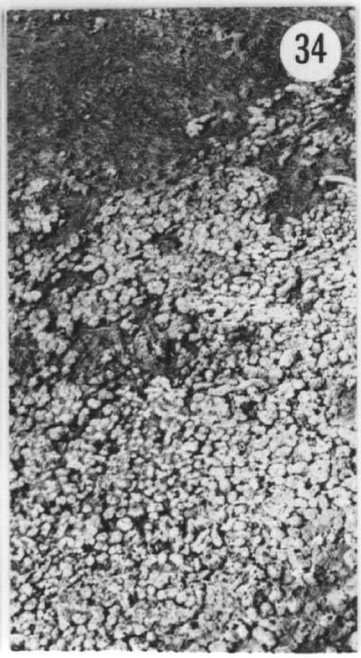

29

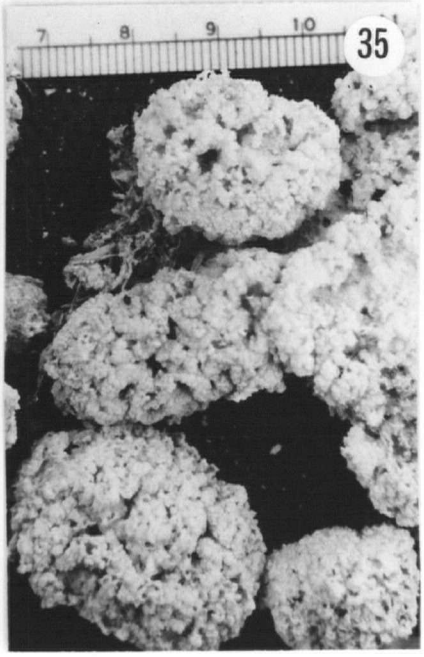

30


31


32
A


33
B. C. STEVENS


34


35

are present throughout the crystal zone in "Cherry Creek Pond" as well as along the margins of the Ruppia zone. The size of these colonies varies from year to year depending upon the length of the growing season. This was apparent in "1st and "2nd Salt Mine Ponds" where these colonies were never greater than 15 cm in diameter. By July, in "Cherry Creek Pond" zoosporangia were absent and many cells were converted in to akinetes. In some extreme habitats, ("1st Salt Mine Pond" and "2nd Salt Mine Pond"), most if not all cells were converted into akinetes by May. Generally there is a noticeable change from a light green to a dark green color when vegetative cells have been completely converted into akinetes. By the end of July, with decreasing water levels, spherical masses of akinetes are stranded along the margins and become encrusted with salt (Fig 34-35). The centers of these encrusted masses, however, remain moist for some time after initial surface encrustment. Cell dimensions of vegetative material also undergo drastic changes with increased seasonal salinity as indicated in table 2. Greatest mean cell length of 88.2μ was obtained in May 1968, collections from "Cherry Creek Pond" with dimensions decreasing as the waters concentrate.

Isolated spherical colonies are often deposited in the semi-moist Ruppia zone in "Cherry Creek Pond" late in the summer. The upper portion of these masses is a sun-bleached orange-brownish color and the lower portion located in the moist sediment, a dark green color. By the end of the summer when the water levels in "Cherry Creek Pond" were extremely low, filamentous chains of akinetes were found approximately 6 cm beneath a translucent crystal layer partially buried in the bottom black ooze. A pinkish-red layer of bacteria was also apparent

Table 2 Monthly Mean Cell Dimensions of Ctenocladus from Field Collections (1968). Collected in Kamloops Area.

MONTH	Range of Cell Size in μ LENGTH	WIDTH	Mean Cell Dimension in μ LENGTH	WIDTH	Mean L:W RATIO	Osmotic Potential in mOsm SURFACE	BOTTOM
MARCH	22.5-75	5.-7	50.4	6.0	8.3	220	705
APRIL	25-105	6-7	65.0	6.0	10.8	330	365
MAY	35-180	5.5-8.5	92.0	6.7	13.8	536	559
JUNE	37.5-120.0	6.5-9.5	53.0	7.3	7.2	690	700
JULY	17.5-87.5	6-9	43.4	7.5	5.7	1237	1258
AUGUST	15.0-37.5	7-12	18.8	9.5	1.9	1612	2382

at the crystal-Ctenocladus zone.

Gametangia formation or development was never observed in field collections from any of the investigated habitats.

During the winter visit (January 1968) fragments of dark green akinete chains were buried beneath 20 cm of snow and 18 cm of ice at the crystal-sediment interface.

Seasonal Occurrence of Other Dominant Organisms

a. Algae

The diversity in algal species inhabiting the saline environments is smaller than expected in a system where salinity extremes do not exist. Greatest diversity of species occurs during spring and early summer, prior to increased salinity levels. Ironmask Lake, "Polygon Pond", "1st Salt Mine Pond" and "2nd Salt Mine Pond" have the least number of total species (generally < 10) with most occurring at the mud-water, mud-crystal interface. Bowers Lake supports the greatest number of species which reflects its relatively lower salinity levels. Dominant species in the saline habitats investigated are shown in Appendix Table XX.

Sediment cores taken from the saline habitats showed no signs of algal spores, possibly reflecting the high pH of the sediments.

Some general seasonal patterns in these habitats were noted for the algae. In the spring when temperatures are low (4-10^{o}C) Ulothrix tenerrima Kutz. was dominant along the margins and in the waters of all habitats. Also Spirogyra sp. was present in "Cherry Creek Pond" in late spring, prior to Ctenocladus blooms. As temperatures increased,

Ctenocladus in "Cherry Creek Pond" and the two "Salt Mine Ponds" becomes dominant. In Bowers Lake and Wallender Lake, Rhizoclonium hieroglyphicum (C.S.Ag.) Kutz. and Cladophora fracta (Dillw.) Kutz. respectively dominate by forming enormous mats that almost cover these lakes in mid-summer. As temperatures increase and water levels drop, Chlamydomonas spp. and Ochromonas vallesiaca Chodat become dominant occuppying isolated pools in these semi-permanent habitats.

By mid to late summer, Chara canescens Desv. & Lois was generally the dominant in Bowers Lake, occupying scattered zones throughout the basin. Chara was not present in any of the other habitats, possibly reflecting the chemical nature of the habitats.

In the fall when temperatures dropped, Ulothrox tenerrima appears again along the margins and among the decomposing masses of Cladophora and Rhizoclonium in Wallender Lake and Bowers Lake respectively.

b. Brine Shrimp

Abundant numbers of Artemia salina (L) occurred in "Cherry Creek Pond", "1st Salt Mine Pond" and "2nd Salt Mine Pond".

Description of Other Investigated Ctenocladus Habitats.

Saline environments investigated in the three arid regions of California and Nevada were similar to the Kamloops area in that minimal precipitation and high temperatures prevailed during the summer months. A typical white ring of efflorescent salts bordered the margins of all habitats. With the exception of Mono Lake (Fig 36), with a mean depth of 19.0 m (Mason 1967), all other saline habitats investigated were

shallow ponds with probable maximum depths after spring runoff and precipitation of less than 1.5 meters (Fig 37-38).

Analysis of saline waters containing Ctenocladus in Nevada and California showed extremely high conductivity levels (Table 3). Other investigated saline habitats used for comparative purposes in the respective area exhibited relatively lower specific conductivity levels (Table 3). Sodium was consistantly the dominant cation making up more than 95% of the total cations in all six habitats. In Mono Lake, both $CO_3^=$ and Cl^- were the major anion constituents making up 44.1% and 38.0% respectively. In "Stateline Pond" waters, $CO_3^=$ alone was the dominant (93.2%). "Hazen Pond" resembles "Cherry Creek Pond" chemically in being predominately Na_2SO_4. All habitats with Ctenocladus had a pH of 9.4 or above, whereas the pH of the other ponds ranged from 8.2-9.2.

Ctenocladus was collected in Mono Lake, "Hazen Pond", and "Stateline Pond", in June 1968. Material from "Stateline Pond" was entirely akinetes (14.0μ x 15.5μ) with large mats covering various substrates (i.e. wooden fenceposts, barbed wire, rocks, etc.) throughout the pond (Fig 39). Spherical masses or balls of Ctenocladus ranging from 10-20 cm. in diameter were present in "Hazen Pond" along the margins as well as attached to Ruppia stems (Fig 40). Many of the cells (at least two-three per filament) had already been converted into akinetes (12.5-14.5μ). Cell dimensions of remaining vegetative cells averaged 8.5μ x 48.2μ. No signs of zoosporangia were observed from these collections in "Hazen Pond". Collections of Ctenocladus from Mono Lake were made along the margins from under the salt encrusted granite rocks next to the mud-water interface.

Table 3. Physico-chemical Analysis of Waters from California and Nevada Area

		K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	HCO_3^-	$CO_3^=$	pH	Specific Cond: millimho
Mono Lake	meq/l	46.0	1108.0	6.8	.55	464.8	218.7	34.0	504.6	9.5	56.6
	%	3.86	95.6	.5	.04	38.0	17.8	41.4	2.8		
"Little Mono Lake"	meq/l	1.1	126.0	.66	1.4	13.8	14.5	-	-	9.2	3.2
		.8	97.6	.5	1.4	-	-	-	-		
"Hazen"	meq/l	21.7	1782.0	58.3	1.1	166.5	1552.0	97.9	333.2	9.4	75.4
	%	.6	95.7	3.1	.6	7.5	73.5	3.5	15.5		
"2nd Hazen"	meq/l	1.8	360.0	1.0	.6	35.4	135.4	-	-	8.4	16.0
	%	.5	99.0	.2	.2	-	-	-	-		
"Stateline"	meq/l	3.7	2260.8	29.1	1.6	44.8	187.5	329.5	2880	9.6	84.9
	%	.02	98.58	1.3	.1	1.8	5.0	9.5	83.7		
"2nd Stateline	meq/l	2.3	86.9	.5	.7	6.1	93.7	-	-	8.7	2.8
	%	2.5	96.1	.6	.8	-	-	-	-		

Figures 36-41. California and Nevada habitats with Ctenocladus.

36. Mono Lake near Lee Vining, California in June.

37. "Stateline Pond" near Klamath Falls, Oregon in June.

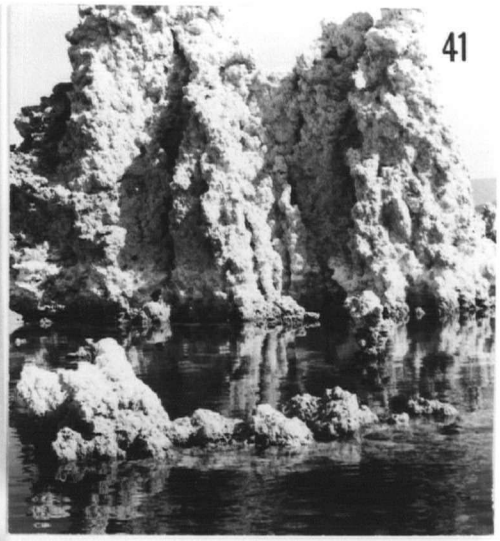
38. "Hazen Pond" near Reno, Nevada during June, with spherical colonies of Ctenocladus along margin as indicated by the arrow.

39. Habitat appearance of Ctenocladus in "Stateline Pond" attached to salt encrusted rotting log.

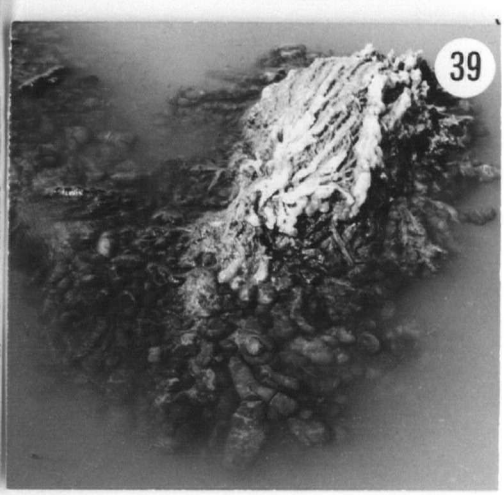
40. Habitat appearance of Ctenocladus in "Hazen Pond" with large spherical colonies (10-20 cm) free-floating or attached to Ruppia maritima L. as indicated by the arrow.

41. Calcareous tufa structures in Mono Lake, California. Ctenocladus attached to these structures in crevices at water level down to ca. 1m.


36


41


37


39


38


40

Additional collections were also taken from calcareous tufa towers, formed by inorganic and organic processes (Dunn 1953; Scholl and Taft 1969) standing along the shoreline at the western end of the lake (Fig 41). These collections were made from crevices at water level down to about 1 m (Fig 41). Material was still in vegetative condition with cell dimensions averaging 41.3μ x 9.5μ. Many cells were converted into akinetes with no zoosporangia present. Artemia salina was abundant in all habitats with Ctenocladus.

EXPERIMENTAL TRANSPLANTS OF CTENOCLADUS

Transplants of Ctenocladus into previously uninhabitated saline water indicated that this alga could not normally grow in such habitats. Transplants made into Bowers Lake showed cells almost entirely converted into akinetes after one month (Fig 42) with those inoculated for 2-month period indicating signs of non-viability. Transplants into Wallender Lake were unavailable for April and May due to predation by Cricotopus sp. larvae, however, the period between May and June showed abnormal growth. Short, irregular cells (17.5μ x 12.0μ) did exist, but with no signs of zoosporangia (Fig 43). Transplants in Ironmask Lake were similar to those of Bowers Lake with the majority of the cells becoming akinetes. Transplants in "Polygon Pond" waters after a month incubation from March-April existed as reduced cells (27.5μ x 1.25μ) with a limited number of zoosporangia. However, individual zoospores within these zoosporangia could not actually be seen. Collections examined after April indicated cells to be short and irregular with many akinetes and no further signs of zoosporangia (Fig 44).

Transplants placed in the crystal zone of "Cherry Creek Pond" showed normal vegetative growth with numerous zoosporangia (Fig 45) and no sign of akinetes except a limited number in the June collections. However, transplants placed in the Ruppia zone of this pond showed vegetative cells to be in similar condition but the number of zoosporangia formed in the March-April period much reduced. Vegetative cells were small and irregular between May and June in the Ruppia zone with many akinetes and no further signs of zoosporangia.

Figures 42-45. Field transplants of Ctenocladus into investigated saline habitats.

