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PREHATCHING INTERACTIONS IN DOMESTIC CHICKENS AND THEIR INFLUENCE ON BEHAVIOR OF THE YOUNG

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PREHATCHING INTERACTIONS IN DOMESTIC CHICKENS AND THEIR INFLUENCE ON BEHAVIOR OF THE YOUNG

by

RAZVAN A. TUCULESCU

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1980

Razvan A. Tuculescu

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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INTRODUCTION

Statement of hypotheses

Recent work in several species of precocial birds (Gottlieb, 1966, 1971, 1975a, 1975b, 1975c, 1978, Impekoven, 1971a, 1976a, Tschanz, 1968, Norton-Griffiths, 1969) suggests that posthatching behavior in a natural brood of chicks has antecedents in prehatching interactions between the embryos and the incubating hen. It is well known that domestic chicks and their parental hen can form a social unit if they interact during the period that follows immediately after hatching (Wood-Gush, 1955, Hess, 1973). The interactions establish social bonds insuring that the young birds and hen continue to associate and interact for an extended period. While chicks readily develop a filial attachment if they interact with the hen early in life, they lose the tendency to show filial behavior if they are isolated. It is possible that some types of vocal interactions between the newly hatched chicks and the hen originate prior to hatching. In addition, the nature and extent of prehatching exposure to maternal stimulation could determine the pattern of posthatching interactions by influencing the response of newly hatched chicks to social stimuli. Until now these hypotheses have been difficult to evaluate because there were no adequate descriptions of the behaviors which occur during incubation in either embryos or the parental hen. To demonstrate that prehatching interactions influence later behavior it is necessary to describe parental and embryonic behaviors during incubation, determine whether interactions occur prior to hatching and then investigate the effects of these interactions. In this study, the behavior of embryos and the incubating hen during the period before hatching is described. The temporal patterning of behavior is analyzed for the presence of interactions. Finally, in order to determine whether prehatching interactions influence later behavior, the behaviors of embryos and chicks with different prehatching experience are compared.

Background

The effects of early experience on the development of social behavior in chicks usually have been inferred from isolation type experiments (Hess and Petrovich, 1973). Typically, chicks are artificially incubated, isolated at hatching and then tested for responsiveness to different forms of stimulation after various isolation treatments (Hess, 1973, Hess and Petrovich, 1973, 1977). Most of these experiments have been performed without taking into account the influence that prehatching experience may have on posthatching behavior. Lately, however, investigators have begun to realize that the egg does not isolate the embryo from external stimuli. Light and sound stimuli penetrate the egg and the embryo appears to react to them (Gottlieb, 1968). It may be that prehatching experience influences the later responses of chicks to social stimuli.

Gottlieb (1975a, 1975b, 1975c, 1978) and others (Impekoven, 1976a, Tschanz, 1968, Norton-Griffiths, 1969, Green and Adkins, 1975) have reported recently that embryonic self-stimulation, passive exposure to parental stimulation and interactions between the embryo and the incubating parent each can modify the responsiveness of the newly hatched bird to social stimuli.

That embryonic self-stimulation influences posthatching behavior was demonstrated by Gottlieb in a series of elegant experiments performed on ducklings (Gottlieb, 1975a, 1975b, 1975c, 1978). He surgically devocalized duck embryos before they started to call and incubated them in sound attenuated incubators. Gottlieb found that after hatching, fewer devocalized ducklings respond to the maternal call than controls which are isolated but not devocalized. The ability of devocalized ducklings to discriminate the species maternal call from other calls is also impaired. When devocalized ducklings are exposed to sibling contentment calls during incubation the proportion of ducklings that respond to the species maternal call approaches that of normal controls. Gottlieb concludes that embryonic self-stimulation with contentment calls enhances posthatching responsiveness to the species maternal call.

Passive exposure to stimulation from outside the egg during incubation also influences the posthatching behavior of some precocial birds. Impekoven (1971a, 1976a) found that the responses of laughing gull chicks to parental calls are influenced by prehatching exposure to such calls. Naturally incubated gull chicks show increased activity when exposed to the parental "croon" call and a mild decrease in activity when exposed to the parental "kow" call. In contrast, artificially incubated naive chicks show no increase in activity when exposed to "croons" and strong behavioral inhibition to "kow" calls. Impekoven demonstrated that the differences in behavior are due to differences in prehatching experience with the parental calls. She exposed artificially incubated gull embryos to recorded parental calls and then observed their posthatching behavior during the playback of the calls. The behavior of the "experienced" chicks was similar to that of naturally incubated birds. Posthatching effects of prehatching exposure to auditory stimulation are also known in other species of precocial birds (Green and Adkins, 1975, Evans, 1973).

Prehatching interactions between embryos and the incubating parent also have a marked influence on posthatching behavior. Tschanz (1968) found that the vocalizations emitted by guillemot embryos after the penetration of their air sac induce the incubating parent to rise and utter the precursor of a feeding call. Conversely, parental vocalizations and other activities during incubation stimulate the embryos to vocalize. Later, during incubation, the embryo responds mainly to the parental food call which is the most frequently occurring call when the embryo is active. After hatching, the guillemot chick responds only to the familiar food call of its own parent. A similar effect of prehatching interactions was found by Norton-Griffiths (1969) in oystercatchers. Before hatching, embryos move and vocalize following parental movements on the nest. In response to embryonic vocalizations and movements the incubating adult emits the parental food call. After hatching oystercatcher chicks respond to the parental food call the first time the parent utters it. In these two instances, the investigators concluded that the prehatching vocal interactions between embryos and the incubating parents influence the development of responsiveness to parental food calls. In guillemots, prehatching interactions increase chick responsiveness to the calls of their own parents while in oystercatchers they insure the reaction to the parental call immediately after hatching.

It appears that there are several ways in which embryonic self-stimulation, exposure to parental stimulation and interactions with the incubating parent influence the behavior of the newly hatched bird. Embryonic self-stimulation may influence the young bird's ability to respond differentially to the species maternal call. Passive exposure to parental stimuli during incubation increases familiarity with such stimuli and may facilitate the expression of appropriate responses immediately after hatching. Prehatching interactions with the incubating parent permit the embryos to perceive the effect of their own behaviors on outside stimulation. They provide a foundation for the development of interactions after hatching.

It is not known whether or not similar factors influence the behavior of newly hatched chicks. For chickens, there is no thorough description available of prehatching behavior in either the incubating hen or the embryos. It is known that chicken embryos start vocalizing long before hatching (Guyomarc'h, 1966). Most of the vocalizations are identical to those emitted by chicks after hatching. It is also known that the incubating hen vocalizes prior to hatching and that the vocalizations are the same before and after hatching (Baeumer, 1962, Guyomarc'h, 1974b, 1975a). This evidence suggests then, that embryos self-stimulate and that embryos and the incubating hen stimulate each other. It is possible, therefore, that prehatching interactions occur and that they influence the later behavior of chicks.

In this study I describe the patterns of maternal and self-stimulation to which embryos are exposed. I analyze the association between embryonic and maternal behaviors to determine what kinds of interactions occur. I determine how the pattern of embryonic self-stimulation is affected by the interactions. Finally, I investigate the influence of prehatching interactions on the later behavior of embryos and newly hatched chicks.

Experimental plan and objectives

The study is comprised of four experiments:

1. The objectives of the first experiment are the following: identify the pattern of embryonic exposure to maternal behaviors and embryonic self-stimulation in natural broods; determine whether or not interactions start before hatching; describe the types of interactions. For this purpose, naturally incubated broods were observed and the embryonic and maternal behaviors under normal conditions of incubation were described. The associations between embryonic and maternal behaviors were then analyzed and the types of interactions described.
2. The objective of the second experiment is to determine how stimulation from an incubating hen alters the pattern of embryonic self-stimulation. The prehatching behaviors of naturally and artificially incubated embryos were observed and compared.

3. The objective of the third experiment is to investigate the effects of prehatching interactions on embryonic responses to maternal stimuli later in incubation. Artificially incubated embryos were tested with recorded maternal stimuli and their responses were compared with those of naturally incubated embryos.
4. The objective of the fourth experiment is to investigate the effects of prehatching interactions on the responses of newly hatched chicks to the maternal hen. The approach and following behavior of naturally and artificially incubated chicks during nest leaving was observed and compared.

EXPERIMENT I

INTERACTIONS BETWEEN EMBRYOS AND THE INCUBATING HEN IN NATURALLY INCUBATED BROODS

Previous investigators have reported that chicken embryos start vocalizing several days before hatching, shortly after they penetrate the air space of the egg (Collias, 1952; Gottlieb, 1965b; Oppenheim, 1973; Guyomarc'h, 1966). It is also known that broody hens start emitting maternal calls well before hatching (Guyomarc'h, 1974b). Although previous research has provided no direct evidence that the maternal and embryonic behaviors are related or that they influence each other the possibility exists that interactions between an incubating hen and her young start prior to hatching.

There are several patterns in which maternal and embryonic behaviors may occur in relation to each other:

a) there may be no systematic association between embryonic and maternal behaviors. In this case the frequency of associations between maternal and embryonic behaviors should approximate that expected by chance alone.

b) sequencing of maternal and embryonic behaviors may occur due to common causal factors (Hinde, 1970). In this case a common factor may activate simultaneously embryonic and maternal behaviors.

c) embryonic and maternal behaviors may be associated because the hen responds to embryonic stimulation and embryos respond to the hen. That is, embryos and the incubating hen interact. The interaction is an exchange of behaviors between different individuals (Dingle, 1969).

To demonstrate that interactions occur it must be shown that: 1) relevant behaviors of the hen and embryos are closely associated in time, 2) the temporal associations between the behavioral events occur more or less often than would be expected by chance alone and 3) reciprocal stimulation occurs between the hen and embryos.

The aim of this experiment is to describe the maternal and embryonic behavior during the later stages of incubation, and to determine if interactions occur between embryos and the incubating hen. Specifically I sought to:

- describe the patterns of embryonic and maternal vocalizations and the changes during the last days before hatching;
- determine if there are nonrandom temporal associations between maternal behaviors and embryonic vocalizations;
- analyze the types of behavioral associations during the hours before hatching;
- determine, by using information analysis, if communication takes place between the incubating hen and embryos.

METHODS

Subjects and housing

In the normal course of reproduction a hen lays a series of fertilized eggs at 1 day intervals and gradually starts incubating them. The number of fertile eggs that are incubated varies from 1 to 13 or more. Chicks hatch after about 20 to 21 days of incubation. The hen remains on the nest almost continuously with only short breaks for eating and drinking. Periodically she rises, turns the eggs and resettles (Olsen, 1930). About one day before hatching she starts to vocalize (Guyomarc'h, 1974a, 1974b). Embryos also move and vocalize during incubation (Oppenheim, 1973, Guyomarc'h, 1966). Embryonic motility starts at about 4 days of incubation. Up until approximately the 17th day of incubation most embryonic behavior consists of jerky, apparently uncoordinated movements. Smooth, coordinated movements begin at day 17th of incubation and increase in frequency until hatching (Provine, 1973, Oppenheim, 1973). Embryonic vocalizations start approximately one day before hatching, a few hours before pipping. In pipping the chick breaks a small opening in the egg shell during the last day of incubation (Oppenheim, 1973).

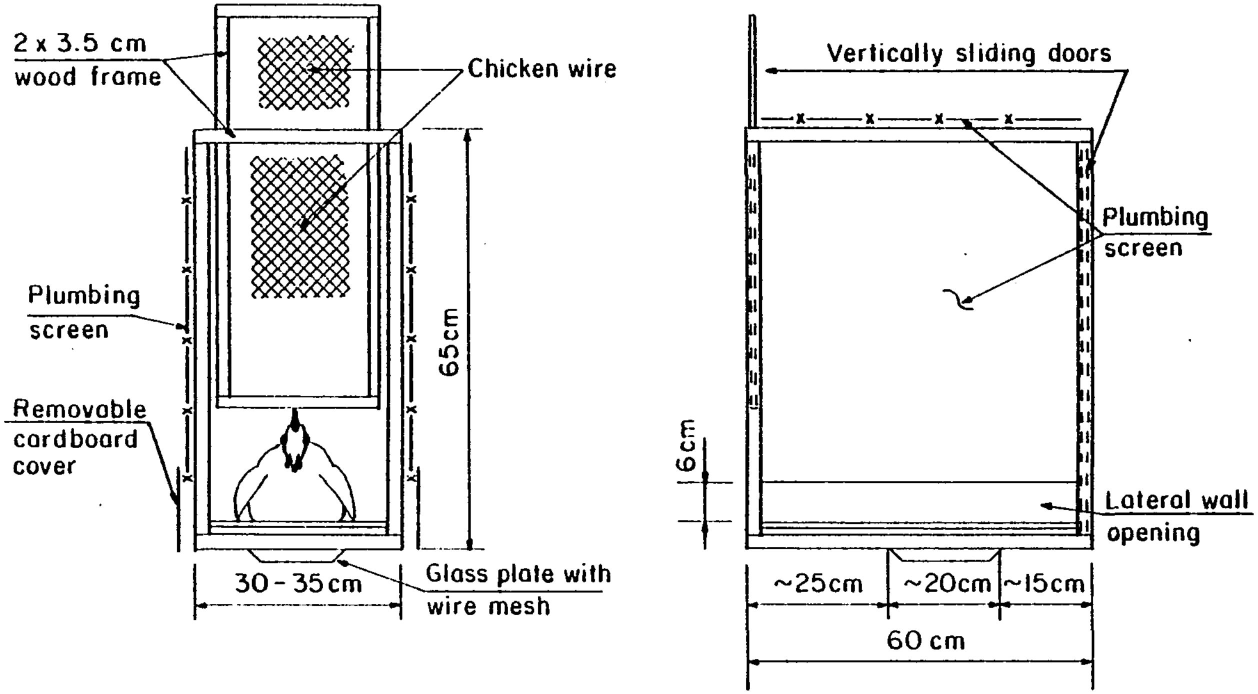
The embryos used in the project developed from fertile White Leghorn eggs procured from a commercial breeder.

Minorca x Rose Comb Bantam hens were used as maternal subjects. Hybrids of this type reliably incubate eggs and care for the young. Four hens with previous maternal experience were chosen from a flock housed in a 2.4m x 3.6m x 2.2m high indoor pen. The bird colony room was kept on a variable 10-14 hour light period at a temperature between 24-30°C. The hens laid and incubated eggs in 65cm x 35cm x 60cm nest cages mounted on the walls of the pen (Fig. 1). Each nest cage had a wood frame with walls and ceiling made of plumbing screen which permitted one-way viewing of the interior. The bottom of the cage was made of plywood outfitted with a glass nest cup 20cm in diameter and 3cm deep. A mirror and light were set beneath the nest cup permitting observation of the hatching process.

Procedure

Broody hens nesting in the experimental cages were monitored daily. Approximately 15 to 18 days after the start of incubation a hen's own eggs were replaced with 6 fertile eggs that had been incubated in a commercial-type incubator for 16 days. On day 17 the nest cage with the incubating hen was moved to the observation room. Sound recordings were started on day 18 of incubation and were continued with only brief interruptions until hatching. The recordings were obtained by placing microphones on the floor of the nest cage within 2cm of the incubating

Fig. 1. Nesting Unit



FRONT VIEW

LATERAL VIEW

FIG. I NESTING UNIT

hen. The microphones were attached to either a Uher Royal series 10,000 or a Sony Model 800B tape recorder. Both recorders operated at a speed of 2.4cm/sec. Hatching was monitored by direct observations or by time lapse filming with a Minolta Super 8 camera at 30 min intervals. The camera was set to take single frame exposures of the mirror that reflected activities in the nest.

Data collection

The analysis was performed on recordings obtained from four different broods of six embryos each. The recordings were made between the eighteenth day of incubation and the emergence of the first chick from its shell. The three-day period was divided into 2-hr intervals and a 30 min continuous sample was taken during each interval. The data were transcribed from the tapes onto paper charts with an Esterline Angus event recorder. Behavioral frequencies, durations and the temporal patterns of succession were retrieved from the charts and tabulated.

RESULTS

Behavioral acts that occur prior to hatching

Tables I and II show seven embryonic behaviors and nine maternal behaviors which occurred frequently during the last days of incubation (embryonic movements and rarely occurring events are not included). Other than the embryonic phioó and soft peep and the maternal intermediate call the remaining embryonic and maternal calls have been described by previous investigators (Collias, 1952; Collias and Joos, 1953; Konishi, 1963; Guyomarc'h, 1966, 1974a). They are presented here for purposes of comparison. The nomenclature of the sounds uses onomatopoetic terms whenever possible. In some cases where onomatopoetic expressions were difficult to apply, descriptive terms based on the usual behavioral context were used instead. The term used for the maternal "intermediate" call describes the relation between its frequencies and those of the maternal cluck and food call. Some problems associated with sound nomenclature are discussed in Appendix I where a brief description of each behavior is provided.

The embryonic vocalizations are grouped into "distress" type calls and "pleasure" type calls according to the system used by Guyomarc'h (1966). Although "pleasure" type calls have been associated with "approach" processes

TABLE I. Embryonic vocalizations in naturally and artificially incubated broods

Distress type calls	Pleasure type calls
Phioo	Twitter
Soft peep	Food call
Peep	Huddling call
Screech	

TABLE II. Maternal behaviors during incubation

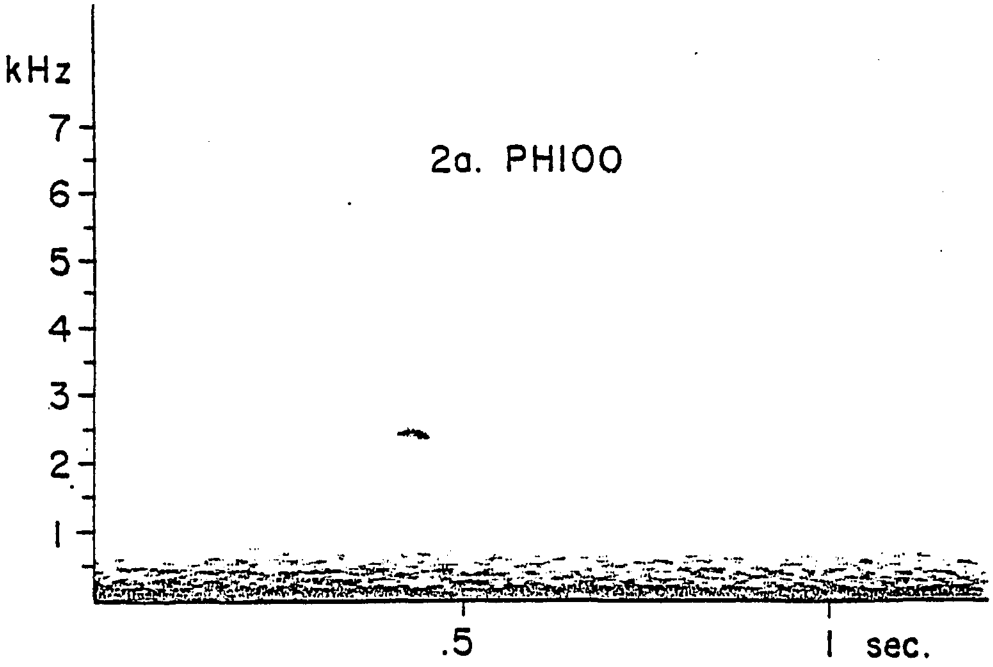
Body Movements	*Head Movements*	*Vocalizations*
Undetermined move	Peck	Cluck
Egg turn	Beak clap	Intermediate call
Resettle		Food call
		Mild alarm call

and "distress" type calls with "withdrawal" processes (Schneirla, 1965), there is no attempt in this study to assume any underlying motivational state. The grouping is based on the spectral characteristics of the sounds and is consistent with conventions currently used in the literature. The "distress" calls group contains vocalizations characterized by a spectrum of descending frequencies while the "pleasure" calls group contains vocalizations with ascending frequencies. This scheme for categorizing chick distress and pleasure calls was proposed by Collias and Joos (1963) and modified by Guyomarc'h (1966). Maternal behaviors were grouped into three categories: body movements, which could stimulate the embryo tactually, vocalizations, and head movements producing tapping sounds which could stimulate the embryo auditorily. The spectral characteristics of the maternal and embryonic vocalizations are shown in Figs. 2,3 and 4.

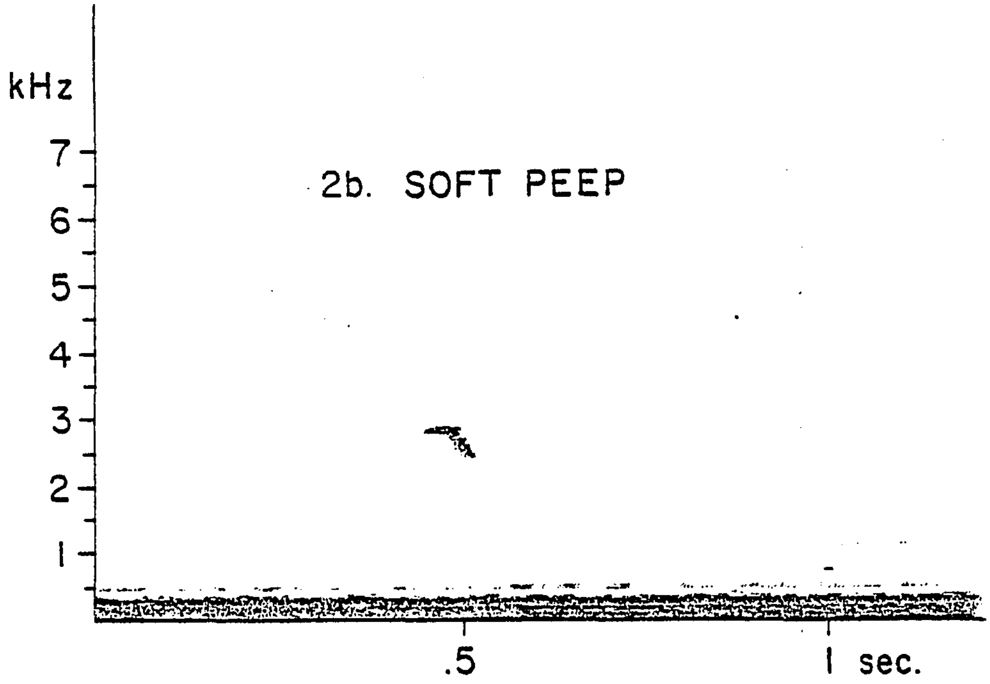
Longitudinal changes in embryonic and maternal behaviors

There is a notable increase in the occurrence of embryonic and maternal vocalizations as hatching approaches. The changes in total durations of maternal and embryonic behaviors are shown in Fig. 5 through Fig. 9. The plotted values are durations estimated by the average number of 1 s intervals during which the behaviors occurred per

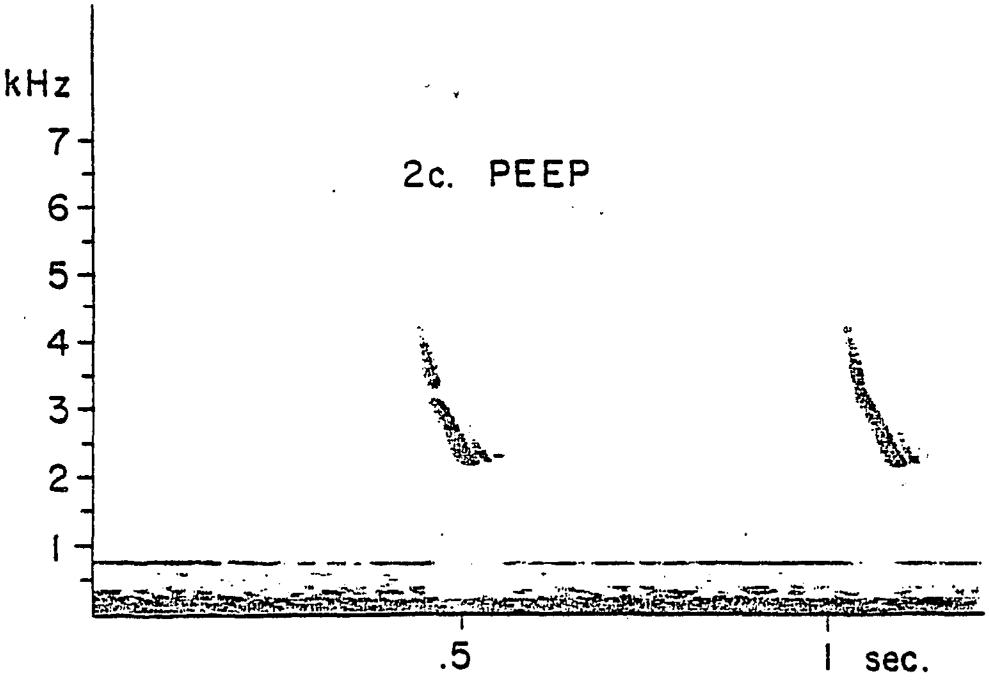
Fig. 2a,b,c,d. Embryonic "distress" type calls. The sonograms were made with a Kay Sona-Graph 7029A, 5-16,000 Hz spectrum analyzer set at the 80-8,000 Hz frequency range.



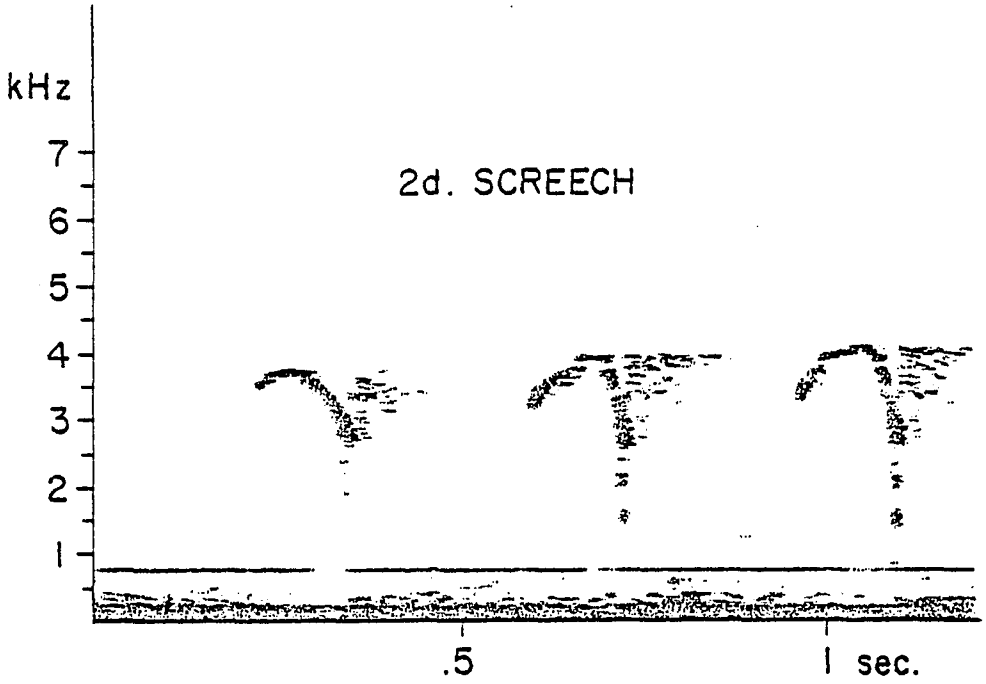
2a. PHIOO



2b. SOFT PEEP

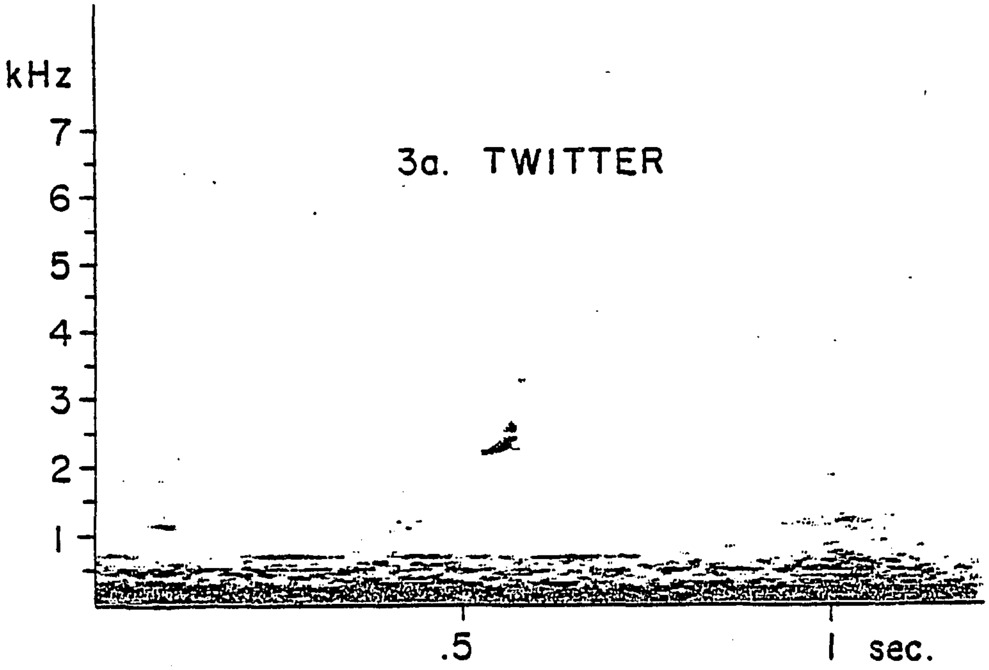


2c. PEEP

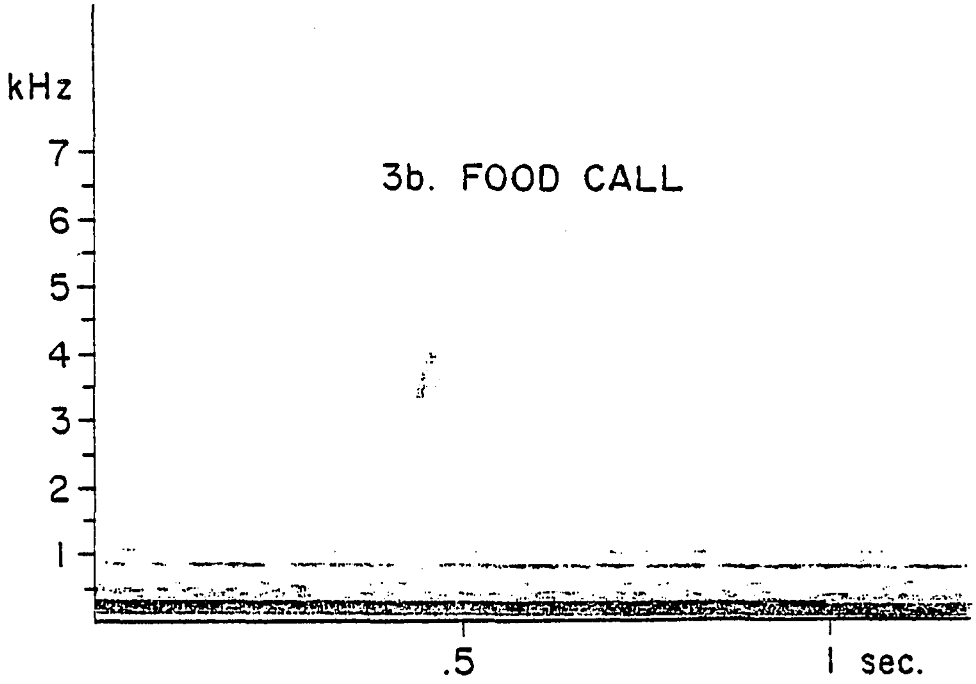


2d. SCREECH

Fig. 3a,b,c. Embryonic "pleasure" type calls. The sonograms were made with a Kay Sona-Graph 7029A, 5-16,000 Hz spectrum analyzer set at the 80-8,000 Hz frequency range.



