

AQUATIC ECOSYSTEMS IN SEMI-ARID REGIONS: IMPLICATIONS FOR RESOURCE MANAGEMENT. 1992.  
R.D. Robarts and M.L. Bothwell (Eds.). N.H.R.I. Symposium Series 7, Environment Canada, Saskatoon.

## IS THE ABSENCE OF ARTEMIA DETERMINED BY THE PRESENCE OF PREDATORS OR BY LOWER SALINITY IN SOME SALINE WATERS?

U. Theodore Hammer

Department of Biology, University of Saskatchewan Saskatoon, Canada S7N 0W0

and Stuart H. Hurlbert

Department of Biology, San Diego State University, San Diego, CA, U.S.A. 92182-0057

### ABSTRACT

Brine shrimp (*Artemia*) are not normally found in lakes with less than 50 g.L<sup>-1</sup> salt (Salton Sea, 40 g.L<sup>-1</sup>; Manito Lake, 29 g.L<sup>-1</sup>) and are absent from some hypersaline lakes (Big Quill Lake, 84 g.L<sup>-1</sup>). Brine shrimp were grown from eggs in filtered water and from mating pairs in waters from the Salton Sea (40, 80 g.L<sup>-1</sup>) and Big Quill Lake (48, 84 g.L<sup>-1</sup>). Mating pairs also produced young in a 25% dilution (28 g.L<sup>-1</sup>) of Little Manitou Lake water (111 g.L<sup>-1</sup>) and Manito Lake water (29 g.L<sup>-1</sup>). Survival was normal at salinities of 38 g.L<sup>-1</sup> or higher but at lower salinities adults died prematurely and young grew very slowly. Water chemistry probably prevents brine shrimp colonization of lower salinity lakes.

In lakes with salinities of 40 g.L<sup>-1</sup> or more, natural predators probably prevent the establishment of *Artemia*. Direct observations of invertebrates capturing and feeding on brine shrimp were made. *Cyclops dimorphus*<sup>1</sup> and *Trichocorixa reticulatae*, natural inhabitants of the Salton Sea, are predators of *Artemia* nauplii. Residents of Canadian saline lakes, *Diaptomus connexus*, *D. nevadensis* and *Cyclops thomasi*, are excellent predators of brine shrimp nauplii. *Trichocorixa interioris* and *Enallagma clausum* consume young *Artemia* rapidly. In unfiltered Big Quill Lake (48 or 84 g.L<sup>-1</sup>) or Manito Lake (29 g.L<sup>-1</sup>) water containing only *Diaptomus connexus* and *Cletocamptus albuquerquensis*, hatching *Artemia* did not survive, but in filtered Big Quill Lake water brine shrimp developed from eggs to maturity. *C. albuquerquensis* apparently prevented *Artemia* populations from developing but was never seen eating brine shrimp.

### INTRODUCTION

This study was initiated after the junior author speculated on the absence of the brine shrimp, *Artemia salina*, in waters of lower salinities in the Bolivian Altiplano. Hurlbert et al. (1984) stated that the Altiplano lakes contained the calanoid copepod *Boeckella poopoensis* Marsh or *Artemia salina* with the latter occurring only at higher salinities, i.e., above 60 g.L<sup>-1</sup>. Hurlbert et al. (1986) suggested that predation by *B. poopoensis* probably excludes *Artemia* from lower salinity waters which the former can tolerate. Croghan (1958b) found that *Artemia* can survive in waters substantially lower in salinity than those where the species is usually found. The senior author has found *Artemia* in Alberta in Gooseberry Lake at 33 g.L<sup>-1</sup> and in Ribstone Lake (35 g.L<sup>-1</sup>) and in British Columbia in Goodenough Lake at 38 g.L<sup>-1</sup> but these are unusual occurrences. In the three western Canadian provinces brine shrimp are usually present in lakes with salinities greater than 50 g.L<sup>-1</sup>, i.e., hypersaline Saskatchewan lakes such as Big Quill Lake ranging in salinity from 40 to 85 g.L<sup>-1</sup> do not have brine shrimp. Mesosaline lakes (20-50 g.L<sup>-1</sup> salt), such as some on the Bolivian Altiplano or the Salton Sea in California, also have no brine shrimp.

A preliminary study by the senior author showed that Big Quill Lake (Saskatchewan) water filtered through 76 µm mesh netting supported brine shrimp from hatching to

<sup>1</sup> *Cyclops dimorphus* Kiefer is a synonym for *Apocyclops dengizicus* (Lepeschkin).

State Water Resources Control Board

Hearing Name IID Transfer - Phase 2

Exhibit: 4

For Ident: \_\_\_\_\_ In Evidence: \_\_\_\_\_

maturity. In unfiltered Big Quill Lake water containing *Diaptomus* (*Leptodiaptomus*) *connexus* Light and *Cletocamptus albuquerqueensis* (Herrick), there were never any *Artemia*.

Scudder (1966) reared *Cenocorixa bifida hungerfordi* Lansbury and *C. expleta* Uhler on *Artemia* adults and subadults. *Trichocorixa verticalis interiores* Sailer is a common corixid in Canadian saline lakes but occurs in very low numbers in lakes with brine shrimp. The Salton Sea contains the corixid *Trichocorixa reticulata* (Guerin-Meneville).

Experiments were designed to determine which invertebrates present in some North American saline waters were potential predators of *Artemia*. The rate of predation was also investigated. The related problem of salinity tolerance by *Artemia* as determined by reproductive success was explored.

The accepted practice is that the North American species of brine shrimp is now referred to as *Artemia franciscana* Kellogg, 1906 (Belk and Bowen 1990; Browne and Bowen 1991). We will simply use *Artemia* since the species of the source material may be questionable.

Each population of shrimps was grown in different dilutions of the source water so the salinity varied but the relative ionic composition was maintained. Each population of shrimps was tested with predators native to the source water.

