ABSTRACT

A code system for describing the postgastrular embryology of teleosts is proposed. The system relies upon the recognition of like conditions in the development of homologous features in different species. Each condition is defined and assigned a number. The condition of the embryo at any point in development is described by a series of twelve such numbers. The ease with which the data can be manipulated is demonstrated in an analysis of the development of three teleost species, Osmerus mordax (Mitchill), Brachydanio rerio (Hamilton-Buchanan) and Perca flavescens (Mitchill). In this analysis heterochronies are illustrated and the rates of somite formation compared. An index of visible change is developed which reflects the degree of development of the embryo at any time. Units are defined which can be used as a time basis in comparative studies and which permit observations to be placed in a developmental sequence. Distribution of like points of eye development within the developmental/time axes is studied. In an appendix the development of the three teleosts is described in a conventional manner and illustrated by photomicrographs.

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For Marilyn

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CHAPTER I

INTRODUCTION

The need for and the difficulties encountered in the clarification of the basic concepts of teleost embryology were pointed out by Legault (32). Previous to this, other workers concerned with facilitating comparative studies were Hubbs (27) and Balon (3). Hubbs emphasized the need for a standard terminology, defining and naming the major periods of teleost development from fertilization to maturity. He defined the embryo as all the developmental stages occurring within the egg; this definition is adopted in the present paper. Balon reviewed the American and European terms used by different authors to label periods of fish development.

Legault (32) proposed a decimal system of defining and labelling phases and stages of teleost development, from the ovum to the end of gastrulation, which he used to make interspecific comparisons. He considered a phase as a period of development dominated by a characteristic process; thus defining fertilization, segmentation, blastulation and gastrulation. Each phase was assigned a digit of ordinal rank. Within each phase he

defined conditions of the embryo on the basis of structure and appearance. These smaller divisions of development he termed 'stages'. A digit of decimal rank was used to label each stage. A number composed of an ordinal and decimal was used to precisely indicate a condition in development.

The last point of development considered by Legault was the completion of keel formation, this being the end of gastrulation. The period considered by the present author is from the end of gastrulation to hatching. There is a slight overlap with Legault's study, as the stage of keel completion is illustrated in two of the species considered.

It is not possible to extend Legault's system to cover the postgastrular development. Many more features make their appearance during this period than in the pregastrular and gastrular development. The relative rates of development of these features are not constant in different species and thus comparable stages are rarely seen in two fishes.

A new, code system was developed by the author to describe the postgastrular development of teleosts. A mathematical presentation was chosen because figures are precise, they make for concise presentation of data in code form and they are easily manipulated for purposes of comparison.

The plan of the thesis is as follows. In the second chapter the system is introduced and its mechanism explained. Methods of obtaining the data to which the system is subsequently applied are described. The third chapter contains four examples of how the data in code form can be handled for the purpose of making interspecific comparisons. Discussion and conclusions regarding the utility of the descriptive method are given in the fourth chapter. An illustrated, conventional account of the development of three teleosts is given in the appendix.

CHAPTER II

PROPOSED CODE SYSTEM

Operation of the system

The proposed system for expressing developmental data depends upon the recognition of like or similar conditions in the development of homologous features in different species. In a consideration of a number of papers dealing with the embryology of teleosts (1,4,7,9, 18,19,26,39,40,44,49,51,52,54), it was noticed that the development of twelve features was often referred to. These features were:

A. Mesoblastic somites.

B. Fish-like body form.

C. Olfactory organs.

D. Visual organs.

E. Auditory organs.

F. Brain.

G. Somatic pigmentation.

H. Heart.

I. Proctodaeum.

J. Pectoral fins.

K. Median fins.

L. Kupffer's vesicle.

Further consideration of papers revealed that within the development of each of the twelve features there were similar conditions observable in different species. For example, the eye was observed to pass through the same conditions, anlage, vesicle, cup and pigmented cup, in all the species for which information is available.

In Table 1, the features A to L are listed. Within each feature the conditions frequently observed are defined, each condition being represented by a number. An embryo is concisely described and labelled, at any moment, by a series of twelve such numbers, the meaning of which can be obtained by reference to Table 1. The first figure refers to the feature A (mesoblastic somites), the second to feature B (fish-like body form) and so on. The mesoblastic somite number is given as observed and not in coded form, for ease of interpretation. When the figure of the first position increases above nine it is set off from the rest by a comma.

When the series of twelve-figure aggregates describing different observations are considered sequentially, they relay to the reader information on the development of the species.

For purposes of comparison it was necessary to devise a method of compensating for the effect of

variations of incubation temperature upon the speed of development of the embryo. This was also necessary for the true positioning of observations, made on embryos developing under slightly different temperatures, in a developmental sequence.

