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EFFECT OF LIGHT AND TEMPERATURE ON THE DEVELOPMENT OF TURBOT EGGS (Scophthalmus maximus L.)

by

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ABSTRACT

Different experiments were done to determine the effects of light and temperature on the embryonic development of turbot eggs (*Scophthalmus maximus* L.) obtained in captivity.

No significant differences have been found between time elapsed from fertilization to hatching in the trials carried out with 24 hours of light and those done in dark conditions,therefore light is not a determining factor in the embryonic development of turbot eggs.

The relation between temperature and time needed to reach each embryonic stage, shows a clear inverse relationship. The exponential-potential equation which relates temperature and the age of the eggs, defined as the time in hours elapsed since fertilization, is:

$$Y = 27.64 \ . \ e^{-0.11\ T\ +\ 0.05\ i} \ . \ i^{1.21} \ ; \qquad r = 0.9904$$

From the application of this equation, this paper also provides the development curves for each embryonic stage for each experimental temperature (in the range 10 ºC to 20 ºC).

INTRODUCTION

Although during the process of incubation of turbot eggs the most important factors for obtaining high hatching percentages are the quality of the eggs themselves, proper hygiene and the characteristics of the sea water used (Barton, 1981; Jones, 1989), there are other diverse factors that can affect embryonic development in an important way.

The kind of flow (closed circuit, semi-open or open) is chosen in most cases depending on the characteristics of the sea water of the area (water quality, salinity, temperature, etc).

Aeration is usually very mild in the incubation tanks to avoid damaging the eggs and to keep them in suspension in the column of water. However, some authors (Kuhlmann y Quantz, 1980; McEvoy, 1984) don't use aeration during the incubation process, so the eggs stay in the upper part of the tank, in a uniform layer at the surface.

The salinity of the water affects the buoyancy of the incubating eggs. When working with sea water which has 30 - 35 ‰ of salinity, the eggs float in static systems, while in values of salinity between 5 and 20 par mil, Kuhlmann and Quantz, 1980, found that the eggs sank to the bottom of the tanks.

Ryland and Nichols (1967) state that the size of the larvae at first feeding is related to initial egg size and the efficiency with which the yolk sac is converted to body tissue. Both factors are a function of the incubation temperature.

Although it is widely documented that water temperature in the incubation process plays an important role in the embryonic development of turbot eggs (Jones, 1972; Kuhlmann and Quantz, 1980; etc), there are only a few papers analyzing the effect of light during the process of incubation.

This paper analyzes the effect of light and temperature on the embryonic development of turbot eggs, also giving the statistical equations that relate both factors.

MATERIAL AND METHODS

This work was carried out in the Aquaculture Station of Vigo which belongs to the Spanish Institute of Oceanography. The female used in this experiment was removed from the broodstock and hand-stripping was used to monitor the sequence of spawning. Milk was obtained from the two males by stripping and artificial dry fertilization of the newly ovulated eggs was carried out. After 15 minutes sea water was added to separate the viable eggs remaining in the upper surface layer, and the fertile percentage was calculated 2 hours after fertilization.

The incubation system was designed to use five different temperatures, each temperature with two replicates in light (24 h) and dark conditions.

Immediately after fertilization, samples of approximately 500 fertilized eggs were placed in 10 glass jars of 1 liter of capacity, 5 in light with an intensity of 54 lux and 5 in dark conditions, and maintained in controlled temperature baths at 10, 13, 15. 18 and 20 ºC. All the experiments were performed in a controlled temperature room at 10 ºC, and the maximum temperature variations observed in the jars were 0.6 ºC.

The sea water used was passed through 1 µm filters before being poured into the incubation system and had a salinity of 32 - 34 ‰ .The water in the jars was partially renewed with that of the same temperature each day after dead eggs had been siphoned off the bottoms of the jars. No aeration was used during the entire process.

Turbot egg development stages were assessed following the description of Jones (1972) and slightly modified for this paper. A total of 10 stages were defined (see Table I).

Table I: Turbot eggs development stages, following Jones (1972) and slightly modificated for this work.

