Canyon Ferry Reservoir zooplankton population dynamics
by Chadwick Lee Martin

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Abstract:
A limnological study involving four stations on Canyon Ferry Reservoir was conducted during 1971 and 1972. This thesis was primarily concerned with zooplankton population analysis. Part of the study involved comparisons of physical, chemical, and biological aspects with a study conducted on the same reservoir during 1956, 1957, and 1958 by Wright (1958, 1959, 1960, 1961, 1965).

Zooplankton production in 1971 and 1972 was less than that of 1957 and 1958.

A difference in production between the summers of 1971 and 1972 was found. This difference was attributed to a 1971 reservoir draw down and reflooding, which released nutrients from the sediments causing increased phytoplankton production, increased zooplankton production and increased predator (Leptodora kindtii) production. These effects were observed to be most pronounced at the upper end of the reservoir near the nutrient release source.

Overall zooplankton concentrations were similar among the four stations. Zooplankton production was greatest at the upper end of the reservoir, where the greatest phytoplankton production was found.

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Salinity tolerance of diapausing eggs of freshwater zooplankton

SARAH A. BAILEY,* IAN C. DUGGAN,* COLIN D.A. VAN OVERDIJK,* THOMAS H. JOHENGEN,† DAVID F. REID‡ AND HUGH J. MACISAAC*

*Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada

†Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, Ann Arbor, MI, U.S.A.

‡National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Ann Arbor, MI, U.S.A.

SUMMARY

1. Many freshwater zooplankton produce diapausing eggs capable of withstanding periods of adverse environmental conditions, such as anoxia, drought and extreme temperature. These eggs may also allow oligostenohaline species to survive increased salinity during periods of tidal flux or evaporation, and here we test the ability of diapause eggs to withstand such conditions.

2. Salinity tolerance may also enable organisms to invade new environments. The increased rate of introduction of non-indigenous species to the Laurentian Great Lakes since 1989, when ballast water exchange regulations (to replace fresh/brackish water at sea with full seawater) were first implemented for transoceanic vessels, has stimulated studies that explore mechanisms of introduction, other than of active animals, in ballast water. One hypothesis proposes that freshwater organisms transported in ballast tanks as diapausing eggs may be partially responsible for the increased rate of species introduction, as these eggs may tolerate a wide array of adverse environmental conditions, including exposure to saline water.

3. We collected ballast sediments from transoceanic vessels entering the Great Lakes, isolated diapausing eggs of three species (*Bosmina liederi, Daphnia longiremis* and *Brachionus calyciflorus*), and measured the effect of salinity on hatching rate. In general, exposure to salinity significantly reduced the hatching rate of diapausing eggs. However, as non-indigenous species can establish from a small founding population, it is unclear whether salinity exposure will be effective as a management tool.

Keywords: ballast water exchange, biological invasion, hatching rates, resting eggs, salinity tolerance

Introduction

The introduction of non-indigenous species is a potent agent of biodiversity change, particularly for lake ecosystems (Sala *et al.*, 2000) and measures are urgently needed to identify and eliminate the vectors that transport them. Ballast water is recognised as the single most important vector for species introduction to aquatic habitats. Approximately 10 billion tonnes of ballast water (and its associated biota) are transferred annually between global ports, providing the primary means of transport and introduction of non-indigenous aquatic biota to ecosystems, including bacteria, dinoflagellates, phytoplankton, zooplankton and fish (Rigby, Hallegraeff & Sutton, 1999; Ruiz *et al.*, 2000). Transoceanic shipping accounts for 77% of the species introduced to the Laurentian Great Lakes since 1970 (Ricciardi, 2001). To reduce this threat, voluntary regulations were enacted in 1989, and mandated in 1993, that effectively require inbound vessels to exchange fresh or brackish ballast water with open-ocean saltwater if that water is to be discharged in the

Correspondence: Sarah A. Bailey, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, N9B 3P4, Canada. E-mail: sarahbailey@canada.com

Great Lakes (United States Coast Guard, 1993). Despite these regulations, the rate at which new species have been recorded in the Great Lakes tripled between 1989 and 1999 compared with the preceding 40 years (Grigorovich *et al.*, 2003). Increased sampling effort and time lags between establishment and discovery of non-indigenous species may partially account for this pattern (Costello & Solow, 2003; Grigorovich *et al.*, 2003), although modelling exercises indicated that ballast water exchange offers only incomplete protection and is least successful for species with benthic or dormant stages contained within ballast sediments (MacIsaac, Robbins & Lewis, 2002). Alternatively, the recent surge in non-indigenous species may be because of the presence of live or dormant organisms contained in the residual ballast of ships declaring 'no ballast on board', which are exempt from the regulations (MacIsaac *et al.*, 2002; Bailey *et al.*, 2003). These vessels carry tonnes of residual sediment and ballast water, and dominate trade inbound to the Great Lakes (Colautti *et al.*, 2003).

Many freshwater zooplankton, including copepods, cladocerans and rotifers, produce diapausing or 'resting' eggs during annual population cycles. These dormant stages probably evolved as an adaptation to periods of adverse environmental conditions, including anoxia, drought and extremely low or high temperature (Gilbert, 1974; Hairston, 1996; Hairston & Cáceres, 1996; Williams, 1998). These eggs could also provide temporal escape from unfavourable salinity, facilitating the intercontinental transfer of freshwater species in sediments of ballast tanks, even those subjected to ballast water exchange. Some of the species recently recorded in the Great Lakes are euryhaline endemics of the Ponto-Caspian region of southeast Europe, which may have been transported as resting stages in ballast water and/or sediments (Ricciardi & MacIsaac, 2000; Reid & Orlova, 2002). While the salinity tolerance of some juvenile and adult freshwater cladocerans and rotifers has been examined (e.g. Miracle & Serra, 1989; Teschner, 1995; Hall & Burns, 2002), very little is known of the salinity tolerance of the diapausing eggs of freshwater taxa. Consequently, it is difficult to infer whether invertebrates capable of producing diapausing eggs could circumvent the salinity 'filter' imposed on potential Great Lakes invaders by ballast water exchange.

