Effect of light and temperature on the development of turbot eggs (*Scophthalmus maximus* L.)

J. Iglesias, G. Rodríguez-Ojea, and J. B. Peleteiro


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 were found between time elapsed from fertilization to hatching in the trials carried out with 24 h of light and those done in dark conditions. Light is not therefore 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 (T) and age of the eggs (Y), defined as the time in hours elapsed since fertilization, is: \( Y = 27.64 \times e^{-0.11T + 0.051} \times t^{0.21} \), \( r = 0.9904 \), where \( t \) is the egg development stage (1 to 10). 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°C to 20°C).

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 have an important effect on embryonic development.

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.).

Ryland and Nichols (1967) stated 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 functions of the incubation temperature.

Salinity and temperature are only two of several factors which can cause interruptions or disturbances in the early development of fish (Rosenthal and Alderdice, 1976). The salinity of the water affects the buoyancy, development rate, hatching, and mortality of the incubating eggs, as well as the number of larval deformities (Alderdice and Forrester, 1968; Kuhlmann and Quantz, 1980; Devauchelle *et al*., 1986). The influence of water temperature on the incubation process and embryonic development is widely documented (Jones, 1972; Kuhlmann and Quantz, 1980; Devauchelle *et al*., 1986), but few studies examine the effects of exposure to light during incubation on marine fish eggs.

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

Materials and methods

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

The incubation system was designed for use at five
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Figure 1. Developmental stages of turbot eggs, following Jones (1972) and slightly modified for this work.

Stages

1. 2–16 cellular divisions
2. Morula stage
3. Blastula stage
4. Gastrula stage
5. First embryonic vestige
6. Embryonic stage E
7. Embryonic stage F
8. Larval stage G
9. Well-formed larval stage H
10. Hatching stage

Immediately after fertilization, samples of approximately 500 fertilized eggs were placed in 20 glass jars of 1 litre capacity, 10 under a light intensity of 60 lux and 10 under dark conditions, and maintained in controlled temperature baths at 10, 13, 15, 18, and 20°C. All the experiments were performed in a controlled temperature room and the maximum temperature variations observed in the jars were 0.6°C.

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 each day with water of proper temperature after dead eggs had been siphoned off the bottom of the jars. No aeration or antibiotics were used during the entire process.

Turbot egg development stages were assessed following Jones’s (1972) description and slightly modified for this paper. A total of 10 stages were defined (see Fig. 1).

Periodic checking of the 10 egg stages was done initially at intervals of 6 h. This was increased after 2 days to an interval of 12 h, which was done until the end of the experiment. Ages were assigned to 10 eggs in each sample using a stereoscopic binocular microscope.

The eggs used for determining the stages were stored in 4% formalin neutralized with Borax, so that results could be confirmed subsequently.

To elucidate the effect of light on the embryonic
Table 1. Mean values of time elapsed in hours from fertilization to hatching under light and dark conditions at five different temperatures of incubation.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>10°C</th>
<th>13°C</th>
<th>15°C</th>
<th>18°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light</strong></td>
<td>175.24</td>
<td>131.00</td>
<td>97.91</td>
<td>83.00</td>
<td></td>
</tr>
<tr>
<td><strong>Dark</strong></td>
<td>165.75</td>
<td>129.75</td>
<td>85.00</td>
<td>82.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mean age (h) observed for each of 10 development stages of turbot eggs, corresponding to the different experimental temperatures.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Stages 10</th>
<th>Stage 13</th>
<th>Stage 15</th>
<th>Stage 18</th>
<th>Stage 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.00</td>
<td>4.38</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>2</td>
<td>25.67</td>
<td>14.73</td>
<td>13.17</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>3</td>
<td>51.58</td>
<td>25.59</td>
<td>25.80</td>
<td>17.00</td>
<td>16.38</td>
</tr>
<tr>
<td>4</td>
<td>38.76</td>
<td>36.53</td>
<td>25.00</td>
<td>21.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>53.33</td>
<td>46.38</td>
<td>33.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>73.67</td>
<td>56.00</td>
<td>41.31</td>
<td>36.90</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100.92</td>
<td>74.37</td>
<td>51.27</td>
<td>47.10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>124.80</td>
<td>92.45</td>
<td>70.38</td>
<td></td>
<td>63.00</td>
</tr>
<tr>
<td>9</td>
<td>155.64</td>
<td>111.00</td>
<td></td>
<td>84.81</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>175.24</td>
<td>131.00</td>
<td></td>
<td>97.91</td>
<td>83.00</td>
</tr>
</tbody>
</table>

development of turbot eggs, the time elapsed from fertilization to hatching was compared in light and dark experiments for each temperature. To do this, the potential regression curves which relate temperature and hatching were calculated for both experiences, and analysed for variance.