Development Stages	Eggs Description
1	Eggs from two to sixteen celular divisions
2	Morula Stage.
3	Blastula Stage.
4	Gastrula Stage.
5	First Embryon vestige.
6	Embryon Stage E from Jones (1972)
7	Embryon Stage F from Jones.
8	Larvae Stage G from Jones.
9	Well formed larvae Stage H from Jones.
10	Hatching Stage.

Periodic checking of the 10 egg stages was done initially at intervals of 6 hours. This interval was increased after two days to an interval of 12 hours, which was maintained until the end of the experiment. Ages were assigned to each egg sample using a stereoscopic binocular microscope.

The eggs taken for determinig the stages were not used again in the experiment, but were stored in 4 % formalin neutralized with Borax so that results could be confirmed subsequently. This avoided possible modification of the results of the experiments by eggs which had been handled.

To find out the effect of light on the embryonic development of turbot eggs, the time elapsed from fertilization to hatching was compared in light and dark experiments for each temperature. To do that, the potential regression curves which relate temperature and hatching were calculated for both experiences, and an analysis of variance was estimated.

With regards to the effect of temperature, in each sample, the number of eggs at each stage of development was counted, and the time elapsed from fertilization recorded. Then the data were statistically weighted to take into account the number of eggs at each development stage in each sample. The mean age in hours derived for each development stage at each incubation temperature was determined from the following equation:

$$\text{Mean time for stage } i = \frac{n_1\, t_1 + n_2\, t_2 + \ldots\ldots\ldots + n_n\, t_n}{n_1 + n_2 + \ldots\ldots\ldots + n_n}$$

Where i = egg development stage; n = number of eggs in each sample checking; t = time for checking.

So it was possible to determine the mean age in hours from fertilization until 50 % of the eggs reached each development stage in the different experiments.

Finally, the data were adjusted to calculate the parameters of the exponential-potential equation for each stage at each temperature. This method was also used by Lo (1985) for similar data concerning anchovy *Engraulis mordax* and Miranda et al. (1990) for the Iberian sardine *Sardina pilchardus*. The equation has the following form:

$$Y = a \cdot e^{b\,T + c\,i} \cdot i^{d}$$

Where Y = age in hours of each development stage; T = mean incubation temperature; i = egg development stage (1 to 10) and a, b, c, d = constants.

With this equation, curves are drawn for each development stage relating age in hours from fertilization to temperature.

RESULTS

Effect of light

In order to find out the effect of light on the embryonic development of turbot eggs, the times elapsed from fertilization until the final phase of the embryonic development process (i.e. until the moment of hatching) were compared. Results obtained in the experiments done in the jars under light conditions (24 h.) and those carried out under dark conditions were as follows:

	10 ºC	13 ºC	15 ºC	18 ºC	20 ºC
LIGHT	-	175.24	131.00	97.91	83.00
DARK	-	165.75	129.75	85.00	82.06

Table II. Mean values of time elapsed in hours from fertilization until hatching under light and dark conditions, at five different temperatures of incubation.

Results at 10 ºC are not given since a total mortality was observed two days after the initiation of the experiment. A possible explanation for this could be that a temperature of 10 ºC may be too low for the incubation of turbot eggs; a second possibility is that this may have been due to the effect of stress caused by the temperature change from fecundation (15 ºC) to incubation (10 ºC).

Although the time elapsed in the five experiments under light conditions show slightly higher values, the potential regression between the values under light and dark conditions gives very similar equations:

$$Y = 1.41 \times 10^{4} \cdot T^{-1.7190} \quad \text{Light} \quad r = 0.9981$$

$$Y = 1.46 \times 10^{4} \cdot T^{-1.7489} \quad \text{Dark} \quad r = 0.9829$$

An analysis of the variance of data shows that the variances and the slopes of both curves are equivalent, therefore it can be concluded that light is not a determining factor in the process of embryonic /

development during the incubation of turbot eggs.

Effect of temperature

Having no significant differences between the experiences carried out under light and dark conditions, the values observed under the 24 hours-light condition were used to calculate the exponential-potential equation that relates each state of egg development with the temperature.