#### MATERIALS AND METHODS

Salton Sea water (42 g.L<sup>-1</sup> salinity as measured with a Reichert refractometer) was filtered through 35 µm mesh netting to remove arthropods but leaving some algae, bacteria and protozoans. San Francisco Bay Brand Inc. brine shrimp eggs were used to initiate cultures following recommended procedures of placing eggs in distilled water for an hour before addition to culture media. However, eggs hatched successfully when placed directly in 40-80 g.L<sup>-1</sup> of Salton Sea derived water. Cultures were established in 50 mL vials, 750 mL Erlenmeyer flasks and 3 L battery jars. The culture containers were covered to prevent evaporation. They were illuminated with two 20 watt cool white fluorescent or GrowLux tubes in order to maintain natural algal populations as food for the shrimp. Aeration was not used to agitate the cultures as it was found to be unnecessary.

*Cyclops* (*Microcyclops*) *dimorphus* Kiefer (900-1100 µm) from the Salton Sea were added to cultures containing young brine shrimp. Interaction of brine shrimp nauplii and metanauplii with *Cyclops* was observed continuously for an hour and intermittently for 2-3 hours in a tissue culture slide (1.6 cm diameter, 0.5 cm deep) with a Wild M8 Stereoscope. Numbers of each group of organisms were varied but usually 1 or 2 adult cyclopoids were used with 3 to 25 brine shrimp. The interactions were allowed to continue for 2-3 days and then observed again.

One or two *T. reticulata* (1080-4000 µm) from the Salton Sea were placed in a 5 cm diameter 5 mm deep dish with up to 35 *Artemia* nauplii. For different experiments *Artemia* ranged from newly hatched nauplii averaging 433 µm long to week old shrimp (X = 2318 µm). Observations made were similar to those for *Cyclops-Artemia* interactions. Tissue culture slides were used for smaller corixids.

San Francisco Bay Brand eggs were initially used to produce *Artemia* nauplii as prey for potential predators from Saskatchewan saline lakes. Later, eggs from Chaplin and Little Manitou lakes were used to initiate cultures. These Saskatchewan eggs hatch optimally at 5 g.L<sup>-1</sup> salt (Vanhaecke and Sorgeloos 1983). Therefore these eggs were first

placed in distilled water, then the mix was introduced on the surface of Saskatchewan saline lake water media. The predators used were *Trichocorixa verticalis interiores* Sailer from Big Quill Lake (48 g.L<sup>-1</sup>), Sayer Lake (28 g.L<sup>-1</sup>), and Waldsea (20 g.L<sup>-1</sup>); *Enallagma clausum* Morse (from Waldsea Lake); *Diatomus connexus* from Marito Lake (29 g.L<sup>-1</sup>); *D. (Hesperodiatomus) nevadensis* Light and *Cyclops (Diacyclops) bicuspidatus thomasi* S. A. Forbes from Sayer Lake (28 g.L<sup>-1</sup>); and *Cletocamptus albuquerqueensis* (Herrick) from Big Quill Lake (48 and 85 g.L<sup>-1</sup>). Techniques for observation of predation were similar to those used for Salton Sea predators.

Longer term experiments involving the actions of two Salton Sea predators on brine shrimp (size given as mean values) were established as follows.

Experiments	Media		Artemia		Predator		Duration	
	Replicates	Salinity	Volume	Number	Size	Number	Species	days
1	5	42 g.L <sup>-1</sup>	43mL	125	823 µm	2	<i>C. dimorphus</i>	8
2	3	-	500mL	275	823	2	<i>T. reticulata</i>	5-19
	3		500mL	275	823	2	<i>C. dimorphus</i>	19
3	3	-	500mL	275	823	0		19
	4		2000mL	6000	823	50	<i>C. dimorphus</i>	26-37
4	3	40 g.L	1500mL	1 mating pr		4,12,0	<i>C. dimorphus</i>	11
	3	80 g.L	1000mL	1 mating pr		4,12,0	<i>C. dimorphus</i>	11
5	4	40 g.L	500mL	2000 eggs		10,10,0,0	<i>C. dimorphus</i>	11
	4	80 g.L	300mL	2000 eggs		10,10,0,0	<i>C. dimorphus</i>	11

Effects were periodically observed visually or by taking subsamples, killing the fauna with formalin, and counting them. At the end of the experiment all the pertinent fauna in each container were killed and counted. In Experiment 3 two of the replicates were allowed to evaporate to determine the effect of higher salinity on predator-prey interactions. The effects of two different salinities (40 and 80 g.L<sup>-1</sup>) were further examined in Experiments 4 and 5.

The effects of different salinities and dilutions of Saskatchewan lake waters as well as filtered (76 µm mesh) and unfiltered waters on *Artemia* growth and predation were examined.

The success of *Artemia* in Salton Sea water was determined by long-term experiments to determine growth rates and maturation times starting with eggs or nauplii or mating pairs. The production of eggs, mating, and the production of young were used to determine success in a particular medium with or without the presence of predators. Waters from a variety of Saskatchewan saline lakes were similarly examined. Mating pairs of *Artemia* were obtained from Burke Lake (89 g.L<sup>-1</sup>) and Little Manitou Lake (111 g.L<sup>-1</sup>).

A Beta videotape of Saskatchewan predators feeding on *Artemia* was produced with a Sony Camera Adaptor, a Sony MF Triniton Colour Video Camera, a Sony Video Cassette Recorder, a Philips Colour Monitor, and a Zeiss Stemi SV8 microscope.

## RESULTS

*Artemia* was highly successful in Salton Sea water (42 g.L<sup>-1</sup>) hatching a day after eggs (230 µm diameter) were added, reaching maturity in as little as 17 days, and then successfully reproducing. Newly hatched nauplii were 430 µm long.