With regard 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 was recorded. The data were then statistically weighted taking 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 using the following equation:

\[
\text{Mean time for stage } i = \frac{n_1t_1 + n_2t_2 + \ldots + n_nt_n}{n_1 + n_2 + \ldots + n_n}
\]

where \( i \) = egg development stage; \( n \) = number of eggs in each periodical checking; \( t \) = time for checking.

With this equation 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 (L.)) and Miranda et al. (1990) for the Iberian sardine (Sardina pilchardus Walbaum). The equation has the following form:

\[
Y = a \times e^{bT + ci} \times t^d
\]

where \( Y \) = age in hours of each development stage from fertilization; \( T \) = 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 hours from fertilization to temperature.

Results and discussion

Effect of light

To find the effect of light on embryonic development of turbot eggs, time elapsed from fertilization until hatching was compared for the two experimental groups. Results obtained in the experiments done in the jars under light conditions (24 h) and those carried out under dark conditions are shown in Table 1.

Results at 10°C are not given since a total mortality was observed two days after starting the experiment. This may have been due to the effect of stress caused by the temperature change from fertilization (15°C) to incubation (10°C).

The time elapsed in the five experiments under light conditions shows slightly higher values. This delayed hatching was also observed by Helvik and Walther (1992), who showed that the hatching process of halibut (Hippoglossus hippoglossus L.) eggs was delayed by light. But the potential regression between our values under light and dark conditions gives very similar equations:

\[
Y = 1.41 \times 10^4 \times T^{-1.7190} \quad \text{Light } r = 0.9981
\]
\[
Y = 1.46 \times 10^4 \times T^{-1.7489} \quad \text{Dark } r = 0.9829
\]

An analysis of the variance of data shows that the variances (F-Snedecor) and the slopes (t-Student's, \( p < 0.05 \)) of both curves are equivalent; it therefore can be concluded that light is not a determining factor in the process of embryonic development during the incubation of turbot eggs. These equations are very similar to that reported by Kuhlmann and Quantz (1980), which state for turbot the relationship between temperature (T) and hours from fertilization to hatching (Y) as

\[
Y = 1.35 \times 10^4 \times T^{-1.760}
\]

Effect of temperature

Not finding significant differences between the experiments carried out under light and dark conditions, the
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Figure 2. Development of turbot eggs for each embryonic stage at each experimental temperature (in the range 10°C to 20°C). Data regarding development at 10°C are extrapolated.

Figure 3. Observed and predicted values of time elapsed from fertilization until reaching each development stage of turbot eggs. The regression equation is: \( Y = 1.000480 \times X - 0.03739; r = 0.99755 \), where \( Y \) is the observed value and \( X \) the predicted value.
values observed under 24 h light conditions were used to calculate the exponential–potential equation that relates each egg development stage to the temperature. This equation will give not only the time needed to reach the hatching stage but the time elapsed from fertilization to reach any of the 10 different egg embryonic stages.

Table 2 gives, for each temperature (10 to 20°C), the mean values observed in hours from fertilization when 50% of the eggs reach each development stage. The effect of temperature on the process of embryonic development is shown. For example, the last embryonic development stage (Stage 10) is reached after 175 h at 13°C, while at 20°C only 83 h are needed.

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

A clear inverse relationship between temperature and time elapsed from fertilization can be observed: as temperature increases, the time needed to reach each development stage decreases. On the other hand, as might be expected, there is a direct relationship between the development stages and the time needed to reach them.

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

\[ Y = 27.6447 \times e^{-0.1108T + 0.0499i} \times i^{1.2088}; \quad r = 0.9904 \]

where \( Y \) is the time in hours from fertilization, \( T \) is the temperature of the experiment, and \( i \) is the egg development stage.

The development curves obtained from this equation for each embryonic stage are shown graphically in Figure 2, where it can be seen that on the first day after fertilization eggs incubated at 20°C reach stage 5, while those incubated at 10°C are still at stage 2. On the second day, stage 7 has already been reached in the group at 20°C, while at 10°C embryonic development is still at stage 3. Three days after fertilization, eggs at 20°C are almost in the last development stage (Stage 10), while those at 13°C 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°C and only about three days after at 20°C, which reveals a clear inverse relation between temperature and time from fertilization.

The data reported in this work (Table 2) are slightly higher than those of Jones (1972) using temperatures between 10 and 13°C and Kuhlmann and Quantz (1980) with a temperature range of 10–20°C. Jones found an incubation time of 215 h at 10°C and Kuhlmann and Quantz 228 h rather than 243 h as in our calculated theoretical value for stage 10. At 15°C we obtain a mean value of 131 h and they state 115 h and 123 h, respectively.

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

Acknowledgements

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References