Table III gives, for each temperature (10 to 20 ºC) the mean values observed in hours from fertilization until 50 % of the eggs reach each development stage. The effect of temperature on the process of embryonic development is clearly shown. For example, the last embryonic development stage (Stage 10) takes 175 hours at 13 ºC while at 20 ºC it takes only 83 hours.

In the experiment carries out at 10 ºC only stage 3 could be reached since a total mortality occurred on the second day of incubation as has been pointed out previously.

Figure 1 shows the graphic representation of data in Table III, and a clear inverse relation between temperature and time elapsed from fertilization can be observed: as the temperature increases, the time needed to reach each state of development decreases. On the other hand, as could be expected, there exists a direct relation between the development stages and the time needed to reach them.

Table III: Mean age (h) observed for each of 10 Stages of development of turbot eggs, corresponding to the different experimental temperatures.

Stages	Temperature (º C)				
	10	13	15	18	20
1	5.00	4.38	3.00	3.00	3.00
2	25.67	14.73	13.17	9.00	9.00
3	51.58	25.59	25.80	17.00	16.38
4	-	38.76	36.53	25.00	21.00
5	-	53.33	46.38	33.00	27.00
6	-	73.67	56.00	41.31	36.90
7	-	100.92	74.37	51.27	47.10
8	-	124.80	92.45	70.38	63.00
9	-	155.64	111.00	84.81	75.00
10	-	175.24	131.00	97.91	83.00

$$\text{Age of stage i (hours)} = \frac{n_1 \cdot t_1 + n_2 t_2 + \ldots\ldots + n_n t_n}{n_1 + n_2 + \ldots\ldots + n_n}$$

Where i = stage; n = number of eggs in that stage; t = time of checking.

The exponential equation for the embryonic development of turbot eggs obtained in captivity was:

$$Y = 27.6447 \cdot e^{-0.1108\,T + 0.0499\,i} \cdot i^{1.2088}; \qquad r = 0.9904$$

Where Y is the time in hours from fertilization, T is the temperature of the experiment and i is the stage of development of the egg.

Using this equation, the development curves for each embryonic stage are shown graphically in Figure 2: It can be seen that in the first day after fertilization, eggs incubated at 20 ºC reach stage 5, while those incubated at 10 ºC are still at stage 2. On the second day, stage 7 has already been reached in the test at 20 ºC, while at 10 ºC embryonic development is still at stage 3. Three days after fertilization, eggs at 20 ºC are almost in the last development stage (Stage 10), while those at 13 ºC have only reached stage 6. Finally, at the extremes of temperature used in this study, hatching would have occurred 10 days after fertilization at 10 ºC and only about three days after at 20 ºC, which reveals a clear inverse relation between temperature and time from fertilization.

For reference use in similar studies, Table IV gives the calculated theoretical values of time elapsed from fertilization until reaching each of the ten stages of embryonic development of turbot in the temperature range from 10 ºC to 20 ºC.

Stages	Temperature (º C)				
	10	13	15	18	20
1	9.59	6.88	5.51	3.95	3.17
2	23.31	16.72	13.40	9.61	7.70
3	40.01	28.69	22.69	16.49	13.21
4	59.55	42.71	34.53	24.54	19.66
5	81.98	58.79	47.11	33.78	27.07
6	107.42	77.04	61.73	44.27	35.47
7	136.04	97.57	78.17	56.07	42.92
8	168.05	120.52	96.57	69.26	55.49
9	203.68	146.08	117.04	83.94	67.26
10	243.18	174.41	139.74	100.22	80.30

Table IV: Mean time expected (in hours) from fertilization until reach each development stage at each temperature.

In conclusion, the exponential- potential equation that relates time from fertilization (Y) to temperature (T) and the stages of development of turbot eggs(i) fits quite well with data obtained in this work as is demonstrated in the graphic representation shown in Figure 3 between values observed and predicted.