Development varies not only with time but with temperature and other environmental factors (11,29) and a unit, the centigrade degree hour (CDH), was defined which takes both time and temperature into consideration. The CDH unit is the product of the age of the embryo in hours and the incubation temperature in degrees. For example, the first observation of the smelt, listed in Table 2, has a CDH value of 600, this was obtained by multiplying the age of the embryo (60 hours) by the incubation temperature (10°C). The units are similar to those used by Wallich (53), and Battle (5), however their units, the product of age in days and temperature in degrees farenheit above freezing, were too difficult to handle for the present purpose.

The age of the embryo at hatching varied in different batches of eggs. In the zebrafish the hatching age was taken to be when half the eggs of a batch of fifty had hatched under standard conditions. In the other two species the time was taken when embryos started to leave the egg mass in large numbers. A collecting sieve filtered the hatched embryos from the

outflow of the Robertson incubator in which the perch were incubated and the contents were checked once a day. The smelt eggs were surveyed daily until large numbers of empty membranes were observed.

An index of visible change of the embryo (IVC) was devised, which reflects the change of the whole embryo in the postgastrular period. The IVC for any particular embryo is derived as follows. The mesoblastic somite number is expressed as a percentage of the number present at hatching. The other twelve digits are summed and the sum expressed as a percentage of the sum of hatching. The two percentages are added and the value of the IVC is obtained. In all species the index runs from zero at the completion of gastrulation to two hundred at the time of hatching.

Experimental work

Three teleosts were studied: the American smelt, Osmerus mordax (Mitchill), the zebrafish, Brachydanio rerio (Hamilton-Buchanan) and the yellow perch, Perca flavescens (Mitchill). The first two species were studied by Legault (32), and were chosen here for the same reasons (viz. availability, phyllogenetic position and the simple incubation techniques involved.) Perca flavescens was substituted for Legault's Percina caprodes semifasciata for reasons of local availability and because a representative of the Order Perciformes

was desired.

O. mordax, was previously studied by Marcotte and Tremblay (40) who reviewed the literature on the species. They devoted a section of their paper to a preliminary account of its development. Due to the presence of the egg membrane their photographs are not clear. In this present study the problem was solved by removing the embryos from their membranes prior to photographing them. Agassiz (1) described the hatching condition of the smelt and Legault (32) described its development up to the end of gastrulation.

Smelt eggs were obtained by stripping ripe females and fertilization was effected in the field. Eggs were extruded from the females onto plastic netting with a mesh-size of 0.5mm., to which they adhered by means of their pedicels. On returning to the laboratory the netting was cut into convenient pieces to facilitate study of the eggs under a microscope. The source of the fish was a small stream running next to McCloskey Road in the Gatineau National Park, Quebec. The stream was visited on the three occasions; 00.30 hrs. on the 4th of May and 00.30 hrs. and 19.00 hrs. on the 5th of May. Maximum spawning activity was reported to occur a few days later.

On the first visit to the stream the water temperature was 7°C, on the second visit 8.5°C and on the

final visit 9.5°C.

Fertilized eggs were kept in the laboratory under two or three inches of running tap-water, the temperature of which increased from 10°C on the 5th of May to 13°C on the 23rd of May. Variations of oxygen concentration, pH and salinity were not measured. There was severe fungal infection of the eggs, however those for study were chosen from localised areas which escaped attack.

The zebrafish, B. rerio, has been studied by embryologists for the last twenty-five years. Experimental (6,12,13,14,15,16,23,24,28), and normal aspects of development (17,18,19,20,22,25,34,38,45,46,47), have received attention.

Fertilized eggs were obtained in the same manner as that described by Legault (31,32), with one minor modification. In place of the white sand, used by Legault to produce different levels within the aquarium, a plexiglass platform was introduced which was easily cleaned.

Eggs were incubated in tap-water contained in finger bowls open to the air. The incubation temperature varied between 24° and 28°C. Variations of oxygen concentration, pH and salanity were not measured. No serious infection by fungi was encountered. Eggs for examination were removed by means of a medicine dropper.

The yellow perch, P. flavescens, has received little attention from the descriptive embryologist and no thorough account of its development is available. Propagation methods have been described (30,40,41) and a short, incomplete description of perch development was given by Mansueti (38), in which he states:

> Knowledge of the early embryology of the yellow perch, nevertheless is quite incomplete.....

Fertilized eggs were obtained by stripping females and males in the laboratory. Ripe perch were obtained from the Ottawa River in the region of Rockland, Ontario, on the 23rd and 26th of April and the 5th of May. One of the females spawned naturally in the laboratory and the appearance and density of the eggs agreed with the descriptions of Leach (30) and Muncy (43).

Eggs were incubated in a Robertson tray, modified according to the description of Legault (33). Running tap-water was used which gradually increased in temperature from 7°C on the 23rd of April to 13°C on the 23rd of May. Variations of oxygen concentration, pH and salanity were not measured. There was only localised fungal infection.

