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Effects of Hypoxia on Egg Development in the Giant Waterbug (Belostoma flumineum)

Miki Furuya

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Effects of hypoxia on egg development in the

giant waterbug (Belostoma flumineum).
(TITLE)

BY

Miki Furuya

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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7/29/92
DATE

31 July 1992
DATE

ABSTRACT

I quantified the oxygen requirements of developing eggs and the adaptive significance of male brooding behavior in the giant waterbug (Belostoma flumineum). Egg pads brooded by males, and egg pads experimentally removed from the dorsa of males and maintained in a metabolic shaker, were tested for hatching success in four oxygen treatments (5%, 8%, 10% and 21%-control) in a closed laboratory system. All of the control male brooded egg pads, and 100% of the eggs/pad, hatched whereas none of the egg pads hatched in the 5% oxygen treatment. Fifteen of 20 (75%) male brooded egg pads in the 10% oxygen treatment hatched 98% of the eggs. Fourteen of 21 (66%) male brooded egg pads hatched an average of only 27% of the eggs/pad in the 8% oxygen treatment; eggs hatched from only the most peripheral three rows of the pad. Most (20 of 26 = 77%) of the male brooded egg pads that did not hatch in the hypoxic treatments were discarded by the male. None of the egg pads in the hypoxic treatments hatched eggs in the maleless experiment; in contrast, 24 of 27 (89%) of the control pads hatched 100% of the eggs/pad. Furthermore, eggs developed more slowly in hypoxic treatments than in controls. These results suggest that the adaptive significance of paternal care in B. flumineum can be attributed, at least in part, to hypoxic conditions commonly found in aquatic habitats inhabited by this species.

ACKNOWLEDGMENTS

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INTRODUCTION

The giant waterbug, Belostoma flumineum (Hemiptera: Belostomatidae) is an interesting species in that females deposit eggs on the backs of males who then brood these eggs (Torre Bueno 1906; Lauck and Menke 1961; Cullen 1969; Smith 1976a). It is one of the few species in the Animal Kingdom that exhibits exclusive post-copulatory paternal care and is often cited as an example of "sex-role reversal" because the reproductive success of females is limited by the availability of male backspace during portions of the breeding season (Kruse 1990).

The evolutionary costs of male brooding include: energetic costs of brooding; increased vulnerability to predators; decreased foraging efficiency; the inability to fly; and loss of polygynous matings (Smith 1976a, 1980; Crowl and Alexander 1989). The primary benefit of male brooding is significant increase in hatching success of offspring (Smith 1976a, 1976b, 1979, 1980) by protecting eggs from desiccation, predators and parasites. This benefit is received only by brooding males because of the effective confidence of paternity adaptations that have evolved in waterbugs (Smith 1979, 1980).

Male B. flumineum brood eggs for 6-13 days depending upon the temperature of the water (Torre Bueno 1906; Kruse 1990). During the brooding period male waterbugs gently stroke the eggs with the hind appendages and position themselves near the air-water interface; the presumptive

functions of these behaviors is to aerate and clean the developing eggs (Cullen 1969; Smith 1976a, 1976b, 1980; Kopelke 1981; Venkatesan 1983; Crowl and Alexander 1989), although this assumption has never been experimentally tested. This study was undertaken in an attempt to quantify oxygen requirements of developing eggs and the adaptive significance of paternal behavior with respect to oxygen supply in B. flumineum.

MATERIALS AND METHODS

General

Waterbugs were collected in several ponds located in Coles Co., IL (USA), during the summer and fall of 1991 and the spring of 1992. Determination of gender was made by examining the genital plate; apical tufts are present in females whereas males lack these structures (Lauck and Menke 1961). Males and females were kept separately in 40 l aquaria that contained a depth of approximately 20 cm of deionized water and plastic vegetation that served as perches. Aquaria were maintained in an environmental chamber (28^{o} C, 14L:10D) and all bugs were fed crickets ad libitum. Following a 10-15 day acclimation period, males and females were placed together in the same aquarium until mating occurred. As soon as males became egg-laden, they, or their newly acquired egg pads, were randomly allocated to treatments described below.

In all experiments, egg-laden males and egg pads were checked twice daily to determine the length of the brooding period. The fate of each egg was (i.e., hatched, did not hatch) determined by microscopic examination of each egg pad. Eggs that hatch can be easily determined by the characteristic rupture in the chorion (Kraus et al. 1989). All bugs and egg pads were used only once so data were statistically independent. Parametric and non-parametric (alpha = 0.05) procedures were followed (Zar 1984).

Experiment 1

This experiment was designed to determine the "hatchability" of eggs attached to a male's back and exposed to oxygen concentrations between 5% and 21% (atmospheric air). The day males became encumbered they were individually placed in 1000 ml Erlenmeyer flasks containing 600 ml of deionized water and a plastic plant. Flasks were sealed with rubber stoppers pierced with stainless steel tubes for delivering the gas of known composition. Four oxygen treatments were used; 5%, 8%, 10% and 21% (control). Composition of the gas mixture was controlled by mixing atmospheric air with pure dry nitrogen. The oxygen tension of the various treatments was continuously monitored with a Puritan-Bennett 252 Airway Gas Monitor, and then supplied via plastic tubing to each flask. Control bugs were supplied with atmospheric air (21%) that was not diluted with nitrogen. All flasks were stored in an environmental chamber (28^{o} C, 14L:10D); the apparatus permitted testing of up to 10 "hypoxic" (5%, 8%, or 10% oxygen) and five "control" (21% oxygen) bugs concurrently.

Experiment 2

This experiment was designed to ascertain the contribution of paternal care on the hatchability of eggs and how the effects of hypoxia are potentially ameliorated by such care. The day males became encumbered, egg pads were carefully removed from the dorsa with a sterilized toothpick

and placed in small petri plates containing a sufficient amount of deionized water to cover the pads. Egg pads were randomly assigned to one of the four oxygen concentrations described in Experiment 1. Petri plates were placed in a sealed plastic container connected to the gas mixing/delivery system described in Experiment 1. Plastic containers were maintained at 28^{o} C and gently agitated in a metabolic shaker. The constant metabolic shaker action and shallow water level in the petri plates was designed to simulate the aeration function of male brooding behavior. Control groups and hypoxic treatments 8% and 10% were repeated using small ($\leq$ 50 eggs) and larger ($\geq$ 60 eggs) egg pads.

