EGG MASS DISTRIBUTION AND CLUTCH PARAMETERS IN *RANA PIPIENS* AT SAWHILL PONDS, COLORADO

Lauren J. Livo
Independant Project
Ecological Methods
Spring, 1978

INTRODUCTION

Organisms may be studied in relation to several types of possible distribution: 2-dimensionsl (surface) distribution, 3-dimensional (surface plus distance above or below some interface) distribution, and distribution in time.

Different reproductive strategies will be reflected in differing utalization of the physical and temporal environment as well as in variable physiological components such as ovum size, clutch volume and ova per clutch. The same individual may vary in its response over its lifetime.

The egg masses of the leopard frog, *Rana pipiens*, were studied in the spring of 1978 to determine what relationships existed between clutch distribution in time and space and clutch volume, ova per clutch, and ovum size.

MATERIALS AND METHODS

Observations of a breeding aggregation in Pond #7 of Sawhill Ponds were made from 17 March 1978 to 14 April 1978 at intervals of from one to three days through 31 March 1978, and approximately weekly thereafter. Limited observations from 1976 and 1977 are also included. Unless noted, the data are from 1978.

The presence of calling males was noted, and a visual search made for clutches along the shore and in the breeding area. When egg masses were discovered the distance from the center of the mass to the nearest center of another mass was measured, as was the water depth and distance from the top of the egg mass to the water surface (water clearance).

The egg mass was put into a graduate cylinder and the volume measured to the nearest mililiter. A numbered tag was then looped around the egg mass and tied to vegetation as near as possible to the site of discovery. A small sample of eggs taken from the mass was counted and placed in a small graduate cylinder and the displacement measured to the nearest tenth of a mililiter. From this an estimate of eggs per clutch was calculated.

The vitellus of ten eggs from each mass was measured with vernier calipers and approximate stage of development (Gosner 1960) recorded.

Developmental stages were checked on two other occassions in the course of the experiment, and the temperature of the egg masses was recorded once.

Pond #7 of Sawhill Ponds is located in T1N, R70W, NE¼Sec 23 at an elevation of about 1570m. The ponds are a series of abandoned gravel pits in the floodplain of Boulder Creek. Construction was initiated in the mid-1950's and the pits abandoned in 1964 (Metger, Colo. Division of Wildlife, personnal communication, 1978). The ponds have been leased since 1973 from the state of Colorado by the City of Boulder.

Most breeding is seen on the south side of the pond in an area of shallow water. The area used for breeding was estimated by pacing.

The weather data were obtained from the National Weather Service at Denver, a site about 42 km from the study area.

RESULTS

Twenty-six clutches were observed; all were probably laid in a two week period (see discussion). Males were heard calling 17 March 1978, and the first clutch discovered 19 March 1978. Fourteen of the 26 clutches were found by 22 March 1978. Ovaposition took place in water 7--17 cm deep, with a mean water depth of 11.2 ± 0.5 cm, n=24. The mean water clearance was 3.4 ± 0.3 cm, n=24. No relationship between water depth and water clearance was observed.

The mean distance between nearest neighbors was 34.1 ± 8.2 cm, n=22. When this information was plotted on a gridded paper, the mean number of clutches in the 36 unit grid per 80 X 80 cm grid unit was estimated to be 0.61 ± 0.29, n=36. The nearest neighbor data fit a negative binomial distribution, while the clutches per grid unit data fit both the Poisson and negative binomial distribution when tested.

The 'k' from the negative binomial was then used to determine the index of aggregation where λ=(mean· V)/2k. V is a function with a Chi Square distribution and its value can be found by looking on a Chi Square table at the 0.5 level with 2k degrees of freedom. If λ is greater than 2, an active process has influenced the aggregation. Environmental features cause the aggregation if λ is less than 2. The index of aggregation for the nearest neighbor method was 23.63, for the grid units it was 0.18.

If the distribution of reflexive pairs was random (a reflexive pair being one in which two masses would be nearest neighbors of each other) the expected proportion of reflexive pairs would be 0.6215. The observed proportion was 12/22 or .545. With one degree of freedom the observed proportion was not significantly different from Poisson. No test was made to determine if the null hypothesis of the observed proportion of reflexive pairs fitting the negative binomial was made.

Using Clark and Evans' (1954) nearest neighbor method, a ratio of observed mean distance to nearest neighbors to the expected mean distance was calculated. The observed mean distance was 34.1 cm, and the expected distance was 56.4 cm when the density was 22 individuals per 2.8 X 10^5 cm^2. The test of significance indicated that there was only a 0.04% chance that the observed distribution was random.

As clutch volume increased, the number of eggs per clutch also increased (·. 6). The mean volume per clutch was 87.7 ± 12.8 ml, n=19, and the mean number of eggs per clutch was 2824 ± 350, n=19.

As clutch volume increased, the mean egg diameter also tended to increase (Fig. 7). When the total volume per clutch of vitellus was plotted against the mean volume of vitellus per single egg it was seen that total volume of vitellus per clutch did not increase much until the mean volume per egg reached about 3.6 mm^3 (Fig. 8). Diameters recorded in this study were within the ranges reported by Wright and Wright (1970) and Ruibal (1955).

As the number of eggs per clutch increased, the egg diameter also tended to increase (Fig. 9).

The greatest number of clutches was ovaposited between 20 March and 23 March 1978. This also was where the total volume of clutches was greatest, the mean volume of clutches was greatest, the mean number of eggs per clutch was greatest and the total number of eggs was greatest for the period for which there is data (see Fig. 10a and 10b). The mean egg diameter was higher in the period between 24 March and 27 March 1978 than for any other period for which there is data.

Calling by males continued from 17 March to at least 8 April 1978. Most males were heard calling from within the main portion of the breeding area, although the center of the chorus appeared to change as the season progressed. Males were occassionally heard from the area near the two small islands north of the main breeding area. When the dispersion of males became evident, about 8 April, sporadic calling was heard from the shore and portions of the pond that had not had any calling from before.

No estimates of hatching success were made from the egg masses laid in the pond, but with one exception they appeared to be high. Larger egg masses seemed to have more dead eggs than smaller egg masses, especially near the center. One egg mass had 0% hatching success and no apparent development of ovum.

DISCUSSION

The breeding season of Rana pipiens is relatively short in duration at Sawhill Ponds, and is within the calling season. The calling season appears to be initiated by temperature, although photoperiod may play some role. A second calling season in the early fall in Texas seems to be initiated by rainfall (Blair 1961).

The calling of males is thought to affect the hormonal state of female anurans (Garton and Brandon 1975, Oldham 1974, Porter 1972, Rabb 1973) and may induce the final ripening of the eggs. The ovum pass into the pseudouturus where, in Rana pipiens, they may remain for up to three days (Oldham 1974) before ovaposition.

A ripe or nearly ripe female enters the breeding area and selects a male. Dominant males may be selectively sought out by females (Rabb 1973) and then lay their eggs within that male's territory (Wells 1976). Territoriality may or may not be present in Rana pipiens but is probably more common in anurans which breed in permanent water than is currently known (Porter 1972: 460-462). In this way a dominant male can increase both the absolute number of descendants and its relative contribution by reducing the number contributed by conspecifics (Verner 1977). Presumably the dominant male would also have its calling territory in the most suitable habitat so that an advantage would be confered on its offspring through differential survival. Some anuran males, however, may not be able to spawn more than once per year (Pseudacris triseriata, Pettus, 1978, personal communication). Whether Rana pipiens males can fertilize multiple clutches is not known.

If Rana pipiens males are territorial and able to fertilize multiple clutches, the aggregation of 7 clutches within a space of 41 cm may be the result of a dominant male being selected. Other possible explainations include a favorable temperature in the area or other grain of the habitat, or the recognition of an area with sperm (which would increase the chance for at least some of the ovum in a clutch getting fertilized even if the male partner is sterile). Crowding as a defense against predators is probably not acting, as there was no disturbance of any clutch by insects, waterfowl, or turtles observed. Salamanders and fish were not present in Pond #7. Fungi was seen on eggs in several masses; this is thought to be saprovoric (Woodruff 1976, Porter 1972, Kramer 1978, Herreid and Kinney 1966).

While most masses appeared to have relatively high hatching success, one egg mass had no successful hatchings and no apparent ovum development. This clutch was probably not successfully fertilized. Woodruff (1976) reports relatively low mortality after development commences, with most mortality after that point occurring during gastrulation. A lower proportion of eggs may be fertilized in anurans that deposit eggs in water (Woodruff 1976). In this study, egg masses that were larger tended to have more dead eggs, especially near the center, than smaller egg masses. Hollenbeck (1978, personal communication) observed this effect with Rana pretiosa. No study indicates any differential with clutch size in anurans that lay all their eggs in one mass as Rana pipiens does. Possible causes for the inviability of eggs near the center of a mass may be that sperm do not penetrate or oxygen is depleted near the center. As the egg masses in this study were reattached to vegetation by being tied, this may have had an effect on viability. If the tendance for more dead eggs in larger egg masses was not an artifact from the egg mass being tied to vegetation, it is possible that selection would still favor an increase in clutch size so long as the number of eggs or survivorship increased at a greater rate than the number of dead eggs in the center of the mass.

