Hydrobiologia 267: 203–209, 1993.
S. H. Hurlbert (ed.), Saline Lakes V.

Salinity tolerance of the copepod *Apocyclops dengizicus* (Lepeschkin, 1900), a key food chain organism in the Salton Sea, California

Deborah M. Dexter
Department of Biology, San Diego State University, San Diego, California 92182, USA

Key words: salinity tolerance, Salton Sea, copepod, *Apocyclops dengizicus*

Abstract

The copepod *Apocyclops dengizicus* is a key item in the food chain of the Salton Sea where the salinity is currently 45 g l^{-1}. The salinity of the Salton Sea may reach 90 g l^{-1} within the next 20 years. This study examined the salinity tolerance of this copepod.

Large copepodite and adult *A. dengizicus* were introduced into various salinities with and without acclimation. The 96 h LC_{50} without acclimation was 101 g l^{-1}. Mortality (at 96 h) without acclimation was low at salinities of 90 g l^{-1} or less.

Copepod cultures were maintained, with successful reproduction of at least one new generation, at salinities of from 0.5 to 68 g l^{-1} for at least 120 days. Copepods maintained at higher salinities, up to 79 g l^{-1}, remained alive up to 90 days, but a new generation was not produced. In laboratory studies of larval production and survivorship, few nauplii were released at salinities of 68 g l^{-1} or higher, and none survived to the copepodite stage.

Introduction

The Salton Sea (33° 25′ N, 115° 50′ W), the largest lake in California, was created between 1905–1907 by the accidental diversion of flood water from the Colorado River. The salinity of the Salton Sea has gradually increased from 3.6 in 1907 (Walker, 1961) to 45 g l^{-1} in 1991. Currently almost all freshwater input is wastewater from agricultural uses. Due to U.S. governmental mandated water conservation measures, the lake salinity may reach 90 g l^{-1} by the year 2010 (Black, 1983). This will create a major change in the lake ecosystem.

The current ecosystem is a simple one consisting of several species of phytoplankton, an unknown number of protozoans, a few species of metazoan zooplankters, at least 3 benthic invertebrates of marine origin, a few species of insects, and 7 species of fish. The Salton Sea also supports the greatest diversity of birds of any U.S. National Wildlife Refuge. Present predictions suggest that a key benthic invertebrate, the polychaete *Neanthes succinea* (Frey & Leuchart) and the 3 main sport fishes – orangemouth corvina, (*Cynoscion xanthulus* Jordan & Gilbert), sargo (*Anisotremus davidsoni* (Steindacher)), and croaker (*Bairdiella icistius* (Jordan & Gilbert)) – will become extinct by the time the salinity reaches 50 g l^{-1} (Black, 1983).

What organisms will survive these predicted salinity changes? Will an adequate food chain exist so that the remaining fish species can support large populations of birds?

The rotifer *Brachionus plicatilis* (Müller) and the copepod *Apocyclops dengizicus* (Kiefer) are permanent members of the zooplankton. *Brachionus plicatilis* is widely distributed occurring on 6 continents in salinities from brackish to 250 g l^{-1} (Hammer, 1986). The major components of the

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merozooplankton are fish larvae, larvae of the barnacle *Balanus amphitritite saltonensis* (Darwin) and larvae of the polychaete *N. succinea*. Studies by Kuhl and Oglesby (1979) suggest that reproduction in *N. succinea* will not be successful at salinities exceeding 50 g l^{-1}. *B. amphitrite* has been collected in salinities up to 75 g l^{-1} (Simmons, 1957), but its tolerance to higher salinities has not been examined.

