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Identifying the developmental stages for eggs of the Australian pilchard, *Sardinops sagax*

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FISHERIES
WESTERN AUSTRALIA

Fisheries research in Western Australia
The Fisheries Research Division of Fisheries Western Australia is based at the Western Australian Marine Research Laboratories, P.O. Box 20, North Beach (Perth), Western Australia, 6020. The Marine Research Laboratories serve as the centre for fisheries research in the State of Western Australia.

Research programs conducted by the Research Division and laboratories investigate basic fish biology, stock identity and levels, populations dynamics, environmental factors, and other factors related to both commercial and recreational fisheries and aquaculture. The Research Division also maintains the State data base of catch and effort fisheries statistics.

The primary function of the Research Division is to provide scientific advice to government in the formulation of management policies for developing and sustaining Western Australian fisheries.

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Identifying the developmental stages for eggs of the Australian pilchard, *Sardinops sagax*

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Abstract

This study outlines the characteristics for identifying the developmental stages for preserved specimens of the eggs of the Australian pilchard (Sardinops sagax). *The report presents the differences between the characteristics of live specimens given in previous publications and pilchard eggs preserved in 5% formalin. The characteristics of eggs that were dead or damaged prior to, or during capture - termed occluded eggs - are also described. A relationship between sea surface temperature and time of capture in a 24 hour period is presented to act as a guide to inexperienced staff for assigning the egg stages to occluded eggs.*

Note: An electronic copy of this report is available at website http://www.wa.gov.au/westfish/res *where parts may be shown in colour where this is thought to improve clarity.*

1.0 Introduction

Obtaining the stock size of commercial fisheries is often the goal of fisheries research institutes. Obtaining these estimates for schooling pelagic fish is difficult because their behaviour makes fishery dependent methods (eg. CPUE) unreliable. Catchability is not constant with stock size and alterations to migration patterns will influence catch rates in an area despite little or no changes in the actual abundance. Consequently, many stocks of pelagic fish are monitored using fishery independent techniques such as the daily egg production method (DEPM) (Parker, 1985). The status of stocks of Australian pilchards (*Sardinops sagax*) are monitored using DEPM.

Put simply, the DEPM assumes that the size of the spawning biomass of a population can be estimated for a particular time using a formula combining:

- the proportion of females which spawned;
- the average weight of females which spawned;
- the average number of eggs each female spawned;
- the daily production of eggs (P_o) over the entire spawning area.

The first three parameters are obtained from sampling of adults. The calculation of P_o includes the rate of egg mortality (z) which is derived from the average number of eggs at different ages. In order to obtain the numbers of eggs at different ages plankton samples must be collected, painstakingly sorted and the number of eggs at each stage of development enumerated. Precise and uniform staging of eggs is therefore required to minimise bias in the estimation of P_o.

There are several factors which can impede accurate and uniform staging of pilchard eggs. Firstly, the characteristics of each stage must be established. There have been several papers which have descriptions of pilchard egg stages. These include Ahlstrom (1950) for *Sardinops sagax caerulea*, Cushing (1957) for *Sardina pilchardus* and Matsuoka and Konishi (1996) who have compared the morphological characteristics of unfertilised and fertilised eggs of the Japanese sardine *Sardinops sagax melanostica*. Baker (1972) provides descriptions for the Australian pilchard, *Sardinops sagax neopilchardus*, but only for live material. There is no published description of development stages of preserved eggs of Australian pilchards.

The second problem is that in order to obtain a daily estimate of egg production, plankton sampling must be conducted in a short period of time. This results in plankton samples being placed in a biological preservative (e.g. formalin) as there is no time for sorting samples and staging eggs in the field. Biological preservatives, such as formalin, can cause morphological changes to preserved material over time due to shrinkage and render material more opaque. Hence there may be discrepancies between descriptions of live material and characteristics of preserved samples.

The third factor which can hinder accurate and uniform egg staging is the condition of eggs in preserved samples. Eggs are often dead or damaged prior to, or during, capture and the percentage of these occluded eggs collected per survey can be as high as 30% (Fletcher & Tregonning, 1992 - describing the eggs as 'opaque'). There can be great difficulty in establishing the correct stage for these eggs as the amount of deterioration can be substantial as well as varied.

Lastly, it is often necessary to establish long term stock monitoring for pelagic fisheries but unfortunately, due to the tedium involved in plankton sorting, there is often a high rate of staff turnover. There is thus a large range of interpretations of published egg descriptions by relatively inexperienced sorters and, in particular, for occluded eggs. This can lead to a large series of samples in a plankton survey being totally mis-identified and having to be staged again.

The aim of this report is to facilitate an increase in the speed, accuracy and uniformity in the staging of pilchard eggs and to identify problem areas and traps for all personnel involved. This report aims to expedite training of new staff and minimise the amount of re-staging work that needs to be done. To this end, the main discrepancies between descriptions of live material by Baker (1972) and material in preserved samples are outlined, together with the characteristics of occluded eggs of each stage. In addition, an analysis of the development rates of pilchard eggs at different temperatures is presented. As there are only a certain number of egg stages which should be present in a sample at any one combination of sea surface temperature and time of capture, this report should assist inexperienced sorters, particularly when assigning egg stages to occluded eggs.

2.0 Methods

Samples were collected over a three year period, with eight separate surveys completed. The area covered in total by all eight surveys extends from Fremantle (32°01′S, 115°44′E) in Western Australia to Adelaide (34°56′S, 138°36′E in South Australia. Samples were initially placed in 5% buffered formalin and seawater, later transferred to 3% buffered formalin and seawater and finally sorted under a dissecting microscope with reflected light.

