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Oxygen Consumption of Zooplankton as Affected by Laboratory and Field Cadmium Exposures

W. Dean Kettle, Frank deNoyelles, Jr. and Chi-Hsiang Lei
Department of Systematics and Ecology, The University of Kansas, Lawrence, KS 66045

Virtually none of the many studies of the responses of aquatic organisms to heavy metals has involved organism response to heavy metals under natural, whole system exposure. The ability of laboratory studies to simulate and predict actual field conditions and responses remains questionable (WINNER and FARRELL 1976, SPRAGUE 1976, STEPHAN and MOUNT 1973).

The effects of cadmium exposure on zooplankton has been measured in laboratory studies (BIESINGER and CHRISTENSEN 1972, MARSHALL 1978a) and in enclosures placed in the field (MARSHALL 1978b). However, studies involving zooplankton subjected to field exposure of cadmium are lacking.

The objectives of this experiment were to measure oxygen consumption, survivorship, and reproduction of Daphnia pulex and Simocephalus serrulatus in response to low level cadmium exposure, in both laboratory and field situations. This design makes possible the comparisons of 1) laboratory and field exposures, and 2) responses of 2 common freshwater zooplankton species.

MATERIALS AND METHODS

1) Description of Study Site

Field studies were carried out on the Nelson Environmental Study Area at the University of Kansas. The experimental pond (0.04 ha) used for this study was divided into four equal sectors by a permanent concrete partition. Just prior to the experiment the pond was drained completely. It was filled with water from a reservoir (0.4 ha) containing a natural plankton community. The filling procedure allowed the water to rise to a level two feet above the partition, which permitted good mixing, then during the night the water was drawn down to 11 centimeters below the top of the partition. Four completely separated but very similar plankton communities were therefore established in the experimental pond. Each sector had a volume of roughly 60,000 liters with a maximum depth of 1.42 meters. Two sectors to serve as controls and two sectors to receive cadmium were chosen randomly. Treatment sectors received cadmium as $CdCl_2 \cdot 2\frac{1}{2}H_2O$ in

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an amount sufficient to create a 5.0 μg Cd/ℓ concentration. All cadmium concentrations in the laboratory experiments were also created with $CdCl_2 \cdot 2\frac{1}{2}H_2O$.

2) Reproduction and Life Span

All animals used in laboratory and field reproductive studies were young produced by several adult females kept in culture in the laboratory. These cultures were started by isolating adults of each species into aquaria containing filtered (86 μm mesh) water from the large reservoir used to fill the divided pond. The water was changed twice weekly. The S. serrulatus adults were isolated from the experimental pond reservoir while the D. pulex were isolated from a collection taken at a nearby lake. No known incident of cadmium exposure has occurred in either of these habitats. Cultures were started a few weeks before the experiment and reproduced favorably. D. pulex young (12 ± 12 hours old) were 0.74 mm in size and S. serrulatus young (12 ± 12 hours old) were 0.61 mm in size.

a) Laboratory - Each experiment was initiated with 10 newborn animals (12 ± 12 hours old). Each cohort of 10 animals was placed in a 300 ml glass stoppered bottle containing water with a cadmium concentration of 0, 5 or 10 μg/L. All water was prepared by pouring water from the control sector of the pond through an 86 μm net (to remove larger zooplankton but allow edible phytoplankton to pass through) and then adding cadmium. Animals were examined every 2 days, the dead and/or young removed, and the water replaced with a freshly prepared quantity. The cohort was followed until all experimental animals had died.

b) Field - Cohorts of 10 newborn animals (12 ± 12 hours old) were maintained in each sector of the experimental pond. They were maintained in a 300 ml Plexiglass cylinder (153 μm netting was permanently affixed to each end with provisions for easy opening of the cylinder into two sections) which was suspended in the sectors at a depth of one meter. Animals were examined every two days, the dead and/or young were counted and removed. The netting was freed of any attached material and the cylinder flushed by gentle agitation in the pond water at that time. We believed the cylinders provided for good exposure to the respective pond conditions.

3) Oxygen consumption

We measured the effects of cadmium exposure on zooplankton oxygen consumption by examining two types of responses: 1) initial response - the response of zooplankters not previously exposed to cadmium (measured in laboratory and control sector animals) and 2) acclimation (long term) response - the response of zooplankton exposed to cadmium for several days (measured from cadmium sector animals).