3a. TWITTER



3b. FOOD CALL

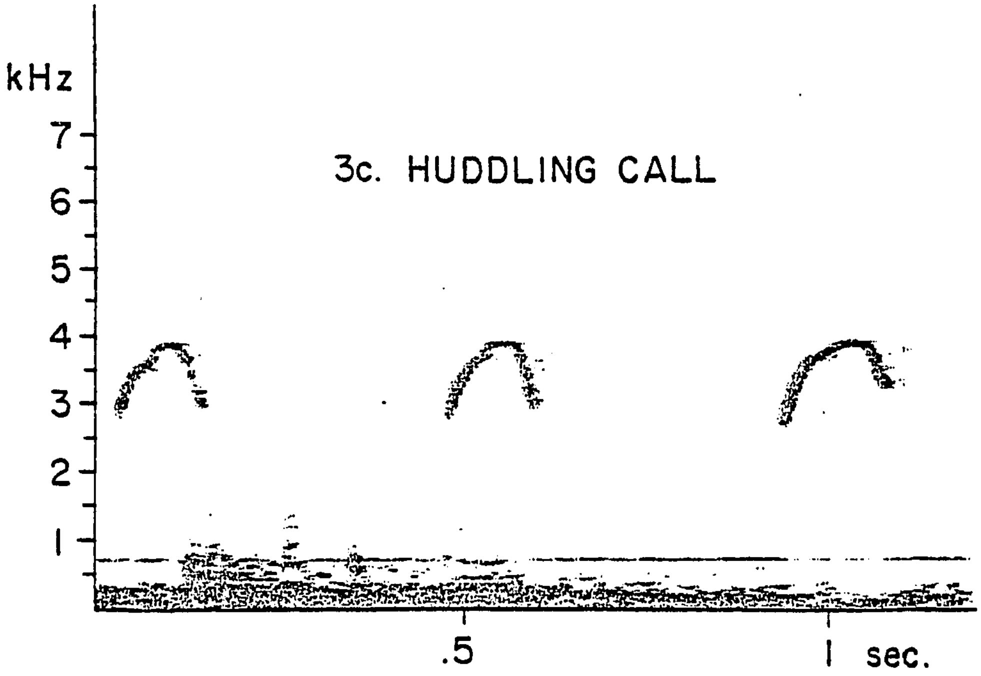
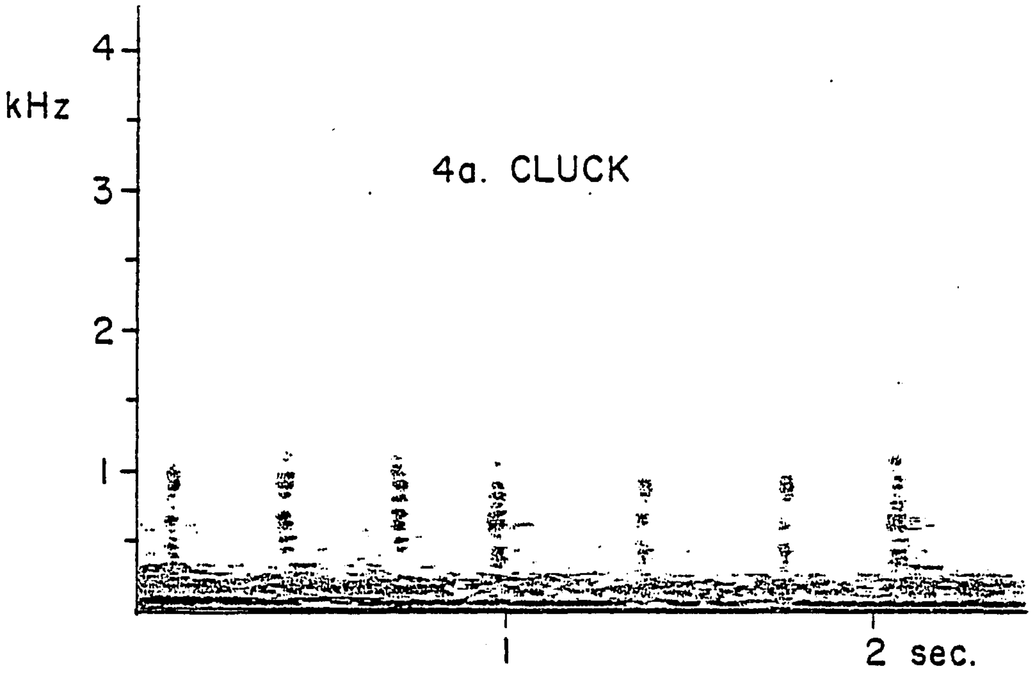
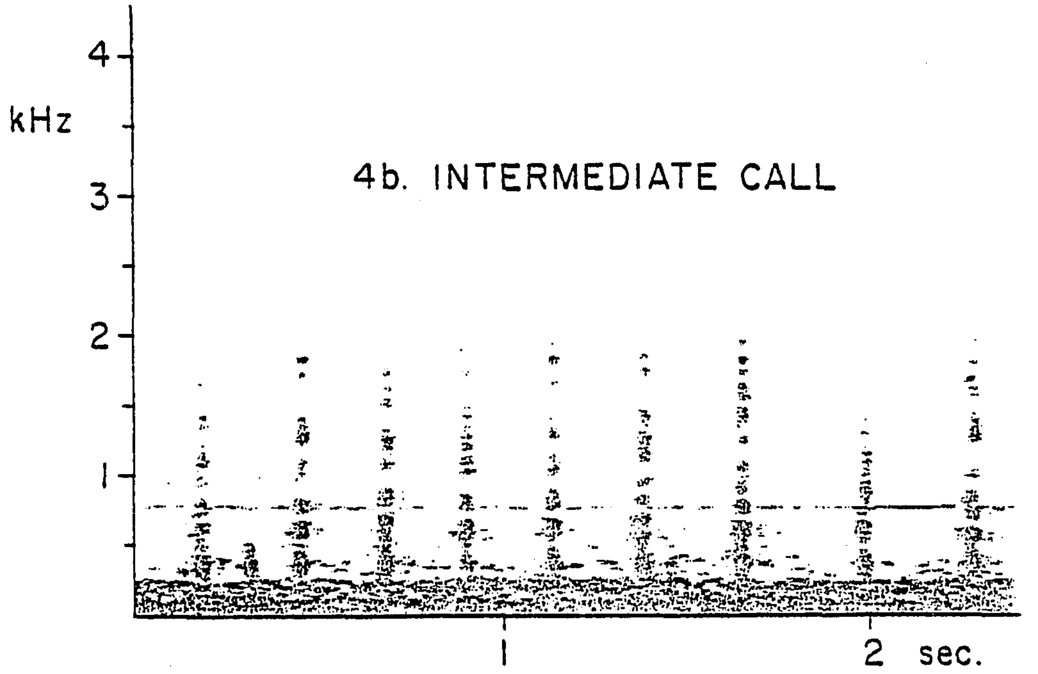

kHz
7
6
5
4
3
2
1
3c. HUDDLING CALL
.5
1 sec.

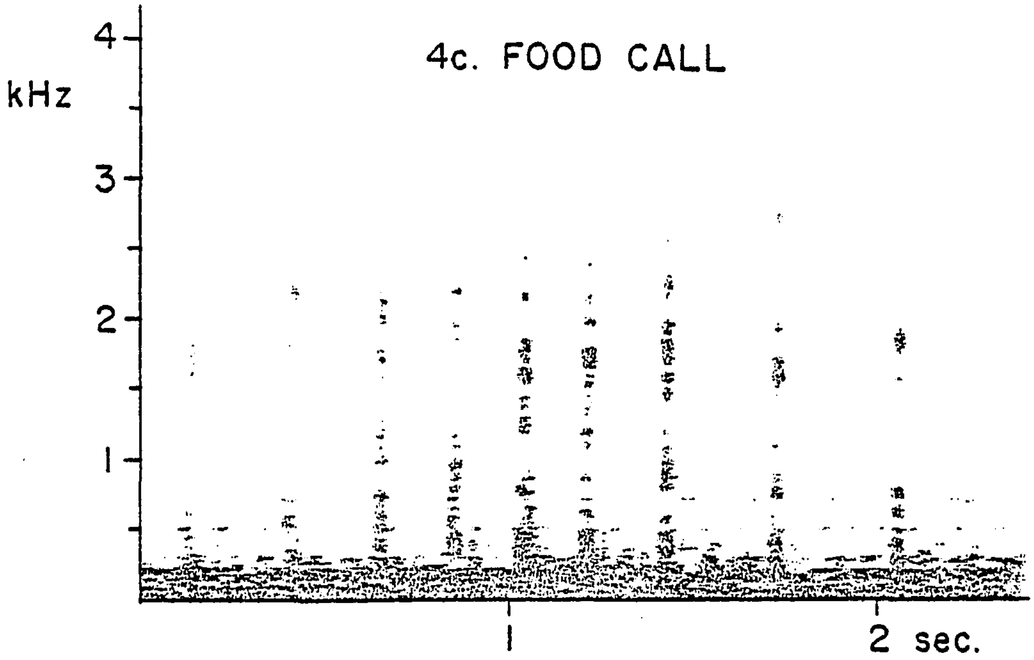
Fig. 4a,b,c,d. Maternal vocalizations. The sonograms were made with a Kay Sona-Graph 7029A, 5-16,000 Hz spectrum analyzer set at the 40-4,000 Hz frequency range.

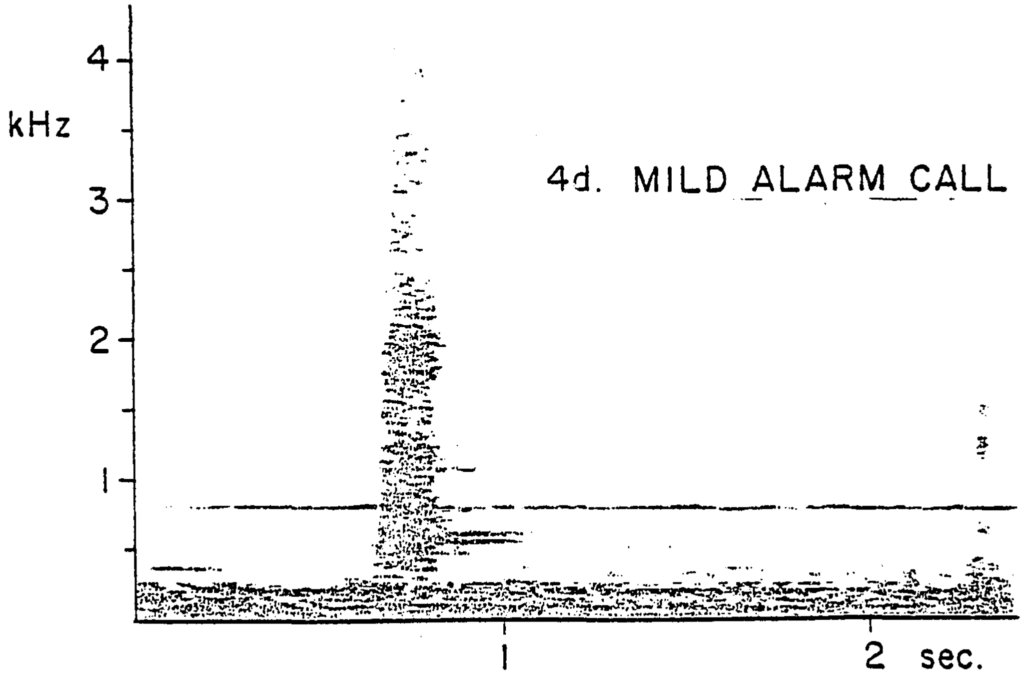


4a. CLUCK



4b. INTERMEDIATE CALL


4c. FOOD CALL


4d. MILD ALARM CALL

30 min sample. One sample per brood was taken at each stage before hatching. The embryonic vocalizations that appear first are distress calls (Fig. 5). As hatching approaches there is a moderate increase in screeches and soft peeps. Peep calls remain at a relatively low level at all stages. Pleasure calls (Fig.6) originate later in development and their duration per sample increases sharply as hatching approaches.

Of the maternal behaviors, some change in relation to embryonic behaviors while others do not. Maternal vocalizations appear after the onset of embryonic distress vocalizations and their total durations increase sharply as hatching approaches (Fig. 7). The start of maternal vocalizations coincides with the onset of embryonic pleasure calls. The movements of the hen on the nest change in an irregular pattern except for undetermined movements ("move") which increase near hatching (Fig. 8). The same irregularity is seen in the changes of maternal pecks and beak claps (Fig. 9).

Embryonic responses to maternal stimuli

Overall embryonic responsiveness to maternal stimulation. The data were analyzed to highlight the stimulus- response relationship between maternal and embryonic behaviors. In order to perform the analysis first it was necessary to establish a criterion for a response. A preliminary analysis of the temporal distribution of embryonic

Fig. 5. Embryonic "distress" type calls (during the 33 hrs before hatching) in broods incubated by a hen. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.

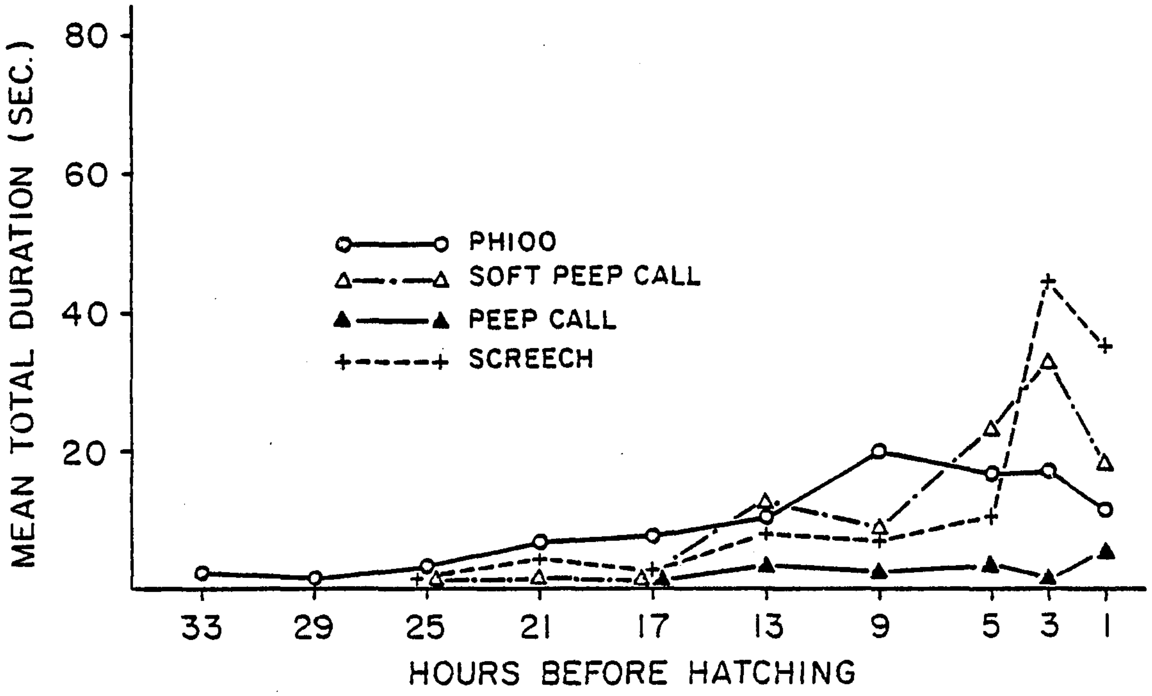

MEAN TOTAL DURATION (SEC.)
80
60
40
20
PHIOO
SOFT PEEP CALL
PEEP CALL
SCREECH
33
29
25
21
17
13
9
5
3
1
HOURS BEFORE HATCHING

Fig. 6. Embryonic "pleasure" type calls (during the 33 hrs before hatching) in broods incubated by a hen. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.

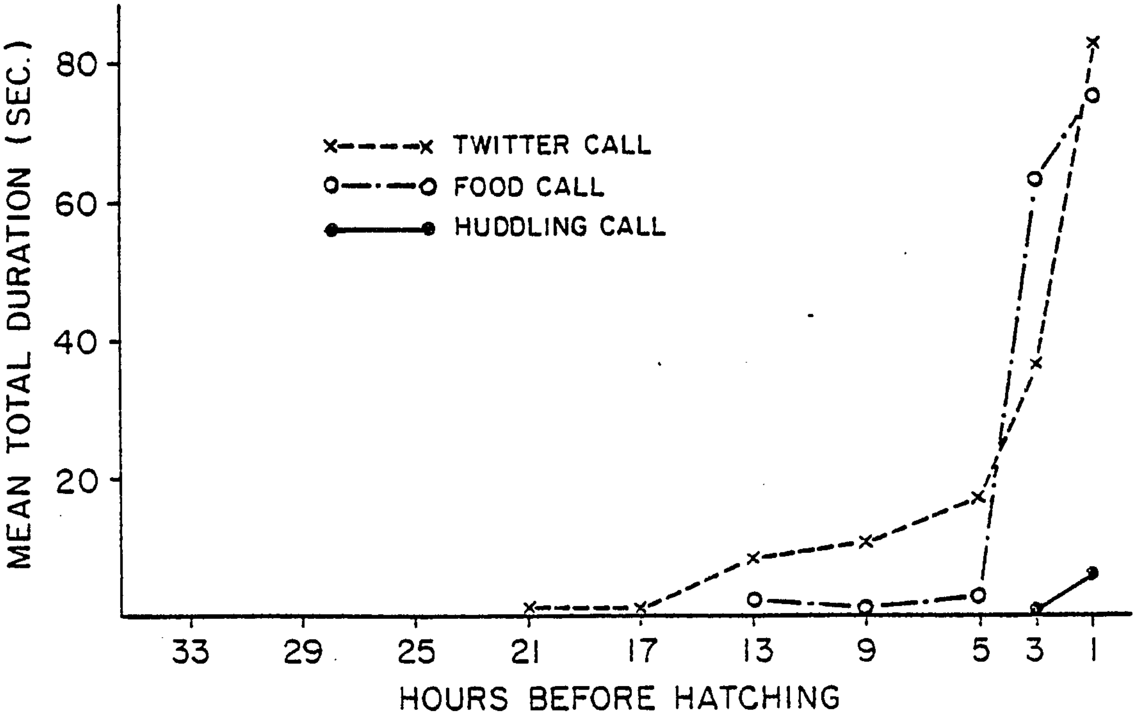

MEAN TOTAL DURATION (SEC.)
80
60
40
20
x----x TWITTER CALL
o-·-o FOOD CALL
●——● HUDDLING CALL
33
29
25
21
17
13
9
5
3
1
HOURS BEFORE HATCHING

Fig. 7. Maternal vocalizations during the 33 hrs before hatching. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.

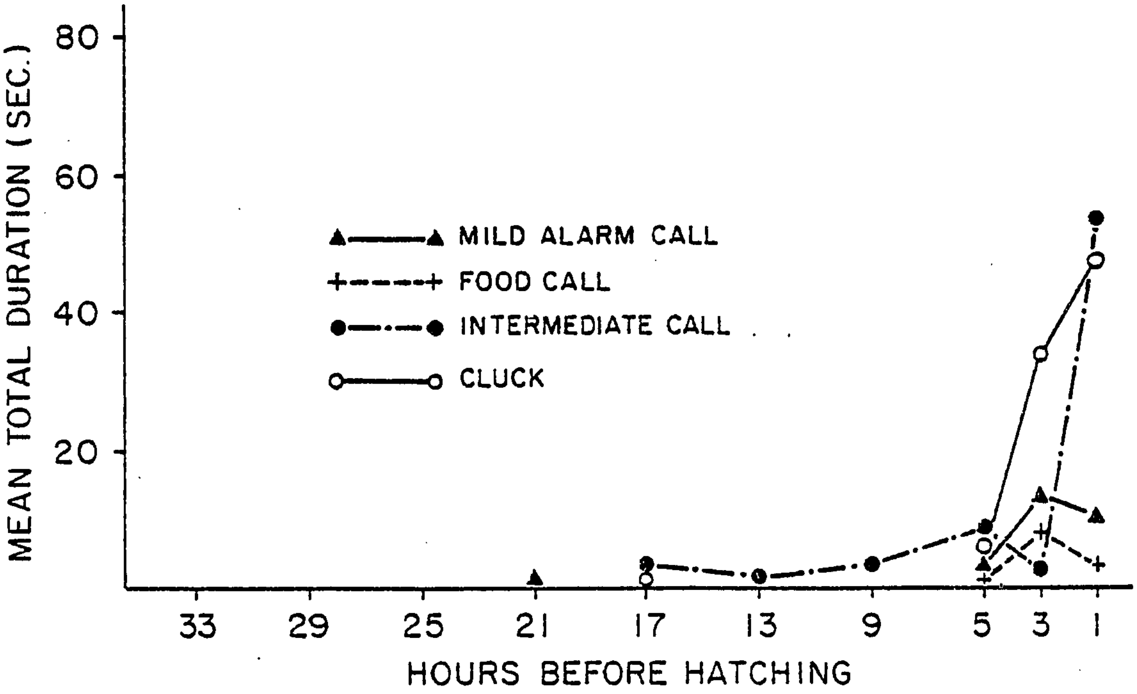

MEAN TOTAL DURATION (SEC.)
80
60
40
20
33
29
25
21
17
13
9
5
3
1
HOURS BEFORE HATCHING
▲——▲ MILD ALARM CALL
+- - - -+ FOOD CALL
●—·—● INTERMEDIATE CALL
○——○ CLUCK

Fig. 8. Maternal movements during the 33 hrs before hatching. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.