Temperature measurements were obtained by one of three methods depending on available resources. In the majority of surveys the sea surface temperature was measured by placing a mercury thermometer in a bucket of water scooped from the sea surface. Other surveys utilised an electronic thermometer deployed over the side of the vessel or by measurements of an onboard conductivity-temperature-depth unit.

The data used to determine the duration of each stage of development for eggs at different temperatures are summarised from samples taken from the above surveys, and from culturing Stage 3 eggs to hatching at 18.5°C temperature. These eggs were captured at 0500 hr and were maintained in small jars for 40 hours. Samples of eggs were regularly removed, fixed in formalin and staged.

Photographs were taken of pilchard eggs using a JVC 1280E colour video camera mounted on a dissecting microscope and linked to an *Amiga* A4000 desktop computer.

3.0 Results

3.1 Staging criteria

Eggs from the Clupeidae family are easily distinguished from other fish eggs, mainly by the large perivitelline space, the segmented yolk and having only a single oil globule. The main characteristics for **live** pilchard eggs (*Sardinops sagax*) are shown below and are summarised from Baker (1972). If a full description of this species eggs is required the reader is referred to Baker (1972).

- egg spherical;
- egg diameter between 1.32 and 1.70 mm, with an average of 1.52 mm;
- large perivitelline space (0.60 to 0.85 mm);
- segmented yolk - the yolk has a rough, granular appearance;
- single oil globule - seen opposite the blastodermal cap (see egg description Stage 3).

Eggs were considered occluded (as distinct from a normal, healthy egg) when the fluid surrounding the yolk and embryo had a white, slightly opaque appearance - in whole or in part - ie. the perivitelline space was not completely clear. This must also be associated with some degree of malformation or shrivelling of the yolk and/or embryo (or blastodermal cells). If the perivitelline space appeared to be opaque in only a small area (<10% of egg volume) and the yolk and embryo appeared normal and healthy, then the egg was not considered occluded.

The staging of occluded eggs is difficult but the same criteria apply in identifying the correct stage for occluded eggs as for normal, healthy eggs. In order to properly determine the stage for an occluded egg, it may be necessary to extract the embryo from the egg case. The yolk of an occluded egg tends to disintegrate or become dissipated, while the embryo tends to shrivel. Therefore, an occluded egg often has only a small amount of yolk and a malformed blastodermal cell mass or embryo. The amount of dissipation or shrivelling and malformation can occur to different degrees depending on the individual egg. The single oil globule in a normal egg may become divided in an occluded egg into several smaller oil globules. The best way to stage an occluded egg is by using a combination of the size and shape of the blastodermal cells or embryo, and also to identify any of the characteristics found in a normal, healthy egg.

Descriptions offered by Matsuoka and Konishi (1996) on unfertilised eggs appear similar to early stage occluded eggs described in this report. These authors describe unfertilised eggs as developing a perivitelline-space (some more narrow than normal eggs) and a blastodisc before disintegrating and becoming cloudy.

Diagrams in this report (see figures of egg stages, page 9-21) were obtained directly from Baker (1972) and are indicated by 'ψ'. These diagrams pertain to descriptions of live pilchard eggs but are also characteristic of preserved material. The diagrams depicting eggs from preserved plankton samples were modified from Baker (1972) (Φ). The digitized images of the eggs show a healthy normal-type egg and an occluded egg in most cases. Note, however, that the difference in appearance of occluded eggs of the same stage can be quite marked. The major descriptions (large bold text) are summarised from Baker (1972) while the note given for each stage is a summary of the characteristics given below.

In order to properly establish the stage of the preserved and occluded eggs please take note of the following points:

Stage 1

We are yet to find any Stage 1 eggs in our samples. This is probably because pilchards spawn at night and plankton samples are typically taken during daylight, several hours after spawning.

Stage 2

The cells of the 8-cell morula are unmistakable in healthy eggs. In late Stage 2 eggs (128-cell morula) the blastoderm is bulbous and distinct from the yolk in colour and shape. The main differences between this stage and Stage 3 are that the blastodermal cells are larger and the blastoderm, as a whole, is more bulbous. Not many Stage 2 eggs were captured, largely because little sampling was completed at night when they are most likely to be found.

Stage 3

The yolk of preserved specimens is not round but is a more elongate, oval shape. Stage 3 eggs are similar in appearance to Stage 2 eggs in having a distinct, bulbous-shaped blastoderm. The blastodermal cells, however, are not as large in Stage 3 as in Stage 2. In occluded eggs, the yolk can deteriorate completely leaving only the blastodermal cells and the oil globule.

Stage 4

In preserved specimens shrinkage tends to make the blastodermal cells cover up to half to two-thirds of the yolk. The germ ring also becomes more evident and is seen as a pronounced ridge between the blastodermal cells and the yolk. A kidney-shaped, transparent area is seen within the blastodermal cells but on the opposite side to the development of the embryonic shield. In occluded eggs, the yolk can deteriorate completely leaving only shrivelled blastodermal cells or, if deterioration is not so advanced, then the blastodermal cells can be seen with a much reduced yolk.

Stage 5a

Stage 5a eggs are easily misinterpreted as the embryonic shield is difficult to see in the early phase. The eggs may appear to be entirely lacking in structure apart from the yolk and a large perivitelline space. Turn the egg on it's 'side' to see the embryonic shield. This appears as a zone on the margin of the yolk slightly more opaque than the rest of the yolk. This zone covers about one-third the diameter of the yolk. The germ ring is still evident in well preserved eggs but is not always obvious. An area of transparency is also visible in Stage 5a eggs opposite embryo development, however this is reduced in size compared to Stage 4 eggs.

