THE EFFECTS OF VARIED LIGHTING REGIMES ON REPRODUCTION IN MINK (*MUSTELA VISON*)

Thesis for the Degree of M. S.

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Larry C. Holcomb

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THE EFFECTS OF VARIED LIGHTING

REGIMES ON REPRODUCTION IN MINK (*Mustela vison*)

by

Larry C. Holcomb

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
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ABSTRACT

THE EFFECTS OF VARIED LIGHTING REGIMES ON REPRODUCTION IN MINK (<u>Mustela vison</u>)

by Larry C. Holcomb

The effects of varied lighting regimes on the reproduction of pen-raised mink (<u>Mustela vison</u>) were investigated by two experimental studies during the breeding season of 1961. Experiment I was conducted at the Experimental Fur Animal Station of the Department of Poultry Science on the campus of Michigan State University; Experiment II was carried out at the Dyer Mink Ranch, a commercial operation in Ingham county, Michigan.

In Experiment I, two color phases of mink, sapphire and dark, were individually confined in standard outdoor pens some of which were equipped with electric lights to provide for the occupants illumination after sunset. The mink were divided into groups consisting of 10 dark and 10 sapphire females illuminated before mating, 10 dark and 10 sapphire females illuminated before and after mating, 11 dark and 10 sapphire females illuminated after mating, and 85 control animals consisting of 15 dark males and 10 sapphire males plus 25 sapphire females and 35 dark females. Ten dark and ten sapphire males were illuminated before and after mating. The illuminated groups received daily 82 minutes of extra "daylight" by electric lights after normal sunset. The

females and males used as a control, received only the light normally available to out-door cages.

In Experiment II, color phases of sapphire and pastel were confined individually in pens housed in a shed. The animals were divided into two groups consisting of 16 pastel females and 21 sapphire females illuminated after mating plus 20 pastel and 42 sapphire females receiving no additional illumination. The females illuminated were exposed daily to 62 minutes of extra illumination from electric light after sunset. Control females were exposed only to the light normally available in the roofed shed. Males employed in matings received only normal light.

Males were already sexually active before Experiment I was started on February 1st. No significant differences were noted in the behavior of illuminated males.

Vaginal smears proved that female mink were brought into estrus earlier than normal after increased illumination before copulation. Females subjected to increased illumination beginning on February 1st were receptive to males beginning on February 15. This was 18 days ahead of the control group which were not willing to mate until March 5th although matings were tried daily beginning February 15th.

Females illuminated after mating had a shorter average gestation period and/or had an average of more kits per litter than the control groups. Females illuminated only before mating had much longer average gestation periods than those illuminated both prior to and following mating. Much of the data was significantly different at the .05 level.

Studies of the control group showed clearly a tendency for shorter gestations in the late-mated females and longer gestations in the early-mated females.

Testes and ovaries from sacrificed animals in Experiment I and from casualty mink at a commercial ranch showed progressive growth of these organs at the onset of breeding. No significant difference was noted in the gonad size of illuminated mink sacrificed and control animals.

The reproductive cycle in the sapphire mink seemed more effected by the increased illumination than those of the dark and pastel mink.

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Mr. James Dyer deserves much credit for the results of Experment II carried out at his commercial mink ranch. Finally, I am indebted to the King Mink Ranch for reproductive organs sent us in January.

This project was accomplished while I held a National Defense Graduate Fellowship grant in Zoology made available by the United States Department of Health, Education, and Welfare.

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LITERATURE REVIEW

Considerable research has been conducted on the effects of various lighting regimes on animal reproduction.

Kirkpatrick and Leopold (1952) demonstrated that a daily quota of 10 hours of light, including a one-hour interruption of light at night, causes gonadal activity in bobwhite quail, (Colinus virginianus). Bissonnette (1935) found that when light was decreased from 17 hours to 6 hours per day that the ferret (Mustela putorious) went into anestrus from estrus. Estrus was again resumed when light was increased. He also found that ferrets came into estrus sooner on 14 hours than on 24 hours of light daily. This indicated a need for both light and dark intervals. Bissonnette and Bailey (1936) by increasing the daily hours of light induced three ferrets to come into estrus --- one each in November, December, and January. Two of them actually bred.

Hammond (1951) reported that when mink were subjected to a constant 7-hour light period followed by a constant 5-hour dark period in winter and then returned to natural light at the end of February, the gonads of both females and males were as small in mid-March (the usual mating season) as in mid-summer. He also found that when ferrets were placed on a constant 14-hour or 24-hour light day, those on the former light period came into estrus sooner than those on the latter light period, thus stressing again the need for light-dark intervals.

Pearson and Enders (1944) subjected female mink to 1.5 hours of added illumination after sunset: (1) prior to and following mating, and (2) after mating. A control group not illuminated had a mean gestation of 54.7 days. Ten females, illuminated before and after mating, had a mean gestation of 49.4 days. Nineteen females, illuminated after mating, had a mean gestation of 50.5 days. When these authors subjected seven females to a shorter photoperiod (1.5 hours less than normal), their gestation periods were not appreciably lengthened from animals receiving only normal light.

Hronopulo (1956) conducted lighting experiments in Russia. Three groups of ten female mink each were subjected to: (1) 15 hours of light per day from mating to whelping (more than the normal amount), (2) seven hours of light per day from mating to whelping (less than the normal amount), and (3) natural daylight conditions. The first group whelped an average of 5.9 kits per female whereas those on a natural light day averaged 5.1 young per female. The females on a 7 hour day had an average of only 4.6 kits per litter.

Pearson and Enders (1944) shortened the gestation period in the marten (Martes americana) by steadily increasing the length of the day by artifical light beginning in September. They had been successful in mating three of eight females under increasing artificial light conditions. This was a high percentage when compared with previous experience with captive marten. They believed that an increase in the length of daylight shortened the implantation period and reduced loss of blastocysts in some females.

Hansson (1947) found that increase in the size of mink ovaries and cornua uteri were coincident with the lengthening of periods of illumination and that the length of gestation was decreased with increased illumination.

INTRODUCTION

The basic objective of the experiments to be described was to study the effect of various lighting regimes on the reproduction of pen-raised mink (Mustela vison) so as to acquire information that might aid mink renchers to obtain maximum production from their herds. Comparisons were to be made between illuminated and control animals on the basis of; 1) comparison of vaginal smears in females, 2) size of the reproductive systems in males and females, 3) readiness of males and females to mate, 4) length of the gestation period, 5) number of kits per litter, and 6) weight of kits at birth.

The relationship between the number of hours of light and the hours of dark per day controls the timing of reproductive processes in many animals. The perception of this daily relationship in mink and other mustelids is through the eyes and the optic nerve and is ultimately received by the anterior pituitary gland which regulates gonadal activity (Pearson and Enders, 1944; Bissonnette, 1935). In mink, and in some other members of the Mustelidae, when the egg is released by the ovary and fertilized, it does not implant in the uterine wall until a varied amount of time has elapsed. In the marten (Martes americana) blastocysts are not implanted for a period of several months. In no other family of mammals does the gestation period vary as much as it does in the Mustelidae. The period in mink according to Hansson (1947) varies generally between 39 and 76 days with the average close to 49 days for once-mated females.