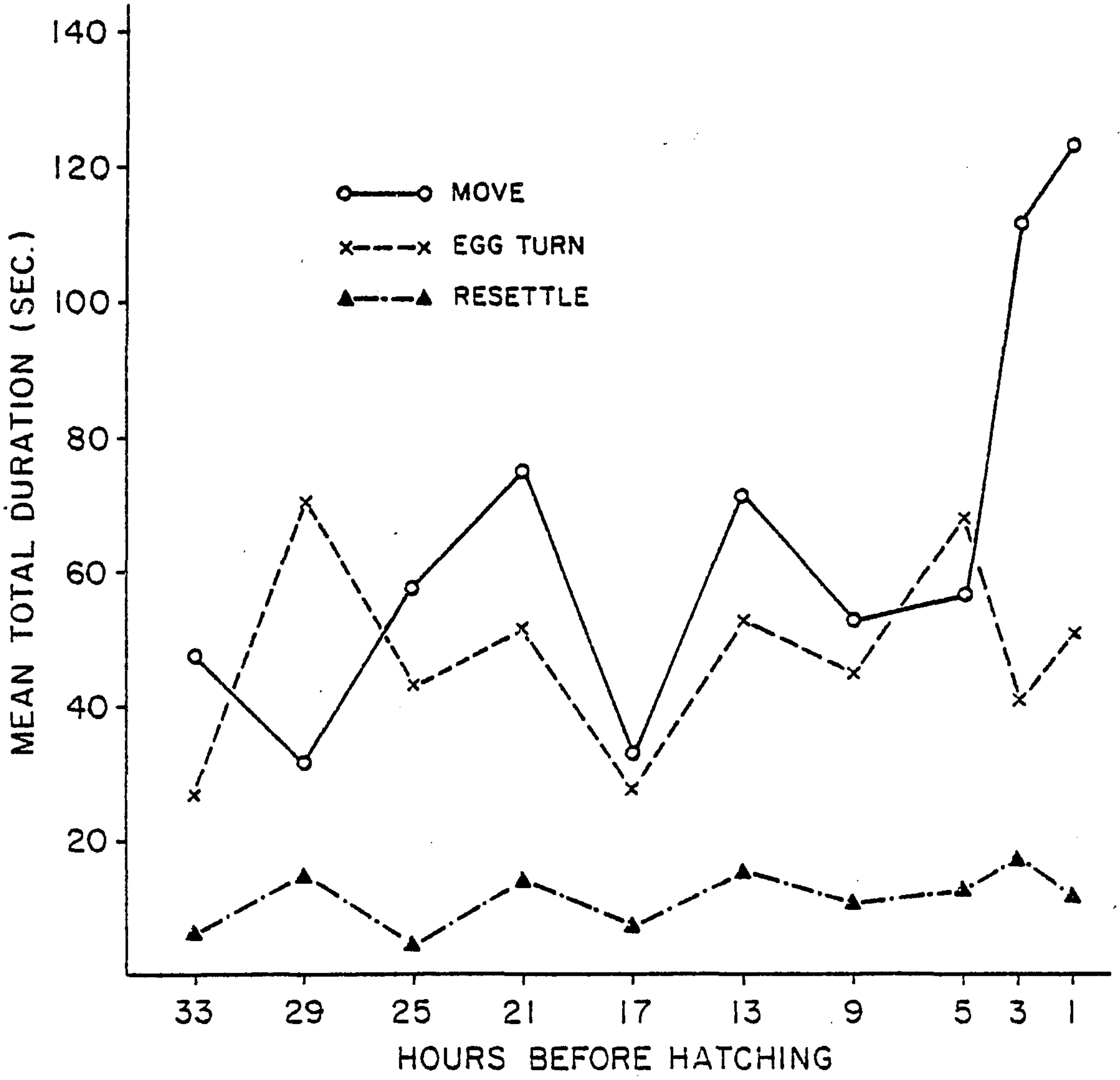
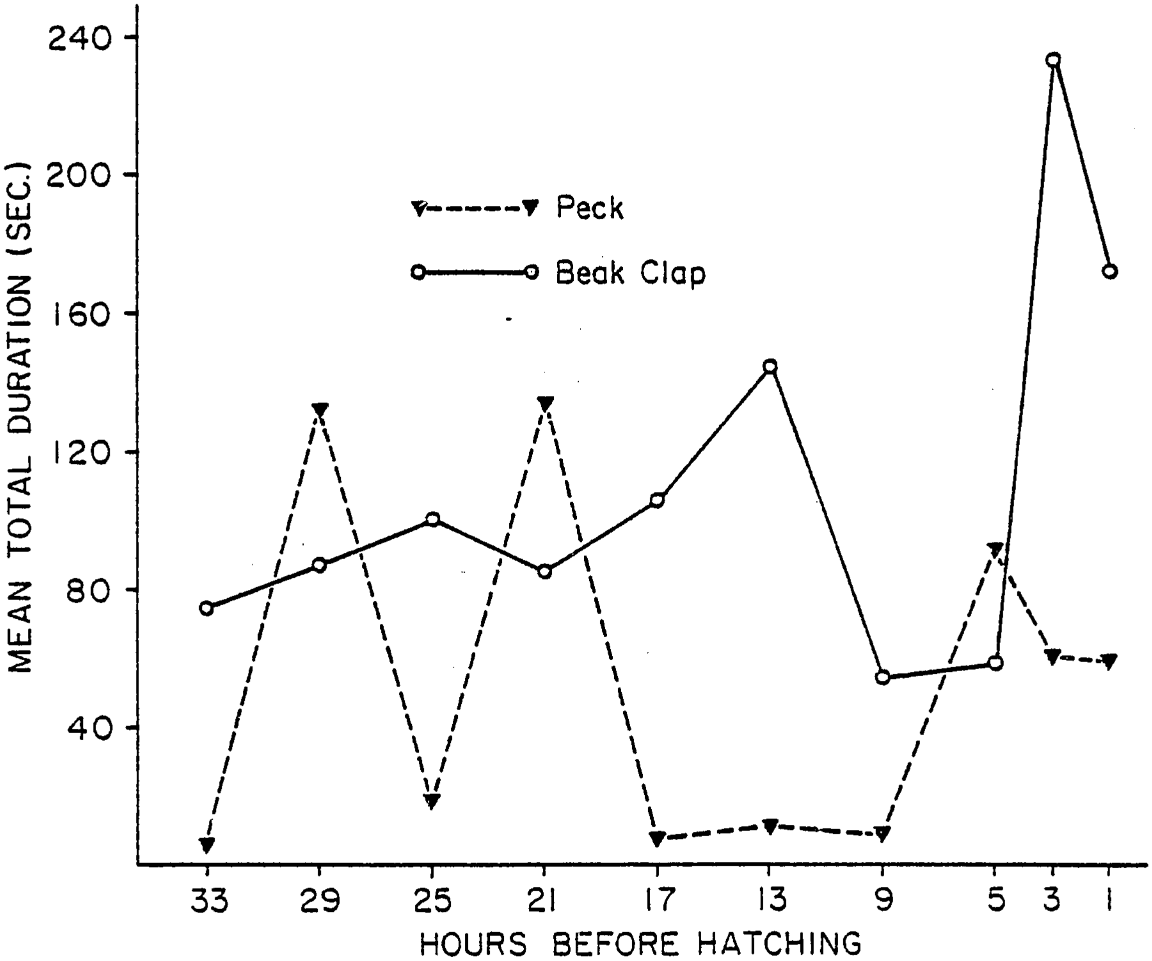

140
120
100
80
60
40
20
MEAN TOTAL DURATION (SEC.)
MOVE
EGG TURN
RESETTLE
33
29
25
21
17
13
9
5
3
1
HOURS BEFORE HATCHING

Fig. 9. Maternal pecks and beak claps during the 33 hrs before hatching. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.


Peck
Beak Clap
MEAN TOTAL DURATION (SEC.)
240
200
160
120
80
40
33
29
25
21
17
13
9
5
3
1
HOURS BEFORE HATCHING

vocalizations following maternal acts revealed that during the two hours before hatching more than 80% of the embryonic calls occur within 15 s after the start of a maternal act. Based on this observation a 15-s interval was used as the criterion for identifying embryonic responses to maternal stimuli.

Embryonic responsiveness to maternal stimulation increases rapidly as hatching approaches. Fig. 10 shows a steady increase in the proportion of embryonic vocalizations that meet the criterion as hatching approaches. There is also an increase in the total number of vocalizations that meet the response criterion (Fig. 11).

In order to determine if the increase in number of embryonic vocalizations meeting the response criterion is due merely to the increased frequency of embryonic and maternal behaviors, the observed number of responses is compared with the number expected by chance alone (Fig. 11 and Table III). To calculate the expected number of embryonic responses, the total number of vocalizations was multiplied by the ratio between the time included in 15-s intervals measured from the start of each maternal act and the total sample time (Fig. 12 and Appendix XI).

At 21 hrs before hatching, the number of embryonic vocalizations that meet the criterion for a response is about equal to the number expected by chance alone. But

Fig. 10. Percentage of embryonic vocalizations that occur within 15 s after the start of a maternal behavior at various stages before hatching.

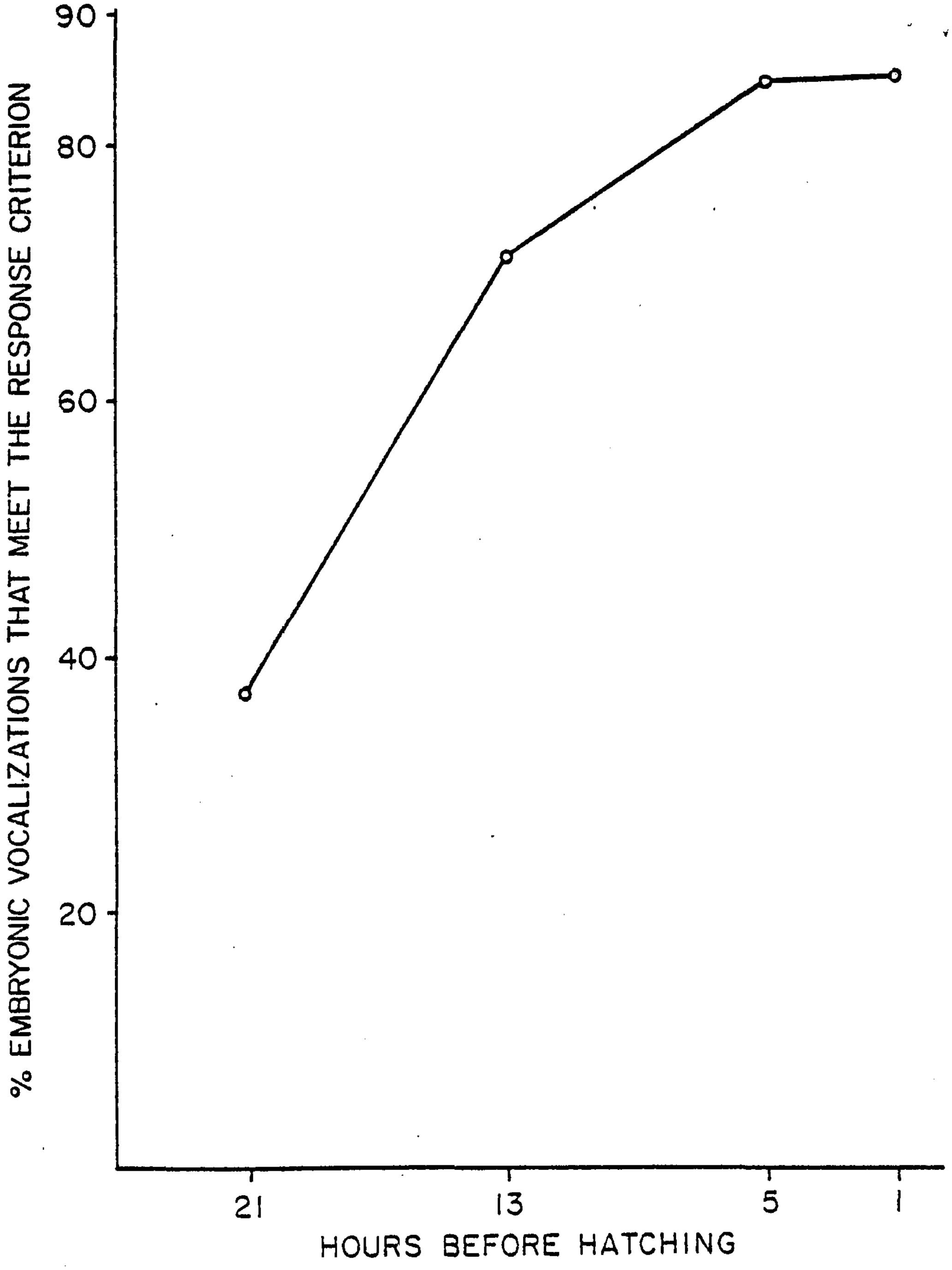

% EMBRYONIC VOCALIZATIONS THAT MEET THE RESPONSE CRITERION
90
80
60
40
20
21
13
5
1
HOURS BEFORE HATCHING

Fig. 11. Changes in the number of embryonic vocalizations that occur within 15 s after the start of a maternal behavior at various stages before hatching. Totals obtained from four 30-min samples are plotted.

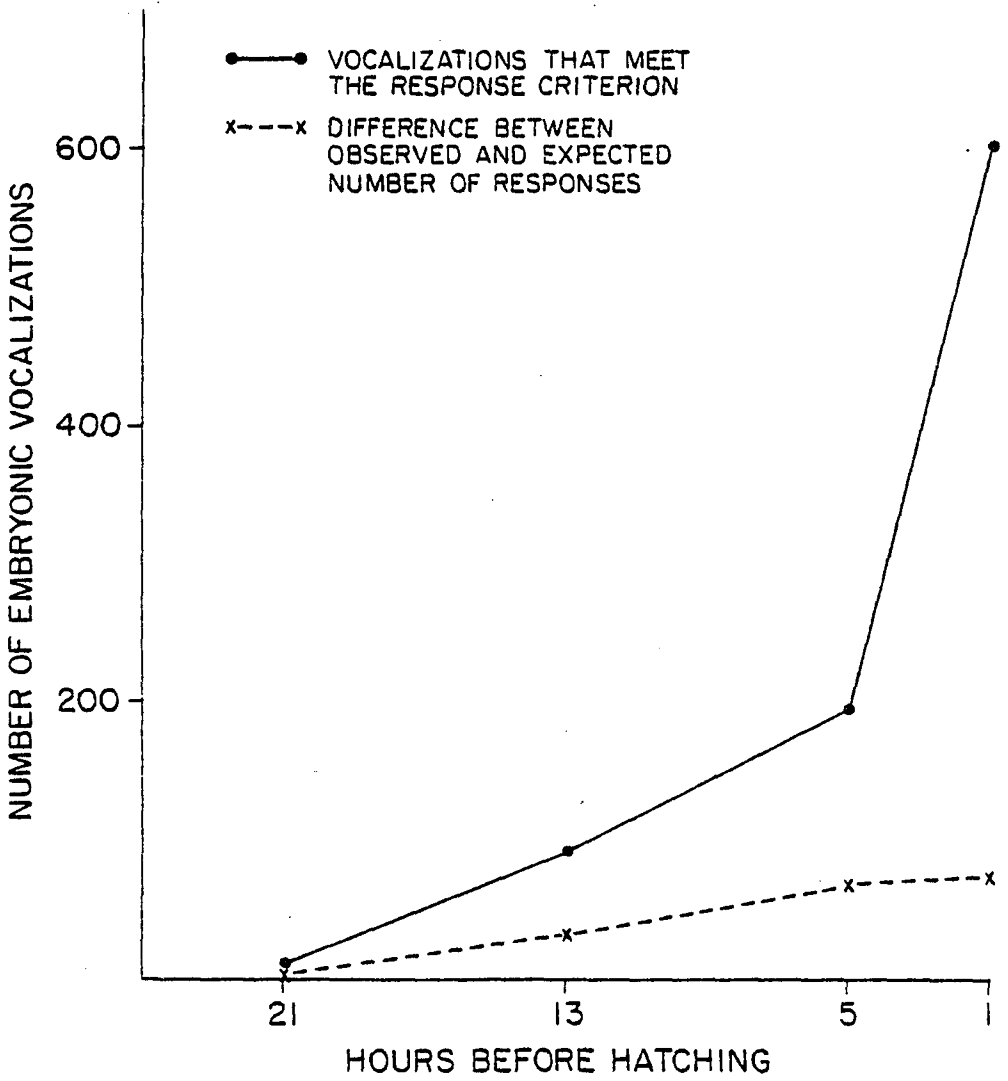

●——● VOCALIZATIONS THAT MEET THE RESPONSE CRITERION
x---x DIFFERENCE BETWEEN OBSERVED AND EXPECTED NUMBER OF RESPONSES
NUMBER OF EMBRYONIC VOCALIZATIONS
600
400
200
21
13
5
1
HOURS BEFORE HATCHING

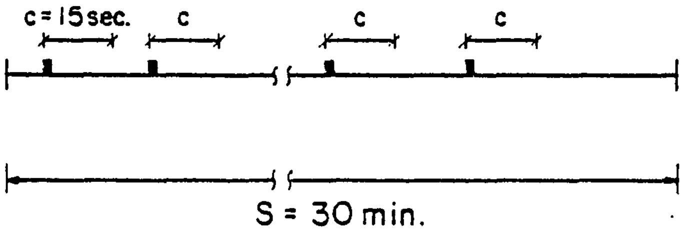
TABLE III. Comparisons between the number of embryonic responses observed and the number expected due to chance alone.

Hours before hatching	Mean number of vocalizations per sample	Mean number of responses per sample		X^{2*}	p
		Observed	Expected		
1	177	151	132	10.69	< 0.001
5	58	49	31	22.25	< 0.001
13	33	23	15	8.20	< 0.01
21	7	2	2	0.00	n.s.

*d.f. = 1 for all comparisons

Fig. 12. Number of embryonic vocalizations expected to follow a maternal act within 15 s from its start.

RESPONSE CRITERION



NUMBER OF EXPECTED RESPONSES: $\frac{\Sigma c}{S} \times N$

N = TOTAL NO. OF EMBRYONIC ACTS

nearer hatching embryonic calls are associated with maternal acts in much higher numbers than expected. While the general trend near hatching is that of an increase in the number of embryonic vocalizations associated with maternal behaviors (Table III and Fig. 11) the frequency of association varies with the type of embryonic vocalization (Table IV). During the hour before hatching distress type calls such as phioos, soft peeps and screeches follow maternal behaviors as often as would be expected by chance, while peeps follow significantly less frequently than expected. In contrast, all types of pleasure calls follow maternal behaviors significantly more often than expected by chance. The nonsignificant value of the heterogeneity X^2 indicates that there is a uniform departure from the expected values for all types of pleasure calls.

The latency of embryonic responses decreases as hatching approaches. Fig. 13 shows the number of embryonic responses that occur at various time intervals after the start of a maternal behavior during the hour before hatching. Most embryonic responses occur within 5 seconds. The proportion of responses that occur within this interval increases gradually as hatching approaches (Fig. 14).

<u>Specific embryonic responsiveness to maternal stimulation</u>. Embryonic vocalizations are temporally associated with maternal behaviors in distinctive patterns

TABLE IV. Comparisons between the number of embryonic responses observed and the number expected due to chance alone during the hour before hatching. The tabulated values were obtained from four samples.

Embryonic Vocalization	Total Number of Vocalizations	Embryonic Responses				
		Observed	Expected		X^2	p
Phioo	43	33	32.0		0.12^1	n.s.
Peep	18	8	13.4		8.50^1	< 0.005
Soft Peep	66	50	49.1		0.06^1	n.s.
Screech	119	97	88.6		3.11^1	n.s.
				TOTAL	11.796^4	< 0.05
	246	188	183.1	POOLED	0.51^1	n.s.
				HETEROGENEITY:	11.283^3	< 0.01
Twitter	201	177	149.6		19.62^1	< 0.001
Food Call	248	225	184.6		34.58^1	< 0.001
Huddling Call	13	13	9.7		4.42^1	< 0.05
				TOTAL	58.62^3	< 0.001
	462	415	343.9	POOLED	57.50^1	< 0.001
				HETEROGENEITY:	1.12^2	n.s.

^{1}d.f. = 1, ^{2}d.f. = 2, ^{3}d.f. = 3, ^{4}d.f. = 4

Fig. 13. Cumulative number of embryonic responses as a function of time from the start of a maternal act. The plotted values are based on four 30-min samples and include only the first response of each type that follows a maternal act.

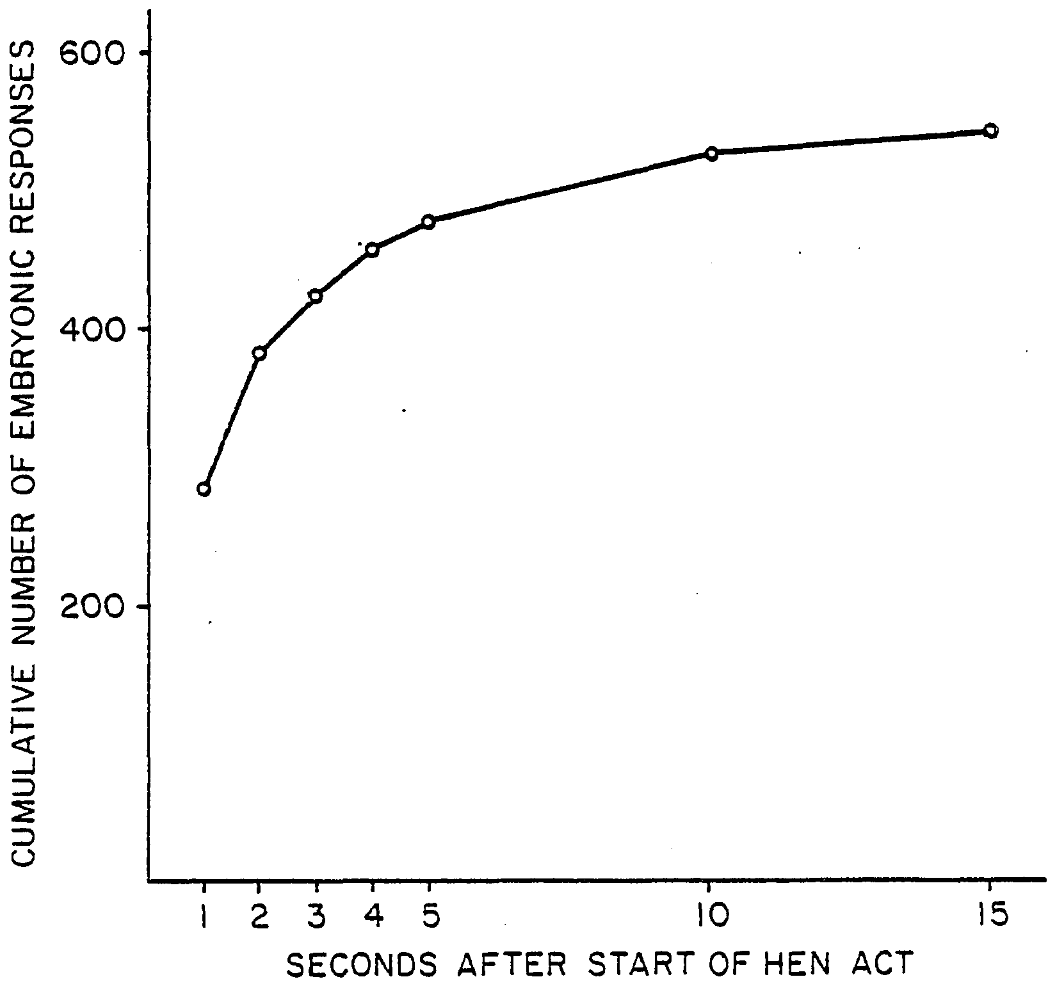
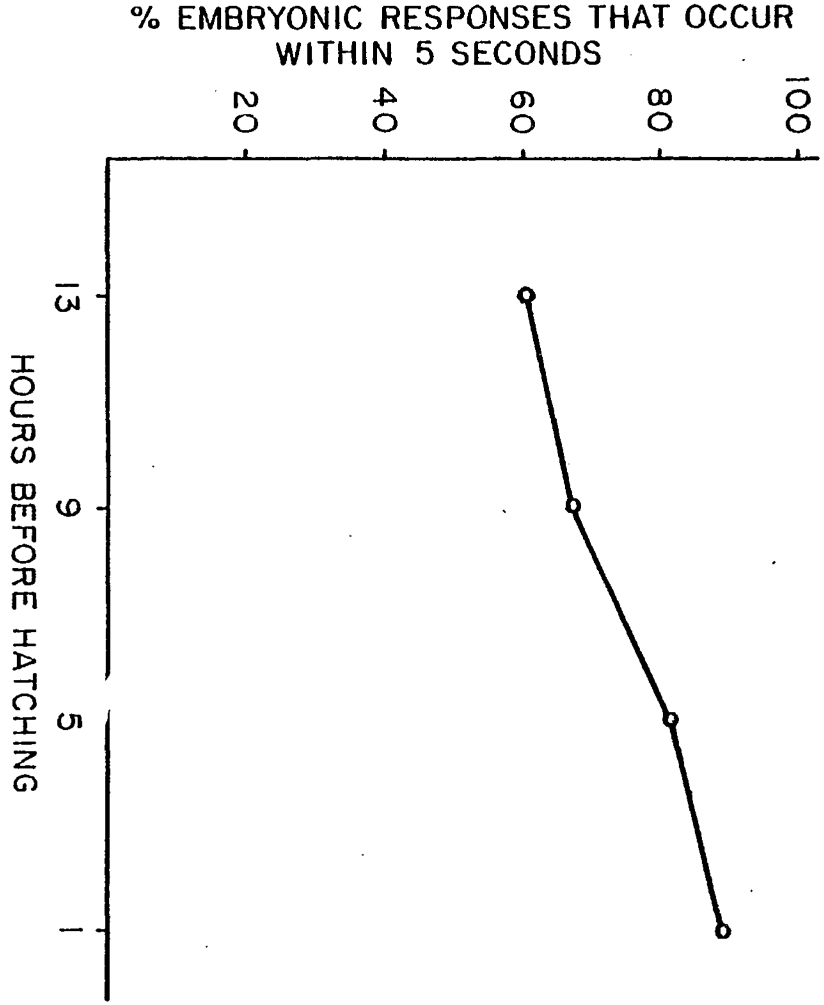

CUMULATIVE NUMBER OF EMBRYONIC RESPONSES
600
400
200
1
2
3
4
5
10
15
SECONDS AFTER START OF HEN ACT

Fig. 14. Changes in the proportion of embryonic responses that have a latency of 5 s or less. The plotted values are based on four 30-min samples at each stage and include only the first response of each type that follows a maternal act.


% EMBRYONIC RESPONSES THAT OCCUR WITHIN 5 SECONDS
20
40
60
80
100
13
9
5
1
HOURS BEFORE HATCHING

and the associations change as hatching approaches (Fig. 15). During early stages, a higher proportion of responses follow maternal movements, whereas nearer hatching the proportions of responses that follow maternal vocalizations and movements are almost equal. A "t" test for percentages (Sokal and Rohlf, 1969, p. 607) shows that the differences between embryonic responses to movements and vocalizations at 1 hr before hatching is significantly lower than at 5 hrs before hatching ($t_s = 1.982$, $p < 0.05$).

During the hours before hatching the type and number of embryonic responses depend on the type of the preceding maternal behavior. The distributions of embryonic responses following various maternal behaviors are shown in Fig. 16. The plotted values are ratios giving the number of embryonic acts per maternal act. Only the first embryonic response following a maternal behavior was counted because subsequent responses would not necessarily be independent of the first.

Embryonic distress type calls occur with a relatively low frequency after all maternal behaviors. In contrast, pleasure type calls such as food calls and twitters have higher frequencies and an uneven distribution. Embryonic food calls are associated most often with maternal vocalizations whereas twitters most often follow maternal movements.

The observed distributions were compared with those

Fig. 15. Change in embryonic responsiveness to maternal movements and vocalizations. Each value is a ratio of the number of embryonic responses divided by the number of maternal acts. Only the first embryonic response following a maternal act was used to compute the ratios.