The other major zooplanktonic species is *Apocyclops dengizicus*. This species was described by Keifer (1931) as *Cyclops dimorphus*; but subsequent taxonomic revisions placed the species in the genus *Apocyclops* (Lindberg, 1940). Kiefer (1967) synonymized *A. dimorphus* with *A. dengizicus* (Lepeschkin, 1900). *A. dengizicus* is also known from inland saline lakes in Australia, Egypt, Haiti, India, Iran, Iraq, and Kazakhstan (Kiefer, 1967). While most species of *Apocyclops* occur at salinities less than 30‰, an undescribed Australian species occurs in salinities as high as 152 g l^{-1} (Hammer, 1986; Timms, 1993). *A. dengizicus* lives in Australian lakes with salinities ranging from 4 to 69 g l^{-1} (Timms, 1993). Carpelan (1961) suggested that development from egg to sexual maturity in *A. dengizicus* took between 10–14 days, and that 10–15 generations of copepods were produced each year at the Salton Sea when it had a salinity of 34 g l^{-1}.

This study focuses on several aspects of salinity relationships in *Apocyclops dengizicus*. These include determination of (1) short term salinity tolerance, (2) long term salinity tolerance, (3) the relationship between the size of gravid females and the number of eggs carried, and (4) the effect of salinity on larval survivorship and metamorphosis.

Methods

Water collected at the Salton Sea was transported to San Diego State University and evaporated outdoors until maximum experimental salinities were obtained. The water was filtered through a 120 μm mesh net, then through a 35 μm mesh net, diluted with de-ionized water to obtain the desired salinities, placed in 4 liter plastic containers, and kept aerated until the start of the experiments. In short term salinity tolerance studies pure de-ionized water was used for the lowest salinity (0 g l^{-1}). In long term salinity cultures water of 0.5 g l^{-1} salinity was obtained by mixing pond water filtered through a 35 μm mesh net as a source for nutrients and phytoplankton with de-ionized water. Phytoplankton were present at all salinities and growth was encouraged with the addition of fish food pellets (Pet Co., Koi's Choice) as a nutrient source.

Salinity was determined with a Reichert-Jung refractometer (0–160‰). This refractometer is calibrated for pure NaCl solutions. Using additions of appropriate salts to, and dilutions, of Salton Sea water and gravimetric determinations, C. Hart & M. Gonzalez (pers.comm.) obtained correction factors for converting to true salinity over the range 10–95 g l^{-1} and these have been used in this study.

Short term salinity tolerance

Salton Sea water was evaporated to obtain a maximum salinity of 102 g l^{-1} in Experiment 1 and 136 g l^{-1} in Experiment 2. Three experimental units (glass dishes) were set up at each salinity in each experiment. Salinities in Experiment 1 were: 0, 11, 23, 34, 45, 57, 68, 79, 90 and 102 g l^{-1}; salinities in Experiment 2 were: 45, 79, 90, 102, 113, 124, and 136 g l^{-1}. Glass dishes 10 cm in diameter filled with 200 ml of water were randomly placed (5 rows of 6 dishes in Experiment 1; 3 rows of 7 dishes in Experiment 2) under a light bank providing 12 hour day/12 hour night cycle with a range of 60–70 microeinsteins m^{-2} s^{-1}. Neither food nor aeration were provided during the experiment. Water temperature during the experiment varied between 23 and 25 °C.

Copepods were collected from Bombay Beach, Salton Sea on Oct. 2, 1990 and maintained in water from that site (45 g l^{-1}) for 2 days (Experiment 1) or 10 days (Experiment 2). Adult and late stage copepodites attracted by light to the top of the container were collected for experimentation.

A 2 ml sample of these concentrated copepods was introduced into each experimental unit in random order; the total number of copepods introduced was determined at the end of the experiment, but averaged 65 (Experiment 1) and 113 (Experiment 2) per container.

At 6, 12, 24, 48, 72, and 96 h experimental units were checked under a dissecting microscope for mortality. Organic debris and molts were removed at each observation, and dead copepods were counted and removed. Upon termination of the experiment the samples were fixed, and the surviving copepods counted.