Stage 5b

In Stage 5b eggs the entire embryonic shield is visible. The anterior region of the embryonic shield is distinct in colour and can be seen to rise above the surface of the yolk. The middle and posterior regions of the embryonic shield, however, remain flush with, and a similar colour to, the yolk. The blastopore closes so the germ ring is no longer evident. The main feature to note to separate this stage from Stage 6, is the absence of the optic vesicles. This stage most likely corresponds to an early phase of Stage 6 in Baker (1972). In a badly shrivelled, occluded egg, it is possible to mistake a Stage 5b egg for an occluded egg of a higher stage of development. This is because the malformation of the blastodermal cells and reduction in yolk tend to make the embryonic shield more obvious and to give it more shape. It is often easier and more accurate to dissect out the yolk and embryo to accurately establish the degree of embryo development.

Stage 6
The presence of the optic vesicles is the best criterion to establish Stage 6 eggs, but these are difficult to see in the early phase. They appear as oval shapes on either side of the head and are best viewed from above. Somites are visible in Stage 6 preserved eggs. Occluded eggs can be easily misinterpreted as being a stage of higher development and extraction of the yolk and embryo from the egg case is advised.

Stage 7
The embryo is a distinct colour from the yolk and protrudes from the surface of the yolk for the entire length of the embryo. The tail does not separate from the yolk at any point. The end of the tail can reach the oil globule. The separation of the tail from the yolk is the best means of establishing whether an egg is Stage 7 or Stage 8. This is the case for both normal and occluded eggs.

Stage 8
The main feature to note is whether the tail has separated from the yolk. This may be seen by only a slight gap which appears just underneath the bulge in the tail.

The best way to determine the development of an occluded egg which is Stage 8 or higher, is to use the criteria outlined for a normal-type egg. It is important to note, however, that the yolk of an occluded egg is in a stage of deterioration and therefore the tail may appear deceptively long.

Stage 9
The tail is separated from the yolk by one-third of the length of the embryo. However, the tail is not bent at an angle away from the dorso-ventral mid-line.

Stage 10
The tail of the developing larva is bent at an angle of 45° away from the dorso-ventral mid-line.

Stage 11
The tail has grown to the extent where the tip may be parallel with the level of the hind-brain. The tip may curve in toward, but does not crossover, the hind-brain.

Stage 12
The tip of the tail is curved in toward the head and crosses over the hind-brain. Specimens of this stage commonly have their tails preserved in positions other than the position the tail grows in the preceding stages. That is, the tail may not be parallel to the trunk and up toward the head, but may be in various other positions relative to the rest of the embryo. Presumably this is a result of the increased ability of the embryo to move just prior to hatching.

3.2 Egg stage development and temperature

Development to hatching took approximately 48 to 50 hours at temperatures less than 16°C. This time decreased approximately 2 hours for every 1°C increase in temperature, so that at 21°C, the time from spawning to hatching took 37 hours. Thus, the assignment of particular egg stages as day 1 eggs or day 2 eggs will vary depending on the temperature of the sea water from which they were collected (Table 1). Nonetheless, there are only a few stages of eggs that will be present in samples taken at each combination of temperature and time (Table 1). Further work on modelling this relationship is currently being undertaken. These results are similar to laboratory-based results obtained for *Sardinops sagax* in South Africa (Le Clus and Malan, 1995).

It is important to note that Table 1 is not intended as a means of identifying egg stages. Rather, this table is presented to reduce misidentification, particularly of occluded eggs by inexperienced sorters. Eggs should be staged primarily by the characteristics outlined above, with Table 1 used as a guide.

4.0 Discussion

This study outlines the characteristics for identifying the developmental stages for preserved specimens of the eggs of the Australian pilchard (*Sardinops sagax*). The report presents the differences between the characteristics of live specimens given in previous publications and pilchard eggs preserved in 5% formalin. It is possible to stage preserved eggs using much the same criteria as those used for live material. However, certain morphological changes that occur to preserved specimens can lead to some confusion in stage identification. The changes result from the shrinkage and increased opacity caused by the 5% formalin, which is generally used by pelagic research institutes in the preservation of planktonic material.

Eggs dead or damaged prior to, or during, capture are difficult to stage and are frequently mis-identified. It is usually easier and more accurate to dissect the yolk and embryo from the egg case when staging these occluded eggs. The degree of deterioration in occluded eggs can be quite varied for different stages. Later stages of occluded eggs are generally easier to identify than earlier stage occluded eggs due to a greater amount of differentiated material (i.e. the embryo) with which to work. It may be necessary to refer to the relationship between egg developmental stage and temperature in order to get an idea as to the stage of an occluded egg.

The relationship between sea surface temperature and time of capture in a 24 hour period acts as a guide for the assignation of the egg stages, particularly for occluded eggs. If an egg appears completely clear and healthy then only the morphological characteristics for the egg should be used to assign egg stage. If an egg is in a high degree of deterioration and the person staging the egg is relatively inexperienced, then Table 1 can be used to point the person in the right direction. The characteristics for occluded eggs can then be used for identifying the correct stage.

Outlining the morphological differences and presenting the temperature, time of capture and egg stage relationship aids in the assignment of egg stages for plankton sorters. This increases the accuracy and uniformity in egg staging and ultimately decreases bias in the estimation of biomass for pelagic fish stocks. This manual will be used by the Fisheries Research Division in future egg surveys in Western Australia to ensure consistency in egg staging.

5.0 References

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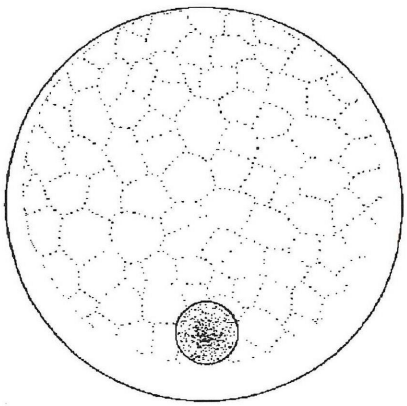
Table 1 Pilchard eggs stages that are likely to be present at each combination of time and temperature. If the eggs are staged outside of these criteria, check again.