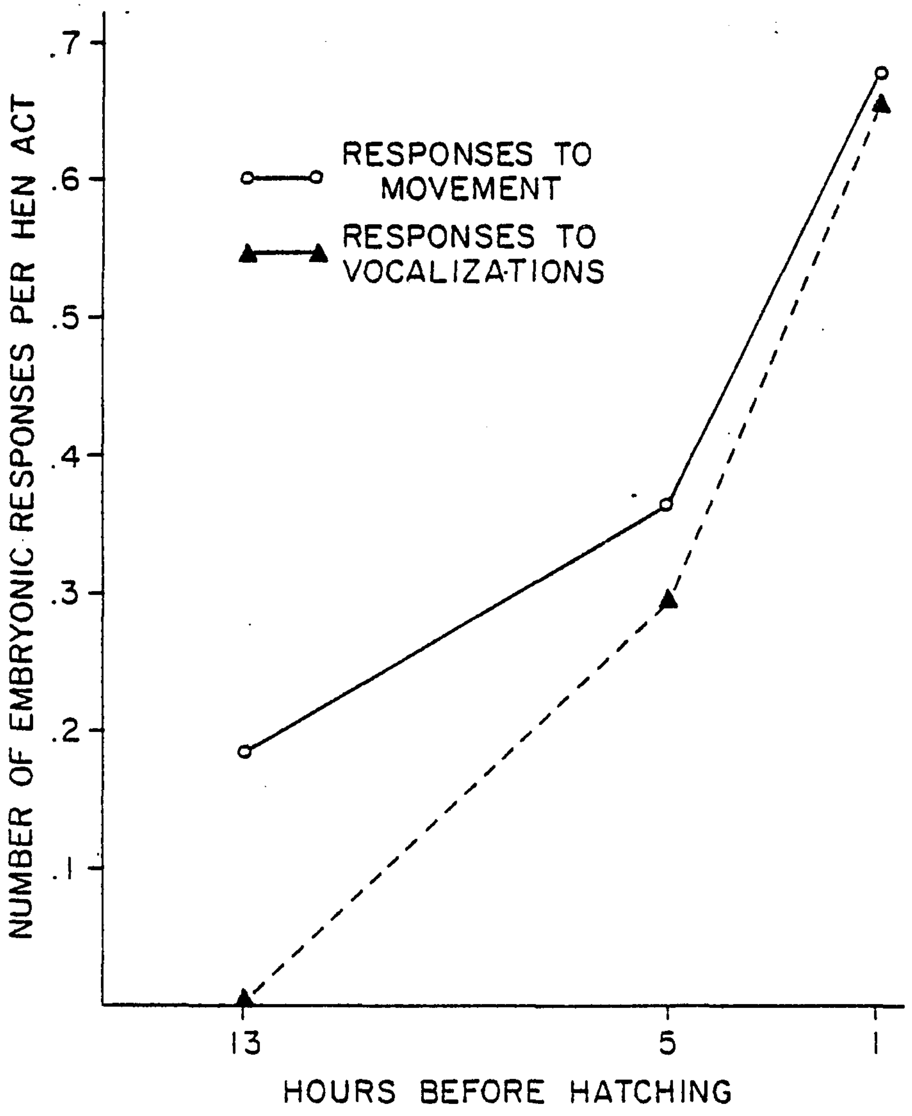
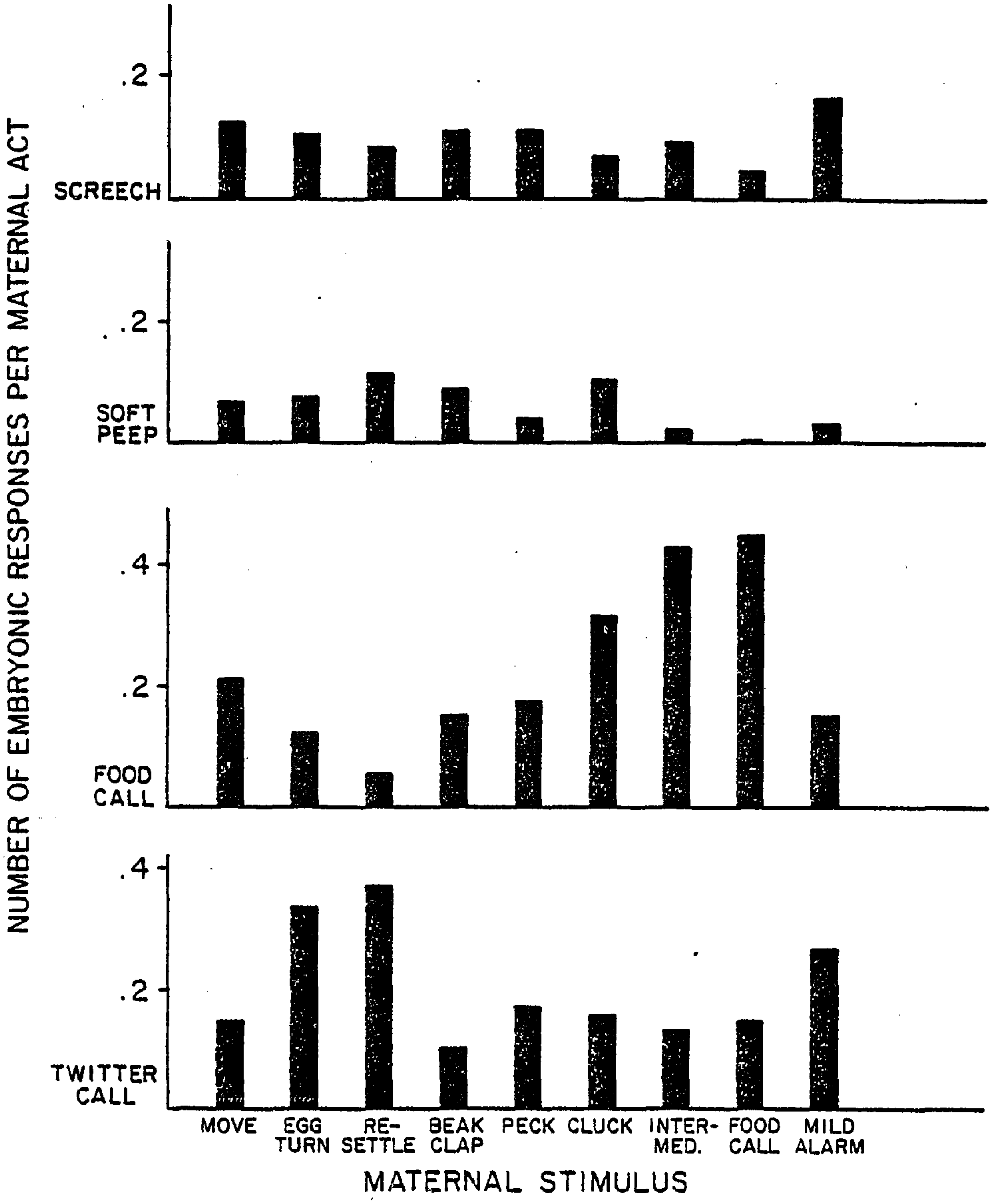
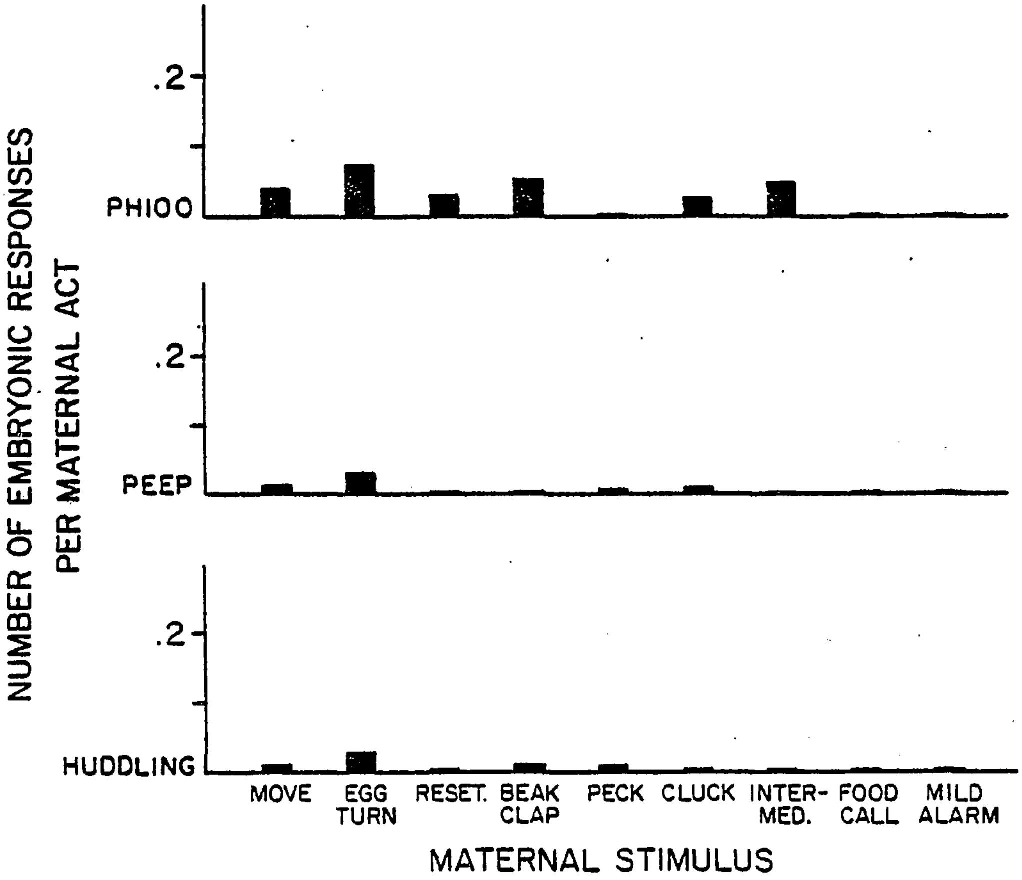

NUMBER OF EMBRYONIC RESPONSES PER HEN ACT
.7
.6
.5
.4
.3
.2
.1
RESPONSES TO MOVEMENT
RESPONSES TO VOCALIZATIONS
13
5
1
HOURS BEFORE HATCHING

Fig. 16. The distribution of embryonic responses following different types of maternal stimulation during the 4 hrs before hatching. The values for each type of embryonic response are computed as follows: the number of responses in one category that followed a certain type of maternal behavior was divided by the total number of maternal acts of that type. The procedure was repeated for each of the maternal behaviors. The data are based on eight 30-min samples.


NUMBER OF EMBRYONIC RESPONSES PER MATERNAL ACT
.2
SCREECH
.2
SOFT PEEP
.4
.2
FOOD CALL
.4
.2
TWITTER CALL
MOVE
EGG TURN
RE-SETTLE
BEAK CLAP
PECK
CLUCK
INTER-MED.
FOOD CALL
MILD ALARM
MATERNAL STIMULUS


NUMBER OF EMBRYONIC RESPONSES PER MATERNAL ACT
.2
PHIOO
.2
PEEP
.2
HUDDLING
MOVE
EGG TURN
RESET.
BEAK CLAP
PECK
CLUCK
INTER-MED.
FOOD CALL
MILD ALARM
MATERNAL STIMULUS

expected to occur by chance (Table V and Appendix III) by using a X^2 for goodness of fit test. The expected frequencies for each type of embryonic vocalization were computed by multiplying the total number of vocalizations of that type by the coefficient of distribution for the various maternal behaviors (Appendix IV). For example, the 12.2 phioos expected to follow maternal moves were obtained by multiplying the total number of phioos (53) by the proportion of maternal behaviors that are moves (0.231). The largest differences were found for pleasure type calls. Observed distributions of both twitters and food calls show a highly significant difference from the expected distributions. Among distress type calls only the distribution of phioo calls differs significantly from that expected.

Several types of maternal behavior have a marked influence on the type and frequency of embryonic vocalizations that follow (Table VI). The influence of each type of maternal behavior is determined by an internal goodness of fit test (Appendix X). It appears that large amplitude movements such as egg turning and resettling on the nest have a strong directive effect on embryonic twitters but tend to inhibit embryonic food calls. Maternal vocalizations have a strong directive effect on embryonic food calls but tend to inhibit screeches and soft peeps. (Note: The terms "directive" and "inhibitory"

TABLE V. Comparison between the observed and expected number of embryonic responses to any maternal act during the four hours before hatching. Only the first embryonic response following a maternal act is included. The data are based on eight 30-min. samples. (See Appendix II for the complete table.)

Embryonic Vocalization	n	x^{2*}	p
Phioo	53	17.73	0.05
Peep	7	5.88	n.s.
Soft Peep	101	13.49	n.s.
Screech	175	6.04	n.s.
Twitter	247	38.45	0.001
Food Call	324	51.38	0.001
Huddling Call	9	2.78	n.s.

*d.f. = 8 for each comparison

TABLE VI. Analysis of the effects of maternal behaviors on each type of embryonic vocalization.

	Embryonic Response						
Maternal Behavior	Phioo	Peep	Soft Peep	Screech	Twitter	Food Call	Huddling Call
Move							
Egg Turn					directive	inhibitory	
Reset					directive	inhibitory	
Beak Clap	directive		directive		inhibitory	inhibitory	
Peck	inhibitory						
Cluck				inhibitory		directive	
Intermediate Call			inhibitory			directive	
Food Call			inhibitory			directive	
Mild Alarm					directive		

do not necessarily imply direct causality.)

Information analysis. Having demonstrated that embryos respond to behaviors of the incubating hen, I proceeded to analyze their communication system by using information theory (see Appendix II). The use of information theory has two important advantages: It makes possible the measuring of the extent to which the hen and embryo communicate in units that permit comparisons with other species of precocial birds and it provides a means to estimate the signal role played in communication by each type of maternal behavior.

To find out if communication takes place between the hen and embryos one has to determine whether or not the distribution of embryonic vocalizations differs from one expected by chance alone due to information transmitted by the maternal behaviors. I therefore calculated the amount of information (degree of uncertainty) present in the distribution of embryonic acts (H) and the information transmission value (T). The amount of information present in the distribution of embryonic acts reaches its maximum (H_{max}) when the distribution is random. The difference between the calculated value and its maximum value measures the effect of maternal behaviors on the distribution of embryonic vocalizations. Transmission is a measure of the constraints imposed by the maternal behaviors on the embryonic vocalizations that follow. It is defined as the mutual reduction of uncertainty and is the amount of information that embryos receive from the incubating hen.

The occurrence of communication is tested with two null hypotheses:

$$H = H_{max}$$

$$T = 0$$

Using the data on two act sequences of maternal and embryonic behaviors (Appendix V) the following information measures are calculated (For details see Appendix II):

a) The amount of information present in the distribution of embryonic acts according to the type of maternal behavior and over all maternal behaviors:

$H(X) = 2.6098$ bits

b) The amount of information contained in the distribution of the embryonic behaviors according to the type of embryonic behavior and over all embryonic behaviors:

$H(Y) = 2.3970$ bits

c) The amount of information present in the individual cells of the transition table: $H(X,Y) = 4.9080$ bits

d) Based on the H values I obtained the following transmission value: $T(X,Y) = 0.099$ bits

A multiple test using "Miller's X^2" is performed in order to test the null hypotheses:

$H(X) = H(X)_{max}$ where: $H(X)_{max} = 3.1699$ bits

$H(Y) = H(Y)_{max}$ $H(Y)_{max} = 3.000$ bits

$T(X,Y) = 0$

The X^2 values obtained (Table VII) indicate that a significant amount of information is transferred. This means that information is communicated by the incubating hen to the embryos.

In order to determine which maternal behavior transmitted the most information to the embryos, partial information measures (J) are used (Appendix II). These estimate the effectiveness of each maternal behavior as a signal. The most effective signals are those for which predictability of the following behavior is highest. Among maternal vocalizations, the intermediate call and the cluck appear to be the most efficient signals (Table VIII). Among the maternal movements, egg turning is the most effective signal.

Maternal beak clapping and pecking also have relatively high transmission values. This is because periods of embryonic silence follow so often after each act (Appendix V). A uniform distribution of frequencies between the cells of a row will result in minimal transmission values whereas the maximal is obtained when a single cell deviates from the overall distribution. In the case of beak claps and pecks the "silent" cells have disproportionately large frequency values explaining their

TABLE VII. "Miller's X^2" values for three null hypotheses: $H(X) = H(X)max$, $H(Y) = H(Y)max$ and $T(X;Y) = 0$. Embryonic responses to maternal stimulation.

Information Measure	Estimate	X^2	d.f.	p
H (X)	2.609	1,054.4	8	< 0.001
H (Y)	2.397	1,135.2	7	< 0.001
T (X;Y)	0.099	186.4	56	< 0.001

TABLE VIII. Amount of information transmitted by maternal behaviors.

Maternal Behavior	J (x;y)	Tp (x;y)
Move	0.0049	0.0012
Egg Turn	0.4361	0.0183
Reset	0.0316	0.0078
*Beak Clap	0.0649	0.02
*Peck	0.076	0.0127
Cluck	0.0844	0.0105
Intermediate Call	0.2635	0.0142
Food Call	0.5905	0.0065
Mild Alarm	0.2057	0.0074
	TOTAL	0.0986 ≈ 0.099

*The transmission values for beak claps and pecks are high due to the high incidence of embryonic silence following their occurence (see Appendix III).

relatively large transmission values.

The extent to which the type of a two act sequence can be predicted by knowing the initial maternal behavior or the following embryonic vocalization may be determined by computing the redundancy coefficient:

$$R = 1 - H/H_{max}$$

The resulting values $R(X) = 0.1767$ and $R(Y) = 0.201$ are relatively low indicating limited predictability. This means that one maternal behavior may be followed by several different embryonic responses.

Maternal responses to embryonic stimuli

Overall maternal responsiveness to embryonic stimulation. Maternal responsiveness to embryonic vocalizations increases rapidly as hatching approaches paralleling the increase in embryonic responsiveness (Fig. 17 and Fig. 18). Using the same 15-s interval as the response criterion, there is a marked increase in the number of maternal responses as hatching approaches. The proportion of maternal behaviors that are responses also increases (Fig 17).

The difference between the observed number of responses and the number expected by chance alone increases toward hatching (Fig. 18). At 21 hrs before hatching few of the maternal behaviors are responses to the embryos: the observed number does not differ significantly from that expected by chance alone (Table IX). Near hatching, maternal behaviors follow embryonic calls significantly

Fig. 17. Percentage of maternal behaviors that occur within 15 s after the start of an embryonic vocalization at various stages before hatching.

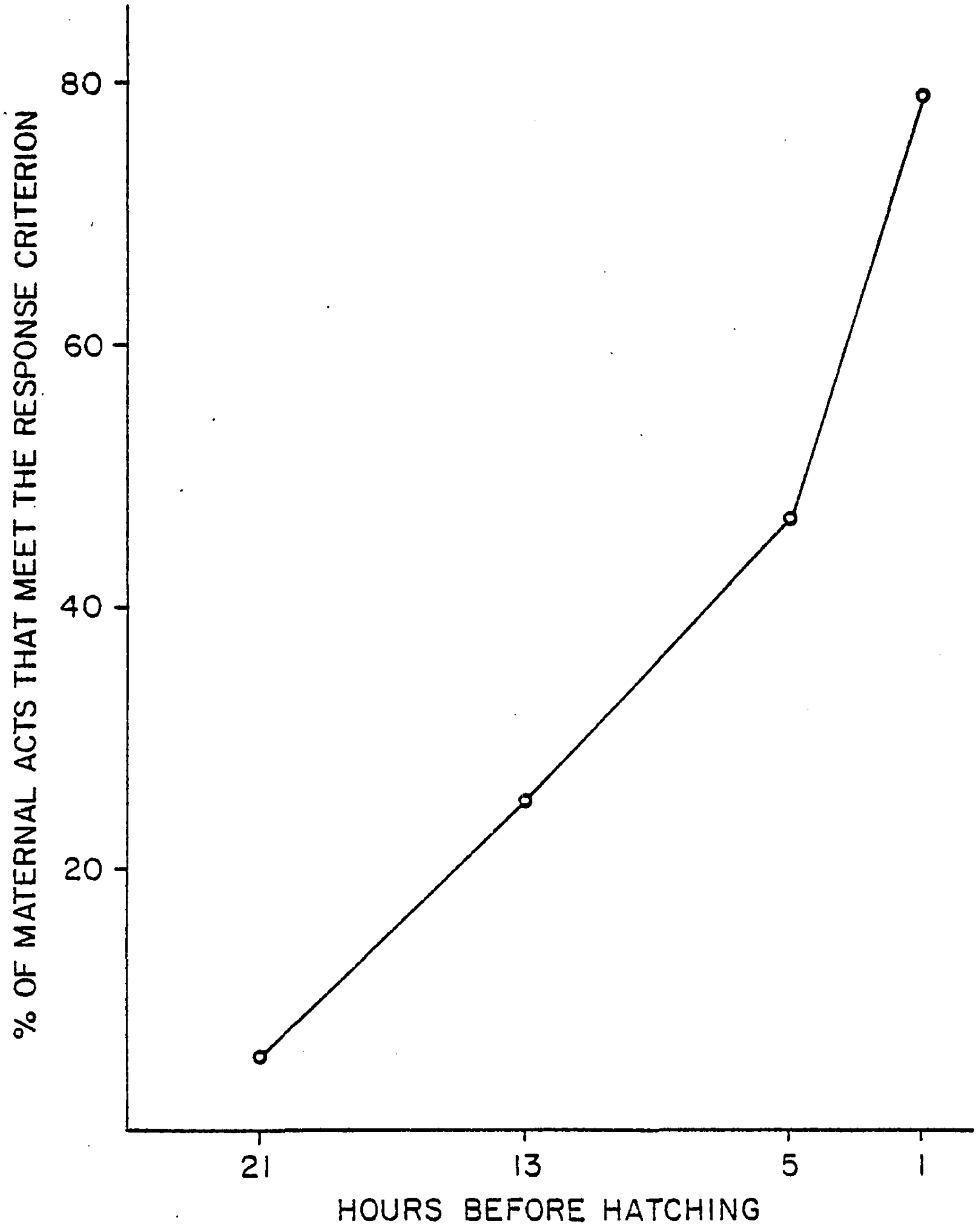


FIG. 12 CHANGES IN MATERNAL RESPONSIVENESS TO EMBRYONIC STIMULATION

Fig. 18. Changes in the number of maternal behaviors that occur within 15 s after the start of an embryonic vocalization at various stages before hatching. Totals obtained from four 30-min samples are plotted.

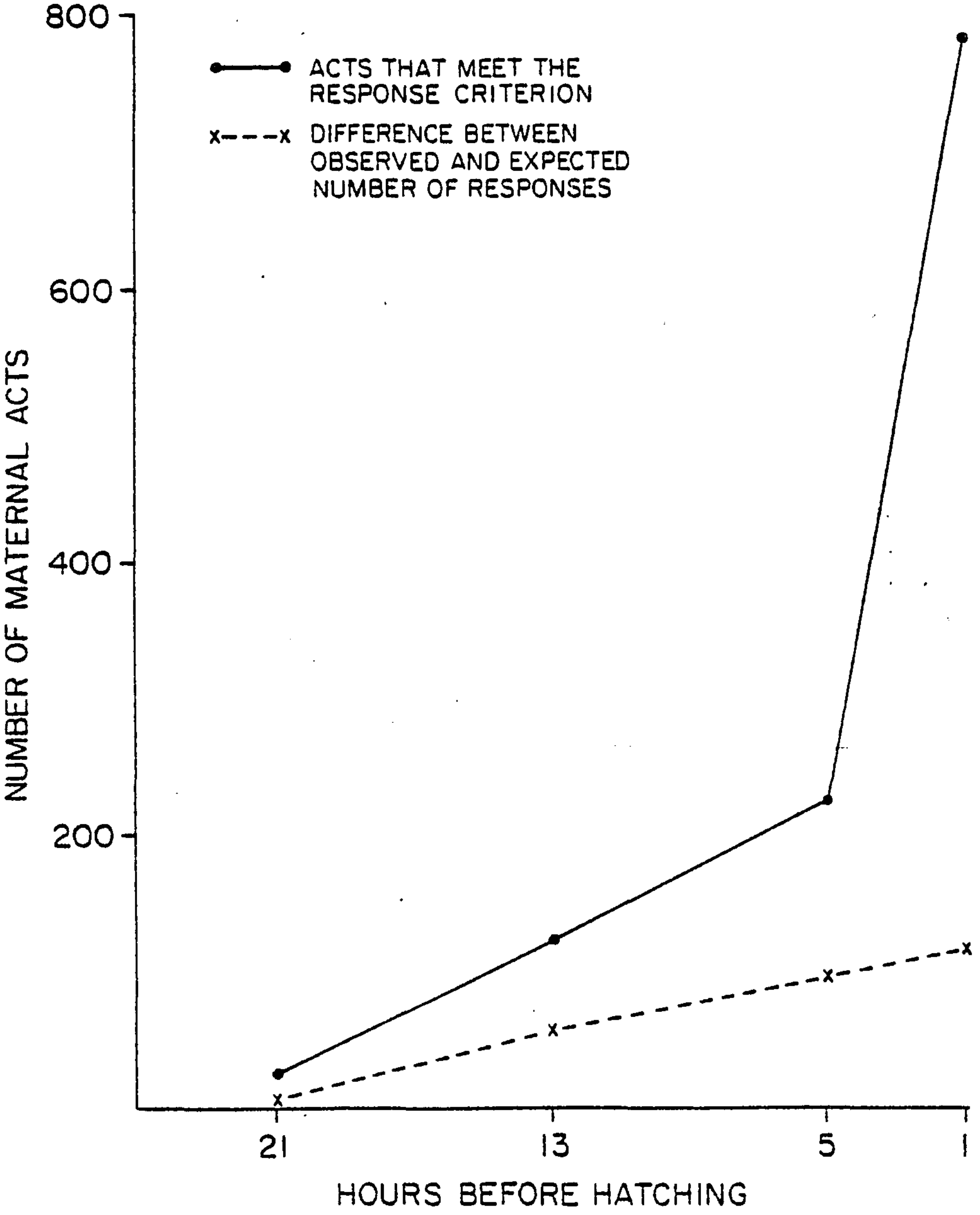

800
600
400
200
NUMBER OF MATERNAL ACTS
ACTS THAT MEET THE RESPONSE CRITERION
DIFFERENCE BETWEEN OBSERVED AND EXPECTED NUMBER OF RESPONSES
21
13
5
1
HOURS BEFORE HATCHING

TABLE IX. Comparison between the number of maternal responses observed and the number expected due to chance alone.

Hours before hatching	Mean number of maternal acts per sample	Mean number of responses per sample		X^{2*}	p
		Observed	Expected		
1	247	196	168	14.34	< 0.001
5	120	56	32	25.52	< 0.001
13	101	30	17	11.87	< 0.001
21	105	6	5	0.08	n.s.

* d.f. = 1 for all comparisons

more often than expected.

The departure of observed from expected frequencies for each maternal behavior is tabulated in Table X. Among the body movements only egg turning follows embryonic vocalizations significantly more often than expected. All maternal vocalizations occur significantly more often than expected. Among them, clucks show the highest level of significance. Both pecks and beak claps follow more often than expected. However, in the case of beak claps this may be due to their frequent association with soft clucks and pecks. Non-significant values of the heterogeneity X^2 indicate that embryonic vocalizations have similar effects on maternal behaviors that belong to the same category.

The latency of maternal responses, measured by the proportion of the responses that follow within 5 s from the start of an embryonic act, shows no consistent change as hatching approaches (Fig. 19).

Specific maternal responses to embryonic stimulation. During the hours before hatching, the type and number of maternal responses depend on the type of the preceding embryonic vocalization. The distributions of maternal responses following various embryonic vocalizations are shown in Fig. 20. The plotted values are ratios giving the number of maternal behaviors per embryonic act. Only the first maternal response was taken into account because subsequent responses may not be independent of the first.

TABLE X. Comparisons between the number of maternal responses observed and expected due to chance alone during the hour before hatching. The data were obtained from four 30-min samples.

Maternal Behavior	Total Number of Acts	Maternal Responses			X^2	p
		Observed	Expected			
Move	213	156	144.5		2.85^{1}	n.s.
Egg Turn	37	34	25.1		9.81^{1}	< 0.01
Reset	17	13	11.5		0.61^{1}	n.s.
				TOTAL	13.27^{3}	< 0.01
	267	203	181.1	POOLED	8.232^{1}	< 0.01
				HETEROGENEITY:	5.038^{2}	n.s.
Beak Clap	281	217	190.6		11.37^{1}	< 0.001
Peck	182	140	123.4		6.94^{1}	< 0.01
				TOTAL	18.31^{2}	< 0.001
	463	357	314.0	POOLED	18.3^{1}	< 0.001
				HETEROGENEITY:	0.01^{1}	n.s.
Cluck	131	116	88.8		25.86^{1}	< 0.001
Intermediate Call	85	67	57.6		4.76^{1}	< 0.05
Food Call	9	9	6.1		4.28^{1}	< 0.05
Mild Alarm	34	30	23.1		6.43^{1}	< 0.05
				TOTAL	41.33^{4}	< 0.001
	259	222	175.6	POOLED	38.08^{1}	< 0.001
				HETEROGENEITY:	3.25^{3}	n.s.

^{1}d.f. = 1, ^{2}d.f. = 2, ^{3}d.f. = 3, ^{4}d.f. = 4

Fig. 19. Changes in the proportion of maternal responses that have a latency of 5 s or less. The plotted values are based on four 30-min samples at each stage and include only the first response of each type that follows an embryonic vocalization.