Table 1 summarizes the experiments involving predation of young *Artemia* by *Cyclops dimorphus* and *Trichocorixa reticulata* from the Salton Sea. Adult *Cyclops* (1070 µm long)

appeared to ignore or even avoid young brine shrimp for periods of 3-15 minutes after they were placed together, although sometimes no attacks occurred during an hour of observation. Stalking of prey took place after some initial period of relatively random activity. *Artemia* nauplii were usually seized by the posterior end of the abdomen, by the dorsal thorax, or by the second antennae. Frequently a nauplius was released after being partially eaten but was often eaten later. The highest frequency of attacks observed was four attacks on 1140  $\mu\text{m}$  nauplii in 13 minutes. Each was released soon after the attack but all died within 30 minutes. Two other Cyclops ate 11 (930  $\mu\text{m}$ ) nauplii within 4.5 hours. The size of *Artemia* predated ranged from newly hatched to three day-old shrimp averaging 1340  $\mu\text{m}$  (range 1170-1500  $\mu\text{m}$ ). No predation was apparent on brine shrimp exceeding 1500  $\mu\text{m}$  in length.

Table 1. Predation of *Artemia* utilizing water and potential predators (*Cyclops* (*Microcyclops*) *dimorphus* and *Trichocorixa reticulatae*) from the Salton Sea, California.

Predator	#	Size ( $\mu\text{m}$ )	Artemia No.	AVG. size ( $\mu\text{m}$ )	Time to 1st capture	Eating time	Duration	No. eaten
Trichocorixa	1	3,500	35	433	-	4 in 7 min	72 hr	35
	1	3,500	16	1,136	2 min	3 in 7 min	1 hr	15
	1	4,000	5	1,746	-	-	72 hr	5
			2	2,318	-	-	72 hr	2
	2	1,080	3	823	-	-	48 hr	3
			3	1,700+	-	-	48 hr	3
	2	1,260	25	623	-	-	72 hr	25
	1	2,000	30	1,338	-	3 in 10 min	48 hr	30
	1	2,000	150	450	-	-	23 hr	144
	Cyclops	1	1,070	8	1,136	3 min	4 in 13 min	1 hr
1		1,070	5	1,700	-	-	72 hr	0
1		1,070	3	450	18 min	3 in 24 min	44 min	3
2		1,070	13	-	12 min	10 min	48 hr	13
2		1,070	11	927	-	-	4.5 hr	11
2		1,070	5	1,338	Note 1	Note 1	48 hr	1

Note 1: 2 1,700  $\mu\text{m}$  *Artemia* left after 48 hr.

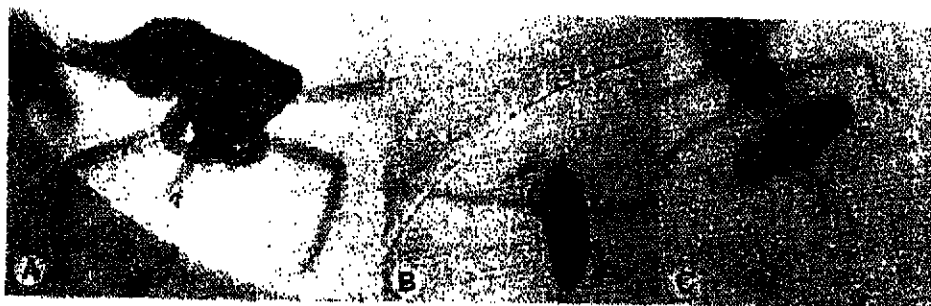


Figure 1. Predation of *Artemia* by (A) *Trichocorixa verticalis interiores*, (B) *Enallagma clausum*, (C) *Diaptomus neoadensis*. Arrows indicate the location of *Artemia* nauplii.

*Trichocorixa*, nymphs (1080  $\mu\text{m}$ ) to an adult 4000  $\mu\text{m}$  long, captured *Artemia* ranging in size from 430 to 2320  $\mu\text{m}$  in length. The time to first capture was as rapid as 2 minutes. One adult corixid ate four 430  $\mu\text{m}$  long nauplii in seven minutes and disposed of 35 nauplii over a 3-day period. It required seven minutes to seize and consume three 3-day old *Artemia* (1140  $\mu\text{m}$ ). When a brine shrimp came in close proximity to the mouth of a corixid, it was seized with the front tarsi and transferred to the mouth (Figure 1). No apparent hunting behaviour was ever observed. Usually the brine shrimp were consumed

before another shrimp was seized. Almost all brine shrimp were eaten over periods of 1-72 hours. Small nymphs (1000-1200  $\mu\text{m}$ ) were relatively unsuccessful at capturing prey in the 5 cm dish compared to the small cavity (1.5 cm diameter) of the tissue culture slide.

Table 2 summarizes the experiments involving some Saskatchewan invertebrates and young *Artemia*. *Trichocorixa interioris* 1300-2000  $\mu\text{m}$  long attacked and ate *Artemia* 470-4260  $\mu\text{m}$  long (Figure 1A). The methods used for capture and behaviour were identical to those of *T. reticulata*. The most rapid capture occurred in 2 minutes when a 800  $\mu\text{m}$  shrimp was devoured in 21 seconds. Most *Artemia* were eaten within 6 hours. *Enallagma clausum* was the largest predator used ranging from 15-25 mm in length. They actively captured any brine shrimp presented from 465-4260  $\mu\text{m}$  long and consumed them quickly (Figure 1B). They also captured *Diaptomus connexus* even though these animals move extremely fast compared to *Artemia*.

Table 2. Predation of *Artemia nauplii* by various Saskatchewan saline lake invertebrates.