The distribution of the egg masses within the breeding area can be considered Poisson (grid units, reflexive pairs) or negative binomial (grid units, nearest neighbor distances, nearest neighbor method of Clark and Evans 1954). Biologically, either intrupretation may be correct, and the mechanisms involved in choosing the site of ovaposition should be known.

Both the water depth and water clearance were normally distributed, but unrelated. There was not a cooresponding increase in water clearance as water depth increased, largely because the egg masses were attached to vegetation. In fact, an egg mass laid in the deepest water had one of the most shallow water clearances. Water near the surface tends to heat up more quickly, which would speed up the development of the eggs. Anoxic conditions may prevail in deeper water, which would also require the eggs to be positioned near the surface (Garton and Brandon 1975). Dessication is probably not usually a serious problem for egg masses.

The data for egg diameter, clutch volume, and eggs per clutch is difficult to analyze without the important datum of what female (or what size female) laid a given egg mass. Ryan (1953) suggests that a few individuals may breed the spring following metamorphosis, while all are probably adults and breed two years after they were eggs. Younger frogs would be putting more energy into growth than older frogs, which might expend somewhat more on maintaince due to their larger body size. If all breeding females were the same size and had the same amount of energy to put into a clutch, partitioning would take place along the number of eggs vs. vitellus size (egg diameter. An increase in volume and energy available for egg production could be utilized in an increase in egg number, egg diameter, or both. Several studies have indicated that as body size increases, both between and within species, both egg size and egg number increase within a given reproductive mode (Pettus and Angleton 1967, Salthe 1969, Salthe and Duellman 1973, Shoop 1974, Wilbur 1977). In this study there seems to be strong pressure to maintain both a minimum number of eggs per clutch and a minimum ovum diameter. Figure 9 appears to indicate that as additional resources become available most individuals increase ovum size before the number of eggs per clutch increases. Egg masses 1, 4, 10, and 14 probably represent the first breeding of females, while masses 8 and 18 are probably females at least a few years old. Mass number 13 is something of an anomily, perhaps the estimate of numbers of eggs in the clutch was too high, or oogenesis began so that the individual was "committed" to a certain number of eggs and was not able to get enough food to bring the diameters up to where they would be expected for a clutch of that size.

The most clearcut relationship was between clutch volume and number of eggs per clutch (fig. 6). As clutch volume increased the number of eggs per clutch also increased. Wilbur (1977) discusses in detail the profit curves to the parents of increasing clutch size and egg number, so that selection would favor an increase in egg number when a linear relationship exists between survival and egg weight, and an increase in ovum size favored when the survuval vs. egg weight curve was a concave upward function.

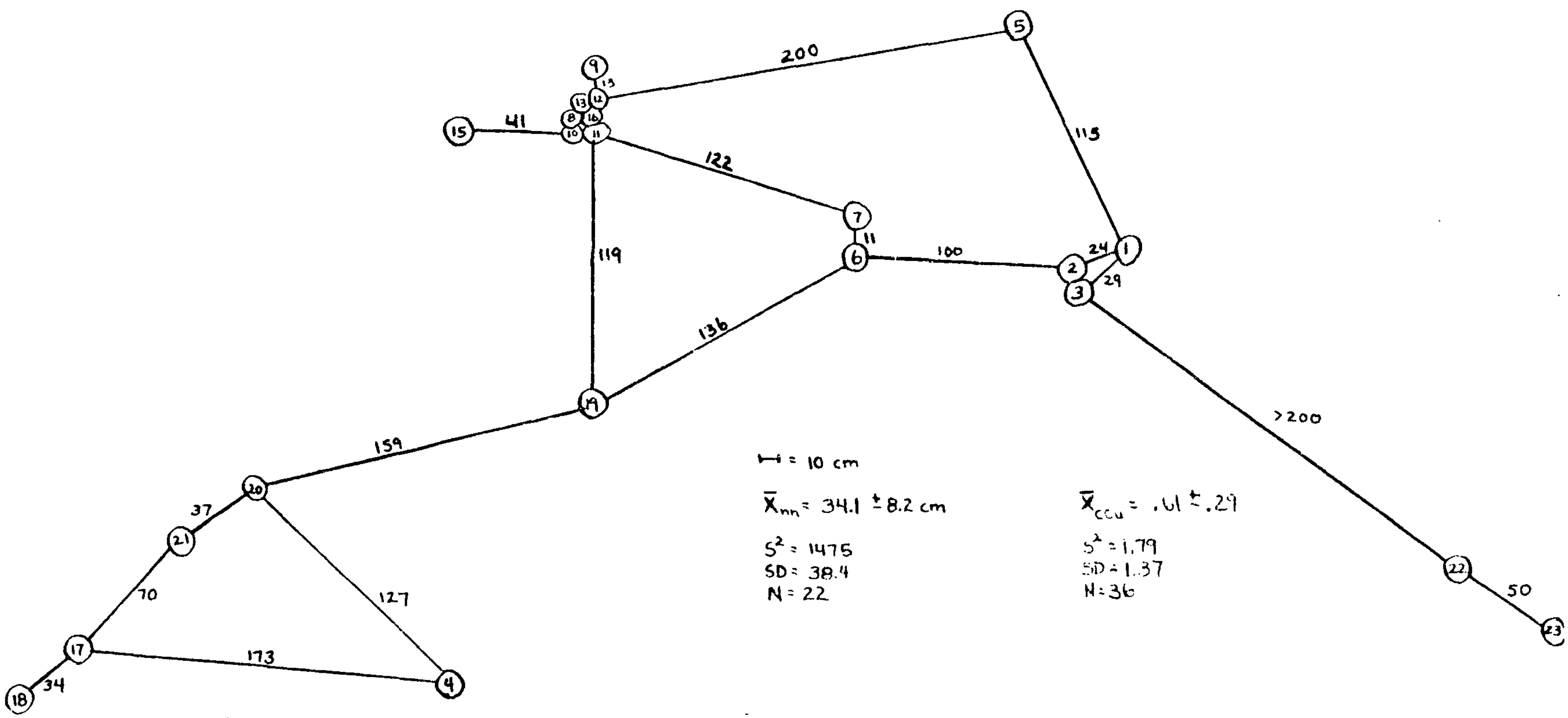
When clutch volume, number of eggs, etc. were grouped acording to the date of oviposition, a peak in all parameters except mean egg diameter occurred between 20 March and 23 March 1978. This might imply that females with more a[illegible]lable resources (older females) were more closely syncronized with regard to breeding than most other females, who tended to oviposition morr randomly with respect to date.

The increase in egg diameter could be increased varience in females whose partitioning of resources was not as predictable as older females.

Over 60,000 eggs were laid in Pond #7, which had less than 60 breeding individuals. Eisenberg (1966) and Shoop (1974) both found that density dependant survival resulted in a relatively consistant number of individuals surviving under different conditions. It would be interesting to determine the standing crop of adults on a yearly basis and compare this with the number of eggs contributed yearly.

In conclusion, several environmental and physiological parameters are in operation with relation to the deposition site and quantity and quality of eggs in Rana pipiens. Many of the details of the life history, such as territoriality in males, selection of dominant males by reproductively ripe females, and female size and age class with respect vitellus partitioning, have yet to be studied.