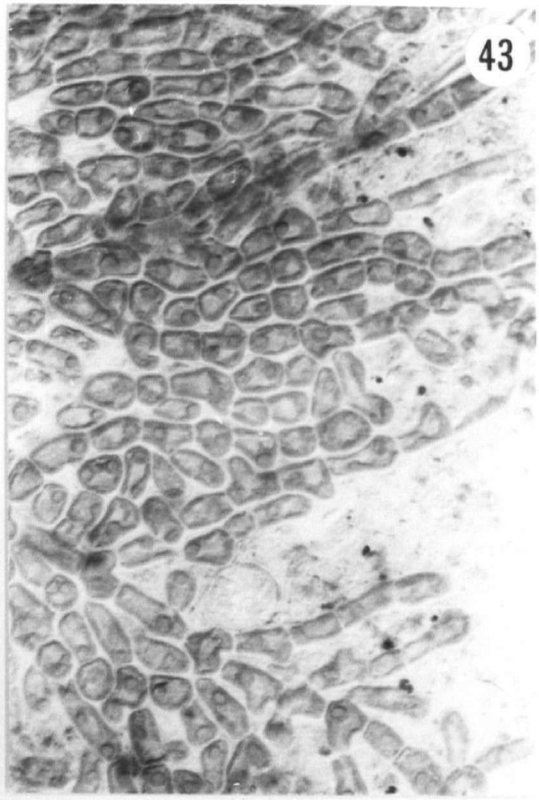
42. Transplants of Ctenocladus into Bowers Lake illustrating mumerous akinetes with remaining reduced cells after one month. (note granular appearance) x1200.

43. Transplant of Ctenocladus into Wallender Lake illustrating short irregular cells (17.5-12.0u) after one month x1200.

44. Transplant of Ctenocladus into "Polygon Pond" illustrating reduced irregular cells x1200.

45. Transplant of Ctenocladus in "Cherry Creek Pond" showing zoosporangia (Z) with zoospores x1200.


42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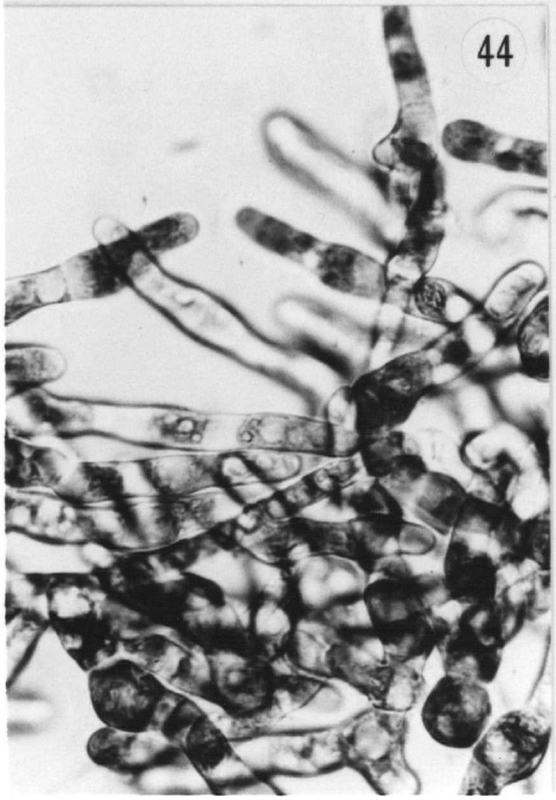
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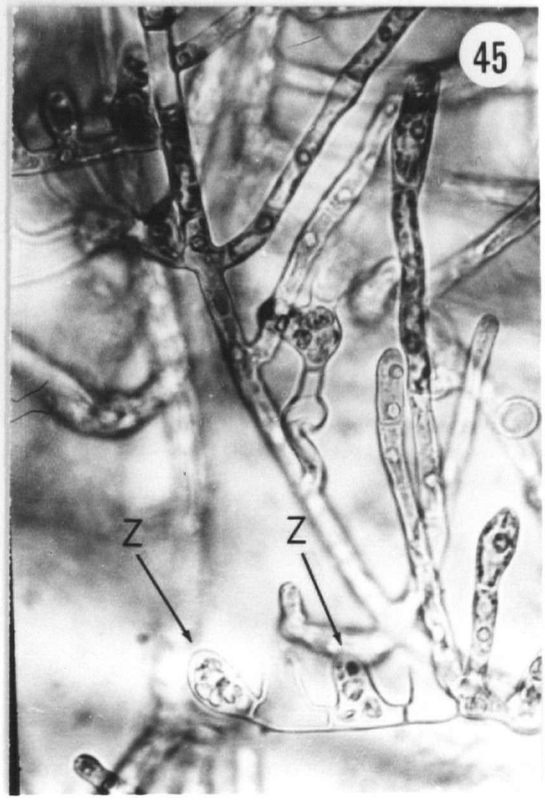

45
Z
Z

LABORATORY RESULTS

General Culture Conditions:

Culture solutions found to be most successful for cultivation of Ctenocladus included: 1.) biphasic saline soil water (biphasic SSW), 2.) Chihara Marine Media (Chihara) and 3.) Provasoli ES enrichment (ES.). Laboratory investigations showed these solutions capable of supporting Ctenocladus in the vegetative condition with production of numerous zoosporangia when transferred every 10-14 days. Zoosporangia were not present beyond six-fold dilutions of Chihara medium even with daily transfers, however, dilutions below this were adequate for normal zoosporangia formation. Major cations of the defined medium prepared specifically for cultivation of Ctenocladus are in similar proportions to those of seawater, whereas proportions of major anionic constituents ($SO_4^=$, Cl^-) are reversed (Appendix Table XXI). Akinete germination (95-100%) with vegetative cells ranging to 180μ in length and 6.5-7.0μ in width were obtained in this culture solutions. Zoosporangia formation, however, could not be induced even with light and temperature variations.

Life History Studies:

Isolations of C. circinnatus were made into both Chihara Medium and SSW for this study. The macroscopic appearance of the organism in the laboratory was similar to that observed in the field. Material consisted of spherical colonies ranging in size from minute to 2-3 cm in diameter. The limited heterotrichous nature of the organism was obscure. It is hidden by the dominant erect system of unilateral branches arising at acute angles from the distal end of the cells. Each cell has one

nucleus and a parietal laminate chloroplast with one-six pyrenoids. Around the pyrenoid are two half-ring or cup-shaped starch bodies. Terminal cells generally possess more pyrenoids (up to six) than intercalary cells (one-three).

Cell dimensions of the vegetative material are extremely susceptible to change depending upon environmental conditions. Average cell dimensions were ca. 105-7.5μ under optimum conditions (<1100 mOsm). Changes in total concentration of the solution brought about drastic variations in average cell length with cells in salinities over twice that of seawater reduced in length three-four times. Consequently, under unfavorable conditions (i.e. increasing salinity, ionic changes, extreme temperatures) terminal cells first undergo rapid division followed by successive intercalary cells forming numerous septa with each segment within the original cell then rounding up into the thick-walled resting akinete.

Cultures may be maintained entirely free of akinetes under optimal conditions if transferred once a week with cells ranging from 6-8μ in width and from 45-220μ in length.

Under optimal conditions akinete germination occurs within 10 hr. The germination tube branches in one or several directions by lateral proliferations at the apical end of the newly formed cells. Each branch grows in a different direction thus giving the plant a radial or spherical macroscopic appearance. Zoosporangia form 6-8 days after akinete germination. This normally begins when the ends of the apical cell round up to form a lateral extension at the upper end. Zoosporangia differ from the normal vegetative cell by the presence of a lateral protrusion (Fig 45), approximately 15-23μ. Eight to 16 biflagellate zoospores (6x8μ) form within the zoosporangia. They are released

singly or in mass through a pore at the end of the lateral protrusion. The zoospores are spherical to subspherical with a conspicuous red eyespot located within the chloroplast at the anterior end. The two equal flagella are approximately as long as the body. Occasionally zoospores were clumped together forming a motile volvocalean type colony. Further development of these structures was not observed, possibly indication that this was an abnormal form of development due to unsatisfactory culture conditions.

Zoospores swim for a period and then settle singly or in groups onto a substrate. Germination of the zoospores begins with formation of a lateral germ tube. This forms a prostrate thallus system of ca. 15-35 cells, each cell capable of branching laterally, which in turn acts as an apical initial giving rise to the erect filamentous portion of the thallus.

Formation of gametangia with development of gametes and zygotes was not observed from Kamloops material in the laboratory. However, gamete formation has been reported by other investigators (Ruinen 1933; and Woronochin and Popova 1929). Zygote germination has never been observed.

Biological Factors:

1. Antagonism

Antagonistic responses between Ctenocladus and dominant algae found in Bowers Lake and Wallender Lake (Rhizoclonium hieroglypyicium and Cladophora fracta) respectively were not detected in the laboratory as noted earlier in the methods section (p. 21). Normal vegetative growth and zoosporangia formation of Ctenocladus occurred with material growing

epiphytically on normal filaments of Rhizoclonium and Cladophora.

2. Predation

Chironomid larvae (Cricotopus sp) introduced into biphasic SSW cultures of Ctenocladus gave strong evidence of predation. After 1 week all slides were denuded of Ctenocladus. In controlled cultures without the larvae, the alga remained on the slides throughout the experiment. Furthermore, periodic laboratory checks of larval gut contents suggested similar results. Predation of Ctenocladus by chironomid larvae in the laboratory studies supports similar observations made at Wallender Lake.

Physico-chemical Experimental Studies

1. Effect of Temperature

Temperature tolerance levels for akinete germination were established in the laboratory under optimum light conditions with temperatures ranging from 0-35°C as illustrated in Fig 46. Akinete germination was indicated by initial rupturing of the thickened wall. Germination occurred from 5-31°C with the optimum between 9.0-26.0°C (Fig 46). Temperatures below 9.5°C and above 34°C produced only one-two celled germination tubes if at all. Possibly this indicates a different optimum for initial germination than for vegetative development. Under various temperatures, the length of time for initial germination was significant. At 19-21°C over 90% of the akinetes had germinated after 6-10 hr whereas nearly 125 hr elapsed for cultures at 10°C before 90% of the akinetes germinated (Appendix Table XXII).

Germination at optimum temperatures after being subjected to temp-

eratures above the optimum range were also examined. The akinetes lost their color and most were nonviable after exposure to temperatures above 34°C (Appendix Table XXIV). Conversely up to 3 months of freezing temperatures as low as -15°C had no adverse effects on akinete viability.

2. Effect of Light

In the laboratory light intensity was found to be a significant factor with respect to akinete germination (Fig 46). In the total absence of light, no germination occurred even though other factors were optimal. Germination could be induced by subjecting akinetes to light intensities as low as 214 lux on a 16 hr light/8 hr dark cycle. Akinete germination remained high (>90%) through various light intensity regimes to approximately 9630 lux. At this intensity, mean germination percentages showed a slight decrease (Fig 46). Akinetes subjected to intensities of 12,305 lux showed a considerable decrease in germination (16.4%).

Akinetes subjected to extreme light conditions prior to transfer to optimal light regimes were also examined for germination. No light, maximum light (12,305 lux) and optimum light (ca. 4280 lux) were examined for germination. Those in darkness up to 1 month showed maximum akinete germination when exposed to 4066-4280 lux illumination. Conversely high light intensity showed adverse affects with the akinetes again losing color and being nonviable (i.e. 11.9% germination) after exposure to 12,305 lux for 10 days. Encrusted dry fragments also exposed to 12,305 lux for 10 days then transferred into filtered SSW extract under optimal light conditions (ca. 4280 lux) demonstrated normal germination with mean percentage values well above 90%. (Appendix Table XXVI).

Akinetes placed in filtered SSW extract from the same encrusted fragments and exposed to 12,305 lux showed considerable reduction in mean germination (<20%).

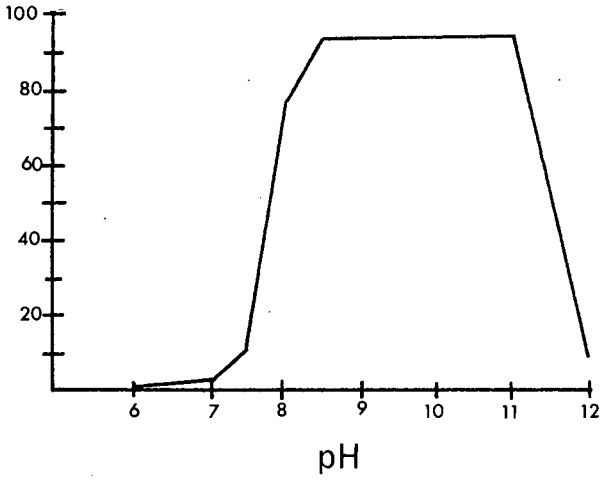
3. Effect of pH

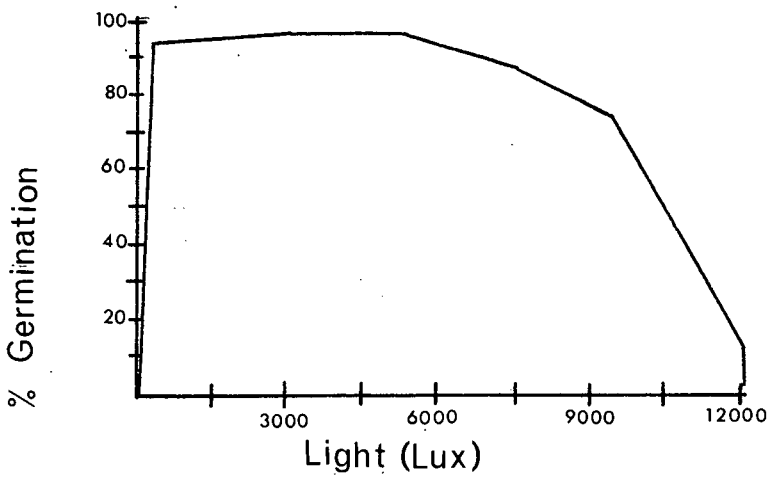
Laboratory studies showed optimal pH conditions for germination of akinetes is between 8.4 and 11.0 as indicated in Fig 46. Values below 7.1 have less than 1% germination. Similarly, pH levels above 11.0 showed reduced germination. In addition, studies indicated that cell dimensions were influenced by hydrogen-ion concentration with solutions below 8.0 inducing shorter cells. That is, at pH below 8.0 the cells averaged 32.4 x 8.5μ whereas cells maintained at pH 8.5-9.0 averaged 82.6 x 7.5μ. The number of cells produced per germination tube was also reduced to less than 3 cells at pH below 8.3.

Akinetes subjected to hydrogen-ion concentrations outside their optimal range for a given period and then transferred to optimal solutions were also investigated as shown in Appendix Table XXVIII. Reduction in mean germination (to 0%) at pH below 7.0 indicates unfavorable conditions to the resting stage at these levels. Similar conditions occur at pH 11.0 with a mean germination of 4.9%.

Attempts to induce akinete germination in the laboratory by increasing the pH of buffered distilled water to optimum levels were unsuccessful. Studies indicated that an increase in pH to levels within the normal optimal range (8.6-10.0) for akinete germination was insufficient to stimulate this process even though light and temperature conditions were optimal.