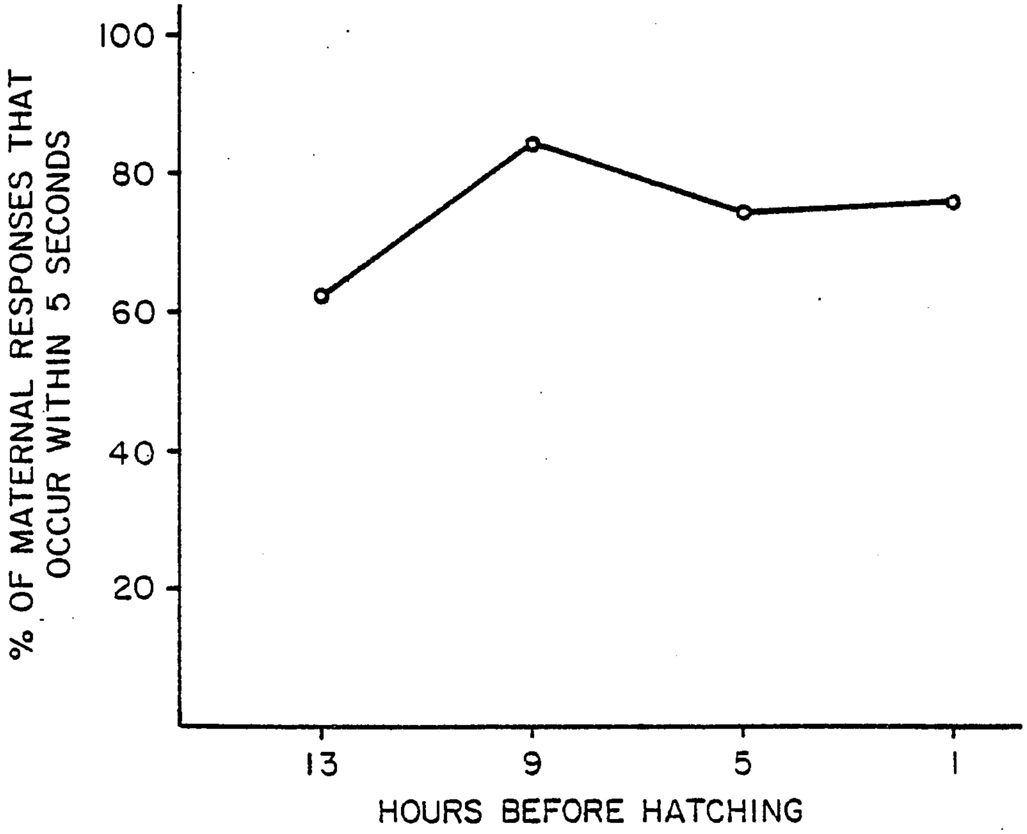
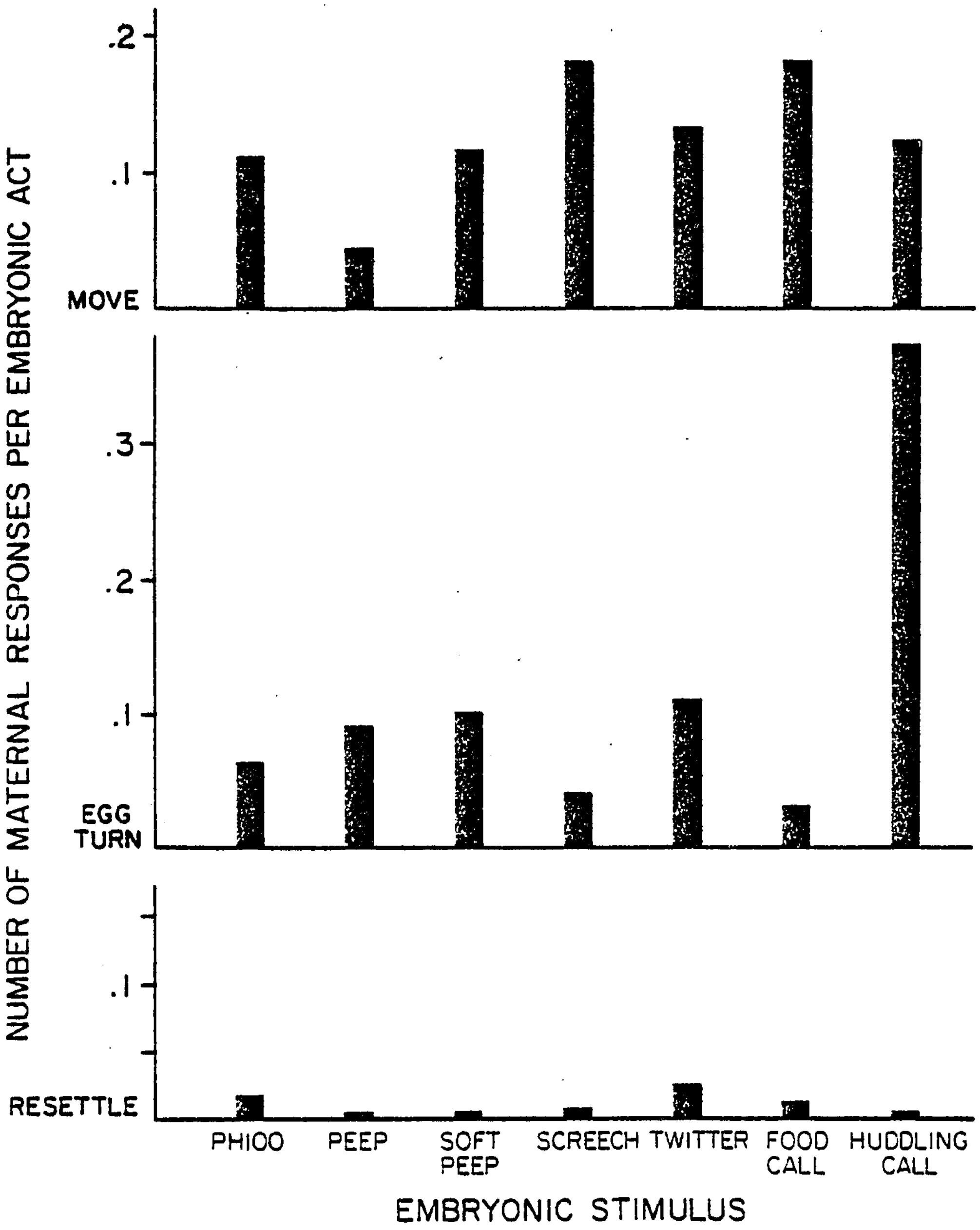
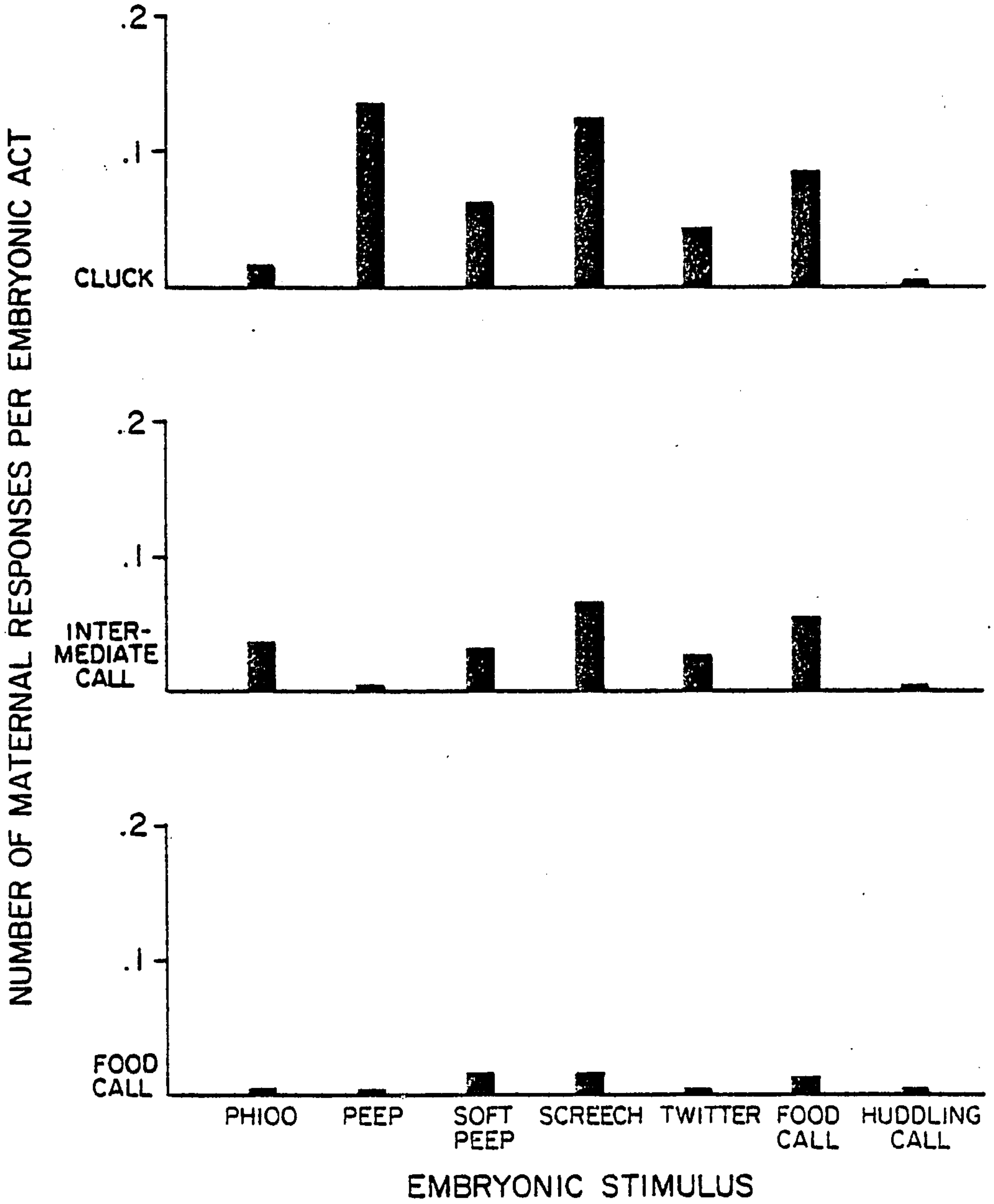
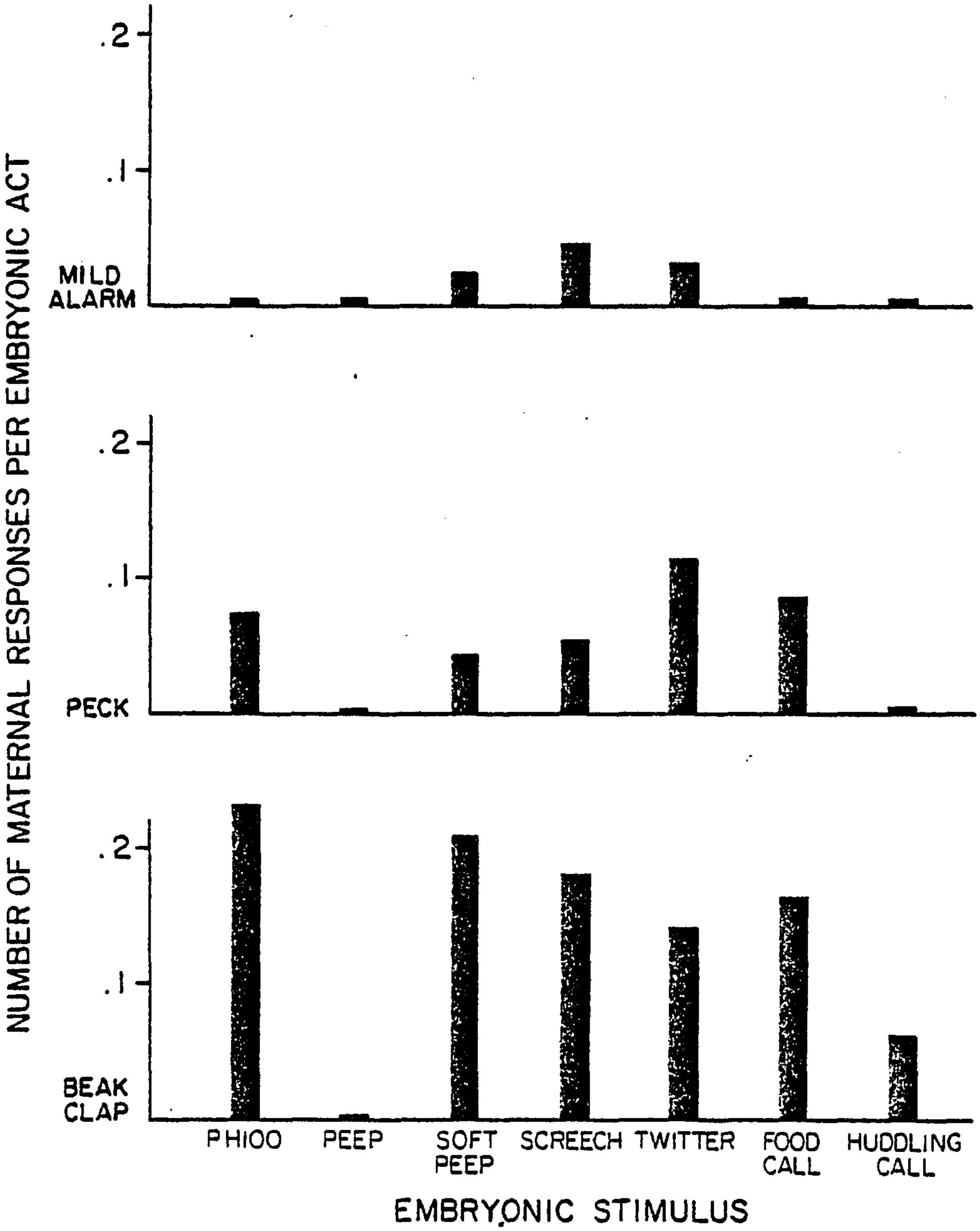


FIG. 13 CHANGES IN THE PROPORTION OF MATERNAL RESPONSES WITH A LATENCY OF 5 SEC. OR LESS.

Fig. 20. The distribution of maternal responses following different types of embryonic vocalizations during the 4 hrs before hatching. The values for each type of maternal behavior are computed as follows: the number of maternal responses in one category that followed a certain type of embryonic vocalization was divided by the total number of vocalizations of that type. The procedure was repeated for each of the embryonic vocalizations. The data are based on eight 30-min samples.


NUMBER OF MATERNAL RESPONSES PER EMBRYONIC ACT
.2
.1
MOVE
.3
.2
.1
EGG TURN
.1
RESETTLE
PHIOO
PEEP
SOFT PEEP
SCREECH
TWITTER
FOOD CALL
HUDDLING CALL
EMBRYONIC STIMULUS


NUMBER OF MATERNAL RESPONSES PER EMBRYONIC ACT
.2
.1
CLUCK
.2
.1
INTER-MEDIATE CALL
.2
.1
FOOD CALL
PHIOO
PEEP
SOFT PEEP
SCREECH
TWITTER
FOOD CALL
HUDDLING CALL
EMBRYONIC STIMULUS


NUMBER OF MATERNAL RESPONSES PER EMBRYONIC ACT
.2
.1
MILD ALARM
.2
.1
PECK
.2
.1
BEAK CLAP
PHIOO
PEEP
SOFT PEEP
SCREECH
TWITTER
FOOD CALL
HUDDLING CALL
EMBRYONIC STIMULUS

The results show few common trends among maternal behaviors that belong to the same category. Unidentified maternal movements follow somewhat more often after screeches and embryonic food calls while egg turns follow very often after huddling calls. In the vocalizations category, clucks most often follow embryonic peeps or screeches whereas intermediate calls follow mostly after screeches. The comparison between the observed and expected number of maternal responses to embryonic vocalizations shows that only egg turns, clucks and mild alarm calls depart from their expected distributions (Table XI and Appendix VI). The distributions for the expected values were obtained by using the distribution coefficients of embryonic vocalizations (Appendix VII). Analysis of the matrix by an internal goodness of fit test shows that the type of embryonic vocalization influences the type of maternal response (Table XII). Distress type calls tend to elicit maternal vocalizations and pleasure type calls to inhibit them.

Information analysis. To find out if communication takes place between embryos and the incubating hen it is determined whether or not the distribution of maternal behaviors differs from random due to information transmitted by the embryonic vocalizations (see Appendix II). First the amount of information (degree of uncertainty) present in the distribution of maternal behaviors (H) is calculated. The difference between the calculated value and its maximum

TABLE XI. Comparison between the observed and expected number of maternal responses to embryonic vocalizations during the four hours before hatching. Only the first maternal response following an embryonic act is included. The data are based on eight 30-min samples. (See Appendix VI for the complete table.)

Maternal Behavior	n	X^{2*}	p
Move	189	7.95	n.s.
Egg Turn	88	44.05	< 0.001
Reset	17	5.34	n.s.
Beak Clap	212	9.37	n.s
Peck	96	12.13	n.s.
Cluck	92	20.34	< 0.01
Intermediate Call	55	7.94	n.s.
Food Call	11	1.91	n.s.
Mild Alarm	26	13.63	< 0.05

* d.f. = 6 for all comparisons

TABLE XII. Analysis of the effects of embryonic vocalizations on each type of maternal behavior. Brackets indicate groupings required by the presence of small values in individual cells. Asterisks indicate borderline values.

	Maternal Response								
Embryonic Vocalization	Move	Egg Turn	Reset	Beak Clap	Peck	Cluck	Intermediate Call	Food Call	Mild Alarm
Phioo				*directive		inhibitory			
Peep	*inhibitory	directive		inhibitory	inhibitory				
Soft Peep									
Screech		inhibitory				directive	*directive		directive
Twitter		directive			directive	inhibitory			
Food Call	*directive	inhibitory							inhibitory
Huddling Call		directive							

value measures the effect of embryonic vocalizations on the distribution of maternal behaviors. Second, the information transmission value (T) is calculated. This is a measure for the amount of information that the incubating hen receives from the embryos. Finally, these information measures are used with the following null hypotheses to determine whether or not communication occurs:

$$H = H_{max}$$

$$T = 0$$

From the data on two-act sequences of embryonic and maternal behaviors (Appendix VIII) we calculate several information measures (see Appendix II):

a) The amount of information present in the distribution of maternal behaviors according to the type of embryonic vocalization over all embryonic vocalizations:
$H(X) = 2.346$ bits

b) The amount of information contained in the distribution of maternal behaviors according to the type of maternal behavior over all maternal behaviors:
$H(Y) = 2.842$ bits

c) The amount of information present in the individual cells of the transition table: $H(X,Y) = 5.082$ bits

d) Based on the H values we calculate the transmission value: $T(X,Y) = 0.106$ bits
The following null hypotheses are tested with a

multiple "Miller's X^2" test:

$H(X) = H(X)_{max}$ where: $H(X)_{max} = 2.807$ bits

$H(Y) = H(Y)_{max}$ $H(Y)_{max} = 3.322$ bits

$T(X,Y) = 0$

The resulting X^2 values (Table XIII) indicate that a significant amount of information is transmitted to the incubating hen and therefore communication occurs.

The effectiveness as signals of various embryonic vocalizations is estimated by their individual transmission values (Appendix II). The relative amounts of information transmitted by each type of embryonic vocalization are listed in Table XIV. According to these values twitters, peeps and screeches are the most effective transmitters of information among embryonic signals. Phioo calls and soft peep calls are the least effective signals.

The predictability (reduction of uncertainty) of a two-act sequence when the embryonic vocalization is known can be estimated by:

$$R(X) = 1 - H(X)/H(X)_{max}$$
$$= 0.164$$

The predictability of the sequence when the following maternal act is known is estimated in a similar manner:

$$R(Y) = 1 - H(Y)/H(Y)_{max}$$
$$= 0.144$$

Both values are relatively low indicating that it is difficult to predict the type of sequence, when either the embryonic or the maternal behavior is known. This means that one embryonic vocalization may be followed by several

TABLE XIII. "Miller's X^2" values for three null hypotheses: $H(X) = H(X)max$, $H(Y) = H(Y)max$ and $T(X;Y) = 0$. Maternal responses to embryonic stimulation.

Information Measure	Estimate	X^2	d.f.	p
H (X)	2.346	681.26	6	< 0.001
H (Y)	2.842	709.34	9	< 0.001
T (X;Y)	0.106	156.65	54	< 0.001

TABLE XIV. Amount of information transmitted by embryonic vocalizations.

Embryonic Vocalization	J (x;y)	Tp (x;y)
Phioo	0.1406	0.0117
Peep	1.0087	0.017
Soft Peep	0.082	0.0107
Screech	0.0855	0.0173
Twitter	0.0845	0.0205
Food Call	0.0505	0.0158
Huddling Call	1.0787	0.0132
	TOTAL	0.1062 ≈ 0.106 bits

different maternal behaviors. For example, an embryonic screech may be followed by either a maternal movement, a beak clap or a cluck.

DISCUSSION

My results confirm previous findings that in domestic fowl, embryonic vocalizations and maternal behaviors occur frequently during the hours that precede hatching. Temporal associations among these behaviors strongly suggest that maternal acts and embryonic vocalizations are reciprocally related. A temporal association between maternal and embryonic behaviors may be explained in several ways:

a) maternal and embryonic behaviors may become temporally associated by chance alone.

b) both embryonic and maternal behaviors could occur in response to commonly perceived stimuli such as external noises.

c) embryonic behaviors occur in response to maternal behaviors.

d) maternal behaviors occur in response to embryonic behaviors.

The first explanation is unlikely because maternal and embryonic behaviors are temporally associated much more often than would be expected by chance (Table III and Table IX). While there are no specific data to refute the second explanation, causal observations reveal that irregularly occurring noises in the laboratory (e.g., door closing, footsteps, etc.) tend to inhibit maternal activity but not embryonic vocalizations.

Embryonic responses

That the temporal associations occur because embryos respond to maternal behavior is supported by several types of evidence. First, the onsets of embryonic and maternal vocalizations are closely associated in time (Fig. 5, 6 and 7). Second, maternal vocalizations and embryonic pleasure calls have a similar pattern of increase as hatching approaches (Figs. 6 and 7). Third, as hatching approaches the synchrony of embryonic vocalizations and maternal acts increases. A higher proportion of embryonic calls follow immediately after a maternal act and their latencies decrease (Figs. 10 and 14). Finally, specific associations between certain embryonic calls and maternal behaviors occur near hatching (Figs. 15 and 16, Appendix III, Table VI).

These lines of evidence taken together indicate that the hen elicits embryonic vocal responses. The stimulating effect of her behaviors is limited to embryonic pleasure type calls (Table IV), specifically twitters and food calls. There is no stimulatory effect on embryonic distress type calls and some appear to be inhibited.

The influence on embryonic vocalizations suggests that maternal behaviors stimulate the embryos differentially and do not simply induce a general arousal. The analysis of associations between individual maternal and embryonic behaviors yields more evidence supporting this suggestion.

Embryonic twitters, food calls and, to a lesser extent, phioo calls have distributions that differ significantly from those expected to follow maternal behaviors by chance (Table V). The selective nature of maternal stimulation is further demonstrated by the specific influence that each type of maternal behavior has on embryonic vocalizations (Table VI). Strong maternal movements such as egg turning and resettling on the nest have a directive effect on embryonic twitters but inhibit food calls. Maternal vocalizations have a directive effect on embryonic food calls but inhibit some distress type calls. It is clear then, that embryos respond selectively and specifically to maternal stimulation.

Information analysis. The analysis of information transfer from the incubating hen to the embryos indicates that communication is initiated prior to hatching. The value of the overall transmission is significantly different from 0 which shows a departure in the distribution of embryonic calls from random. The maternal behaviors most effective in eliciting embryonic responses are egg turning, intermediate calls and clucks (Table VIII).

Some of these transmission values may not reflect the total amount of information transmitted. The transmission values are just minimum estimates of the transferred information. The actual values of the transferred information may largely exceed the values computed above. The fact that the types of distress calls are roughly equal in number to the types of pleasure calls increases the uniformity in responsiveness and lowers the overall estimates of transmission. Transmission values decrease as uniformity among responses (uncertainty) increases. In the case of maternal intermediate calls and clucks the transmission values would be much higher if the number of different embryonic distress calls (which were inhibited) was not similar to the number of different pleasure calls (which were activated).

The influence of maternal acts on the distribution of embryonic vocalizations is further demonstrated by the significant differences between the values of H and H_{max}. The uncertainty ratio (H/H_{max}) is relatively high resulting in low redundancy values. This means that it is rather difficult to predict the type of embryonic vocalization that will follow a certain maternal behavior. The implication is that embryos respond in a similar way to a number of maternal stimuli within the same category. In other words, one type of embryonic pleasure call may be stimulated by several different maternal calls. Clucks and intermediate calls both stimulate embryonic food calls. Egg turning and resettling stimulate embryonic twitters. Similarly, all maternal behaviors reduce the incidence of distress calls.

Maternal responses

The analysis of sequences in which maternal behaviors follow embryonic vocalizations show that the incubating hen responds to embryonic stimuli and that responsiveness increases toward hatching. An increasing proportion of maternal behaviors follow embryonic vocalizations as hatching approaches; many more than would be expected by chance alone (Table IX). The number of maternal behaviors that follow embryonic vocalizations also increases toward hatching and the difference between the observed and expected numbers widens (Fig. 17).

Maternal responsiveness develops more rapidly than embryonic responsiveness. Maternal responses reach a relatively constant latency soon after their onset (Fig. 19). The latency of embryonic responses continues to decrease until hatching (Fig. 14).

Embryonic vocalizations have a general stimulatory effect on the hen. Most maternal behaviors are activated by embryonic vocalizations (Table X). This includes all kinds of vocalizations, egg turning, beak claps and pecks.

When only the first maternal behavior following an embryonic vocalization is considered, the responses appear rather selective (Fig. 20 and Table XI). In this case only egg turns, clucks and mild alarm calls differ significantly in their overall distribution from that expected by chance alone. Clucks and mild alarm calls depart from the expected distributions mainly because of the strong directive effect of embryonic screeches (Table XIV).

The responses of the incubating hen are similar to those observed after hatching. Clucks are known to be emitted by the broody hen for maintaining and reestablishing contact with chicks (Collias and Joos, 1953) when the chicks distress call (Bruckner, 1933). Taken together these findings lead to the conclusion that the hen begins responding selectively to chick vocalizations before hatching.

Information analysis. According to the results the total transmission value is significantly different from zero indicating that information is communicated by the embryos to the incubating hen. Among the most effective embryonic signals are the intense distress type calls such as peeps and screeches (Table XIV). Screeches stand out due to their activating effect on maternal clucks, beak claps and undetermined movements (Appendix VIII). Peeps have a high signal value, mostly because they inhibit maternal responses or, to a lesser extent, stimulate clucking. The main effect of embryonic twitters, which also have a relatively high signal value, is to inhibit maternal activity. The hen seems to listen.

The measures for the amount of information present in the response distribution (H) are significantly different from their maximum values but estimates of redundancy (R) are quite low. So although information is communicated by the embryos to the incubating hen, embryonic vocalizations and maternal responses are quite variable in their association patterns. Predicting the maternal response based on the preceding embryonic vocalization is difficult.

CONCLUSIONS

It is concluded that communicatory interactions between chicks and the maternal hen start prior to hatching:

1) Embryos and the incubating hen respond to each other.
2) Both embryonic and maternal responsiveness increase as hatching approaches.
3) Embryonic and maternal responses are influenced by the type of stimulus received although the specific type of response is difficult to predict.

EXPERIMENT II

PREHATCHING VOCALIZATIONS OF ARTIFICIALLY INCUBATED EMBRYOS

The results obtained from my observations of naturally incubated broods suggest that maternal stimulation influences the patterning of embryonic vocalizations. Embryonic pleasure calls follow maternal behaviors significantly more often than expected, whereas distress type calls follow either less often or as often as expected (Table IV). It appears that maternal behaviors elicit embryonic pleasure calls and either inhibit or have no effect on distress calls. If this is the case, the patterning of embryonic vocalizations should change when embryos are deprived of the normal stimulation from the incubating hen. Artificially incubated embryos, which lack maternal stimulation, should emit fewer pleasure type calls and, possibly, more distress calls than naturally incubated embryos.

The aim of the present experiment is to determine the types and frequencies of embryonic vocalizations in artificially incubated embryos and compare them with those observed in naturally incubated embryos.

METHODS

Subjects were embryos developed from eggs of the same strain used in the previous study. The handling procedures and incubating schedules were similar except that the eggs were artificially incubated. On the 17th day of incubation, six fertile eggs were moved to the observation room and placed in the nest cup of a nesting cage (Fig. 1). They were covered with a transparent incubating bell 30 cm in diameter and 14 cm high equipped with a 30 W heating unit. Recordings of the embryonic vocalizations started on the 18th day of incubation and continued until hatching. The microphones were placed near the eggs under the incubator. Data collection and analysis were performed in a manner identical to that described earlier for the naturally incubated embryos.

RESULTS

Longitudinal changes in embryonic vocalizations. The types of vocalizations recorded from artificially incubated embryos are identical to those of naturally incubated embryos (Table I). Distress type calls appear early and become frequent as hatching approaches (Fig. 21a). The values plotted in the graphs are durations estimated by the average number of 1-s intervals during which the behaviors occurred per 30-min sample. Peeps and soft peeps show a moderate increase at earlier stages, and near hatching become the

Fig. 21.

a) Embryonic "distress" type calls during the hours before hatching in naturally and artificially incubated embryos.

b) Embryonic "pleasure" type calls during the hours before hatching in naturally and artificially incubated embryos.

The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample.