In this study, we examine the effect of salinity on the hatching rate of diapausing eggs of the cladocerans *Bosmina liederi* De Melo & Hebert and *Daphnia longiremis* Sars, and the rotifer *Brachionus calyciflorus* Pallas, common inhabitants of ballast sediments of transoceanic vessels entering the Great Lakes. While these three species are native to the Great Lakes, their presence in residual ballast sediments suggests that they are representative of the types of organisms that pose a potential risk of invasion. Bailey *et al.* (2003) tested the viability of diapausing eggs recovered from ballast sediments and noted a tendency for reduced viability with high pore water salinity, although this relationship was not tested directly. Here, we test the hypothesis that diapausing eggs of freshwater zooplankton will be destroyed by exposure to saline water.

Methods

Sample collection

Residual sediments were collected from five transoceanic vessels entering the Great Lakes in 'no ballast on board' status in December 2000, May, August and December 2001, and in June 2002. Ships were sampled at the ports of Hamilton, Thorold and Toronto, Ontario, Canada, and Cleveland, OH, U.S.A. Residual sediment was collected from at least one ballast tank per ship, with additional tanks sampled depending upon availability and the ease and safety of access. Sediments were collected along longitudinal shell frames that trapped sediment in areas away from drainage flows. Approximately 4 kg of sediment (in total) were collected from at least five areas within each tank and placed in a single container. These composite samples were stored in the dark at 4 °C until experimentation. The salinity of residual sediment pore water, separated from sediment by centrifugation at approximately 3300 *g* (approximately 32 360 m s^{-2}) for 15 min, was measured using an optical refractometer (F. Dobbs, Old Dominion University, Norfolk, VA, U.S.A.).

Egg density counts

After thorough mixing, four 40-g subsamples (wet weight) were taken from each sample and preserved in 95% ethanol. Subsamples were each washed

through a 45 μm sieve to remove fine sediment. Diapausing eggs were subsequently separated from the coarse sediment using the colloidal silica Ludox® HS 40 (Burgess, 2001) and counted under a dissecting microscope.

Hatching experiments

Sediments were stored in plastic containers in the dark at 4 °C for at least 4 weeks to allow a refractory period before hatching experiments commenced (see Grice & Marcus, 1981; Schwartz & Hebert, 1987). After this time, diapausing eggs were removed from sediment using a sugar flotation method (Bailey *et al.*, 2003). Briefly, sediment was processed through a 45 μm sieve and washed into centrifuge tubes using a 1 : 1 (w : v) mixture of sucrose and water. After centrifugation (5 min at approximately ~27 *g*) the supernatant was decanted and rinsed thoroughly with water through 45 μm mesh before being transferred to a counting dish. Diapausing eggs were immediately recovered from the supernatant and sorted by size and gross morphology under a dissecting microscope, selecting only fully intact, apparently healthy eggs. A single, replicated experiment was conducted on the most abundant egg type (*Brachionus* or *Daphnia* species) for each of five tanks. For sediment from ship 1, in which *Brachionus budapestinensis* Daday eggs dominated (see Table 1), experiments were attempted using *B. budapestinensis*, but were abandoned owing to loss of eggs over time because of their extremely small size. Experiments were therefore conducted on a subdominant species, *B. liederi*. All other eggs were incubated at 0‰ (parts per thousand salinity) for identification purposes only. Occasionally, two or three species hatched during a single trial (see Table 1). These secondary species always contributed <1% of the total number of hatchlings. In total, 11 species hatched, although only three were used in the replicated experiments. Four trials were conducted with the rotifer *B. calyciflorus* and one trial each for the cladocerans *D. longiremis* and *B. liederi*.

Eggs used in the experiments were separated into 20 replicates of 20 eggs each, and placed into vials containing 15 mL of sterile medium (0, 8, 16, or 32‰) representing incremental efficiencies of ballast exchange. Five replicates were placed into each

Table 1 List of species hatched from ballast sediments through quantitative and qualitative hatching studies

Ship	No. of replicates	Species	No. eggs per 40 g	Pore water salinity (‰)
1 (FP)	N/A	*Brachionus budapestinensis*	92	2
	5	*Bosmina liederi*	56	
	N/A	*Brachionus calyciflorus*	52	
	N/A	*Daphnia longiremis*	6.3	
	N/A	*Daphnia ambigua* Scourfield*		
2 (DB)	5	*Daphnia longiremis*	391	10
	N/A	*Daphnia ambigua**		
3 (DB)	5	*Brachionus calyciflorus*	100	35
	N/A	*Brachionus quadridentatus* f. *rhenanus* (Lauterborn)*		
	N/A	*Brachionus urceolaris* Müller*		
	N/A	*Brachionus budapestinensis*	56.5	
	N/A	*Brachionus angularis* Gosse*		
4 (DB)	5	*Brachionus calyciflorus*	57.8	4
	N/A	*Daphnia magna* Straus	1.5	
	N/A	*Diaphanosoma* sp.	<1	
4 (FP)	5	*Brachionus calyciflorus*	119.5	20
5 (DB)	5	*Brachionus calyciflorus*	187.8	10
	N/A	*Asplanchna brightwelli* Gosse	1.5	

Species with N/A replicates were not used during experimentation, and were hatched only for identification purposes. Ship tanks are identified by type: FP, forepeak tank; DB, double-bottom tank.

*Denotes secondary species hatched from single morphological egg type listed immediately above.

salinity treatment at 20 °C (photoperiod 16 h light : 8 h dark), resulting in an experimental design using 400 eggs per trial. The 0‰ treatment was considered a control to assess maximum viability for these freshwater species. Synthetic pond water (Hebert & Crease, 1980) or diluted, filtered, natural seawater (collected from a vessel transiting the Great Lakes loaded with ocean water ballast, filtered through 0.2 μm Whatman number 5 paper filter) were used as hatching media. Vials were checked for emergence every 24 h for 10 days, with all hatched individuals removed daily. Media were refreshed on day 5. On day 10 all remaining eggs were transferred to synthetic freshwater media by pipette to examine hatching rates after salinity exposure. Again, the number of hatchlings was recorded daily. Negative controls containing only treatment media were kept in each treatment group to detect any introductions of organisms from the environment. We chose the 10 day hatching period after exposure for two reasons. First, previous experiments indicated that 96% of hatching occurs within the first 10 days of trials run for 20 or 30 days in the manner described above (Bailey *et al.*, 2003; unpublished data). Secondly, the typical transit time of a 'no ballast on board' vessel carrying Great Lakes water within the lake system is 7–10 days. If the uptake of Great Lakes water does induce diapausing eggs contained in ballast sediments to hatch (as suggested by Bailey *et al.*, 2003), this is the period of greatest risk.