Experiment 3

This experiment was designed to investigate the effects of brief periods of hypoxic conditions on the development of waterbug eggs brooded by males. The day males became egg-laden, randomly selected individuals were placed in Erlenmeyer flasks as described in Experiment 1, that received 5% oxygen gas for 24, 48, or 72 hours, after which time, the gas mixture was changed to pure atmospheric air (21% oxygen).

Experiment 4

This experiment was designed to investigate the effects of varying periods of hypoxia on the development of egg pads experimentally removed from male dorsa and placed in petri plates containing a small amount of water and continuously shaken (see Experiment 2). Egg pads were removed and randomly allocated to 5% or 8% oxygen treatments for exposure

periods ranging from 24 to 216 hours (1-9 days). Following the hypoxic treatment, sealed containers containing the petri plates and egg pads received pure atmospheric air (21% oxygen).

RESULTS

Experiment 1 (Male Experiment)

The fate (i.e. hatched at least one egg or failed to hatch) of the egg pads brooded by males in each oxygen treatment is shown in Fig. 1(A). All of the control males hatched eggs whereas none of the males in the 5% treatment successfully hatched eggs. In general, hatching success declined with lowering oxygen tension. Significantly more controls hatched eggs than did any of the hypoxic treatments (control vs 10% - $P = 0.041$; control vs 8% - $P = 0.011$; control vs 5% - $P < 0.001$). Males in the 10% and 8% treatments hatched egg pads in statistically equal proportions ($P = 0.410$), however both treatments hatched more egg pads than the 5% treatment (10% vs 5% - $P < 0.001$; 8% vs 5% - $P < 0.001$).

Males exposed to hypoxic treatments often discarded egg pads before the completion of brooding and this accounts for most (80% - 10% O_2; 71% - 8% O_2; 79 % - 5% O_2) of the "unhatched" egg pads [Fig. 1(B)]. In a few instances, males died or the egg pads became infected by fungus.

An alternative way to access the effect of hypoxia on egg hatchability is to compare the percentage of eggs per pad that hatched in each oxygen concentration. All (100%) of the eggs on every pad in the control group hatched, and nearly all (98%) of the eggs in the 10% treatment hatched [Fig. 1(C)]. In contrast, only 27%, on average, of the eggs in the 8% oxygen treatment hatched. It is interesting to note that

nymphs hatched successfully from only the three most peripheral rows of the egg pads in the 8% oxygen treatment. As expected, the mean percentage hatched differed among the three oxygen treatments (F_{cal} = 766, P < 0.0001); a mean comparison procedure indicated that the hatching percentage did not differ between the control and the 10% treatments (P > 0.05) but both hatched a significantly higher percentage (P < 0.01) of eggs than the 8% oxygen treatment.

Experiment 2 (Maleless Experiment)

The fate of the small and large egg pads in each oxygen treatment is shown in Fig. 2(A & B). None of the eggs hatched in the hypoxic treatments while most of the egg pads (87.5% - small; 91.0% - large pads) in the control hatched. Of the 24 control pads that hatched, 100% of the eggs on every pad hatched.

In order to investigate the differences in hatching success rates between Experiment 1 and 2 at the same oxygen level, the proportion of egg pads that hatched at least a single egg was compared. There was no significant difference between the controls (P = 0.24) or the 5% treatments (P = 1.0); however, a significantly higher proportion of the egg pads brooded by males hatched than their maleless counterparts (8% - P = 0.0009; 10% - P = 0.0002).

Experiment 3

The fate of egg pads brooded by males exposed to 5% oxygen for 24 - 72 hours is shown in Fig. 3. None of the males exposed to 5% oxygen for 72 hours hatched eggs (all

were discarded); whereas two of five males exposed to 5% oxygen for 24 hours, and one of five males exposed to 5% oxygen for 48 hours, hatched eggs. All of the successful males hatched 100% of the eggs. The proportion of males that hatched eggs following 24 and 48 hours exposures to 5% oxygen did not differ significantly ($P = 0.26$) from those exposed to the same hypoxic condition for 72 hours.

Experiment 4

The fate of egg pads exposed to 5% oxygen in the metabolic shaker for varying time periods is shown in Fig. 4. None of egg pads exposed to hypoxic conditions for 72 or more hours hatched; most (83.3%) of the pads that experienced 5% oxygen for 24 hours hatched some eggs and three of six (50%) eggs pads that received 5% oxygen for 48 hours hatched eggs. The proportion of egg pads that hatched at least some eggs was significantly higher for the 24 and 48 hour 5% treatment than $\geq$ 72 hours ($P = 0.015$ and $P = 0.0003$, respectively), however, the 24 and 48 hour proportions did not differ significantly ($P = 0.27$). Relatively few of the eggs on each pad hatched following the 24 and 48 hour exposure to the 5% oxygen treatment (24 hours - 24.5%; 48 hours - 8.6%).

Eggs that received 8% oxygen for varying lengths of time showed similar results to the results just described; all of the four egg pads, and 100% of the eggs/pad, hatched after 24 and 48 hour exposures but none of the nine egg pads hatched a single egg that received this treatment for 72 or

more hours. The proportion of egg pads that hatched from the 24 and 48 hour exposures was significantly greater ($P = 0.001$) than those exposed for periods $\geq$ 72 hours. Six control (21% oxygen) pads were run concurrently with the 5% and 8% exposure experiments and 11 of 12 (91.6%) of the pads hatched 100% of the eggs.

In order to ensure that the number of eggs per pad did not bias the results in Experiments 1-4, the mean number of eggs in each oxygen treatment for each experiment was compared; with a single exception, egg pad sizes did not differ significantly among treatments within experiments (Table 1).

