KW: amphib, hell, thesis

RESPONSES OF CRYP'I'OBPANCl-JllS l\LLEGAt,IE!,JSIS ALLEGANIENSIS TO DIFFEf<EN'r OXYGr N CONCENTrtATIONS' TEMPE!,A'l'URES AND P!IO'l'OPERIOD

A Thesis

Submitted to tl1e Department of Life Sciences

and the Faculty of the Graduate School

Southwest 1issouri State llniversity

In Partial Fulfillment

of the P.equirements for the Degree

Master of Arts

by

David Arthur Beffa

July, 1976

RESPONSES OF CRYPTOBRANC f!US AJ,LI,Gi\NIENSIS Al,L}:GANIENSIS

TO LJI}"FEREN'l' OXYGEN CO!lCENTRATIONS,

TEI-IPERATURES AND PIIOTOPERIOD

Approvecl:

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(Dean of the Graduate Djvj_sion)

RESPO:\:SES Of' CRYl)'l'OBrtANCHUS ALJ,EGA!'JIENSIS J\TaLEGANIENSIS
'l"O f)IFFEREN'l' C_JXYGEN CONCENTR}\'l'IONS,
'l'E'•lPt:RA'rlJRES AND PllO'I'OPf:RlOD

David Artl1t1r Bcffc1
Soutch'.,est 1-lissouri Stc1te llniv2rsi_ty, July, 197G

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Cryptobranchus 21lJ.cgaI1ier1si.s, a large ,1.c1t1at.ic sal_a-111,"3_ncic.r, vlaS stuc}j_cd to detern1ine i.f it_s uric1eiwater IttC)\lC--':rnc:- r1t.s are rc spirc.itory in £ur1ct.ion. Tl1ese arli_n·1als also cx_hj_bit a side-to-side swaying motion, ar1c_l ıJ dr j_cJus authors .ı1zı1,ıc sug-gested that tl1is ro:::kirig ıs lo produce a fl_ow of water over the skin in order to RJ1ha11c:e the exchange of CjdSCS. Trac-ings cif -Lhe salamanders' movements were made on a physio-graph, then the d;-:1t:.a v;c;re subjected to Cill anc1lvsi_::-; o f vari-2.I1C(:'. Ei.ghtcen salan1c.nders were Lcsted. Tl1cv werP c]ivi.ded in"Lo threc g YOIl[:>s of ,1 x 0 JJi llli::1l.s , and each group was sub-jected to four different oxygen saturation levels (25 per-cent, 50 percent, 75 percent, 100 percent) and day and night photoperiods. 1'1intl tc s IIIQ\TID9, mi11utcs rocking, periods of _r·ock ing, pc,riods ofTTLC)Vi11g, periods ()f qui_c::scenc'e, dTIC'1 11llITl-ber ofJrocJcs were recorded for eacl1 fiftcen-1ninute PPr.iod of c,Cl.C 11 two-hour experi_rr1er1t. !,esul ts .indicate that roc.kir1cr and moving both may have a respiratory function.

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approved as t:o form ar1d content.

Robert P. Wilkinson Jr.

Chairman, Advisory Committee

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INTRODUCTION

Much work has been done on respiration and respiratory mechanisms in the Amphibia. Anurans have been studied quite comprehensively and urodeles to a lesse:r· degree. l\t least one supposed urodelan respiratory mechanism, howe,Jer, has need of further research. The hellbender, Cryptol)ranchus alleganiensis, a large aquatic salamander, sometimes exhibits a side to side swaying or rocking motion when placed in aquaria. Since these urodeles prefer cold, highly oxygenated, lotic water, some a1_1thors have s11ggested that this rocki11g is a response to reduced oxygen saturation levels in the aquaria (Nickerson et al., J.972).

Noble (1931) stated that Cry ptobranchus has a highly vascular lateral fold, and Guimond (1970) showed that over 90 percent of the gas exchange takes place through the skin. Lenfant and Johansen (1967) suggested that n1ovement of a respiratory surface could increase the efficiency of gas exchange in aquatic media. Thus the theory of rocxing as a function of respiration has support. However, other investigators have proposed that the rocking motion rrught be associated with ski.11 shedding, sexual attraction, or some facet of the breeding period (Grote, 1877 and Metter, 1976). It therefore appears just as likely tl1at tcl1is rock.ing n,otion is a response to son1e factor otl'1er tr1ar1 u.noxia.

SUI{\'EY OF Tl!E LITERATURE

Initially, rocking ,,as not associated ,vith respiration. Grote (1877), one of the first authors to mention hellbender rocking, described it as "an intermittent swaying motion from side to side." He attributed the cause to skin shedding, sexual a.ttraction, or the breeding period. Rocking was also n1entioned by Willey (1920). He described the motion, but suggested that oropharyngeal irrigation and air breathing are responsible for respiration. Gage and Gage (1886) discussed respiratory movements of Cryptobr211chus, but they described pharyngeal motion and made no mention of rocking.

Smith (1907) gave a description of the activities of hellbenders i.n .ciqt1aria.· He stated that hellbenders show uneasiness and discomfort in a lentic situation. lie mentioned that Cryptobranchus swims restlessly about, frequently surfaces for air, and refuses food, but he made no mention of rocking except as part of their breeding behavior. lie said that both the male and female make "swaying lateral and vertical movements of the posterior part of the body," just prior to the act of spawning.

Noble (1931) stated that the lateral folds of Cryptobranchus act as gills and that capillary divcrticula penetrating almost to the surface of these folds afford ideal conditions for cutaneous respiration. rle equated the lateral folds to gills. He also said that some urodele larv0e respond to anoxic conditions by an increase in respiratory movements, and that changes in the rate of buccopharyngeal movement may be a result of sudden illumination, moving images, vibrations, or temperature change. Lenfant and Johansen (1967) cited the waving motion of gill filaments n Necturus as an aid to respiration. They stated "the long external gills are intermittently waved to stir the water. This waving increases the efficiency of gas exchange as indicated by the redness of the gill filaments."

A relationship between roc,king and respiration is suggested by Bisl1op (1941) and Nickerson and !-lays (1973). Bishop suggested that Cryptobranchus roclcs to aerate its egg masses, and to facilitate cutaneous respiration. He also mentioned the use of lungs in still water, but did not discuss their relative importance.

Cross, Murdaugh and Robin (1966) studied gas exchange in Cryptobranchus. They recorded blood gas pressures and pH in hellbenders exposed to both air and water, but made no me11tion of rocking. Guimond (1970) studied aquatic and aerial respiration in four species of salamanders, ineluding Cryptobranchus. He reported several instances of rocking, but did not mention rocking in his experimental animals. This is probably due to the system of physical restraints that he use·d. He confirmed the skin as the main respiratory organ, finding that hellbenders use their skin for more than 90 percent of their gas exchange.

Reese (1906) subjected Necturus and Cryptobranchus to varying colors and intensities of light, and to variou_s temperatures. He omitted detailed descriptions of the results, but did state whether or not the hellbenders exhibited a violent reaction to a rapid change in temperature (in one instance he noted an individual exhibiting slight discomfort after being placed in 26° C water, when the animal had been acclimated to 14° C). Hellbenders showed "violent reaction" to being placed in 42°C water after being acclimated at 18° C and 26° C, but no reaction was noted when these hellbenders were placed in water at 33°C or cooler. Nickerson and !•lays (1973), however, observed violent thi·ashing and biting when hellbenders were placed in ice water after capture from streams at 18 - 22° C.

According to Noble (1931), normal reflexes are depressed at low teniperatures. He suggested that the critical thermal minimum is lower for amphibians adapted to cool temperatures than for those adapted to warm temperatures. Whitford and Hutchison (1965) studied the affects of temperature on gas exchange in Ambystomidae, Salamandridae, and Plethodontidae. They indicated that some of the variation in oxygen uptake through the skin may be due to the opposing forces of decreased oxygen binding properties of hemoglobin, and increased heart rate at higher temperatures.