Variation in the cumulative proportion of diapausing eggs hatched between treatments was analysed using a one-way ANOVA with repeated measures using Systat 8.0 (SPSS, 1998; SPSS, Chicago, IL, U.S.A.). Tukey's multiple comparison test was performed on the total proportion of eggs hatched to determine the impact of salinity on hatching rate. As emergence was inhibited at higher salinities, analyses were conducted on the 10-day hatching segment for each treatment, depending on the timing of emergence (i.e. days 0–10 for 0 and 8‰ treatments were compared with days 10–20 for 16 and 32‰; if no hatching occurred during days 0–10 for the 8‰ treatment, then days 10–20 were used). Only days when hatching occurred in at least one of the replicates were analysed. The proportion of eggs hatched was normalised using an arcsine square root transformation before analysis.

Results

Hatching experiments were conducted on zooplankton diapausing eggs isolated from residual ballast sediment collected from six tanks on five vessels. Salinity of sediment pore water varied from 2 to 35‰ (Table 1). Diapausing egg densities of dominant taxa were high (>50 eggs per 40 g sediment, Table 1). Eggs were induced to hatch in all experiments, with hatching rates ranging from 16 to 89% in the 0‰ treatment (Fig. 1). In each experiment the proportion hatching declined with increasing salinity. No organisms were recorded in the negative control vials. *Brachionus calyciflorus* generally began to hatch within 24 h of incubation at 0‰, while for the cladocerans *B. liederi* and *D. longiremis* emergence began at day 3 (Fig. 1). Development also began promptly in the 8‰ treatments, with *B. calyciflorus* hatching in three out of four trials by day 5. In addition, development of eye-stage embryos was recorded by day 5 in the 8‰ treatments for 50 and 90% of *Daphnia* and *Bosmina* eggs, respectively (see Fig. 2a). Apparently, these species could not tolerate emergence into brackish water, as development always stopped before emergence from eggs was complete. None of the eye-stage embryos recorded in 8‰ treatment continued development after the medium was replaced with 0‰ water on day 10. Conversely, no organisms hatched or completed significant development during the 10 days of exposure at either of the two higher salinities (i.e. 16 and 32‰, Fig. 2b,c), although some lipid accumulation was noted in the 16‰ treatment. Rather, emergence occurred in these treatments only after brackish or saltwater media were exchanged for 0‰ water (Fig. 1). After exchange, hatching rates among experiments varied between 0–31 and 0–78% for the 16 and 32‰ treatments, respectively. *Bosmina liederi* was the only species tested for which no hatching occurred after exposure to any of the salinity (>0‰) treatments.

The difference in hatching rate between treatments was highly significant for all trials ($P < 0.05$, ANOVA, Table 2). All trials exhibited divergence of hatching rates over time, as time × treatment interaction terms were significant ($P < 0.0001$, ANOVA, Table 2). The proportion of eggs hatched was higher in the 0‰ treatment for eggs recovered from ships 1–4 (Fig. 1a-e; $P < 0.05$, Tukey *post hoc* test). The hatching rates of *B. calyciflorus* for the 0 and 32‰ treatments for ship 5