Brooding period lengths for Experiments 1-4 are shown in Table 2. In general, eggs in hypoxic treatments developed more slowly than control eggs. The brooding period for the three oxygen treatments in Experiment 1 differed statistically (Kruskal-Wallis Statistic = 6.99, $P = 0.0302$); control male brooding times were shorter than those in hypoxic treatments. Brooding times did not differ between male controls ($n_1 = 16$) from Experiment 1 and maleless controls ($n_2 = 24$) in Experiments 2 (two-tailed Mann-Whitney U-test, $U_1 = 233.5$, $P = 0.25$). Hypoxic eggs, irrespective of experiment (i.e., $n_1 = 44$, pooled data from all experiments), developed more slowly than control eggs ($n_2 = 40$, two-tailed Mann-Whitney U-test, $U_1 = 528$, $P = 0.0016$).

DISCUSSION

Belostoma flumineum eggs, like other members of the family, are extremely large and ovoid shaped; consequently, they have a small surface area to volume ratio. Therefore, it is not surprising that a high percentage of waterbug eggs detached from the male dorsum do not hatch when placed in static water (Voelker 1968; Cullen 1969; Smith 1976a; Venkatesan 1983) because the eggs lack sufficient surface area for adequate gas exchange. However, it is interesting that, even though waterbugs eggs have a relatively small surface area to volume ratio, they rapidly desiccate when placed in air (Venkatesan and Rao 1980). Baker (1987) describes the chorionic morphology of Belostoma lautarium eggs but the detailed mechanisms of embryonic respiration in belostomatids remains unknown.

The critical oxygen concentration for the survival and development of B. flumineum eggs appears to be between 5% and 8% oxygen. Not a single egg hatched in the 5% oxygen atmosphere whether brooded on a male's back or not. Eggs that experienced relatively brief exposures to hypoxic conditions, in general, did not hatch. At 8% and 10% oxygen levels, the brooding behavior of the male significantly affected the hatchability of eggs. Under comparable hypoxic conditions, most of the brooding male's egg pads hatched at least some eggs while none of the maleless eggs survived to hatching. Of the 16 maleless egg pads exposed to 21% oxygen (controls) maintained in the metabolic shaker, only two

became covered with fungus and failed to hatch; this result was not significantly different from the 100% survival in the 21% oxygen male-brooded controls. The continual water movement over the developing egg pads provided by the metabolic shaker simulated the aeration function of male brooding behavior.

These results strongly suggest that male B. flumineum brooding behavior is contributing to the survival of developing eggs when exposed to hypoxic conditions. Physical laws dictate that oxygen diffuses much more slowly in water (>300,000 times) than air. Given that males and egg pads are situated in identical hypoxic conditions, egg-laden males can expose the eggs directly to the atmosphere, which would greatly facilitate oxygen exchange, and explain why brooded eggs hatched and the non-brooded egg pads covered with a thin layer of water in the metabolic shaker did not. Hatching success from male brooded eggs in 8% oxygen further corroborate this notion; only eggs that had the most surface area exposed (i.e., the three most peripheral rows) hatched. Furthermore, hypoxic treated egg pads developed more slowly than controls. These results are largely congruent with those reported by Milne and Calow (1990) who showed that the developmental rates of non-brooded glossiphoniid leech eggs and the survival and weight of hatchlings were reduced at 25% oxygen levels. Furthermore, slowed development attributable to lack of oxygen/metabolic waste exchange of eggs located within thick egg masses has been demonstrated in gastropods

and polychaetes (Chaffee and Strathmann 1984), marine fishes (Giorgi and Congleton 1984), frogs (Salthe and Mecham 1974) and turtles (Ackerman 1981).

Waterbug eggs imbibe water through the chorionic hydropyle (Madhavan 1974; Venkatesan 1983; Kraus et al. 1989) during development and, therefore, grow in size. Freshly laid B. flumineum eggs are less than 1 mm long but grow to lengths exceeding 3 mm immediately before hatching (unpublished data). Microscopic examination of egg pads that became infected with fungus during/following hypoxic treatments revealed that eggs did not grow appreciably in length suggesting that the eggs died from hypoxia rather than from a fungal infection.

Egg-laden male waterbugs exposed to hypoxic conditions often discarded egg pads unhatched. Previous studies (Smith 1976a; Kraus et al. 1989; Kight and Kruse In Press) have observed male waterbugs discarding egg pads unhatched and suggest that the cessation of paternal care is adaptive under certain conditions as predicted by theory (Williams 1966; Clutton-Brock 1991). It appears that when egg-laden male waterbugs are stressed by hypoxia, they discontinue brooding.

Aquatic habitats that contain B. flumineum undoubtedly experience considerable fluctuations in oxygen concentrations from variations in temperature, atmospheric pressure, photosynthetic rates, organic decomposition and other factors (Wetzel 1983). Smith (1976a) reports that egg-laden B. flumineum males have been collected from ponds where the

oxygen concentration is only 1 ppm. Egg-laden waterbug males probably never encounter hypoxic conditions like those found in this study; in nature they can escape to the water surface and expose themselves and the eggs directly to atmospheric oxygen.

In conclusion, results from this study suggest that the adaptive significance of paternal care in B. flumineum, at least in part, can be attributed to the selective pressure of occasional low oxygen concentrations in aquatic habitats inhabited by this species.

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Table 1. Number of eggs per pad in Experiments 1-4.

	n	Mean	SE	F	P
Experiment 1.					
5%	14	107.2	5.75		
8%	21	99.6	4.56		
10%	20	98.6	5.80	0.92	0.44
control	16	109.4	6.55		
Experiment 2.					
Small egg pads					
5%	10	36.0	3.40		
8%	11	27.5	3.70		
10%	7	38.7	9.00	1.50	0.23
control	14	42.4	5.70		
Large egg pads					
8%	6	62.2	8.65		
10%	6	66.2	13.87	1.98	0.17
control	10	89.4	9.95		

Table 1. (cont.). Number of eggs per pad in Experiments 1-4.

	n	Mean	SE	F	P
Experiment 3.					
24 hours	5	90.4	10.57		
48 hours	5	93.0	12.12	0.02	0.98
72 hours	5	90.6	10.03		
Experiment 4.					
5%					
24 hours	6	102.3	11.56		
48 hours	6	103.5	11.95	0.59	0.56
≥ 72 hours	15	90.7	7.60		
8%					
24-48 hours	4	67.3	11.05		
≥ 72 hours	9	93.1	6.35	5.84	0.03

Table 2. Brooding period length (days) in Experiments 1-4. Brooding period = time from oviposition completion to cessation of nymph emergence.