Temperature (°C)	< 16.0		17.0		18.0		19.0		20.0		21.0	
Time (24 hr)	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
200	2, 3	7, 12	2	7	2	8	2, 3	8	2	9	2	9, 10
400	2, 3	7, 8	2, 3	8	3	8, 9	2, 3	8, 9	2, 3	9, 10	2, 3	10
600	3	8 (9)	3, 4	8, 9	3, 4	9	3, 4	9, 10	3, 4	9, 10	3, 4	10, 11
800	4	8, 9	3, 4	8, 9	4	9, 10	4	9, 10	3, 4, 5	10, 11	4, 5	10, 11, 12
1000	4	(8), 9	4	9, 10	4, 5	9, 10, 11	4, 5	10, 11	4, 5	10, 11	4, 5, 6	11, 12
1200	5	9, 10	4, 5	9, 10	4, 5	10, 11	5, 6	11, 12	5, 6	11, 12	5, 6	12
1400	5	9, 10	5, 6	10	5, 6	11, 12	5, 6	11, 12	6	12	6, 7, 8	**hatch**
1600	(5), 6	9, 10, 11	5, 6	10, 11	5, 6	11, 12	5, 6, 7	12	6, 7	**hatch**	7, 8	
1800	6	10, 11	6, 7	11	6, 7	12	6, 7	**hatch**	7, 8		7, 8	
2000	6, 7	10, 11	6, 7	11	6, 7	**hatch**	6, 7		7, 8		8, 9	
2200	7	11, 12	7	12	2, 7		2, 7		8		8, 9	
2400	2, 7	12	2, 7	**hatch**	2, 7, 8		2, 7, 8		8, 9		9	

7.0 Figures

Egg Stage 1

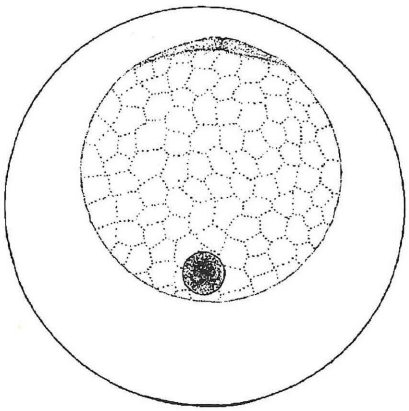


live Ψ

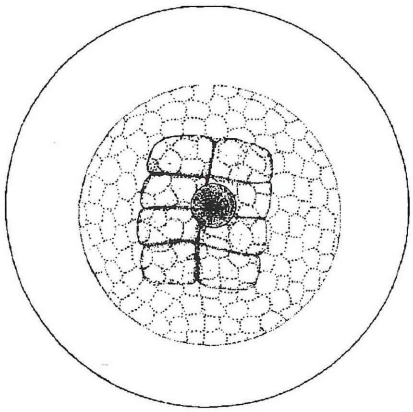
- **small perivitelline space**
- **no cleavage**

Note: Egg diameter is usually small (1 mm). Egg is either recently fertilised or unfertilised. This stage is either difficult to identify or develops rapidly to stage 2.

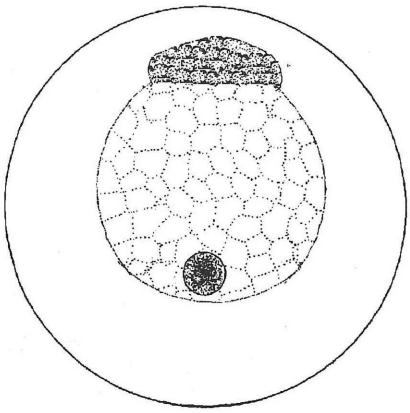
Egg Stage 2



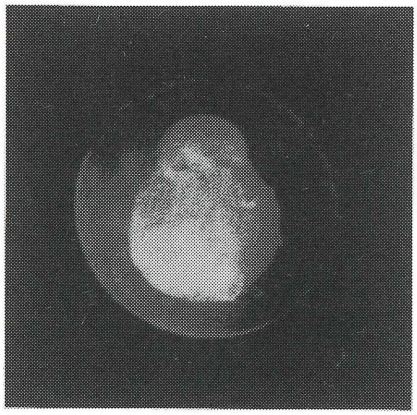
live - lateral view (early) Ψ



live - top view (early) Ψ



preserved - lateral view (late) Φ

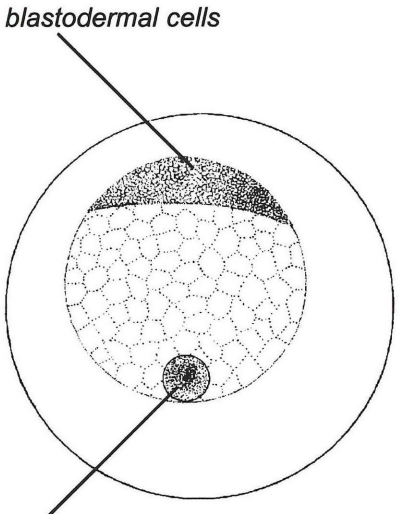


normal - lateral view

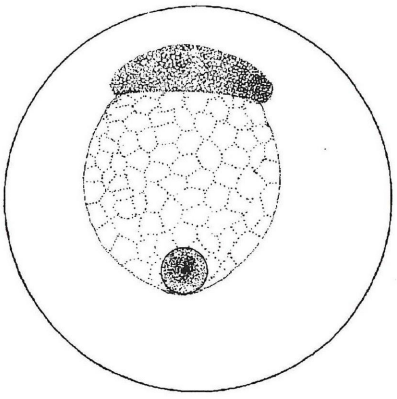
- **first cleavages**
- **to 128-cell morula**

Note: Blastoderm cells are large, prominent and easily identified in the early phase of this stage. Yolk is more oval shaped than round. The late phase of stage 2 is similar in appearance to stage 3, however, the blastodermal cells are larger in the former.