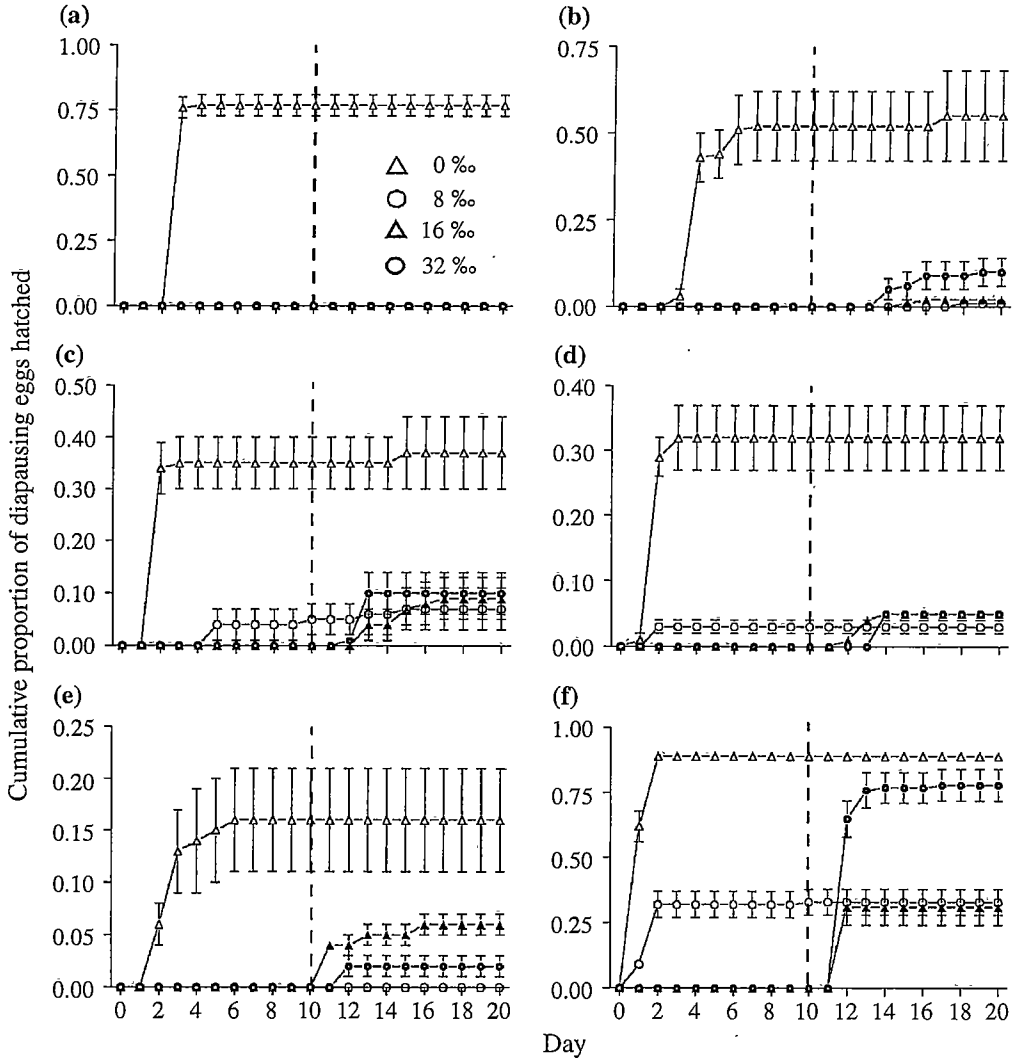

Fig. 1 Mean (±SE) cumulative proportion of diapausing eggs hatched under salinity treatments, by species. (a) *Bosmina liederi* (ship 1), (b) *Daphnia longiremis* (ship 2), (c) *Brachionus calyciflorus* (ship 3), (d) *B. calyciflorus* (ship 4-DB), (e) *B. calyciflorus* (ship 4-FP) and (f) *B. calyciflorus* (ship 5). After 10 days (dotted vertical line) all unhatched eggs in each treatment group were transferred to 0‰ media. Note scale difference for each ordinate. Error bars <0.03 are hidden by graph symbol.

were significantly higher than for the other two treatments (Fig. 1f; $P < 0.001$, Tukey *post hoc* test).

Discussion

To date, investigations of the salinity tolerance of freshwater zooplankton have been limited to measuring direct effects on growth and survival (e.g. Miracle & Serra, 1989; Teschner, 1995; Hall & Burns, 2002), or examining species richness and composition in waterbodies of varying salinity (Frey, 1993; Brain, Fourie & Shiel, 1995). These approaches have not considered diapausing egg stages, probably resulting in an underestimate of the range of salinities a particular taxon can tolerate, particularly in instances where salinity varies temporally. In this study, we have demonstrated that the hatching rate of diapausing eggs is reduced by exposure to saline conditions. The ability of diapausing eggs to tolerate fluctuations in salinity may stem from an evolutionary history in temporary habitats, which generally fluctuate more in their physical and chemical environment than adjacent, permanent ones (Williams, 1998).

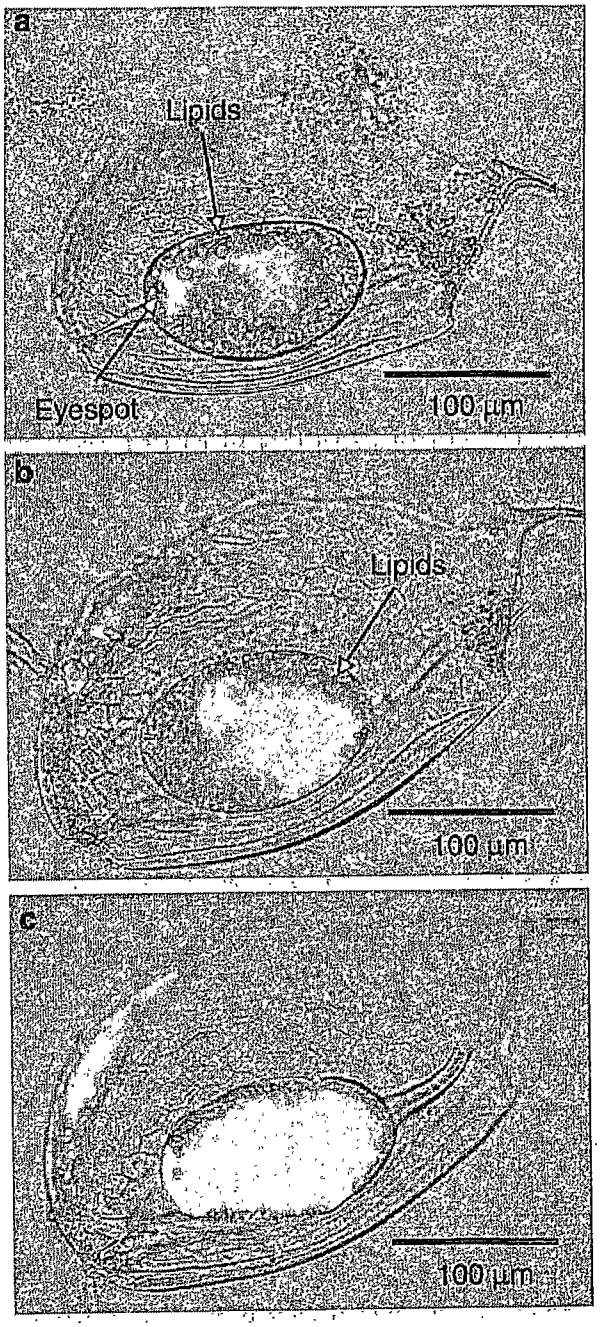

Fig. 2 Condition of diapausing eggs of *Bosmina liederi* after 10 days at each treatment. (a) 8‰, aborted eyed-embryo, (b) 16‰, little differentiation and (c) 32‰, no change. Scale bars (100 µm) are included on each image.

Of the species examined here, diapausing eggs of *B. liederi* appear to exhibit the lowest salinity tolerance with no hatching after exposure to saltwater. *Daphnia longiremis* exhibited a modest degree of salinity tolerance, as a small proportion of diapausing eggs hatched following saltwater exposure. *Brachionus calyciflorus* demonstrated the widest tolerance, with up to 78% eggs hatching after saltwater exposure. Interestingly, *B. calyciflorus* also displayed a wider salinity tolerance as adults than either *B. liederi* or *D. longiremis*. Although typically considered to 'prefer' freshwater, *B. calyciflorus* is frequently observed in brackish waters (e.g. Brain *et al.*, 1995; Park & Marshall, 2000). It is also the only species in these trials that successfully hatched into 8‰ salinity during days 0–10, albeit at rates significantly lower than in freshwater. This finding is consistent with observations of Snell *et al.* (1991), who reported an approximately 40% reduction in the hatching rate of *B. calyciflorus* at 8‰ as compared with 2‰ growth media.

