

Oxygen and the Infectivity of *Romanormis culicivorax*

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Abstract: The effects on infectivity by parasitic *Romanormis culicivorax* resulting from four exposure times to four concentrations of oxygen at three temperatures were studied. Low oxygen (1.8 and 3.1 mg/liter) slowed losses of infectivity. Losses of infectivity were similar under aerobic (6 mg/liter) and microaerobic (0.4 mg/liter) conditions for the first 24 h but thereafter were slower in the microaerobic group. No interactions between temperature and oxygen were found over the ranges tested (15-27 C; 0.4-6.0 mg/liter). **Key Words:** Mermithid nematodes, mosquitoes, *Culex pipiens*, anoxybiosis.

Since *Romanormis culicivorax* Ross and Smith (14) is used increasingly in mosquito management programs, there is an urgent need to know more about the ways in which physical environment affects the infectivity of this mermithid nematode so that it can be used more effectively. Previous studies on density-independent factors have dealt with the effects of temperature (2, 7, 10) and salinity (3, 10) on the infectivity of this nematode. The present study concerns the effects of oxygen concentrations that might be encountered in fresh and polluted waters on infectivity as measured by recovery of the postparasitic stage.

MATERIALS AND METHODS

R. culicivorax was propagated in a hybrid strain of autogenous *Culex pipiens* Linnaeus (12). Preparasitic nematodes were obtained from sand cultures as described previously (2).

A factorial experiment was designed to determine the effects of four exposure times to four oxygen concentrations at three temperatures on the infectivity of *R. culicivorax* for *C. pipiens*. There were four replications at each treatment level. Each exposure period was repeated twice, each time on a different day.

All treatment replications were conducted in 300-ml BOD bottles. Water with the three lowest levels of oxygen was obtained from an oxygen-fractionating column constructed of plexiglass similar to that described by Fry (4). Water with the highest oxygen concentration was obtained by bubbling air through tap water in a 24-liter

polyethylene bucket for 20 min and allowing the water to stand for 12 h. The bottles were filled by standard methods (1). Immediately after each bottle was filled, its oxygen concentration was measured with an oxygen electrode (Beckman Fieldlab oxygen analyzer) which had been zeroed in a stream of nitrogen and calibrated with the Winkler-Azide titration method (Hach Chemical Company). One drop of 1% sodium thiosulfate was then added to the water at the bottom of each bottle to remove any remaining chlorine. The bottles were stoppered and placed at the appropriate temperatures for 12 h, and the oxygen concentration then measured again. Preparasites (150 ± 20) were then injected with a 1-ml plastic syringe with an 18-gauge needle into the water at the bottom of each bottle. The bottles were placed at the three temperatures for specified exposure times. Upon completion of exposure, the oxygen level in each bottle was measured and the contents of the bottle were poured into a 500-ml polystyrene cup containing (30 ± 1) first-instar *C. pipiens* in 50 ml of water. The mosquitoes were fed and reared at 27 C for 10 days as described previously (2). After 10 days, the postparasites and adult mosquitoes in each container were counted.

Included in each experiment were zero-time controls consisting of eight replicates of 150 preparasites and 30 mosquito larvae in 350 ml tap water (oxygen = 5-7 mg/liter) at 27 C.

The exposure periods run on the two different days were averaged. The zero-time controls were used to calculate a postparasite recovery index (RI) for the experiment. This was calculated in a manner similar to Petersen's Susceptibility Index (11). The number of postparasites recovered in each replication was divided by the mean number of postparasites recovered in the

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zero-time-control replicates at that exposure period:

$$RI = \frac{\text{mean number of postparasites recovered per treatment}}{\text{mean number of postparasites in control}} \times 100$$

Results were analyzed by analysis of variance and Duncan's multiple-range test (15).

RESULTS

The average concentration of oxygen present when the preparasites were added to the BOD bottles in all experiments was used for analyzing the data. These concentrations (\pm standard deviation) were 0.4 (\pm 0.15), 1.8 (\pm 0.18), 3.1 (\pm 0.20), and 6.0 (\pm 0.60) mg/liter. Oxygen decreased slightly in some of the bottles during the exposure periods, but never more than 0.3 mg/liter.