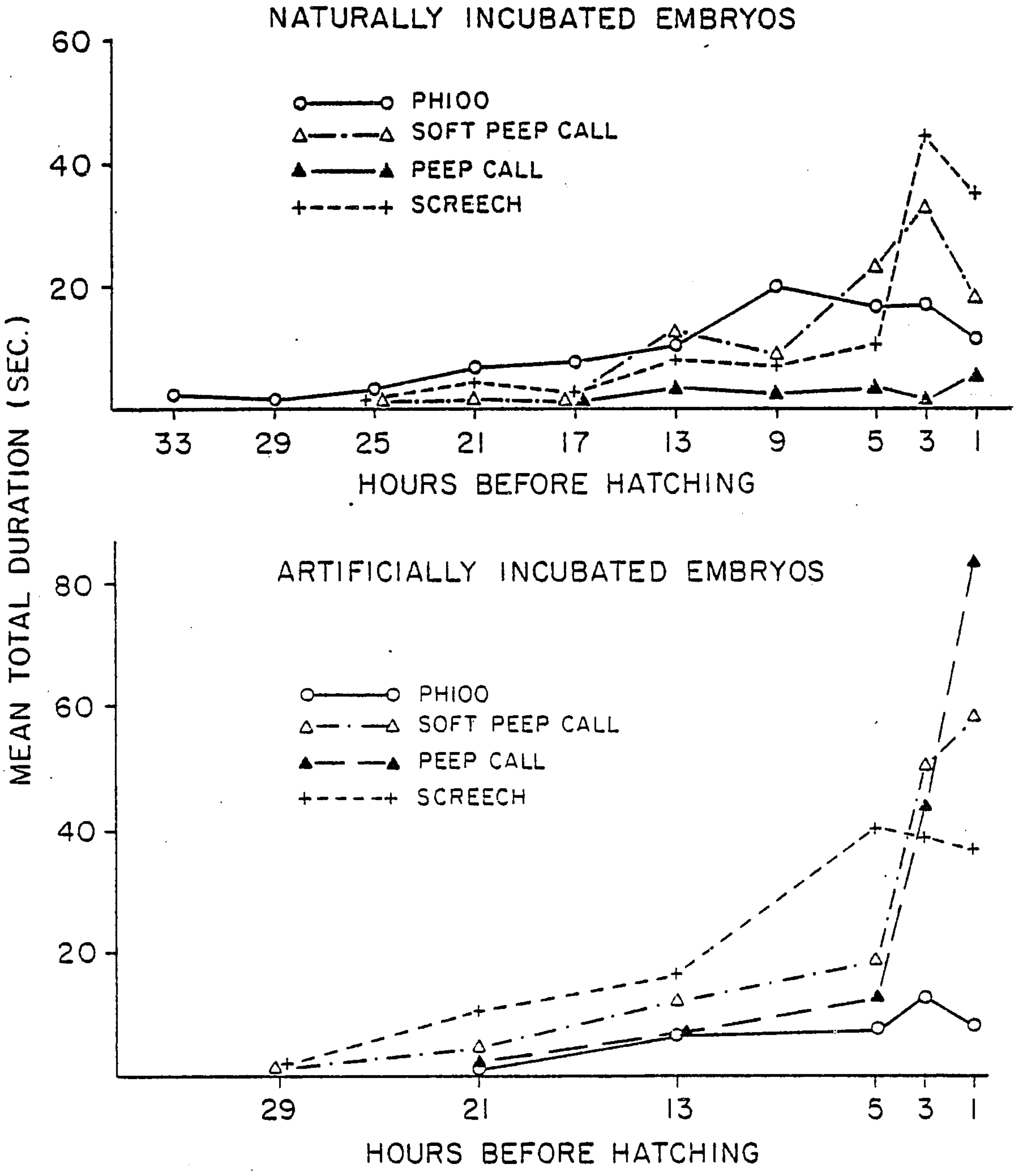


FIG. 21 a. EMBRYONIC VOCALIZATIONS IN ARTIFICIALLY AND NATURALLY INCUBATED BROODS. DISTRESS TYPE CALLS.

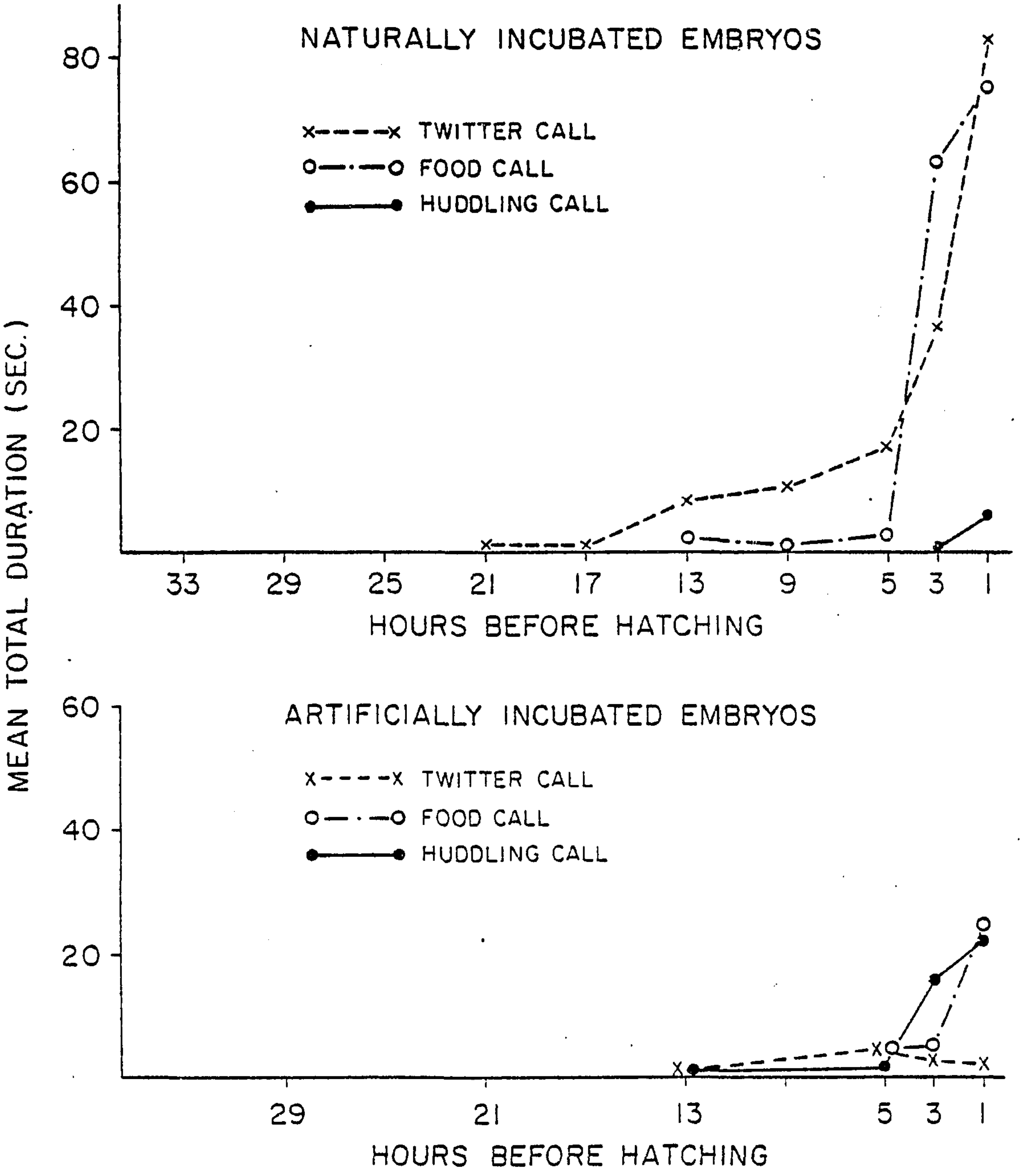


FIG. 21b.EMBRYONIC VOCALIZATIONS IN ARTIFICIALLY AND NATURALLY INCUBATED BROODS. PLEASURE TYPE CALLS.

dominant vocalizations.

The onset of pleasure type calls is late during incubation (Fig. 21b). Food and huddling calls show a small increase near hatching while twitters remain at a low level.

Embryonic vocalizations in artificially and naturally incubated embryos. The pattern of embryonic vocalizations in broods incubated artificially differs markedly from that of broods incubated by hens (Fig. 5, 6, 21a, and 21b). In naturally incubated broods the most frequent vocalizations are pleasure type calls while distress type calls are the most frequent vocalizations in artificially incubated broods. Near hatching, embryonic food calls and twitters dominate in naturally incubated broods, peeps and soft peeps in artificially incubated broods. Huddling calls, which are very rare in naturally incubated embryos, occur more often in artificially incubated embryos. Screeches and phioo calls exhibit similar patterns of occurrence.

The total durations per sample of embryonic vocalizations (Fig. 22) are markedly different in naturally and artificially incubated broods. A chi-square test for independence was applied to the mean number of one-second intervals covered by each type of vocalization during the 30-min sampling time (Table XV). The test shows that although the total duration per sample of all embryonic vocalizations taken together is similar for the two groups,

Fig. 22. Embryonic vocalizations during the 4 hrs before hatching in naturally and artificially incubated broods. The mean total duration of each behavior is estimated by the average number of 1-s intervals in which the behavior occurred during a 30-min sample. Two samples were taken for each brood.

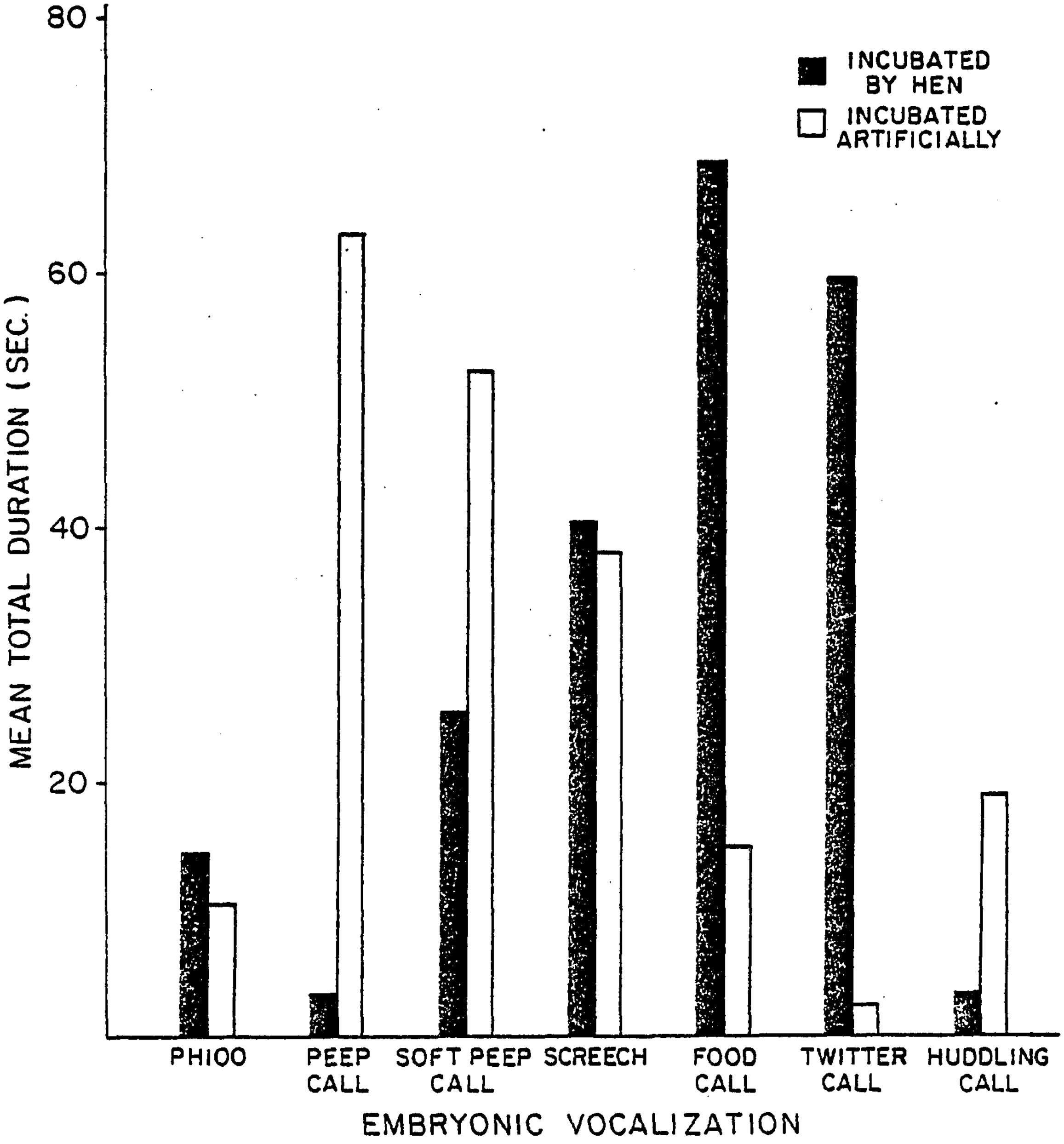

80
60
40
20
MEAN TOTAL DURATION (SEC.)
INCUBATED BY HEN
INCUBATED ARTIFICIALLY
PHIOO
PEEP CALL
SOFT PEEP CALL
SCREECH
FOOD CALL
TWITTER CALL
HUDDLING CALL
EMBRYONIC VOCALIZATION

TABLE XV. Number of 1-s intervals covered by embryonic vocalizations during the 4 hrs before hatching in naturally and artificially incubated broods. The tabulated values are means per 30-min sample based on eight samples, two for each brood. The values in parentheses are expected frequencies.

	Vocalization									
Type of Incubation	Phioo	Peep	Soft Peep	Screech	Twitter	Food Call	Huddling Call	Total	X^2	p
Natural	14 (12.83)	3 (34.38)	26 (41.05)	40 (40.02)	60 (32.33)	69 (43.10)	3 (11.29)	215		
Artificial	11 (12.17)	64 (32.62)	54 (38.95)	38 (37.98)	3 (30.67)	15 (40.90)	19 (10.71)	204		
TOTAL	25	67	80	63	84	78	22	419	163.4	< 0.001

there are highly significant differences between the relative durations per sample in individual categories.

Each type of vocalization was analyzed separately by using a "t" test for percentages (Sokal and Rohlf, 1969, p. 607). As shown in Table XVI, the total durations per sample of most vocalizations are significantly different. Near hatching, naturally incubated embryos emit predominantly food calls and twitters while artificially incubated embryos emit mainly peeps, soft peeps and huddling calls.

DISCUSSION

These results support the hypothesis that maternal behaviors elicit embryonic pleasure type calls while inhibiting distress type calls. With maternal stimulation embryos emit frequent food calls and twitters and very few peeps. The reverse is true when maternal stimulation is absent. To a lesser extent, soft peeps are also inhibited by the presence of the hen. This last effect was not as apparent earlier when associations between maternal and embryonic behaviors were analyzed in naturally incubated broods. Embryonic screeches and phioo calls remain unchanged whether maternal stimulation is present or not, which is consistent with my earlier findings.

There is an obvious similarity between the factors that

TABLE XVI. Comparison between the total durations per sample of embryonic vocalizations in naturally and artificially incubated broods. Tabulated figures represent 1-s intervals per 30-min sample.

Vocalization	Natural Incubation	Artifical Incubation	ts	p
Phioo	14	10	0.827	n.s.
Peep	3	64	8.930	< 0.001
Soft Peep	26	54	3.216	< 0.001
Screech	40	38	0.229	n.s.
Twitter	60	3	8.565	< 0.001
Food Call	69	15	6.335	< 0.001
Huddling Call	3	19	3.72	< 0.001

influence embryonic vocalizations and those that influence chick vocalizations after hatching. Twitters and food calls are emitted in response to stimulation normally provided by social companions. After hatching, twitters and food calls occur typically during group activity, such as foraging, when interactions between members of the group are frequent. Before hatching, these calls are emitted during interactions with the incubating hen. Peep calls seem to be emitted in the absence of social stimulation (Andrew, 1964, Guyomarc'h, 1966, Collias, 1952, Montevecchi et al., 1973, Kaufman and Hinde, 1961). Chicks reared in isolation cease emitting peeps and start emitting pleasure calls when presented with stimulation of the type normally provided by the social companions. That peeps occur in the absence of adequate stimulation is suggested also by the finding that chicks are more active and emit more peeps in an environment that provides a low level of stimulation than in a stimulus-rich environment (Bateson, 1964d, Montevecchi et al., 1973). Similar causal factors are suggested for embryonic peeps. The absence of maternal stimulation during incubation results in an increased rate of peeping.

The factors that influence screeches before and after hatching are also similar. After hatching, screeches are induced by forcibly preventing a chick from moving. In embryos, screeches appear to be closely connected with the process of hatching. They accompany strong embryonic movements that eventually break the shell (Guyomarc'h, 1972). It seems then that screeches are related to physical restraints imposed on embryos by the shell and the surrounding membranes. Such restraints have no systematic relationship to maternal behaviors. This may account for the similar frequencies of screeches in naturally and artificially incubated embryos.

The higher incidence of huddling calls in artificially incubated embryos may be explained by the absence of proximal stimuli from the incubating hen. After hatching, huddling calls accompany attempts by the chick to approach and establish direct physical contact with the hen. Their higher frequency in artificially incubated embryos suggests similar contact searching behavior by the embryo.

It is concluded that the major differences between the patterns of vocalizations emitted by naturally and artificially incubated embryos indicate that maternal stimulation has a marked influence on the emission of embryonic vocalizations.

EXPERIMENT III

ANALYSIS OF EMBRYONIC RESPONSES TO MATERNAL STIMULI

I. Artificially incubated embryos.

My observations of naturally incubated broods revealed that the selective responsiveness to maternal stimuli exhibited by chicks after hatching is already present prior to hatching. Two important experiential factors that could influence embryonic responsiveness are maternal stimulation and embryonic self-stimulation. To investigate the role of maternal stimuli I tested the responsiveness of artificially incubated embryos to recorded maternal vocalizations and to movements imitating those of an incubating hen.

METHODS

The eggs were incubated for the first 16 days in the commercial-type incubator. On the 17th day of incubation, the eggs were moved to the experimental room and placed under bell shaped plastic incubators (see earlier description). Testing began after the embryos reached the pipping stage. Pipping occurs about 6-16 hours before hatching when the embryo breaks a 3-5 mm hole in the shell near its beak. (For a more complete description see Oppenheim, 1973). The hole in the egg shell facilitates the recording of embryonic vocalizations.

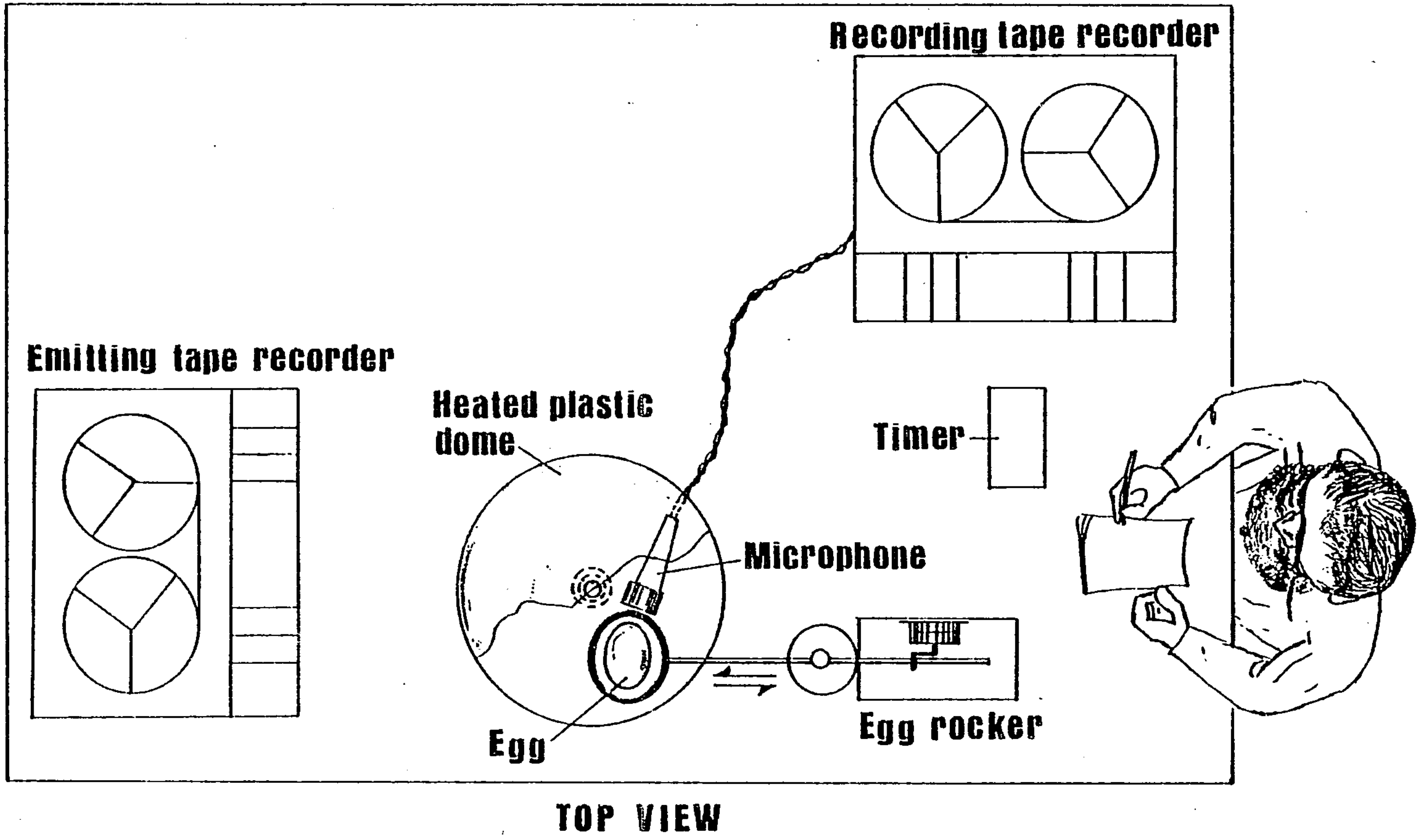
The embryos were tested for responsiveness to four different stimuli: tape-recorded maternal clucks and food calls plus slow and fast movements applied to the egg. Each embryo was tested once with only one type of stimulus. The prerecorded maternal vocalizations were presented using a Sony Model 800 B recorder. The sound pressure level at the location of the embryo was 60-65 dB as measured by a Triplett Model 370 sound pressure level meter. Measurements were taken on the B weighted scale. The movement stimuli were applied with an "egg rocker" (Fig. 23). A foam padded ring surrounding the egg was moved back and forth with a push-rod system driven by an electric motor. Two types of movement were applied: a slow, large amplitude movement and a fast, lower amplitude movement (see Appendix IX). The slow movement simulates egg turning by the hen while the faster movement approximates the stimulation during resettling.

The embryo was placed in the experimental incubator at least 15 min before the beginning of the test. At the end of this introductory period, the tests proceeded as follows:

(a) Playback of recorded maternal vocalizations. The test started with a 2-min pre-stimulation period. It continued with four 30-s periods of stimulation separated by 30-s silent intervals. The test ended with a 2-min post-stimulation period.

(b) Movements applied to the egg. The test started with a 2-min pre- stimulation period and continued with three 30-s periods of stimulation separated

Fig. 23. Experimental apparatus for recording embryonic responses.



EXPERIMENTAL SET-UP FOR RECORDING EMBRYONIC RESPONSES.

by 40-s intervals. The test ended with a 2-min post-stimulation period.

Embryonic vocalizations and large amplitude movements were recorded. Recordings started at the beginning of the pre-stimulation period and were continued until the end of the post-stimulation period. Embryonic vocalizations were recorded through a microphone placed about 1 cm from the egg, close to the pipping hole. The microphone was attached to a Sony Model 800 B tape recorder. The occurrence of embryonic movements was recorded by direct observations.

The analysis of responses to recorded maternal vocalizations was performed on the number of embryos that moved or vocalized in the 2-min interval before, during, and after stimulation. The analysis of responses to movements applied to the eggs was performed on the number of embryos that vocalized in the 1-min interval before, during, and after stimulation. In most cases a chi-square test was used to test for independence. In borderline cases and in cases where expected values were too low, independence was tested by using the binomial distribution. Embryonic vocalizations were treated per category, as pleasure type or distress type calls, according to their ascending or descending frequency spectrums.

RESULTS

The number of embryos that emit pleasure calls during the stimulation periods with recorded clucks is significantly higher than the number calling before of after stimulation (Table XVII). Although more embryos emitted pleasure calls during stimulation than outside the stimulation period only 39% of all embryos called. The number of embryos that emit distress calls is significantly lower during stimulation than beforehand and remains low afterwards. There are no significant differences between the number of embryos that move during stimulation as compared to before and after.

The influence of maternal food calls on embryonic movements and vocalizations is more limited (Table XVIII). The number of embryos emitting pleasure calls during stimulation is significantly higher than before or after but only 28% of them called. Neither the number of embryos that move nor the number that emit distress calls change significantly over the 3 periods.

Only the fast movements applied to the eggs have a noticeable effect on the embryonic vocalizations (Table XIX). There is a significantly smaller number of embryos that emit distress calls during the stimulation period as compared to before and after.

DISCUSSION

None of the stimuli has a strong effect on the behavior of inexperienced embryos. The most noticeable effect is produced by recorded clucks. Their enhancement of embryonic pleasure calling and inhibition of distress calling is

TABLE XVII. Number of artificially incubated embryos that move or vocalize during the test with recorded clucks (n = 18).

	TEST PERIOD				
TYPE OF BEHAVIOR	BEFORE STIMULATION (2 min)		DURING STIMULATION (2 min)		AFTER STIMULATION (2 min)
MOVEMENT	16		16		15
		n.s.		n.s.	
PLEASURE TYPE CALL	2		7		2
		$p < 0.05$		$p < 0.05$	
DISTRESS TYPE CALL	11		6		7
		$p < 0.05$		n.s.	

TABLE XVIII. Number of artificially incubated embryos that move or vocalize during the test with recorded maternal food calls (n = 18).

	TEST PERIOD				
TYPE OF BEHAVIOR	BEFORE STIMULATION (2 min)		DURING STIMULATION (2 min)		AFTER STIMULATION (2 min)
MOVEMENT	13		12		15
		n.s.		n.s.	
PLEASURE TYPE CALLS	2		5		1
		$p < 0.05$		$p < 0.05$	
DISTRESS TYPE CALLS	5		4		4
		n.s.		n.s.	

TABLE XIX. Number of artificially incubated embryos that vocalize during the test with movements applied to the egg (n = 30).

TYPE OF STIMULUS	TYPE OF VOCALIZATION	TEST PERIOD				
		BEFORE STIMULATION (1 min)		DURING STIMULATION (1 min)		AFTER STIMULATION (1 min)
FAST MOVEMENT n = 15	PLEASURE TYPE CALLS	0	n.s.	2	n.s.	1
	DISTRESS TYPE CALLS	5	$p < 0.05$	1	$p < 0.05$	4
SLOW MOVEMENT n = 15	PLEASURE TYPE CALLS	0	n.s.	0	n.s	0
	DISTRESS TYPE CALLS	4	n.s.	3	n.s.	2

similar to the effect of maternal clucks on naturally incubated embryos. The effect of recorded food calls is also comparable to that of maternal food calls in naturally incubated broods, stimulating embryonic pleasure calls and slightly depressing distress calls.

Fast movements applied to the eggs have an inhibitory effect on distress type calls. Although I found no comparable effect of maternal movements in naturally incubated embryos it may be that the overall level of distress calling was so low that no particular inhibition by individual movements could be detected.

The results indicate that the artificially incubated embryos show some selective responsiveness to maternal behavior even though they had no prior experience with it. The low overall responsiveness does suggest that prior exposure to maternal behaviors promotes the development of selective responding.

II. Naturally incubated embryos.

The limited influence of the recorded maternal vocalizations on the behavior of naive embryos suggests that prior experience with maternal stimuli is important for the development of embryonic responsiveness. This experiment was designed to investigate that possibility by testing the response of naturally incubated embryos to recorded maternal clucks. I chose clucks because of their effectiveness in eliciting responses as demonstrated in our previous experiments.

METHODS

Fertile eggs were incubated for 16 days in a commercial incubator. On day 17 of incubation the eggs were removed from the incubator and placed under an incubating hen. When the embryos reached the pipping stage three days later they were tested for responsiveness to recorded clucks. The embryos were tested individually in the dome-shaped incubator described earlier (Fig. 23). The testing procedure and data analysis were identical to those used for artificially incubated embryos.

RESULTS

There is a significant increase in the number of embryos that emit pleasure calls during the stimulus period as compared to the periods before and after stimulation (Table XX). There is a nonsignificant increase in the number of embryos that emit distress calls. The number of embryos that move is high in all three periods but remains unchanged during the entire test.

Significantly more naturally incubated embryos emitted distress calls during stimulation with clucks than did artificially incubated ones (Table XXI). There is no significant difference in the number of embryos that emitted pleasure calls or moved during the stimulation period.

TABLE XX. Number of naturally incubated embryos that move or vocalize during test with recorded clucks (n = 18).

	Test Period				
Type of Behavior	Before Stimulation (2 min)		During Stimulation (2 min)		After Stimulation (2 min)
Pleasure Type Calls	1	$p < 0.05$	6	$p < 0.05$	0
Distress Type Calls	7	n.s.	10	n.s.	7
Movement	17	n.s.	18	n.s.	18

TABLE XXI. Number of artificially and naturally incubated embryos that move or vocalize during stimulation with recorded hen clucks (n = 18).

TYPE OF BEHAVIOR	TYPE OF INCUBATION		
	NATURAL	ARTIFICIAL	p
PLEASURE TYPE CALL	6	7	n.s.
DISTRESS TYPE CALL	10	6	<0.05
MOVEMENT	18	16	n.s.