Figure 46. Effect of pH, light and temperature on germination of Ctenocladus akinetes. (n = 800)


100
80
60
40
20
6
7
8
9
10
11
12
pH


% Germination
100
80
60
40
20
3000
6000
9000
12000
Light (Lux)

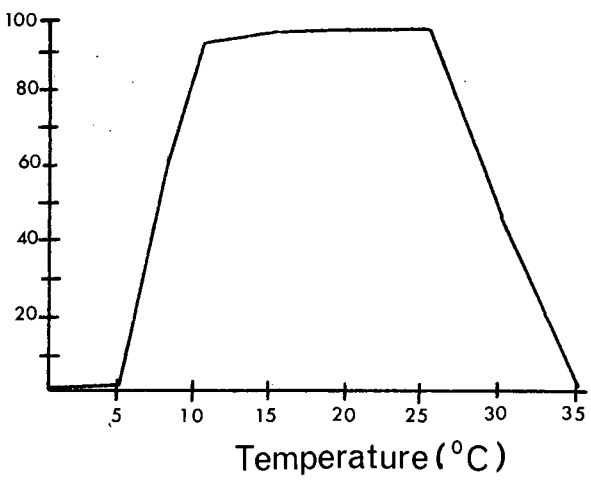
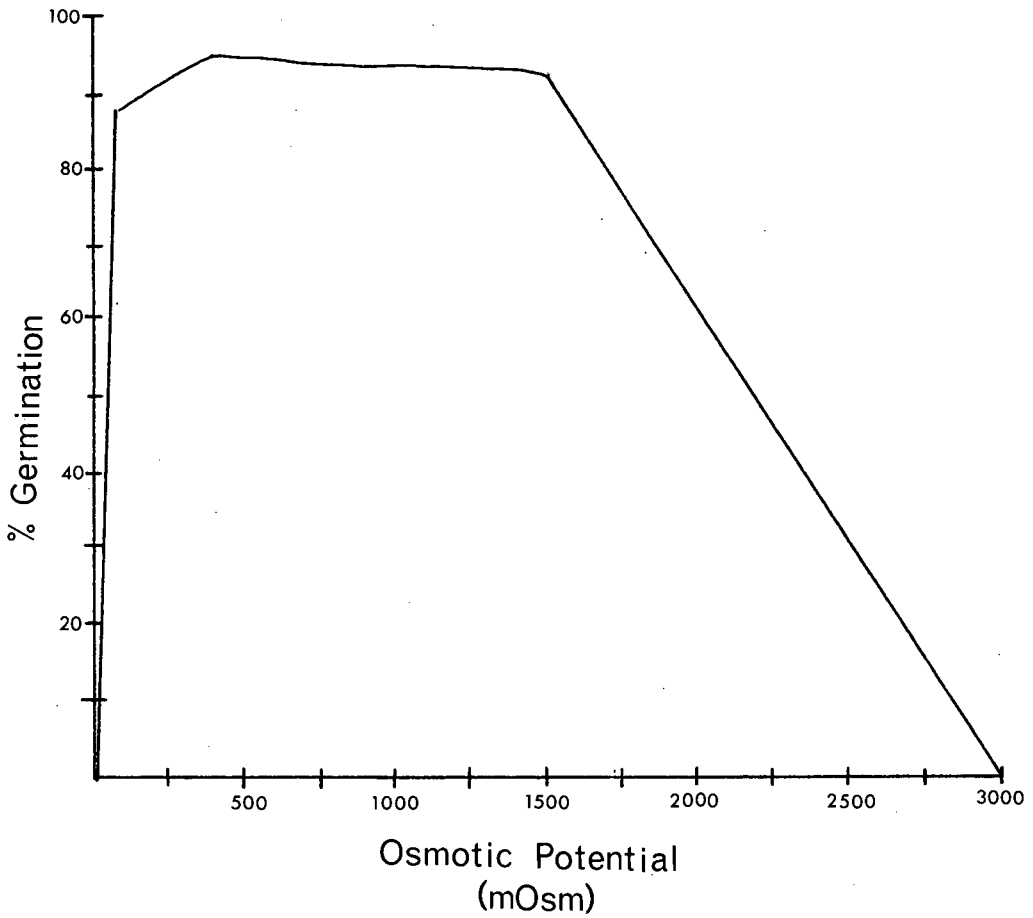

100
80
60
40
20
5
10
15
20
25
30
35
Temperature (°C)

Figure 47. Effect of dilution of "1st Salt Mine Pond" water with distilled water on germination of Ctenocladus akinetes. (At 20°C, 4066-4280 lux. 16 light/8 dark cycle)


100
80
60
40
20
% Germination
500
1000
1500
2000
2500
3000
Osmotic Potential
(mOsm)

4. Effect of Osmotic Potential

Waters collected from various investigated lakes and ponds with different ionic proportions and concentrations showed the optimal osmotic potential for akinete germination to be below 2383 mOsm (Appendix Table XXIX). Above this level, germination rapidly decreased. Mean germination as high as 98% was achieved at 1700 mOsm of ca. two times that of seawater. Germination was also drastically reduced with dilutions of more than 200 times or less than 40 mOsm as shown in Fig 47. The number of cells produced per germination tube was greatly reduced at extreme osmotic potential regimes with < 2 cells produced at levels 2540 mOsm and 20 mOsm (Appendix Table XXX).

Similar to field measurements, osmotic potential appeared significant in altering vegetative cell dimensions as shown in Table 4. Dilutions of "1st Salt Mine Pond" water with distilled water showed an increasing length to width ratio with decreasing osmotic potential.

Dilutions of "Cherry Creek Pond" water, ranging from 1626 mOsm to 255 mOsm, indicated no zoosporangia fomation at 1385 mOsm and above (Appendix Table XXXI). Zoosporangia were formed in all cultures with osmotic potential values below 1385 mOsm.

Akinete germination could not be induced by increasing the osmotic potential of distilled water with the other factors optimal (pH, temperature, light) to values as high as 375 mOsm with Polyethylene Glycol.

5. Effects of Specific Ions.

Laboratory studies showed that akinete germination could be induced only by the addition of Na^+ salts while no germination occurred with

Table 4. Effect of Salinity on Cell Dimensions measured in Microns (N = 200) Temperature = 19-21°C; Light ca. 4280 lux 16 hr light/8 hr dark.

Dilution Factor	Osmotic Potential in mOsm	Range of Cell Size in μ		Mean Cell Dimension in μ		L:W Ratio
		Length	Width	Length	Width	
1.42	2500-2550	22.5-50.0	9.0-13.0	36.25	11.0	3.3
2.00	1500-1540	30.0-100.0	8.0-11.0	65.0	9.5	6.8
3.33	1020-1050	50.0-162.5	8.0-10.0	106.0	9.0	11.7
10.0	350-375	47.5-152.5	7.0-8.5	100.0	7.7	12.9

Cl^- and $SO_4^=$ salts of the other three major cations (Appendix Table XXXII). Greatest akinete germination percentages were achieved by the addition of Na_2SO_4 (2.0 g/l) and NaCl (1.0 g/l) with decreasing germination percentages occurring above these concentrations. After 6-8 days in Mg^{++}, Ca^{++} and K^+ salt solutions, akinetes became colorless and non-viable. This was most apparent in Mg^{++} salt solutions.

The addition of Na^+ salts to filtered SSW extract from Bowers Lake suggested the importance of either an Na:Mg ratio or monovalent:divalent cation ratio (M:D). Soil water extract from Bowers Lake had an Na:Mg ratio of .45 as indicated in Appendix Table XXXIII, and supported Ctenocladus only as akinetes. With the addition of various concentrations of either Na_2SO_4 or NaCl, consequently increasing the Na:Mg ratio as shown in Appendix Table XXXIII, a significant change in the condition of the organism occurred. At an Na:Mg ratio of 1.3, vegetative cells first appear as short irregular cells (25.9 x 8.8μ). As the ratio increased, corresponding increases in cell length to width ratios occurred. Zoosporangia were formed only when the Na:Mg ratio was 13.7. This may indicate the significance of the Na:Mg or M:D ratio prior to zoosporangia formation as well. The significance of (M:D) cation ratios may possibly be eliminated in this study since similar experiments with K^+ salts showed this ion to be extremely toxic with increased K:Mg ratios.

Similar manipulations of Na:Mg ratios obtained with Chihara Medium are indicated in Appendix Table XXXIV. Material in solutions with equal concentrations of Na^+ and Mg^{++} showed short, irregular cells with many cells forming akinetes (Fig 48). In addition, branching appeared to range from acute angles to nearly 90^o (Fig 49). Solutions with Mg^{++} higher than Na^+ (.5 Na:Mg) showed nearly all cells converting

to akinetes (Fig 50). Conversely, with Na^{+} increased to three times that of the Mg^{++}, as shown in Appendix Table XXXIV, normal vegetative cells (91.9 x 8.1μ) with acute branching were observed (Fig 51). Similar results were obtained with additions of either $MgCl_2$ or $MgSO_4$ salts as indicated in Appendix Table XXXIV.

6. Effect of Natural Saline Waters

a. Saline Soil Water Extracts

Major cation analyses of sediment extracts (Appendix Table XXXV) from the various saline habitats appear similar to those in solution in the overlying water column (Appendix Table V - IX). One noticeable difference did occur however, in that relatively high percentages of Ca^{++} occurred in these sediments compared to percentages of Ca^{++} in the water column. This is probably due to continued precipitation as Ca^{++} salts, particularly $CaCO_3$, have a fairly low solubility constant (Langbein 1961).

Ctenocladus in all of these sediment extracts except from "Cherry Creek Pond" only occurred in the form of akinetes with normal vegetative cells (Fig 52) maintained in "Cherry Creek Pond" extracts. Noticeable differences in zoosporangia were noted between the two zones in the pond. Sediment extract from the "crystal zone" consistently produced more zoosporangia, whereas few to none occured on extracts from the "Ruppia zone". As noted in field results (p.32), chemical differences in the water column between the two zones also exsisted.

b. Other Saline Pond Water

Vegetative filaments of Ctenocladus transferred into untreated

Figures 48-53. Condition of Ctenocladus when subjected to various natural solutions and Na:Mg ratios.

48. Ctenocladus akinetes illustrating condition after subjected to Na:Mg ratios of less than 1.0 (note granular appearance) x1200.

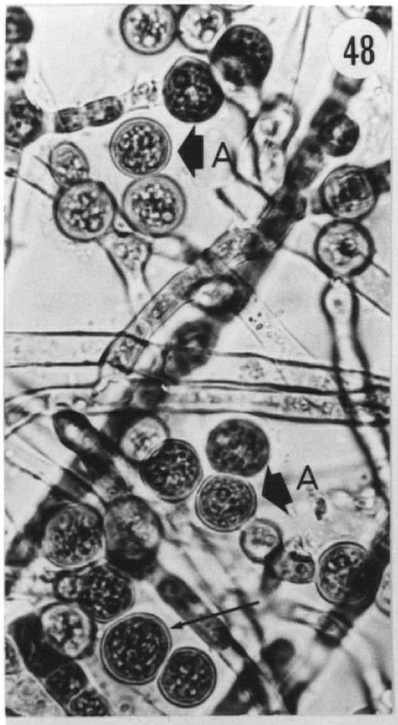
49. Ctenocladus illustrating reduced cells with right angle branching after subjected to Na:Mg ratios of less than 1.0 x1200.

50. Ctenocladus after subjected to solutions with magnesium concentrations higher than sodium with most cells as akinetes x1200.

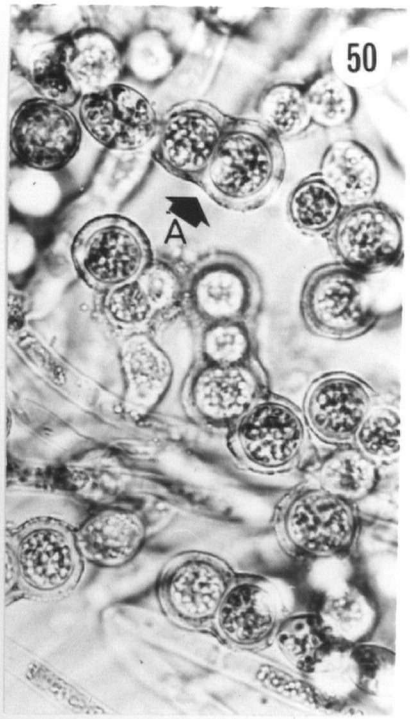
51. Normal vegetative cells of Ctenocladus with normal acute branching in culture solutions with Na:Mg ratio of 3 : 1 x1200.

52. Ctenocladus illustrating normal vegetative cells with limited akinete formation x300.

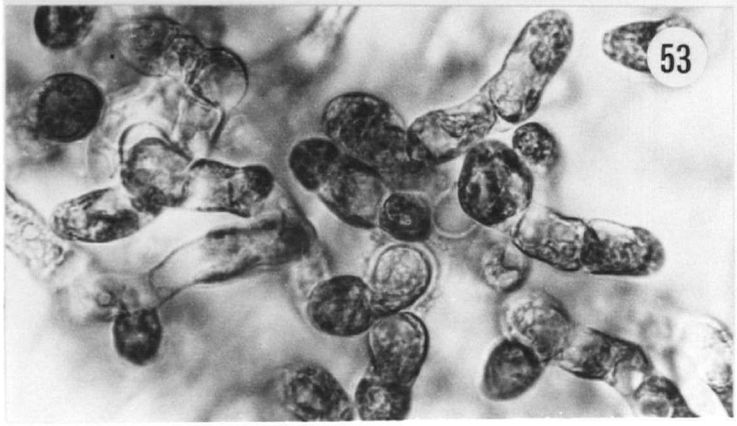
53. Akinete germination with reduced number of cells in natural solutions from Bowers Lake x1200.


48
A
A


49


50
A


51


53


52

water (unfiltered and unsteamed) lost their color within 10-12 days in waters collected from all habitats except "Cherry Creek Pond" and the two "Salt Mine Ponds". In water from "Cherry Creek Pond" normal vegetative cells with numerous zoosporangia were observed.

Akinete germination occurred in all saline waters collected during spring and early summer. Germination tubes existed with a limited number (less than 3 cells) of irregular cells (Fig 53) in steamed and filtered waters from other than Ctenocladus habitats. Material in Wallender and Ironmask Lakes usually remained in this condition; whereas, that in Bowers Lake and "Polygon Pond" formed akinetes after 14-20 days.

Condition of vegetative material when transferred into steamed and filtered water collected from Bowers Lake (April 1968) was similar to experimental field transplants into this lake in that akinetes were immediately formed as shown in Fig 47, later losing their color. Best growth occurred in steamed and filtered water collected from "Cherry Creek Pond", as would be expected, and numerous zoosporangia were formed. Treated waters from Ironmask Lake, "Polygon Pond" and Wallender Lake from April 1968, indicated these waters to be unsatisfactory for normal vegetative growth, as the majority of the cells converted into akinetes within one month, with transfers every 14-16 days. Dilution of all waters by a factor of three showed no significant change in growth indicating that specific ions or proportions of these ions may be more significant than total concentrations.

Herbarium Material

Collecting data as well as general condition (i.e. vegetative

or akinetes) of material is noted for each specimen. This is shown in Appendix Table XXXVI.