Guimond (1970) stated that the rate of gill beats of Necturus maculosus show a significant increase with a rise in temperature. He also mentioned that preferred temperatures for amphibians are based upon thei.r ability to extr2.ct oxygen from the medium at higher temperatures.

Whitford and Massey (1970) found that ArnlJystoma tigrinum activity seems to be controlled primarily by tern perature, and secondarily by oxygen. Fifteen degrees centigrade seems to be the preferred temperature of this salamander. They stated that diel activity is regulated by both light and temperature.

Hutchison, Engbretson and Turney (1973) studied thermal acclimation and tolerance of Cryptobranchus. Tl1e critical thermal maximum was deterrnined to be 32.7°C, 32.99°C, and 36.57°C at 5°C, 15°C, and 25°C, respectively. The time required for acclimation from 5°C to 25°C was approximately four days, and that required for acclimation from 25°C to 5°C was about eight days. They stated that these acclimation times are slo,ver than those for any an1phibians previously studied in the same temperature range.

Kenny and Rose (1974) suggested that one of two possible alternatives could result ,,,hen amphibians are subjected to higl1 temperatures and low oxygen. First, activity levels could be lowered to minimize oxygen demand; second, respiratory stress could cause an increase in respiratory movement in order to increase the influx of oxygen.

Wassersug and Siebert (1975) stated tl1at the response to anoxic stress can be both physiological and behavioral. Also, anatomical modifications arc associated vii th anoxi a. They found tl1e rate of bobbing of several species of anuran tadpoles to be correlated with dissolved oxygen concentrations below a critical level.

MATERIALS AND METHODS

All hellbenders used in a pilot study and experiments were collected from the Niangua River in Dallas County, Missouri. The salamanders \vere collecterl by wading the river and using potato rakes to overturn rocks, then if a hellbender ,vas sighted, it would be guided into a c1ip net. The animals used in the pilot study were collected during July, 1975. The animals used in the experiments were collected during August, September and October, 1975. llellbenders were transported on j_ce to the lab, then placed in 19 liter plastic temporary holding tanks filled with tap water at 15 to 18° C. The animals were maintained in the tanks for a minimum of twenty-four hours, during which time they usually regurgitated and defecated. Compressed air ,,as bubbled through the tank water at all times. All salamanders were acclimated for at least eight days to the respective temperatures at which tl1ey were to be tested. The photoperiod vJas regulated at sixteen hours light and eight hours dark. No food was offered during acclimation or testing.

PolyethyJ_ene intravenous tubi.ng, with annealed stainless steel wire inserted, was surgically implanted in the left lateral fold of the hellbenders, then looped and tied to form a ring (Figure 1). Trjcaine (Ethyl-M-An1ino

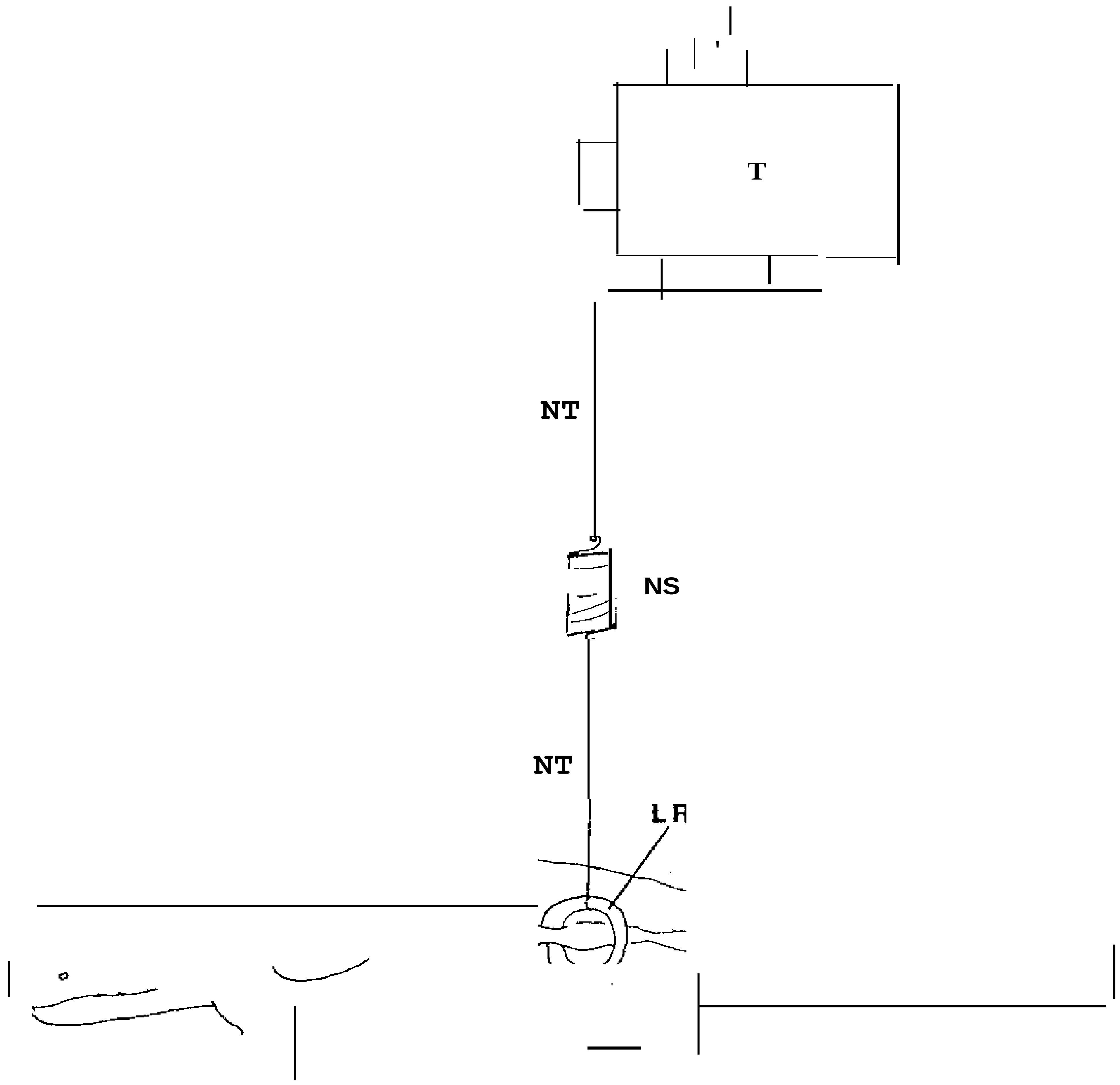

Figure 1. Hellbender attached to physiograpl1 transducer: T, transducer; tlT, nylon thread; NS, Nichrome spring; LR, lateral fold ring.

Benzoate) obtained from the Sigma Chemical Company, St. Louis, Missouri, was used as the anesthetizing agent. The hellbenders were placed one at a time in a solution of 0.5 g Tricaine dissolved in 3.8 liters of tap water. They were considered anesthetized when they failed to respond to toe pinching. After surgery, the hellbenders were weighed, measured, and sexed. The method described by Bishop (1941) was used to determine sex.

The salamanders were then placed in chambers containing tap water adjusted to the temperatures at which the animals were to be acclimated. The 5° group, comprised of six hellbenders, was acclimated at 5°C in a 114 liter aquarium placed in an environmental chamber. The 12° group, also composed of six hellbenders, was acclimated in a galvanized milk cooler at 12°C. The six hellbenders that made up the 20° group were acclimated in the six 38 liter aquaria in which the experiments were performed. The experimental temperatures were within the range of temperatures recorded for the North Fork of the White River (Nickerson and Mays, 1973) and the Niangua River (6° - 22°C) (Wilkinson, 1975). A minimum of eight days temperature acclimation time was allowed, and according to Hutchison et al. (1973) this should have been sufficient.

Control of photoperiod was better in the 5° and 12° groups than in the 20° group. The latter was exposed to reflected diffuse light from a mercury vapor fixture across the street and from a dim light in the hallway outside the room.