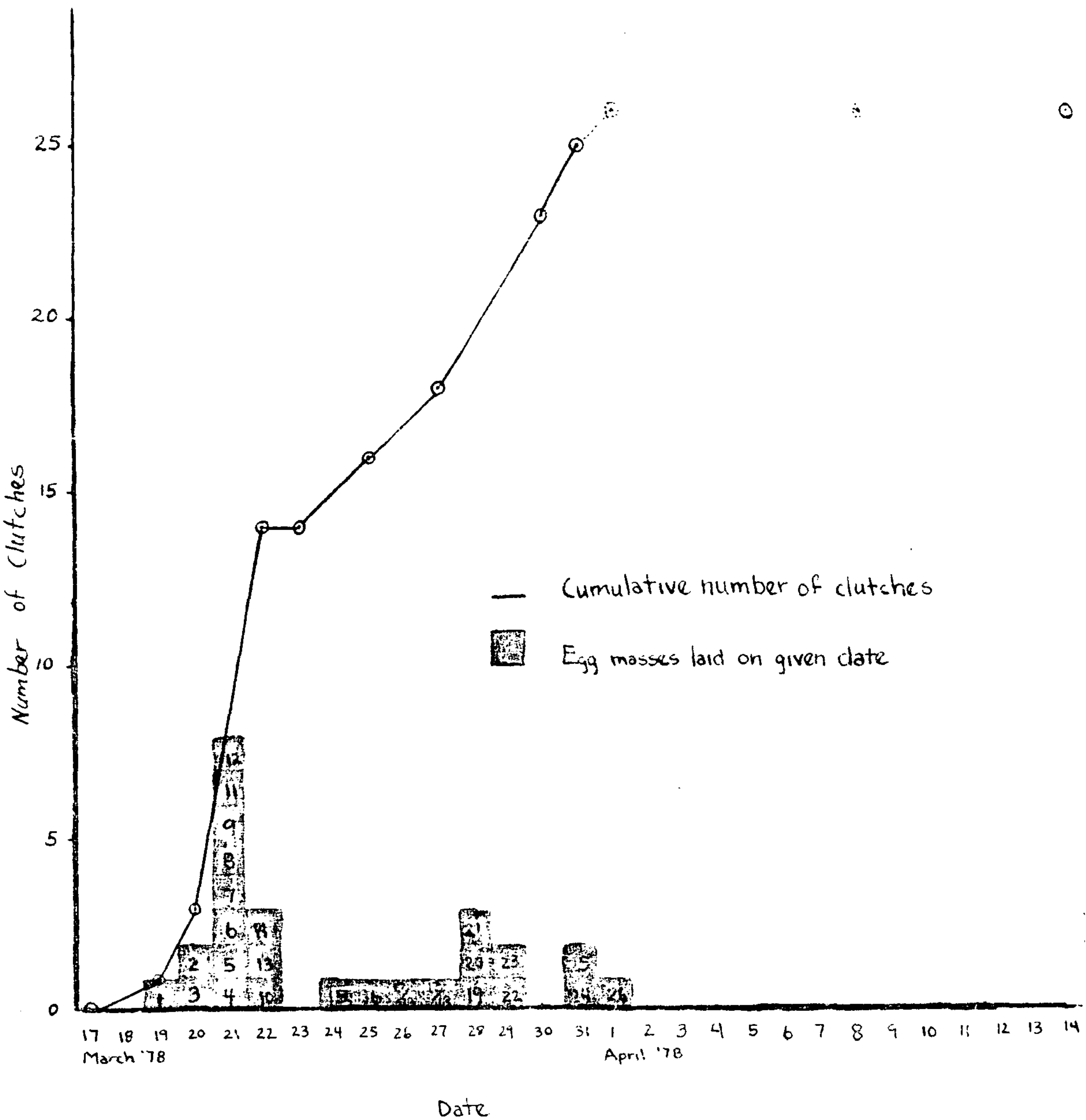
FIGURES



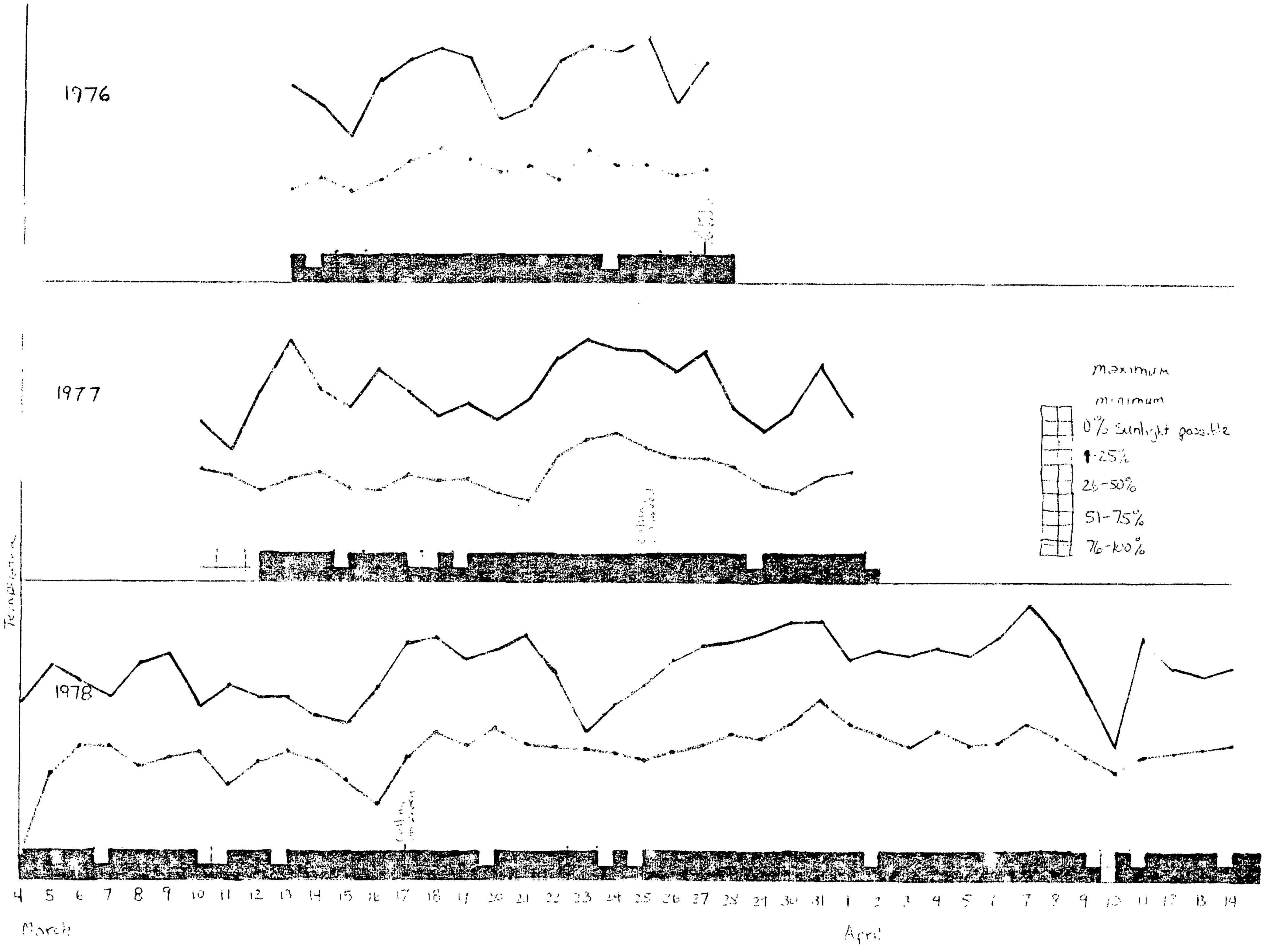
Distribution of egg masses in breeding area

Figure 1

Figure 2

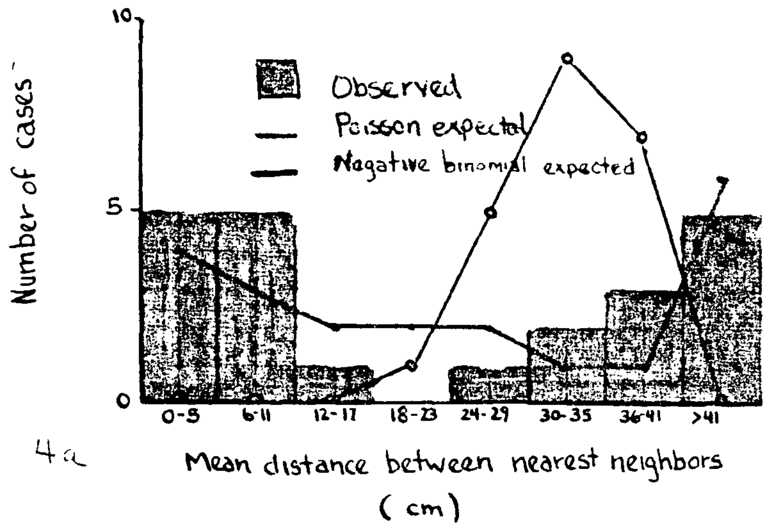


Cumulative number of clutches
Egg masses laid on given date
Number of Clutches
Date
March '78
April '78

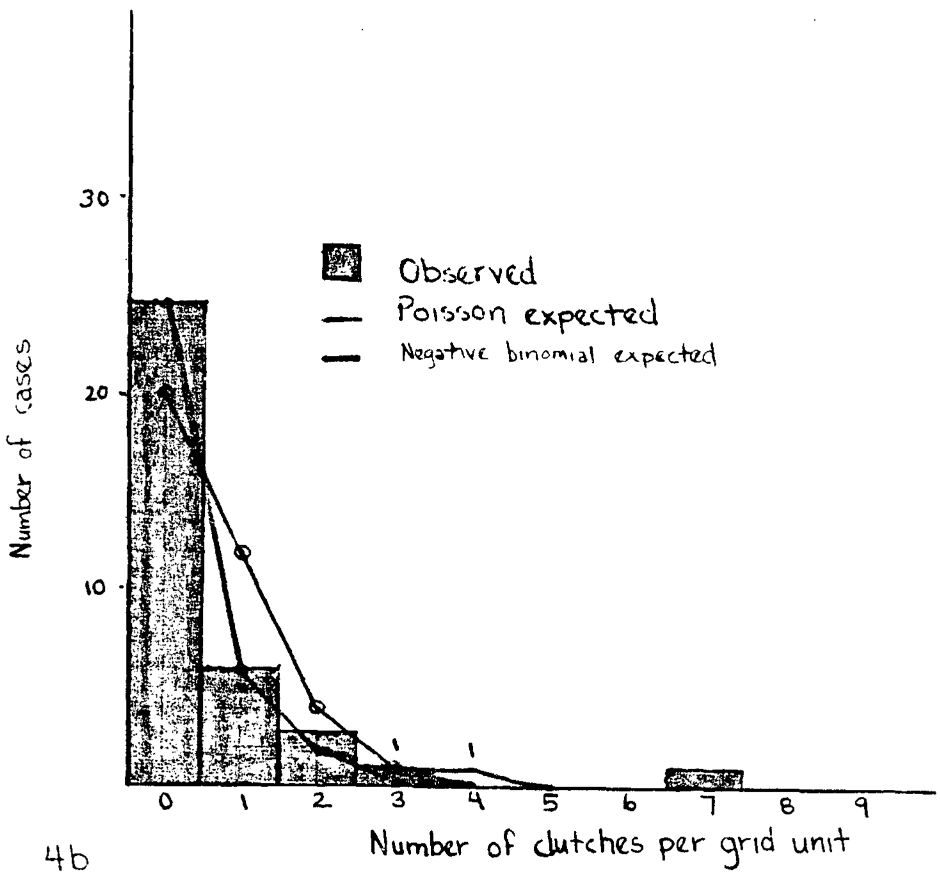


1976
1977
1978
maximum
minimum
0% Sunlight possible
1-25%
26-50%
51-75%
76-100%
Temperature
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14
March
April