Predator	No.	Size ( $\mu\text{m}$ )	<i>Artemia</i> number	Average size ( $\mu\text{m}$ )	Time to 1st capture	Eating time	Duration	No. eaten
<i>Trichocorixa interioris</i>	1	2,000	9	-	41 min	-	15 hr	9
	1	1,300	7	802	2 min	21 sec	3 hr	7
	3	1,426	18	672	16 min	4 min	3 hr	14
	1	1,500	8	465	-	-	6 hr	8
<i>Enallagma clausum</i>	1	15,000	12	802	5 min	21 sec	4 hr	12
	1	25,000	14	465-4,260	-	-	22 hr	14
<i>Diaptomus connexus</i>	1	1,050	10	780	-	-	7 hr	0
	2	1,500	6	450	-	-	6 hr	6
<i>D. nevadensis</i>	2	2,940	11	672	80 min	< 1 min	3 hr	6
<i>Cyclops thomasi</i>	4	1,300	24	465	83 min	1 min	3.5 hr	8

*Diaptomus connexus* 1500  $\mu\text{m}$  long captured and ate newly hatched *Artemia* but smaller forms (1050  $\mu\text{m}$ ) did not attack brine shrimp which exceeded 780  $\mu\text{m}$  in length. *D. nevadensis*, which is twice as large as *D. connexus*, readily attacked (Figure 1C) and ate *Artemia* 670  $\mu\text{m}$  long. Ingestion in one case took less than a minute. When brine shrimp over 1000  $\mu\text{m}$  long were added to cultures containing *D. nevadensis*, the shrimp were quickly eliminated.

The cyclopoid copepod *Cyclops thomasi* which occurs in many Canadian saline lakes (Hammer unpubl.) also attacks and eats small (470  $\mu\text{m}$ ) brine shrimp. The harpacticoid *Cletocamptus albuquerqueensis* also present in more saline lakes was never observed to have any effect on *Artemia nauplii*. However, when *Artemia* eggs were added in distilled water to saline lake water with only this harpacticoid present, no living brine shrimp were ever observed. If *Cletocamptus* was removed and *Artemia* eggs added, young brine shrimp were observed within two days.

Experiment 1 (Table 3) showed little difference between numbers of *Artemia* after 8 days (about 10% survival) even when one vial had no cyclopoids remaining. Mortality was high over the first day (14-48% in 5 replicates and 90% in the 6th). After 5 days the 6th replicate had only 3 *Artemia* left and was the only vial which still had two cyclopoids. The control still had 60 *Artemia* on day 5.

Experiment 2 (Table 3) tested the survival of 125 *Artemia* with either 2 *Trichocorixa* nymphs or 6 *Cyclops* in unfiltered Salton Sea water. More brine shrimp survived when corixids were added than when cyclopoids were the predators. Many *Cyclops* were present in both systems when the experiment was terminated. The filtered controls all had many more *Artemia* than the systems with the predator added.

Table 3. Experiments on *Artemia* (823  $\mu\text{m}$ ) predation by Salton Sea invertebrates. Salton Sea water (42 g.L<sup>-1</sup>) was used as media.

Experiment Replicate	Medium volume	Artemia added	Predators added		End salinity	Duration (days)	Numbers observed		
			<i>Cyclops</i>	<i>Trichocorixa</i>			<i>Artemia</i>	Corixid	<i>Cyclops</i>
1 A	45 mL filtered	125	2	0		6	10		1
B	"	"	2	0		8	12		1
C	"	"	2	0		8	9		1
D	"	"	2	0		8	11		1
E	"	"	2	0		8	11		0
F	"	"	2	0		5	3		2
control	"	"	0	0		5	60		0
2 A	500 mL unfiltered	275	0	2		19	10	1	many
B	"	"	0	2		5	0	1	many
C	"	"	0	2		19	20	0	many
D	"	"	6	0		19	7		many
E	"	"	6	0		19	0		many
F	"	"	6	0		19	3		many
G	500 mL filtered	"	0	0		19	30		
H	"	"	0	0		19	30		
I	"	"	0	0		19	30		
3 A	2 L	5900	50	0	(28 g.L <sup>-1</sup> )	26	124		19
B	Filtered	"	50	0	(78 g.L <sup>-1</sup> )	26	167		0
C	"	"	50	0	(35 g.L <sup>-1</sup> )	37	104		48+
D	"	"	50	0	(125 g.L <sup>-1</sup> )	26	111		0

Experiment 3 (Table 3) tested the interaction of large numbers of *Artemia* and *Cyclops*. In the replicates which retained the original salinity, *Artemia* numbers were reduced to about 2% of the initial populations after 26 days. Much *Cyclops* reproduction occurred in replicate C which was terminated 11 days after replicate A. Two of the four battery jars were allowed to evaporate and thus concentrate the salts. After 26 days the salinity had increased to 78 and 125 g.L<sup>-1</sup> and no *Cyclops* survived, but *Artemia* populations were similar to those in Salton Sea salinities.

Table 4. The interactive effects when *Cyclops* and mating pairs of *Artemia* are added to media of two different salinities derived from the Salton Sea.

Expt Rept	Media		Cyclops Number	Observed Organisms Present	
	Salinity	Volume		after 9 days	after 11 days
A	80 g.L <sup>-1</sup>	1 L	4	1 pr <i>Artemia</i>	2 ♀, 2 ♂, 3 young <i>Artemia</i> , 3 <i>Cyclops</i>
B	"	"	12	1 pr <i>Artemia</i>	
C	"	"	0	1 pr <i>Artemia</i>	2 ♀, 1 ♂, 5 young <i>Artemia</i> , 6 <i>Cyclops</i>
D	40 g.L <sup>-1</sup>	1.5 L	4	1 pr. 480 nauplii	
E	"	"	12	1 pr. 200 nauplii	2 ♀, 1 ♂, 4 young <i>Artemia</i> , 55 nauplii
F	"	"	0	1 pr <i>Artemia</i>	3 adult <i>Cyclops</i> , <i>Cyclops</i> nauplii 16 adult <i>Cyclops</i> , <i>Cyclops</i> nauplii 0 ♀, 2 ♂, 3 <i>Artemia</i> nauplii (490 $\mu\text{m}$ )

In experiment 4 (Table 4) a pair of mating *Artemia* was added to each replicate of two sets of battery jars to test the effects of two salinities and *Cyclops* on brine shrimp reproductive success. After 4 days many nauplii appeared in the lower salinity jars but not in the control! No young brine shrimp were present in the higher salinity jars. After 11 days young *Artemia* were present in the high salinity media and the low salinity control but there were only *Cyclops* in the two low salinity replicates. *Cyclops* were still present in the high salinity replicates.

