INFLUENCE OF SOME ENVIRONMENTAL FACTORS UPON THE EMBRYONIC DEVELOPMENT OF THE ARGENTINE ANCHOVY *ENGRAULIS ANCHOITA* (HUBBS, MARINI)[1]

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An extensive literature exists on the problem of the influence exerted by several environmental factors upon the early stages of development in fishes. Other workers have dealt with this subject either from the point of view of obtaining a deeper knowledge of the biology of the species under research, or from the standpoint of solving some practical problems through their studies. Thus the majority of these publications refer to species of economical importance.

The object of the present work was to study the influence exerted upon the embryonic development of the Argentine anchovy *Engraulis anchoita* by the following environmental conditions: temperature, salinity, light, and mechanical factors. Special attention was given to the temperature, since this factor undergoes greater changes during the spawning period of the anchovy. The reproductive season of this species is quite long (Dz. de Ciechomski, 1965; Fuster de Plaza, 1964), lasting for approximately 9 to 10 months, and therefore the temperature varies considerably throughout this period. The investigation herein described was aimed at the determination of the developmental rate at different temperatures, and at an establishment of the optimal values and limits within which the embryonic development of the anchovy normally takes place.

In the case of salinity, an attempt was also made to establish the limiting values within which the normal embryonic development of the anchovy could take place. It was also the aim of this research to obtain salinity data in order to compare them with the values recorded for other related species of great salinity tolerance.

In the case of light, it seemed to be of interest to determine whether this factor exerts any influence upon the embryonic development of the anchovy.

Upon noting the great mechanical susceptibility of the eggs of *Engraulis anchoita,* it was considered of interest to study this phenomenon in detail. The investigation was thus directed towards a determination of the developmental stages of the embryo at which this susceptibility manifested itself with the greatest intensity. A study of this problem is of particular interest since high seas and storms may have some influence upon the fate of anchovy embryos which happen to be in development under those circumstances. Rollefsen (1930), in his work on the cod, has shown that intense wave movement does have an effect on the embryonic eggs of fishes. According to this worker, the years of very good catches were always preceded by years of light winds and fine weather during the reproduction season of this species.

MATERIALS AND METHODS

As basic material for the experiments, eggs obtained by artificial fecundation as well as eggs collected from plankton were used. Artificial fecundation was accomplished using the method usually employed to this end. The eggs obtained from plankton were selected so as to use those which were in early stages of development, (i.e., up to the beginning of the blastula stage).

For the experiments concerning the influence of temperature, the eggs obtained from artificial fecundation were placed in different aquaria, some being kept within a temperature ranging from 19° to 20° C., some within 14° to 15° and others at 4° C. The temperature of 19° to 20° C. was obtained by means of a thermostat; the 4° C. by placing the aquarium inside a refrigerator, whilst the 14°–15° C. corresponded to the room temperature at that time. For technical reasons, it was impossible to obtain temperatures ranging from 8° to 10° C.

In the experiments made concerning salinity, the following procedure was used: to obtain salinities lower than normal, distilled water was added to the filtered sea water; to obtain salinities higher than normal, a suitable amount of sodium chloride was added. Experiments were made with the following salinity values: 3.9 ‰; 8.4 ‰; 16.8 ‰; 25.2 ‰; 33.5 ‰; 50 ‰, and 60 ‰.

To study the influence of light, an aquarium containing eggs in development was kept in complete darkness, another one was kept under continual illumination, whilst a third was maintained with natural light as a control.

The study of the mechanical susceptibility of the embryos was made by submitting the embryos at different stages of development to the effects of different pressure, and of shocks resulting from their being dropped from different heights. To study the pressure factor, the eggs were placed between two slides, three at a time, arranged so as to form a triangle. Progressively larger weights were then placed upon the slides. After each manipulation the eggs were examined under the microscope in order to determine the resistance of the membrane and of the embryo. These

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weights which produced the rupture of the membrane, or which produced signs of destruction in the embryos, were considered to be critical weights. This method is similar to the one used by Galkina (1957), in her work on the herring of the Okhotsk Sea. In order to submit the eggs to shocks, they were taken in approximately equal amounts by means of a pipette, and were dropped upon a nylon tulle tautly extended on the concave side of a petri dish. The eggs were dropped from heights of 25 and 50 cm. The embryos used were in the following stages of development: the beginning of blastula, early gastrulation, the stage at which the blastoporic ring exceeds the equator of the eggs, and embryos in the stage of tail growth. The eggs submitted to shock were placed in aquaria and the following day the mortality rate was determined. A similar method was employed by Rollefsen (1932) for determining the susceptibility of cod embryos.