	Exp. 1 (Male Brooded)			Exp. 2 (Maleless)	Exp. 3 (Male brooded) 24-48 hrs	Exp. 4 (Maleless) 24-48 hrs
	Control	10%	8%	Control	5%	5% & 8%
N	16	15	14	24	3	12
$\bar{X}$	7.2	7.4	7.9	7.6	8	8.83
SD	0.66	0.63	0.62	0.92	0	0.57
Range	6-8	6-8	7-9	7-10	8-8	8-10

Fig. 1A. Results of male brooded egg pads in varying oxygen levels in Experiment 1.

Solid bars = egg pads that hatched at least a single egg; striped bars = unhatched egg pads (which includes those discarded, fungus growth and males that died).

A.

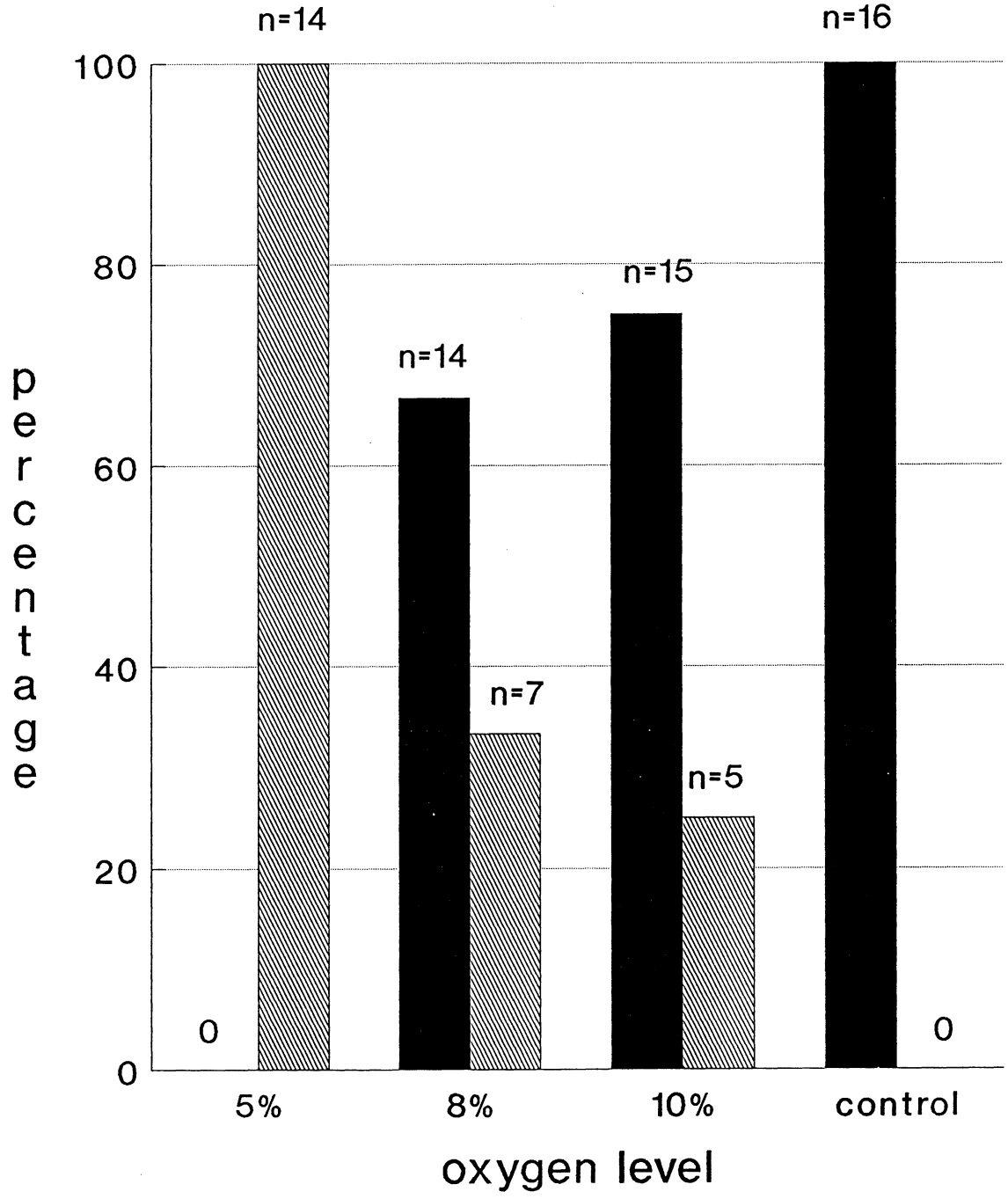

n=14
n=16
100
80
n=15
n=14
60
40
n=7
n=5
20
0
0
0
percentage
5%
8%
10%
control
oxygen level

Fig. 1B. Results of unhatched male brooded egg pads in Experiment 1.

Solid bars = egg pads discarded;

striped bars = egg pads infected by fungus;

open bars = males died.

B.