The number of available specimens (27) from various herbaria gives an indication of the limited distribution of the organism throughout the world. Most specimens were collected from saline habitats in arid regions in North America with a few scattered collections taken from similar habitats in Siberia, Italy and Peru.

In general, most of the material was in the akinete stage. However, seasonal patterns with regard to condition of material were noted from collections in Mono Lake, California. In 1940, early summer collections (June 12) were primarily vegetative, whereas late summer collections (September 6) showed almost all cells as akinetes.

DISCUSSION AND CONCLUSION

In this study, as in most ecological investigations, it is extremely difficult to separate one factor as the single governing "regulator" responsible for a species inhabiting a given environment. The presence of an organism in a habitat is based on an interrelated complex of factors, each exerting an influence directly or indirectly, when expressed outside the tolerance levels of that species. In stress environments, such as inland saline habitats, a limited number of "regulatory" factors may prevail due to existing extreme conditions. Species occurring in such environments may be unable to compete in more desirable habitats, or existing conditions in the enviroment may fulfill their requirements.

The resting stage, or akinetes, appear to be the dominant stage (present 70-90% of the time), and the most important in the distribution and survival of Ctenocladus in these extreme saline environments. Therefore laboratory experiments designed with environmental extremes were normally conducted on akinetes. Zoosporangia production is probably not significant with respect to distribution and will be examined briefly along with the absence of sexual stages, after discussion of physico-chemical factors affecting vegetative growth and akinete germination and formation. Physico-chemical and biological factors of the investigated saline habitats as correlated with laboratory studies will be discussed both individually and in combination for a better appreciation of the ecology of this interesting alga.

Salinity:

In this study difficulties encountered with water analysis were due to the extreme salinities, consequently dilutions were necessary for final analyses. Oxygen determinations, especially during the latter part of the season, by the Winkler method (American Public Health Association 1965) may be questioned since violent bubbling occurred due to the high carbonate content. Similar difficulties were encountered by Anderson (1958) during his study of two saline lakes in Washington.

Concentration of salts in a closed saline environment eliminates organisms when levels exceed the optimum for germination of resting spores or when ionic concentration or the osmotic stress of the solution is detrimental to the spores. Extreme alkalinity or salinity of the sediment may reflect the age of the system and also be characterized by the total lack of spores in sediment cores. Consequently it is nearly impossible to reconstruct the history in many of these extreme saline habitats.

Several workers have proposed mechanisms which buffer the continued concentration of salts apparent in these habitats. Removal of dried salt by wind action from the margins as well as throughout the entire basin is considered most significant in regulating the salt economy of a saline lake system (Langbein 1961). This was observed in those shallow habitats which were nearly dry by early summer. In addition, selective salt depletion by wind transport has been suggested by Jones and Van Denburgh (1967). This is based on the idea that dried efflorescent salts composed primarily of carbonate mineral are more powdery than the platy sulfate rich materials or the hard irregular crusts dominated by halite.

The Ctenocladus habitats ("Cherry Creek Pond", "1st Salt Mine Pond" and "2nd Salt Mine Pond") in the Kamloops region occupied small depressions (less than 0.2 hectare) with extensive salt encrustation throughout the basin. Other collections of Ctenocladus were made from larger lakes as Mono Lake (51.5m) as described by Mason (1967). Sites in the Kamloops region with Ctenocladus were morphometrically distinct. These habitats were sheltered depressions, whereas those without Ctenocladus were large expanded and exposed basins. This is particularly significant in the shallow semi-permanent habitats ("Polygon Pond" and Ironmask Lake) in which surface evaporation rates are considerably greater due to lower surface area to volume ratios. The fact that evaporation rates are relatively greater in habitats such as Ironmask Lake and "Polygon Pond" is significant as the optimum period of vegetative development is reduced or eliminated due to increasing salinity levels early in the season. Consequently, some environments may be too concentrated for akinete germination when all other factors are optimum (i.e. pH, light, temperature, specific ions, etc.). This could occur in such habitats as the "2nd Salt Mine Pond" where osmotic potential values were measured at 2283 mOsm as early as mid May 1968, consequently minimizing the period for optimal vegetative development. Laboratory studies showed that cells generally start forming akinetes at levels around 1600-1800 mOsm and are converted entirely into akinetes above 2400 mOsm. Extreme salinities, however, appear to have no detrimental effects on Ctenocladus since cells merely convert into akinetes when total concentration is above the tolerance limits. The mechanism utilized by this alga to tolerate these extreme osmotic conditions while still in the vegetative condition is

intriguing and requires further investigation.

Concentration levels above the optimum for vegetative growth may explain why Ctenocladus has not been reported from habitats such as the Great Salt Lake (salinity nearly 4 times that of seawater) even though other conditions appear to be suitable for its existence (Adams 1964). However, one might predict Ctenocladus in parts of this lake in the future, since the lake is now divided by a railroad embankment with one side getting more salty and the other more dilute. A smaller volume of saline water flowing into one portion in addition to a greater evaporation rate accounts for the differences in salinity in two bodies (Adams 1964). The disappearance of Ctenocladus from habitats as the waters become more and more concentrated through the years is also feasible. Hammer (personal communication) reported the absence of Ctenocladus in Little Manitou Lake, Saskatchewan, in 1968. The lake is three-four times saltier now than when Ctenocladus was originally collected (Kuehne 1941). The disappearance of Ctenocladus from such habitats as Little Manitou Lake may result from total concentration of ions or reflect major shifts in dominance of major ions. Hutchinson (1957) indicated that as saline habitats age and concentrate over many years there may be changes in dominant cations with Mg^{++} concentrations increasing. Such a phenomenon was observed in the temporary saline habitats investigated in this study only on an annual time scale, with spring dilution restoring the process each year. Field studies showed significant seasonal fluctuations in major cations, particularly Na^{+} and Mg^{++} in two of the temporary habitats (Ironmask Lake and "Polygon Pond") with Na^{+} dominant in the early spring and Mg^{++} in the late summer. Seasonal shifts in major cations, due to selective precipitating of the

ions, in temporary saline habitats has been suspected but not reported (Cole 1968). In larger, permanent saline lakes it is generally assumed that the mean relative ionic proportions for the various lakes remain substantially constant despite large fluctuations in salinity (Bayly and Williams 1966). The two permanent lakes in this study (Wallender Lake and Bowers Lake) demonstrate limited cation ratio fluctuations, while the temporary habitats showed large seasonal fluctuations in cation dominance.

The significance of these annual and permanent shifts may be enhanced through examination of experimental data. The cationic proportions of the water in the investigated saline habitats seem to be extremely important in the distribution of Ctenocladus as indicated by experimental field transplant studies. Transplants of Ctenocladus were maintained only in habitats with marked Na^+ dominance. All habitats personally investigated as well as those investigated by other workers (Cole and Whiteside 1965; Rawson and Moore 1944; Wetzel 1964; Mason 1967) indicated Na^+ to be the dominant cation. Laboratory studies substantiate this since akinetes remain viable and germinate only in solutions of Na^+ salts (Appendix Table XXXII) whereas akinetes in solutions of other major cations (Mg^{++}, Ca^+, K^+) were non-viable, ultimately becoming colorless. In addition, laboratory results indicated that Na:Mg ratios as well as total monovalent:divalent (M:D) cation ratios are important in the distribution of Ctenocladus.

Ratios of Na:Mg of at least 1.5-20 were required for akinete germination and growth. Several investigations (Wetzel and McGregor 1968; Wetzel 1964 and Talling and Talling 1965) have indicated the significance of total (M:D) cation ratios to vegetative growth of algae

in various lake systems. However, (M:D) cation ratios did not appear to be significant except when involving Na^+, as increased germination and vegetative development occurred only with the addition of Na^+ salts of either $SO_4^=$, $CO_3^=$ or Cl^-, with adverse effects occurring when these ratios were increased by adding similar K^+ salts.

Additional field observations in Lyons Lake enhanced the significance of Na:Mg ratios (Northcote and Halsey 1969). In this lake these ratios increased with depth. Ctenocladus has been collected at 12 meters and below, which coincides with higher Na:Mg ratios at these levels.

Absolute Na^+ concentration does not appear to be as important as ratios of the particular cations present, as analysis of habitats where Ctenocladus is absent indicates comparable concentrations of Na^+ to those in Ctenocladus habitats. Sodium, however, is important and possibly required for development as akinete germination was only initiated by the addition of Na^+ salts. Sodium as such, is generally not required for autotrophic plants, however, several species of blue-green algae require it for normal logarithmic growth (See Kratz and Myers 1955; Allen 1955). Droop's (1958) studies with euryhaline algae also suggested that the concentration of Na^+ is single most important factor with the use of salinity as an ecological factor perhaps masking this possibility.

pH:

Seasonal pH values in all investigated habitats in the Kamloops region was above 8.0 with values in some as high as 10.2. Thus pH may not limit Ctenocladus as values showed similar seasonal fluctuations

in all habitats with Ctenocladus. However, laboratory studies showed pH below 7.0 to be extremely detrimental to akinetes, ultimately causing loss of color. Consequently, hydrogen-ion concentrations are indirectly significant in restricting the organism from neutral and acidic habitats. Also pH reflects the ionic constituents of the solutions. Changes in hydrogen-ion concentration of buffered distilled water to optimum levels (8.5-10.0) were not sufficient for akinete germination. However, pH is significant in overall growth as only akinetes or short irregular cells were present below pH 8.4.

Hydrogen-ion concentration is important in the availability of certain nutrients, especially in alkaline waters where micronutrient deficiencies are common (Shutte 1964). One important deficiency in saline waters is a decrease in available iron with increasing pH. Hence the ecological significance of chelators in these aquatic systems may play an important role in the availibility of such micronutrients as iron. Schelske (1960) and Schelske, et al. (1962) noted that the iron complex of EDTA increased the photosynthetic uptake of ^{14}C in several marl lakes. Normal growth of Ctenocladus was obtained in laboratory by adding EDTA-Na_2 (.015 g/l) to seawater, whereas untreated seawater showed marked reduction in akinete germination and little vegetative development. This may explain the absence of Ctenocladus in marine environments, since salinity, temperature and major ionic constituents are suitable for normal development. Preliminary studies of the response of Ctenocladus to various concentrations of iron showed no significant results in growth and development.

Temperature:

Large seasonal and daily fluctuations in temperatures occur in aquatic habitats in the temperate region. These values are augmented in habitats with exposed shallow expanded basins. Diurnal fluctuations in freshwater habitats have been investigated by Klimowicz (1961) and Bamforth (1962) with daily temperature amplitudes as high as 10.2°C depending upon depth.

Extreme temperatures were recorded in both Ironmask Lake and "Polygon Pond" where temperatures approached 40°C or nearly 10°C higher than other habitats investigated. Corresponding laboratory investigations showed that akinetes were extremely susceptible to temperatures above 34°C with the majority becoming non-viable. These extreme temperatures could play an important role in the absence of Ctenocladus in such habitats. In addition, water levels drop at a much greater rate in these expanded shallow basins (Ironmask Lake and "Polygon Pond") and reach higher temperatures earlier in the season, with significant reductions in availability of dissolved gases, particularly oxygen. Concentrations of dissolved oxygen are indirectly proportional to temperature (Ruttner 1966) consequently minimum oxygen values are achieved earlier in the season than in other more sheltered or permanent habitats.

Temperature stratification in the early spring in the saline habitats investigated makes it tempting to correlate higher bottom temperatures with akinete germination allowing this process to occur early in the season. Some investigators (Guseva 1947) suggest that forms predominating at the beginning of the spring period are usually forms without known

resting stages. Her view is that forms overwintering as spores appear later in the year because of the time period needed to reach the stage at which germination is possible. This is definitely not true for organisms such as Ctenocladus which inhabit saline environments, since germination begins early in the spring as soon as the ponds are ice-free and temperatures are above 5-6°C. Germination at this time is important since conditions (i.e. temperature, salinity, etc.) of temporary or semi-permanent habitats would be too extreme for germination later in the season.

Light:

Laboratory studies indicated that light was required for germination and subsequent growth. This suggests that a photosynthetic mechanism is involved in the actual germination process. Normal akinete germination could be induced, however, at extremely low light intensities (215.2 lux) on a 16/8 hr light-dark cycle. This may be correlated with the location of the alga in the pond during spring germination (i.e., buried in the upper layers of sediment and under salt encrustment), yet normal germination occurs.

Water levels in saline habitats drop to a minimum and even dry up completely during certain years. Consequently Ctenocladus akinetes exist along the margins as encrusted spherical masses or buried beneath precipitated salt crystals. Therefore direct exposure to sunlight is not common. Light intensity was not measured in the field, however it appears that the intensity would be very high, with reflected light from the dried white efflorescent salt similar to that of a snowfield.

Laboratory studies indicated that akinetes were very sensitive to relatively high light intensities (11,770 lux) causing breakdown of the chloroplast. This again corresponds with field observations on the position and condition (buried in the sediment, buried in the salt crystals or encrusted) of the akinetes during the period when extreme light intensities occur. Light may therefore by an ecologically significant factor in reducing populations by destroying akinetes when they are exposed to levels above 11,770 lux during peaks in the summer. Wetzel and McGregor (1968) also found that increasing light intensity of over 9000 lux in carbonate lakes suppressed germination of Chara zygotes with very poor development of pigments. Further studies on photoperiod and light quality need to be conducted for a more critical analysis of the total effect of light on akinete germination and existence of the resting stage.

Biological:

The presence of the brine shrimp, Artemia salina, in habitats with Ctenocladus appears to be consistent. Croghan (1958) showed that survival of this anostracan was possible only in media where certain Na^{+} salts (principally NaCl) predominate, with toxic levels occuring in solutions dominated by Mg^{++}, Ca^{++} and especially K^{+}. Similar ionic proportions in media utilized by D'Agostino and Provasoli (1968) for Artemia and media used in cultivating Ctenocladus (Appendix Table XXI), suggest similarities between chemical requirements for these two organisms in nature. Presence of these two organisms in a saline habitat therefore may be used as an indicator of the major cationic

conditions of the environment. Brine shrimp have been collected in many habitats (Cole and Brown 1967) without Ctenocladus, but the reverse has not occurred. Normally later in the season large numbers or Artemia concentrate around clones of Ctenocladus (primarily as akinetes) possibly capitalizing on the limited photosynthetic oxygen released by the alga.

Sulphur bacteria, common in saline habitats, undoubtedly play an unforeseen role in the physico-chemistry of the entire aquatic ecosystem. Interactions between the mud-water interface are extremely significant in shallow habitats and are undoubtedly regulated to a large extent by bacterial activity.