The eggs used for the light, salinity, pressure and shock experiments were kept at a temperature ranging between 14° and 15° C. The embryos used in all the experiments developed from eggs of anchovies which approached the shore for reproduction in October. This last observation is necessary, especially in regard to experiments on the influence of temperature, since it is known that embryos from anchovies spawning in summer (for instance) at much higher temperatures of the water, can show different physiological characteristics with respect to the temperature factor.

INFLUENCE OF TEMPERATURE

As is well known, the rate of the embryonic development of a given species is directly related to the temperature. The higher the temperature, the more rapid the developmental processes take place (Leiner, 1923; Vucetîć, 1957, etc.). In this respect the anchovy follows the general rule. At the temperature of 14°–15° C., hatching occurs 69 to 72 hours after fertilization; at 19°–20° C., 50 to 53 hours after fertilization. The rate of development within the aforementioned temperatures is shown in Table 1.

Besides the differences in developmental rate, other phenomena related to temperature became apparent.

TABLE 1

THE EMBRYONIC DEVELOPMENT OF THE ANCHOVY AT DIFFERENT TEMPERATURES

Stage of development	Hours of development	
	14–15° C.	19–20° C.
First cell formation	0 h 35 m	0 h 35 m
Two—blastomere stage	1 h 10 m	1 h 00 m
Four—Blastomere stage	1 h 40 m	1 h 20 m
Early blastula	2 h 25 m	2 h 00 m
Blastula	5 h 00 m	4 h 20 m
Early gastrula	16 h 45 m	15 h 30 m
Closing of the blastopore	33 h 30 m	29 h 00 m
Blastopore closed. Stage of Kupffer vesicle	39 h 00 m	32 h 30 m
The embryo occupying ⅗ of perimeter	56 h 30 m	44 h 30 m
The embryo occupying ¾ of perimeter	66 h 00 m	50 h 00 m
Hatching	69 – 72 h	51 – 53 h

Within 14° to 15° C., 90 to 95% of hatching is obtained with very few anomalous cases, whilst within 19° to 20° C., 80 to 90% of the eggs hatch, and anomalies appear with greater frequency. These anomalies consist mainly in axial deviations, especially in the region of the tail. Differences in the length of the larvae born at these different temperatures have not been observed. The influence of the temperature was manifested also in the heart beat. The heart beat of an embryo immediately prior to hatching, at temperatures within 14° to 15° C., was on the average 65 to 70 beats per minute, whilst at temperatures between 19°–20° C., it was about 100 beats per minute.

A temperature of 4° C. was lethal for the embryos of anchovy. Eggs placed at 4° C. in the early blastula stage showed a complete halt in their development. After 5 to 6 days, and always in the same stage, they died and became opaque. When eggs which had been kept for 1 to 2 days at 4° C. were transferred to aquaria at a normal temperature (14° C.), their death occurred more quickly. Thus the lower temperature seems to cause irreversible changes.

From the aforementioned results, there seem to be grounds for making certain assumptions with regard to the optimal temperatures for the embryonic development of the anchovies reproducing in spring.

As has been mentioned in a previous publication (Dz. de Ciechomski) the anchovy begins to spawn in a rather intensive way at 10° to 11° C. The greater spawning intensity is achieved within 11.5° to 13.8° C. Thus a temperature of 10° C. may be considered as the lower limit of the optimal temperature range for development. It has been noted that development at a temperature within 19°–20° C. does not take place in a completely normal manner, and thus there seems to be an upper temperature limit to the optimal range. The assumption which can be made from these data is that the anchovy which spawns in the spring has its optimal developmental range within 10° C. and 16°–17° C. These values might be somewhat different for individuals of the same species that reproduce in summer at higher water temperatures.

INFLUENCE OF SALINITY

A large number of species of the clupeid group show great tolerance in respect to the salinity factor. For the embryonic development of *Clupea harengus,* Ford (1928) gives salinity limits from 4.8‰ to 37.8‰. Holliday and Blaxter (1960) give the values 5.9‰ to 52.5‰ (for the same species). Demir (1963) observed that *Engraulis encrasicolus* in European waters can develop normally within salinities from 9‰ to 37.5‰. For the species of the family *Engraulidae* which live in waters close to the American Continent, no sufficient data are available at present on this problem.

In the case of the Argentine anchovy (*Engraulis anchoita*) it has been possible to establish that it does not possess as great a tolerance in respect to salinity thresholds for its embryonic development.