The 'maximum' hatching rates observed at 0‰ ranged from 16–89%. Bailey *et al.* (2003) suggested that pore water salinity might be negatively correlated with hatching success. Our study also found a low hatching rate for eggs recovered from sediments with a pore water salinity of 20‰ or higher (16 and 37%). However, lower pore water salinity (≤10‰) did not guarantee a high hatching rate (i.e. 32% hatch for 4‰), so other factors (such as age of eggs, duration of diapause or hatching cues) are probably involved. In addition, the effects seen in both studies may be impacted by the wide salinity tolerance of *B. calyciflorus* (i.e. 10‰ is only slightly above the natural range for this species, resulting in a high hatching rate at intermediate salinity). Alternatively, pore water salinity measured at the time of collection may not be a good indicator of egg history; eggs may have been retained in ballast sediments for years, experiencing widely varying salinity, of which only the most recent may be reflected by pore water salinity. Nevertheless, while it is possible that the 'maximum' hatching rate (and subsequent reductions in hatching rate) measured in this study may be affected by previous exposures, our results do indicate the effectiveness of ballast water exchange because the sediments carried in transoceanic vessels originate from ports of varying salinity.

Hatching during days 10–20, following transfer to 0‰ medium, occurred mainly in the 16 and 32‰ treatments. Very little hatching occurred during this period in either the 0 or 8‰ treatments, with individuals emerging only from eggs that had not visibly developed during the first 10 days. This suggests that

Table 2 Analysis of variance with repeated measures demonstrating the effect of salinity treatment on the hatching rate of diapausing eggs

Ship	Organism	ANOVA effects [*F* value (d.f.)]		
		Treatment	Time	Time × treatment
1 (FP)	*Bosmina liederi*	32.56 (3,16)***	43.48 (3,48)***	16.98 (9,48)***
2 (DB)	*Daphnia longiremis*	368.97 (3,16)***	353.05 (9,144)***	350.90 (27,144)***
3 (DB)	*Brachionus calyciflorus*	19.78 (3,17)***	41.08 (7,119)***	10.39 (21,119)***
4 (DB)	*Brachionus calyciflorus*	36.60 (3,16)***	47.26 (4,64)***	15.10 (12,64)***
4 (FP)	*Brachionus calyciflorus*	6.15 (3,16)*	14.97 (6,96)***	6.54 (18,96)***
5 (DB)	*Brachionus calyciflorus*	35.25 (3,16)***	322.04 (4,64)***	22.47 (12,64)***

Data were arcsine square root transformed prior to analysis. Significance levels for *F*-values: *$P < 0.05$; ***$P < 0.0001$. Ship tanks are identified by type: FP, forepeak tank; DB, double-bottom tank.

the *Bosmina* and *Daphnia* eye-stage embryos that developed by day 5 at 8‰ were no longer viable. It is possible that a salinity of 8‰ is sufficiently low for the initiation of egg development in freshwater species, but too high for complete development and emergence to occur. In contrast, no development in these genera was apparent in eggs exposed to 32‰ water, thus there remained a 'bank' of viable embryos left to emerge following transfer to freshwater media. Although both *B. liederi* and *D. longiremis* displayed this trend, we cannot explain why only *Daphnia* eggs hatched after exposure to 32‰. However, this trend was also observed for *B. calyciflorus*, with a higher emergence rate after exposure to higher rather than to lower salinity during the latter half of the experiment, particularly for eggs from ship 5. A similar phenomenon was observed by Lutz, Marcus & Chanton (1994), who exposed copepod resting eggs to variable oxygen conditions. They noted that low oxygen concentrations were more detrimental to egg viability than total anoxia because metabolism was completely shut down during anoxia but not under low oxygen conditions. Thus, there appears to be greater interaction between the embryo and the environment under nearly favourable conditions than under extreme conditions. However, it is also possible that the transfer of eggs from 32 to 0‰ acted as a stronger hatching cue than the transfer of eggs from 8 or 16 to 0‰. If this is the case, then subjecting diapause eggs to ballast water of 32‰ may actually promote mass hatching once the eggs are returned to freshwater conditions.

Charmantier & Charmantier-Daures (2001) suggested that rehydrated *Artemia* embryos are protected from high salinity by the cyst envelope that is permeable to water but impermeable to ions. However, salinity and temperature are known to interact in their effects on tolerance, with temperature affecting metabolic rate, ion uptake rate, and membrane permeability (Lee & Bell, 1999). Our experiments explored salinity tolerance at 20 °C, arguably a more challenging environment than exposure at a lower temperature for temperate species. It will be necessary to conduct future trials at a variety of temperatures to deduce the interaction between temperature and salinity on diapausing egg viability.

The variation in hatching rate seen among *B. calyciflorus* trials after exposure (day 10–20) may have resulted from the disparate histories of the populations tested, as indicated by pore water salinity of ballast sediments. Of particular interest was the hatching rate of *B. calyciflorus* collected from ship 5, as 78% eggs hatched successfully after exposure to salinities up to open-ocean levels (i.e. 32‰). In contrast, hatching rates of the other three *B. calyciflorus* populations were significantly reduced after similar exposure (<10%). It is possible that salinity experienced during diapause egg formation may influence the range of salinities eggs can survive while dormant, much like it affects the optimal salinity for the initiation of hatching for the euryhaline rotifer *Brachionus plicatilis* Müller (Gilbert, 1974). We were unable to explore this hypothesis, as the origins of the diapausing eggs in this study are unknown. Future studies using clonal populations from both permanent and temporary habitats may help clarify this possibility.

In general, <10% of *Daphnia* and *Brachionus* eggs hatched after salinity exposure in our experiments. Nevertheless, considering the high egg density in ballast sediments (10^4–10^5 eggs m^{-2} using 1.6 g cm^{-3}

conversion factor for wet sediment), large populations of viable zooplankton eggs may remain after salinity exposure. While it is possible that a longer exposure may have reduced egg viability further, the length of transoceanic crossings will generally not permit longer exposure regimes. As only a small 'seed population' is necessary to establish a cohort of reproductive individuals, and given that the maximum density of diapausing eggs in natural populations ranges between 10^3 and 10^6 eggs m^{-2} (Hairston, 1996), new populations could establish when salinity returns to favourable values (i.e. when the vessel subsequently loads freshwater, or if the eggs get flushed into a freshwater environment). Hall & Burns (2001) suggest that resting eggs of *Boeckella hamata* Brehm, a freshwater copepod, are responsible for the recolonisation of the tidally-influenced Lake Waihola, New Zealand, after seasonal salinisation up to 4.8‰. The average hatching rate for resting eggs of *B. hamata* was only 2.3% under optimal conditions in the laboratory. Therefore, while ballast water exchange may reduce the viability of diapausing eggs by as much as 90% for some taxa, it apparently does not offer complete protection against non-indigenous species entering the Great Lakes by this mechanism. Interestingly, our study indicates that ballast water exchange using brackish water (e.g. 8‰) may have a larger impact on diapausing egg viability than 32‰; however, this effect would have to be weighed against the possibility of introducing live euryhaline species in water of lower salinity, for which ballast water exchange of 32‰ is decidedly more effective (Locke *et al.*, 1993; MacIsaac *et al.*, 2002).