Figure 4

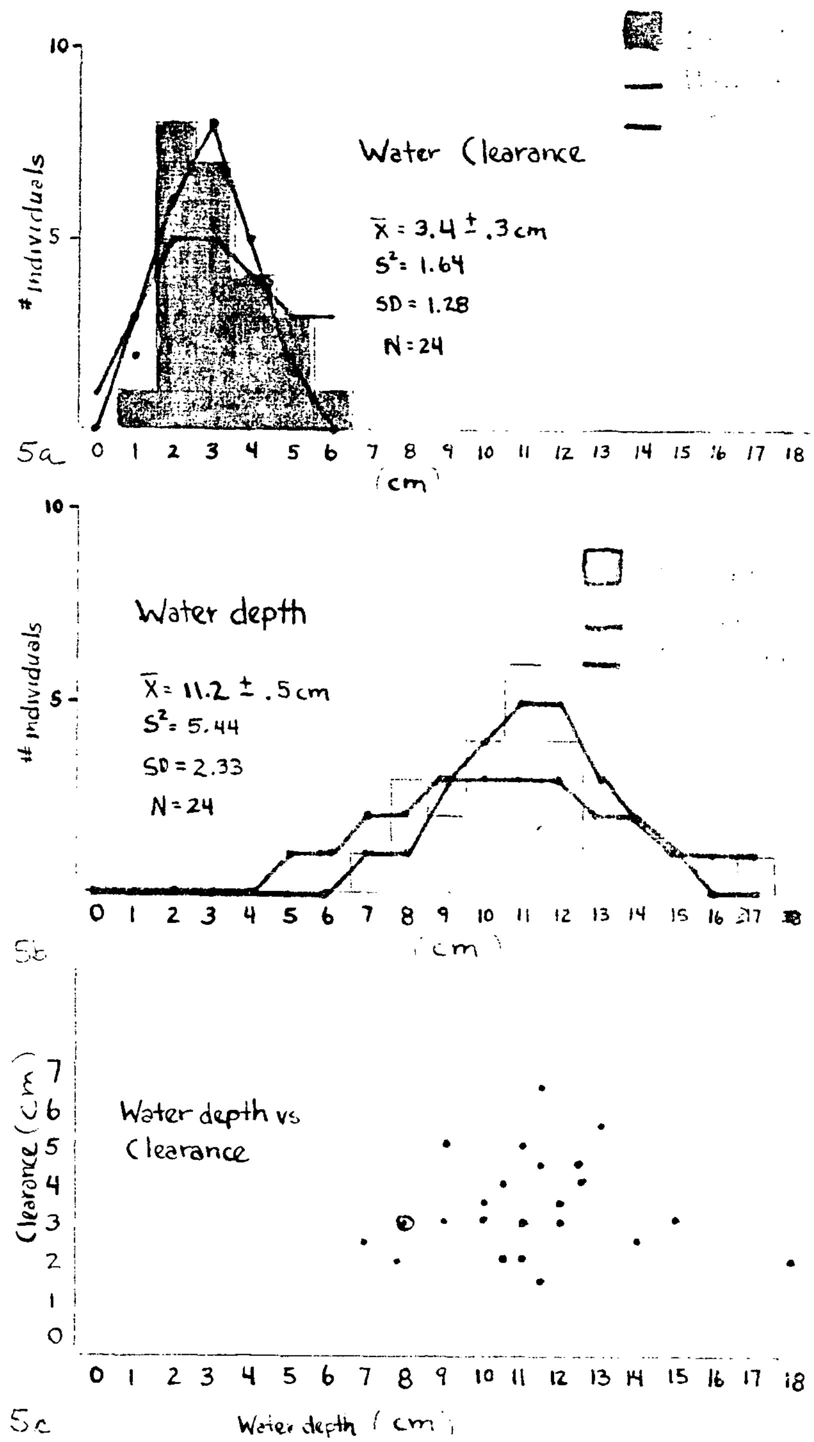


4a



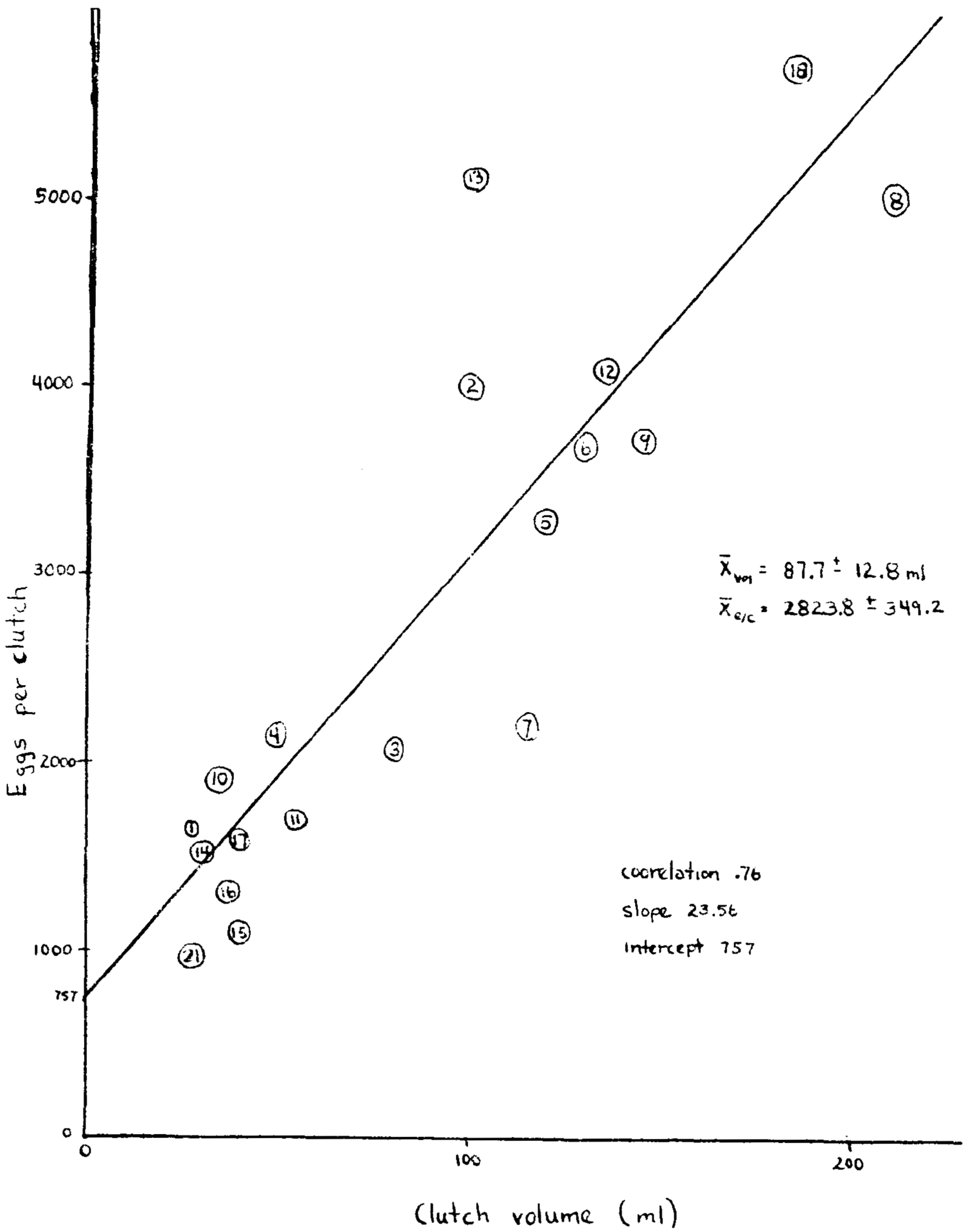
4b

Figure 5



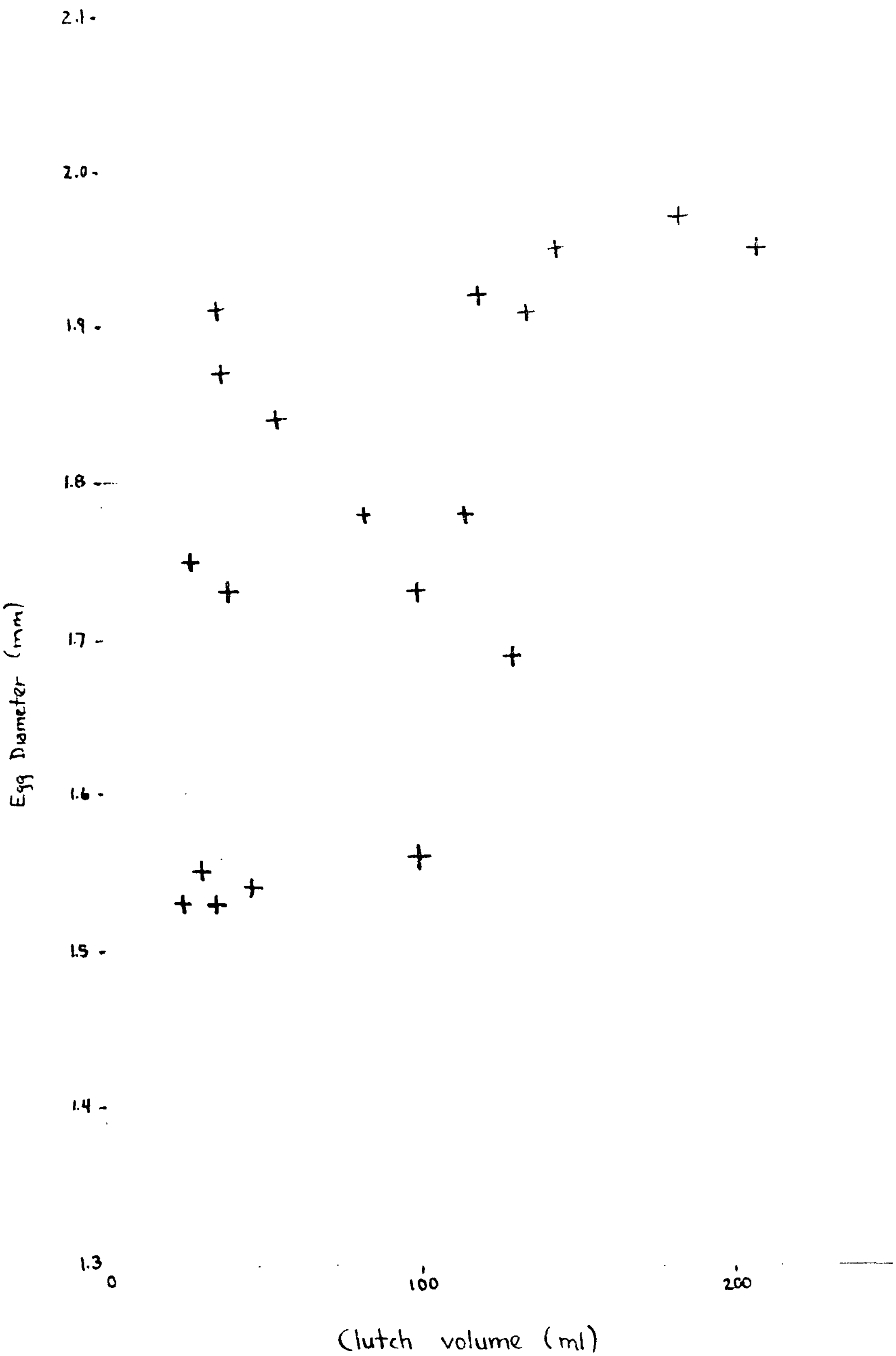
Water Clearance
X̄ = 3.4 ± .3 cm
S² = 1.64
SD = 1.28
N = 24
# individuals
5a
(cm)
Water depth
X̄ = 11.2 ± .5 cm
S² = 5.44
SD = 2.33
N = 24
# individuals
5b
(cm)
Water depth vs Clearance
Clearance (cm)
5c
Water depth (cm)

Figure 6



Eggs per clutch
5000
4000
3000
2000
1000
757
0
Clutch volume (ml)
0
100
200
$\bar{X}_{vol}$ = 87.7 ± 12.8 ml
$\bar{X}_{e/c}$ = 2823.8 ± 349.2
coorelation .76
slope 23.56
intercept 757

Figure 7



2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
Egg Diameter (mm)
0
100
200
Clutch volume (ml)

Table 1 after Clark and Evans, 1954

$$\bar{r}_A = \frac{\sum_{i=1}^{N} r_i}{N}$$

$\bar{r}_A$ = mean observed distance between nearest neighbors = 3[illegible]

r_i = i^{th} nearest neighbor

$$\bar{r}_E = \frac{1}{2\sqrt{d}}$$

N = number of individuals = 32

$\bar{r}_E$ = mean expected distance = 5[illegible]4 cm

$0 \leq R \leq 2.1491$

$$R = \frac{\bar{r}_A}{\bar{r}_E} = .60$$

R = 0 max agg
R = 1 random
R = 2.1 repulsed

d = density = 32/(2 × 10^5 cm^2)