Anchovy eggs which are transferred at any stage to water of a lower than normal salinity sink and continue their development whilst lying on the bottom of the aquarium. Upon transferring the eggs at the blastula stage to water with a salinity of 3.4‰, most of the embryos die within a short time. Many eggs increase in size and take the shape of a very wide barrel. This phenomenon is probably caused by perturbations in osmoregulation. Some of the embryos reach the Kupffer visicle stage, all of them showing anomalies in the axial part. The axis of the embryo takes the form of an "S" or a sinuous shape, as is shown in Figure 1. At this stage all of the eggs die. When the transfer has been made while the embryos are still in the stage at which the tail has already been separated from the vitellus, the embryos continue their development only for a short time. Then they come to show axial alterations as in the preceding case, and they die. The embryos transferred in the

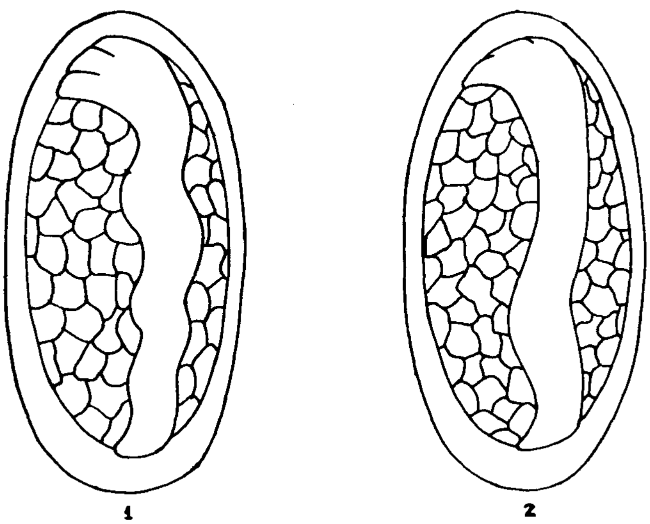

FIGURE 1. Embryos of the anchovy maintained at salinity 3.4‰ showing anomalies in the axial part.

blastula stage to water with a salinity of 8.4‰ continue their development up to the stage in which the embryo occupies about $\frac{2}{3}$ of the perimeter of the egg. From this moment on the eggs all die. An increase in the size of the eggs is not observed as at 3.4‰ salinity, but most of the embryos show the same axial alterations.

The embryonic development seems to lag a little as compared to the controls. In water with a salinity of 16.8‰, the embryos develop at a somewhat slower rate than the controls and most of them (60 to 70%) are able to hatch. Among the resulting larvae a few appear to be normal but die soon after hatching. Most of the larvae hatch in an earlier stage of development than the controls and many of them show abnormalities, especially in the region of the tail. These latter results offer grounds for assuming that low salinity has a greater effect on the development of the embryo than upon the action of the hatching enzymes. With water of 25.8‰ salinity, normal hatching of approximately 60% of the eggs is obtained. These larvae do not differ from the controls and appear to be normal. From the remaining 40% a small portion does not reach the hatching stage and most of the larvae show small abnormalities. Eggs in any stage of development, when kept in high salinity water, remain buoyant close to the surface. The value 33.5‰ corresponds to the normal salinity at which the anchovy spawns in the sea.

Observing the embryonic development of the anchovy in salinities higher than normal, several results are obtained. In water with 50‰ salinity, development continues in a more or less normal fashion. The hatching rate is high (90–95%) and few abnormalities are observed. The developmental rate appears to be to a certain degree higher than that of the controls. In water of 60‰ salinity, about 70% of the embryos reach the hatching stage. Most of the embryos that hatch are abnormal, and in most cases, show anomalies in the region of the tail (Figure 2). Some monsters are born almost without a tail. These

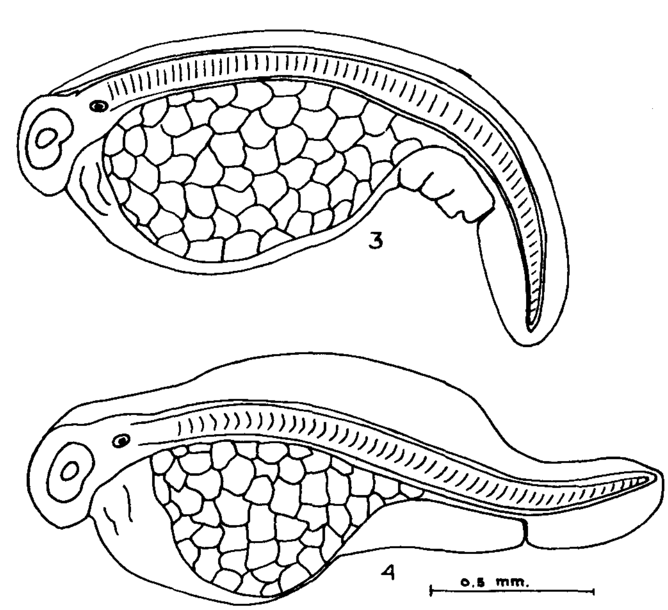

FIGURE 2. Monsters of the anchovy born in water of 60‰.