The long gestation period in some female mink seems to be due to delayed implantation of the blastocyst. It has been determined that blastocysts may not be implanted for as many as 16 days (Hansson, 1947) or 20 days (Pearson and Enders, 1944) after conception. However, Pearson and Enders (1944) noted that gestation periods are shortest when the females conceive late in the breeding season. Also, these authors determined that the shorter the gestation period the larger the number of offspring produced. This variability in the number of offspring suggests that some blastocysts are never implanted, since Hansson (1947) found that females released an average of $8.73 \pm .30$ ova but produced only an average of $4.37 \pm .005$ young.

This variation may have a logical explanation. Pearson and Enders (1944) hypothesized that the increasing length of days in early spring induces the liberation of follicle-stimulating hormones (FSH) from the anterior pituitary gland, resulting in growth of ovarian follicles and indirectly the preparation of the uterus for implantation. Copulation stimulates secretion of luteinizing hormone (LH) from the pituitary causing mature follicles to ovulate. If copulation occurs early in the breeding season, the FSH secretion at this time from the pituitary is not great enough to raise the estrogen level to a height necessary to prepare the uterus for implantation. These changes probably do not take place until longer days stimulate release of more FSH. Consequently, the implantation of the fertilized egg is delayed. Mink mated late in the breeding season have already obtained sufficient estrogen to prepare the uterus for rapid implantation.

The changes in the photoperiod to which animals are subjected are gradual and continous. For regions outside the tropics, there is a gradual increase in the number of hours of light per day for six months of the year. There is then a gradual decrease in the number of hours of light per day for the other six months of the year. These changes become greater the further one proceeds away from the Equator. The annual regularity of these light cycles is striking. This constancy is far from the case with regard to other environmental factors such as temperature, humidity, air movements, and rainfall. It would not be surprising then, that in the course of evolution, the daily light changes, especially in temperate and frigid zones have been adapted to by animals to insure that the young are born at the most suitable season of the year for survival and development.

So far, it has not been determined whether it is merely the increasing length of the days that effect the mink reproductively or whether it is the constant rate of increase of light per day ranging from early February to early May. There is an increase of from 4 to 19 minutes of light per week from the 6th of January to the 12th of February. From then until May 6 there is a weekly increase of not less than 19 or more than 22 minutes of light. The ranges of increase and decrease in light on both ends of this interval change much faster. An example is the range of from 4 to 19 minutes increase of light per week between January 1 and February 6 and an increase per week of 18 to 3 minutes between May 6 and June 24. (Fig. I)

There are 713 minutes between sunrise and sunset on March 15th and 795 minutes on April 15th -- a difference of 82 minutes. Between February 15th and March 15th and between April 15th and May 15th there are also differences of about 82 extra minutes of daylight, respectively. (Fig. I) Earlier, Hansson (1947) determined that there was a maximum of stimulation to the mink's reproductive tract from the effects of light when there was about 90 extra minutes of illumination per day.

Mink generally mate from early March to early April. This season varies with latitude. Mink farmers in Michigan plan to begin their breeding program between the 5th and 10th of March.

If March light conditions could be produced in February, the breeding season for the mink might be induced to start at an earlier date. Hammond (1951) reported finding sperm in male mink late in December. The object of the experiment was to determine the reproductive capacity of both males and females during February. It was desirable then to compare the mating behavior of illuminated and control mink. Would increased light stimulate gonad growth in both sexes and hasten the breeding season in mink? Could female mink be brought into estrous faster with increased illumination?

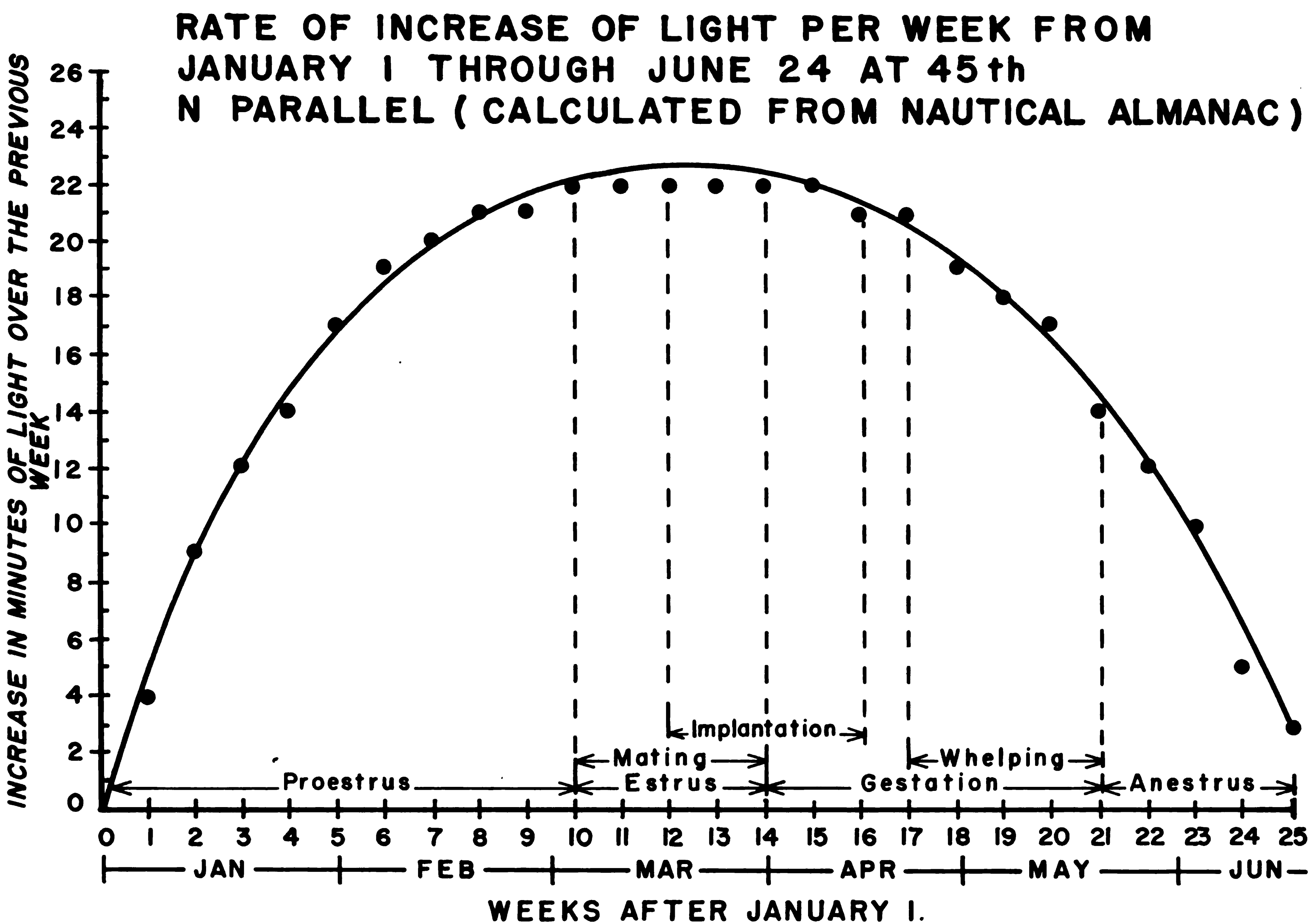

RATE OF INCREASE OF LIGHT PER WEEK FROM JANUARY 1 THROUGH JUNE 24 AT 45th N PARALLEL (CALCULATED FROM NAUTICAL ALMANAC)
INCREASE IN MINUTES OF LIGHT OVER THE PREVIOUS WEEK
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Implantation
Mating
Whelping
Proestrus
Estrus
Gestation
Anestrus
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
JAN
FEB
MAR
APR
MAY
JUN
WEEKS AFTER JANUARY 1.