Anionic constituents do not appear to be significant in restricting Ctenocladus from saline habitats, since the alga was found in all of the major anionic solutions in nature (Cl^-, $SO_4^=$, $CO_3^=$ and $Cl^-/CO_3^=$), and the addition of any of the anionic salts in the laboratory did not show significant changes in growth and development. The absence of free CO_2 in all Ctenocladus habitats investigated is, however, ecologically significant in that it indicates that this alga is among the few which can utilize at least the HCO_3^- portion of combined CO_2 as a photosynthetic carbon source. Dissolved phosphate and nitrate concentrations also do not seem to be significant in the habitats investigated in this study. Interesting studies of successional patterns of species in saline habitats could be conducted, because reverse patterns of seasonal nutrient cycling occurs in these habitats with an extreme build-up of phosphate concentrations in the late summer.

Other considerations such as the drainage pattern of the basin as well as general characteristics of the basin itself may prove to

be significant following more critical investigations. In the Kamloops study area, one major geological intrusive, rich in hornblende, (Ironmask Batholith) (Cockfield 1961) exists within the volcanic material, which may reflect the differences in the major cationic constituents of the habitats. Those habitats located within the drainage basin of the Ironmask Batholith showed considerably higher Mg^{++} concentrations than others outside this formation. However, since it was beyond the scope of this investigation to study the lithologic characteristics of the drainage basin, the importance of these drainage patterns, based on other investigations, can only be brought to the readers attention through speculation. In addition, continued salt precipitation on the bottom of the basin may have a significant influence on the occurrence of Ctenocladus in crystal vs. non-crystal zones. This may be extremely significant where $SO_4^{=}$, through anaerobic decay of organic materials results in losses of sulphate by reduction as H_2S, with possible toxic conditions existing at the mud-water interface. The importance of H_2S also needs further examination since techniques for critical analysis of H_2S and the significance of its occurrence in such habitats were not perfected or fully exploited.

The influence of physico-chemical factors on zoosporangia formation does not appear to be significant in excluding Ctenocladus from a habitat, since populations can be maintained through formation of akinetes in a population within a given habitat. Zoosporangia formation is inhibited by temperatures above 26°C and osmotic potential values above 1153 mOsm. Consequently populations in extremely concentrated habitats ("1st Salt Mine Pond" and "2nd Salt Mine Pond") may only rarely be able

to produce zoosporangia at times when water is diluted below the optimum temperature and salinity levels for such reproduction.

The lack of sexual reproduction in cultures of Ctenocladus from the Kamloops region may partially account for its restriction to a very narrow range of physico-chemical factors or conditions. Also, difficulty in developing a suitable culture medium for laboratory cultivation reflects upon its narrow tolerance limits or specific requirements. The resting stage or akinete substitutes for the resting zygote, which appears to be a more efficient mechanism for maintaining a population in these extreme environments. However, without genetic recombination, variability is drastically limited and unable to draw upon the genetic pool. Consequently, Ctenocladus may have restricted itself through the years to a very narrow ecological niche.

Diversity of algal species within the investigated saline habitats was markedly reduced, although population densities were very high. This is the same as reported by other investigators of saline environments (Flowers 1934; Hutchinson 1937; Kuehne 1941; Anderson 1958). Cyanophyceae and Bacillariophyceae are very common in both the sediment and water column, with chlorophyceans rarely collected, with the exception of Ctenocladus. Most forms were at the mud-water interface, since the vertical distance of the water column is limited with those species unable to maintain themselves in the water column soon dropping to the bottom.

Bowers Lake displayed the greatest diversity of species, which reflects upon its relatively lower salinity compared to the other habitats investigated. Wallender Lake had comparable salinity levels yet had a limited number of species. The low diversity of algal species

in this lake may be due to the oxygen depleted layer which exists in the lower levels during most of the year.

Seasonal periodicity of algal species in the water column of semi-permanent habitats is extremely difficult to predict due to yearly fluctuations in salinity levels. A more accurate prediction can be made with benthic species, which appears to be a more stable environment.

The period for optimal vegetative growth of Ctenocladus in semi-permanent or temporary habitats appears to be extremely short since it must occur between the minimum temperature values for akinete germination in the spring and maximum salinity tolerance levels later in the season. This optimum period fluctuates from year to year depending upon weather condition, (i.e. winter snowfall, spring rain, temperature). Consequently, Ctenocladus may have been overlooked in a number of habitats since it may only be in the akinete stage, buried in the sediment or encrusted at the time of examination.

Field and laboratory studies also suggest that cell dimensions are not valid criteria for separating species of Ctenocladus as proposed by some authors (Woronochin and Popova 1929; Printz 1964), because physico-chemical factors (i.e. osmotic fluctuations, pH) drastically alter the cell length:width ratios. Consequently, it appears then that at least two species of Lochmiopsis (L. siberica and L. Printzii) may be one with Ctenocladus a monotypic genus displaying several physiological variants or ecotypes responding to environmental conditions of this extreme environment. Based on personal observations in the field and laboratory, it is likely that, seasonal environmental conditions may be responsible for differences in branching as well.

SUMMARY

1. Ctenocladus circinnatus Borzi is restricted to saline waters in arid regions where sodium is the dominant cation, with at least a 1.5 Sodium:Magnesium ratio.

2. Morphometric features of saline environments appear to be significant in the distribution of Ctenocladus. Those habitats with deeper sheltered basins are more desirable for Ctenocladus than open expanded basins with excessive evaporation rates.

3. Two semi-permanent habitats without Ctenocladus showed seasonal shifts in dominant cations, dominated in the spring by sodium and in the fall by magnesium. These seasonal shifts in major cation dominance may be very significant in the distribution of Ctenocladus as magnesium dominated solutions both in the field and laboratory were found to be detrimental to vegetative development.

4. Optimum pH (8.5-10.0) for akinete germination and development, established in the laboratory, coincide with the pH of the occupied habitats. There is some indication that hydrogen-ion concentration also influenced cell length:width ratios.

5. Laboratory studies showed akinete germination was initiated at very low temperatures with relatively higher temperatures causing breakdown of akinetes. This may be ecologically significant since extreme temperatures are achieved in some of the broad exposed basins, possibly reducing populations of the alga.

6. Different levels of light intensity gave varying responses with respect to akinete germination. No germination occurred in absolute

darkness with only extremely low light intensities (215 lux) needed to initiate normal germination. Relatively higher light intensities (10,780 lux) were extremely detrimental to the akinetes, which may be significant since it is assumed that comparable high light intensities are achieved in exposed habitats investigated.

7. In temporary or semi-permanent habitats, the optimum period of vegetative development for Ctenocladus is remarkably short and variable depending upon the year, as it must occur between minimum temperatures for akinete germination in the spring diluted waters and maximum salinity tolerance levels later in the season.

8. Seasonal variation in cell length:width ratios, due to increasing salinities, emphasizes the possibility of physiological races or variants responding to varying environmental conditions rather than distinct species.

9. Consistent association of Ctenocladus and Artemia salina (L) suggests similar physico-chemical requirements with their coexistence possibly used as an indicator of the general catonic composition of the habitats.

10. Field and laboratory observations of chironomid larvae feeding on Ctenocladus populations suggested the possible importance of predation in the distribution of Ctenocladus.

11. The lack of genetic recombination in isolates of Ctenocladus from the Kamloops region suggests that akinetes have substituted for the resting zygote with this loss of variability restricting the organism to a very narrow ecological niche over the years.

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APPENDIX TABLES I - XXXVI

Table I. Reported Collections of Ctenocladus Including Date, Collector, and Dominant Ions.

LOCATION	DATE	COLLECTOR	DOMINANT CATION	DOMINANT ANION
MESSINA, SICILY	1881	A. BORZI	Na^+	$CO_3^=$
BOLSHOYE PETUKHOVSKOYE LAKE SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
LAKE MALOYE SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
LAKE BELENKOYE SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
KUPALNOYE LAKE SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
LEBYAZHYE LAKE (RUBTSOVSKOGO OKR.) SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
MALOYE USHKALY LAKE SLAVGOROD DIST.; OMSK REGION, USSR	1927	N.N. WORONICHIN & T.L. POPOVA	Na^+	$CO_3^=$
LAKE BOZA CUZCO, PERU	1/1943	A. MALDONADO	-	-
LIMA, PERU	12/1940	A. MALDONADO	-	-
MONO LAKE, CALIFORNIA	9/6/1940	M.J. GROESBECK	Na^+	Cl^-
MONO LAKE, CALIFORNIA	8/24/1942	A. CARTER	Na^+	Cl^-
MONO LAKE, CALIFORNIA	6/11/1942	M.J. GROESBECK	Na^+	Cl^-
BORAX LAKE, CALIFORNIA	1964	R.J. WETZEL	Na^+	$Cl^- - CO_3^=$
MARINA, CALIFORNIA	1929	L.G.M. BASS-BECKING	Na^+	Cl^-
BERMUDA; MARINE LAB.	3/1954	R. LEWIN	(Na^+)	(Cl^-)
GREEN POND, APACHE COUNTY ARIZONA	1964	G. COLE, M. WHITE-SIDE & R.J. BROWN	Na^+	$CO_3^= - Cl^-$
RED POND, APACHE COUNTY ARIZONA	1964	G. COLE, M. WHITE-SIDE & R.J. BROWN	Na^+	$CO_3^= - Cl^-$
ABERT LAKE, OREGON	1967	G.W. PRESCOTT	Na^+	Cl^-
"STATE LINE POND" 22 mi. S of KLAMATH FALLS, OREGON	6/1968	D.W. BLINN	Na^+	$SO_4^=$
"HAZEN POND" 23 mi. W of FALLON, NEVADA	6/1968	D.W. BLINN	Na^+	$SO_4^=$
LITTLE MANITOU LAKE SASKATCHEWAN, CANADA	9/12/1940	P.E. KUEHNE	Na^+	$SO_4^=$
LYONS LAKE, BRITISH COLUMBIA	7/1961	T.G. HALSEY & T.G. NORTHCOTE	Na^+	$SO_4^=$
WHITE LAKE, BRITISH COLUMBIA		J.R. STEIN	Na^+	

Table 1 Continued

LOCATION	DATE	COLLECTOR	DOMINANT CATION	DOMINANT ANION
"CHERRY CREEK POND" 7.6 mi. W OF KAMLOOPS, BRITISH COLUMBIA	5/1961	J.R. STEIN	Na^+	$SO_4^=$
"1ST SALT MINE" 8.1 mi. W OF KAMLOOPS, BRITISH COLUMBIA	5/17/1968	D.W. BLINN	Na^+	$CO_3^=$-$SO_4^=$
"2ND SALT MINE" 7.2 mi. W OF KAMLOOPS, BRITISH COLUMBIA	5/17/1968	D.W. BLINN	Na^+	$SO_4^=$

Table II. Procedures Used in Water Analysis

Na^+, K^+, Ca^{++}, Mg^{++}	Perkin-Elmer Atomic Absorption Spectrophotometer from .45μ Millipore filtered sample.
Cl^-	Mohr method with "Hach" Chemicals (Hach Chemical Company, Ames, Iowa)
$SO_4^=$	Barium sulphate turbimetric method with "Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
NO_2^--NO_3^-	Alpha Naphtylamine and Sulfanic acid with "Hach" Chemicals (Hach Chemical Company, Ames, Iowa)
NH_4^+	Nesserlerization method with "Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
Ortho-$PO_4^{\equiv}$ and Total-$PO_4^{\equiv}$	Stannous Chloride with "Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
HCO_3^- and $CO_3^=$	Potentiometric titration (Standard Methods) American Public Health Association, 1965)
O_2	Winkler Method with "Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
CO_2	Sodium Hydroxide titrametric method with "Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
H_2S	"Hach" chemicals. (Hach Chemical Company, Ames, Iowa)
Specific Conductivity	Radiometric-Copenhagen Conductivity Meter at 25°C.
Osmotic Potential	.45μ Millipore filtered sample on Precision Osmette Osmeter.
Total Dissolved Solids	Evaporation at 103-105°C of 50 ml sample (Standard Methods, American Public Health Association, 1965)
pH	Metrohm pH meter.

Table III. Media Used for Cultivation of Ctenocladus.

Chihara Marine Medium (personal communication)

Seawater	1 l
$NaNO_3$	0.2 gm
$NaH_2PO_4 \cdot H_2O$	0.025 gm

Minor Element Mix 2 ml

$FeCl_3 \cdot 6H_2O$	.00032 gm
$MnCl_2 \cdot 4H_2O$	.00048 "
$ZnCl_2$	.00006 "
$CoCl_2 \cdot 6H_2O$	.00002 "
$CuCl_2 \cdot 2H_2O$	.000005 "
H_3BO_3	.00240 "
$Na_2MoO_4 \cdot 2H_2O$	.00020 "
EDTA - Na_2	.01500 "
Distilled Water	1 l

Provasoli ES Enrichment (Provasoli, 1968)

Seawater	1 l
$NaNO_3$	3.5 g
Na_2 glycere-Phosphate	500 mg
Vitamin B_{12}	100 g
Biotin	50 g
Thiamin	5 mg
Tris Buffer (Sigma Co.)	5 g

P II Metal Mix 1ml/1

H_3BO_3	.114 gm
$FeCl_3 \cdot 6H_2O$	.0049 "
$MnSO_4 \cdot 4H_2O$	.0164 "
$ZnSO_4 \cdot 7H_2O$	.0022 "
$CoSO_4 \cdot 7H_2O$	.00048 "
EDTA - Na_2	.001 "
Distilled Water	1 l

Table IV. Seasonal Drop in Water Levels for Kamloops Habitats Measured from Calibrated Stake.

Period Measured	"Cherry Creek Pond"	"Polygon Pond"	Ironmask Lake	Bowers Lake	Wallender Lake
10vi-1vii67	12.0cm	-	-	11.0cm	12.0cm
1vii-23vii67	8.0	-	-	14.0	12.0
23vii-12viii67	10.0	-	-	11.0	9.5
12viii-31viii67	8.0	-	-	14.0	12.5
31viii-23ix67	Dry	-	-	2.5	3.0
26iii-20iv68	1.0	-	-	-	-
20iv-19v68	6.0	7.5cm	8.0cm	9.5	4.5
19v-15vi68	4.0	3.5	4.2	2.5	2.5
16vi-19vii68	13.0	14.0	Nearly	16.5	13.0
19vii-29viii68	-	3.0	Dry	15.0	12.0

Table V. Chemical Analysis of Major Ions in Kamloops Area for June, 1967.