Furthermore, most transoceanic vessels currently trading on the Great Lakes declare 'no ballast on board' status (Colautti *et al.*, 2003), and thus are exempt from ballast water exchange regulations (United States Coast Guard, 1993). MacIsaac *et al.* (2002) suggested that these vessels, collectively, may pose a higher invasion risk than vessels entering the system with saline ballast water owing to the abundance of viable diapausing eggs contained within residual sediments. Our results suggest that the risk posed by diapausing eggs present in sediments of these vessels could be reduced, but not eliminated, by introducing a lens of saltwater into the 'empty' ballast tanks similar to ballast exchange.

Sala *et al.* (2000) suggested that lakes will experience very steep declines in biodiversity this century owing to biotic exchange, land use change and climate change. The salinity of endorheic freshwater habitats is likely to increase during summer months as water inputs decline and evaporation increases (Schindler, 1997, 2001). In addition, coastal lakes and freshwater habitats upstream from tidal estuaries may suffer periodic salinisation as pulsing surges of saltwater seep inland owing to evaporation and anthropogenic diversion of freshwater (Jones, 1994; Hall & Burns, 2003). The persistence of populations through salinity fluctuations by means of diapausing eggs could have profound implications on the extent of biodiversity loss during habitat change. Species incapable of tolerating changing salinity could be replaced by taxa tolerant of brackish or saline conditions (Schindler, 1997); however, this study demonstrates that some populations may be capable of tolerating enhanced fluctuations in habitat salinity, providing a mechanism for enriching biodiversity if the habitat returns to freshwater conditions.

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CANYON FERRY RESERVOIR ZOOPLANKTON POPULATION DYNAMICS

by

CHADWICK LEE MARTIN

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Botany

Approved:

John C. Wright
Chairman, Examining Committee

James M. Pickett
Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

February, 1975

Vita

The author, Chadwick Lee Martin, was born in Billings, Montana on May 28, 1950 to Robert H. and Hattie U. Martin.

He graduated from Billings Senior High School, Billings, Montana in 1968. In September 1968 he entered Montana State University at Bozeman, Montana. He received a Bachelor of Science degree in Fish and Wildlife Management in June 1972. In June 1972 he began graduate studies at Montana State University.

In August 1972 he was married to Christine Lee Neff.

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Evening near the river side -

A scene for a painter,

Throwing on his straw raincoat,

The fisherman returns home.

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ABSTRACT

A limnological study involving four stations on Canyon Ferry Reservoir was conducted during 1971 and 1972. This thesis was primarily concerned with zooplankton population analysis. Part of the study involved comparisons of physical, chemical, and biological aspects with a study conducted on the same reservoir during 1956, 1957, and 1958 by Wright (1958, 1959, 1960, 1961, 1965).

Zooplankton production in 1971 and 1972 was less than that of 1957 and 1958.

A difference in production between the summers of 1971 and 1972 was found. This difference was attributed to a 1971 reservoir draw down and reflooding, which released nutrients from the sediments causing increased phytoplankton production, increased zooplankton production and increased predator (Leptodora kindtii) production. These effects were observed to be most pronounced at the upper end of the reservoir near the nutrient release source.

Overall zooplankton concentrations were similar among the four stations. Zooplankton production was greatest at the upper end of the reservoir, where the greatest phytoplankton production was found.

INTRODUCTION

Canyon Ferry is a main-stem impoundment on the Missouri River between Helena and Townsend, Montana. The dam was constructed during 1949 - 1954 and storage began in March of 1953. The reservoir was filled for the first time during 1955. Since that time the reservoir has become an increasingly popular recreational site and a large number of summer and permanent homes have been built along its shores.

A major objective of the study was to compare 1971 and 1972 production to that of 1957 and 1958 (Wright 1958, 1959, 1960, 1961, 1965; Kaiser, 1971).

Dr. Ron Rada, in partial fulfillment of a Doctor of Philosophy degree, analyzed physical, chemical, and phytoplankton paramaters.

Hall, Cooper, and Warner (1970) said, "Food chain dynamics and the role of zooplankton have been neglected except for the fundamental work done on ponds in Czecheslovakia and Poland". Thus, comparison of various zooplankton related parameters between the summers and among the stations were made.

DESCRIPTION OF THE STUDY AREA

Canyon Ferry Dam is located 24 kilometers east of Helena, Montana (Fig. 1, Rada, 1974). Its major uses are for irrigation, power generation, flood control and recreation. The reservoir receives water from a 41, 191 square kilometer drainage area. The reservoir is approximately 40 kilometers long with a mean width of 3.5 kilometers. At maximum operating pool level (1157 meters m.s.l.) the reservoir has a capacity of 24.01×10^8 meters^3, a surface area of 13,936 hectares, a maximum depth of 49.2 meters and a mean depth of 18.0 meters.

Samples were taken at four stations 8 kilometers apart. Stations one, two, three, and four were approximately 50, 35, 25, and 20 meters deep, respectively (Fig. 1).

Stations one and two became thermally stratified by July 1. Station three rarely became stratified. Station four never became stratified because of its shallow depth. Wind and wave action increased from station one through four as a result of the prevailing westerly winds. Turbidity increased from station one through four (Fig. 2, Rada, 1974).

Figure 1. Map of the study area, Canyon Ferry Reservoir, Montana (Rada, 1974).