For the experiments conducted on the various days, recovery of postparasites in the zero-time controls ranged from 36 to 46% (Table 1) of the preparasites added, which was similar to the variation in infectivity found in previous studies (2, 3).

Analysis of variance showed that differences occurred between the overall means of the four oxygen levels and between the four exposure periods. Also evident was a significant oxygen-exposure interaction. No differences or interactions occurred with respect to the 3 temperatures, so in subsequent comparisons the data for each exposure time at different temperatures were combined.

Nematodes retained their infectivity better at oxygen concentrations of 1.8 and 3.1 mg/liter than at 0.4 and 6.0 mg/liter, a difference which increased with increasing exposure (Table 1). There were no significant differences in infectivity between 1.8 and 3.1 mg/liter. At 12, 24, and 36 h, infectivity was lower at the lowest level of oxygen tested than at the two intermediate levels but greater than at 6.0 mg/liter. These data are significantly different from each other at both 12 and 36 h. The data showed a general though not statistically significant trend toward decreased recovery of nematodes with longer exposure (24 and 36 h).

When the data from Table 1 were plotted as individual curves for each tem-

TABLE 1. Effect of aging at four concentrations of oxygen and subsequent infectivity of *Romanomermis culicivorax* (averaged over 15, 21, and 27 C).^x

Exposure period (h)	Oxygen concentration (mg/liter)	Post-parasites recovered (mean \pm standard deviation)	RI ^y
6	0.4	61 \pm 24	88 cdef ^z
	1.8	71 \pm 16	107 ab
	3.1	77 \pm 18	111 a
	6.0	64 \pm 23	91 bcde
	Control	69 \pm 10	—
12	0.4	53 \pm 22	81 defg
	1.8	66 \pm 15	101 abc
	3.1	71 \pm 17	108 ab
	6.0	40 \pm 18	61 h
	Control	66 \pm 13	—
24	0.4	60 \pm 26	69 gh
	1.8	69 \pm 41	79 efg
	3.1	73 \pm 35	81 defg
	6.0	54 \pm 41	56 h
	Control	54 \pm 22	—
36	0.4	39 \pm 15	72 fgh
	1.8	52 \pm 18	95 abcde
	3.1	54 \pm 17	98 abcd
	6.0	8 \pm 13	14 i
	Control	56 \pm 20	—

^xInfectivity was measured by recovery of postparasites. Each point is the mean of 12 replicates with 150 preparasites/replication.

^yRI = Recovery Index = $\frac{\text{mean number of postparasites recovered/treatment}}{\text{mean number of postparasites in control}} \times 100$

^zValues followed by the same letter are not significantly different from each other at the 5% level of confidence by Duncan's multiple-range test (15).

perature (Fig. 1), general parabolic trends were seen, with much overlapping of the parabolas for exposures at 15 C and 21 C, and a complete separation of the parabolas at 6 and 12 h from those at 24 and 36 h at 27 C. The separation of the curves for the longer from the shorter exposures began to be apparent at 21 C, with complete separation of the longer (24 and 36 h) from the shorter exposure times (6 and 12 h) occurring at 27 C, and proportionally less postparasite recovery at the long exposure times.