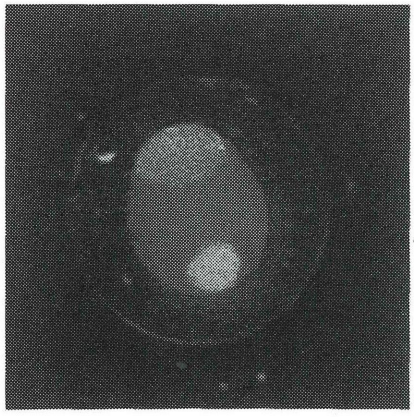
Egg Stage 3



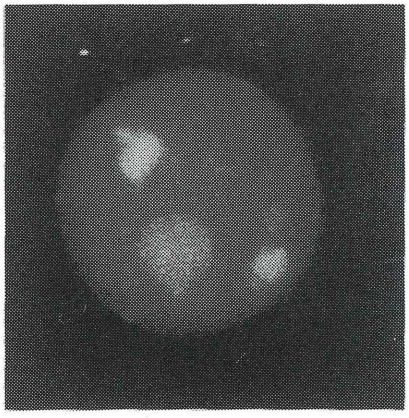
live - lateral view Ψ



preserved - lateral view Φ



normal - lateral view

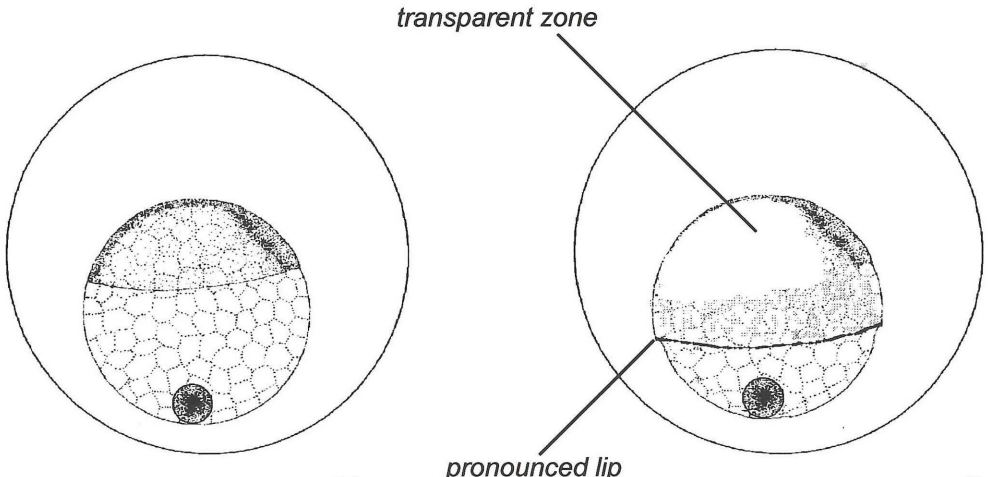


occluded

- **blastodermal cells very small**
- **blastodermal cells in the shape of a lenticular cup**

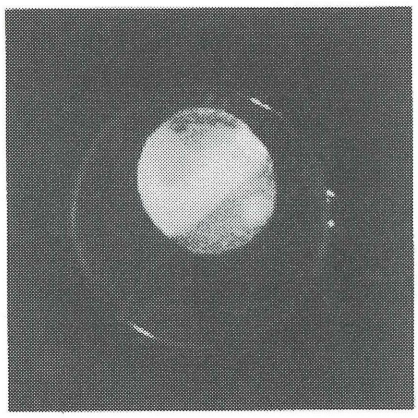
Note: Shrinkage in preserved specimens leads to the yolk being a more oval shape and the blastodermal cells having a bulbous appearance. Occluded eggs may have only the blastodermal cells and oil globule discernable. The egg yolk tends to become dissipated.