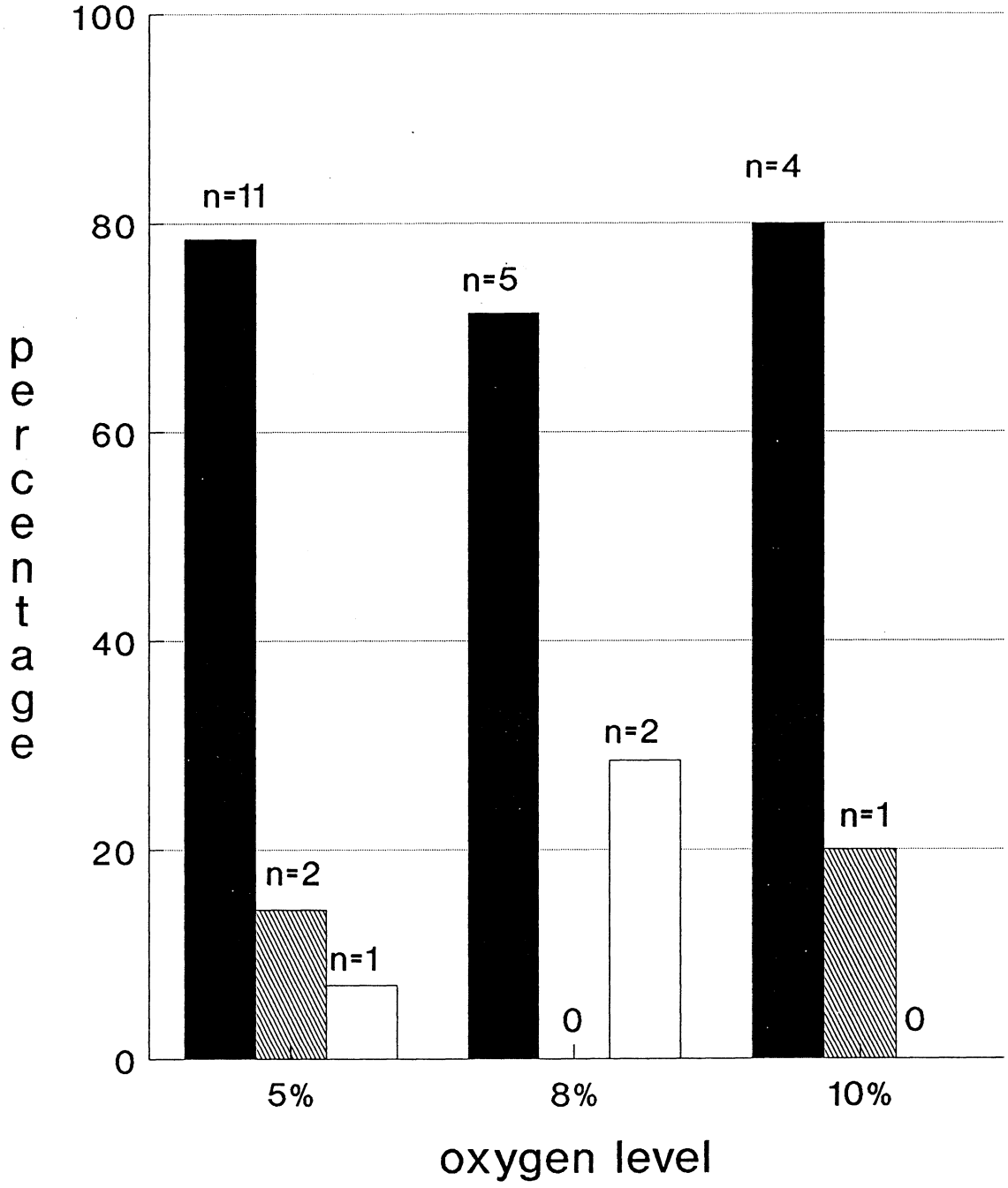
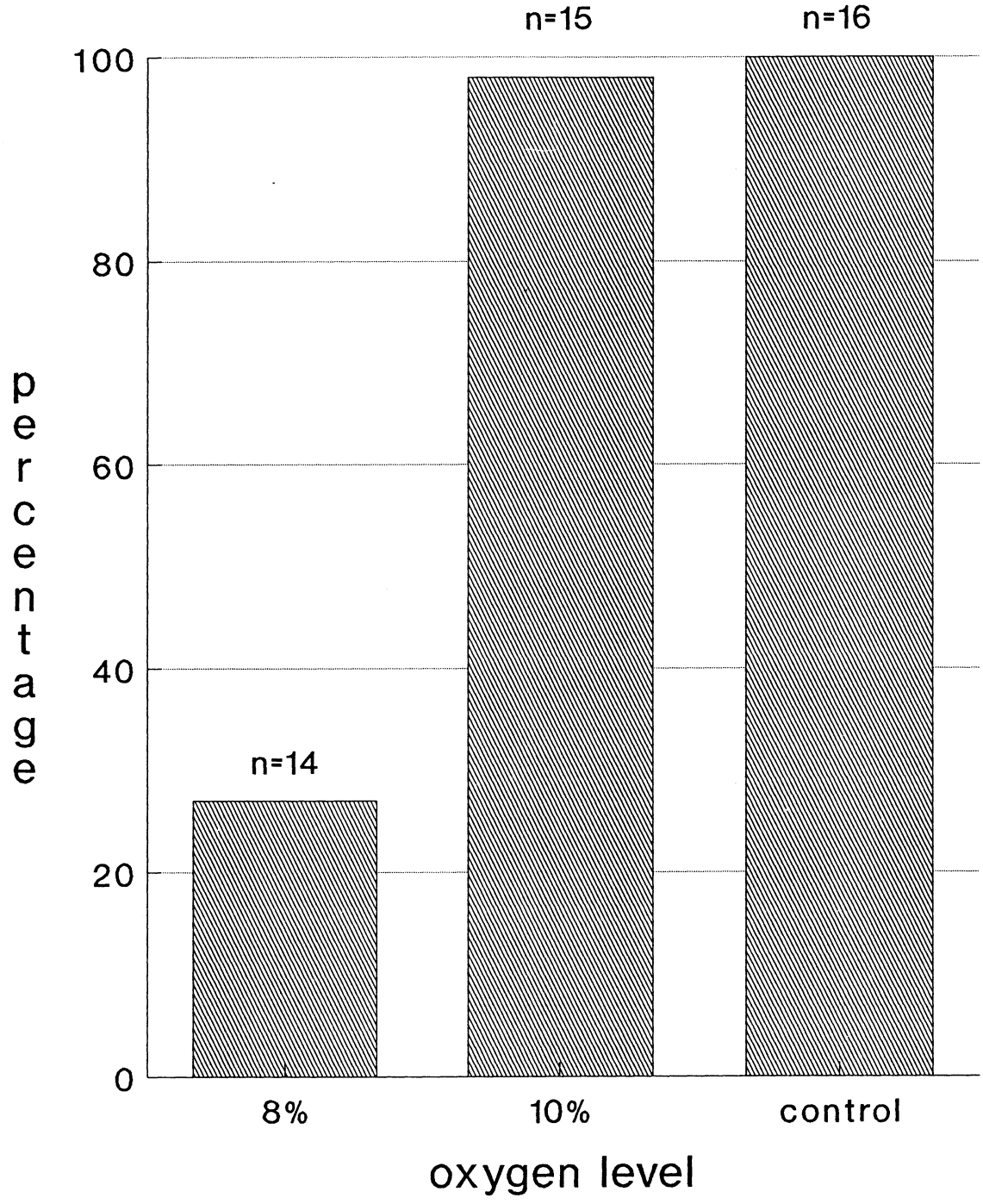