Figure I

If the female mink could somehow be artificially given extra light, would the fertilized ova implant quicker? Would the female have a shorter gestation period and more kits per litter because of the decreased implantation period? These were some of the questions which various lighting regimes might help to answer.

METHODS AND PROCEDURES

Experiment I was carried out at the Fur Animal Experiment station under the supervision of the Department of Poultry Science and on the campus of Michigan State University. Experiment II was conducted at the Dyer mink ranch near Lansing, Michigan.

The mink on Experiment I were in an outside environment. They were in standard mink breeder cages about 2' by 2' by 18", and were exposed to the weather, except for a nest box and a sheet of tar paper roofing over the top of the cage. Mink in Experiment II were in similar cages to those in Experiment I but they were inside a shed-type building. Mink in both Experiments were fed the same commercial mink ration.

Experiment I was started on February 1, 1961 and Experiment II on March 10, 1961. According to Yeates (1954) artificial light, if produced too quickly, is not effective on males. Consequently, precautions were taken in both experiments so that the increase in daylight time was done slowly over a ten day interval. Only those females lighted after mating in Experiment I, were subjected to the 82 minutes of extra light per day from the start.

To make a lighting schedule, it was necessary to find sunrise and sunset at the latitude of East Lansing, Michigan. The mink were given the extra illumination at the evening hour only. Yeates (1954) and Stevenson (1946) believe that civil twilight is the barrier between stimulation and non-stimulation by light. This is that time at dusk and sunrise when outside work can be carried on without artificial light. One or two foot candles seem to be all that is necessary. The mink received the sunrise light but not the extra amount which they would have received after sunset, since the lights were turned on exactly at sunset. It was later determined that after sunset natural light, equal to or more than 2-foot candles, was present from 10 to 20 minutes after sunset, depending on the weather conditions.

The light used on Experiment I were out-door, 7-watt, white incandescent bulbs. They were spaced along the front of the cages at least one to each cage and provided a minimum of 2-foot candles of light at the opening of the nestbox. The mink received 5-foot candles when they were near the light.

In Experiment II, 75-watt, bulbs were spaced, one above each five cages throughout the shed building. There was a minimum of 2-foot candles of light.

The lights were turned on and off manually in both experiments and checked daily for burned out bulbs. Light intensity in both experiments was checked with a light meter. There was some difficulty at the onset of Experiment I for the animals liked to chew through the electric wires. Fortunately this did not happen when the lights were on.

March 5th was the only day on which the lights were not turned on (Experiment I). On April 18th the lights were left on all night. Both of these incidents were accidental and it is impossible to ascertain their effect, if any, on the results of the experiment.

Males and females of both sapphire and dark mink were used on Experiment I. Males and females of both sapphire and pastel mink were used on Experiment II.

The mink chosen for Experiment I and for different aspects of the experiment were selected by the use of random digit tables and numbered coins so as to prevent biased selection.

In Experiment I the female mink were divided into four groups: 10 sapphire and 10 dark females lighted before mating, (LN); 2) 10 sapphire and 11 dark females to be lighted after mating, (NL); 3) 10 sapphire and 10 dark females plus 10 sapphire males and 10 dark males lighted both before and after mating, (LL); 4) and 85 control animals consisting of 15 dark males and 10 sapphire males plus 25 sapphire females and 35 dark females receiving no extra light (NN). (See table I) The lighted groups in this experiment were all given 82 minutes of illumination after sunset. Under these conditions a daily light schedule 30 days in advance was simulated.

The mink on Experiment II were divided into two groups; (1) 16 pastel and 21 sapphire females to be lighted after mating (NL), and (2) 20 pastel and 42 sapphire females to receive no extra light (NN). The males on Exp. II received no extra light. The lighted groups in this experiment were given 62 minutes of illumination after sunset. This advanced the daily light schedule by 21 days.

In subsequent discussions, the above symbols will be used with one asterisk if mated once; with two asterisks if mated twice. Comparisons were made between like color phases. There were only four mink in some classes because of limited cage space and numbers of mink available (see Table I).

Vaginal smears from illuminated and control females in Experiment I were checked weekly to determine the onset of estrus. The same animals from each group were checked each time to determine progression in development. Five animals in the illuminated group and five animals from the control group were picked at random for this purpose.

In taking the vaginal smears one man held the female mink with thick leather gloves. A second man inserted a few drops of saline solution into the vagina. To get a good smear, the contents from the vagina were placed on a slide and allowed to dry around the edges. It was then labeled and placed in a solution of alcohol-ether-water fixative until ready to stain by the Papanicolaou method.

Readiness of males and females to mate was determined by placing a male with a female, always of the same color phase, and observing the behavior of each animal. Beginning February 8 and continuing until March 4 (the date which control groups normally began mating freely), special attention was given to observing mating behavior between a sample of lighted females and control females when placed with either lighted or control males.

TABLE I. Female Mink Mated In Each Experiment (Experiment I)

Color phase	(LL)*	(LL)**	(LN)**	(NL)**	(NN)*	(NN)**
Dark	5	4	8	11	12	23
Sapphire	5	4	10	10	11	14

Experiment II:

Color Phase	(NL)*	(NN)*
Pastel	16	20
Sapphire	21	42

Unfortunately, proven males were not chosen to be illuminated in Experiment I. All of the ten dark males and six of ten sapphire males picked randomly were yearlings and had never experienced a mating. This had a decided effect on the number of mating attempts, for even though the young males often-times attempted a mating, they were easily discouraged by the females and were generally unsuccessful.

If one mating had taken place, efforts were made in all cases to mate the female a second time. At least two matings are needed to help insure a fertile mating (Hansson, 1947, and Johansson and Venge, 1951). The second mating sometimes occurred the day following the first mating. Most frequently, once mated females were serviced again after an eight day interval. In drawing conclusions from the data obtained, these twice mated females in the different groups were compared.

In Experiment I, if copulation took place between a male and female, the female was checked to make absolutely sure that sperm were present in the vagina. This was determined by use of the vaginal lavage procedure. The vaginal material was placed on a glass slide, covered with a cover glass and observed for sperm under a microscope. In Experiment II, sapphire females were checked for sperm but the pastels were not. The average number of kits per litter was based on total kits whelped and total females mated in each group.