The 38 liter experj_rnental aquaria were insulated on four sides and the bottom with 2.54 cn1 thiclc s tyrofoan, **insu-** lation board. 0110 end of each aquaritrm was ir1sulated only one-half of the distance fro111 botton1 to top to facilitate. observation. The aquaria measured 50 cm in length by 30 cm in heighth by 25 cm *in* widtl1.

A Magi-Whirl heating and cooling water bath, manu-. factured by the Blue M Electric Company, Blue Island, Illinois, was used to provide a constant ternperature source for the aquaria. Two model CP5000 Little Giant reciprocal pumps were attached to an alternating series of rubber tubing and aluminu1n conduit in order to heat or cool the aquaria water (Figure 2). The reciprocal punips were im- mersed in the reservoir of the water bath and the11 attached to one end of the rubber tubing. The aluminum conduit was submerged in the aquaria a11d the rubber tubing was attached to the conduit ends between adjacent aquaria. Three aquaria were regulated per pump.

Each hellbender was placed in a 6 mm mesh hard,,are cloth cage that measured 23 cm in width by 40 cm in length by 20 cm in heighth. The cages were used to prevent the animals from becoming entangled in the temperature control tubing during testing. A 7.5 cm by 15 cm slot in the top of the cages permitted the salamanders to be attached to a physiograph transducer (Figure 1).

Oxygen saturations of the aquaria water were ad- justed to the desired levels immediately before recording.

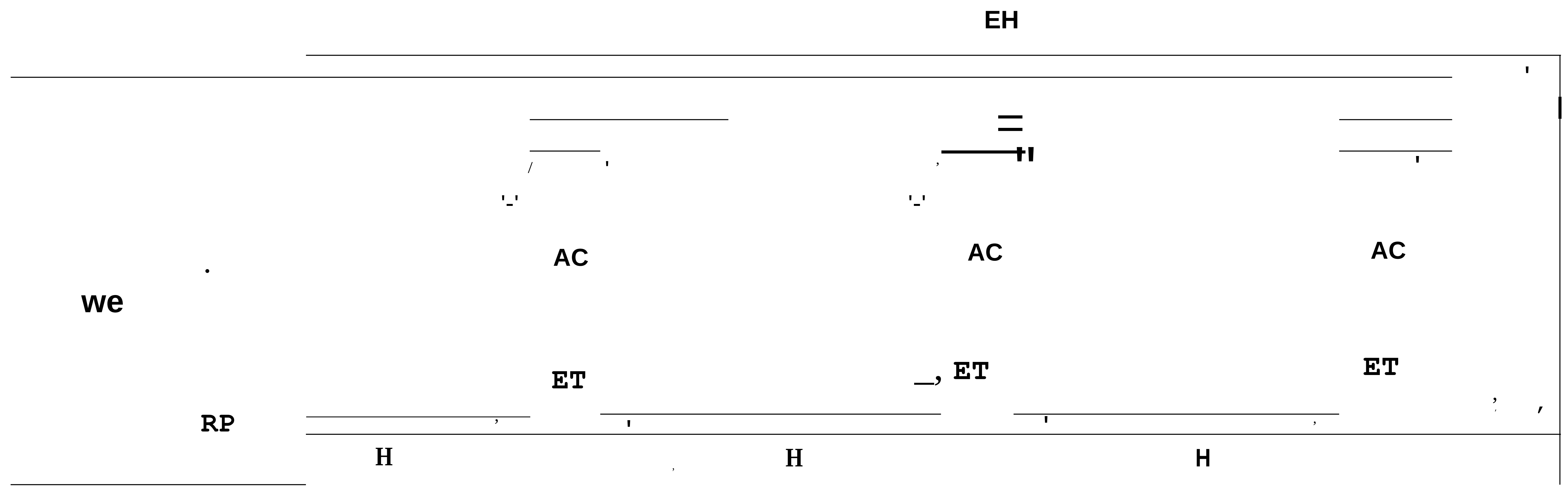

Figure 2. Three experimental tanks and cooling apparatus: WB, water bath; RP, reciprocal pump; H, hose; AC, aluminum conduit; ET, experimental aquaria; EH, effluent hose.

A portable oxygen meter and probe (Yellow Springs Instrument Company) was used to monitor oxygen levels. Readings were taken in parts per million and then converted to percent saturation using a Rawson Nomogram. The four oxygen saturation levels used for testing were: 25 percent, 50 percent, 75 percent, and 100 percent. The saturation levels were maintained within a plus or minus 5 percent range by the addition of compressed gases at various times throughout recording. Oxygen was bubbled through the water using an air stone to increase the saturation level, and nitrogen was used to decrease the saturation level. At all times prior to experiments, the hellbenders were maintained at normal saturation levels of 80 - 85 percent dissolved oxygen. Normal saturation levels were obtained by bubbling a constant supply of compressed air into the holding chamber water.

Responses were recorded on a six channel E & M physiograph using "B" type transducers at a paper speed of 0.1 cm/min. The amplitude varied according to amplifier and transducer and was not analyzed. The salamanders were attached to the transducer by braided nylon string hooked to the lateral fold ring by means of a piece of Nichrome wire bent into a "U" shape. A Nichrome wire spring was used to enable the physiograph transducer to record with maximal and minimal tension placed upon the transducer, at the same time allowing the hellbenders a greater freedom of movement than otherwise possible.

A total of eighteen salamanders was initially used in the experiments and they were tested at four oxygen '.saturation levels and three temperatures during the light and dark portions of their photoperiod. Six hellbenders' ,,ere recorded simultaneously, but the data from one of these six was discarded after testing. A random numbers table (Rohlf and Sokal, 1969) was used to choose the discard.

Each experimental period 1-1as approximately two hours in length. After testing, the recording paper was divided into eight 90 cm lengths, each representing a fifteen-minute period.

The following data were recorded for each animal during each fifteen-minute period: minutes of random n1oving, minutes of rocking, periods of moving, periods of rocking, periods of quiescence, and number of rocks. Random moving was defined as tracings of an irregular nature or without any observable pattern (Figure 3a). Rocking was defined as one or more characteristic tracings made in a regular or irregular sequence (Figure 3b). A period of rocking was defined as one or more rocks that were not separated by more than 9 cn1 (Figure 3c). A period of movement was defined as a series of random tracings not separated by more than 9 cm (Figure 3d). A period of quiescence was defined as a period of at least 9 cm during which no movement or rocking occurred (Figure 3e). A rock was defined as one characteristic tracing separated from a similar characteristic tracing by a