*Artemia* nauplii were present in every flask of experiment 5 a day after the eggs were added (Figure 2). By the 4th day higher concentrations of *Artemia* were present in the high salinity (80 g.L<sup>-1</sup>) than in the low salinity (40 g.L<sup>-1</sup>) media. After 11 days the lowest concentrations of brine shrimp occurred in the low salinity media. Nevertheless, there was the same variability between the controls and at least one of the replicates in each salinity.

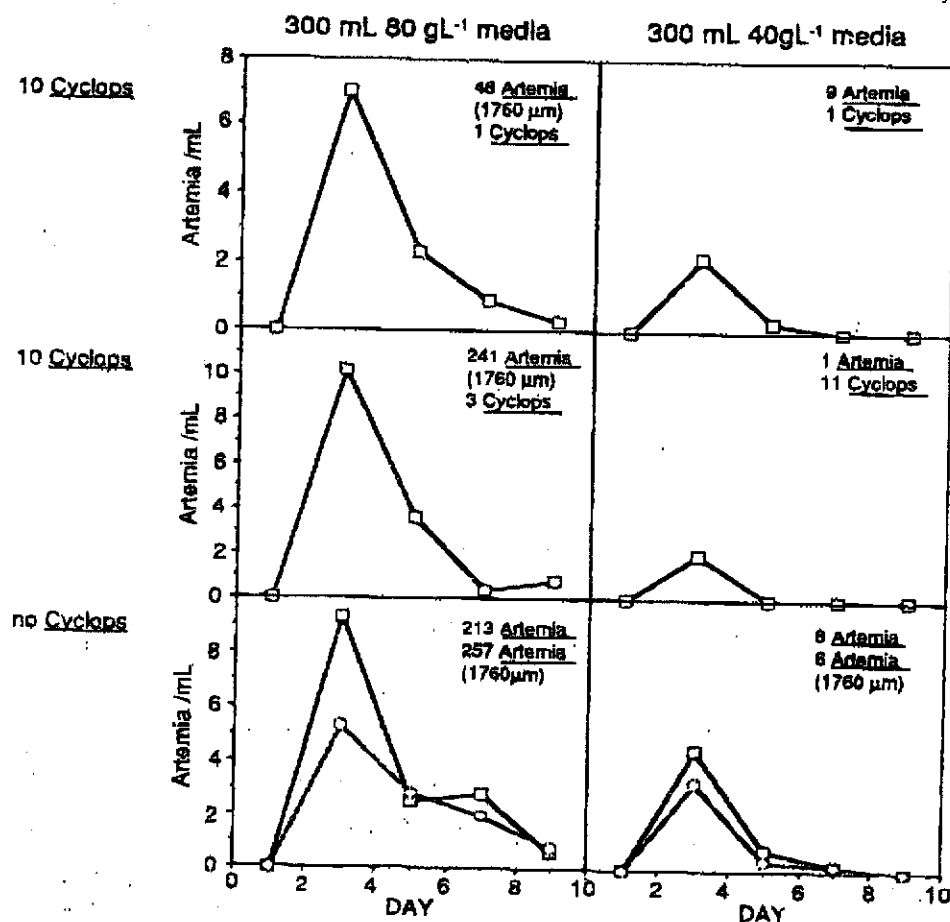


Figure 2. The interactive control of *Artemia* populations by the presence of *Cyclops dimorphus* in two different salinities. Data in each block refer to the numbers above on day 9.

The rate of growth of *Artemia* was determined under different salinities of Salton Sea water. Figure 3 illustrates the results. The brine shrimp grew more slowly at the lowest salinity (38 g.L<sup>-1</sup>) and best at the intermediate salinity (78 g.L<sup>-1</sup>).

Table 5 summarizes the results of experiments relating to salinity tolerance of *Artemia*. The most rapid rate of development from eggs to adulthood took place in Salton Sea water with young produced as early as 20 days. Mated pairs produced young after only 1 day into Big Quill Lake water (84 g.L<sup>-1</sup>) as early as 2 days after introduction in Salton Sea water, and after 6 days in concentrated Salton Sea water and Manito Lake water (29 g.L<sup>-1</sup>).

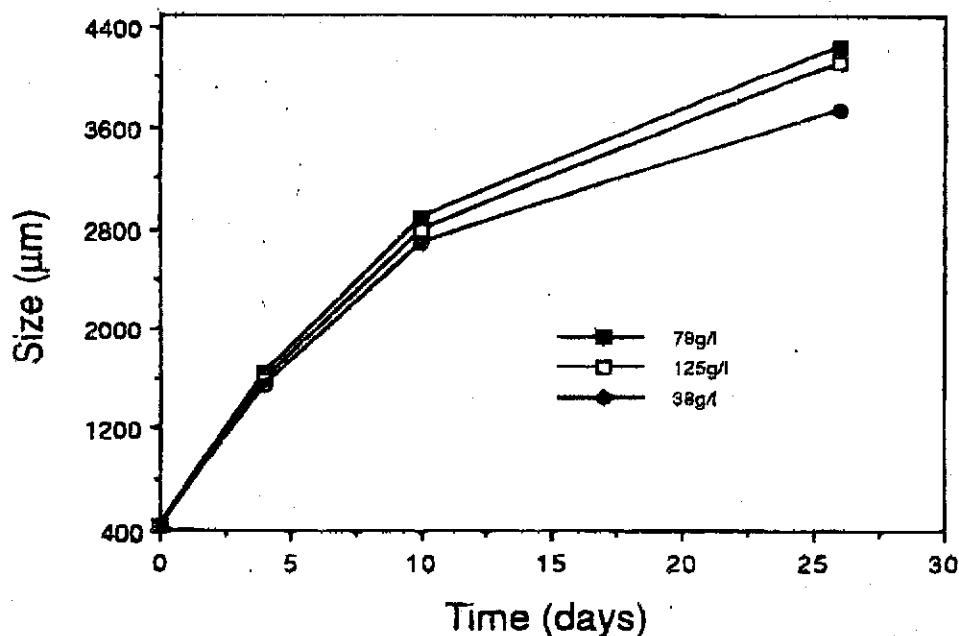


Figure 3. The growth of *Artemia* in three different salinities derived from the Salton Sea water.