The significance of the effect of the increased illumination was tested with the t-distribution, using a one-sided test at the .05 level. Also, the 80 and 95 per cent confidence intervals of the difference between the various lighting regimes were calculated to determine whether or not the increased illumination offered sufficient improvement in the normally variable reproductive cycle to be economically useful to the commercial mink rancher.

RESULTS

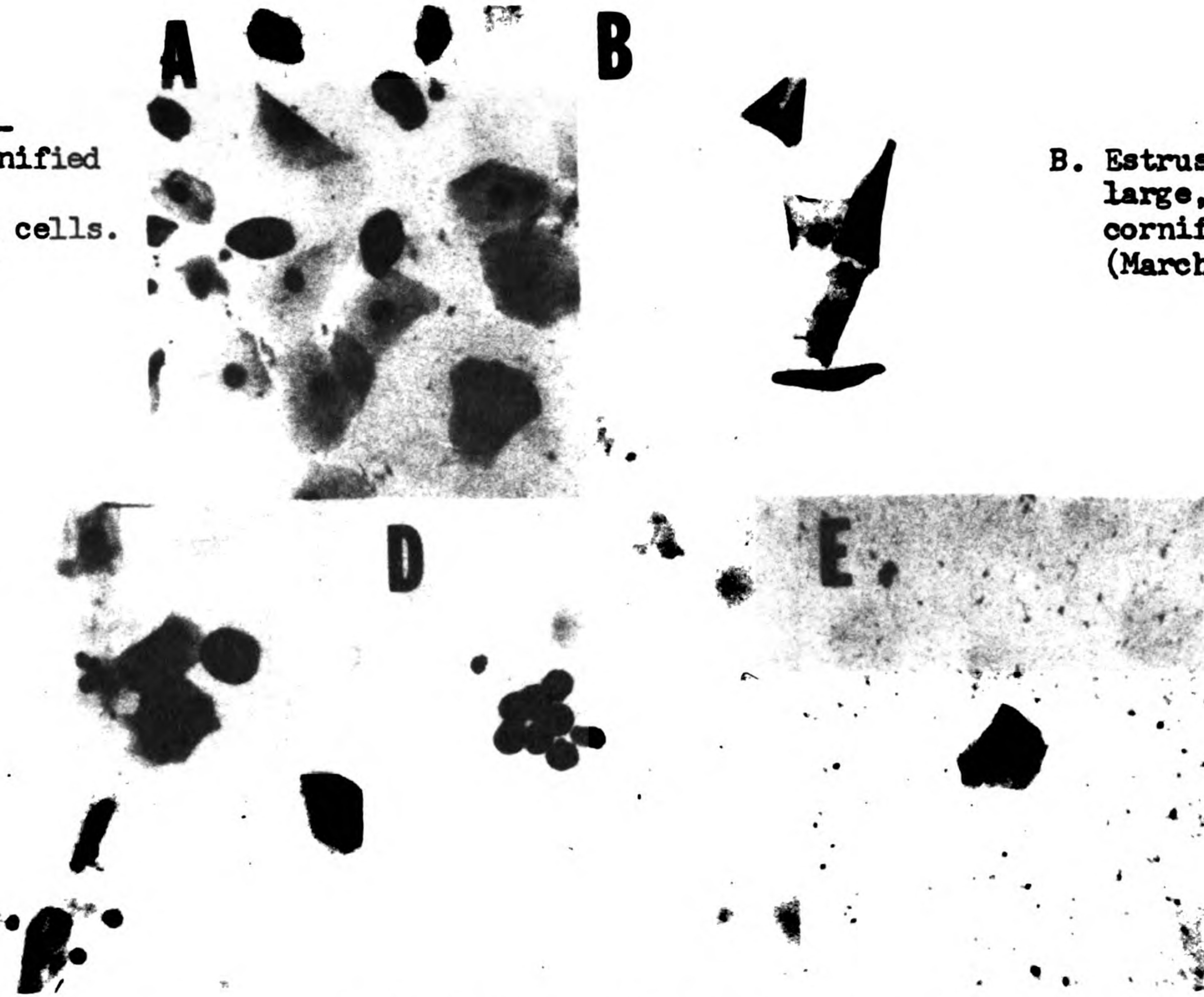
Vaginal Smears - Smears were taken at least once a week from February 1 through April 25. Earlier, in September to November, according to Hansson (1947), females are in anestrous and have little vaginal secretion. Some epithelial cells, a few leucocytes and cell detritus are present (see Hansson for a thorough discussion of materials in smears).

Toward the end of January, this anestrous stage passes gradually into a proestrous stage. On smear slides we found that delamination and secretion of cells was on the increase and larger cornified cells beginning to appear dark blue when stained.

From proestrous on to estrous the vaginal secretion changes and cornified cells increase. These cells have a thinner appearance and when stained they have a tendency to roll up at the corners. The changes in proestrus are very slow indeed.

After ovulation there is a marked tendency for an increase in the cornified irregular shaped cells that persists for 7 or 8 days. This is characteristic of the estrus picture.

Before the final disappearance of the estrus picture there is a considerable increase in the cornified cells in the smear. This is followed by an invasion of leucocytes. At this time, small, round, epithelial cells are clearly visible. During pregnancy the contents of the vagina resemble that of anestrous.



A. Proestrus – large, cornified and small epithelial cells. (February)

B. Estrus – large, thin cornified cells. (March)

C. Early Leucocytic Invasion – groups of polymorphonuclear among cornified cells. (Late March)

D. Late Leucocytic Stage – enlargement, scattering of the leucocytes. (Early April)

E. Anestrus – lack of cells; nucleus may or may not be visible. (July)

Plate I. Cells in vaginal smears of mink at different stages during the reproduction cycle.

Microscopic observations of slides confirmed mating results. The first of the (LL) and (LN) females showed indication of early estrus on February 15th. On this day, three of the (LL) and (LN) females were mated. Previous to this time all of the females had been in a proestrous condition.

By March 1st all of the (LL) and (LN) mink from which smears were taken had shown signs of estrus. All but four of the (LL) and (LN) groups had been mated. By March 8th, the smears indicated that vaginal cells were going through the leucocytic invasion stages. The others were in late estrus. By March 15th all the (LL) and (LN) females had leucocytes present in smears. From then through April 25th there were changes resulting in what seemed to be anestrus.

Smears from (NN) females first showed indications of estrus on March 8th. Matings took place first on March 5th. By March 15th most females had reached late estrus. Many of the smears indicated the leucocytic stage. From March 23rd through April 25th there was a gradual change resembling that taking place in the (LL) and (LN) females.

Gonad Development - There was an increase in size of testes and ovaries taken from males and females as the reproductive organs matured. Eight female casualties in January from a commercial mink ranch had individual ovaries ranging from 36 to 112 mgs. A female sacrificed on February 1st had ovaries weighing respectively, 140 mgs. and 146 mgs. A (LL) female that had a broken leg was sacrificed March 3rd and had ovaries averaging 283 mgs. A (NN) female sacrificed March

3rd had ovaries averaging 434 mgs.