Water from a control sector was filtered through a 0.45 μm Millipore filter, aerated for 24 hours at the test temperature, and added to 50 ml stoppered bottles. Cadmium concentrations of 0, 5 or 10 μg/ℓ were then created in the bottles. Immature female zooplankton of a known size class were added to some bottles at each concentration with a complement of bottles receiving no zooplankton. Bottles were placed in an environmental chamber for 24 hours with a 15 hour photoperiod. The amount of dissolved oxygen in bottles was measured with the azide modification of the Winkler technique (using 0.001 N sodium thiosulfate). Sample size for each concentration was six (n = 6). The difference between the oxygen content of bottles with and without zooplankton at each cadmium level was taken as the amount of oxygen consumed by the animals. The amount of oxygen consumed was then converted to a per animal per hour value (μg O_2/animal/hour).

Field animals used in oxygen consumption studies were immature females that were produced by adults that had been held in cylinders in the control and cadmium sectors for 14-15 days. These test animals were separated as young from the adults and kept in cylinders in their respective sectors for two days until testing. The D. pulex test animals were isolated as young on DAY 15 (DAY 0 = cadmium addition), but the S. serrulatus could not be isolated until DAY 20. Because all S. serrulatus (those introduced on DAY 0) in the cadmium sectors were initially killed but were successfully reintroduced on DAY 5. Parent stock for both species of test animals were exposed for 14-15 days, but initiation of this exposure period had to be delayed 5 days for S. serrulatus. Cadmium concentration dropped rapidly in the pond to a very low level by the end of the first week and were similar to control concentrations on DAY 15 and DAY 20.[1] Laboratory animals used for oxygen consumption studies were obtained from the same laboratory culture as previously described.

Significant differences in all experiments were determined by using an analysis of variance, fixed effects model. Mean separation for oxygen consumption studies was accomplished by using the Student-Newman-Keuls (SNK) test. Statements in the text relative to significance always refer to the 0.05 probability level or less.

RESULTS

The oxygen consumption of S. serrulatus (TABLE 1) showed no response at the 5.0 μg level in any of the experiments regardless of exposure history. Respiration remained more or less constant. At 10.0 μg, however, oxygen consumption was lower than that of the 0 μg level. This decrease at 10.0 μg expressed as a mean of all

[1]Cadmium analyses were performed by stable isotope dilution using surface ionization mass spectrometer for isotope ratio measurements.

TABLE 1

Mean Oxygen Consumed (µg/animal/hour) by Simocephalus serrulatus of Various Exposure Histories and Cadmium Concentrations, ± Standard Deviation. Numbers in parentheses are 10 µg/ℓ values expressed as a percent of the control.

TEST	EXPOSURE HISTORY	CONCENTRATION (µg/ℓ) 0	5.0	10.0
1	Laboratory animals[a]	0.2433 ±0.0385	0.2521 ±0.0088	0.1654* (68) ±0.0192
2	Field animals from cadmium sector[b]	0.2033 ±0.0242	0.1934 ±0.0148	0.1798 (88) ±0.0039
3	Field animals from control sector[c]	0.1638 ±0.0429	0.1664 ±0.0473	0.1271 (78) ±0.0593
4	Field animals from cadmium sector[d]	0.3180 ±0.0742	0.3269 ±0.0310	0.2980 (94) ±0.0579

* indicates significance from control at $p < 0.05$ within a test
a animals size 1.28 ± .03 mm incubated at temperature 20°C
b c animals size 1.03 ± .06 mm incubated at temperature 24°C
d animals size 1.28 ± .08 mm incubated at temperature 24°C

the experiments is 18%, with the value of experiment 1 having statistical significance. Field animals from the cadmium sectors appear to have a higher level of respiration than those from the control sectors (compare test 2 vs 3, cadmium sector animal respiration is 20% higher at 0 µg, 15% higher at 5.0 µg, and 20% higher at 10.0 µg). The trend of a lowered level of oxygen consumption at the 10.0 g level is apparent in control and cadmium sector animals.

D. pulex (TABLE 2) from the control sector increased respiration significantly when exposed to cadmium. The oxygen consumption at the 5.0 µg level is nearly twice that of the control. The increase from the 5.0 to 10.0 µg level is not significant, indicating that an additional level elicits no further increase in response. D. pulex from the cadmium sector did not significantly increase oxygen consumption when exposed to additional cadmium levels.