100
80
60
40
20
0
percentage
n=11
n=2
n=1
n=5
0
n=2
n=4
n=1
0
5%
8%
10%
oxygen level

Fig. 1C. Proportion of eggs that hatched from male brooded egg pads in Experiment 1.

C.


n=15
n=16
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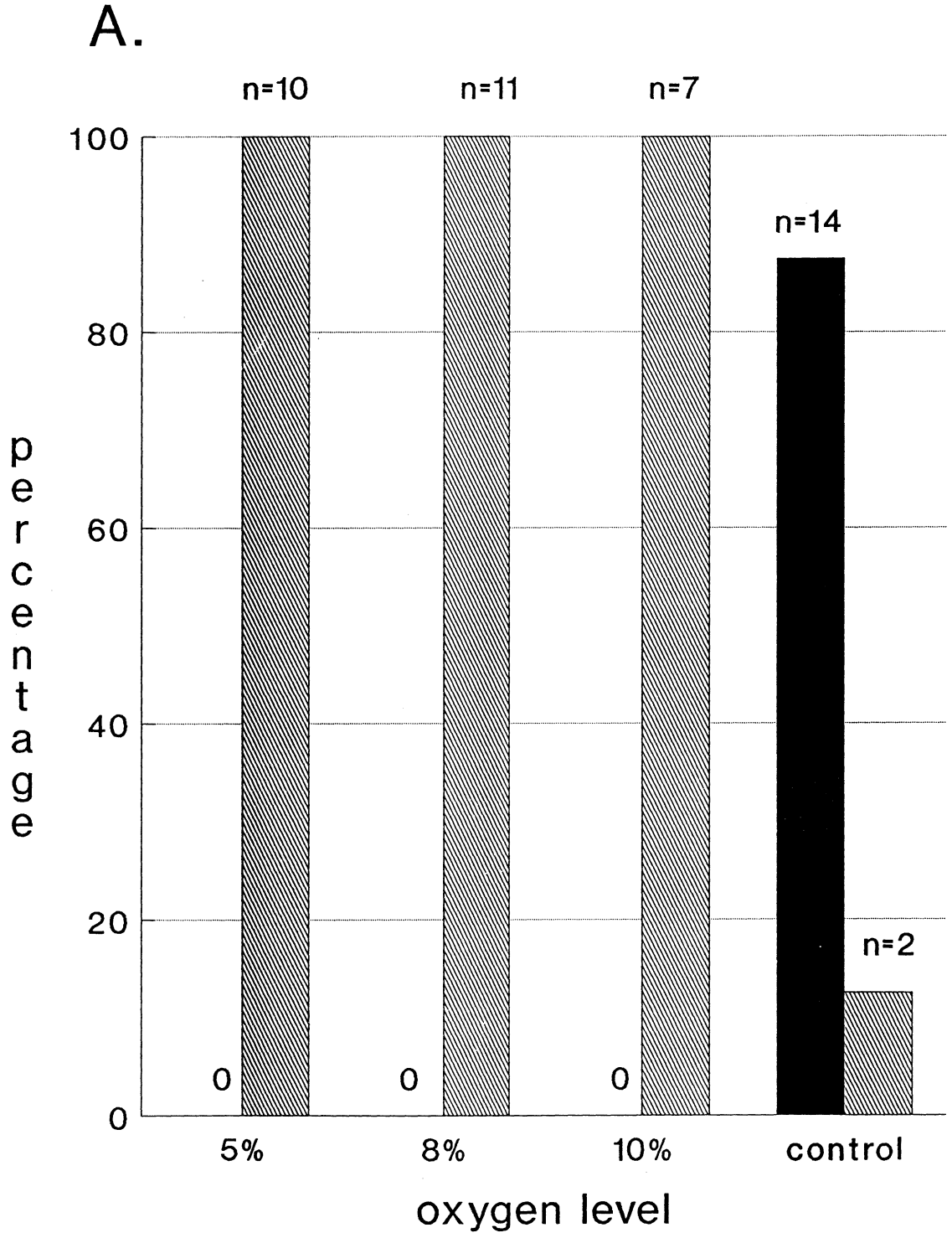
80
60
40
20
0
percentage
n=14
8%
10%
control
oxygen level

Fig. 2. Results of removed egg pads in varying oxygen levels in Experiment 2.

Solid bars = egg pads that hatched at least a single egg; striped bars = unhatched egg pads.

(A) Small egg pads ($\leq$ 50 eggs/pad).

(B) Large egg pads ($\geq$ 60 eggs/pad).