A male sacrificed on February 1 had testes weighing an average of 1796 mgs. During the breeding season in March, 1961, male mink had individual testes with a minimum weight of 2001 mgs. and a maximum weight of 3512 mgs. From a male sacrificed in September, 1961, testes were taken weighing only 496 and 611 mgs.

Early Mating Results: - Experiment I - Detailed observations of early matings and attempts were made only in Exp. I. On February 8th, 8 days after extra light was first added, varied combinations of matings of male-female mink were attempted. These meetings, were between (LL), (LN), and (NN) females and (LL) and (NN) males. Of 12 meetings, not one female was at all interested in mating. Of the six (NN) males used, two tried hard to mate; the other four seemed indifferent. Of the six (LL) males, only one tried to mate.

On February 15th, 12 meetings of males and females once more took place. Of six (LL) and (LN) females, four seemed ready and three actually mated. The vaginas of these females were found to contain sperm. The six (NN) females were unreceptive; one of them was mated without conceiving by an older, experienced male.

On February 19th, 11 meetings of males and females took place. The five (NN) females were not willing to mate. Three of six (LL) and (LN) females were mated and their vaginas contained sperm. The males all seemed willing to mate. On February 21st, six matings were tried on (NN) females.

All of the males tried to mate but the females were not receptive.

February 26th was the last date on which full observations were taken of 12 meetings between (LL) and (NN) males and females. The six (NN) females were unwilling to receive the males. Five of the six (LL) females were mated; their vaginas contained sperm. All of the males eagerly tried to mount the females.

As of March 4th, all of the (LL) and (LN) females had been mated at least once except for two females. One had a broken leg; the other was killed by the male in the mating attempt.

A total of 68 matings in 200 attempts took place on the 38 (LL) and (LN) females between February 8 and March 4th. This was 34% success. Thirty-two of these matings were by (NN) males and 36 by (LL) males. Attempted matings were tried on (NN) females every day while the illuminated females were mating. However, only three matings took place from a total of 81 attempts on the (NN) females before March 5th. This was only 4% success. Even more significant results may have been possible had males which were proven breeders been used with greater frequency on the (LL) and (LN) females. (LL) males were used whenever possible.

There were three matings in the (LL) and (LN) groups on February 15th -- approximately three weeks ahead of the normal mating time. (see Table II) Mating of these groups proceeded at a regular rate and increased to a maximum on February

23 when nine matings took place. The final mating in these groups was on March 4.

Fifteen days after there was an increase in illumination on the (LL) and (LN) groups, there took place a mating season quite normal in appearance. This season had a duration of 17 days.

In the (NN) group, there was one mating on the 15th, one on the 21st and one on the 23rd of February. None of these resulted in pregnancy. There were no more matings until March 4th when this group seemed to come into their regular estrous cycle. (see Table II.)

In all of the dark female groups, except for the (LN) females, at least 75% of the matings were made by (NN) males. In the group of (LN) females, 50% were mated with lighted males. Surprisingly, this group was the one which had a higher kit average than any of the others even though females in the group had the longest average gestation period. (see Table IV)

Although the majority of matings were made by (LL) males in the sapphire groups, the litters that were a direct result of these matings had a tendency to be smaller than those that were the result of matings by (NN) males.

Females mated early in the breeding season had a tendency for longer gestation periods while females mated late in the breeding season had a tendency toward a short gestation. (Figures II and III) Time of mating and whelping, gestation period length, kits per litter, and wt. of kits at birth in Experiments I and II are shown in Tables II through V.

THE EFFECT OF ARTIFICIAL ILLUMINATION ON THE

MATING AND WHELPING DATES OF MINK

Table II.

Experiment I:

Groups	Range of Dates of Matings	Range of Dates of Whelping
(LL)	Feb. 19 - Mar. 7	Apr. 11 - May 4
(LN)	Feb. 15 - Feb. 28	Apr. 11 - May 8
(NL)	Mar. 5 - Mar. 22	Apr. 25 - May 6
(NN)	Mar. 6 - Mar. 26	Apr. 23 - May 15

Experiment II:

(NL)	Mar. 7 - Mar. 27	Apr. 27 - May 13
(NN)	Mar. 7 - Mar. 25	Apr. 29 - May 21

EFFECT OF VARIOUS LIGHTING REGIMES

ON REPRODUCTION IN MINK (SAPPHIRE)

Table III
Experiment I

		Sapphire females				
Times mated	No. bred	Gestation period*	No. whelped	% whelped	Litter av.**	Av. wt. of kits at birth
Once (LL)	5	56.3	3	60	2.8	9.75
Twice (LL)	4	48.0	4	100	4.3	8.99
Twice (LN)	10	62.0	4	40	1.3	9.88
Twice (NL)	10	45.3	8	80	4.8	10.14
Once (NN)	11	49.4	7	64	2.5	9.28
Twice (NN)	14	44.3	9	64	2.6	9.43

* Based on last mating

** Based on total females mated

EFFECT OF VARIOUS LIGHTING REGIMES

ON REPRODUCTION IN MINK (DARK)

Table IV
Experiment I

			Dark females			
Times mated	No. bred	Gest. period*	No. whelped	% whelped	Litter av.**	Av. Wt. of kits at birth gm.
Once (LL)	5	48.0	3	60	3.4	8.76
Twice (LL)	4	42.3	3	75	4.5	10.19
Twice (LN)	8	54.8	8	100	5.8	8.73
Twice (NL)	11	44.9	11	100	5.2	9.67
Once (NN)	12	49.6	11	92	4.7	9.56
Twice (NN)	23	46.2	19	87	3.8	8.57

* Based on last mating

** Based on total females mated

EFFECTS OF VARIOUS LIGHTING REGIMES

ON REPRODUCTION IN MINK

Tabel V
Experiment II

Color phase	Times mated	No. mated	Gest. period*	No. whelped	% whelped	Litter av.**
Pastel (NL)	once	16	52.2	13	81	4.2
Pastel (NN)	once	20	56.7	16	80	4.2
Sapphire (NL)	once	21	52.0	13	62	3.1
Sapphire (NN)	once	42	52.0	13	31	1.4

*Period in days

**Based on total females mated

DISCUSSION

According to Hansson (1947), the last mating is the one from which the litter of kits generally originates. Occasionally, there are cases of superfetation in which a split litter occurs, but he considered this quite rare. In the experiments where males of different mutations were used to determine whether the first or second mating was the one from which the litter arose, the last mating proved to be the one in which final pregnancy occurred in 25 of 27 cases.

Johansson and Venge (1951) also used different mutant strains to determine which mating produced the offspring. They found more kits resulted from the second mating if the latter was eight days after the first mating. However, there was a percentage of the kits from the first mating in every case. This would establish superfetation as the rule rather than the exception.

There were six basic measures with which we tried to interpret results of the experiment:

1. Comparison of vaginal smears in females.
2. Size of the reproductive systems in males and females.
3. Readiness of males and females to mate.
4. Length of the gestation period.
5. Number of kits per litter.
6. Weight of kits at birth.

The change from proestrus to estrus as shown by vaginal smears is a fine distinction. There are sometimes fewer small, epithelial, rounded cells in the estrous smear than in the proestrous smear.