Table 5. Salinity tolerance of *Artemia* as expressed by survival, growth and reproductive success in a variety of natural or modified waters.

Lake	Salinity (g.L <sup>-1</sup> )	Artemia inoculum	Time to eggs	Time to mating	Time to first young	Largest Survival		
						Time	Size	(days)
Salton Sea	42	eggs*	7 days	17 days	20 days	-	-	>48
(concentrated)	40	mated pres	present	-	2-11 days	-	-	>11
Big Quill	80	mated pres	present	-	6-9 days	-	-	>11
	48	eggs*	-	-	-	19 days	9642 µm	<29
	84	713 µm	25 days	25 days	-	25 days	9827 µm	>47
	84	611 µm	16 days	21 days	-	14 days	10881 µm	>51
(diluted)	84	mated pres	present	-	1-15 days	-	-	>24
	42	568 µm	-	-	-	15 days	5797 µm	>43
Little Manito	111	mated pres	present	no reproduction	no reproduction	-	-	>30
(diluted)	55.7	mated pres	present	no reproduction	no reproduction	-	-	>30
(diluted)	28	mated pres	present	-	21 days	-	-	>30
Manito	29.4	450 µm	-	-	-	16 days	2077 µm	<23
	29.4	mated pres	present	-	6 days	-	-	<23
Waldsee	20.1	756 µm	-	-	-	11 days	1135 µm	<20
	20.1	mated pres	present	-	-	-	-	<2

\* San Francisco Bay Brand; most results from Little Manito or Burke (88 g.L<sup>-1</sup>)

TABLE 5



Mated pairs only produced young (after 21 days) in the lowest dilution (28 g.L<sup>-1</sup>) of Little Manitou Lake water. Eggs appeared in ovisacs as early as 7 days after eggs were introduced into Salton Sea water and 14 days after young nauplii were placed in Big Quill Lake water. In the most saline Big Quill Lake water the largest shrimp (10881 µm) grew from a young nauplius after only 14 days. Salinities half as great (48-42 g.L<sup>-1</sup>), either natural or diluted Big Quill Lake water, produced shrimp only in the 5000-6000 µm maximum range after 15-19 days. At still lower salinities (Manito 29 g.L<sup>-1</sup> Waldsea 20 g.L<sup>-1</sup>), brine shrimp grew much more slowly. In Waldsea water the size obtained in 11 days was about equal to that in Salton Sea water in three days. Mated pairs introduced into Waldsea water failed to survive for even a day. In Manito Lake water females succumb fairly quickly (2-3 days); males survived 12 to more than 16 days but were not present after 23 days.

## DISCUSSION

### Predation of *Artemia*

There appears to be little specific published information on *Artemia* predation. Scudder (1966) used brine shrimp as food for corixids in culture. It is, therefore, not surprising that the corixids *Trichocorixa reticulata* and *T. interiores* readily capture and consume *Artemia*. What was surprising, however, was that the *Artemia* nauplii had to come in close proximity to the fore-tarsi of the corixid before they were seized and consumed. Larger corixids were capable of predating *Artemia* over 4 mm long. Since these corixids may be abundant (up to 1000 m<sup>-2</sup>) in salinities of 37-53 g.L<sup>-1</sup> (Sayer, Gooseberry, Big Quill) (Hammer et al. 1990), they could have a considerable effect on a newly introduced population of *Artemia*. The availability of the damselfly *Enallagma* which has a broad salinity tolerance permitted us to show that it too is a potential predator of *Artemia*. Even though it has a voracious appetite, its numbers are probably too small (except in 37 g.L<sup>-1</sup> Sayer Lake) to have much influence on a brine shrimp population.

Kristensen (1963) made the general statement that "a brine shrimp population will be exterminated if certain fishes, cyclopoid copepods or rotifers enter the pond". He stated that two fish species (mollies) would eliminate brine shrimp from a pond "in a week or so". Brine shrimp are widely used as tropical fish food and in aquaculture, but salinities in these systems are lower than the ones we are concerned with. These waters, except for the Salton Sea, generally do not have fish populations. The Salton Sea has a number of species derived from the Gulf of California as well as a *Tilapia* species. Some Canadian prairie lakes between 20 and 30 g.L<sup>-1</sup> (e. g., Deadmoose, Waldsea) have littoral populations of two sticklebacks, *Culaea inconstans* (Kirtland) and *Pungitius pungitius* (L.) but Waldsea waters were lethal to *Artemia*.

Fryer (1957) cited Jurine (1820) as being the first to mention that cyclopoids were predators. Fryer's research showed that larger species of cyclopoids (*Macrocyclus*, *Acanthocyclus*, *Cyclops*, *Mesocyclus*) were carnivorous on cyclopoids, calanoids, nauplii, and some cladocerans. Two species of *Macrocyclus* attacked crustaceans in more than 50% of attacks. However, he considered small species such as *Microcyclus* herbivores. In our experiments *C. (Microcyclus) dimorphus* attacked and ate *Artemia* nauplii as large as itself. Kristensen (1963) reported observing cyclopoid predation of *Artemia* adults and nauplii under a microscope but did not identify the predators specifically. McQueen (1969) found that *Cyclops thomasi* (copepodids IV and V and adults) was a very effective predator of its own nauplii, and those of diatoms, eating more than 30% of the standing stock in Marion Lake, B. C. These predatory activities characterized this species when feeding on brine shrimp nauplii in our experiments. Brandl and Fernando (1978) found that *Cyclops vicinus* and *Mesocyclus edax* preyed on 23 and 12 species, respectively, including all species

of rotifers, copepods, and cladocerans coexisting with these predators. They generally preferred rotifers and larval stages of copepods (including their own) and some cladocerans. This type of predation would tend to allow the survival of large bodied species (Kerfoot 1977) and presumably adults the size of *Artemia* should coexist. Kerfoot (1978) stated that predatory copepods are capable of sophisticated behaviour: sensing prey approaching from a distance, judging approach angle and prey speed, and being able to reorient during attack. Cyclopoids in our experiments appeared capable of such behaviour, and could thus capture and handle *Artemia* nauplii successfully.