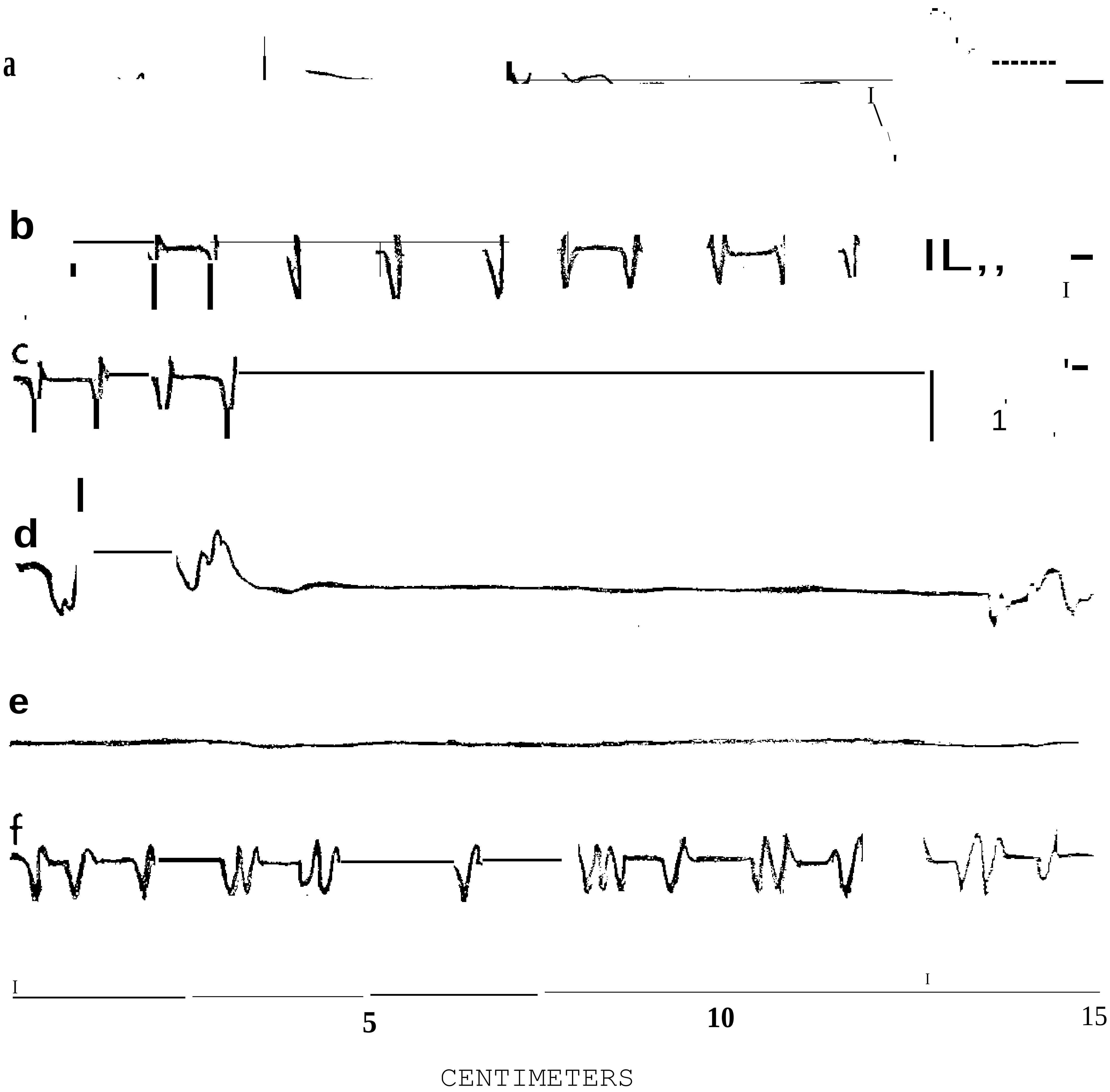

Figure 3. Samples of various hellbender responses: a, random moving; b, rocking; c, two periods of rocking; d, two periods of moving; e, quiescence; f, multiple rocks.

clearly discernable horizontal line on the recording paper (Figure Jf).

Water temperature, oxygen saturation levels (percent), day or night, animal number, period number, sex,., weight, total length, snout-to-vent length and number of minutes in the period were recorded for each hellbender and each fifteen-minute experimental period. Some periods we,e less than fifteen minutes in duration, although the data were all corrected to an equivalent fifteen-minute period prior to analysis.

Data were subjected to computer processed Balanova and multiple regression analyses. Unless otherwise stated, significance should be assumed to represent P<.01.

RESULTS

Table l sl1ows sex, weight, total length and snout-to-vent length of the experimental animals. The first group was acclimated and tested at 5° C, the second group at 12° C, and the third group at 20° C. Female weights ranged from 506 to 923 g. Male weights ranged from 3,14 to 849 g. Female total lengths ranged from 427 to 478 mm, and male total lengths ranged from 357 to 528 mm. According to multiple regression analysis, sex, weight, and lengths had no signj_ficant effect on the types of movements studied.

The dependent variables considered were minutes of random moving (M/>1) , minutes of rocking (MR), periods of moving (PM), periods of rocking (PR), and number of rocks. The only treatments or -independent variables to be discussed wiJ_l be temperature, oxygen (O_2) saturation, and photoperiod. Only those treatments having a sig1lificant effect on any dependent variable will be mentioned.

Table 2 shows that treatments having a significan effect 011 M1-1 were oxygen (P<.05), and the interaction of photoperiod and temperature (P<.OS). Random moving decreased with increased oxygen saturation from a mean MM of 2.5 min. at 25 percent saturation to 1.5 min. at 100 percent saturati_on (Figure 4).

TABI,E 1. Hellbender Data (Weights in Grams, Lengths in Millimeters)

	Number	Sex	Weight	Total Length	Snout - Vent Length
Group 1	1	Male	558	528	357
	2	Female	798	478	317
	3	Female	506	427	287
	4	Male	748	475	319
	5	Male	394	389	258
Group 2	1	Female	923	472	326
	2	Male	849	500	342
	3	Male	344	357	244
	4	Male	542	444	299
	5	Female	865	463	317
Group 3	1	Male	427	410	280
	2	Female	584	446	294
	3	Female	700	465	320
	4	Male	559	415	277
	5	Male	429	393	270

Group 1 tested at 5°C, Group 2 tested at 12°C, Group 3 tested at 20°C.

1

TABLE 2. Analysis of Variance for Minutes Moving (MM)

Treatment	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Significance
Terr.perature	2	41.7	20.9	0.8	N. S.
Oxygen	3	239.8	79.9	3.0	P<.05
Photoperiod	1	13.1	13.1	0.5	N. S.
Temperature X Oxygen	6	315.4	52.6	2.0	N. S.
Temperature X Photoperiod	2	205.4	102.7	3.9	P<.05
Oxygen X Photoperiod	3	142.1	47.4	1.8	N.S.
Temperature X Oxygen X Photoperiod	6	241.9	40.3	1.5	N.S.