The results from the three highest salinities in both experiments were used to determine the 96 h LC_{50} using probit analysis (Litchfield, 1949), *i.e.* the estimated salinity at which exactly 50% of the original individuals would remain alive after 96 h.

Long term salinity tolerance

Zooplankton tows were taken with a 110 μm net along the Salton Sea shoreline, usually at Red Hill Marina. Within 24 hours of collection, 100 ml of concentrated zooplankton, mostly *A. dengizicus*, were introduced into aerated plastic containers with 1.6 liters of Salton Sea water adjusted to various salinities (methods of obtaining salinities described above). Each container received 300 ml of mixed phytoplankton culture previously maintained at that salinity for at least 2 weeks. In initial cultures copepods were introduced without acclimation and without replication into salinities of 0.5, 1, 6, 11, 17, 23, 28, 34, 40, 45, 51, 62, 68, 73, 79, 85, 90, 96, 102, and 107 g l^{-1}. In subsequent cultures, three replicates were set up at 0.5, 1, 11, 28, 45, 51, 68, and 79 g l^{-1}, and single cultures at 90, 102, and 107 g l^{-1}. All copepods to be maintained in cultures at salinities greater than 57 g l^{-1} were first introduced into a salinity of 57 g l^{-1}. Salinity was increased by approximately 11 g l^{-1} every 3–7 days by salt addition until final salinities were attained.

Salinities were monitored every 15 days and adjusted by addition of deionized water. Water temperature varied between 20 and 25 °C. Phytoplankton and protozoans were present at all salinities and growth was encouraged with the addition of approximately 0.2 grams biweekly of fish food pellets as a nutrient source. At 30, 60, 90, and 120 days, each culture was gently filtered through a 35 μm mesh net, examined under a dissecting microscope for abundance and presence of life history stages, and returned to its respective container.

Gravid females maintained in the long term 2 liter batch cultures at salinities of 1, 11, 28, 45, 57, and 68 g l^{-1} for at least 60 days were collected for examination. Twenty gravid-females selected haphazardly from each salinity were isolated into depression slides, and methyl cellulose was added to slow movement. Right and left egg clutches were separated under dissecting microscope at 60 × and counted at 120 ×. Egg size was not measured. The female was placed under a compound microscope at 100 × and the length of the cephalothorax measured with an ocular micrometer to the nearest 12 μm.

Larval survivorship and metamorphosis

A total of 124 gravid females freshly collected from Red Hill Marina, Salton Sea were isolated within 24 h of collection and directly immersed into small dishes containing 6 ml of Salton Sea water adjusted to salinities of 1, 11, 45, 57, 68 and 79 g l^{-1}. Containers were examined daily to determine the presence of nauplii. Females were removed from the containers as soon as nauplii were observed, as predation upon nauplii by adults is well known. Nauplii were followed until metamorphosis into the copepodite stage or death (0–40 days).

Results

Short term salinity tolerance

After 96 h, mean cumulative mortality was 10% or less for all salinities < 102 g l^{-1} (Fig. 1). It was

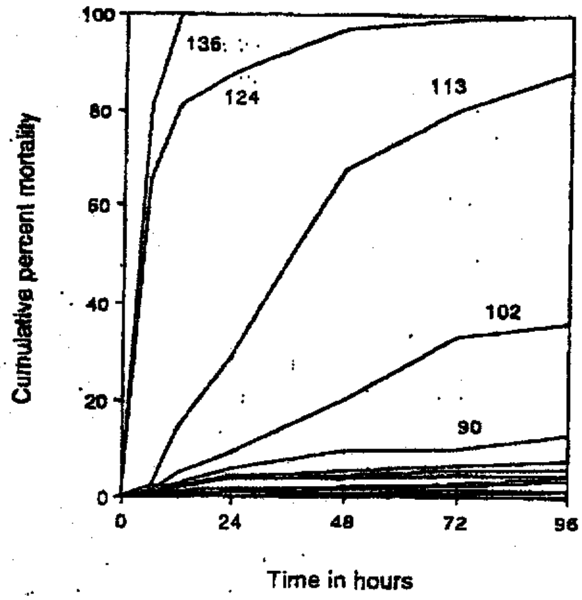

Fig. 1. Cumulative mortality of *Apocyclops dengizicus* through time at various salinities. The lowermost curves are for 79, 0.5, 68, 57, 45, 11, 23, and 34 g l^{-1}, respectively, and are not detectably different from each other.