A.
n=10
n=11
n=7
n=14
n=2
100
80
60
40
20
0
percentage
0
0
0
5%
8%
10%
control
oxygen level

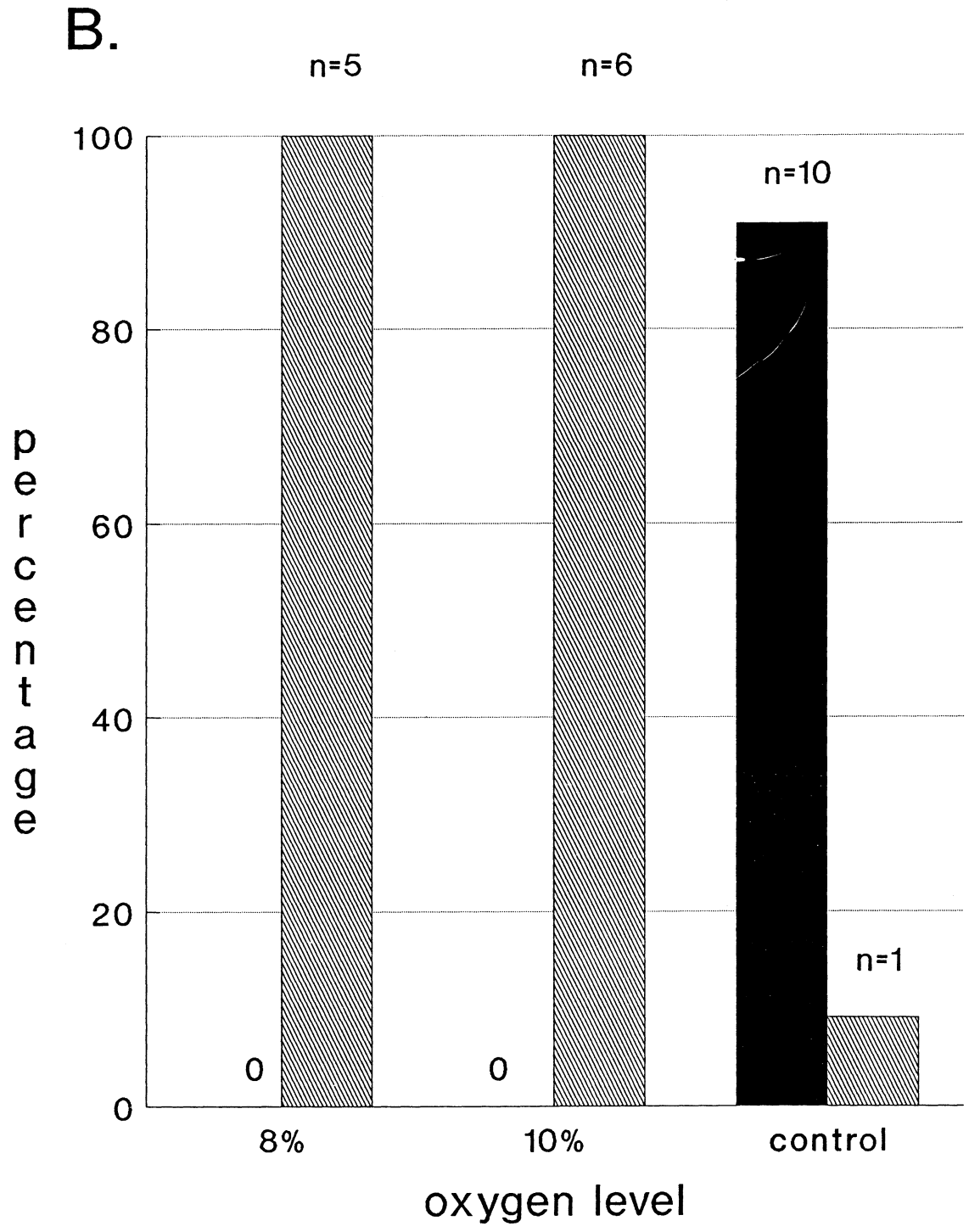

B.
n=5
n=6
n=10
n=1
100
80
60
40
20
0
percentage
0
0
8%
10%
control
oxygen level

Fig. 3. Results of male brooded egg pads in varying lengths of hypoxic (5%) treatment in Experiment 3.

Solid bars = egg pads that hatched at least single egg; striped bars = unhatched egg pads.