The entire smear record indicated that the (LL) and (LN) females were further progressed at all stages than the (NN) females. From data observed, there can be no doubt that there is an increase in the size of reproductive organs at the onset of the breeding season although there was no discernable significant differences between the illuminated and control groups for either males or females.

It was difficult to determine whether (LL) males showed a significantly greater mating drive than the (NN) males. It is very possible that all the males were in breeding condition when the experiment was started since the male sacrificed February 1st had sperm in the testes.

<u>(LL) and (LN) Females</u> - That the mating season could be hastened by illuminating females before mating was shown both by smear observations and mating results. The decrease in light on the (LN)** females by exposing them only to normal daylight after mating, lengthened the average gestation period in both the dark and sapphire females.

Dark Females, Exp. I - The difference between the average gestation periods in the (LL)** and (LN)** dark females indicates variation in time of implantation. The 42.3 day average gestation period in the (LL)** group and the 54.8 day average gestation period in the (LN)** group were significantly different at the .05 level. The upper and lower 95% confidence limits for the differences between the means of the gestations in the (LL)** and (LN)** groups were respectively

20.8 and 4 days. Both (LL)** and (LN)** groups of dark females had high litter averages. In the (LL)** group this could be explained as possibly due to a high estrogen level. The average larger size of the kits in the (LL)** group also indicates that the fertilized ovum may have been implanted sooner due to an increase in the estrogen level.

Sapphire Females, Exp. I - There were longer average gestation periods in the (LN)** sapphire females than in (LL)** females. The differences in the lengths of the average gestation periods of these two groups were significantly different at the .05 level. The upper and lower 95% confidence limits for the differences between the means of the gestation periods in the (LL)** and (LN)** groups were respectively 27.2 and .79 days. The 4.3 litter average in the (LL)** group and the 1.3 litter average in the (LN)** group were significantly different at the .05 level. The upper and lower 95% confidence limits for the differences between the means of the number of kits per litter in the (LL)** and (LN)** groups were respectively 5.35 and .55 kits. Both the decreased length of the average gestation period and increased average number of kits per litter in the (LL)** females when compared to those of the (LN)** females may have been due to an increased estrogen level permitting faster implantation of fertilized ova.

The increase of illumination to the (LL)** group of females of both dark and sapphire color phases may have resulted in an increase in follicle stimulating hormone production from the anterior pituitary and a resultant increase of estrogen level that readied the uterus for rapid implantation of fertilized ova. Those female mink subjected to normal daylight after mating may have had a decrease in estrogen as a result of the decreased stimulation of the anterior pituitary and therefore the uterus may not have been readied for implantation until longer periods of daylight produced the needed stimulation.

Sapphire and Dark Females, (LL)* and (LN)* - The (LL)* sapphire and dark females had longer average gestation periods than comparable twice-mated females. The once-mated females also averaged less kits per litter than twice-mated females. The longer average gestation period in the once-mated females may have resulted from the early mating of these females when the uterus was not ready for fast implantation. Less average kits per litter in the once-mated females may have been a result of intrauterine mortality because of the longer average gestation period. (Hansson, 1947)

<u>(NL) and (NN) Females</u> - Females illuminated after mating were compared with those used as controls to discover if the increased illumination had shortened the implantation period and thus produced shorter gestation periods, more kits per litter, and larger kits from the illuminated females.

Dark Females, Exp. I - The average gestation period was shorter, the average litter size was larger, and the average kit weight was larger in the (NL)** dark females when compared to the (NN)** dark females. The (NL)** females had 1.4 more kits per litter on the average. The litter average in the (NL)** and (NN)** females was very close to being significantly different at the .05 level. The upper and lower 80% confidence limits for the difference between the means of the number of kits per litter in the (NL)** and (NN)** groups were respectively 2.11 and .69 kits per litter. At the .05 level, the kit weights were significantly different in the (NL)** and (NN)** group. The upper and lower 95% confidence limits for the difference between the means of the weights of kits in the (NL)** and (NN)** groups were respectively 2.07 and .23 grams.

Sapphire Females, Exp. I - The gestation length was much the same in the (NL)** and (NN)** sapphire females. The average litter size was larger, and the kits were larger in the (NL)** females than the (NN)** females. The upper and lower 80% confidence limits for the difference between the means of the kits per litter in the (NL)** and (NN)** groups were respectively 3.31 and .65 kits per litter. The upper and lower 80% confidence limits for the difference between the means of the weights of kits in the (NL)** and (NN)** groups were respectively 1.38 and .09 grams.

Once-mated Females, Exp. I - There was not a great deal of difference in production of (NN)* and (NN)** dark females. The same situation held true in the (NN)* and (NN)** sapphire females. The average gestation periods were somewhat shorter in the twice-mated females, possibly because the second mating was later when the estrogen level may have been higher.

Pastel Females, Exp. II - The (NL)* pastel females had a shorter average gestation period than the (NN)* females. The (NL)* and (NN)* females both had an average of 4.2 kits per litter.

Sapphire Females, Exp. II - The (NL)* sapphire females had a shorter average gestation period than the (NN)* females. The (NL)* females had a significantly different litter average than the (NN)* females at the .05 level. The upper and lower 95% confidence limits for the difference between the means of the kits per litter in the (NL)* and (NN)* groups were respectively 2.91 and .47 kits per litter.

Compared Results of Years 1959 and 1961 at M.S.U. - From records kept in past years at the Fur Experimental Station at Michigan State University, comparisons were made of the production in 1959 and 1961. Dark (NN)** females in 1959 had an average gestation period of 44.9 days and a litter average of 3.94 kits. At birth, these kits only weighed an average of 7.4 grams. The average number and weight of the kits were somewhat less than that of the mink that were illuminated after mating in 1961. The average gestation periods for dark (NN)** and (NL)** females were almost identical; 44.91 days in 1961 as compared with 44.9 days in 1959. However, the (NL)** females in 1961 were the first mink mated in this group and all of them had mated the second time before the 16th of March. Pearson and Enders (1944) showed progressively shorter gestations for animals breeding later in the season. Figures II and III bear resemblance to a graph by Pearson and Enders (1944). Since these (NL)** females were mated early in the season they probably would have longer gestation periods due to a longer delayed implantation period. Those (NN)** females in 1959 all were mated by March 30th. These later mated females would probably have a shorter delayed implantation and thus a shorter gestation period. (NL)** females in 1961 may have had a much shorter average gestation period had they been mated later in the season.

The sapphire (NN)** females in 1959 at Michigan State University had an average gestation period of 44.5 days and had an average of 4 kits per litter, each averaging 7.4 grams in weight. Comparable (NL)** females in 1961 had an average gestation period of 45.3 days, and an average litter size of 4.8 kits averaging 10.14 grams in weight. The average gestation period in the (NL)** females in 1961 was longer than (NN)** females in 1959, however those (NL)** females in 1961 were mated much earlier in the season than those (NN)** females in 1959. Those mated later in the season may already have a high estrogen level and this may allow faster implantation. Even though the mink in 1961 were illuminated after mating it might take some time for this extra illumination to take effect and raise the estrogen level by increased FSH production from the pituitary gland.