DISCUSSION

Of the three temperatures tested, one

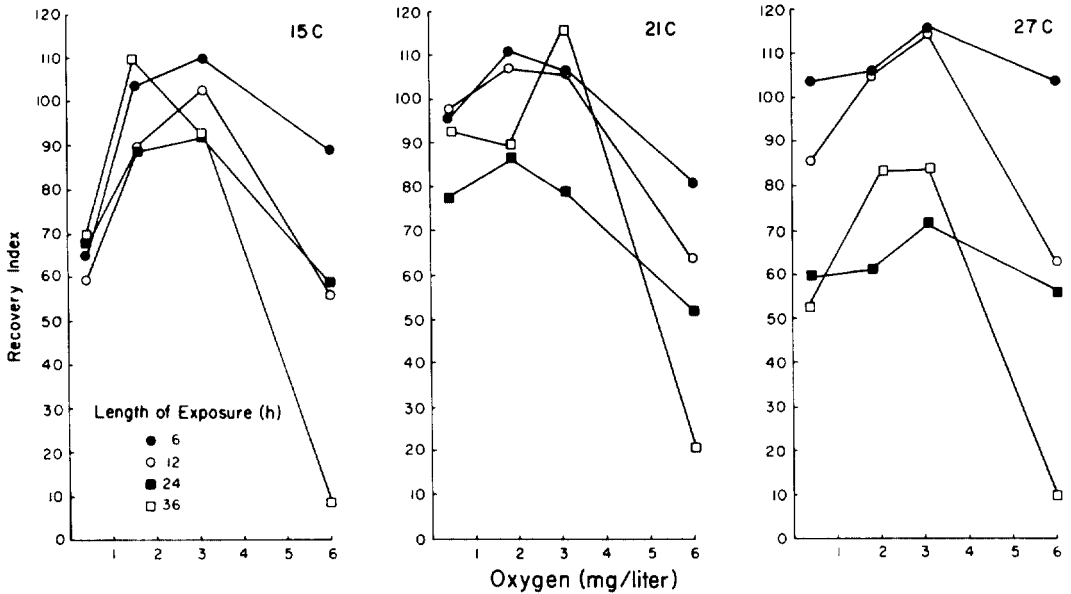


FIG. 1. Recovery index for *Romanomermis culicivorax* after four different exposure periods of preparasites to four levels of oxygen at three temperatures. Each point is the mean of four replicates with 150 preparasites/replication.

(15 C) was below the optimum range for infectivity and development of this nematode, and two (21 and 27 C) were within it (2, 6, 7). These temperatures were chosen as representative of temperatures encountered in field studies with *R. culicivorax*. Nevertheless, no significant interactions of temperature with oxygen were found. The results reported were due mainly to the effects of oxygen, which were independent of temperature effects.

The oxygen levels were chosen to represent the range commonly found in fresh and polluted waters where *R. culicivorax* might be used. The National Technical Advisory Committee (17) has stated that dissolved-oxygen concentrations should be above 5 mg/liter for a diversified warm-water biota. Dissolved oxygen in water bodies with much organic productivity often fluctuates widely in response to biological activity. Streams with large loads of organic material may have oxygen-consuming reactions that deplete oxygen to near anaerobic levels (5).

These experiments have shown that populations of *R. culicivorax* preparasites can survive low levels of oxygen for at least 36 h with most individuals remaining infective. Since most bodies of water do not have prolonged periods of low oxygen, low

levels of oxygen will not seriously hinder use of this nematode and might actually be beneficial by prolonging its infective period. Preparasitic nematodes could be stored, if necessary, for at least 36 h at low levels of oxygen before being applied in the field.

The physiological mechanisms that contribute to survival in environments of low oxygen include energy from fermentative metabolism, reduced metabolism, and ability to use oxygen under very low partial pressures (16). Since *R. culicivorax* retains almost 100% of its infectivity after 36 h of exposure at low (3.1 and 1.8 mg/liter) levels of oxygen, we suggest that the nematode enters a quiescent state of reduced metabolism under these conditions. It is improbable that quiescent *R. culicivorax* would penetrate mosquito larvae, although the actual infective ability of *R. culicivorax* at low oxygen levels has not yet been determined. *C. pipiens* larvae could not be tested under the conditions used in these experiments since they became inactive within 5 min in BOD bottles. Numerous other authors have found that the activity of certain nematode species decreases with lack of aeration and that activity resumed upon return to higher oxygen levels (8, 9, 16).

The infectivity of *R. culicivorax* differed little between microaerobic conditions (0.4

mg O₂/liter) and aerobic conditions for the first 6 h, but thereafter was greater (significant at 12 and 36 h) under microaerobic conditions, suggesting a beneficial effect probably mediated by the quiescent state (as in the intermediate groups). Van Gundy et al. (18) found that populations of 4 species of plant-parasitic nematodes were significantly reduced at 0% oxygen but tolerated low oxygen concentrations for several days.

The two nonparasitic life-cycle stages of *R. culicivorax* differ significantly in habitat. Preparasites characteristically swim close to the surface, where levels of oxygen are likely to be high, whereas postparasites live at the bottom of pools, which are likely to be anaerobic or microaerobic at times. Preliminary biochemical evidence indicated that the postparasitic larval stage was able to function metabolically under anaerobic conditions (13; J. Imbriani, personal communication). Physiological investigations will be required to determine the metabolic adaptations of the preparasitic stages.

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