38.5% at 102 g l^{-1}, 92.4% at 113 g l^{-1}, and 100% at both 124 and 136 g l^{-1}. The 96 h LC_{50} was estimated to be 101 g l^{-1} with a 95% confidence interval of 89–112 g l^{-1}. The mean number of copepods initially present per experimental unit, determined at the end of the experiment, was 63.5 (range 31–105) in Experiment 1 and 112.8 (range 41–256) in Experiment 2.

Long term salinity tolerance

Copepod cultures at salinities from 0.5 to 45 g l^{-1} contained individuals of all life history stages (nauplii, copepodites, males, females, and gravid females) throughout the duration of the 120 day experiment (Table 1). Density of copepods was low in cultures at 0.5 g l^{-1} in which chironomid larvae, introduced as a contaminant with the pond water, were present. When the rotifer *Brachionus plicatilis* was dense in cultures at various salinities, the density of *A. dengizicus* was often low. Otherwise, throughout the 120 days, densities > 100 copepods l^{-1} were maintained at salinities from 1 to 45 g l^{-1}.

Mating was observed at salinities from 1 to 68 g l^{-1}, but gravid females were extremely rare at salinities of 68 g l^{-1}. Cultures survived for 120 days at this salinity, but at very low densities. When adults maintained at 68 g l^{-1} for more than 60 days were returned to 45 g l^{-1}, however, successful reproduction and larval development occurred and within 30 days, all life history stages were present, and overall density was high. At

Table 1. Long term salinity tolerance of *Apocyclops dengizicus*.

Life history stage	Abundance[1] at				Salinity (g l^{-1})
	30 days	60 days	90 days	120 days	
Gravid females	++	++	++	++	0.5, 1, 6, 11, 17, 23, 28, 34, 40, 45, 51
	+	+	+	+	57, 62, 68
	0	0	0	0	73, 79, 85, 90, 96, 102, 107
Nauplii larvae	+++	+++	+++	+++	0.5, 1, 6, 11, 23, 34, 40, 45, 51
	++	+	+	+	57, 62, 68
	0	0	0	0	73, 79, 85, 90, 96, 102, 107
Small copepodites	+++	+++	+++	+++	0.5, 1, 6, 11, 17, 23, 28, 34, 40, 45, 51
	++	+	+	+	57, 62, 68
	+	0	0	0	73, 79, 85, 90, 96, 102, 107
Large copepodites and adults	+++	+++	+++	+++	0.5, 1, 6, 11, 17, 23, 28, 34, 40, 45, 51
	+++	+++	++	+	57, 62, 68, 73, 79
	++	+	0	0	85, 90, 96, 102, 107

[1] Abundance categories are denoted as follows: 0 not seen, + 1–5/container, ++ 6–20/container, +++ > 20/container.