<u>Discussion of Combined Results</u> - One sapphire female had a shorter gestation period than any previously reported. She had a gestation of 33 days, was in the control group and had a litter of five small but otherwise healthy kits.

Why the sapphire mink responded reproductively with more

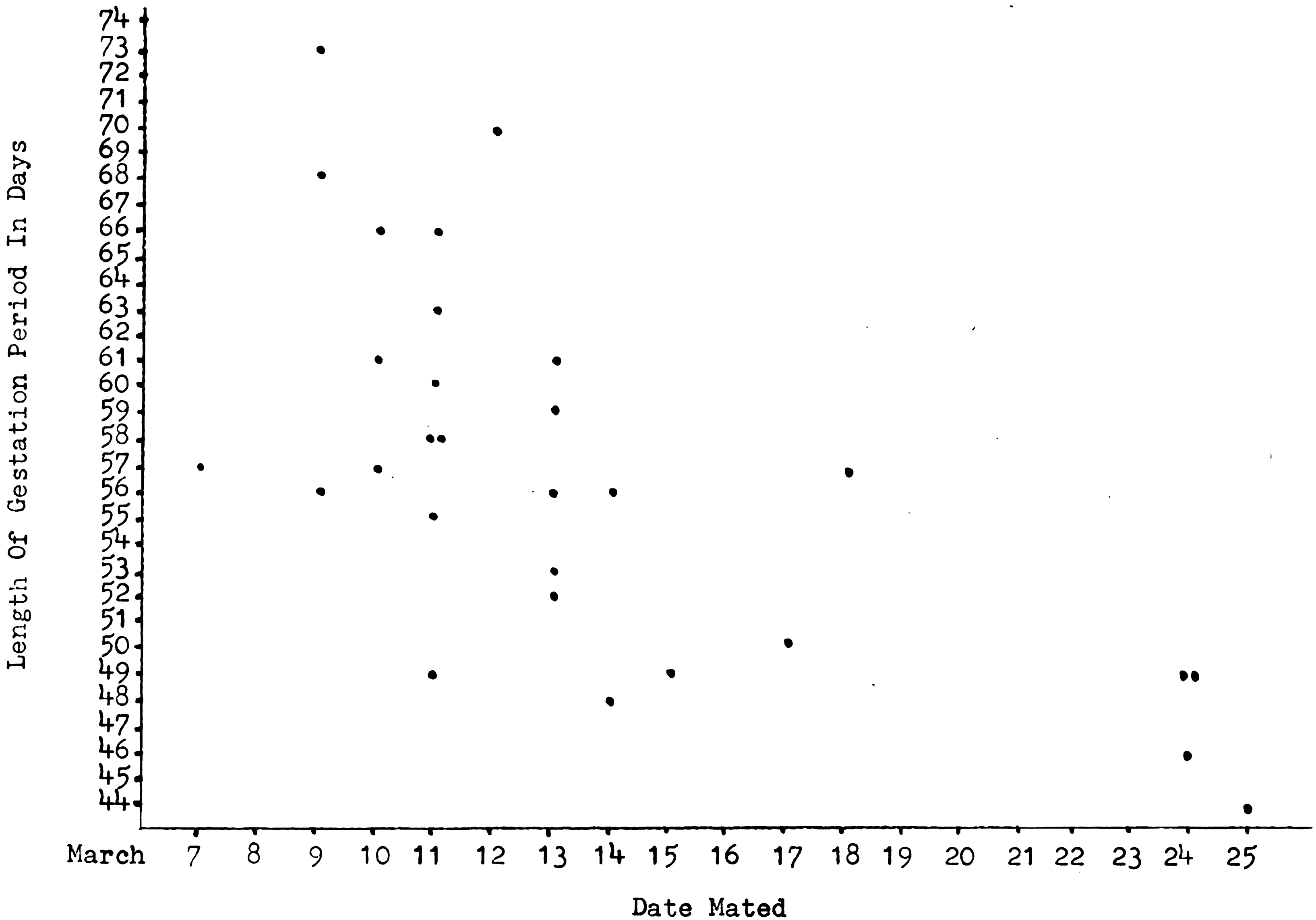


FIGURE II. Comparison Of Date Mated And Length Of Gestation Period For (NN)* Mink In Experiment II (1961)