	K	Na	Mg	Ca	Cl	SO_4	HCO_3	CO_3
"Cherry Creek Pond"								
Surface								
meq/l	10.8	705.6	43.3	9.3	13.7	750.0	-	-
%	1.4	91.4	6.09	1.2	-	-	-	-
Bottom								
meq/l	8.9	708.0	180.0	10.1	31.7	822.9	-	-
%	1.0	73.1	19.8	1.1	-	-	-	-
"Polygon Pond"								
meq/l	8.2	1086.0	1666.0	26.6	77.2	2958.0	-	-
%	.3	39.0	59.8	.9	-	-	-	-
Ironmask Lake								
meq/l	21.2	1565.0	1750.0	21.3	63.3	2260.0	-	-
%	.6	46.6	52.2	.6	-	-	-	-
Bowers Lake								
Surface								
meq/l	6.9	86.9	183.0	14.7	5.9	250.0	-	-
%	2.4	29.8	62.8	5.0	-	-	-	-
Bottom								
meq/l	9.0	89.1	191.7	15.3	4.3	260.0	-	-
%	2.9	29.3	62.8	5.0	-	-	-	-
Wallender Lake								
Surface								
meq/l	8.3	252.0	145.8	9.6	20.4	375.0	-	-
%	2.0	60.5	35.1	2.3	-	-	-	-
Bottom								
meq/l	12.9	286.9	233.3	14.6	21.4	541.0	-	-
%	2.0	54.2	41.0	2.8	-	-	-	-

Table VI. Chemical Analysis for Major Ions in Kamloops Area for August, 1967

	K	Na	Mg	Ca	Cl	SO_4	HCO_3	CO_3
"Cherry Creek"								
meq/l	35.8	2804.0	116.0	140.0	67.5	3968.0	20.1	46.1
%	1.2	90.6	3.7	4.5	1.7	96.7	.5	1.1
"Polygon Pond"								
meq/l	340.0	2478.0	4325.0	870.0	337.0	8020.0	77.6	-
%	4.2	30.9	54.0	10.9	4.0	95.1	.9	-
Ironmask Lake								
meq/l	276.0	3322.0	3333.0	940.0	133.8	7291.0	65.6	39.6
%	3.5	42.2	42.4	11.9	1.0	91.8	6.7	.5
Bowers Lake								
meq/l	13.2	113.9	140.0	24.6	8.7	293.0	.85	4.4
%	4.5	39.1	48.0	8.4	2.8	95.5	.3	1.4
Wallender Lake								
meq/l	15.3	320.0	261.6	28.8	25.3	625.0	1.5	16.3
%	2.4	51.1	41.9	4.6	3.8	93.5	.3	2.4

Table VII. Chemical Analysis of Major Ions in Kamloops Area for March 26, 1968

	K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	HCO_3^-	$CO_3^=$
"Cherry Creek:								
Surface								
meq/l	1.8	180.0	21.0	1.5	6.1	214.0	1.4	.62
%	.9	88.1	10.2	.8	2.7	96.4	.63	.27
Bottom								
meq/l	-	791.0	75.0	6.5	15.1	854.0	3.2	.73
%	-	90.7	8.6	.74	1.8	97.8	.37	.08
Wallender								
Surface								
meq/l	7.6	165.0	125.0	5.1	6.9	218.0	2.2	3.2
%	2.5	54.4	41.5	1.6	2.9	94.8	.9	1.4
Bottom								
meq/l	-	326.0	261.0	7.1	33.0	510.0	13.9	3.1
%	-	54.9	43.9	1.2	5.3	92.0	2.2	.5
"Polygon"								
meq/l	10.2	173.0	141.0	6.4	4.3	333.0	1.45	.93
%	3.1	52.3	42.6	2.0	1.3	97.9	.42	.27
Ironmask								
meq/l	16.0	123.0	118.0	14.5	7.3	312.0	3.4	1.24
%	5.8	45.4	43.4	5.4	2.3	96.3	1.1	.38
Bowers								
Surface								
meq/l	12.1	93.0	112.0	17.5	5.2	302.0	2.2	3.1
%	5.2	39.6	47.7	7.5	1.9	97.9	.01	.2
Bottom								
meq/l	12.8	230.0	116.0	18.5	8.1	406.0	-	-
%	3.4	61.0	30.7	4.9	-	-	-	-

Table VIII. Chemical Analysis of Major Ions in Kamloops Area for May 18, 1968

	K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	HCO_3^-	$CO_3^=$
"Cherry Creek"								
Surface								
meq/l	4.1	508.0	71.0	4.1	9.3	510.0	5.1	12.4
%	.75	86.4	12.1	.75	1.8	94.83	.97	2.4
Bottom								
meq/l	5.1	530.0	100.0	4.9	9.4	625.0	7.4	9.6
%	.8	82.8	15.6	.79	1.4	96.0	1.1	1.5
Wallender								
Surface								
meq/l	5.1	217.0	183.0	12.3	15.8	395.0	3.4	8.0
%	1.2	52.0	43.9	2.9	3.7	93.6	.81	1.9
Bottom								
meq/l	9.4	426.0	350.0	14.5	29.9	687.5	4.3	15.5
%	1.2	53.3	43.8	1.8	4.1	93.9	.58	2.1
"Polygon"								
meq/l	20.0	831.0	1011.0	29.0	18.3	1979.0	7.6	0
%	1.1	43.9	53.5	1.5	.9	98.7	.38	0
Ironmask								
meq/l	8.8	391.0	395.0	6.3	15.8	833.0	7.1	9.6
%	1.1	48.8	49.3	.8	1.8	96.2	.82	1.1
Bowers								
Surface								
meq/l	15.0	186.9	283.0	19.2	6.4	479.0	6.0	.62
%	2.9	37.2	56.1	3.8	1.3	97.4	1.2	.13
Bottom								
meq/l	15.8	217.0	333.0	19.0	6.5	500.0	6.9	-
%	2.7	37.2	56.9	3.2	1.3	97.4	1.3	-
"1st Salt Mine"								
meq/l	10.5	2752.0	37.5	.4	76.8	708.0	131.0	1546.0
%	.4	98.3	1.3	.01	3.1	28.8	5.3	62.8
"2nd Salt Mine"								
meq/l	7.5	2130.0	58.3	.3	14.1	1896.0	54.0	320.0
%	.3	97.0	2.7	.01	.6	83.0	2.4	14.0

Table IX. Chemical Analysis of Major Ions in Kamloops Area for August 29, 1968.

	K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	HCO_3^-	$CO_3^=$
"Cherry Creek"								
Surface								
meq/l	23.0	1826.0	350.0	16.8	35.8	2400.0	9.0	25.6
%	1.0	82.4	15.8	.76	1.4	97.1	.45	1.05
Bottom								
meq/l	23.0	2278.0	266.0	16.1	53.7	1500.0	10.8	28.5
%	.89	88.2	10.3	.62	2.1	96.4	.5	1.0
Wallender								
Surface								
meq/l	23.0	380.0	375.0	14.5	29.0	697.0	1.9	17.4
%	2.9	47.9	47.3	1.8	3.9	93.6	.26	2.3
Bottom								
meq/l	30.8	578.0	733.0	24.5	51.2	1343.0	22.6	0
%	2.3	42.3	53.6	1.8	3.6	94.8	1.6	0
"Polygon"								
meq/l	40.4	1304.0	3083.0	47.5	184.0	4125.0	22.3	27.0
%	.9	32.3	65.7	1.1	4.2	94.7	.51	.62
Ironmask								
meq/l	100.4	955.9	1348.0	46.6	22.4	2558.0	9.9	10.6
%	4.1	39.0	51.0	1.9	.75	98.6	.33	.35
Bowers								
Surface								
meq/l	39.3	298.9	469.6	46.1	11.1	822.9	2.4	5.3
%	4.6	35.0	55.0	5.4	1.3	97.7	.3	.63
Bottom								
meq/l	22.4	330.0	700.0	28.9	12.8	1125.0	-	6.3
%	2.2	30.5	64.7	2.6	1.1	98.3	-	.6
"1st Salt Mine"								
meq/l	41.0	4260.0	183.0	1.5	214.0	2395.0	55.2	2613.0
%	.9	94.9	4.2	.03	4.1	45.4	1.0	49.5
"2nd Salt Mine"								
meq/l	45.0	4434.0	233.0	1.15	100.0	3291.0	54.6	1633.0
%	.95	94.2	4.9	.02	1.9	64.8	1.1	32.5

Table X. Seasonal Monovalent:Divalent (M:D) Total Cation Ratios and Na:Mg Ratios for Kamloops Habitats (1968).

Saline Habitats	26 iii	18 v	29 viii
"CHERRY CREEK POND"			
Crystal Surface			
(M:D)	8.8	6.8	5.3
Na:Mg	8.8	7.1	5.3
Crystal Bottom			
(M:D)	-	5.4	8.1
Na:Mg	10.5	5.3	8.8
Ruppia Surface			
(M:D)	6.6	3.8	-
Na:Mg	7.0	3.9	-
Ruppia Bottom			
(M:D)	-	-	-
Na:Mg	4.9	-	-
"1st SALT MINE POND"			
(M:D)	-	75.9	22.3
Na:Mg	-	75.6	22.5
"2nd SALT MINE POND"			
(M:D)	-	36.0	19.4
Na:Mg	-	35.9	19.2
"POLYGON POND"			
(M:D)	1.2	.81	.49
Na:Mg	1.2	.82	.49
IRONMASK			
(M:D)	1.0	.99	.81
Na:Mg	1.0	.98	.76
WALLENDER LAKE			
Surface			
(M:D)	1.3	1.1	1.0
Na:Mg	1.3	1.1	1.0
Bottom			
(M:D)	-	1.1	.8
Na:Mg	-	1.2	.8
BOWERS LAKE			
Surface			
(M:D)	.81	.66	.65
Na:Mg	.83	.68	.63
Bottom			
(M:D)	1.8	.66	.47
Na:Mg	1.9	.65	.47

Table XI. Seasonal Values for Nitrogen (NO_2^--NO_3^- and NH_4^+) measured in mg/l for Kamloops Habitats During 1967 and 1968.

Saline Habitats	19v67	19vi67	23vii67	11viii67	30viii67	23ix67
"CHERRY CREEK POND"						
NO_2-NO_3						
Surface	.12	.11	trace	trace	.02	---
Bottom	.07	.18	trace	trace	---	---
BOWERS LAKE						
NO_2-NO_3						
Surface	.02	.04	.06	trace	.03	.03
Bottom	---	.06	.06	trace	.02	.02
POLYGON POND						
NO_2-NO_3	.52	.30	2.25	.08	.03	---
IRONMASK LAKE						
NO_2-NO_3	.08	.11	.11	.12	.02	.03
WALLENDER LAKE						
NO_2-NO_3						
Surface	.05	.07	.05	.04	.03	.04
Bottom	---	.06	.02	.03	.01	.01

Table XI Continued

Saline Habitats	26iii68	20iv68	18v68	15vi68	19vii68	29viii68
"CHERRY CREEK POND"						
NO_2-NO_3						
Surface	.07	.05	.12	.16	.16	.09
Bottom	.34	.09	.10	.16	.18	.10
NH_4						
Surface	5.25	2.68	2.50	5.3	2.4	-
Bottom	6.81	3.47	3.30	7.2	2.5	-
BOWERS LAKE						
NO_2-NO_3						
Surface	.07	.07	.04	.06	.06	.04
Bottom	.05	.07	.03	.08	.07	.06
NH_4						
Surface	19.2	6.9	2.5	2.4	.2	-
Bottom	23.0	9.0	5.4	11.5	12.0	-
POLYGON POND						
NO_2-NO_3	.10	.04	.18	-	-	-
NH_4	5.25	7.50	49.25	48.30	11.25	-
IRONMASK LAKE						
NO_2-NO_3	.07	trace	.34	.45	-	-
NH_4	8.75	10.75	13.10	10.50	8.75	-
WALLENDER LAKE						
NO_2-NO_3						
Surface	.11	.08	.04	.08	.04	.04
Bottom	.12	.09	.04	.07	trace	-
NH_4						
Surface	9.25	3.0	3.05	2.5	.75	-
Bottom	10.23	6.10	10.10	9.8	26.0	-
"1st SALT MINE"						
NO_2-NO_3	-	-	trace	-	-	-
NH_4	-	-	4.30	3.0	6.25	-
"2nd SALT MINE"						
NO_2-NO_3	-	-	.16	-	-	-
NH_4	-	-	3.30	2.50	3.75	-

Table XII. Seasonal Values for Phosphorus (Ortho-phosphate=O-$PO_4^{\equiv}$ and Total-phosphate=T-$PO_4^{\equiv}$) measured in mg/l for Kamloops Habitats During 1967 and 1968.

Saline Habitats	19v67	19vi67	23vii67	11viii67	30viii67	23ix67
"CHERRY CREEK POND"						
O-PO_4						
Surface	1.41	.84	.96	1.86	11.0	-
Bottom	.44	.78	.95	1.86	-	-
T-PO_4						
Surface	1.54	1.10	1.25	2.14	12.40	-
Bottom	.50	1.07	1.41	2.08	-	-
BOWERS LAKE						
O-PO_4						
Surface	.04	.09	.09	.07	.07	.07
Bottom	-	.14	.24	.07	.06	.06
T-PO_4						
Surface	.19	.78	.68	1.79	3.20	3.51
Bottom	-	.43	.72	1.70	3.14	3.81
POLYGON POND						
O-PO_4	2.01	-	69.5	71.75	165.0	-
T-PO_4	2.05	-	101.0	124.25	177.3	-
IRONMASK LAKE						
O-PO_4	.32	10.0	13.75	30.75	40.50	41.85
T-PO_4	.38	10.84	14.50	33.50	45.20	43.40
WALLENDER LAKE						
O-PO_4						
Surface	1.41	1.38	.92	.84	1.16	1.21
Bottom	-	2.34	1.30	4.20	4.30	5.28
T-PO_4						
Surface	1.54	1.54	1.34	1.31	1.21	1.28
Bottom	-	2.66	1.61	4.35	4.48	5.41

Table XII. Continued

Saline Habitats	26iii68	20iv68	18v68	15vi68	19vii68	29viii68
"CHERRY CREEK POND"						
$O\text{-}PO_4$						
Surface	.28	.78	.55	.44	1.23	2.20
Bottom	.34	.90	.81	1.10	1.81	3.90
$T\text{-}PO_4$						
Surface	.30	.90	.56	.47	1.28	2.27
Bottom	.41	1.10	.81	1.14	1.87	3.99
BOWERS LAKE						
$O\text{-}PO_4$						
Surface	.12	.50	.06	.06	.12	.11
Bottom	.57	.06	.23	.26	.12	.06
$T\text{-}PO_4$						
Surface	.78	1.16	.21	.23	.30	.57
Bottom	.59	.87	.25	.78	.92	1.12
POLYGON POND						
$O\text{-}PO_4$	.68	2.41	6.80	34.5	59.2	75.0
$T\text{-}PO_4$	1.01	2.45	16.97	45.5	72.1	86.2
IRONMASK						
$O\text{-}PO_4$	.92	.74	.17	.26	8.35	10.60
$T\text{-}PO_4$	.98	.95	.20	.30	8.75	11.10
WALLENDER LAKE						
$O\text{-}PO_4$						
Surface	1.98	1.41	2.20	1.50	.71	2.20
Bottom	2.14	2.37	2.34	3.80	7.85	14.25
$T\text{-}PO_4$						
Surface	2.41	2.51	2.48	1.52	.74	2.35
Bottom	2.31	2.39	2.44	3.91	7.98	14.70
"1st SALT MINE"						
$O\text{-}PO_4$	-	-	.07	.19	.42	12.50
$T\text{-}PO_4$	-	-	.09	.20	.45	13.75
"2nd SALT MINE"						
$O\text{-}PO_4$	-	-	46.50	75.0	220.0	345.0
$T\text{-}PO_4$	-	-	47.20	75.5	270.0	350.0

Table XIII. Seasonal Total Dissolved Solids (measured in g/l) for Kamloops Habitats, 1967 and 1968.