Although *Cletocamptus albuquerqueensis* was never observed to feed on brine shrimp larvae, the latter never appeared when this harpacticoid was present. It may be that the shrimp larva was eaten just after the shell of the cyst split when it was contained in a delicate transparent membrane sac (Jennings and Whitaker 1941). Rotifers such as *Brachionus plicatilis* Muller were also present but Kristensen (1963) stated that rotifers compete with *Artemia* for food and sometimes eliminate them competitively in some scenarios.

#### Salinity tolerance

The results show that *Artemia* is capable of thriving in some lake waters where they are now absent. These lakes vary from the sodium chloride waters of the Salton Sea to the sodium (magnesium) sulphate waters of Saskatchewan lakes. Successful maturation and reproduction took place at salinities of 38 g.L<sup>-1</sup> or higher. High levels of salinity (>100 g.L<sup>-1</sup>) were not investigated. Hammer (1986) reviewed *Artemia* salinity tolerance. Brine shrimp occur naturally in highly saline Saskatchewan sulphate lakes (Whiteshore 308 g.L<sup>-1</sup>, Muskiki 342 g.L<sup>-1</sup>, Chaplin West 214 g.L<sup>-1</sup>), and in chloride lakes such as Great Salt (North) Lake, U.S.A. (332 g.L<sup>-1</sup>, Post 1977); Laguna Colorado, Bolivia (292 g.L<sup>-1</sup>, Hurlbert et al. 1984); and Lake Elton, U.S.S.R. (260 g.L<sup>-1</sup>, Zhadin and Gerd 1961). Croghan (1958a) found that *Artemia* survived indefinitely in 218 g.L<sup>-1</sup> NaCl solution and were able to adapt to solutions of 0.26% NaCl to crystallizing brine (Croghan 1958b) due to its efficient osmoregulatory abilities.

The success of *Artemia* in lower salinities in the natural environment (Gooseberry, Ribstone, Goodenough) is probably related to water chemistry. These lakes have relatively high pH, from 9.6 to 10.9, and high alkalinities. Goodenough Lake had pH 10.3, 14.8 g.L<sup>-1</sup> CO<sub>3</sub>, 5.6 g.L<sup>-1</sup> HCO<sub>3</sub> with *Artemia* in May; and pH 10.9, 18.5 g.L<sup>-1</sup> CO<sub>3</sub> and 7.3 g.L<sup>-1</sup> HCO<sub>3</sub> but with no *Artemia* in August 1990. Croghan (1958a) found that *Artemia* could not tolerate a M-NaHCO<sub>3</sub> medium; the bicarbonate ion was probably responsible for toxicity. This was discounted by Cole and Brown (1967) who found *Artemia* in naturally high carbonate waters in Arizona, California, and Nebraska lakes. In western Canadian lakes, the carbonates are usually subdominant and probably antagonized by other ions present. However, when Lake Goodenough salinity rose from 38 to 44 g.L<sup>-1</sup> through evaporative concentration, *Artemia* disappeared. In 3-Mile Lake, which has lower pH and alkalinity, *Artemia* survived as the salinity rose from 50 to 109 g.L<sup>-1</sup>. Bowen (1964) found the Mono Lake water (54 g.L<sup>-1</sup>) with pH 10 and high carbonate was lethal to Great Salt Lake brine shrimp, but supported an endemic species of *Artemia*. Such waters were not available for experimental work but would be useful in assessing *Artemia* survivability to highly alkaline waters. In our experiments only Manito Lake water (28 g.L<sup>-1</sup> TDS, pH >9.6, 1.2 g.L<sup>-1</sup> CO<sub>3</sub>, 2.5 g.L<sup>-1</sup> HCO<sub>3</sub>) was available and *Artemia* survival was limited.

Boone and Baas-Becking (1931) found that the osmotic effect was completely overshadowed by the chemical influence of particular salts. Sodium salts are most favourable and Croghan (1958a) stated that only where "sodium salts (principally NaCl) predominate do *Artemia* survive indefinitely". In western Canada this is usually the case

but the dominant salt is often sodium sulphate with little NaCl present. Although magnesium salts are predominant in some Saskatchewan lakes, these are usually lower in salinity; i. e.,  $< 30 \text{ g.L}^{-1}$ . However, some saline lakes which contain brine shrimp (Little Manitou, Aroma) have magnesium present as the highest milliequivalent percentage of the cations. It seems, therefore, that *Artemia* can be successful in a broad range of natural waters but salinity, cation, and anion ranges in some types may limit the species. More specific experimental work is required to establish these ranges.

#### Growth and development.

Observations on growth and development were a by-product of studies of salinity tolerance and predation.

The largest size reached was 10.8 mm after 14 days in Big Quill Lake water ( $84 \text{ g.L}^{-1}$ ). When  $42 \text{ g.L}^{-1}$  Big Quill media was used the maximum size was only 5.8 mm in 15 days; in the Manito Lake medium ( $29 \text{ g.L}^{-1}$ ) newly hatched brine shrimp only reached 2 mm in length after 16 days. Gilchrist (1960) found that a Californian brine shrimp stock reached 8 mm in average length in 23 and 31 days in 35 and  $140 \text{ g.L}^{-1}$  salinities, respectively. Maximum sizes of 10.7 and 8.8 mm were attained after 47 and 44 days, respectively, in the two salinities. The growth rates in Saskatchewan lake salinities below  $50 \text{ g.L}^{-1}$  (Table 5) appear to be slower than this although they compare favourably at higher salinities.