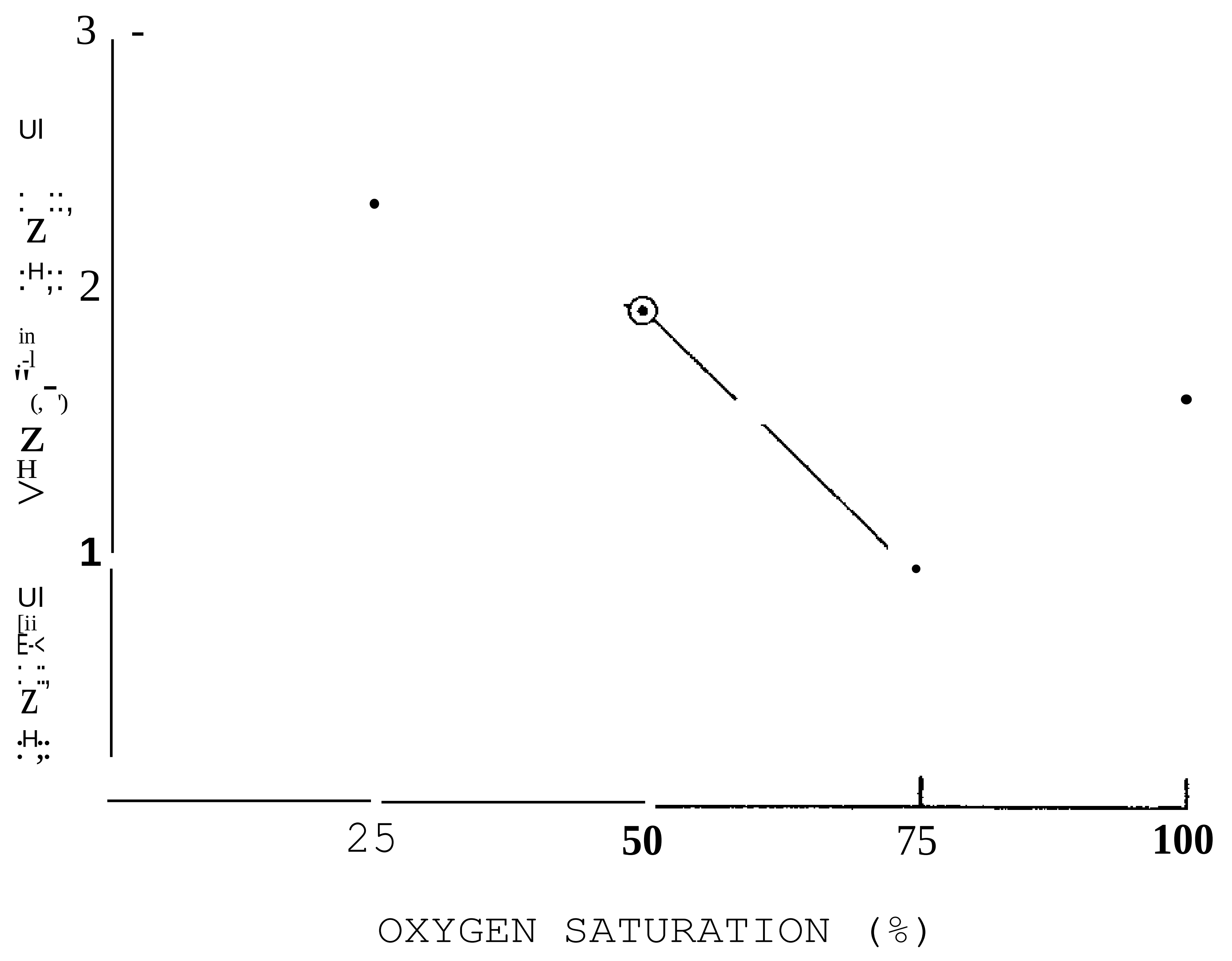

Figure 4. Minutes Moving at four oxygen saturation levels. Each circled point represents a mean of 240 observations.

Figure 5 shows !'1M in response to temperature in ligl1t and dark. MM decreased from a mean of 1.9 min. to a· mean of 1.2 min. during light testing. The opposite was true during dark conditions. Moving increased from 0.8 min. at S°C to 2.3 min. at 20°C. An apparent synergism is indicated here. Neither temperature nor photoperiod by themselves have a significant effect on MM. 1-Iowever, the two treatments considered together show a significant effect. The types of random movement observed included walking around the perimeter of the hardv1are cloth restraining cage, occasionally thrusting the nares and anterior portion of the head out of the water, and a viole·nt thrashing a11d t,,isting motion. If this violent behavior occurred, the test was temporarily stopped. The observation then continued if the more convel·,tional behavior·returned. This thrashing seemed to be a response to the pressure of the physiograph l1ookup on the lateral ring. Only two hellbenders exhibited this behavior.

The interaction of temperature and oxygen had a very highly significant effect (P<.001) on MR (Table 3). !1R increased from a low of 1.8 min. (average of the four oxygen saturation levels) to 10. 4 min. as temperature increased from 5° to 12° (Figure 6). MR was increased by a factor of 5.8 due to the temperature increase. The affect of oxygen was opposite that of temperature. Generally, IR increased at all oxygen levels from 5° to 20° C. From 12° C to 20° C, however, MR at 75 percent and at 100 percent decreased. The

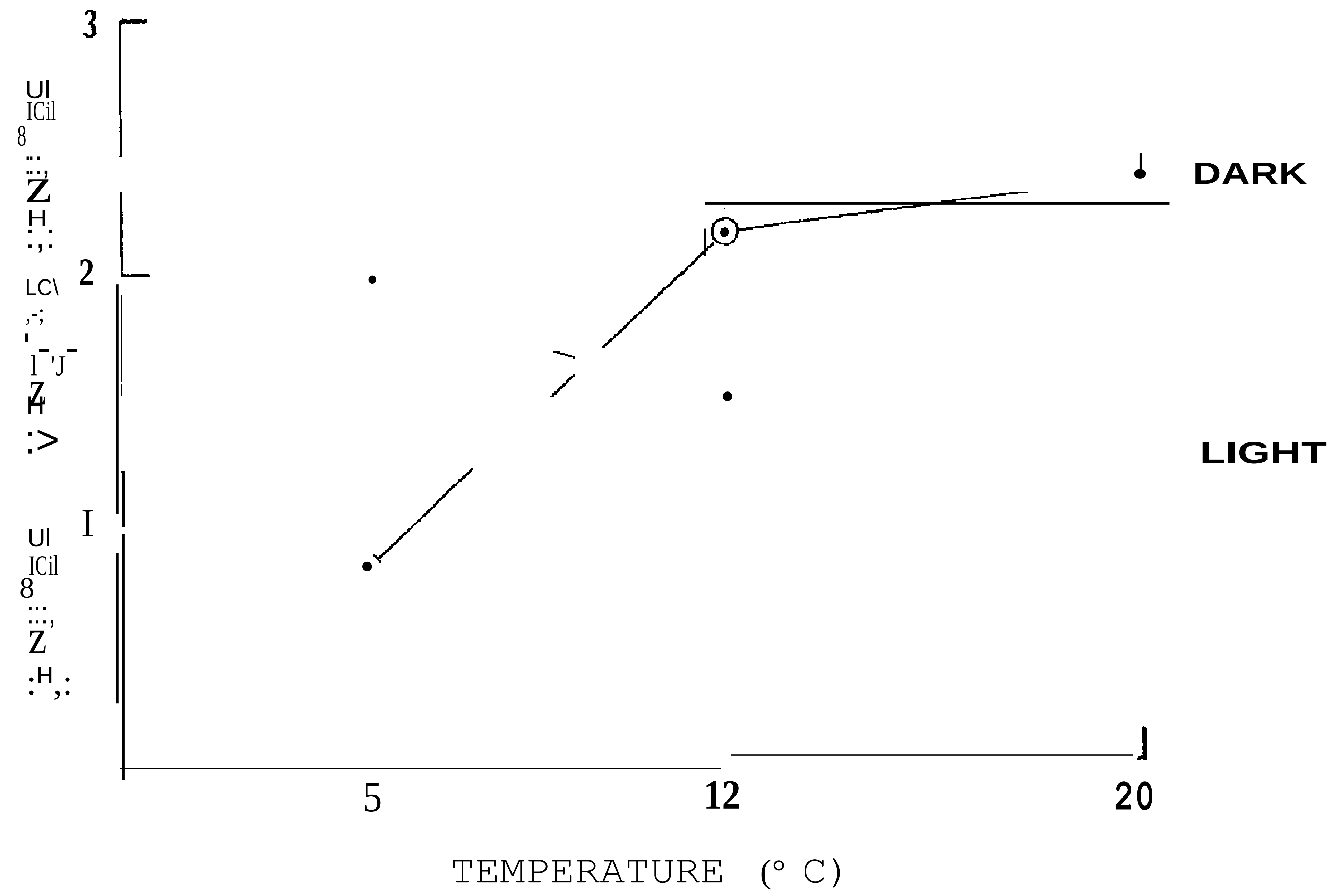

Figure 5. Minutes loving at three temperatures and light and dark testing. Each circled point represents a mean of 160 observations.

TABLE 3. Analysis of Variance for Minutes Rocking (MR)

'rreatment	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Significance
Temperature	2	16400.2	8200.1	91.7	P<.001
02'.ygen	3	1454.2	484.7	5.4	P<.005
Photoperiod	1	26.0	26.0	0•2	N.S.
Temperature X Oxygen	6	2341.0	390.2	4.3	P<.001
Temperature X Photoperiod	2	78.9	39.4	0•4	N.S.
Oxygen X Photoperiod	3	326.3	108.7	1.2	N.S.
Ternperature X Oxygen X Photoperiod	6	520.3	86.7	0•9	N.S.