DISCUSSION

The testing of naturally incubated embryos yielded some unexpected results. According to the hypothesis that prior experience with maternal stimuli enhances embryonic responsiveness to such stimuli it was predicted that clucks would have a stronger directive effect on embryonic pleasure calls and an inhibiting effect on distress calls. Neither of these predictions was confirmed. In fact, more of the naturally incubated embryos distress called during stimulation than artificially incubated ones. These results contrast with my previous findings which show that maternal behaviors inhibit embryonic distress calls. A possible explanation is that naturally incubated embryos detect a difference between the recorded clucks and those produced by their own hen. The playback cluck used in the test was not that of the hen incubating the eggs. Moreover, there is always a certain amount of change inherent in the tape recording of a sound. That embryos are able to discriminate between different clucks is confirmed by the findings of Guyomarc'h (1974a, 1975b). Chicks that were exposed to a particular cluck prior to hatching showed a significant preference for the familiar cluck in a choice discrimination test with a different cluck.

The emerging hypothesis is that chicken embryos interact with the incubating hen and, near hatching, are capable of discriminating the calls of their own mother from those of an unfamiliar hen. The relative influence of maternal stimulation and embryonic self-stimulation on the development of such discrimination abilities deserves further investigation.

EXPERIMENT IV

THE INFLUENCE OF PREHATCHING INTERACTIONS ON THE BEHAVIOR OF THE CHICKS AT NEST LEAVING

A possible consequence of early interactions between embryos and the incubating hen is enhanced responsiveness of the chicks to maternal vocalizations after hatching. Increased responsiveness to vocal stimuli could, in turn, influence responsiveness to associated visual stimuli. This stimulus association might facilitate filial attachment (imprinting).

The aim of this experiment is to determine if prehatching interactions increase the responsiveness of newly hatched chicks to maternal stimulation during nest leaving. Nest leaving is the first instance when filial responses are expressed by approach and following behavior. During nest leaving, chicks have to make a choice between following the hen and continuing to stay near the familiar nest and siblings. This event provides a good opportunity for observing differences between naturally and artificially incubated chicks.

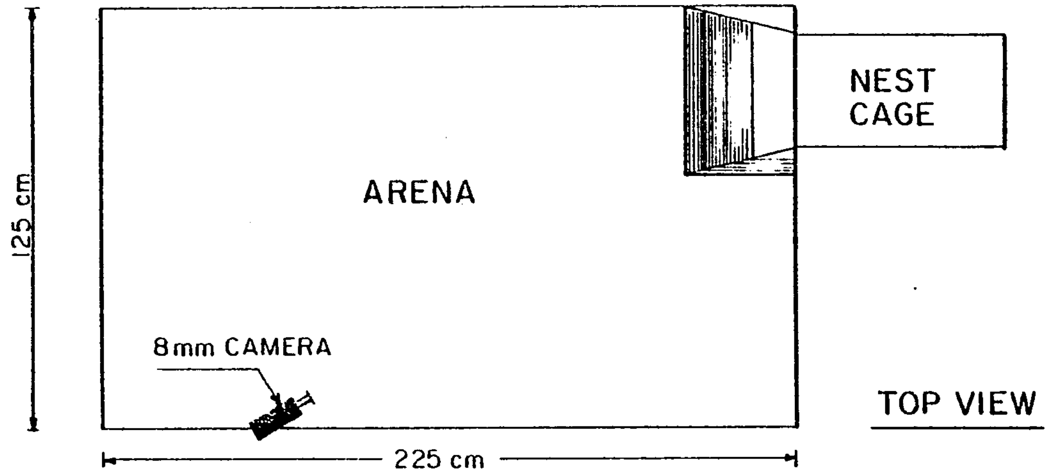
METHODS

I used hens of the same Minorca x Rose Comb variety as in the previous experiments. The hens layed and incubated eggs in nest cages suspended from the walls of the colony pen (see earlier description). After 15-20 days of incubation one nest cage was moved to the observation room and attached to an open field arena (Fig. 24). The bottom of the cage was higher than the floor of the arena and the connection was made by a ramp. The ramp facilitated observations of the hen and chicks during nest leaving. I observed six different broods each containing three artificially incubated and three naturally incubated chicks.

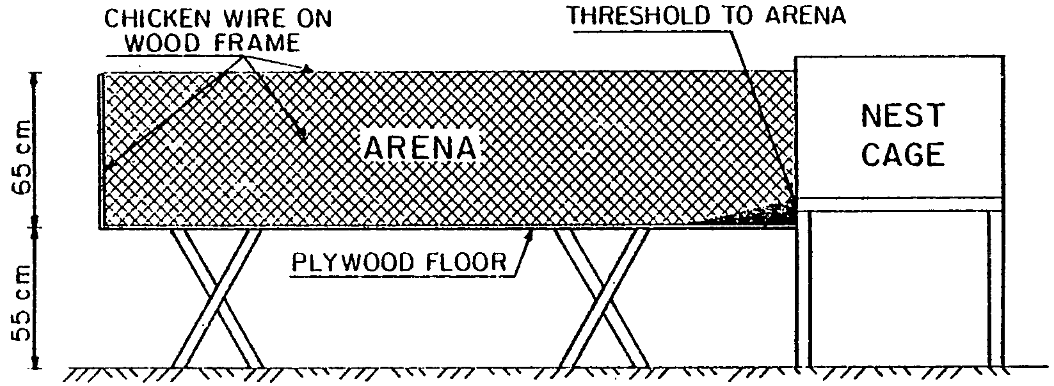
Procedure. Fertile, White Leghorn eggs were incubated for 16 days in the laboratory incubator. On the 17th day five eggs were removed and placed under the incubating hen. Beginning on the 20th day, we monitored chicks hatching under the hen and in the incubator by direct observations. When a chick hatched under the hen, an additional chick that had hatched in the incubator was color marked for identification and placed under the hen. The incubator hatched chick was chosen so that its hatching time was within one hour of that of the naturally hatched chick. The procedure was repeated until six chicks, three of each type, were obtained. When the last incubator hatched chick was placed under the hen, all unhatched eggs were removed from the nest and the observations were begun.

Data collection. The order and latency of nest leaving for each chick were recorded beginning the moment the hen passed the threshold of the nest cage and entered the arena. Nest leaving was monitored by direct observations and by

Fig. 24. Experimental enclosure for observations of nest leaving behavior.



TOP VIEW



LATERAL VIEW

filming with a Super 8 mm Minolta camera at a speed of one frame per second.

RESULTS

Naturally incubated chicks have a tendency to follow the hen out of the nest sooner (Table XXII). The difference between their position in the group and that of artificially incubated chicks barely misses significance in a Wilcoxon two-sample test ($U_s = 190$, $p = 0.065$) and is significant if a Kolmogorov-Smirnov test is applied ($D = 0.375$, $p < 0.05$; Sokal and Rohlf, 1969, page 393, 573). The latency for nest leaving is significantly lower in naturally incubated chicks than in artificially incubated ones ($\overline{X} = 159.3$ s for artificially incubated and $\overline{X} = 106.8$ s for naturally incubated chicks; $F = 3.01$; d.f. = 17, 15; $p < 0.05$). The data for two naturally incubated chicks were discarded because they had great difficulties locomoting. Both chicks had been accidentally injured by the hen prior to their departure from the nest.

DISCUSSION

While these results are consistent with the hypothesis that prehatching interactions influence the behavior of chicks during nest leaving, our observations suggest that other factors have an important influence as well. First, even among normal chicks there are differences in their locomotory abilities. Such differences seem likely to

TABLE XXII. Comparison between the order of nest leaving by naturally and artificially incubated chicks.

Order at Nest Leaving	Number of Chicks	
	Naturally Incubated n = 16	Artificially Incubated n = 18
1st	3	3
2nd	5	1
3rd	4	2
4th	1	5
5th	1	5
6th	2	2

affect the order of chicks following the hen. Second, the order of nest leaving appears to be influenced by which birds are out of the nest cup when the hen leaves the nest cage. The chicks may come out of the nest cup and move around the hen before the hen leaves the nest cage. I observed that in three out of six broods the hen left the nest when artificially incubated chicks had come out of the nest cup and had approached the entrance to the arena. This happened in two of the three cases when the first chick to follow the hen was artificially incubated. In most broods, the first chicks to come out of the nest cup were naturally incubated chicks. After a few minutes of locomoting and pecking around the hen, these chicks crawled back under the hen to be brooded. At about the same time, artificially incubated chicks would start to come out of the nest. Soon after their emergence the hen would rise and walk away from the nest, leaving the naturally incubated chicks behind. Finally, during the period between their placement in the nest and nest leaving, the artificially incubated chicks were exposed to intensive maternal stimulation. This, probably, helped increase their responsiveness to maternal stimuli at nest leaving.

The tests show that artificially and naturally incubated chicks respond differently to the hen at nest leaving. However, my observations revealed that in addition to prehatching experience several other factors may be important. Further tests are required to determine the relative influence of these factors.

GENERAL DISCUSSION

The study reveals that embryos and the incubating hen stimulate each other and interact prior to hatching. Prehatching vocal interactions are similar to those that occur after hatching. Exposure to maternal stimuli and interactions with the hen influence the patterns of vocalizations in naturally incubated embryos and affect their responsiveness to maternal stimuli. Artificially incubated embryos respond selectively to maternal stimuli but their responses differ from those of naturally incubated embryos. Also, during nest leaving, chicks from the two groups exhibit differences in filial behavior.

These findings suggest the following hypotheses:

1) Different prehatching experiences produce chicks that react differently to certain posthatching stimuli. The responsiveness of newly hatched chicks to social stimuli is determined by their prehatching experience with maternal and self-produced stimulation. For instance, prehatching experience with the hen facilitates the coordination of the social unit after hatching. At hatching naturally incubated chicks are already familiar with maternal stimuli and respond to them in ways similar to those observed later on. Coordination in behavior between the young and their parent is already apparent before hatching.
2) Embryonic responsiveness to maternal vocalizations

develops even in the absence of prehatching interactions with an incubating hen. Self-stimulation with distress calls may influence the development of selective responses to the maternal cluck.

3) Although responsiveness to maternal stimuli may develop in the absence of an incubating hen, exposure to maternal stimulation can affect responsiveness in three different ways:
    a) it can increase the chick's familiarity with certain types of stimuli
    b) it can improve the chick's ability to discriminate among stimuli
    c) it can enhance the attractiveness of some stimuli

The present findings also lend support to the following hypotheses:

4) Embryonic responsiveness to tactile stimulation develops first followed by responsiveness to auditory stimulation. This sequence parallels the maturational processes of the respective sensory systems.

5) Prehatching interactions influence the development of maternal behavior in the incubating hen.

1. Prehatching processes that influence later behavior.

The study of prehatching experiences that could influence the development of behavior in embryos proceeds in stages. To demonstrate that prehatching exposure to stimulation affects later behavior, it is necessary to describe the parental and embryonic behaviors during incubation, determine the factors that may influence embryonic stimulation and then establish the influence of these factors. After prehatching stimulation is shown to influence later behavior it becomes possible to investigate processes that mediate development of the stimulus-response relations.

The present study shows that the prehatching experience of domestic chicks is influenced by the same three factors which act in other species of precocial birds (Gottlieb, 1975a, 1975b, 1975c, 1978, Impekoven, 1971a, 1976a, Tschanz, 1968, Norton-Griffiths, 1969). First, maternal stimulation induces embryonic responses that change the pattern of embryonic self-stimulation from one dominated by distress calls to one which is largely pleasure calls. Secondly, passive exposure to maternal stimuli expands the embryo's experience in both the tactile and auditory modalities. Artificially incubated embryos totally lack this exposure. Thirdly, naturally incubated embryos interact with the incubating hen and perceive the influence of their own behavior on the pattern of outside stimulation. Artificially incubated embryos do not have that experience.

Based on current theories of development (Kuo, 1967, Schneirla, 1965, Sluckin, 1970, Hinde, 1970, Gottlieb, 1976a, 1976b, Bateson, 1976) one would predict that such differences in prehatching experience would lead to differences in behavior later on. The results of my experiments show that this is indeed the case. Embryonic experience with parental stimuli alters the way embryos respond to maternal clucks and the readiness of chicks to follow the hen at nest leaving.

Previous research suggests two ways in which early experiences can influence later behavior. In processes akin to conditioning, a stimulus acquires eliciting properties through systematic association with an effective stimulus or with the response. In processes akin to perceptual learning, repeated exposure to a stimulus is enough to affect the response (Zajonc, 1971, Hinde, 1970). Schneirla (1965) hypothesized that the maternal cluck becomes attractive to the newly hatched chick through a conditioning process. The cluck may become effective in eliciting embryonic responses through association with effective embryonic self-stimulation or with maternal tactile stimulation. At the present time we have no direct support for Schneirla's hypothesis, but we have evidence that the factors necessary for conditioning are present during incubation. My observations show that chick embryos respond to maternal tactile stimuli and interact vocally with the incubating hen. There is also evidence that conditioning can occur in chick embryos (Reviewed by Gottlieb, 1968 and Impekoven, 1976b).

In addition to effects through conditioning, effects of mere repetitive exposure to clucks or to similar low frequency sounds could enhance responsiveness to these stimuli and attraction towards their source (Zajonc, 1971). According to Gottlieb (1976a) early experience may influence development in three different ways: it could maintain ongoing development, facilitate the rate of development and/or induce development along certain channels. Evidence for the maintianing and facilitative roles of prehatching experience with auditory stimuli is provided by Gottlieb's work on devocalized ducklings that was described earlier (Gottlieb, 1975a, 1975b, 1975c, 1978).

Prehatching experience has similar influence in chickens although it is not clear whether the effects are the result of maintenance, facilitative or inductive processes. The results of my experiments show that artificially incubated embryos approach and follow the clucking hen less readily during nest leaving than chicks incubated by the hen. Impekoven (1976b) found that chicks emit more pleasure calls when exposed to clucks if they experienced the same clucks prior to hatching. Moreover, it was reported by Guyomarc'h (1974a, 1975b) that newly hatched chicks discriminate between a particular cluck that they experience before hatching and a different cluck. It is conceivable that in some of these instances conditioning occurs too but the possibility that clucks are systematically associated with other effective stimuli is remote.

Prehatching experiences of chick embryos may have effects which are not apparent right after hatching. According to the primacy concept (Reviewed by Hess, 1973) early experiences have a determining influence on later behavior. This influence may be due either to "foundational" experiences (primordial experiences which lay the grounds for reactivity to future experiences, Sluckin, 1970), or to early experiences that occur during certain sensitive periods (Scott, 1962). My study shows that, during artificial incubation, the absence of a hen and of the hen-induced changes in embryonic self-stimulation coincides with a period when embryos are quite responsive to stimulation. As a result, differences in experience with maternal and self-produced stimuli may have lasting effects on later behavior.

The results of the study show that the prehatching experiences of artificially and naturally incubated chicks are very different. Artificially incubated chicks could be considered deprived subjects because they lack experiences with maternal stimulation and with their own pleasure calls. They also experience an abnormally high number of distress calls. Artificially incubated embryos also lack the opportunity to interact with the hen and do not experience changes in the environment linked to their own behavior. It appears then, that artificially and naturally incubated embryos follow different developmental paths because of different stimulus experiences. Whether or not the behavior of artificially incubated chicks is representative of that following development under natural conditions is open to serious question.

2. Self-stimulation and the development of selective responses to the hen. Chicks respond selectively to maternal

vocalizations even in the absence of previous interactions with the incubating hen (Collias, 1952, Baeumer, 1962, Impekoven, 1976b, Snapp, 1969). One factor that could determine the development of selective responsiveness without exposure to maternal calls is vocal self-stimulation. Schneirla (1965) and Guyomarc'h (1973) suggested that embryonic self-stimulation with pleasure calls determines the development of responsiveness to the maternal cluck. Evidence provided by my study indicates that embryonic pleasure type calls are not likely to influence the development of embryonic responsiveness. It is possible though that early exposure to distress calls influences later responses to maternal vocalizations.

The results of my experiments show that artificially incubated chicken embryos respond to maternal vocalizations in a selective fashion. Similar evidence has been obtained by other investigators. Collias (1952) reported that artificially incubated chicks having no previous experience with maternal calls tend to approach and stay near a speaker emitting maternal clucks. There is no difference between the proportion of artificially and naturally incubated chicks that approach the speaker (Impekoven, 1976b). Collias (1952) also found that artificially incubated chicks start emitting pleasure calls when exposed to clucks. If naive chicks are exposed to clucks as they distress call, their vocalizations change to pleasure calls (Baeumer, 1962).

The selectivity of chick responsiveness has been demonstrated by Ramsay (1951) who reported that naive, freshly hatched chicks, tend to approach a vocalizing broody hen rather than a mallard or muscovy duck in a simultaneous choice test. That the selective approach of the hen is due to the cluck stimulus and not to the hen's visual configuration is supported by the finding that naive chicks approach a speaker emitting clucks more readily than a stuffed maternal replica (Gottlieb, 1971). Gottlieb (1971) also observed that chicks follow only objects that emit clucks when subjected to a choice test with objects emitting maternal calls of other species.

Earlier, Schneirla (1965) proposed that embryonic self-stimulation with contentment calls plays an important role in the development of responsiveness to maternal calls. Schneirla's hypothesis is supported by Gottlieb (1965, 1965a, 1966, 1971, 1976) and Reynolds (1977) who found that artificially incubated ducklings respond selectively to the species maternal call. After investigating the experiential factors that may determine the development of responsiveness to the maternal call, Gottlieb (1975a, 1975b, 1975c, 1978) concluded that self-stimulation with contentment calls by the duck embryo plays a major role.

That embryonic self-stimulation with pleasure calls may play a similar role in chicks was suggested by Schneirla (1965) and Guyomarc'h (1973). Guyomarc'h compared the frequency spectrum of the maternal cluck with that of the chick twitter and concluded that there are enough similarities between the two calls to support this hypothesis. He even suggested that twitters are precursors of clucks (Guyomarc'h, 1972).

My observations cast some doubt on the validity of the self-stimulation hypothesis for chicks. Artificially incubated chicks have little experience with pleasure calls and yet they are strongly attracted to the cluck call of the mother. Pleasure type calls appear late in incubation and are seldom emitted by artificially incubated embryos. It is therefore quite unlikely that pleasure calls play an essential role in the initial development of a preference for the maternal cluck.

It appears from my observations that the only form of vocal self-stimulation that could promote the development of selective responsiveness are distress calls. Distress calls start quite early and occur very frequently after the 18th day of incubation when embryos are already responding to external stimulation (Vince, 1973, Hamburger, 1973). These calls however are very different in spectral qualities from the cluck and there is evidence suggesting that chicks can not perceive their high frequencies until the 19th day of incubation (Saunders et al., 1973, Saunders et al., 1974). This is primarily because the middle ear is filled with fluid and does not transmit high frequency vibrations. Lower frequencies, on the other hand can be detected much earlier.

One way in which distress calls could be detected by the embryo is through distortion frequencies of the original sound that fall within a lower range. Distortion frequencies are caused by combination tones transmitted by the middle ear fluids and the bony labyrinth (Wever and Lawrence, 1954). Experiments on transmission of sound vibrations through resonance of the auditory meatus and the skull performed on ear models (Von Bekesy, 1960) reveal that there is a sound pressure peak on the eardrum produced by sounds within the 1,800 to 2,500 frequency range. A similar pressure peak may occur on the eardrum of the chick embryo as a result of combination frequencies produced by embryonic distress calls. Vibrations of the eardrum in response to these high frequency sounds would probably be in a much lower range. There is evidence from other species that the ear structures transmit low frequency vibrations. Thus, the auditory meatus of the cat transmits frequencies around 1,000 Hz which are in its resonance range (Wever and Lawrence, 1954). Vibrations in a similar low range could be transmitted by the chick embryo's auditory meatus and detected by its cochlea. Embryonic detection of combination frequencies around 1,000 Hz may help to explain the development of sensitivity and preference for maternal clucks.

It appears that the auditory stimulation provided by prehatching interactions between the embryo and the incubating hen is not necessary for the initial development of responsiveness to the maternal cluck. It is still not known whether or not vocal self-stimulation by the chick embryo contributes to the initial development of this responsiveness, but a mechanism is proposed in which self-stimulation by distress calls could enhance responsiveness to maternal clucks.

3. Possible effects of prehatching experience with maternal stimulation. Although naive chicks are capable of responding selectively to species-specific stimuli, their responses are different from those of naturally incubated chicks. There are three possible explanations for these differences. The interactions that occur during natural incubation could permit familiarization with maternal stimuli; they could influence the selectivity of responsiveness; or they could enhance the attractiveness of some stimuli.

The results of my experiments show that exposure to maternal vocalizations changes the behavior of embryos. It seems that the change in behavior is due largely to increased familiarity with the stimulus and not just to the attractiveness of the stimulus as is often assumed. For instance, the results show that in naturally incubated embryos unfamiliar recorded clucks tend to elicit distress calls while familiar clucks of the incubating hen tend to inhibit them. These findings are consistent with results obtained in several earlier investigations. Impekoven (1976) found that chicks exposed to clucks prior to hatching emit significantly more pleasure calls than naive chicks when they hear clucks after hatching. This also applies to stimuli other than the species specific ones. In separate experiments Grier (1967) and Rajecki (1974) exposed chick embryos to a 200 Hz sound and tested their response after hatching to the familiar 200 Hz sound and a novel 2,000 Hz sound. More chicks approached the speaker and emitted pleasure calls to the familiar than to the novel sound. In contrast, naive chicks behaved the same towards the two sounds. Lien (1976) and Evans and Knapton (1977) performed similar experiments and found that newly hatched quail chicks behave differently towards familiar and unfamiliar sounds.

Changes in behavior produced by increased familiarity with the stimulus have their counterpart in the behavior shown by chicks when exposed to novel stimuli. There is some evidence that novel stimuli have a generalized inhibitory effect on chick activity (Montevecchi et al., 1973, Andrew, 1963). When introduced into a novel environment chicks show very low levels of activity. With repeated experience in the same environment their activity increases. Russock (1971) reported that chicks show a "freezing" reaction when exposed initially to maternal food calls played through a loudspeaker. Only after two or three exposures do chicks start approaching the speaker. A similar reaction to novel stimuli is shown by embryos at an early stage. Gottlieb (1971) reported that the first presentation of maternal calls inhibits embryonic beak claps and vocalizations in duck embryos. On subsequent presentations, the maternal call enhances embryonic activity. Similar inhibitory effects were found in wood ducklings by Heaton (1972) and in herring gull chicks by Evans (1973). Impekoven (1976a) found that the effects of parental calls on the activity of newly hatched laughing gulls depend on whether or not they had previous experience with the calls.

It is clear then that novelty influences the response of the young bird regardless of how potentially attractive is the stimulus. However, due to the limited number of criteria currently used for judging responsiveness, it is difficult to separate the effects of stimulus familiarity from those of stimulus attractiveness. A newly hatched bird, that otherwise shows locomotory competence, may not approach a stimulus because of inhibition, lack of attractiveness or both. In this case, using the locomotory response as a sole criterion limits the ability of the investigator to differentiate among the effects. Increased resolving power could be obtained by extending the range of behavioral criteria used in measuring responsiveness. If vocalizations are considered with locomotory responses the larger number of combinations will permit more precise determinations. For example, a chick that distress calls and does not approach a source of stimulation is probably not attracted to it. But, if the chick remains immobile and silent it is probably inhibited by the stimulus.

Increased familiarity with the stimulus is only one of the effects of prehatching interactions. Another major effect is on the selectivity of embryonic responsiveness to different maternal stimuli. The results of my observations show that during the hours before hatching naturally incubated embryos respond selectively to various maternal stimuli. I also found that naturally incubated embryos respond differently to the clucks of the incubating hen than to those recorded from a different hen. There is also evidence that, after hatching, chicks discriminate between the individual cluck of their parental hen and the clucks of other broody hens. Ramsay (1951) observed that chicks are able to recognize their parental hen from other broody hens on the first day after hatching. Collias (1952) found that chicks from different broods that are mixed in the dark show a tendency to approach their own parental hen. That prehatching interactions influence this discrimination ability is also suggested by the findings of Guyomarc'h (1974a). Guyomarc'h exposed different groups of embryos to clucks recorded from different hens and then tested the responses of the newly hatched chicks to the familiar and unfamiliar clucks. He found that most chicks approached the source of the familiar cluck. A similar effect was found by Tschanz (1968) in guillemots. Guillemot chicks learn to recognize the individual call of their parent as a result of prehatching interactions.

At the species level, prehatching interactions influence the ability of the newly hatched bird to discriminate the calls of their own species from those of other species. Gottlieb (1971, 1975a, 1975b, 1975c) demonstrated that exposure to species specific calls prior to hatching enhances the ability of the newly hatched ducklings to discriminate between their species maternal call and calls of other species.

It is apparent from the examples cited above that a third major influence of prehatching interactions is that they may enhance the attractiveness of certain stimuli. Some of the auditory stimuli perceived during incubation become not only familiar but also more attractive. Newly hatched chicks approach more readily the source of a cluck heard previously than that of a novel cluck (Guyomarc'h, 1974a, 1975). While it is not possible to show that attractiveness can be increased without increasing familiarity the opposite can be shown. There are stimuli which fail to become attractive even though they are frequently perceived during incubation. For instance, chick distress type calls which occur frequently before hatching are not attractive to newly hatched chicks (Gottlieb, 1966).

I conclude with the suggestion that there are three different factors that influence the responsiveness of newly hatched chicks to auditory stimuli: the familiarity with the stimulus, the ability to discriminate between different stimuli and the attraction to the stimulus.

Unfortunately in many instances the effects of these factors have been confounded. It will be helpful in future investigations to distinguish among the different factors.

4. Development of embryonic responsiveness parallels maturation of sensory systems. I found evidence that embryonic responsiveness to tactile and auditory stimuli develops as the respective sensory systems mature (Reviewed by Gottlieb, 1968, Impekoven, 1973, 1976, Vince, 1973).

In chick embryos, sensitivity to cutaneous tactile stimulation may be shown as early as the 6th day of incubation. Overt responses to tactile stimuli however appear much later. It is believed that tactile stimuli start influencing embryonic movements by the 16th to 17th day of incubation (Vince, 1973, Hamburger, 1973, Provine, 1973). Onset of the auditory function is later in development than that of the tactile function (Saunders et al., 1973, Saunders, 1978). Although the cochlea becomes functional by days 11-13 of incubation there is no clear evidence of sound effects on embryonic motility before the 18th day of incubation. My results show that at early stages the largest proportion of the embryonic vocal responses are given to tactile stimulation from the hen. Later, during the hours just before hatching, the proportion of responses given to maternal vocalizations increases. A similar type of chronological sequence in embryonic responsiveness was observed by Tschanz (1968) in guillemots and Impekoven (1976) in laughing gulls.

It was suggested by Schneirla (1965) that stimuli of a later maturing sensory system may become effective through association with stimuli of a sensory system that matured earlier. Embryonic responsiveness to certain calls may develop through their association with familiar tactile stimuli. Several investigators (Impekoven, 1976, Clements and Lien, 1976) have attempted to test Schneirla's hypothesis but the evidence is still unclear. To test the hypothesis, I am exposing artificially incubated embryos to maternal vocalizations while simultaneously moving the eggs. Subsequently I test the effect of the maternal calls on embryonic vocal responses. To isolate the effect of mere arousal caused by tactile stimulation, I am using an alternate way of arousing the embryos, with loud noises, before presenting the maternal calls.

5. Prehatching interactions and the development of maternal behavior. Evidence provided by this study supports the hypothesis that prehatching vocal interactions between embryos and the incubating hen influence the development of maternal behavior. My observations reveal that the hen starts emitting maternal calls after the onset of embryonic vocalizations and frequently in response to them. The number of maternal responses increases steadily as hatching approaches.

The incubating hen responds to embryonic calls in a manner similar to that in which broody hens respond to their chicks after hatching. For instance, embryonic distress calls have a strong stimulatory effect on maternal clucks. There is additional evidence that supports this hypothesis (Baeumer, 1962, Collias, 1952, Maier, 1962, Guyomarc'h, 1975a). According to Baeumer (1962), Collias (1952) and to my observations the hen continues to incubate long after the usual period if the eggs are not fertile and embryos do not develop. Moreover, the hen attacks and kills newly hatched chicks if not exposed first to embryonic stimuli. Maier (1962) showed experimentally that chick vocalizations can induce maternal behavior in nonbroody hens. These pieces of evidence suggest that the end of incubation and the initiation of maternal behavior are determined by stimuli provided by the embryos and the newly hatched chicks.