Egg Stage 4

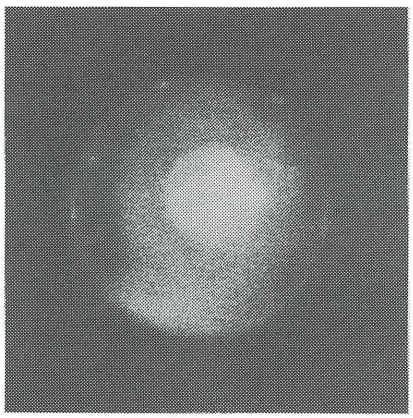


live - lateral view Ψ

preserved - lateral view Φ



normal - lateral view

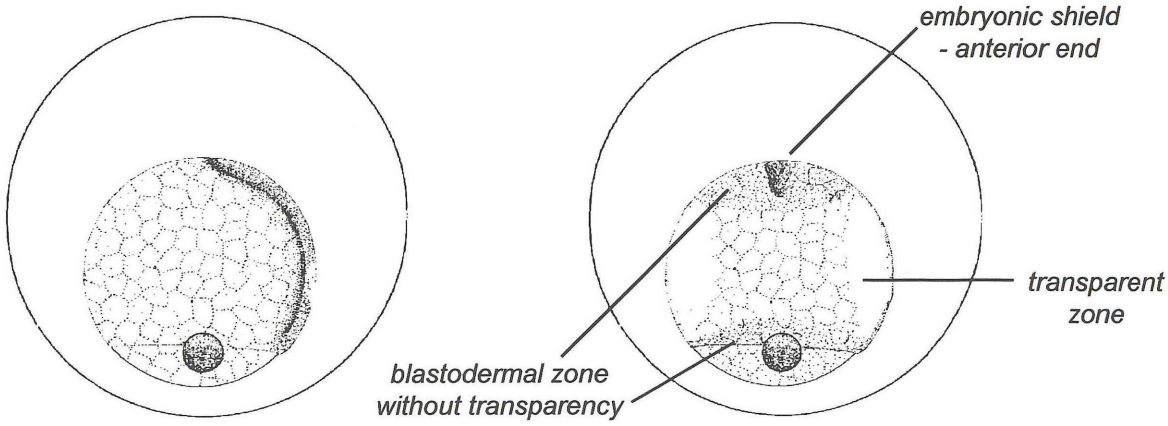


occluded - lateral view

~ blastoderm covers one-third (1/3) of yolk

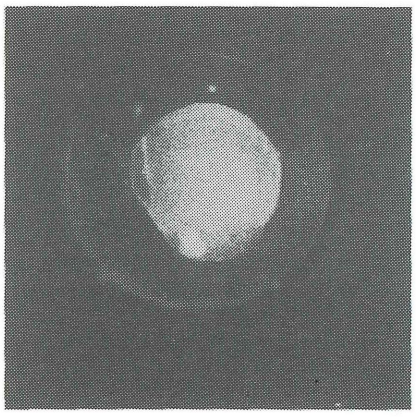
Note: The blastoderm may appear to cover up to half or two-thirds of the yolk. Germ ring pronounced. Area of transparency obvious in blastoderm, opposite to embryonic shield development. In occluded eggs only a small amount of yolk may be discernable.

Egg Stage 5a

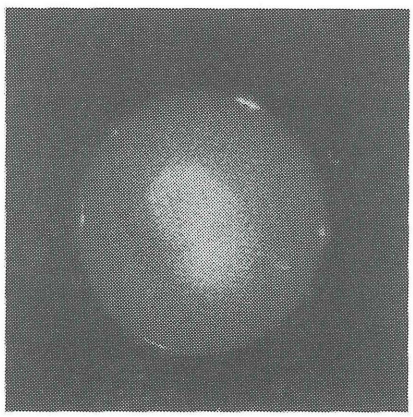


live - lateral view Ψ

preserved - ventral view Φ



normal - lateral view

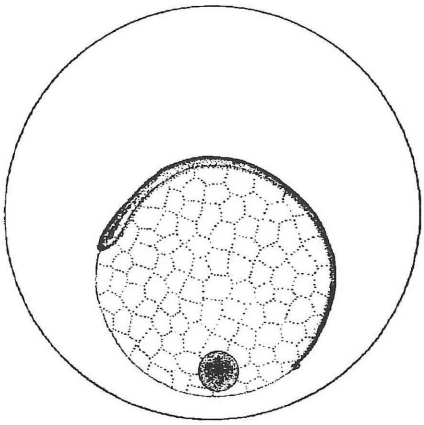


occluded - lateral view

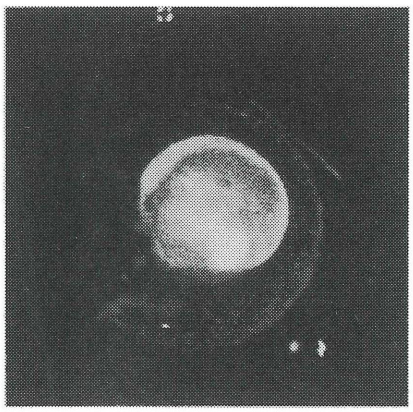
- **embryonic shield visible**
- **blastoderm covers four-fifths of yolk**

Note: The embryonic shield appears as an opaque zone on the margin of the yolk. This zone covers about one-third the diameter of the yolk. A kidney-shaped, transparent zone is apparent opposite to embryonic shield development but reduced in size compared to stage 4. Germ ring still evident.

Egg Stage 5b



live - lateral view Φ

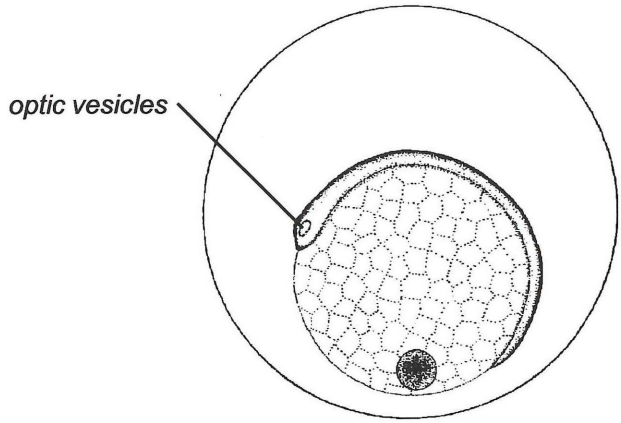


normal - lateral view

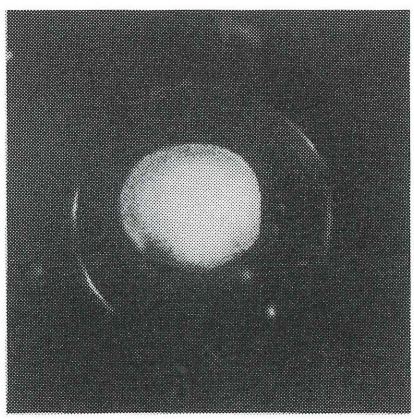
~ blastopore closes

Note: The embryonic shield appears as an opaque zone on the margin of the yolk. The anterior region of the embryonic shield is distinct in colour and rises above the surface of the yolk but the middle and posterior regions remain flush with the yolk surface and are of a similar colour. Germ ring may no longer be evident.