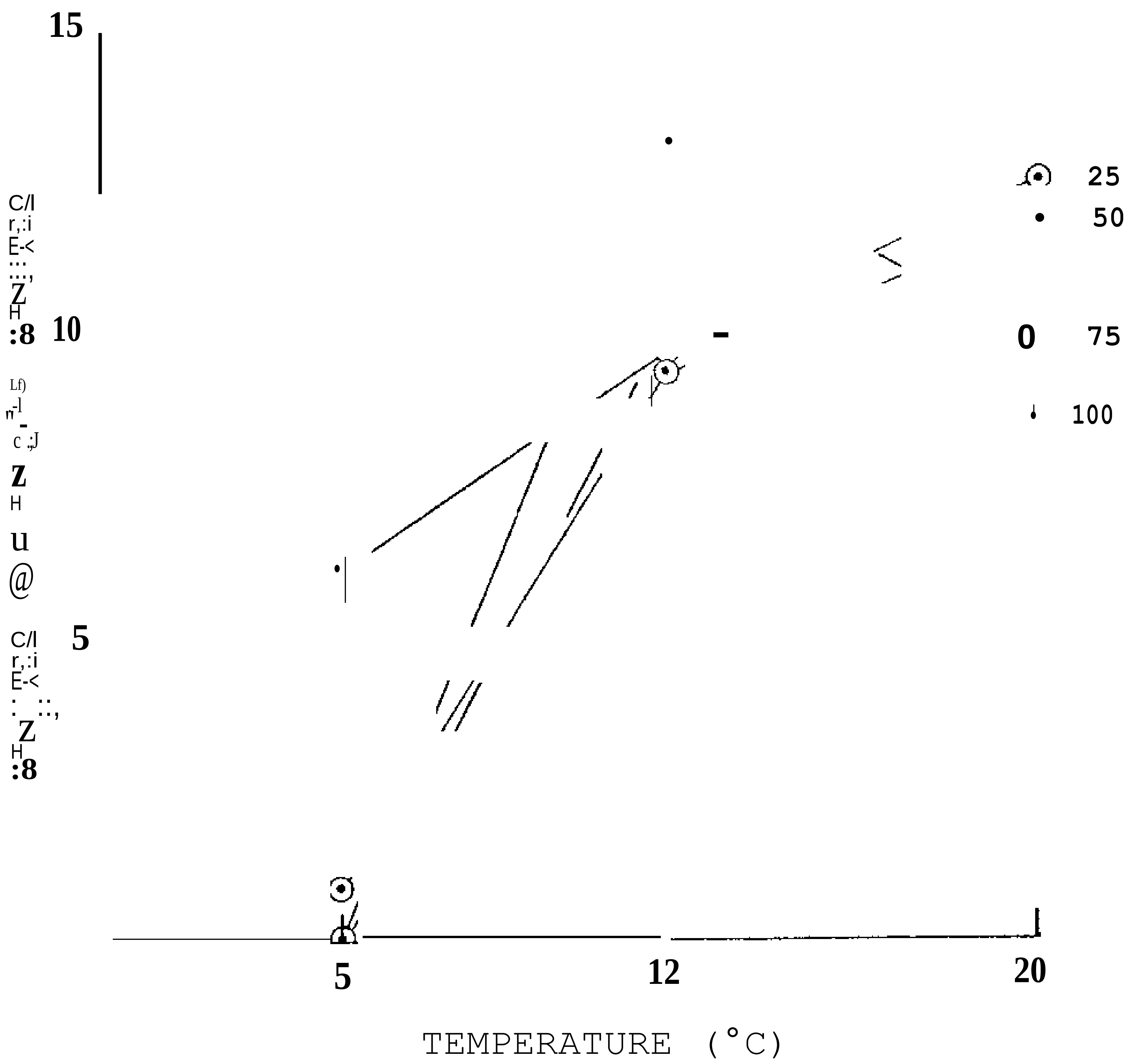

Figure 6. Minutes Rocking at three temperatures and four oxygen saturation levels. Each circled point represents a mean of 80 observations.

rocking decreased from a mean of 12.8 min. to 9.8 min. at 75 percent saturation and from 9.9 min. to 8.6 min. at 10 percent saturation. A comparison between MM and 1R shows that as oxygen saturation increases both MM a11d MR decrease, but the effect is more noticeable on MR.

Table 4 shows that periods of moving (PM) were significantly affected by temperature, oxygen, and photoperiod ($P<.05$). Hellbenders averaged 0.5 PM at 5°C and increased to an average of 1.4 PM at 20°C at all oxygen saturations during both light and dark (Figure 7). The moving response was increased by a temperature increase. Conversely, an increase in oxygen saturation caused a decrease in PM. The average for all temperatures during both light and dark decreased from 1.2 PM at 25 percent oxygen saturation to 0.6 PM at 75 percent saturation. A greater rate of increase of PM became apparent at dark than at light by averaging the results of oxygen saturation and comparing the effects of temperature and photoperiod. Figure 7 sl1ows the effects of temperature and oxygen on PM during both light and dark. The effects, while significant ($P<.05$), are not immediately obvious. However, there is a tendency for hellbenders to suppress the n1oving response when subjected to an increase in oxygen saturation at all temperatures and during both light and dark.

Treatment effects on periods of rocking (PR) (Table 5) show similarities to those of PM. A comparison between Figure 7 and Figure 8 shows that rate of change is

TABLE 4. Analysis of Variance for Periods of Moving (PM)

Treatment	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Significance
Temperature	2	149.6	74.8	35.5	P<.001
Oxygen	3	70.7	23.6	11.2	P<.001
Photoperiod	1	5.6	5.6	2.6	N.S.
Temperature X Oxygen	6	5.6	.9	0.4	N.S.
Temperature X Photoperiod	2	20.7	10.4	4,9	P<.01
Oxygen X Photoperiod	3	1.0	.3	0.1	N.S
Temperature X Oxygen X Photoperiod	6	36.7	6.1	2.9	P<.01