Gilchrist (1960) found that two stocks of *Artemia* became sexually mature in 15-17 days in 35 and  $140 \text{ g.L}^{-1}$  salinity media. In our studies females with egg sacs appeared only 7 days after eggs were added to Salton Sea water ( $42 \text{ g.L}^{-1}$ ), but mating did not occur for another 10 days. Starting with day-old nauplii 14 days elapsed before females were carrying eggs and 21 days before they mated in Big Quill media ( $84 \text{ g.L}^{-1}$ ). Times to maturity appear to be fairly similar in these three studies.

The effect of different salinities on the time required by introduced mating pairs to produce young is difficult to interpret. No comparable data exist. It may be that mating pairs vary considerably in their degree of maturity and this in turn gives variable results for time to first young. Our data are insufficient to warrant specific conclusions.

#### CONCLUSIONS

Predation by resident invertebrates such as corixids and copepods can probably prevent the introduction and establishment of brine shrimp populations in some saline lakes. Brine shrimp eggs are easily transported by birds and wind so there should be no impediment to lake access by *Artemia*. Although Salton Sea waters are amenable to the successful growth of brine shrimp, many western Canadian waters below  $40 \text{ g.L}^{-1}$  are less likely habitats unless they are high in pH and alkalinity. Marginal habitats are probably unsuitable if any predators are also present.

#### ACKNOWLEDGEMENTS

The investigation was supported by NSERC Grant No. 1412 to U.T.H. and a Grant-in-Aid by the SDSU Foundation to S.H.H. Thanks are extended to Karen Hagel for her help with experiments on predation in Saskatchewan media and invertebrates and for making the video film, to Dr. T. Gilmour for providing the equipment for making the video and to Dennis Dyck for drawing the figures and providing photos from the video. Specific thanks go to Sarane Bowen for advice regarding *Artemia* species nomenclature.

## REFERENCES

- BELK, D. and S. T. BOWEN. 1990. *Artemia franciscana* Kellogg, 1906 (Crustacea, Branchiopoda): proposed conservation of the specific name. Bull. Zool. Nomenclature. 47:178-183.
- BOONE, E. and L. G. M. BAAS-BECKING. 1931. Salt effects on eggs and nauplii of *Artemia salina* L. J. Gen. Physiol. 14:753-763
- BOWEN, S. T. 1964. The genetics of *Artemia salina*. IV. Hybridization of wild populations with mutant stocks. Biol. Bull. 126:333-344.
- BRANDL, Z. and C. H. FERNANDO. 1978. Prey selection by the cyclopoid copepods *Mesocyclops edax* and *Cyclops vicinus*. Verh. Internat. Verein. Limnol. 20:2505-2510.
- BROWNE, R. A. and S. T. BOWEN. 1991. Taxonomy and population genetics of *Artemia*, p. 221-235. Chapter 9 In: *Artemia Biology*. CRC Press, Boca Raton, Florida.
- COLE, G. A. and R. J. BROWN. 1967. The chemistry of *Artemia* habitats. Ecology. 48:858-861.
- CROGHAN, P. C. 1958a. The survival of *Artemia salina* (L.) in various media. J. Exp. Biol. 35:213-218.
- 1958b. The osmotic and ionic regulation of *Artemia salina* (L.) J. Exp. Biol. 35:219-233.
- FRYER, G. 1957. The food of some freshwater cyclopoid copepods and its ecological significance. J. Animal Ecol. 26:263-286.
- GILCHRIST, B. M. 1960. Growth and form of the brine shrimp *Artemia salina* (L.) Proc. Zool. Soc. London. 134:221-235.
- HAMMER, U. T. 1986. Saline lake ecosystems of the world. Monographiae Biologicae 59, Dr. W. Junk, Publishers, Dordrecht, The Netherlands. 616 p.
- HAMMER, U. T., J. S. SHEARD, and J. KRANABETTER. 1990. Distribution and abundance of littoral benthic fauna in Canadian prairie saline lakes. Hydrobiologia. 197:173-192.
- HURLBERT, S. H., W. LOAYZA, and T. MORENO. 1986. Fish-flamingo-plankton interactions in the Peruvian Andes. Limnol. Oceanogr. 31:457-468.
- HURLBERT, S. H., M. LOPEZ, and J. O. KEITH. 1984. Wilson's phalarope in the Central Andes and its interaction with the Chilean Flamingo. Revista Chilena de Historia Natural. 57:47-57
- JENNINGS, R. H. and D. M. WHITAKER. 1941. The effect of salinity on the rate of excystment of *Artemia* Biol. Bull. 80:194-201.
- KERFOOT, W. C. 1977. Implications of copepod predation. Limnol. Oceanogr. 22:316-325.
- KERFOOT, W. C. 1978. Combat between predatory copepods and their prey: *Cyclops*, *Epsichura* and *Bosmina*. Limnol. Oceanogr. 23:1089-1102.
- KRISTENSEN, I. 1963. Ecology of salines. p. 20-22. In: Association of island marine laboratories, 4th Mtg., Curacao, Nov. 18-21, 1962.
- MCQUEEN, D. J. 1969. Reduction of zooplankton standing stocks by predaceous *Cyclops bicuspidatus thomasi* in Marion Lake, B.C. J. Fish. Res. Bd. Canada. 26:1605-1618.
- POST, F. J. 1977. The microbial ecology of the Great Salt Lake. Microbial Ecol. 3:143-165.
- SCUDDER, G. G. E. 1966. The immature stages of *Cenocorixa bifida* (Hung.) and *Cenocorixa expleta* (Uhler) (Hemiptera: Corixidae). J. Entomol. Soc. Brit. Columbia. 63:33-40.
- VANHAECKE, P. and P. SORGELOOS. 1983. International study on *Artemia*. XIX. Hatching data for ten commercial sources of brine shrimp cysts and reevaluation of the "hatching efficiency" concept. Aquaculture. 30:43-52.
- ZHADIN, V. I. and S. V. GERD. 1961. Fauna and flora of rivers, lakes and reservoirs of the U.S.S.R. Trans. from Russian by Israel Programme of Sci. Translations Ltd., Jerusalem 1963. 626 p.