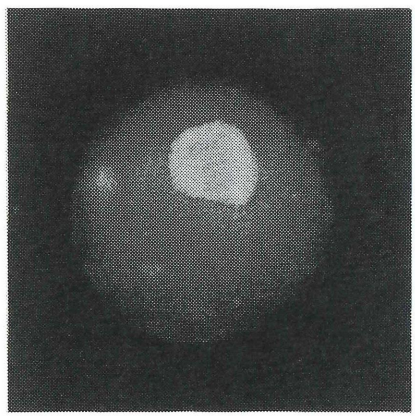
Egg Stage 6



live - lateral view Ψ



normal - lateral view

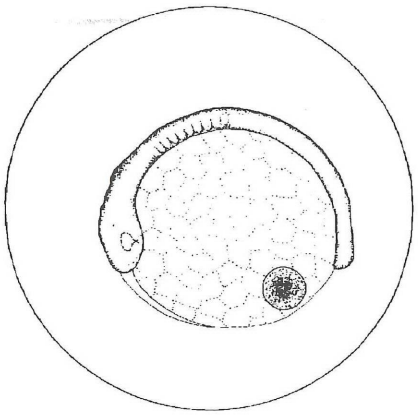


occluded - lateral view

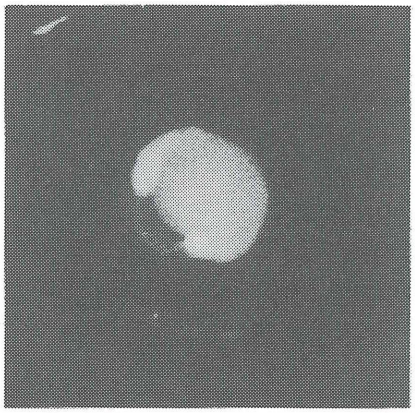
~ optic vesicles visible

Note: The middle region of the embryo remains mostly flush with the yolk surface. The anterior and posterior regions bulge from the yolk surface and the optic vesicles appear as transparent bulges in the head region. Somites may be visible.

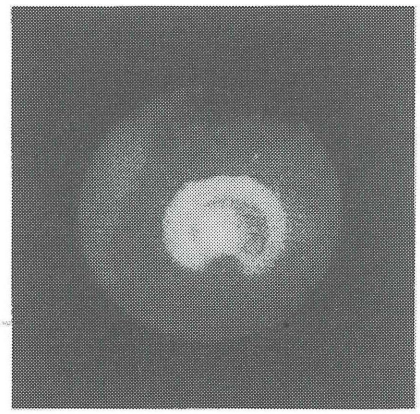
Egg Stage 7



live - lateral view Ψ



normal - lateral view

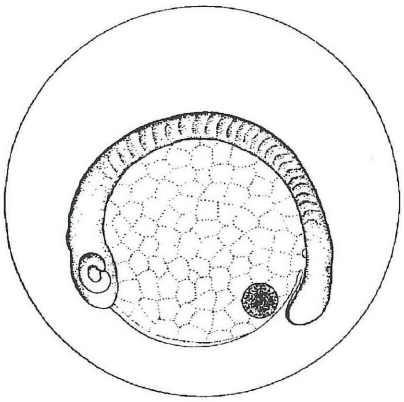


occluded - lateral view

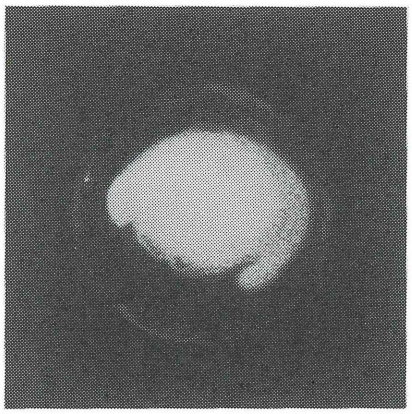
- **swollen tail of embryo almost reaches oil globule**
- **somites visible**

Note: The embryo is obvious above the yolk surface along the whole length of the body. Tail has not detached from the yolk. End of tail may appear to reach oil globule.

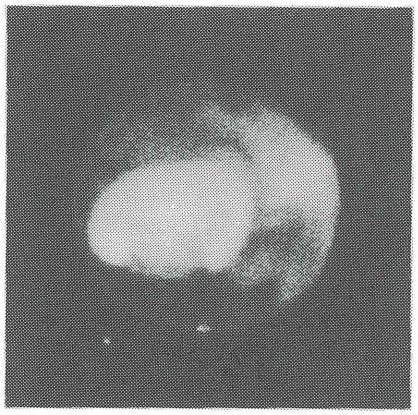
Egg Stage 8



live - lateral view Ψ



normal - lateral view

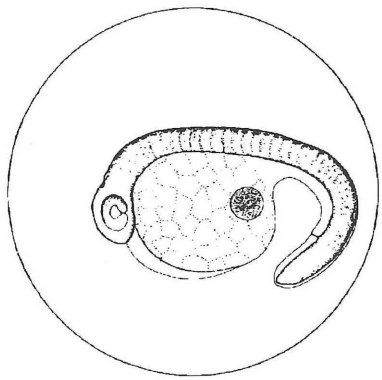


occluded - lateral view

- **tail separates from yolk**
- **tail extends to oil globule but not beyond**

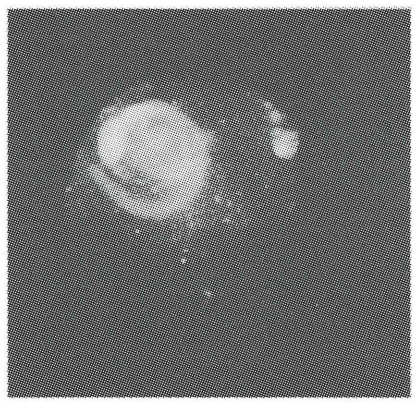
Note: Tail is detached from yolk. This may be seen by only a slight gap which appears just underneath the bulge of the tail.