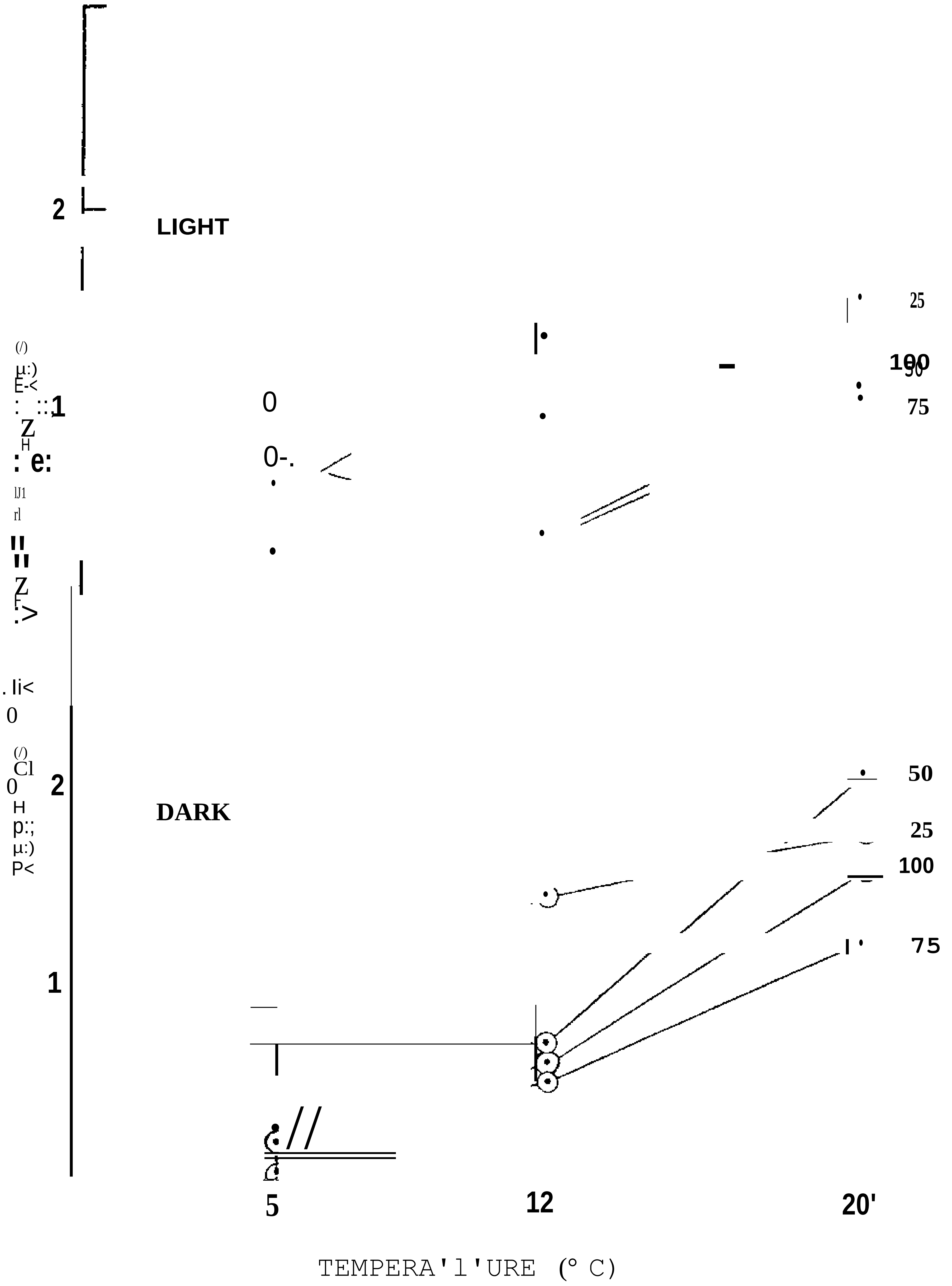

Figure 7. Periods of Moving at four oxygen saturations, photoperiod and three temperatures. Each circled point represents a mean of 40 observations.

TABLE 5. Analysis of Variance for Periods of Rocking (PR)

Treatment	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Significance
Temperature	2	470.8	235.4	218.9	P<.001
Oxygen	3	74.3	24.8	23.0	P<.001
Photoperiod	1	.5	.5	0.4	N.S.
Temperature X Oxygen	6	24.1	4.0	3.7	P<.005
Temperature X Photoperiod	2	12.4	6.2	5.7	P<.005
Oxygen X Photoperiod	3	5.6	1.9	1.7	N.S.
Temperature X Oxygen X Photoperiod	6	29.2	1.1	4.5	P<.001

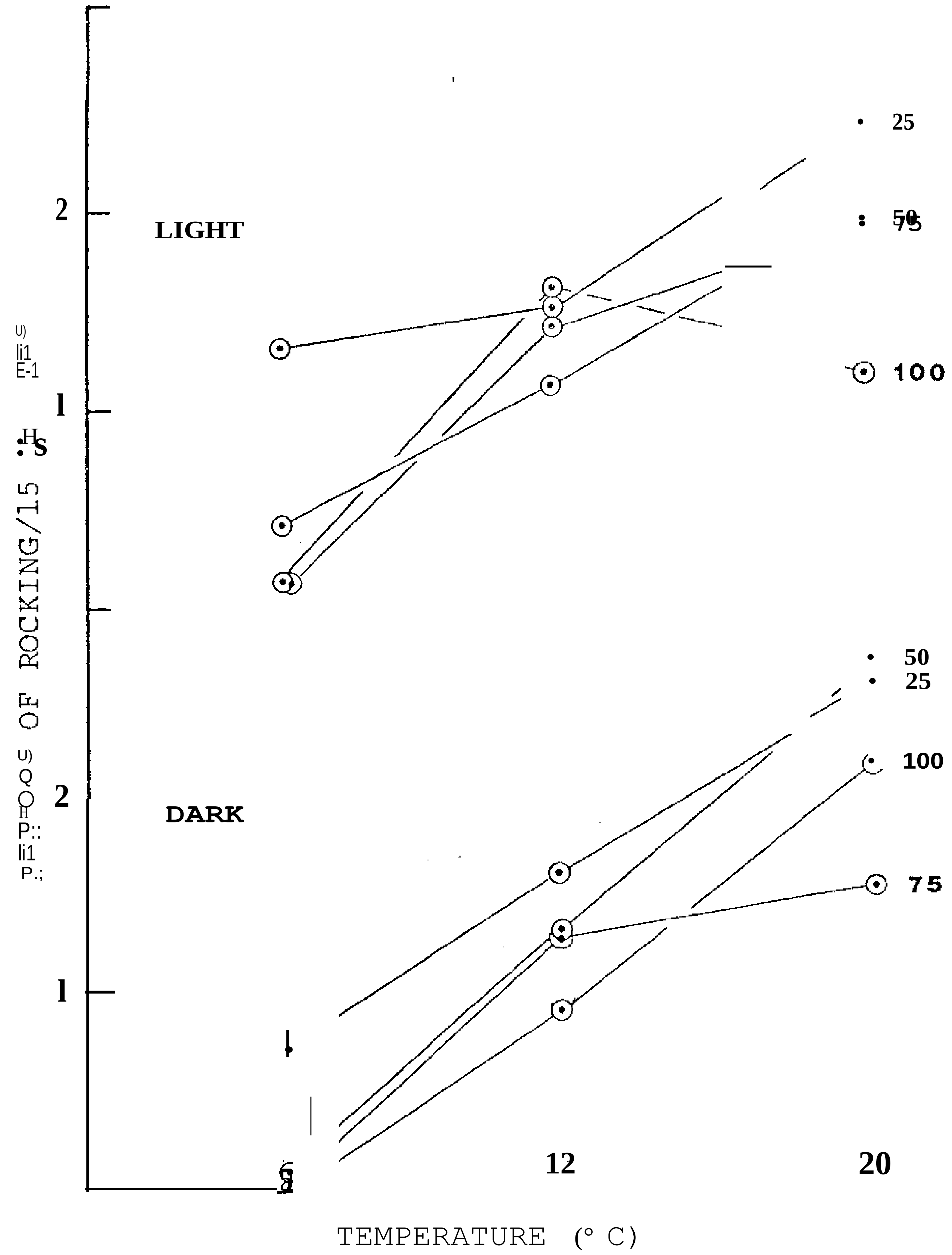

Figure 8. Periods of Rocking at four oxygen saturations, photoperiod, and three temperatures. Each circled point represents a mean of 40 observations.

greater for periods of rocking than for periods of moving. PR increases by a factor of 1.7 from 5°C to 20°C (Figure 8) while PM increases by a factor of 0.9 under the same conditions (Figure 7). A net decrease of 0.7 PM occurs from 25 percent to 100 percent oxygen saturation for both light and dark, and all temperatures. The corresponding decrease in PM is 0.6. Similarities again become evident if we compare the effects of temperature and photoperiod for the two variables. Both P ! and PR increase as temperature increases, but the magnitude of increase is again greater for PR. The net increase for light and dark combined is 1.8 PR compared to a 0.9 PM increase. The total effects of temperature, oxygen, and light or dark are very similar on both PM and PR (Figure 8). A gradual decrease in both variables occurs during both light and dark conditions as oxygen saturation **increases.**

Hellbenders significantly increase their total number of rocks as temperature increases and oxygen decreases (Table 6). An average of oxygen levels and photoperiod shows that rocking increased from a mean of .5 rocks/15 min. at 5°C to a mean of 62 rocks/15 min. at 20°C. The effect of oxygen on rocking was less pronounced. The mean number of rocks/15 min. decreased from 61 at 25 percent oxygen to 28 at 100 percent saturation. Figure 9 shows that frequency of rocking increases at all temperatures and at 25 and 50 percent oxygen saturation. Frequency, however, decreases at 20°C for 75 percent and 100 percent saturation levels. The

TABLE 6. Analysis of Variance for Numbers of Rocks

Treatment	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Significance
Temperature	2	641776.9	320888.4	58.6	P<.001
Oxygen	3	140783.3	46927.8	8.5	P<.001
Photoperiod	1	19.3	19.3	0.0	N.S.
Temperature X Oxygen	6	135586.3	22597.7	4.1	P<.001
Temperature X Photoperiod	2	6546.2	3273.1	0.5	N.S.
Oxygen X Photoperiod	3	27812.9	9270.9	1.6	N.S.
Temperature X Oxygen X Photoperiod	6	9088.6	1514.8	0.2	N.S.