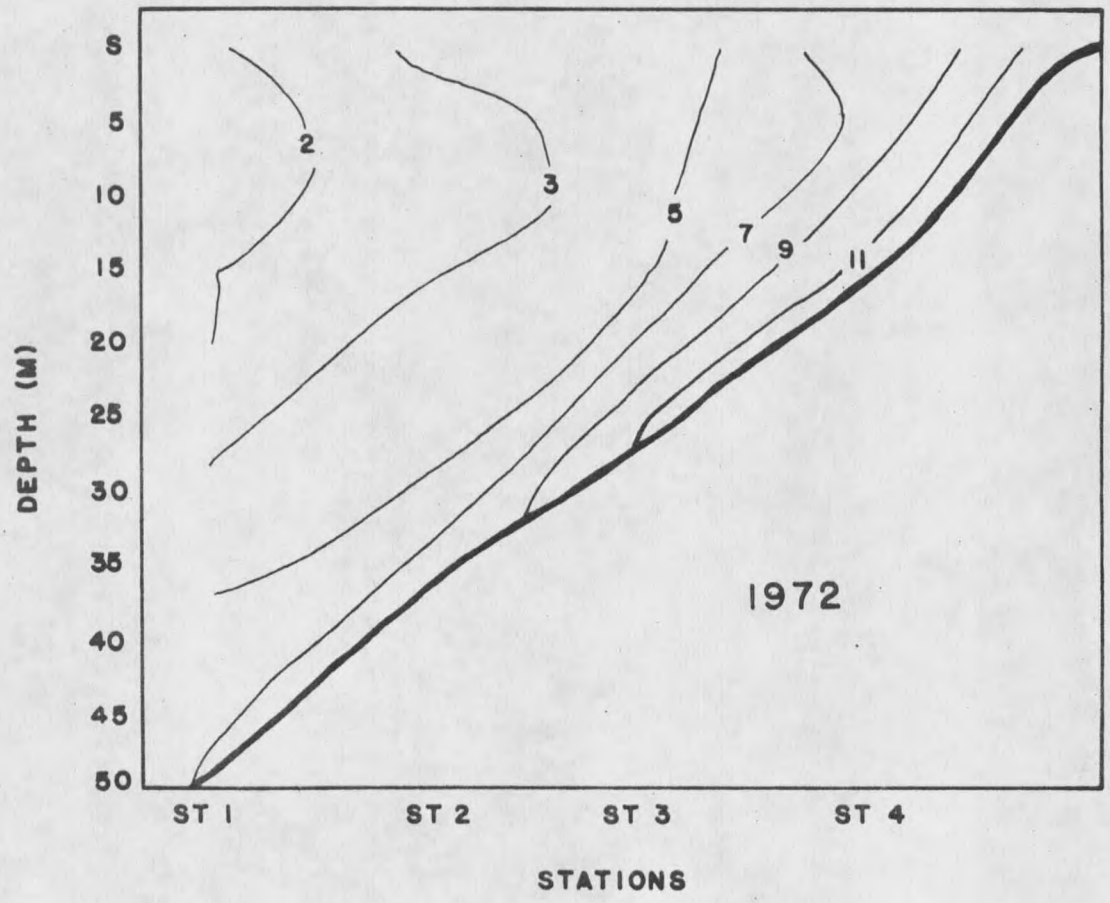

Figure 2. Isoclines of average Jackson Turbidity Units in Canyon Ferry Reservoir during 1972 (Rada, 1974).

MATERIALS AND METHODS

Zooplankton samples were collected during the 38 weekly summer cruises between May and September, 1971 and May and September, 1972. Tows were made in the center of the reservoir at each of the four stations (Fig. 1). Bottom to surface oblique tows were made with a Clarke-Bumpus plankton sampler using a number 20 net and cup (Clarke and Bumpus, 1950). Generally, between 100 and 250 liters were filtered. The number of liters per revolution of the plankton sampler was calculated with the help of Mr. Ted Williams of the Montana State University Civil Engineering Department.

The filtered zooplankters were placed in 100 ml French square bottles, killed with 10 ml 95% ethanol, and then preserved with 10 ml of 95% formalin. Zooplankters were identified to genus and species using a 45 X binocular microscope and Edmondson (1959) as a taxonomic reference. All Leptodora kindtii of the sample were counted. Subsamples were retrieved in a manner recommended by Edmondson (1971). Aliquots of 1 or 2 ml were taken with a Hensen-Stempel pipette and placed in a rotary counting chamber (Ward, 1955). All zooplankters of each aliquot were counted until approximately 100 Daphnia spp. were counted.

Population dynamics of Daphnia spp. were estimated following the methods described by Edmondson (1960), Hall (1964), and Wright (1965) except instantaneous birth rates (b), which were calculated using Caswell's (1972) correction.

The equations for these parameters are:

B = finite birth rate

$= E/DN_o$

where D = egg duration in days

N_o = number of juvenile + adult Daphnia spp. per liter

E = number of eggs per liter,

r = instantaneous growth rate

$= (\ln N_t - \ln N_o)/T$

where T = time interval in days,

b = instantaneous birth rate

$= rB/(e^r-1)$.

Also, instantaneous death rate (d) was calculated as b minus r to give a positive number.

The following counts were made of the Daphnia schodleri and Daphnia galeata mendotae populations: eggs, juveniles, adults and number of eggs per adult. For the copepod species, Cyclops bicuspidatus thomasi and Diaptomus leptopus, counts were made of egg sacs, copepodids, and male and female adults. Nauplii of both species were combined because of inability to determine species differences of nauplii. Only total numbers were counted for Leptodora kindtii, Bosmina sp., Alona sp. and Macrothrix sp. The 1972 data also included size estimation of all zooplankters and number of eggs per egg sac of D. leptopus and C. bicuspidatus.

Relative concentrations of C. bicuspidatus nauplii in 1971 were determined by the following formula:

$$N.CC/(CC+DC) = CN$$

Where CN = estimated number of C. bicuspidatus nauplii

N = total number of nauplii

CC = number of C. bicuspidatus copepodids

DC = number of D. leptopus copepodids.

Relative 1972 C. bicuspidatus nauplii concentrations were determined by the following formula:

$$N.SCC/(SCC+SDC) = CN$$

Where CN = C. bicuspidatus nauplii

SCC = number of stage one + two C. bicuspidatus copepodids

SDC = number of stage one + two D. leptopus copepodids.

Because many eggs were ejected from the carapaces upon preservation the relative number of Daphnia galeata mendotae eggs were determined by the following formula:

$$TN.ND/(ND+NDS) + NDE = TDE$$

Where TDE = total number of D. galeata eggs

NDE = number of enclosed D. galeata eggs

TN = total number of free Daphnia spp. eggs

ND = number of D. galeata juveniles + adults

NDS = number of D. schodleri juveniles + adults.