larvae reach a length of only 1.25–1.35 mm, whilst the length of the controls is about 3 mm. Malformation in the embryonic fin are observed in some larvae just after hatching, and an anomaly is also observed in the existence of a very large cavity in the anterior part of the vitellum. Similar abnormalities have been found by Nakai (1962) in the case of eggs of *Sardina melanostica* kept in salinities much higher than normal.

It can be assumed from these data that the Argentine anchovy does not show as high a tolerance in respect to salinity thresholds as do some related species, such as *Engraulis encrasicolus.* The limiting salinities, within which the embryonic development of *Engraulis anchoita* can take place more or less normally, would be fixed at 25.8‰ and 50‰. This phenomenon might be due to the fact that the Argentine anchovy lives in a habitat undergoing slight changes in salinity, and that, therefore, it has not had to need to develop adaptative trends in respect to this factor. The present author (Dziekonska, 1958), as well as other workers, holds the view that individuals of one species from water of a certain salinity might reproduce in waters whose salinity is lethal for other individuals of the same species living in waters of lower salinity (Ivlev, 1940).

INFLUENCE OF LIGHT

Experiments have shown that the influence of light is not a factor of great importance in the embryonic development of *Engraulis anchoita.* Eggs which were kept in complete darkness developed and hatched in a normal way, and no differences were observed in respect to the controls.

In aquaria kept under continual illumination, hatching took place earlier and a greater number of anomalies was observed, although this latter fact is possibly the result of a temperature increase due to illumination rather than to the effect of light itself.

The relative indifference of the embryos in respect to light is probably due to the fact that the eggs of the anchovy are planktonic and thus they stay fairly close to the surface of the sea, and are exposed to the rays of the sun as well as to darkness. Also, their development is quite rapid and, therefore, both the presence and absence of light acts upon their development during a brief period of time. In the case of some species of salmonids, for instance, it has been shown that light generally has a negative influence. In the case of this species, its development requires a long time and takes place in natural conditions, in darkness, under sand or pebble beds (Eisler, 1957; Willer, 1928). The embryos of Pleuronectes, which develop under natural conditions similar to those of the anchovy, also appear to be indifferent to the effect of light. (Johansen, Krogh, 1914).

INFLUENCE OF MECHANICAL FACTORS

Anchovy eggs which are submitted to pressure react in different ways, according to the developmental stage which the embryos have reached. The results of the experiments are shown in Table 2. As may be observed, the embryo appears to be more susceptible to pressure during the early stages of its development.

TABLE 2

RESISTANCE OF THE ANCHOVY EMBRYOS AND EGG MEMBRANE SUBMITTED TO THE EFFECTS OF DIFFERENT PRESSURE DURING VARYING STAGES OF DEVELOPMENT (WEIGHT IN GMS)

Stage of development	Maximum w. membrane	Maximum w. embryo
Early blastula	160	slide
Early gastrula	--	slide
The blastop. ring exceeds the equator of the egg	110	slide
Closing of the blastopore	200	slide
Embryo—Kupffer's vesicle	170	3–5
Commencement of the separation of the caudal region of the vesicle sac	140	10
Embryo occupying ⅔ of perimeter	130	16
Embryo prior to hatching	75	40

Up to the closing of the blastopore, the weight of the slide alone is sufficient to destroy the embryo. After the vitellus is completely enveloped, the resistance of the embryo increases. Close to the hatching stage, the weight of the slide together with a weight of 40 gm is necessary for the destruction of the embryo. This phenomenon seems to have its explanation in the fact that the vitellus is protected by the envelopment of embryonic tissues, and therefore offers greater resistance. This slight susceptibility of the anchovy embryo before hatching does not agree with the observations of Schaperclaus (1940), upon the eggs of *Esox lucius.* This author found that the resistance of the embryos of this species in respect to the pressure factor decreases sharply just before hatching.

As is shown in Table 2, the resistance of the egg membrane becomes greatest at the stage corresponding to the closing of the blastopore, and decreases sharply immediately prior to hatching. This phenomenon may be attributed to the effect of the hatching enzymes, which at this stage act by lyzing the chorion.