For D. pulex field animals with no further cadmium additions, the cadmium sector animals had a respiration level that was significantly higher than control sector animals. As has been shown (RICHMAN 1958, BUIKEMA 1972), small animals have a higher weight specific level of metabolism, but a lower total metabolism

TABLE 2

Mean Oxygen Consumed (μg/animal/hour) by Daphnia pulex of Differing Exposure Histories and Cadmium Concentrations, ± Standard Deviation.

TEST	EXPOSURE HISTORY	CONCENTRATION (μg/ℓ) 0	5.0	10.0
1	Field animals from control sector[a]	0.1128[†] ±0.0609	0.2020 ±0.0228	0.2224 ±0.0122
2	Field animals from cadmium sector[b]	0.2249 ±0.0256	0.2426 ±0.0507	0.2440 ±0.0372

† this value is significantly smaller ($p < 0.05$) than all other values, of which none are significantly different from each other

a animals size 1.38 ± .07 mm incubated at 24°C

b animals size 1.06 ± .06 mm incubated at 24°C

TABLE 3

Longevity (days) and Reproduction (number young/female/lifetime) of D. pulex and S. serrluatus. Values given are those of animals exposed to 5.0 μg/ℓ cadmium, expressed as a percent of the control animals.

TEST	ORGANISM	EXPOSURE SITUATION	LONGEVITY	REPRODUCTION
1	D. pulex	Laboratory[a]	30*	38*
2		Field[a]	ns	ns
3	S. serratus	Laboratory[b]	5*	0*
4		Laboratory[c]	30*	0*
5		Field[b]	15*	0*

* indicates signficant difference at $p < 0.05$ level

ns indicates non-significance at $p < 0.05$ level

a animals 0.5 ± 0.5 days old, size 0.74 mm

b animals 0.5 ± 0.5 days old, size 0.61 mm

c animals 4 ± 1 days old, 1.2 mm size

than larger animals within the same species. The control animals were slightly larger than the cadmium sector animals. Therefore, when size differences are considered, the difference in rate of oxygen consumption between control and treatment sector animals becomes even more distinct.

The effects of cadmium on reproduction and longevity of D. pulex and S. serrulatus are shown in TABLE 3. D. pulex in the laboratory had a shortened life span (30% of control) and produced fewer young per female (38% of control). D. pulex exposed to cadmium in the field did not respond significantly different from the control sector animals.

S. serrulatus (12 ± 12 hours old) in the laboratory were very sensitive to 5.0 µg of cadmium (life span of less than 3 days). Reproduction was completely inhibited in these animals. Larger sized S. serrulatus were tested (1.2 mm). These animals also had life spans shorter than controls and likewise failed to produce young. In the pond sectors, cadmium exposed S. serrulatus (12 ± 12 hours old) lived less than 3 days and did not reproduce, a highly different response than that of control sector animals.

DISCUSSION

The two cladoceran zooplankton species differed greatly in their responses to cadmium. These variations in response are likely due to physiological differences between the species that allow the animals to handle (transport, excrete, etc.) toxins in a different manner or degree. Regardless of the mechanism producing the differential responses between the species, the potential problem of using toxicological responses of one "representative" zooplankton species to predict the result of toxicant exposure on the entire zooplankton community is illustrated.

SIGMOND (1979) and KLEKOWSKI AND ZVIRGZDS (1971) state that oxygen consumption is a useful measure of sublethal effects because energy processes serve as an indicator of overall physiological state. If one used the response of a respiration rate at the 5.0 µg level to predict the effects of a 5.0 µg cadmium exposure on S. serrulatus, it would be a very poor predictor. No change in S. serrulatus respiration rate would imply no effect on the population. Yet it would be completely eliminated at that level. D. pulex on the other hand exhibited a large response in oxygen consumption, but was not affected reproductively in the field. Clearly the use of respiration rates in response to toxicants can be useful for predicting ecological consequences, but only of limited value, unless integrated with an overall approach that considers long term responses such as longevity and reproductive parameters.

When examining the energy budget of zooplankton (RICHMAN 1958, COMITA 1964) the consequences of an elevated respiration rate, such as exhibited by D. pulex in the treated pond sector, could be less energy for reproduction. With a high respiration rate, relatively more of the assimilated energy will go into maintenance with less available for production. When food availability is high, the organism may tolerate the high respiration level. But, under conditions of food stress, elevated respiration may be expressed as an impaired reproductive rate. D. pulex reproduced in the treated pond sector equal to that of the control, even though a high level of respiration occurred. High food availability in the pond may have accounted for this unimpaired reproductive rate.

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