The remaining free eggs were added to the eggs enclosed in carapaces

of D. schodleri.

Cowell (1967)and Hall, Cooper, and Warner (1970) decided that comparison of stations was more accurate if averages rather than values for individual cruises were used. The author used this method.

Analysis of averages for the second half of the summers often yielded patterns which were not obvious for either the first half or total summer. Therefore, patterns for the first half, second half and total summer were analyzed. Midsummer was defined as July 11.

The probability values (p-values) for the Analysis of Variance (ANØV) program indicate the probability of observing a difference between averages larger than the difference actually observed given that the averages follow a normal distribution.

Nonparametric statistical analysis has some advantages over Analysis of Variance. Nonparametric analysis may be much more meaningful for non-normally distributed averages. Also, nonparametric analysis can often point out the true patterns in a manner much easier to understand than ANØV. A nonparametric test called the sign test was used to compare 1971 averages to 1972 averages and the first half of the summer averages to the second half of the summer averages (Mosteller and Rourke, 1973). This test is based on the statistic of the number of stations for which the 1971 average is larger than the 1972 average. The calculated p-values for this test are the probability of getting a statistic as or more extreme than the one measured, given the

difference in averages is equally likely to be positive or negative. This p-value is based on the number of "exceptions" to the expected pattern.

A special nonparametric statistical analysis was set up by the author to analyze relative increase or decrease of values from station one through four. Basically, possible relative orders of values for the four stations were ordered from increasing from station one through four to decreasing from station one through four (Table 1). Then these were divided into groups (called number of exceptions) indicating the relative increase or decrease.

The p-values for the possibility of getting a value as or more extreme, given actual random occurrence were determined. Table 2 shows the number of combinations of orders, given the number of "exceptions" for each summer. For example, if 1971 averages were 1, 2, 4, 3 and 1972 averages were 1, 2, 3, 4 for stations one through four, this order would be in the 0, 1 group, which has 6 equivalent orders of 0, 1 and 1, 0 "exceptions".

Table 3 shows the number of orders and subsequent probabilities for individual and cumulative number of "exceptions" for the two summers given each order is equally likely to occur. Table 4 gives the cumulative probability values for the nonparametric tests.

Table 1. Possible combinations of four different numbers in groups ranging from increasing to decreasing.

	Number of Exceptions (Relative Group)						
	0	1	2	3	4	5	6
Ordered Values	1	1 2 1	1 2 3 2	1 1 3 3 2 2 4 4	3 3 2 4	3 4 4	4
	2	2 1 3	3 1 1 3	4 4 2 1 3 4 1 1	2 4 4 2	4 2 3	3
	3	4 3 2	4 4 2 1	2 3 1 4 4 1 2 3	4 1 3 1	2 3 1	2
	4	3 4 4	2 3 4 4	3 2 4 2 1 3 3 2	1 2 1 3	1 1 2	1

Table 2. The number of possible ordered values per two groups of "exceptions".

Groups of Exceptions Including Reverse																											
Order	0	0	1	2	2	2	3	3	3	3	4	4	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6
	0	1	1	0	1	2	0	1	2	3	0	1	2	3	4	0	1	2	3	4	5	0	1	2	3	4	5	6
Number of Ordered Values	1	6	9	8	24	16	16	48	64	64	8	24	32	64	16	6	18	24	48	24	9	2	6	8	16	8	6	1

Table 3. The possible number of ordered values per total number of exceptions with corresponding individual and cumulative probabilities, assuming each order is equally likely to occur.

Total Number of Exceptions	Number of Ordered Values	Individual probabilities	Cumulative probabilities
0	1 = 1	.0017	.0017
1	6 = 6	.0104	.0121
2	9+8 = 17	.0295	.0417
3	24+16 = 40	.0694	.1111
4	16+48+8 = 72	.1250	.2361
5	64+24+6 = 94	.1632	.3993
6	64+32+18+2=116	.2014	.6007
7	64+24+6 = 94	.1632	.7639
8	16+48+8 = 72	.1250	.8889
9	24+16 = 40	.0694	.9583
10	9+8 = 17	.0295	.9878
11	6 = 6	.0104	.9982
12	1 = 1	.0017	.9999

Table 4. Cumulative probability values for the nonparametric tests.

Test	Number of Exceptions	Cumulative Probability Value	Test	Number of Exceptions	Cumulative Probability Value
Comparison	0	.062		0	.0017
of 1971	1	.312		1	.0121
and 1972	2	.688		2	.0417
	3	.938		3	.1112
	4	1.000	Analysis	4	.2361
Comparison	0	.004	of the	5	.3993
of the	1	.035	Four	6	.6007
first half	2	.145	Stations	7	.7639
of the	3	.363		8	.8889
summers	4	.637		9	.9583
to the	5	.855		10	.9878
second	6	.965		11	.9982
half of the	7	.996		12	1.0000
summers	8	1.000			

RESULTS AND DISCUSSION

The major zooplankton taxa, excluding rotifers, encountered during the study were Daphnia schodleri Sars, Daphnia galeata mendotae Birge, Cyclops bicuspidatus thomasi S. A. Forbes, Diaptomus leptopus S. A. Forbes, and Leptodora kindtii Focke. These were the major species found during 1957 and 1958 (Wright 1965). As found by Wright (1965), Daphnia schodleri was Canyon Ferry's major zooplankton producer. Bozmina sp., Macrothrix sp., and Alona sp. were rarely found.

During June of 1971 the water level of Canyon Ferry was lowered to an elevation below the lowest water level of 1972 (Fig. 3). The 1971 water level was then raised to a level comparable to that of 1972. Thus, during 1971 more reservoir bottom was exposed and then reflooded than during 1972. Apparently, dissolved nutrients, algal populations, and zooplankton populations were affected differently in 1971 than 1972. The effects of the 1971 drawdown on the zooplankton populations were most prominent during the second half of the summer, after July 11.

DAPHNIA SCHODLERI

Daphnia schodleri was the dominant Canyon Ferry zooplankton species. The 1971 and 1972 summer populations were bimodal in appearance (Fig. 4). Stations one and two showed this pattern more clearly than stations three and four. The minimum between the two peaks occurred in early July for both summers.