Prehatching vocal interactions with the embryos may help the hen learn the calls of individual chicks within her brood. Maier (1962) observed that hens becoming broody as a result of being exposed to chicks show maternal behavior only toward familiar chicks. Ramsay (1951) reported that hens are capable of recognizing the calls of their own chicks the first day after hatching. Unfamiliar chicks that are transferred to the brood are attacked by the hen only if they start vocalizing.

Additional research is necessary in order to clearly identify the effects of prehatching interactions on the initiation of maternal behavior and the development of individual attachments between the hen and the newly hatched chicks.

SUMMARY

Evidence from several species of precocial birds suggests that the behavior of newly hatched chicks is influenced by embryonic self-stimulation, passive exposure to maternal stimuli and prehatching interactions with the incubating hen. In this study prehatching interactions in naturally incubated broods are analyzed and the influence of the incubating hen on the later behavior of the chicks is investigated. In the first experiment naturally incubated broods were observed and the behavior of embryos and the incubating hen was described. The associations between embryonic and maternal behaviors were analyzed. The embryos and the incubating hen were found to interact and the type of interactions were identified. In the second experiment the behaviors of naturally and artificially incubated embryos were compared. The incubating hen was found to influence embryonic self-stimulation by increasing the frequency of pleasure type calls and decreasing the frequency of distress type calls. In the third experiment, the responses of artificially and naturally incubated embryos to recorded maternal vocalizations were compared. It appears that interactions with the incubating hen influence subsequent embryonic responses to maternal calls. A higher proportion of naturally incubated embryos distress called when exposed to unfamiliar recorded clucks. In the fourth

experiment, filial responses of newly hatched artificially and naturally incubated chicks were compared. The results show that, during nest leaving, naturally incubated chicks follow the hen more readily than artificially incubated ones.

It is concluded that, as a result of different prehatching experience, the behaviors of naturally and artificially incubated chicks might not be comparable. Although artificially incubated chicks are capable of responding selectively to the species-specific maternal stimulation, prehatching exposure to the incubating hen leads to familiarization, increased attractiveness and differentiation between stimuli. It is hypothesized that embryonic self-stimulation with distress type calls could influence the development of selective responsiveness to maternal calls in artificially incubated chicks.

APPENDIX I

A description of embryonic and maternal behaviors that occur during incubation.

Embryonic and maternal vocalizations are described by using onomatopoetic expressions or contextual characteristics. Onomatopoetic nomenclature has the advantage of avoiding motivational or functional connotations. However, many times it is difficult to provide onomatopoetic expressions that accurately describe certain sounds. This is especially true when different sounds have close frequency spectrums. In such cases the use of similar onomatopoetic expressions for the different sounds may imply functional relationship. Another difficulty is presented by the fact that different people may not interpret a particular expression in the same way. In addition, one sound may have different functions depending on the context. The use of nomenclature that describes the context in which a sound is usually emitted provides valuable information for its identification. When onomatopoetic expressions are difficult to find short-hand nomenclature by context seems to be a reasonable substitute.

In order to best identify the recorded sounds brief descriptions are provided which include: acoustic characteristics, names used by other investigators, description of the usual context, and, if known, possible functions.

Embryonic vocalizations

Phioo: This is a low intensity whistle-like sound of very short duration (Fig. 2a). Its frequency range is limited to the 2,400-2,600 Hz band. Although it occurs at a slightly lower frequency, it is similar to the first part of a soft peep (Fig. 2b). It appears early during incubation, increases in frequency as hatching approaches, but then decreases in frequency right before hatching. This embryonic vocalization has not been described previously. The context in which it usually occurs, the factors that trigger it and its function are unknown.

Peep: This is a high intensity, high pitch call of short duration, with a spectrum of sharply descending frequencies (Fig. 2c). It is repetitive, with a variable number of repetitions at approximately 0.5s intervals. This call was described previously as "distress" call or "cheep" by Collias and Joos (1953). Guyomarc'h (1966) described it as the "searching call of the isolated chick". It appears early, well before pipping (Guyomarc,h, 1966). Before hatching it occurs mainly in the absence of external stimulation and in response to low environmental temperature. After hatching, it is triggered by a variety of factors such as: isolation from social companions, lowering of the environmental temperature, a decrease in the level of light in the environment and unfamiliar surroundings. It is believed that its function is to elicit vocal responses in social companions that may serve as cues for their location (Guyomarc'h, 1966).

Soft peep: This is a low intensity call with a short spectrum of descending frequencies (Fig. 2b). It is close to a subdued "screech" or "peep" call but its frequency range is different and it is not repetitious. This embryonic vocalization has not been previously described. The call starts early during incubation and it occurs frequently especially in artificially incubated embryos. It seems to occur more often in the absence of external stimulation. The function of this call is unknown.

Screech: This is a high intensity call with a spectrum of descending frequencies similar to the "peep" call. It is longer in duration than the peep due to an initial portion of ascending frequencies (Fig. 2d). This is the most intense call during the period that precedes hatching. It was previously described by Guyomarc'h (1966, 1972) as the "escape-predation" call. Its emission often coincides with powerful embryonic movements accompanied by shell cracking noises. After hatching the vocalization is emitted by chicks that are forcibly prevented from moving (Guyomarc'h, 1966). The function of this call remains obscure although Guyomarc'h (1966) suggested that its role is to elicit brood defense behavior in the hen and escape behavior in the siblings.

<u>Twitter</u>: This is a low intensity call with a spectrum of gradually ascending frequencies (Fig. 3a). It appears relatively late during incubation and its frequency of occurrence increases after pipping. It was first identified in newly hatched chicks as a "pleasure note" or "twittering" by Collias and Joos (1953). Guyomarc'h (1972) described it as a call of "psycho-physiological equilibrium". The call may be elicited in the embryo by movements applied to the egg. After hatching, it is the dominant type of vocalization when the chick is in a group of social companions. The function of the call is not known.

<u>Food call</u>: This is a moderate intensity call with a spectrum of sharply ascending frequencies followed by a short descending portion (Fig. 3b). The call appears during the last period of incubation. In artificially incubated embryos (Guyomarc'h, 1972) it often follows after strong movements and shell cracking noises. Previous investigators (Collias and Joos, 1953, Guyomarc'h, 1966, 1972) did not

differentiate between twitters and food calls and classified them together as pleasure calls. They acknowledged, however, the presence of calls with different frequency spectrums in the pleasure calls category. After hatching this vocalization is emitted frequently during foraging and is usually associated with feeding bouts. The function of the call is not known.

Huddling call: This is a moderate intensity call with a spectrum containing both ascending and descending frequencies, the ascending portion being more pronounced (Fig. 3c). Guyomarc'h (1972) named it "cri de blottissement" which translates as "huddling call". This call appears very late, right before hatching. At hatching there is a sudden increase in its frequency and it becomes the dominant call during the hours following hatching. After hatching this call accompanies approach and contact seeking movements by the chick toward the ventrum of the hen. Its function is not known.

Maternal vocalizations

Cluck: This is the characteristic call of a broody hen (Fig. 4a). It has been described by several investigators (Collias and Joos, 1953, Baeumer, 1962, Guyomarc'h, 1972, Rush, 1978). It consists of low frequency pulses with the loudest frequencies at the low end of the spectrum. Broody hens start emitting low intensity clucks long before hatching. Clucks become more frequent after hatching and are usually intense when the brood is moving and exploring the environment. The clucks reach their highest intensity when a chick is isolated from the hen and emits peep calls (Guyomarc'h, 1972). The apparent function of the cluck is to maintain the contact between the hen and the chicks.

Food call: This is a brief, repetitive, high intensity call with a wide frequency range (Fig. 4c). The onset of this call is during the last few hours before hatching and its frequency remains relatively low until after hatching. The food call has been described repeatedly by various investigators (Collias and Joos, 1953, Konishi, 1963, Guyomarc'h, 1974). It is always accompanied by tidbitting movements or head lowering and functions to attract the chicks when the hen discovers a source of food (Stokes, 1971, Guyomarc'h, 1974).

Intermediate calls: This call ranges in spectral characteristics between the cluck and the food call (Fig. 4b). Its upper frequencies are intermediate between those of the cluck and food call. It is a frequent call during incubation. After hatching it is emitted when the hen sits brooding the chicks. It is not accompanied by tidbitting movements or head lowering and does not appear to be related to feeding or environmental exploration. This call has not been previously described. Its function seems to be to attract the chicks when the hen is sitting to brood them.

Mild alarm: This is a relatively short call composed of successive pulses that closely follow each other and form a blurred spectral image (Fig. 4d). This vocalization was described by Collias and Joos (1953) under the name of "alerting call" while Rush (1978) called it the "chirr". It is emitted under a variety of conditions, most often when the hen is pecking at an unusual object or when the brood is approached by a person. The function of this call is probably to alert the chicks to a change brought by a novel object in the environment.

Maternal movements

Egg turn: This is the egg rolling movement performed by the hen during incubation (Olsen, 1930). The hen catches the egg with her beak and through a ploughing motion of the head induces egg rolling. The movement of the eggs produces an intense rasping sound.

Resettle: This is a brief waggling movement used by the hen to adjust its position on the eggs. Olsen (1930) described it as "a kind of shuffling motion" of the body. It often follows after egg turning bouts. This movement produces a characteristic rhythmic scratching sound.

Move: Under this designation are grouped those low amplitude movements of the hen on the nest that could not be clearly identified or assigned to the other categories. These movements produce brief scratching sounds of moderate intensity.

Peck: Pecks that are delivered to the ground or to objects in the vicinity by the sitting hen produce a tapping sound when the beak hits the target.

Beak clap: This is a rapid closing of the mandible that occurs in connection with such activities as feeding on large and hard pieces of food and preening. In addition, beak clapping is often associated with very soft, almost imperceptible clucks. The movement of the mandible produces a clattering sound.

APPENDIX II

A summary of information theory based on material published by Dingle (1969, 1972), Steinberg and Conant (1974), Steinberg (1977) and Losey (1978).

The amount of information received by an observer when one event X_i occurs, out of n equally probable events is computed with the formula:

$$H(X) = \log_2 n \text{ (bits)}$$

This may also be written:

$$H(X) = \log_2 \left(\frac{1}{p}\right)$$

where: p is the probability of one event; H is the amount of information that is provided by the event and it represents the degree with which uncertainty is reduced by the event.

Information is measured in "bits". A bit is the amount of information required in order to choose between two equally probable events.

If the events are not equally probable and the probability of an event X_i is:

$$P(i) = \frac{n_i}{N}$$

where: n_i is the number of occurrences of event X_i

N is the total number of occurrences of all events

then: $H(X) = \sum_{i=1}^{n} P(i)\log_2(\frac{1}{p(i)}) = -\sum_{i=1}^{n} P(i)\log_2 P(i)$

or: $H(X) = \frac{1}{N} [N\log_2 N - \sum_{i=1}^{n} n_i \log_2 n_i]$

In cases where the event is defined by the joint occurrence of two different acts such as a two act sequence of a contingency table the information is:

$$H(X,Y) = \frac{1}{N} [N\log_2 N - \sum_{j=1}^{m} \sum_{i=1}^{n} n_{ij} \log_2 n_{ij}]$$

where: n_{ij} are the individual cell entries

This formula gives the value of the "joint" information based on the number of two act sequences found in the cells of the contingency table.

The maximum value of H, designated Hmax, is reached when all events are equally probable (uncertainty is maximal). Therefore, as shown above:

$\text{Hmax} = \log_2 n$

where n is the number of equiprobable events

The minimum value of H is zero and is obtained when only one out of n events always occurs. A high value of H then indicates little predictability of the specific event that is going to occur. In the latter case the occurrence of one specific event provides a large amount of information (thus reducing the high uncertainty). The ratio H/Hmax represents the relative uncertainty or relative information present. This ratio can be used to measure the extent to which diversity (some events occur more often than others) in the occurrence of different events is used to carry information. When diversity increases the ratio decreases.

Redundancy (R) is defined as:

$R = 1 - H/Hmax$

and is zero when all events are equally probable. A high redundancy value (close to 1) is an indication of strong predictability. In the analysis of two act sequences an increased value of R would indicate better predictability of the second act when the first act is known.

"Conditional information" is the information provided by an event when another event has already occurred. H is defined by:

$$H\ (Y/X) = \Sigma\ P_{ij}\ \log_2 P_{j/i}$$

where: $P_{j/i}$ is the probability of occurrence of event Y_i given that event X_i has just occurred

P_{ij} is the joint probability of occurrence of events X_i and Y_j.

In the analysis of two act sequences performed by two different animals the conditional information estimates the amount of information provided by the second act when the first act in a sequence is already known. The higher the value of $H(Y/X)$ the smaller is the effect of the first act on the occurrence of the second act in a sequence.

The difference between the amount of information provided by an act in a succession without knowledge of the act preceding it and the information provided by the same act when the act preceding it is known, is the information transmitted or communicated (or simply "transmission"):

$$T(X:Y) = H(Y) - H(Y/X) = H(X) - H(X/Y)$$

Transmission is a measure of the constraints between acts in a succession and equals zero when the acts succeeding each other are independent or H when the acts are totally dependent on each other. $T(X:Y)$ is a minimum estimate of the information transmitted per act. In the case of communicating animals the transmission value is a measure for the effects that the behavior (X) of one animal has on the subsequent behavior (Y) of another animal. The value of transmission may also be computed with the following formula:

$$T(X;Y) = H(X) + H(Y) - H(X,Y)$$

The relative contribution of each event (X_i) to the overall transmission between events X and Y is determined by the weighted average of the information carried by each event. Partial transmission is computed as:

$$Tp(x;y) = \sum_{x} P(x)\ J(x;y)$$

where: $P(x)$ is the probability of event X

and $J(x;y) = \sum_{y} P(y/x) \log_2 \frac{P(y/x)}{P(y)}$

where: $P(y/x) = \frac{P(x,y)}{P(x)}$

In a contingency table with behavioral sequences that result from communication between two animals the $Tp(x;y)$ values indicate the degree to which each initial behavior (row heading) is an effective signal. A behavioral act is an effective signal when the act following it is highly predictable.

Hypothesis testing:

There are two null hypotheses that could be tested to determine whether communication occurs:

a) $T = 0$

b) $H = Hmax$

In both instances, "Miller's Chi-Square" can be used:

a) $X^2 = 1.3863\ NT$, d.f. = $(Nx-1)(Ny-1)$

b) $X^2 = 1.3863\ N\ (H-Hmax)$, d.f. = $Nx,\ y-1$

where: T is "transmission"

H is the amount of information present

Nx is the number of rows

Ny is the number of columns

A significant X^2 value resulting from the testing of the first null hypothesis ($T = 0$) would indicate that the amount of information transmitted is significantly larger than zero

($T \neq 0$) and that communication is occurring. Similarly, one can conclude that communication is occurring when $H \neq Hmax$. A value of H which is significantly lower than Hmax indicates that the amount of uncertainty present is reduced by communication.

Embryonic responses to maternal behaviors. The data on two act sequences of maternal and embryonic behaviors (Appendix V) can be used to calculate various parameters of information transfer and sharing between the incubating hen and embryos. The first measure determined is the amount of information (degree of uncertainty) present in the distribution of the embryonic acts according to the type of the initial maternal act:

$$H(X) = \log_2 N - \frac{1}{N} \sum_{i=1}^{n} n_i \log_2 n_i$$

where: N is the grand total

n_i are the row totals

The resulting value is:

$H(X) = 2.6098$ bits

The amount of information contained in the distribution of the following acts according to the type of following act is determined in a similar manner:

$$H(Y) = \log_2 N - \frac{1}{N} \sum_{j=1}^{n} m_j \log_2 m_j$$

where: m_j are the column totals

The resulting value is:

$H(Y) = 2.3970$ bits

The "joint" information, that is the information present in the individual cells of the matrix, is computed next:

$$H(X,Y) = \frac{1}{N}\left[N \log_2 N - \sum_{j=1}^{m}\sum_{i=1}^{n} n_{ij} \log_2 n_{ij}\right]$$

where: n_{ij} are the individual cell entries

The resulting value for the "joint" information is:

$H(X,Y) = 4.9080$ bits

Based on the information measures computed above, the "transmission" is determined as:

$$T(X:Y) = H(X) + H(Y) - H(X,Y)$$

The transmission measures the amount of information that embryos receive from the incubating hen.

The resulting transmission value is:

$T(X;Y) = 0.0990$ bits

This value may be compared with zero in order to establish if a significant amount of information is transmitted.

It can also be compared with transmission values obtained from other species of precocial birds.

In order to determine which maternal behavior transmitted the most information to the embryos partial information measures are used:

$$J(x;y) = \sum_{y} P(y/x) \log_2 \frac{P(y/x)}{P(y)}$$

where: $P(y/x) = \frac{\text{cell entry}}{\text{row total}}$

$P(y) = \frac{\text{column total}}{N}$

J(x;y) measures the information transferred by each kind of maternal behavior. This permits an estimate of signal effectiveness for each type of maternal behavior, that is, the effectiveness in transmitting information. The most effective signals are those for which predictability of the following behavior is highest.

The partialized transmission values (Table VIII) are obtained as follows:

$$Tp\ (x;y) = \sum_{x} P(x)\ J(x;y)$$

$$\text{where:}\quad P(x) = \frac{\text{row total}}{N}$$

The transmission values are weighted with the coefficient P(x) in order to eliminate the discrepancies created by events that are very rare. If not weighted by their probability of occurrence coefficient, rare events will carry large transmission values of doubtful statistical validity. The sum of the weighted partial transmissions should equal the value of the overall transmission.

The occurrence of communication between the incubating hen and the embryos can be demonstrated by testing several null hypotheses:

$H(X) = H(X)\ \max$

$H(Y) = H(Y)\ \max$ and

$T(X;Y) = 0$

A multiple test using "Miller's X^2" can be performed in order to test these hypotheses:

$$X^2_{H(X)} = 1.3863\ N\ [H(X) - H(X)max]$$

$$X^2_{H(Y)} = 1.3863\ N\ [H(Y) - H(Y)max]$$

$$X^2_{T} = 1.3863\ NT(X;Y)$$

where: H(X), H(Y) are the information measures for rows and columns

Hmax = $\log_2 n$, n being the number of rows or columns

T(X;Y) is the overal transmission

N is the matrix grand total

In our case: H(X)max = 3.1699, H(Y)max = 3.0000

The X^2 values obtained (Table VII) indicate that a significant amount of information is transferred to the embryos and therefore communication is occurring.

Maternal responses. The amount of information (degree of uncertainty) present in the distribution of maternal acts is determined from the data on two act sequences of maternal behaviors following embryonic vocalizations (Appendix VIII). The first measure determined is the amount of information present in the distribution of maternal acts according to the type of the preceding embryonic vocalization. From row totals is obtained:

$$H(X) = \log_2 N - \frac{1}{N} \sum_{i=1}^{n} n_i \log_2 n_i$$

$$= 2.346 \text{ bits}$$

The amount of information contained in the distribution of the maternal acts according to the type of maternal act is obtained from column totals:

$$H(Y) = \log_2 N - \frac{1}{N} \sum_{j=1}^{m} m_j \log_2 m_j$$

$$= 2.842 \text{ bits}$$

The joint information present in the individual cells of the matrix is computed as:

$$H(X,Y) = \frac{1}{N} [N \log_2 N - \sum_{j=1}^{m} \sum_{i=1}^{n} \log_2 n_{ij}]$$

$$= 5.082 \text{ bits}$$

The amount of information transferred by embryonic vocalizations to the incubating hen is determined as follows:

$$T(X;Y) = H(X) + H(Y) - H(X,Y)$$

$$= 0.106 \text{ bits}$$

This value represents the amount of information received by the hen as indicated by the behaviors performed by the hen.

The relative amounts of information transmitted by each type of embryonic vocalization are calculated using the expression:

$$Tp(x;y) = \sum_{x} P\ (x)\ J\ (x;y)$$

$$\text{where: } J(x;y) = \sum_{y} P(x/y) \log_2 \frac{P(y/x)}{P(y)}$$

The resulting values are listed in Table XIV.

In order to determine if the values obtained for the measures of information are due to communication between embryos and the incubating hen the following null hypotheses are tested using "Miller's X^2":

$H(X) = H(X)\text{max}$ where: $H(X)\text{max} = \log_2 7$

$H(Y) = H(Y)\text{max}$ $= 2.807$

$H(Y)\text{max} = \log_2 10$

$T(X;Y) = 0$ $= 3.322$

The resulting X^2 values (Table XIII) indicate that information is indeed communicated by the embryos to the incubating hen.

APPENDIX III. Comparison between the observed and expected number (in parentheses) of embryonic responses to various maternal acts during the 4 hrs before hatching (only the first embryonic response following a maternal act is included). The data are based on eight 30-min samples.

	Maternal Act											
Embryonic Vocalization	Move	Egg Turn	Reset	Beak Clap	Peck	Cluck	Intermediate Call	Food Call	Mild Alarm	$\sum$	X^2	p
Phioo	14 (12.2)	5 (2.2)	1 (1.2)	24 (16.1)	0 (9.1)	5 (6.7)	4 (2.8)	0 (0.7)	0 (2.0)	53	17.73	<0.05
Peep	3 (1.6)	2 (0.3)	0 (0.2)	0 (2.1)	1 (1.2)	1 (0.9)	0 (0.4)	0 (0.1)	0 (0.3)	7	5.88	n.s.
Soft Peep	25 (23.3)	5 (4.2)	4 (2.2)	42 (30.7)	11 (17.3)	11 (12.8)	1 (5.3)	0 (1.3)	2 (3.8)	101	13.49	n.s.
Screech	45 (40.4)	7 (7.4)	3 (3.9)	55 (53.2)	32 (29.9)	14 (22.2)	8 (9.1)	1 (2.3)	10 (6.7)	175	6.04	n.s.
Twitter	54 (57.0)	22 (10.4)	13 (5.4)	50 (75.1)	46 (42.2)	32 (31.4)	11 (12.8)	3 (3.2)	16 (8.9)	247	38.45	<0.001
Food Call	77 (74.8)	8 (13.6)	2 (7.1)	73 (98.5)	48 (55.4)	63 (41.1)	35 (16.8)	9 (4.2)	9 (12.3)	324	51.38	<0.001
Huddling Call	2 (2.1)	2 (0.4)	0 (0.2)	3 (2.7)	2 (1.5)	0 (1.1)	0 (0.5)	0 (0.1)	0 (0.3)	9	2.78	n.s.

APPENDIX IV. Coefficients for the distribution of various maternal acts. The coefficients are the proportion from the total number of maternal behaviors represented by each type of behavior.

	Maternal Behaviors								
Move	Egg Turn	Reset	Beak Clap	Peck	Cluck	Intermediate Call	Food Call	Mild Alarm	$\sum$
361	65	35	475	268	198	82	20	59	1,563
0.231	0.042	0.022	0.304	0.171	0.127	0.052	0.013	0.038	1

APPENDIX V. Frequency distribution of maternal-embryonic two act sequences from eight 30-min samples taken during the 4 hrs before hatching.

Initial Behavior	Following Behavior								Total
	Phioo	Peep	Soft Peep	Screech	Twitter	Food Call	Huddling Call	Silent	
Move	14	3	25	45	54	77	2	97	317
Egg Turn	5	2	5	7	22	8	2	6	57
Reset	1	0	4	3	13	2	0	9	32
Beak Clap	24	0	42	55	50	73	3	172	419
Peck	0	1	11	32	46	48	2	87	227
Cluck	5	1	11	14	32	63	0	43	169
Intermediate Call	4	0	1	8	11	35	0	14	73
Food Call	0	0	0	1	3	9	0	2	15
Mild Alarm	0	0	2	10	16	9	0	12	49
Total	53	7	101	175	247	324	9	442	1,358

APPENDIX VI. Comparison between the observed and expected number (in parentheses) of maternal responses to embryonic vocalizations during the 4 hrs before hatching. The data are based on eight 30-min samples and include only the first responses.

Maternal Act	Embryonic Vocalization									
	Phioo	Peep	Soft Peep	Screech	Twitter	Food Call	Huddling Call	∑	x^2	p
Move	12 (16.5)	1 (3.4)	19 (24.8)	43 (36.4)	40 (45.5)	72 (60)	2 (2.4)	189	7.95	n.s.
Egg Turn	7 (7.7)	2 (1.6)	18 (11.5)	10 (16.9)	33 (21.2)	12 (28)	6 (1.1)	88	44.05	< 0.001
Reset	2 (1.5)	0 (0.3)	0 (2.3)	2 (3.3)	8 (4.1)	5 (5.4)	0 (0.2)	17	5.34	n.s.
Beak Clap	25 (18.5)	0 (3.8)	34 (27.8)	45 (40.8)	42 (51.1)	65 (67.4)	1 (2.7)	212	9.37	n.s.
Peck	8 (8.4)	0 (1.7)	7 (12.6)	13 (18.5)	34 (23.1)	34 (30.5)	0 (1.2)	96	12.13	n.s.
Cluck	2 (8)	3 (1.6)	10 (12)	30 (17.7)	13 (22.1)	34 (29.2)	0 (1.2)	92	20.34	< 0.01
Intermediate Call	4 (4.8)	0 (1)	5 (7.2)	16 (10.6)	8 (13.2)	22 (17.5)	0 (0.7)	55	7.94	n.s.
Food Call	0 (1)	0 (0.2)	3 (1.4)	4 (2.1)	0 (2.6)	4 (3.5)	0 (0.1)	11	1.91	n.s.
Mild Alarm	0 (2.3)	0 (0.5)	4 (3.4)	11 (5)	9 (6.3)	2 (8.3)	0 (0.3)	26	13.63	<0.05

APPENDIX VII. Coefficients for the distribution of embryonic vocalizations. The coefficients are the proportion from the total number of embryonic vocalizations represented by each type of vocalization.

	Embryonic Vocalizations						
Phioo	Peep	Soft Peep	Screech	Twitter	Food Call	Huddle	$\sum$
108	22	162	238	298	393	16	1,237
0.087	0.018	0.131	0.192	0.241	0.318	0.013	1

APPENDIX VIII. Frequency distribution of embryonic-maternal two act sequences from eight 30-min samples taken during the last 4 hrs before hatching.

Initial Behavior	Following Behavior										
	Move	Egg Turn	Reset	Beak Clap	Peck	Cluck	Inter-mediate Call	Food Call	Mild Alarm	Silent	Total
Phioo	12	7	2	25	8	2	4	0	0	29	89
Peep	1	2	0	0	0	3	0	0	0	12	18
Soft Peep	19	18	0	34	7	10	5	3	4	39	139
Screech	43	10	2	45	13	30	16	4	11	42	216
Twitter	40	33	8	42	34	13	8	0	9	71	258
Food Call	72	12	5	65	34	34	22	4	2	83	333
Huddling Call	2	6	0	1	0	0	0	0	0	4	13
Total	189	88	17	212	96	92	55	11	26	280	1,066

APPENDIX IX

Characteristics of the movements applied to the embryo

Fast movement. One movement consisted of pushing the egg forward for a distance of approximately 1.5 cm in 0.6 s and then pushing it back to its original location in 0.3 s. Stimulation was applied in three series of three movements each. The total time for each series was approximately 8 s. The interval between series was 40 s.

Slow movement. A complete movement consisted of pushing the egg forward for a distance of 3 cm in approximately 2 s, then 4 s later, pushing it back to its original location in 2 s. Three complete movements were applied. They were separated by 40-s intervals.

APPENDIX X

Internal goodness of fit test for a transition table (Bishop et al., 1975, p. 136; Fagen and Young, 1978)

The influence that each initial act of a transition table has on each of the following acts can be determined with the expression:

$$|Y| > \sqrt{X^2_{(0.05,\ d.f.)}/R^2}$$

where: $$Y = \frac{x_{ij} - m_{ij}}{(m_{ij})^{1/2}}$$

x_{ij} = Observed frequency in cell(i,j)

m_{ij} = expected frequency in cell (i,j)

R^2 = number of cells in the table

When $Y > 0$ the initial act is directive to the following act whereas

When $Y < 0$ the initial act tends to inhibit the following act.

APPENDIX XI

Computation of the number of behaviors expected to follow within 15 s in two-act sequences

Let us assume that 13 maternal behaviors took place within the S = 30 min sample (See Fig. 12). The total time covered by these behaviors according to the 15 s criterion is Σ c = 195 s. This represents

$$\frac{\Sigma c}{S} = 10.83\%$$

of the total sample time. If 30 embryonic behaviors took place during the sample time one would expect

$$\frac{\Sigma c \times N}{S} = 30 \times 0.1083 = 3.2$$

of them to follow maternal behaviors within 15 s by chance alone.

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