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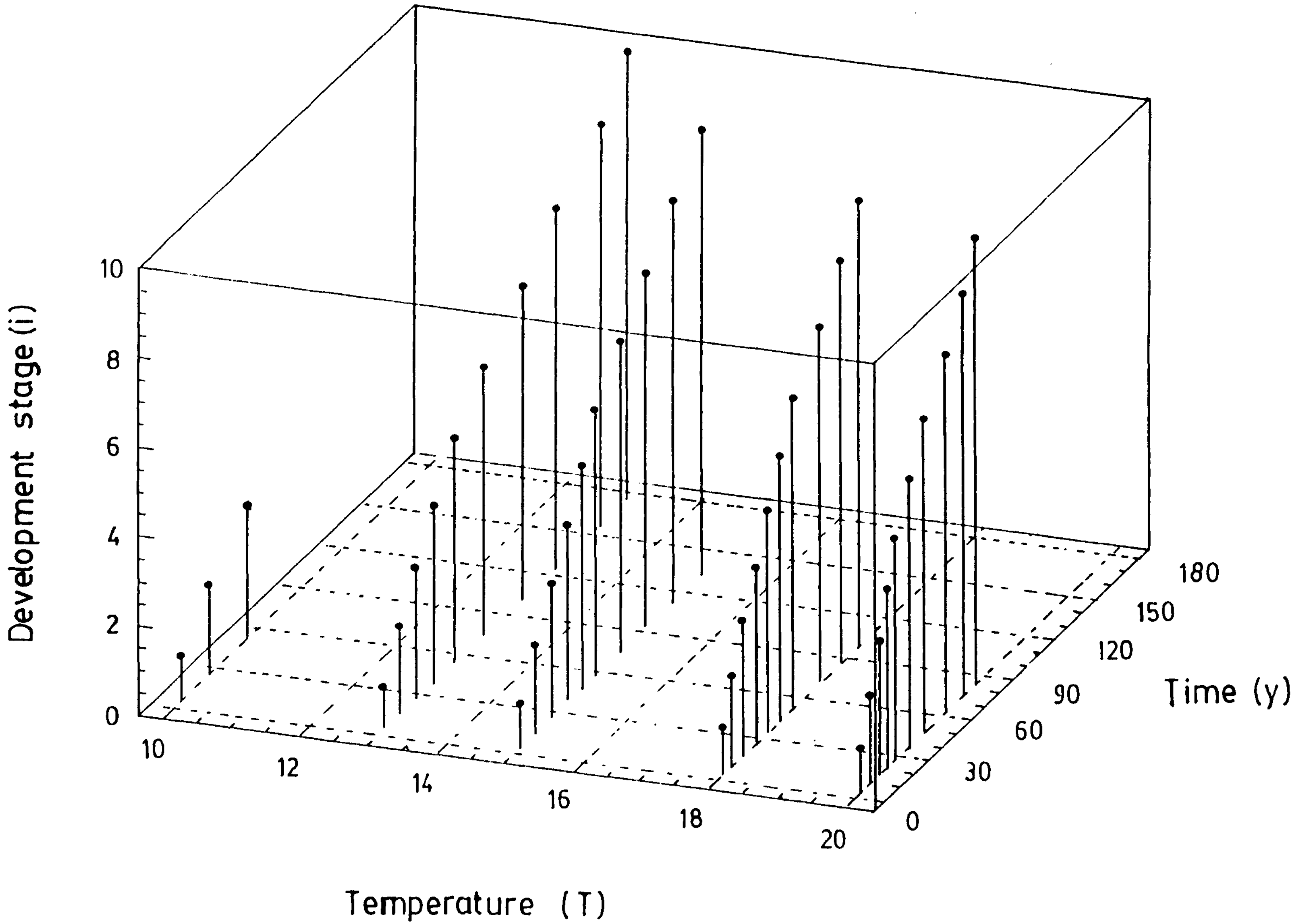
FIGURE LEGENDS:

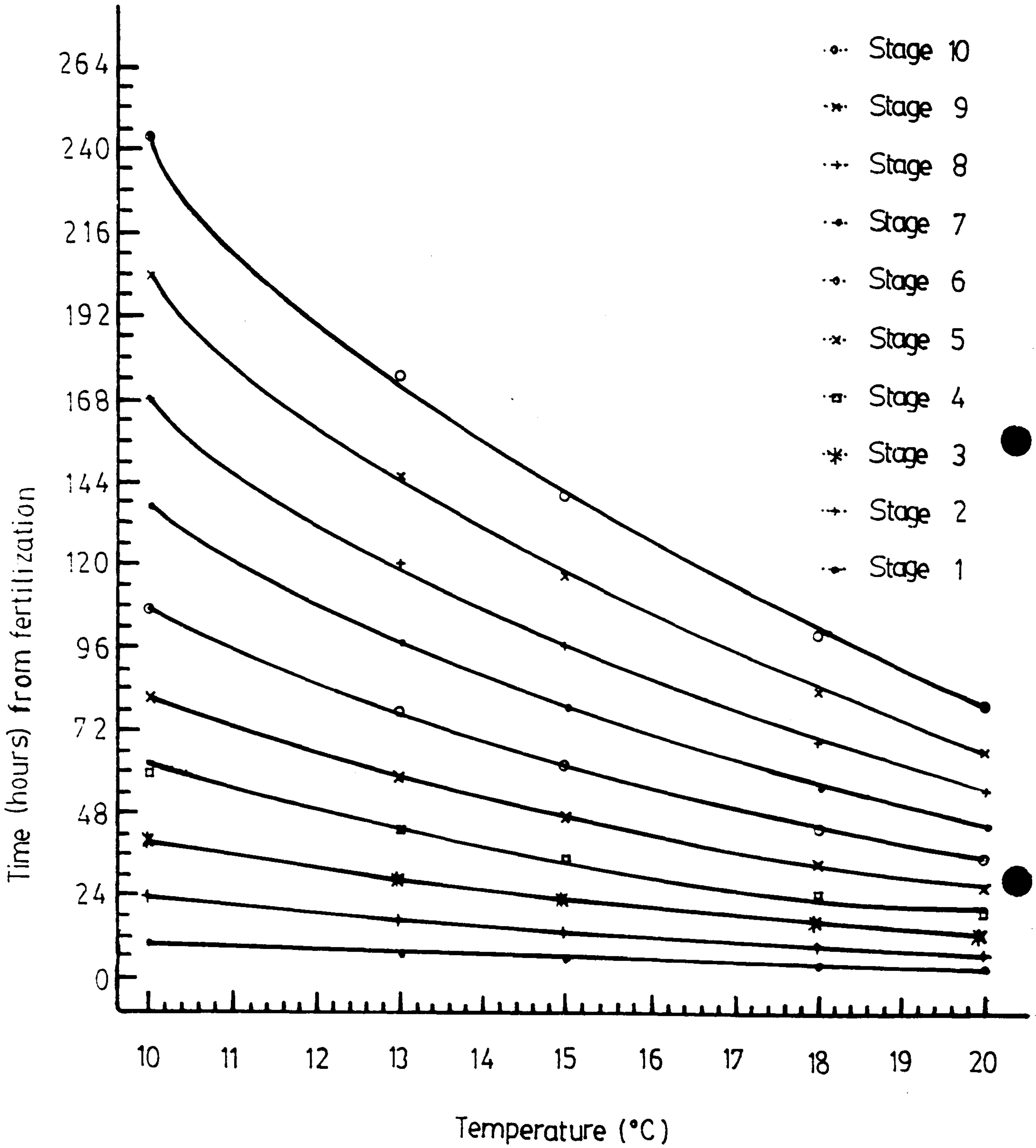
Figure 1: Relation between temperature (T), development stages of turbot eggs (i) and time in hours until reaching each stage (Y).

Figure 2: Development curves of turbot eggs for each embryonic stage at each experimental temperature (in the range of 10 ºC to 20 ºC).

Figure 3: Observed and predicted values of time elapsed from fertilization until reaching each development stage of turbot eggs.