Egg Stage 9



live - lateral view Ψ

normal - lateral view

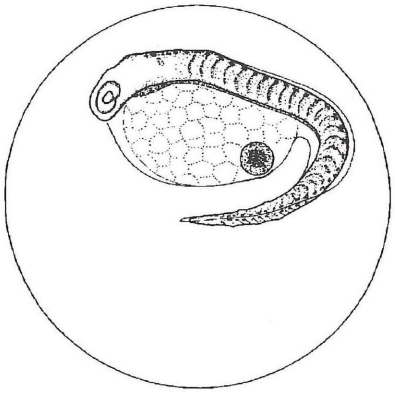


occluded - lateral view

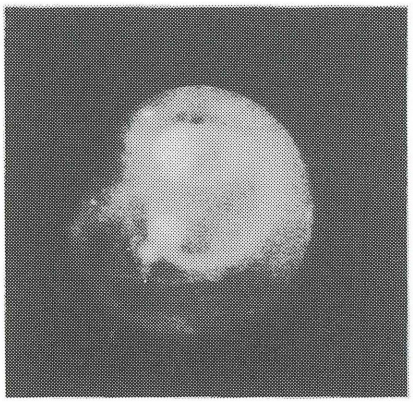
- **free tail one-third body length**
- **melanophores appear dorsally**

Note: Most notable is that the tail remains in line with the trunk. That is, it is not bent at an angle away from the dorso-ventral line of the body.

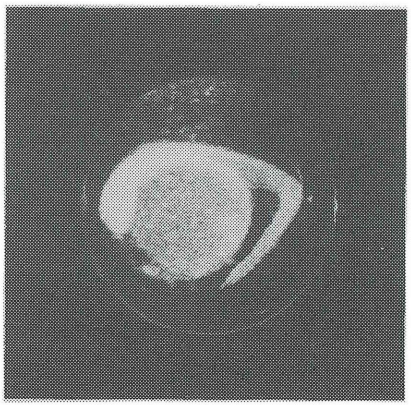
Egg Stage 10



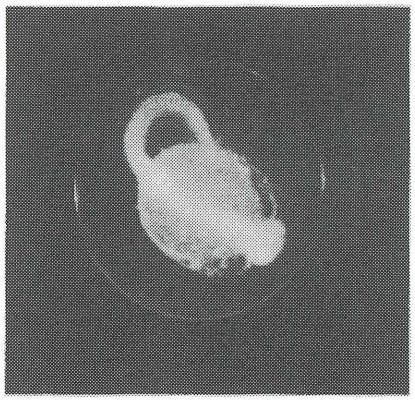
live - lateral view Ψ



occluded - lateral view



normal - lateral view

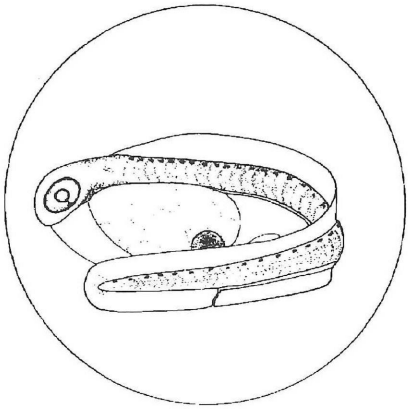


normal - top view

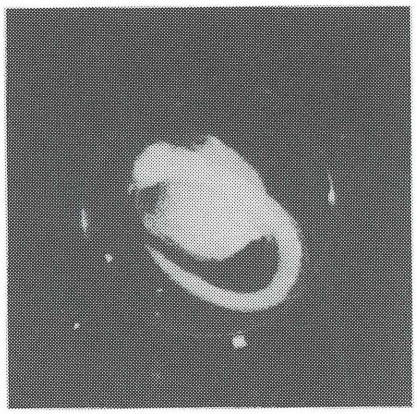
- **tail bent at 45 degree angle to trunk**
- **fin folds one-third depth of body**

Note: Tail is bent at 45 degree angle to dorso-ventral midline.

Egg Stage 11



live - lateral view Ψ



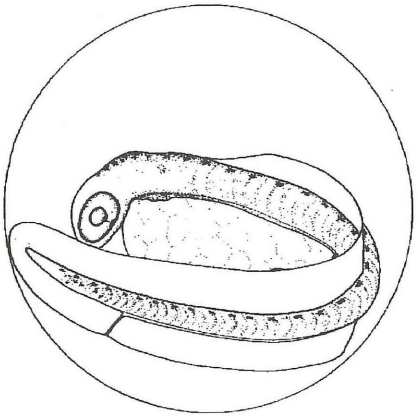
normal - top view

occluded - top view

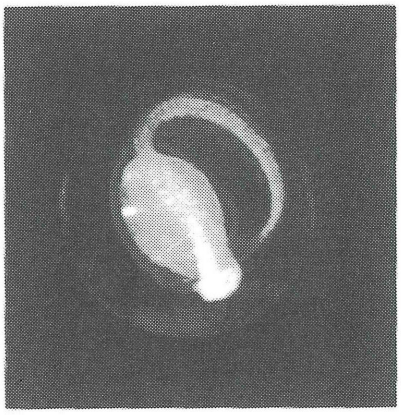
- **~ tail parallel with trunk**
- **~ tip of tail level with hindbrain**

Note: Tip of tail is not curved in toward head.

Egg Stage 12



live - lateral view Ψ



normal - top view

~ tail overlaps head

Note: The tip of the tail is curved toward the head region and crosses the hindbrain. However, tail may be in position other than parallel to trunk.