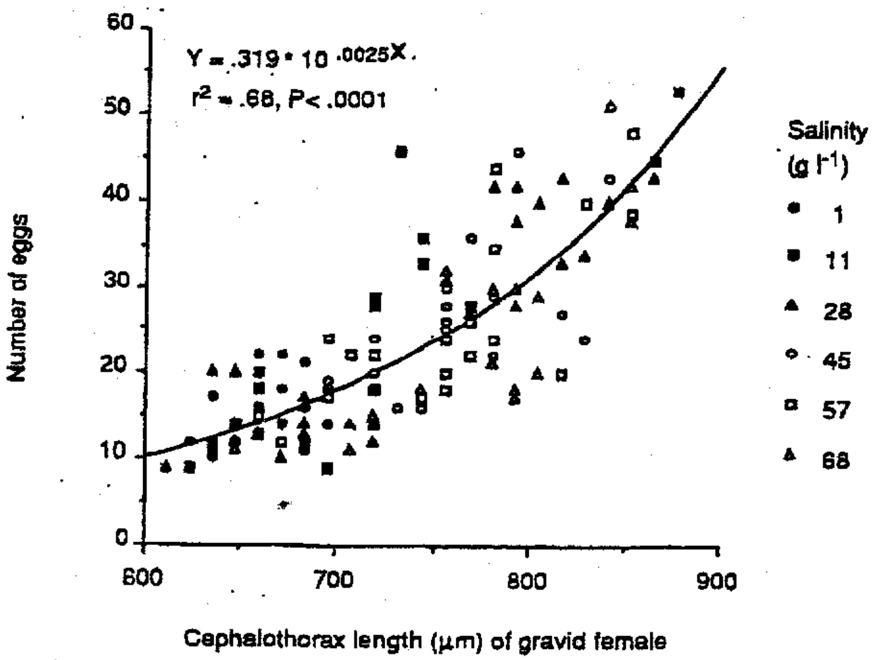

Fig. 2. The relationship between size of gravid *Apocyclops dengizicus* females, number of eggs, and salinity.

salinities ≥ 79 g l^{-1} only large copepodites and adults were present after 30 days, and neither gravid females nor nauplii were seen. Copepods survived salinities up to 79 g l^{-1} for 90 days, and up to 107 g l^{-1} for 60 days, but density rapidly declined with time.

Size of gravid females, number of eggs carried, and relationship to salinity

The number of eggs carried by a female increased with female size at all salinities examined (Fig. 2). Egg number ranged from 9 to 53 and gravid fe-

Table 2. Larval release, survivorship, and metamorphosis, in relation to salinity.

Salinity g l^{-1}	Number of gravid females	Number of nauplii released	Mean days until metamorphosis (range)	Number of copepodites	% of nauplii surviving to copepodite stage
1	27	110	15.2 (5–31)	5	4.5
11	15	104	10.1 (4–18)	63	60.5
45	15	88	7.9 (6–15)	11	16.4
57	18	84	17.8 (6–25)	23	27.4
68	30	68	–	0	0
79	19	1	–	0	0

males ranged from 612–876 μm in cephalothorax length. Salinity did not seem to effect the relationship between female size and number of eggs produced.

Larval survivorship and metamorphosis

There was variability in survivorship of gravid females, nauplii, and copepodites at all salinities (Table 2). Release of nauplii was low at 68 g l^{-1} and only 1 nauplius was released at 79 g l^{-1}. Duration of the nauplius stage ranged from 5–31 days before successful metamorphosis to the copepodite stage. There was no metamorphosis into the copepodite stage at salinities ≥ 68 g l^{-1}.

Discussion

Some of the life history features seen in this study differ from those suggested in earlier studies. Carpelan (1961) reported that *A. dengizicus* was found in the offshore zooplankton in the Salton Sea only during the warmer months of the year (June through December/early January). My studies indicate that it is present all year in the shallow waters along the shoreline, although its density is reduced during in the colder months. Secondly, Carpelan (1961) suggested that generation time was between 10–15 days. In the laboratory experiments *A. dengizicus* took as little as 2 weeks and as long as 2 months to complete the progression from nauplius to sexual maturation and production of a new generation. Thirdly, Johnson (1953) reported a range of 12–16 eggs/sac while during my studies as many as 27 eggs/sac were observed. These differences, the slower developmental rate, higher fecundity, and year round presence, suggest a more flexible life history than originally reported.