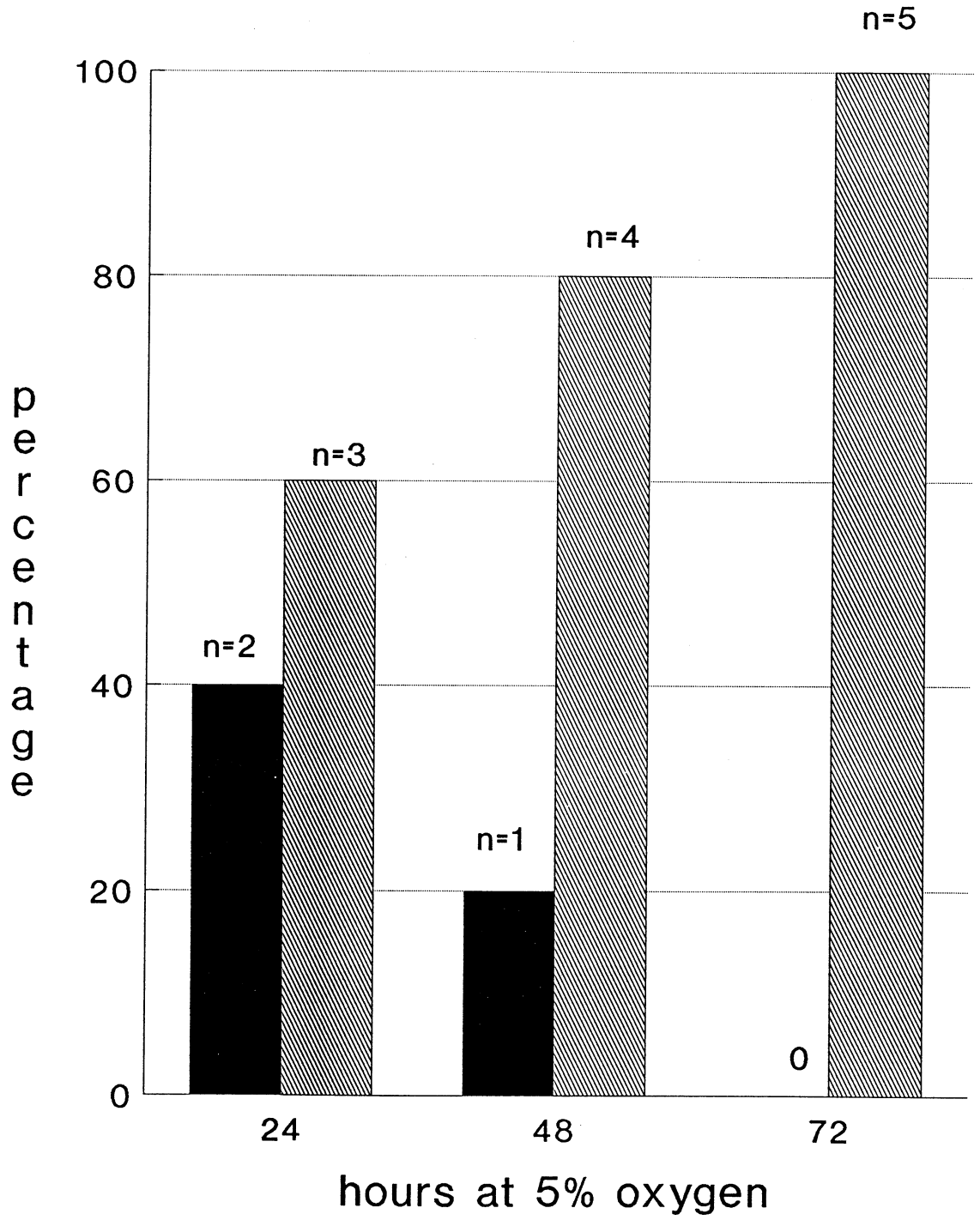
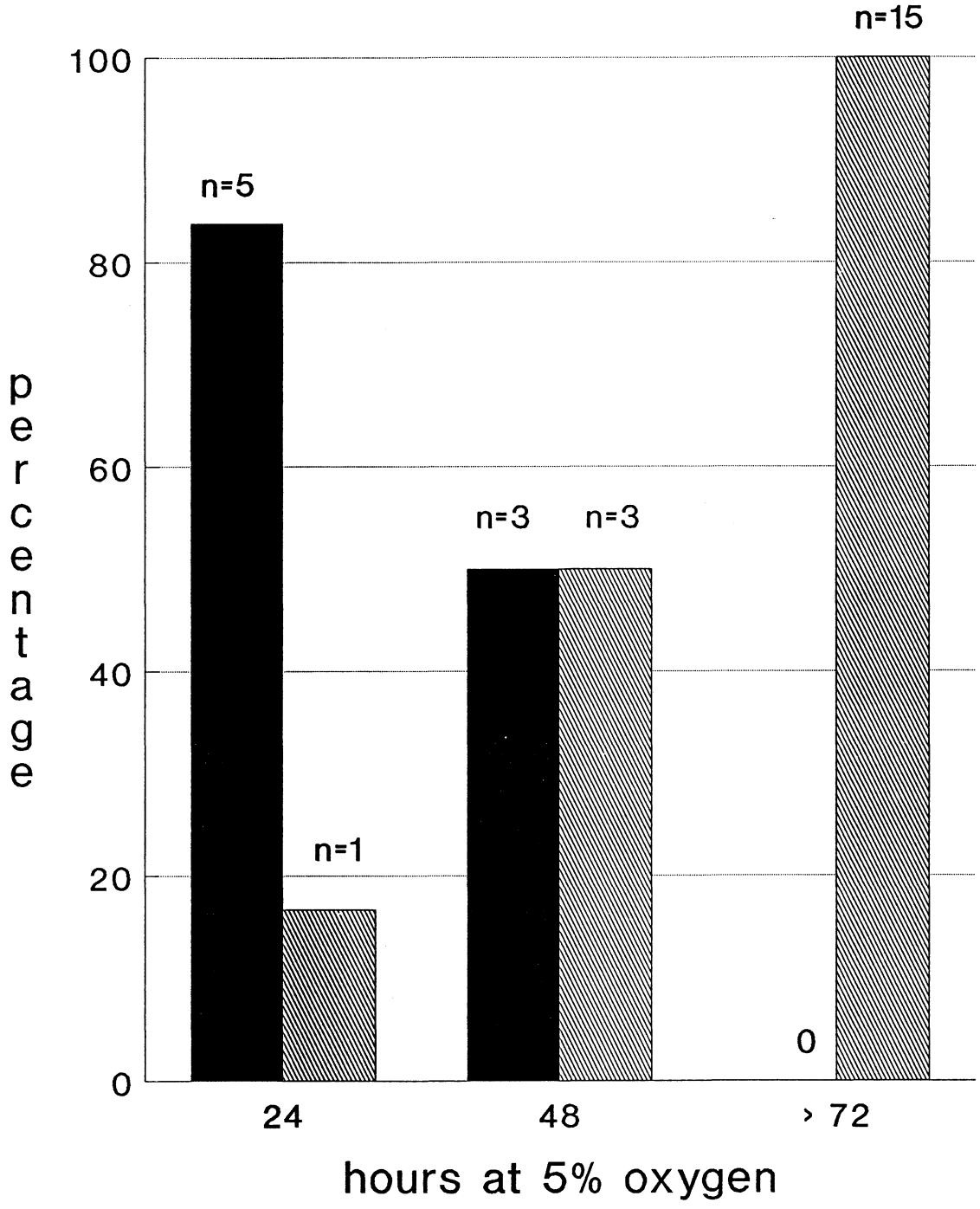

n=5
100
80
n=4
n=3
60
n=2
40
n=1
20
0
0
24
48
72
percentage
hours at 5% oxygen

Fig. 4. Results of removed egg pads in varying lengths of hypoxic (5% & 8%) treatments in Experiment 4. Solid bars = egg pads that hatched at least a single egg; striped bars = unhatched egg pads.


n=15
100
n=5
80
60
n=3
n=3
40
n=1
20
0
0
24
48
> 72
percentage
hours at 5% oxygen