The experiments concerning the effect of shock in eggs dropped from different heights leads to conclusions analogous to those suggested by the pressure experiments. The results are depicted in Figure 3. As

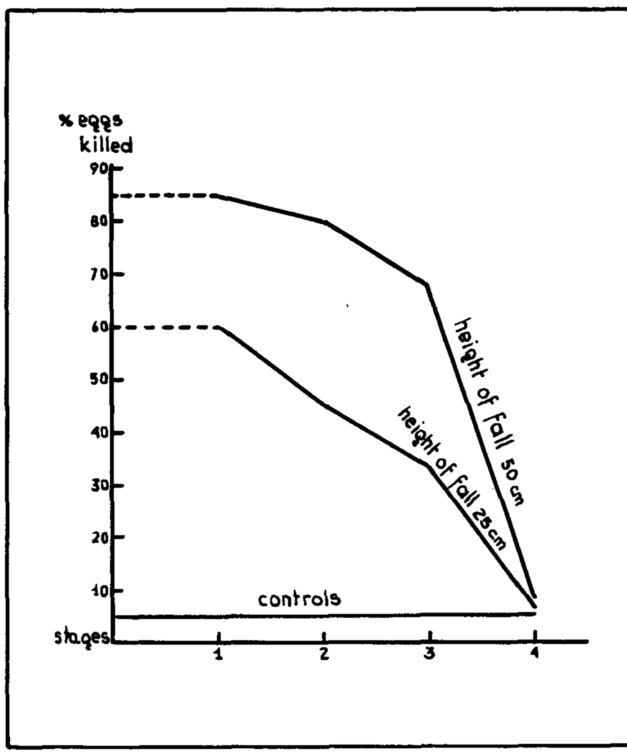

FIGURE 3. Percentage of anchovy eggs at differing stage of development killed after falls from different heights.

it was impossible to obtain suitable materials for performing experiments upon the early cleavage stages, no conclusions will be drawn on the susceptibility of the embryos during the early stages of development. The earliest stage at which the embryos were submitted to shock was the early blastula. In this stage, eggs dropped from a height of 50 cm had a mortality rate of 85%, while those dropped from 25 cm had a mortality rate of 60%. As development continues, the susceptibility, though diminishing slightly at the beginning, remains at a high rate, and as the enveloping of the vitellus progresses, the susceptibility decreases. At an advanced embryonic stage, the resistance increases so much that little difference is observed between the mortality rate of the embryos submitted to the experiment and that of the controls. These results agree with the observations made by Rollefsen (1932), who, in the case of the genus *Gadus,* also recorded an increase of the resistance throughout the development of the embryo. This observation does not agree, however, with the data given by Lindroth (1942) for the embryos of *Salmo salar*. In his experiments, he reached the conclusion that the resistance of the embryo in this species is not related to the enveloping of the vitellus, and that it can decrease in the last stages of development.

The data which have been obtained, showing the high susceptibility of anchovy embryos to mechanical factors, lead to the assumption that high seas and storms might have an influence upon the fate of developing embryos which are in the sea at that time. This assumption has grounds, too, in the observations made by other workers (Rollefsen, 1930; Carruthers, Parrish, 1951), who have shown that high seas can kill the embryos of some species of the genera *Gadus* and *Pleuronectes* at a critical stage of their development. Thus, they were able to forecast on the basis of meteorological conditions, especially wind data, the volume of the catches of gadids two years prior to their entry into commercial fishing.

SUMMARY

The influence of temperature, salinity, light and mechanical factors upon the embryonic development of the Argentine anchovy *Engraulis anchoita* (Hubbs, Marini) has been established.

1) The embryonic development of the anchovy within the temperature range 14°–15° C. lasts from 68 to 72 hours, whilst within 19°–20° C., it lasts from 50 to 53 hours. A temperature of 4° C. was found to be lethal. The optimal temperature range for the anchovy hatching in the spring appears to be from 10° to 17° C.

2) Embryonic development takes place normally at salinities ranging from 25.8‰ to 50‰. Salinities above or below this range produce very pronounced abnormalities in the embryos.

3) Light does not appear to have any particular influence upon the embryonic development of the anchovy.

4) Anchovy embryos show great susceptibility to mechanical factors (pressure, shock). This susceptibility becomes more pronounced during the earlier stages of development, including early gastrulation. The assumption is made, on these grounds, that high seas and storms can destroy the developing embryos. The resistance of the egg membrane shows its greatest intensity in the stage corresponding to the closing of the blastopore and diminishes sharply immediately prior to the hatching of the egg.

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