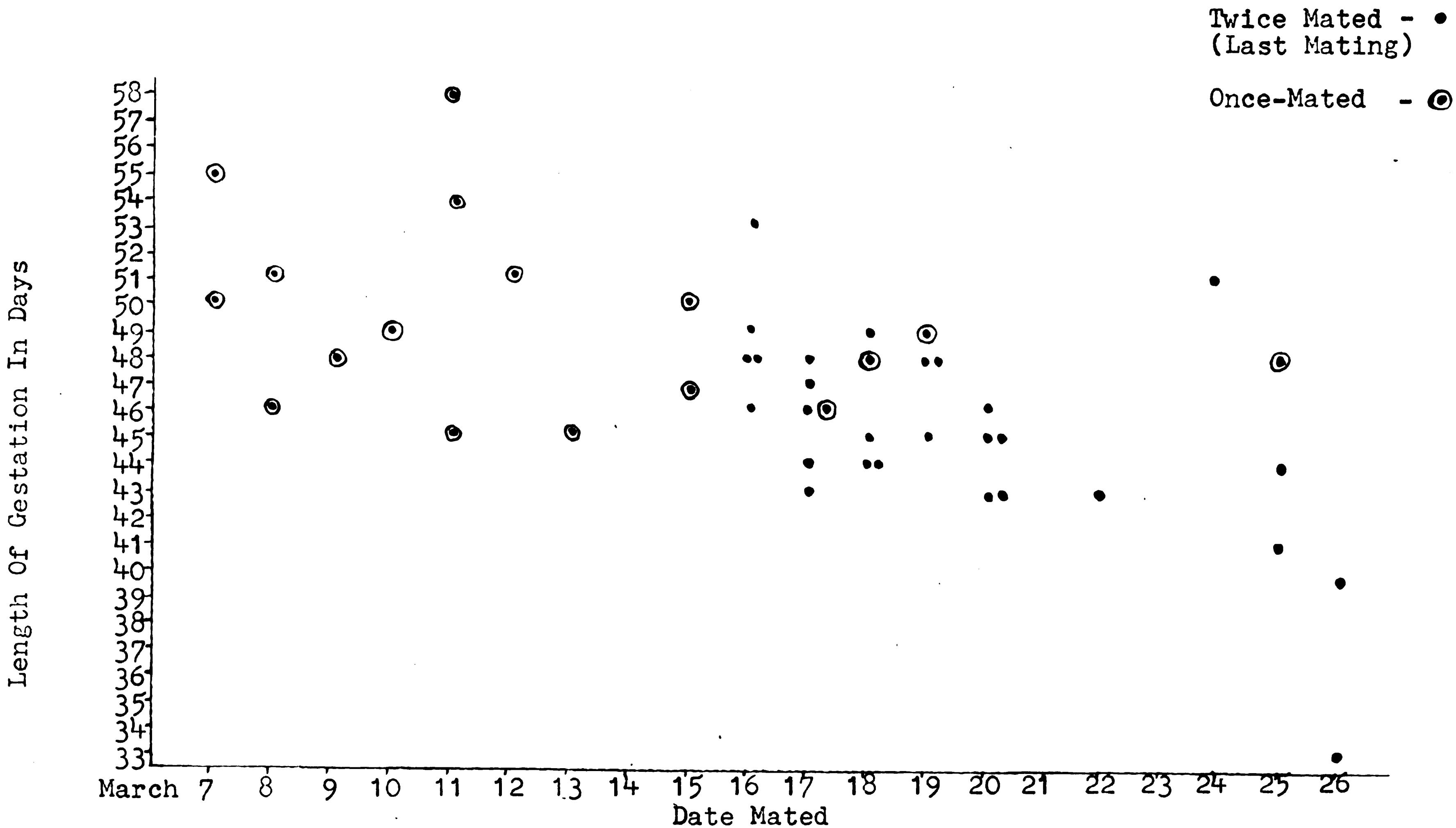


FIGURE III. Comparison Of Date Mated And Length Of Gestation Period

For (NN)* And (NN)** Mink In Experiment I (1961) vigor to illumination than the dark and pastel females is not fully understood. It is possible that because less pigmentation is present in the eye of this pink-eyed mutation, the animals may respond quicker to an increase of light and thus the effects show more readily. The dark and pastel mink have a dark eye with normal pigment present. This would allow only normal amounts of light to enter the eye and a change in duration of light might not be as noticeable to the animals.

The changes in light duration were reacted to with vigor when sapphire females in Experiment I were removed from extra illumination after mating. Of eight dark (LN)** females taken off light after mating, all eight whelped and had a high litter average of 5.8 kits but they had a long gestation period of 54.8 days as compared with the 42.3 day average gestation of the (LL)** dark females. This was the reverse of the results expected concerning the number of kits produced in the dark (LN)** females. Of ten sapphire (LN)** females only four whelped and of those having kits, there were but 1.3 kits per litter on the average. These animals had an average gestation period of 62 days as compared with that of the 48 day average gestation period for (LL)** sapphire females. These results seem to indicate that the sapphire females may have detected the light duration change with greater rapidity through the eyes and were thus more effected.

Hansson (1947) found that there were fewer eggs ovulated in mink at the beginning than at the middle of the mating season. Females mated early would ovulate less eggs to be fertilized. If a female were mated before the estrogen level was high enough for quick implantation, there might be a lengthened gestation period and less kits per litter as a result. The female has few kits per litter as compared to the eggs ovulated. This may be due to re-absorption of the fertilized ovum and intrauterine mortality. (Hansson, (1947) Fertilized ovum are possibly reabsorbed while lying in wait for implantation. Blastocysts that are implanted may be physiologically unfit for further development due to a long delay in implantation. The increased illumination after mating may have resulted in an increase of hormonal production from the anterior pituitary which may have caused less delay in implantation and a shorter gestation period. As a result more and larger kits were produced in most cases.

Some of the results discussed previously which were not significantly different at the .05 level in favor of the illuminated groups, were very near to being significant. The small numbers involved and the large variances in litter sizes and kit weights were factors effecting signigicance of differences. When results of the experiments were subjected to tests involving 80% and 95% confidence intervals of the differences between lighting regimes, there was but one instance when at least 80% confidence was not found in favor of added illumination for better production. This one exception was in the Experiment II pastels. The (NL)* pastel females in Experiment II may have had a larger litter average than the (NM)* females if they had only been exposed to a longer illumination period. The 62 minutes of extra illumination they did receive may not have been sufficient for extra stimulation to the pituitary for promotion of any noticeable differences.

The results indicate that the use of added illumination would be of practical importance to the mink rancher because there is a good possibility that he would get more and larger kits from the illuminated (sufficiently) females with very little extra financial output.

SUMMARY

Mink (<u>Mustela vison</u>) were the subjects in experiments using varied lighting regimes. Some mink were exposed to increased illumination 1) before and after mating, 2) before mating only, and 3) after mating only. Control groups were kept on normal light. Some males also were illuminated.

Two experiments were completed. Experiment I was at the Fur Experimental Station under the direction of the Department of Poultry Science at Michigan State University where illuminated mink received 82 minutes of extra light after sunset. Experiment II was at a commercial mink ranch where illuminated mink received 62 minutes of extra light after sunset. These experiments started on February 1, 1961 and were terminated by May 21, 1961.

Dark mink and sapphire mutations were used in Experiment I. Sapphire and pastel mink were employed in Experiment II.

Female mink were in normal size breeder cages in both Exps. Mink in Exp. I were outside and mink in Exp. II were sheltered by a shed.

Mink in Exp. I were illuminated with white outdoor, 7-watt incandescent lights. Seventy-five-watt bulbs were placed at 15 foot intervals in the shed for illumination of Exp. II females. At least 2-foot candles of light reached the mink at the opening of the nest box.

Results of Exp. I were to be dependent on:

1. Comparison of vaginal smears in females.
2. Size of reproductive systems in males and females.

3. Readiness of males and females to mate.

4. Length of gestation periods.

5. Litter size.

6. Weight of kits at birth.

Results of Experiment II were to be analyzed by comparison of length of gestation period and litter size.

The effects of illumination on the males in Experiment I were not noticeable. It is supposed that they were in breeding condition when the added illumination was started on February 1st because the male sacrificed had sperm in the testes.

The estrous condition in the female mink can be followed by smear slides showing an increase in cornified cells from the vagina. The estrous condition was brought on earlier by the use of extra illumination per day and was coincident to the hastening of the mating season.

Quantitative study of gonads indicated an increase of growth in testes size and ovary size at the onset of the breeding season. It was not determined if increased illumination had an effect in stimulating growth.

In Experiment I, dark females illuminated only before mating had longer average gestation periods but averaged more kits per litter than dark females illuminated both before and after mating. Those sapphire females illuminated both before and after mating had shorter average gestation periods and averaged more kits per litter than those illuminated only before mating.

In Experiment I, females illuminated only after mating averaged more kits per litter than the control groups. In Exp. II, sapphire females illuminated after mating averaged more kits per litter than the control group, while the pastels illuminated after mating had an identical average litter size as those in the control group.

Weights of kits from litters of Exp. I females illuminated after mating were noticeably larger, although these females had a short average gestation period. This may have been due to quicker implantation of the fertilized ovum because of the effects of the increased illumination on the gonadal hormone production of the anterior pituitary.

Statistical support from our data strengthens the hypothesis that increasing and decreasing illumination is the regulating factor controlling reproductive growth, initiation of breeding season, and implantation of the fertilized ovum in the uterus.

Sapphire females seemed to respond with more vigor reproductively to the increase in illumination than darks and pastels.

Females mated early in the breeding season had longer gestations than those mated later.

The results of experiments I and II when subjected to 80% and 95% confidence intervals of the differences between lighted and unlighted groups indicate that the use of added illumination would be of practical importance to the mink rancher.

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