	10vi67	23vii67	30viii67	23ix67
"Cherry Creek Pond"				
Surface (g/l)	50.4	161.6	478.0	-----
Bottom (g/l)	54.5	163.6	-----	-----
Bowers Lake				
Surface (g/l)	16.6	18.8	84.0	88.2
Bottom (g/l)	19.6	21.0	82.0	85.0
Wallender Lake				
Surface (g/l)	28.0	36.4	91.6	96.0
Bottom (g/l)	35.8	37.2	103.2	106.0
"Polygon Pond"				
Surface (g/l)	205.6	560.0	568.0	-----
Ironmask Lake				
Surface (g/l)	218.0	472.5	569.0	520.0

	1i68	26iii68	20ix68	18v68	19vii68	29viii68
"Cherry Creek Pond"						
Surface (g/l)	----	14.5	21.8	39.0	95.7	139.3
Bottom (g/l)	----	59.6	28.4	45.0	113.2	225.0
Bowers Lake						
Surface (g/l)	78.4	17.2	27.2	31.0	42.1	51.0
Bottom (g/l)	-----	26.2	27.6	34.6	53.4	65.5
Wallender Lake						
Surface (g/l)	112.0	16.5	20.0	27.6	40.7	48.8
Bottom (g/l)	-----	42.0	56.4	52.8	63.4	82.5
"Polygon Pond"						
Surface (g/l)	-----	24.3	40.8	123.8	278.0	271.5
Ironmask Lake						
Surface (g/l)	217.0	20.5	33.2	60.5	234.5	198.0
"1st Salt Mine Pond"						
Surface (g/l)	-----	-----	-----	148.7	298.8	295.4
"2nd Salt Mine Pond"						
Surface (g/l)	-----	-----	-----	142.5	312.0	315.4

Table XIV. Seasonal Osmotic Potential for 1968 in (mOsm) for Kamloops Habitats.

MONTH	CHERRY CR. POND		BOWERS		WALLENDER		POLYGON	IRONMASK
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Surface
MARCH	220 mOsm	705 mOsm	240 mOsm	470 mOsm	108 mOsm	521 mOsm	245 mOsm	211 mOsm
APRIL	330	365	281	285	228	615	465	374
MAY	536	559	355	322	312	629	1240	715
JUNE	690	700	368	344	353	673	1720	1242
AUGUST	1612	2382	510	599	535	682	>3000	>3000

Table XV. Seasonal Specific Conductivity Values (measured in millimhos) for Kamloops Habitats, 1967 and 1968.

	19v67	10vi67	23vii67	11viii67	30viii67	23ix67
"Cherry Creek Pond"						
Surface (millimho)	32.7	37.7	78.9	101.9	80.2	-----
Bottom (millimho)	32.8	38.8	80.9	-----	----	-----
Bowers Lake						
Surface (millimho)	10.6	11.1	14.2	18.9	29.9	30.5
Bottom (millimho)	10.2	11.2	13.8	19.2	27.8	31.5
Wallender Lake						
Surface (millimho)	17.5	18.2	25.5	34.0	35.0	35.9
Bottom (millimho)	19.0	23.4	29.8	41.5	42.8	40.8
"Polygon Pond"						
Surface (millimho)	77.9	72.4	48.9	36.8	37.9	-----
Ironmask Lake						
Surface (millimho)	72.3	64.2	56.6	49.1	45.3	46.2

	1i68	26iii68	20iv68	18v68	15vi68	19vii68	29viii68
"Cherry Creek Pond"							
Surface (millimho)	----	8.6	16.2	30.0	40.5	64.1	73.5
Bottom (millimho)	----	40.6	19.1	31.1	41.0	66.9	90.2
Bowers Lake							
Surface (millimho)	20.8	12.2	18.9	20.7	22.6	27.3	28.3
Bottom (millimho)	----	20.1	19.3	21.5	22.6	29.8	33.0
Wallender Lake							
Surface (millimho)	30.2	7.5	13.4	19.8	26.4	27.4	29.2
Bottom (millimho)	----	30.1	35.8	37.7	35.9	37.9	34.9
"Polygon Pond"							
Surface (millimho)	----	13.3	30.2	63.5	77.0	60.3	70.5
Ironmask Lake							
Surface (millimho)	----	13.6	23.6	30.1	56.7	77.3	50.2
"1st Salt Mine Pond							
Surface (millimho)	----	----	----	82.0	105.7	101.9	94.3
"2nd Salt Mine Pond							
Surface (millimho)	----	----	----	77.3	101.9	107.5	95.6

Table XVI. Seasonal Values for Oxygen (measured in mg/l) for Kamloops Habitats, 1967, 1968. (None Det. indicates not measurable due to extreme salinity.)

Saline Habitats	19v67	19vi67	23vii67	11viii67	30viii67	23ix67
"Cherry Creek Pond"						
Crystal						
Surface	9.0	8.0	3.0	1.0	0.0	0.0
Bottom	9.0	7.5	---	---	---	---
Ruppia						
Surface	12.0	11.0	3.5	---	---	---
Bottom	12.0	11.0	---	---	---	---
Bowers Lake						
Surface	5.0	5.0	6.5	5.5	5.0	5.0
Bottom	---	---	6.0	5.5	---	---
"Polygon"						
Surface	None Det.	None Det.	None Det.	None Det.	None Det.	None Det.
Ironmask	"	"	"	"	"	"
Wallender Lake						
Surface	6.0	4.0	6.0	3.0	3.0	3.5
Bottom	---	.5	1.0	1.0	.5	.5

	26iii68	20iv68	18v68	15vi68	19vii68	29viii68
"Cherry Creek Pond"						
Crystal						
Surface	11.0	8.5	6.5	6.0	8.5	4.5
Bottom	8.5	8.5	6.5	6.0	.5	1.0
Ruppia						
Surface	12.0	9.0	8.0	8.5	8.5	---
Bottom	7.5	9.0	8.0	---	---	---
Bowers Lake						
Surface	8.5	3.0	3.0	5.0	6.5	2.0
Bottom	8.5	3.0	2.5	3.5	6.0	.5
"Polygon Pond"						
Surface	9.0	6.5	None Det.	None Det.	None Det.	None Det.
Ironmask	8.0	6.0	"	"	"	"
Wallender Lake						
Surface	8.5	6.0	4.0	5.0	6.5	1.5
Bottom	2.5	1.5	1.5	.5	.5	.5

Table XVII. Seasonal pH for Kamloops Habitats 1967 and 1968.

Saline Habitats	19v67	10vi67	23vii67	11viii67	30viii67	23iv67
"Cherry Creek Pond"						
Crystal						
Surface	9.2	9.6	9.9	8.9	8.3	-
Bottom	9.4	9.6	9.9	8.9	8.3	-
Ruppia						
Surface	9.5	10.2	-	-	-	-
Bottom	9.5	10.4	-	-	-	-
Bowers Lake						
Surface	8.8	8.6	8.5	9.0	8.7	8.6
Bottom	8.8	8.6	8.6	9.0	8.7	8.5
Wallender Lake						
Surface	9.7	9.9	9.9	9.8	9.8	9.9
Bottom	9.7	9.9	8.7	9.0	8.8	8.7
Polygon Pond						
Surface	9.9	8.5	8.2	8.3	8.1	-
Ironmask						
Surface	8.9	8.6	8.2	8.4	8.6	9.1

	12i68	26iii68	20iv68	18v68	15vi68	19vii68	29viii68
"Cherry Creek Pond"							
Crystal							
Surface	-	9.4	8.9	9.1	10.2	9.6	9.0
Bottom	-	9.4	9.0	9.0	10.2	9.4	8.8
Ruppia							
Surface	-	9.2	9.0	9.4	10.3	9.7	-
Bottom	-	9.6	9.0	-	-	-	-
Bowers Lake							
Surface	8.1	8.5	8.4	8.6	9.2	9.1	9.4
Bottom	8.2	8.5	8.5	8.6	9.3	9.1	9.3
Wallender Lake							
Surface	9.3	9.0	9.1	9.2	10.0	10.2	9.6
Bottom	9.0	8.8	9.2	9.5	9.2	8.8	8.0
Polygon							
Surface	-	9.9	8.4	8.35	8.3	8.2	8.4
Ironmask							
Surface	9.0	8.9	8.8	8.9	9.1	8.5	8.6
"1st Salt Mine"							
Surface	-	-	-	9.4	9.8	9.8	10.2
"2nd Salt Mine"							
Surface	-	-	-	9.5	9.6	9.6	9.7

Table XVIII. Seasonal Water Temperatures for Kamloops Habitats (1967 - 1968)

MONTH	"CHERRY CREEK POND"				BOWERS		WALLENDER		"POLY"	"IRON"	1ST	2ND
	"CRYSTAL"		"RUPPIA"									
1967	S	B	S	B	S	B	S	B	S	S	S	S
My 19	14°C	14°C	14°C	14°C	15°C	15°C	14°C	14°C	14°C	15°C		
Je 10	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	16.0	17.0		
Jul 23	18.0	18.0	18.0	---	18.5	17.5	20.0	19.5	37.5	32.0		
Aug 11	22.0	---	---	---	18.5	18.0	20.5	19.5	27.0	23.0		
Sept 23	18.5	---	---	---	15.0	14.0	16.0	15.5	---	19.0		
1968												
Jan 12	-4.0	---	---	---	-3.0	-3.0	-3.0	-3.0	---	-5.0		
Mar 26	4.5	11.5	5.2	11.5	7.5	12.0	7.0	8.5	6.0	6.5		
Apr 20	8.0	9.0	8.0	8.0	10.0	10.5	9.5	9.5	9.5	9.0		
My 18	17.0	17.0	17.0	17.0	16.0	16.0	15.0	17.0	17.5	16.0	20.5	21.0
Je 15	21.0	21.0	21.0	---	19.5	19.5	15.0	17.0	22.5	23.0	25.0	25.5
Jul 19	19.5	19.5	19.5	---	21.0	20.0	15.5	15.5	26.0	25.5	28.0	28.0
Aug 29	26.0	---	---	---	18.5	20.0	15.0	14.0	31.5	27.5	25.0	24.0

Table XIX. Trace Analysis for Surface Waters for Kamloops Habitats (May 1968) in mg/l. (LDL = lowers detection limit of test; ND = not determined). Spectrographic analysis by Coast-Eldridge, Vancouver, British Columbia.

ELEMENT	LDL	CHERRY CR.	1ST	2ND	BOWERS	WALLENDER	POLYGON
Al	.3	32.0	400.0	400.0	16.0	26.0	100.0
Sb	3.0	ND	ND	ND	ND	ND	ND
As	25.0	ND	ND	ND	ND	ND	ND
Ba	2.0	ND	ND	ND	30.0	ND	ND
Be	.03	ND	ND	ND	ND	ND	ND
Bi	.3	ND	ND	ND	ND	ND	ND
B	.3	4.0	100.0	9.0	21.0	1.5	1.0
Cd	3.0	ND	ND	ND	ND	ND	ND
Cr	.1	ND	ND	ND	ND	ND	ND
Co	.3	ND	ND	ND	ND	ND	ND
Cu	.1	.4	trace	trace	trace	.3	trace
Ga	.3	ND	ND	ND	ND	ND	ND
Fe	.3	20.0	150.0	400.0	16	12	65.0
Pb	.3	ND	ND	ND	ND	ND	ND
Mn	1.0	ND	ND	ND	ND	ND	ND
Mo	.3	trace	trace	trace	trace	trace	trace
Nb	.3	ND	ND	ND	ND	ND	ND
Ni	.3	ND	ND	trace	ND	ND	ND
Si	.3	28.0	300.0	400.0	21.0	25.0	130.0
Ag	.03	trace	ND	ND	ND	ND	trace
Sr	.3	20.0	ND	14.0	32.0	50.0	40.0
Sn	.3	ND	ND	ND	ND	ND	ND
Ti	.3	4.0	40.0	40.0	3.0	9.0	40.0
W	25.0	ND	ND	ND	ND	ND	ND
V	.3	1.2	15.0	4.0	.3	1.5	10.0
Zn	3.0	ND	ND	ND	ND	ND	ND

Table XX. Dominant Benthic and Planktonic Algal Species in Kamloops Habitats 1967, 1968. (Pg = Polygon, Im = Ironmask, Bo = Bowers, Wa = Wallender, CC = Cherry Creek Pond, 1S = 1st Salt Mine, 2S = 2nd Salt Mine).

Species	Bo	Wa	Im	Pg	CC	1S	2S
Anabaena spp.	X	X	X	X	X	X	X
Merismopedia tenuissima Lemm.	X	-	-	-	-	-	-
Nodularia spumigena Mertens	X	X	X	X	X	X	X
Oscillatoria spp.	X	X	X	X	X	X	X
Navicula sp.	X	X	X	X	X	X	X
Pinnularia sp.	X	-	-	-	X	-	-
Surrurilla sp.	X	-	-	-	X	-	-
Synedra fasiculata (C.A.Ag). Kutz	X	-	-	-	X	-	-
Cladophora fracta (Dillw.) Kutz.	-	X	-	-	-	-	-
Chlamydomonas spp.	-	-	X	X	X	X	X
Ctenocladus circinnatus Borzi	-	-	-	-	X	X	X
Rhizoclonium hieroglyhium (C.A.Ag.) Kutz.	X	-	-	-	-	-	-
Ulothrix tenerrima Kutz.	X	X	X	X	X	-	-
Phacus sp.	X	-	-	-	-	-	-
Euglena spp.	X	-	-	-	-	-	-
Cryptomonas sp.	X	-	-	-	-	-	-
Gymnodinium sp.	X	-	-	-	-	-	-
Ochromonas vallesiara Chodat	X	-	-	-	-	-	-

Table XXI. Defined Medium Recipe for Ctenocladus Compared with Major Ions of Sea Water (Harvey, 1963).