Robertson *et al.* (1974) concluded that clutch size in *Cyclops vernalis* varied with age of the female, and that food quality influenced fecundity. I made no attempt to determine clutch order in the experiments on *A. dengizicus*, so perhaps some of the variation in the relationship between size of female and number of eggs is caused by differences in clutch order. Differences in fecundity at the various salinities have not been statistically analyzed, because food quality and quantity were not held constant across salinity levels.

Such variables can confound salinity effects on survival, generation time, and other life history features. Food originated from the Salton Sea at all salinities except at 0.5 and 1 g l^{-1}. Presence and abundance of the rotifer *B. plicatilis* in the cultures was unpredictable and was not correlated with salinity levels. *B. plicatilis* is suitable food for *A. dengizicus* but in cultures where it was very dense, density of *A. dengizicus* was reduced. Perhaps some competition for phytoplankton occurs between these species or perhaps it was the decreased predation by *A. dengizicus* that allow *B. plicatilis* to increase.

Although *A. dengizicus* is able to complete its life cycle at low salinities (0.5 and 1 g l^{-1}) the results suggest poorer performance at these salinities (higher short term mortality – see Fig. 1; lower densities in long term cultures, fewer nauplii surviving to copepodite stage – see Table 2, and small gravid females – see Fig. 2). Greater physiological stress at low salinities is a possible explanation. It is most probable that the experimental conditions were not as good at the lowest salinities. Sources of water (de-ionized and pond) and food (pond phytoplankton) differed from all other cultures where Salton Sea water and phytoplankton were mixed with de-ionized water.

A. dengizicus is quite tolerant to a wide range of salinities. Successful reproduction, larval development, sexual maturation, and production of additional generations occurs easily at salinities from 0.5 to 45 g l^{-1}, and less frequently at salinities up to 68 g l^{-1}. This species has survived short term introduction under laboratory conditions into salinities 2.5 times the salinity in which they were collected. With acclimation *A. dengizicus* adults can live in salinities as high as 107 g l^{-1} for at least 60 days. Completion of the entire life history at salinities above 68 g l^{-1} was not observed in the laboratory studies.

If *A. dengizicus* was introduced into the Salton Sea shortly after its creation, then it survived a

10-fold increase in salinity over a period of 85 years. The predicted increase in salinity at the Salton Sea with subsequent loss of at least 3 of the 7 fish species now present (Black, 1983) will cause a decline in fish predation on *Apocyclops*. Given its short length of life, the occurrence of many generations yearly, and reduced predation it is likely that *A. dengizicus* will remain in the plankton of the Salton Sea for at least a few years.

These initial salinity laboratory experiments on *Apocyclops* show successful acclimation to increased salinity. Whether there is sufficient genetic variability to provide genetic adaptation of this species to even higher salinities is unknown. Natural selection for tolerance to increasing salinity is likely, considering the recent history of the species in the Salton Sea and its short generation time. What is the maximum limit? My data indicate that acclimation allows survival of this species up to salinities of at least 68 g l^{-1}. However, laboratory conditions provide stable physical conditions, constant food, absence of predation, etc. and thus are more optimal for survival than the abiotic and biotic conditions experienced in the natural habitat. Furthermore, as the salinity of the Salton Sea increases, it will likely be colonized by more salt tolerant invertebrates which may prey upon or outcompete *A. dengizicus*. Additional studies are required for accurate predictions of the effect of increasing salinities on this and other Salton Sea species.

Acknowledgements

This research was supported in part by University of California Water Resources Center Grant No. S22625(07) to S. H. Hurlbert. I thank J. Green, U. T. Hammer, D. B. Herbst, S. Twombly, and B. Timms for their positive critical review of this manuscript. The numerous corrections required by Stuart Hurlbert, some accepted by the author only under extreme duress, extended manuscript preparation by at least 6 months, disrupted the serene disposition of the author, and otherwise provided a challenge to maintenance of a long standing friendship.

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