Development stage (i)
0
2
4
6
8
10
10
12
14
16
18
20
Temperature (T)
0
30
60
90
120
150
180
Time (y)

(Figure 1)



Stage 10
Stage 9
Stage 8
Stage 7
Stage 6
Stage 5
Stage 4
Stage 3
Stage 2
Stage 1
264
240
216
192
168
144
120
96
72
48
24
0
Time (hours) from fertilization
10
11
12
13
14
15
16
17
18
19
20
Temperature (°C)

(Figure 2)

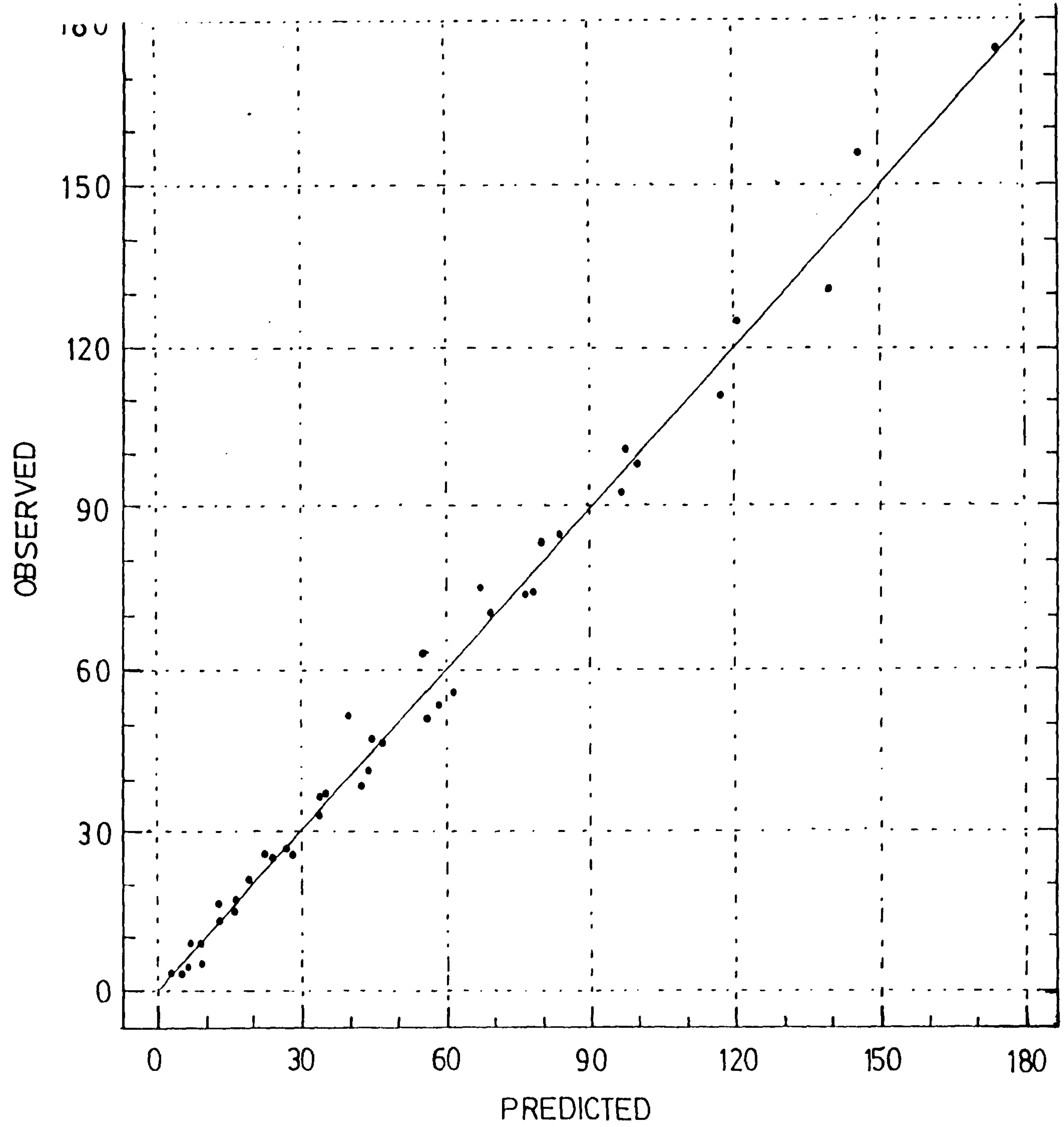


Figure 3.- Time (hours) from fertilization