$$\sigma_{\bar{r}_E} = \frac{.26136}{\sqrt{N \cdot d}} = 6.24$$

$$c = \frac{\bar{r}_A - \bar{r}_E}{\sigma_{\bar{r}_E}} = 3.55$$

absolute deviation = .[illegible]
0.04% chance that distribution is random

Figure 8

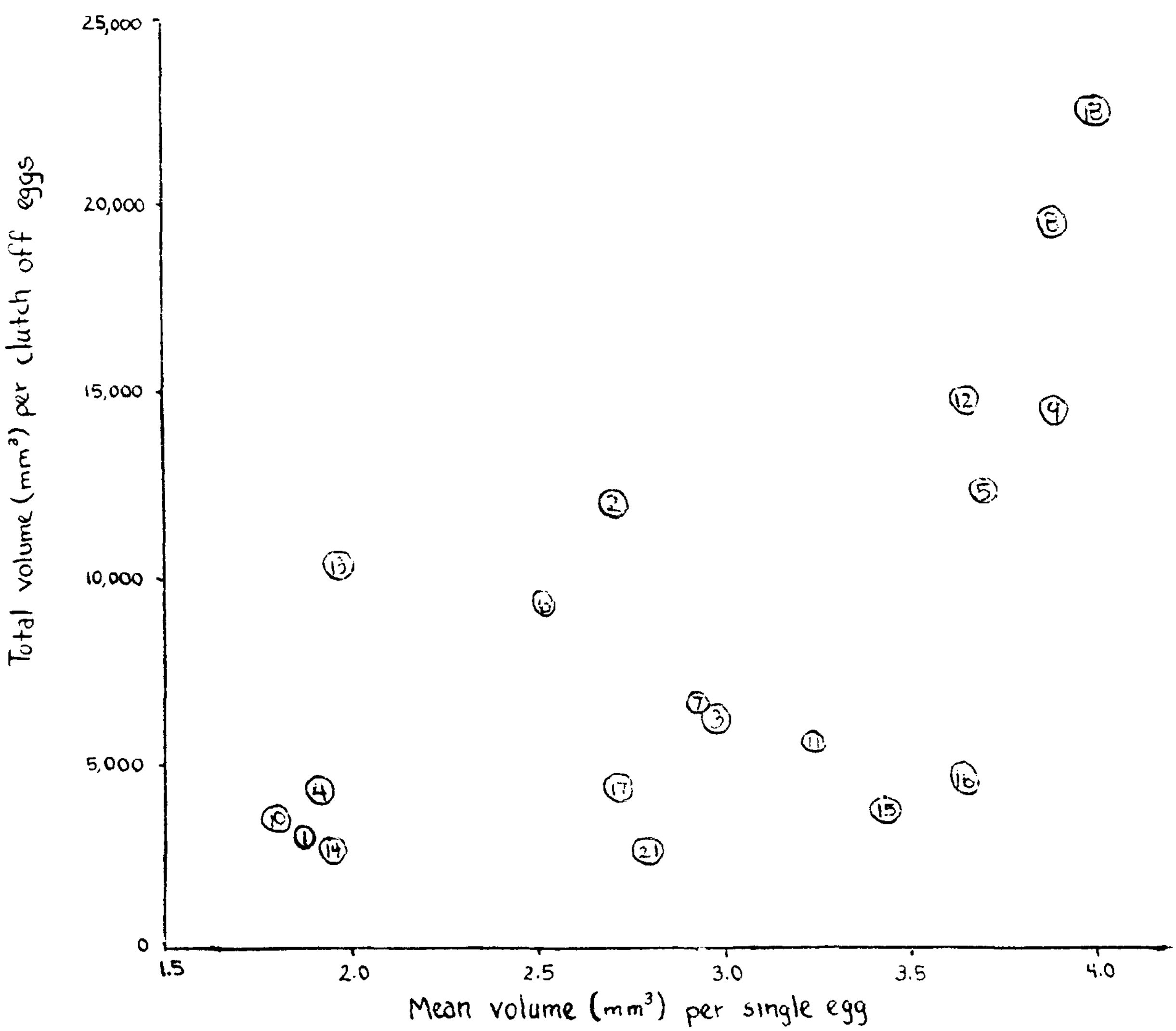


Figure 9

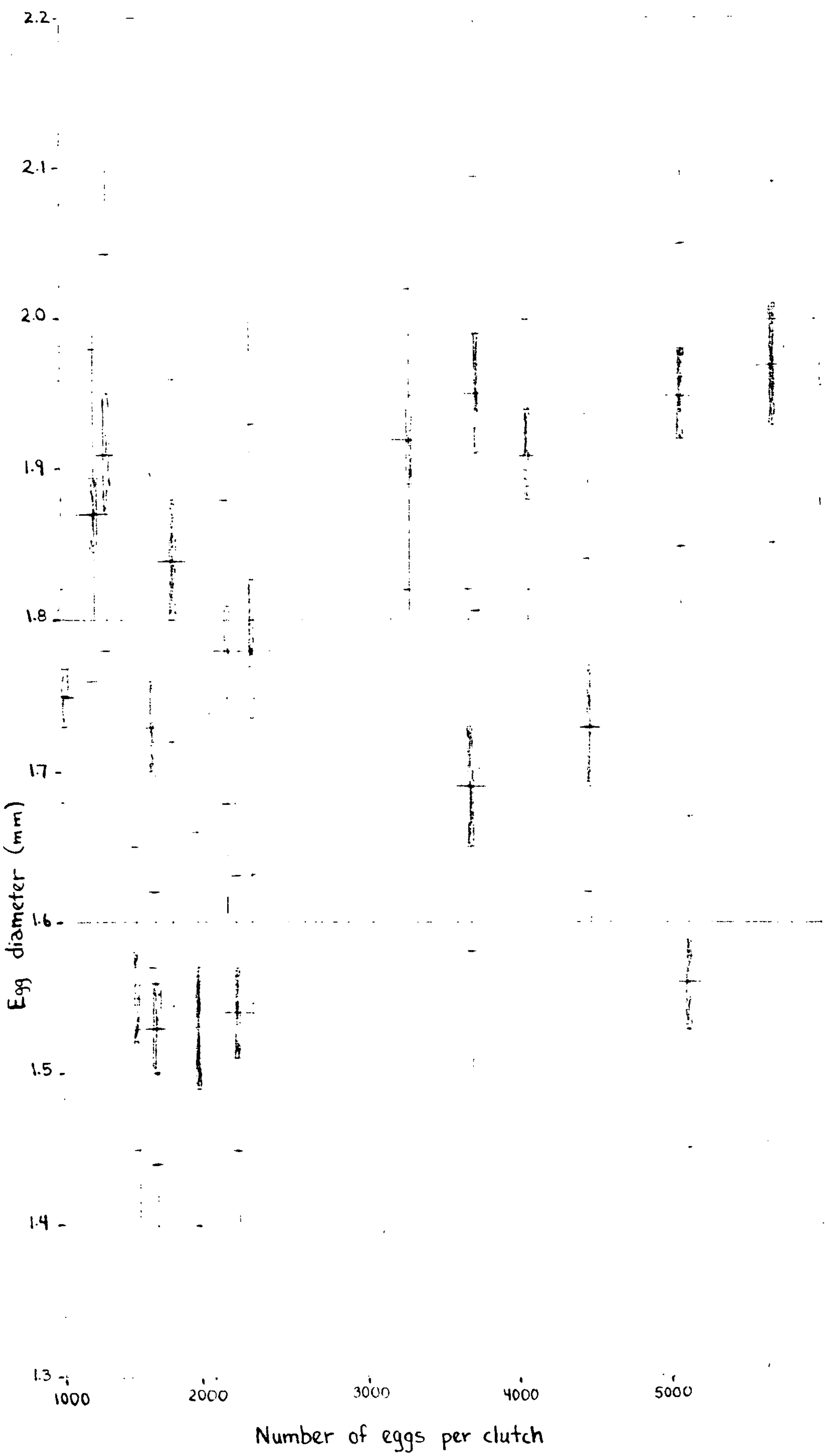
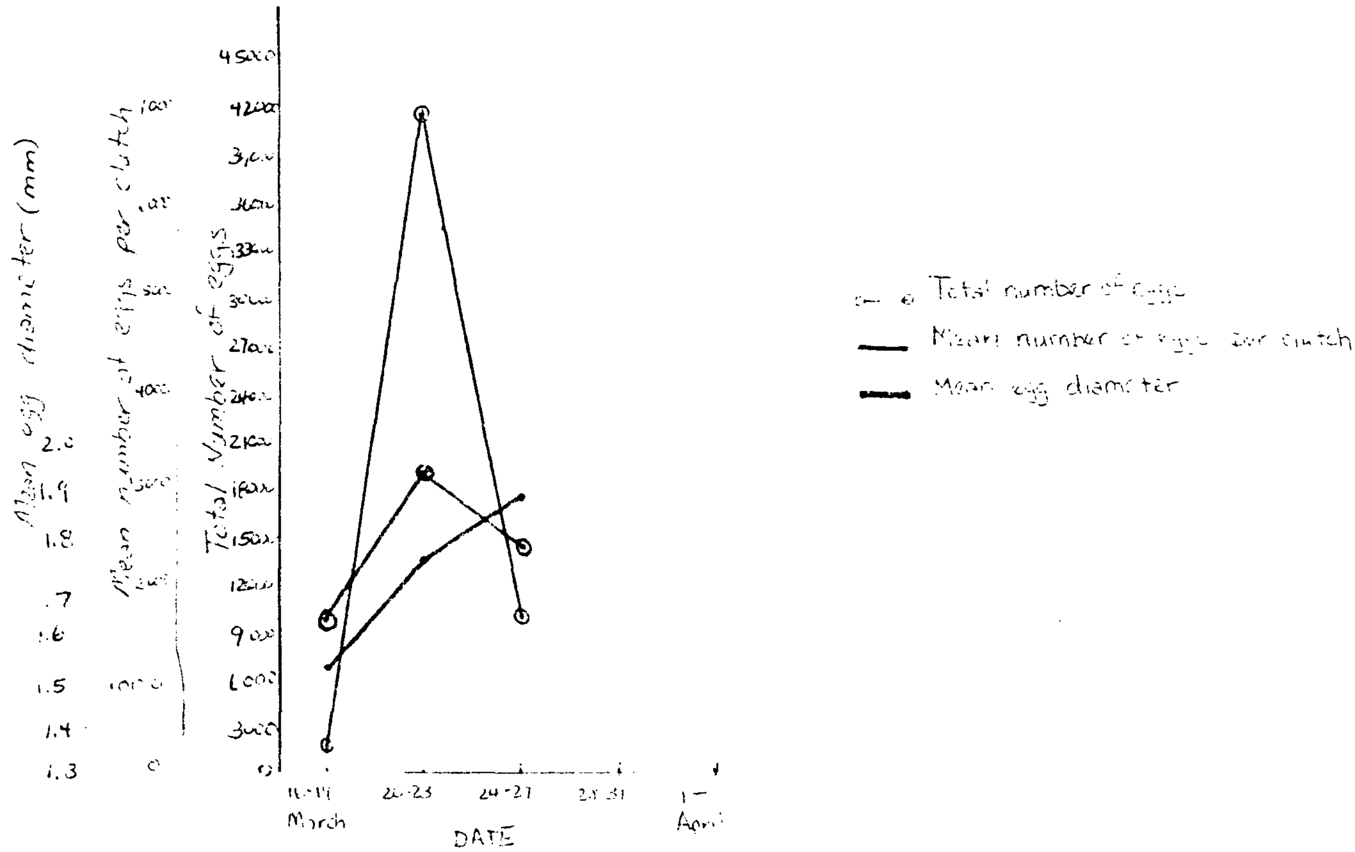
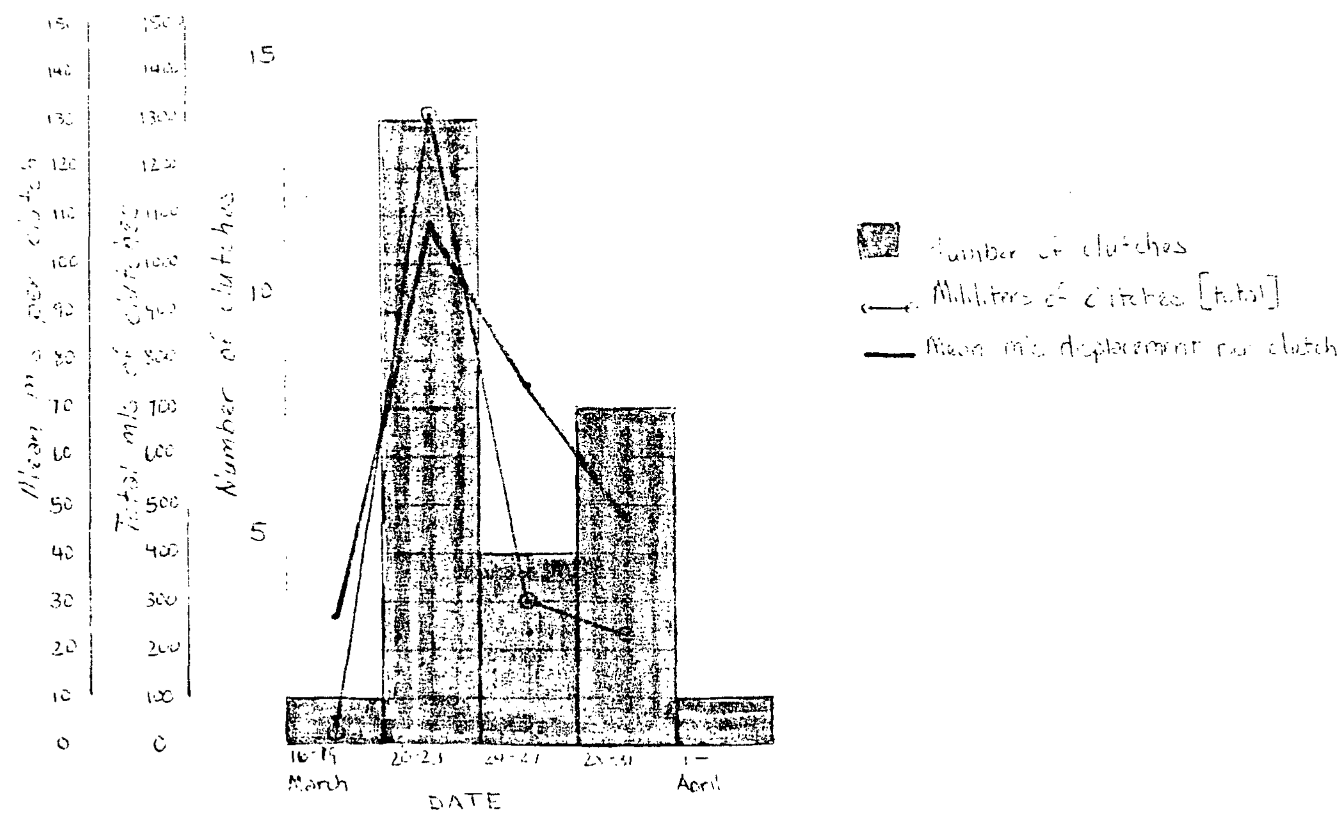
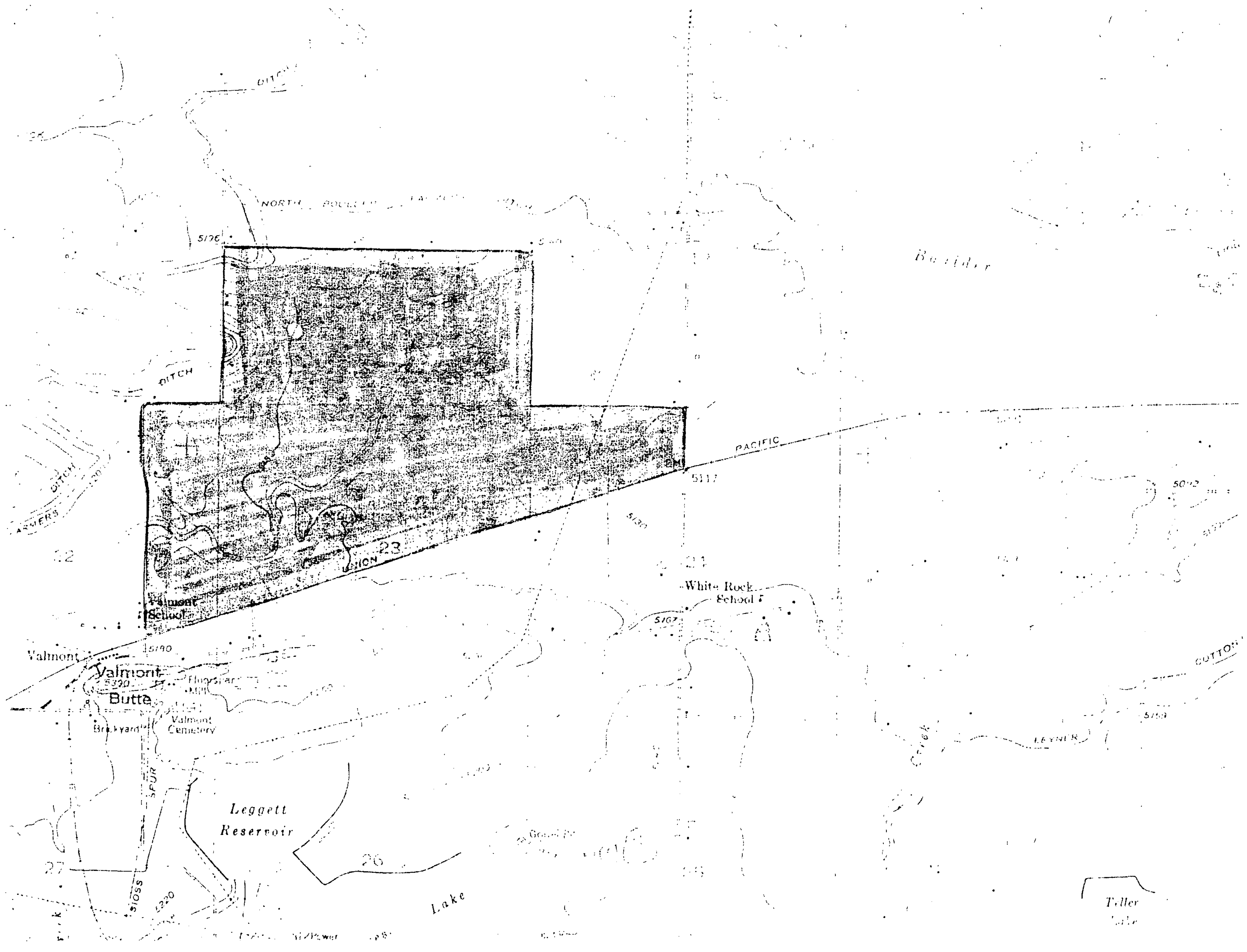
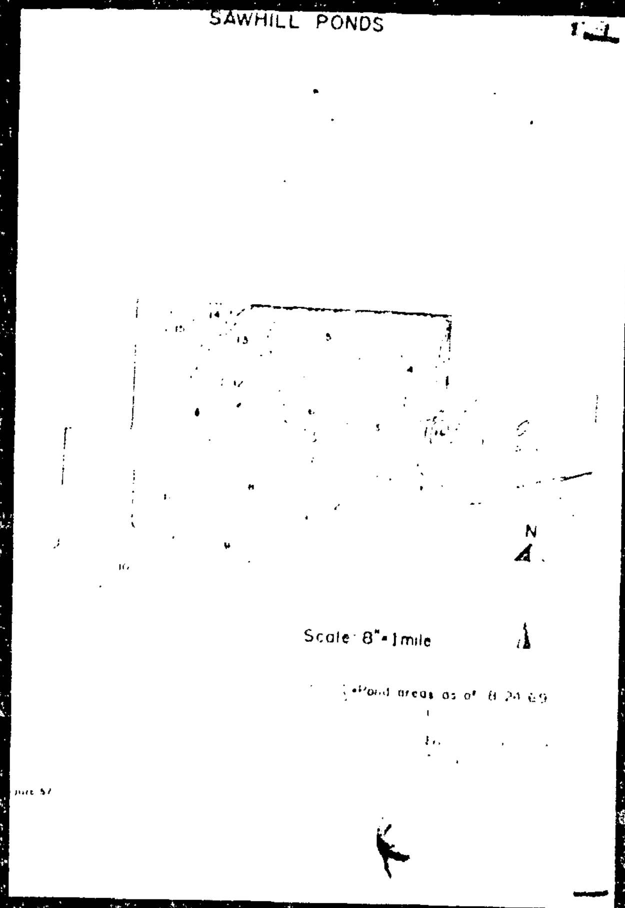