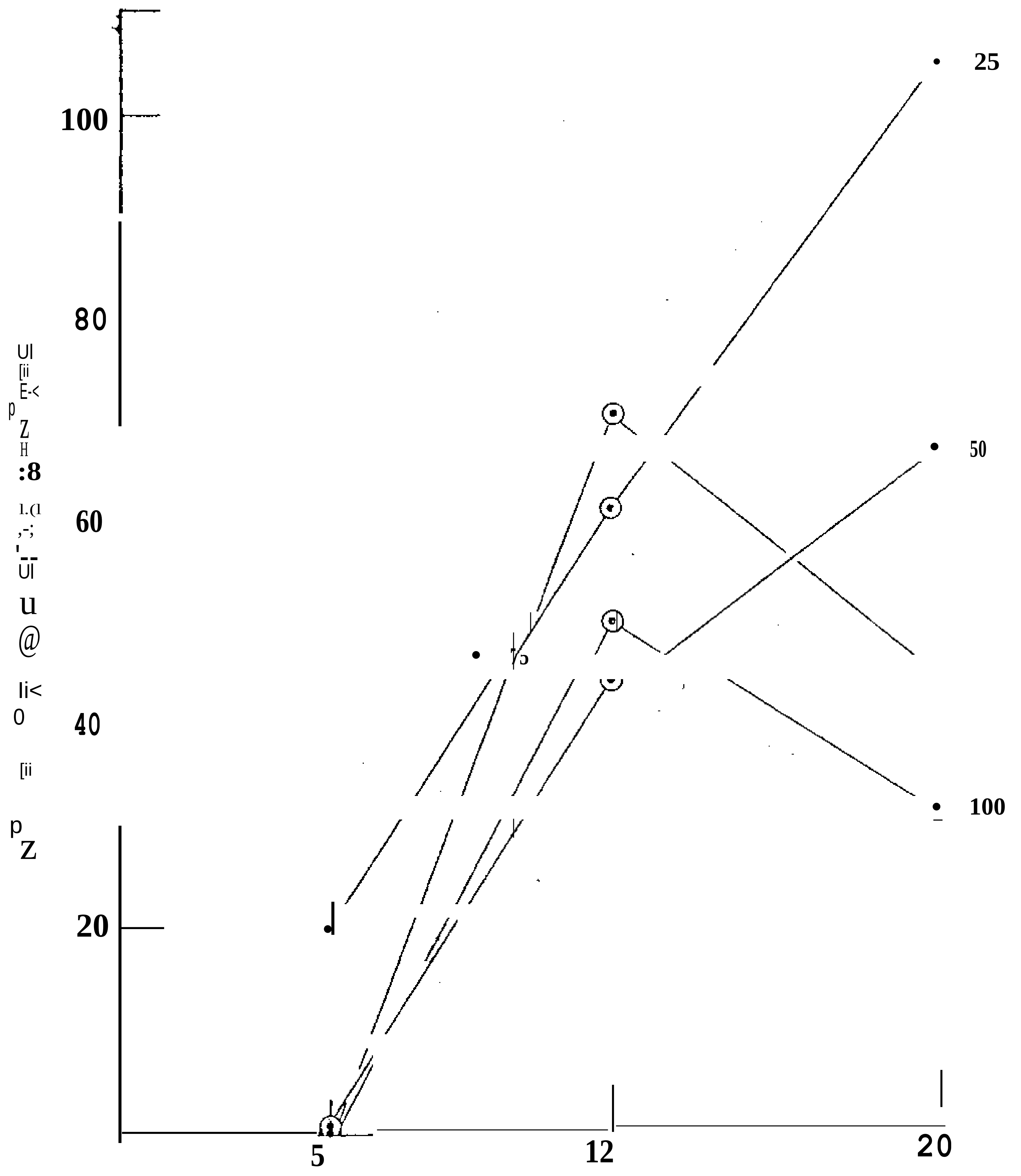

Figure 9. Number of rocks/15 min at three temperatures and four oxygen saturations. Each circled point represents a mean of 80 observations.

overall trend is significant ($P<.005$), with the mean number of rocks/15 min. ranging from a high of 61 at 25 percent saturation to 28 at 100 percent saturation.

Comparison of the effects of oxygen on b!R, PR, and nwnber of rocks showed some similarities. All three variables show the same trend, which is a decrease in rocking as oxygen saturation increases. The same holds true to these three variables and the effects of temperature. All three measured kinds of rocking increase as temperature increases. When one considers the combined effects of temperature and oxygen, however, the 75 percent and 100 percent oxygen saturations depart from the expected t-rend at the 12°C temperature level for both MR and number of rocks.

MR and MM, when compared, both show a tendency to decrease as oxygen levels increase.

DISCUSSION

Random moving, rocking, and quiescence were the three primary types of behavior observed during testing and acclimation. 1'1oving included walking, sv1imming and surfacing for inspiration. Rocking responses were similar, but. some triple and double rocks were observed (Figure 3f). Multiple rocks were counted as single rocks for purposes of calculation. Amplitude or vigor of rocking varied but was not considered. Quiescence was simply an absence of measurable response, and will not be discussed because it, in effect, is a complement of moving and rocking. On a few occasions the hellbenders would rise to the surface and lie quietly for a few moments with their nares above the surface film, but 110 inspirations were observed during these periods of quiet.

Random moving was studied to ascertain whether any similarities or differences could be noted between rocking and moving. 'l'he act of moving could perform the same supposed function as that of rocking. Rocking theoretically aids cutaneous respiration by causing a fl.ow of aerated water to corne in contact with the skin. Moving could incidently do L e same. Temperature, apparently, is one of the factors causing moving.

The data indicate that both the number of periods of moving (PM) and the time spent moving (MM) are decreased by at least half as oxygen saturation increases from 25 perce11t to 75 percent. MM and Pi'! both increase again at the 100 percent saturation level. Since the normal oxygen saturation of the rivers in wl1icl1 these salamanders are found is usually around 100 percent (llickerson and Mays, 1973), this decrease in movement at the 75 percent saturation level may be a response of the type hypothesized by Kenny and Rose (1974), whereby the hellbender depresses its activity level to minimize the oxygen demand of the tissues. At 50 percent and 25 percent saturation levels, whereby the oxygen availability is further decreased, the hellbenders may respond b}' moving to avoid the anoxic conditions or to facilitate the respiratory exchange.

The interaction of temperature and photoperiod ha•;e a significant effect on movenient. Higher temperatures increase movement and the rate of increase is greater during dark than during ligl1t. Mrl increases at higher temperatures during dark and decreases at higher temperatures during light. These trends agree with the observations that <u>Cryptobranchus</u> is nocturnal (Bishop, 1941). A combination of higher temperature and lower oxygen during dark increases the moving response. As determined experimentally, moving appears to be a response to unfavorable or stressful conditions with the excepti·on of darkness, which may cause move111er,t as ?c,rt of the nuLrt,al 11octurnal activity pattern of Cryptobra11c11us.

Rocki11g is increased by high temperature or low oxygen in light or dark. The most obvious trend is the increase in periods of rocking as temperature increases. A comparison of periods of rocking at all temperatures and at **all** oxygen levels shows the rate of change most affected by temperature.

The same trends are evident when the effects of oxygen and temperature on minutes of rocking are considered. High temperatures produced much more rocking response than low oxygen saturation levels.

Frequency of rocking is affected by temperature and oxygen in the same way as periods of rocking. The greatest similarity, however, exists between frequency of rocking and minutes of rocking. The exception to this similarity is oxygen. Oxygen seems to exert more of an influence on frequency than on time rocking. Figure 9 shows that from 12°c to 20°C at 75 percent and 100 percent saturation levels frequency of rocking decreases noticeably. T\,elve degrees centigrade appears to be a threshold for the relative importance of oxygen and temperature on rocking.

Since low oxygen and hig11 temperatures cause an increase in moving and rockir1g, both responses could be due to /respiratory stress.

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