SALT	CONCENTRATION
Na_2SO_4	13.5 g/l
$NaHCO_3$	.098
$CaCl_2 \cdot 2H_2O$	.100
$MgCl_2 \cdot 6H_2O$	.05
KCL	.05
NaCl	13.75
$NaNO_3$	.175
$NaH_2PO_4 \cdot H_2O$	.100
$FeCl_3 \cdot 6H_2O$	.00032 g/l
$MnCl_2$	.00048
$ZnCl_2$	.00006
$CoCl_2 \cdot 6H_2O$	.00002
$CuCl_2 \cdot 2H_2O$	.000005
H_3BO_3	.00240
$Na_2MoO_4 \cdot 2H_2O$	.00020
Tris-buffer	1.0 g/l
EDTA-Na_2	.015

ION	SEAWATER	DEFINED
Na^+	10.76 g/l	15.75 g/l
K^+	.387	.052
Mg^{++}	1.29	.014
Ca^{++}	.413	.047
Cl^-	19.353	16.780
$SO_4^=$	2.712	9.140
HCO_3^-	.142	.071

Table XXII. Influence of Temperature on Akinete Germination and Zoosporangia Formation at ca. 4280 lux, 16 hr light/ 8 hr dark cycle (+ = zoosporangia; - = no zoosporangia)

TEMPERATURE	TIME FOR INITIAL GERMINATION	90% 2-CELL STAGE	ZOOSPORANGIA
0-1°C	none	nil	-
4-6°C	none	nil	-
7-9°C	106-125 hr	nil	-(+)
9-11°C	25-38 hr	80-95 hr	+
14-16°C	22-30 hr	60-72 hr	+
19-21°C	6-10 hr	24-36 hr	+
25-27°C	<10 hr	nil	-(+)
29-31°C	<10 hr	nil	-

Table XXIII. Effect of Temperature on Ctenocladus Akinete Germination (N=800) ca. 4280 lux, 16 hr light/8 hr dark cycle.

Temperature	I	II	III	IV	$\bar{X}$ Germ %	$\bar{X}$ Cell # per germ tube
0-1°C	0	0	0	0	0	0
3-4	1.0%	0	0	0	.25%	0
5-7	47.0	59.0%	38.5%	61.5%	51.4	2
7-9	54.0	63.5	70.0	59.0	61.7	2
9-11	96.0	92.0	98.0	89.5	93.9	>2
15-16	97.0	95.0	99.0	93.0	96.0	>2
19-21	99.0	98.5	95.5	98.0	97.3	>2
25-26	91.0	99.5	98.0	96.5	96.3	>2
30-31	60.5	55.0	41.5	32.0	46.8	>2
34-35	6.0	0	0	0	1.5	0

Table XXIV. Tolerance of Ctenocladus Akinetes to Various Temperatures (Initial germination % after 8 days exposure to initial temperature conditions; optimum germination % measured 8 days after transferred to optimum temperature (19-21°C).

Initial Temperature	Optimum Temp.	$\bar{x}$ Initial %	$\bar{x}$ Optimum %
0-1°C	19-21°C	0%	98.0%
3-4	"	0	97.9
5-6	"	56.0	97.0
7-9	"	52.9	94.4
15-16	"	98.3	96.2
19-21	"	97.8	97.4
30-31	"	60.9	85.4
34-36	"	3.4	4.7

Table XXV. Influence of Light Intensity on Ctenocladus Akinetes (N=800) 19-21°C.

Light Intensity	Duration*	I	II	III	IV	$\bar{x}$ germ %
0 lux	0	0	0	0	0	0
214	16/8	81.5%	96.5%	92.5%	97.5%	95.0%
535	"	92.0	97.5	89.5	96.0	93.8
1070	"	95.0	90.0	88.0	100	93.3
3210	"	97.5	99.0	92.0	100	97.1
5350	"	96.0	98.0	92.0	98.0	96.0
7490	"	82.5	86.0	93.5	92.0	88.3
9630	"	79.5	62.0	81.5	88.5	77.9
12,305	"	17.0	26.0	7.0	15.5	16.4

*light/dark cycle

Table XXVI. Tolerance of Ctenocladus Akinetes to Various Light Intensities (Initial germination % after 6 days exposure to initial conditions; optimum germination % measured 6 days after transferred to optimum light conditions (4280 lux)

Initial Light Quantity	Duration* 1 day	$\bar{x}$ Initial %	Optimum Light	Optimum %
0 lux	0	0	4280 lux	95.3
3210	16/8	96.7%	"	95.7
12,305	16/8	9.3%	"	11.9

*light/dark cycle

Table XXVII. Effect of Hydrogen Ion Concentration on Ctenocladus Akinetes Germination (N=800) (19-21°C, ca. 4280 lux 16 hr light/8 hr dark cycle)

pH	I	II	III	IV	$\bar{x}$ Germ %
6.0-6.3	0	0	0	0	0
6.9-7.1	0	0	1.0%	0	.25%
7.4-7.6	9.0%	16.0%	6.5%	12.0%	10.9%
7.9-8.1	85.5%	72.0%	69.5%	82.5%	77.5%
8.4-8.6	97.0%	92.0%	98.5%	92.5%	94.9%
8.9-9.1	90.0%	97.0%	88.0%	92.5%	91.9%
9.8-10.1	96.0%	93.5%	86.5%	89.0%	91.3%
10.8-11.1	91.5%	98.0%	94.5%	99.0%	95.8%
11.6-12.1	4.5%	18.0%	13.0%	6.5%	10.6%
8.5-8.7 (control in SSW)	97.0%	99.5%	100.0%	94.0%	97.7%

Table XXVIII. Tolerance of Ctenocladus Akinetes at Various Hydrogen Ion Concentrations (Initial germination % after 7 days at initial pH conditions; optimum germination % measured 8 days after transferred to optimum pH- 8.5-9.0)

Initial pH	$\bar{x}$ Initial %	Optimum pH	Optimum %
6.0-6.3	0	8.8-9.2	5.8
6.9-7.1	1.3%	"	55.3
7.4-7.6	48.9%	"	83.8
7.9-8.1	82.4%	"	92.9
8.5-8.7 (control in SS)	96.4	8.5-8.7	96.4

Table XXIX. Effect of Natural Waters on Ctenocladus Akinete Germination n=400. (19-21°C, ca. 4280 lux 16 hr light/ 8 hr dark cycle

Solution Source	Date of Collection	Osmotic Potential (mOsm)	$\bar{x}$ Germination
"Cherry Creek Pond"	14vi68	690	94.3%
"2nd Salt Mine"	19v68	1587	94.6
Mono Lake	1vi68	1700	89.3
"Polygon Pond"	15vi68	1720	92.7
"1st Salt Mine"	19v68	2383	18.7
"Hazen Pond"	2vi68	2611	19.7
"Polygon Pond"	19vii68	2672	26.4
"Stateline Pond"	3vi68	2914	2.3
"2nd Salt Mine"	14vi68	>3000	0
"1st Salt Mine"	14vi68	>3000	0

Table XXX. Effects of Dilution of "1ST SALT MINE POND" Water with Distilled Water on Ctenocladus Akinete Germination. ($\bar{x}$ germination % based on 2 trials of 200 akinetes each 19-21°C; ca. 4280 lux 16 hr light/8 hr dark cycle)

Dilution Factor	Osmotic Potential in mOsm	$\bar{x}$ No. Cells per germination tube	$\bar{x}$ % Germination
0	>3000 mOsm	0	0.0%
1.1	>3000	0	0.0
1.42	2540	1-2	31.5
2.00	1510	many	93.5
3.33	1050	many	92.5
10.00	372	many	96.0
20.00	221	many	91.3
50.00	108	many	95.2
100.00	41	1-2	88.7
200.00	20	1	20.1
1000.00	<10	0	0.0

Table XXXI. Effects of Dilution of "CHERRY CREEK POND" Water with Distilled Water (August 29, 1968) 19-21°C; ca. 4280 lux 16 hr light/8 hr dark cycle.

DILUTION FACTOR	OSMOTIC POTENTIAL	ZOOSPORANGIA FORMATION
Normal	1625 mOsm	NIL
1.40	1385 "	NIL
1.45	1153 "	NUMEROUS
1.73	997 "	NUMEROUS
2.00	925 "	NUMEROUS
2.66	726 "	NUMEROUS
4.00	483 "	NUMEROUS
8.00	255 "	NUMEROUS

Table XXXII. Effect of Specific Ions on Akinete Germination (n=200) 19-21°C, ca. 4280 lux 16 hr light/8 hr dark cycle) (KCl, K_2SO_4, $MgSO_4$, $MgCl_2$ at all concentrations (.05-6 mg/l) gave 0% germination)

Salt Solution	Concentration in mg/l	$\bar{x}$ Germination %
NaCl	.05	0.0
"	.1	0.0
"	.5	10.0
"	1.0	77.0
"	2.0	5.0
"	4.0	.5
"	6.0	0.0
Na_2SO_4	.05	0.0
"	.1	7.5
"	.5	63.5
"	1.0	83.2
"	2.0	2.5
"	4.0	0.0
"	6.0	0.0

Table XXXIII. Effect of Various Na:Mg Ratios In Bowers Sediment Extract on Reproduction (A = Akinete, Z = Zoosporangia, 0 = Neither) (19-21°C, 4280 lux, 16 hr light/8 hr dark cycle) (Na^+ source = Na SO)

	A	B	C	D	E	F	G	H
meq/l Mg^{++}	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7
meq/l Na^+	5.1	6.6	9.4	15.7	42.3	109.3	214.7	13.7
Na:Mg Ratio	.45	.56	.80	1.3	3.6	9.3	18.3	13.7
Reproduction	A	A	A	0	0	0	Z	Z
$\bar{x}$ Cell								
Length (μ)	11.5	11.5	11.5	25.9	54.2	95.3	91.6	102.5
Width (μ)	12.5	12.5	12.5	8.8	7.5	7.35	7.12	7.3
Range in Cell Size								
Length (μ)	10.0-12.5	10.0-12.5	10.0-12.5	12.5-37.5	25.0-87.5	37.5-155.0	35.0-147.5	47.5-210.0
Width (μ)	12.0-13.5	12.0-13.5	12.0-13.5	7.5-10.0	6.5-8.0	6.0-8.1	6.2-7.9	6.5-7.9
Length:Width Ratio	-	-	-	2.9	7.2	12.9	12.8	14.0

Table XXXIV. Effect of Various Na:Mg ratios in Chihara Medium on branching (ac = branches arising acutely, rt. = branches arising at right angles) and cell dimensions (N=200). At 19-21°C, ca. 4280 lux on 16 hr light/8 hr dark cycle.

Salt	Mg^{++} meq/l.	Na^{+} meq/l.	Branching	Range of Cell Size in Length	Width	$\bar{x}$ Cell Size in Length	Width	Mean Ratio L:W	Approximate Na:Mg Ratios
$MgSO_4$	157	140	ac-rt	17.5-50.0	6.9-8.7	32.9	7.9	4.1	1:1
$MgSO_4$	280	140	ac-rt	15.0-40.0	7.2-9.0	24.5	8.2	2.9	1:2
$MgSO_4$	157	482	ac	50.0-127.5	7.5-8.4	91.9	8.1	11.3	3:1
$MgCl_2$	164	140	rt	20.0-52.5	7.0-9.0	34.5	7.9	4.3	1:1
$MgCl_2$	312	140	ac-rt	17.5-42.5	7.4-10.0	26.4	8.5	3.1	2:1
$MgCl_2$	164	498	ac	37.5-120.0	6.4-7.6	71.4	7.2	9.9	1:3
Chihara Solution	21	140	ac	30.0-197.5	6.6-7.2	89.5	6.8	13.1	1:7

Table XXV. Analysis (in meq/l) of Sediment Extracts from Investigated Habitats (CC-C = "Cherry Creek" Crystal Zone, CC-R = "Cherry Creek" Ruppia Zone, B = Bowers Lake, W = Wallender Lake, I = Ironmask, P = Polygon Pond) Showing Effects on Ctenocladus Reproduction. (Temp. = 20°C, Light = ca. 4280 lux 16 hr light/8 hr dark cycle, pH = ca. 8.5-9.1.

Extract	Mg^{++}	Ca^{++}	K^{+}	Na^{+}	Na:Mg Ratio	Cation M:D Ratio	Reproduction
CC-C	.95	.325	.25	13.1	13.7	10.4	many zoosporangia
CC-R	24.8	4.3	1.47	172.0	6.9	5.98	few-no zoosporangia
B	11.7	10.4	.875	5.3	.45	.28	Akinetes
W	2.75	2.2	.525	2.7	.98	.65	Akinetes
I	13.8	11.9	2.2	18.9	1.4	.82	Akinetes
P	6.45	6.6	.100	4.8	.74	.37	Akinetes

Table XXXVI. Collections of Ctenocladus Deposited in Various Herbaria with Collecting Data. (Abbreviations after Lanjouw, J and Stafleu, F, 1956.)

Herbarium	Herbarium #	Location	Date & Collector	Condition of Material
Cryptogamic Herbarium Chicago (F)	----------	Little Manitou Lake Sask. Canada	P.E. Kuehne 12ix41	Akinetes
"	----------	Marina, California Monterey County	L.G.M. Bass Becking, 1929	Undet.
"	1074231	Lima, Peru	A. Maldonado 12/1940	Vegetative
"	1123022	Laguna Boza, Lima Peru	A. Maldonado 1/1943	Akinetes
"	1049310	Mono Lake, California	M.J. Groesbeck 12vi40	Vegetative
"	1115929	Mono Lake, California	A. Carter 24viii42	Vegetative-Akinete
"	1051587	Mono Lake, California	M.J. Groesbeck 6ix40	Akinete
"	1049303	Mono Lake, California	M.J. Groesbeck 11vi40	Undet.
"	1049308	Mono Lake, California	M.J. Groesbeck 12vi40	Vegetative
Farlow Herbarium Cambridge, Mass. (FH)	301b	Laguna Boza, Lima	A. Maldonado 1/1943	Undet.
Naturhistorische Museum Austria, Wein (W)	9938	Little Manitou Sask. Canada	P.E. Kuehne 12ix40	Akinetes
Rijksherbarium Herbarium Leiden, Netherlands (NBV)	L-3085 166	Little Manitou Sask. Canada	P.E. Kuehne 12ix40	Akinetes
"	L-3085 173	Marina, California	L.G.M. Bass Becking, 1929	Vegetative

Table XXXVI Continued

Herbarium	Herbarium #	Location	Date & Collector	Condition of Material
Rijksherbarium Herbarium Leiden, Netherlands (NBV)	L-3085 170	Marina, California	L.G.M. Bass Becking, 1929	Undet.
"	L-3085 171	Laguna Boza, Lima, Peru	A. Maldonado 1/1943	Vegetative-Akinete
"	L-3085 172	Lima, Peru	A. Maldonado	Vegetative-Akinete
"	L-3085 168	Mono Lake, California	M.J. Groesbeck 11vi40	Undet.
"	L-3085 164	Mono Lake, California	M.J. Groesbeck 6ix40	Akinete
"	L-3085 165	Mono Lake, California	M.J. Groesbeck 12vi40	Vegetative
"	L-3085 167	Mono Lake, California	M.J. Groesbeck 12/640	Vegetative
"	L-3085 169	Mono Lake, California	A. Carter 24viii42	Vegetative-Akinete
Univ. California Herbarium Berkeley, California (UC)	953416	Marina, California	L.G.M. Bass Becking, 1929	Undet.
"	680585	Laguna Boza Lima, Peru	A. Maldonado 1/1943	Akinete
"	641536	Cuzco, Peru	A. Maldonado 12/1940	Undet.
"	674693	Mono Lake, California	A. Carter 24viii42	Vegetative-Akinete