2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
Egg diameter (mm)
1000
2000
3000
4000
5000
Number of eggs per clutch

Fig 10


Mean egg diameter (mm)
Mean number of eggs per clutch
Total Number of eggs
DATE
March
April
Total number of eggs
Mean number of eggs per clutch
Mean egg diameter

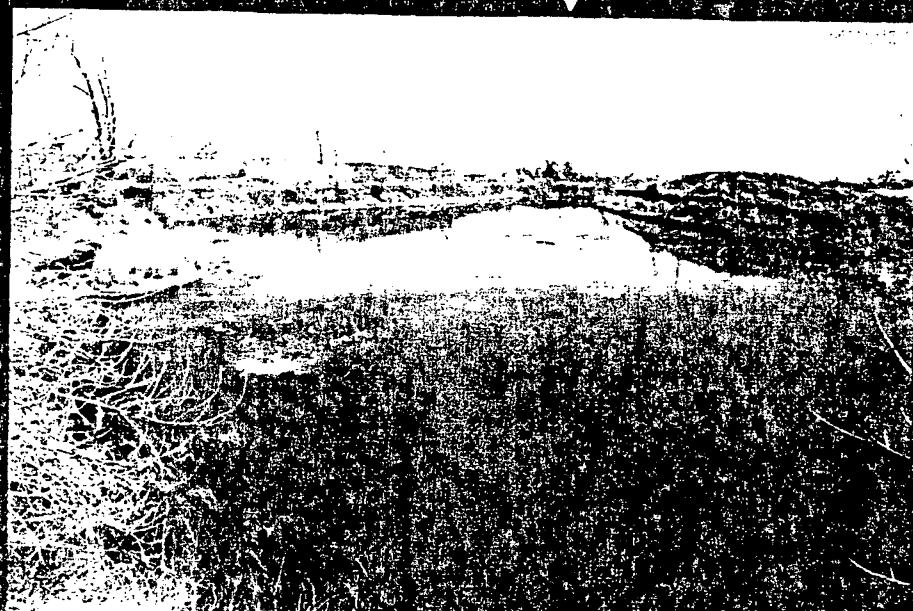

Total mls of clutches
Number of clutches
DATE
March
April
Number of clutches
Mean mls displacement per clutch


DITCH
NORTH BOULDER
5126
Boulder
DITCH
FARMERS
PACIFIC
5117
5092
5130
23
UNION
White Rock
School
5167
Valmont
School
Valmont
5180
Valmont
5330
Butte
Fluorspar
Mill
Brickyard
Valmont
Cemetery
COTTON
5159
LEYNER
Creek
SPUR
Leggett
Reservoir
26
27
Lake
Teller
5220

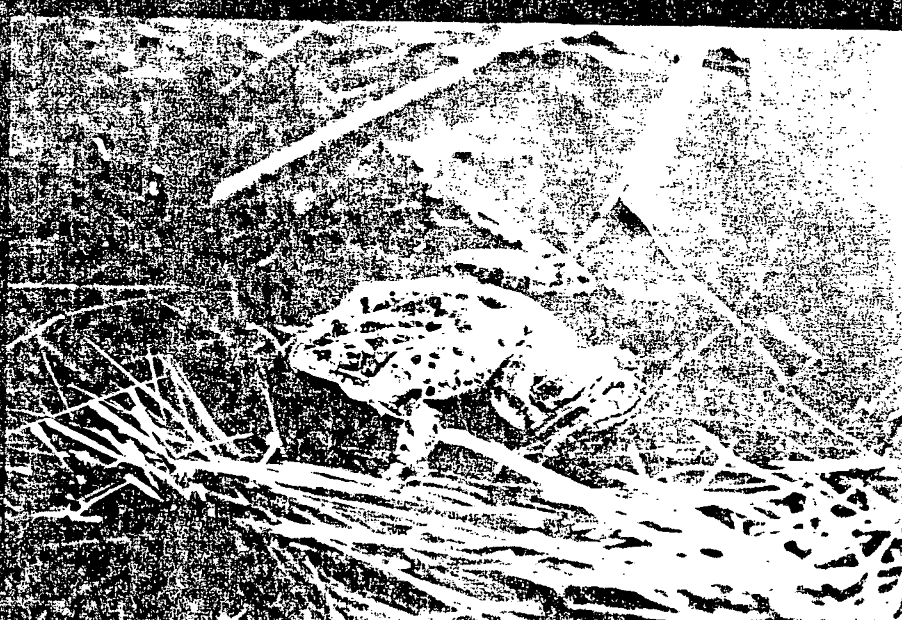


Rana pipiens breeding area studied was in Pond 7

Main breeding area in Pond 7

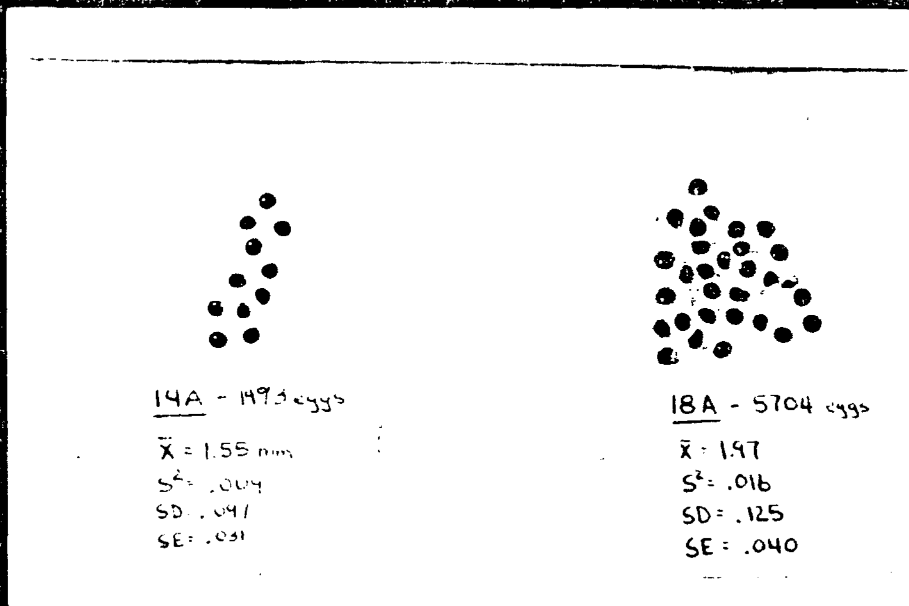


Pond 7. Arrows at side and top endicat main breeding area. An egg mass was also found in the area of shallow water near islands and another at the far end of islands.

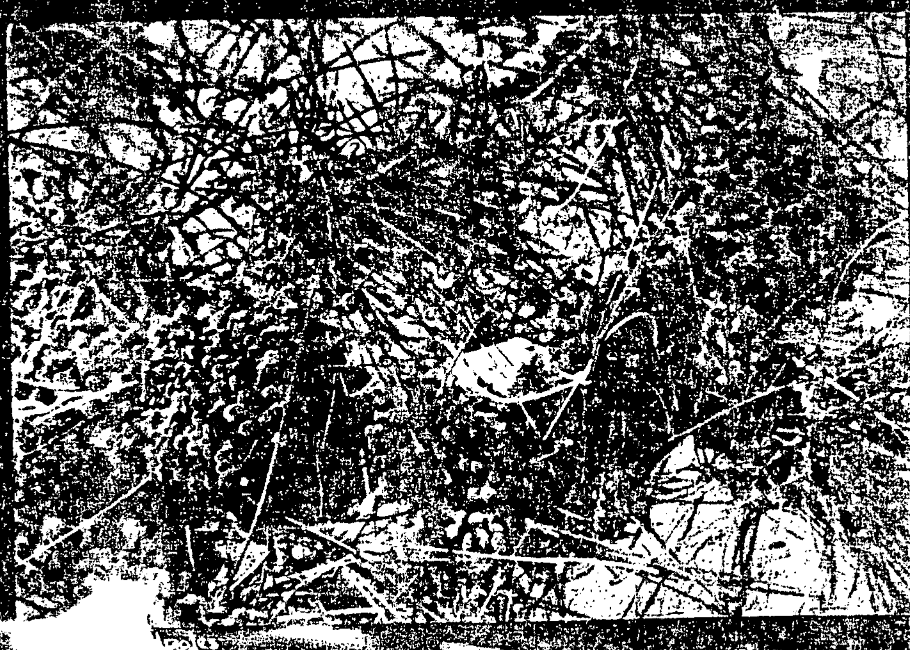


Male *Rana pipiens* with inflated vocal pouches

Aggregation of seven egg masses



Comparison of eggs from two clutches



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[illegible]

[illegible]
[illegible] Parks
[illegible] 701
[illegible]

Dear [illegible],

Please find enclosed a copy of the paper I did on the breeding population of _Rana pipiens_ at [illegible] Ponds. I [illegible] forward to continuing in greater detail the work that I started [illegible].

Thank you for your help.

Sincerely,

[illegible]
[illegible]
Denver, Colorado 80210
[illegible]

12 Feb. 1979

Ron Donahue
Boulder Parks
Box 791
Boulder, CO 80306

Dear Mr. Donahue,

Last year, with the permission of the Boulder Parks Department, I made observations of a breeding aggregation of Leopard Frogs at Sawhill Ponds as part of an independant project for a graduate Biology course. The results and the questions raised have interested me so much that I am again asking permission to study this population's breeding behavior.

The data from this more intensive study will be the basis of my proposed Master's Thesis at the University of Colorado at Denver. A summary of the procedures is enclosed. If any additional information is needed, or if you would like a summary of my results to date, please call or write.

Thank you very much for your consideration.

Sincerely,

Lauren J. Livo
1215 S. Osceola Street
Denver, Colorado 80219
936-0440

Summary of Procedures

A grid will be set up in the breeding area with stations at intervals of 1 meter. Male calling stations, temperature gradients, egg mass deposition, water depth, etc will be recorded in relation to these stations. The area is in Pond #7 (according to map at Sawhill).

Observations will be taking place from dawn to dusk every day for approx. a month after initiation of breeding activities. If necessary there may be some nighttime observations, but I will inform designated persons in writing ahead of time.

Small samples will be taken from each observed egg mass, and a small sample of hatchlings will also be collected (under the renewal of Colo. Division of Wildlife Scientific Collection Permit #78-179) and preserved. I calculated that over 60,000 eggs were laid in the pond last spring, and the quantity that I would preserve would be negligible.

All frogs possible would be toe-clipped, sexed, and measured. Some tadpoles will be marked when they reach stage 31 continuing through metamorphosis (Gosner, 1960) to estimate the number of tadpoles that survive to metamorphosis.

The above procedures are the only ones in the study that entail alterations or consumptive uses of the area. Other procedures--tape recordings, photographs, etc.--are nonconsumptive.