Development of all the above species was observed under a sterobinocular microscope (Wild M5) with a maximum magnification of 50X. Photomicrographs were taken within a similar limit of magnification by means of a Zeiss 35mm. still camera, attached to one occular

of a Spencer dissecting microscope by an adapter similar to that described by Caldwell and Carlin (10). Mobile embryos were anaesthetised with methyl penatol before being photographed.

Observations are expressed in code in Tables 2, 3 and 4, and in conventional manner in the appendix. Many of the observations were made on only one individual, however sometimes it was possible to observe similar conditions of development in more than one embryo, in which case the results were pooled. The number of embryos considered is given at the start of each paragraph of the conventional account in the appendix.

CHAPTER III

MANIPULATION OF THE DATA

In this chapter, the ease with which digital data can be manipulated and the utility of CDH units and the IVC are demonstrated by an analysis of the post-gastrular embryology of the species. The first use to which the coded data are put is in the comparison of relative rates of development of features in the different teleosts. The second application involves comparisons of the rate of development of the same feature (mesoblastic somites) in relation to time. The third part of the chapter is given to a comparison of the rate of change of the whole embryo (IVC), in the three teleosts. Finally both the IVC and CDH units are used to study the dispersion of like points of development of the eye within the development time axes.

Heterochronies

1. General.

Rugh (47) defined heterochrony as: 'An alteration and reversal of the sequence of the stages of ontogeny.' In this context 'stage' is taken to refer to a condition in the development of a feature rather than of the whole

organism. The alterations and reversals, of which Rugh speaks, are due to differences in the relative speeds of development of the different features. An example of heterochrony is seen in the differential existing between the rate of development of the eye and ear of the clingfish, described by Runyan (49), and the zebrafish, described by Hisaoka and Battle (18). The otoliths of the ear develop before the retina is pigmented in the zebrafish (Hisaoka and Battle's stage 21 and 22 respectively). On the other hand the eye is pigmented in the clingfish before the otoliths are formed (Runyan's stage 13 and 14 respectively). In other words there is an alteration of the sequence of the stages of ontogeny.

By using the data, presented in a code form, heterochronies of this type are easily found. The data are manipulated as follows:

a) Conditions are chosen arbitrarily in the development of the features A to L. For example the condition chosen in the B feature is when the embryo has just lifted from the yolk posteriorly.

b) The first observations of each of the conditions chosen are underlined in the appropriate columns of Tables 2,3, and 4.

c) These first observations are then considered, for the present, to be <u>points</u> in development.

d) The temporal relationship between any two of these points is established for each species.

e) The temporal relationships between pairs of points are compared in the three species.

f) Where there is a difference in this last relationship, between two or more species, then this is considered to be an example of heterochrony.

The following points, underlined in Tables 2, 3 and 4, were chosen in the twelve features:

A. First somite pair(s) fully formed.

B. Tail bud elevated from the yolk.

C. Olfactory placodes first observed.

D. Optic vesicle first observed.

E. Otic placodes first visible.

F. Cerebellar fold first seen.

G. Scattered melanophores visible.

H. Heart first observed as a straight tube.

I. Gut opens posteriorly.

J. Pectoral fins present.

K. Median finfold observable.

L. Kupffer's vesicle present.

In Table 5 (i, ii and iii), the temporal relationships existing between each of these points is presented for each species. Where the point in development of any feature (A to L) in the left hand column (X), precedes in development the point chosen in the horizontal (Y)

series (A to L), this is represented as 'a', i.e., antedates. When the converse is true, 'p' is used, i.e., postdates. If the two points appear to occur simultaneously, 's' is used. Each table is divided into two halves by the line, both halves having the same meaning.

In Table 6, the Tables 5i, 5ii and 5iii are superimposed. All letters retain the same meaning as in Table 5. For each position in the Table there are three letters given. The first refers to the smelt, the second to the zebrafish and the third to the perch.

Where the letters of any trio are the same, this means that the temporal relationships between the two points are the same in all three species. If the trio is heterogenous, containing different letters, this indicates heterochrony. For example, the trio 'aap' indicates that in the smelt and perch a point in development of one feature is achieved before a point in a second feature, while in the perch the order of attainment is inverted.

Where the configurations 'xxs', 'xsx', 'sxx', 'ssx', 'sxs', or 'xss' are seen, where 'x' can be either 'a' or 'p', this is discounted as an indication of heterochrony, unless both 'a' and 'p' are present. The apparent temporal coincidence indicated by 's', may be an artefact of the code method of recording development. If observations had been made closer together then a

difference might have been found in the order of attainment of the conditions.

The heterogeneous trios remaining after the dubious ones have been removed are decoded below:

A:L. aap. The first somites make their appearance in the smelt and zebrafish before the formation of Kupffer's vesicle. The opposite sequence of events occurs in the perch.

B:C. aap. Lifting of the caudal mass from the yolk precedes the first observation of olfactory placodes in the smelt and zebrafish, but this order is inverted in the perch.

B:F. paa. The relationship between the start of caudal lifting and the time of formation of the cerebellar fold is the same in the zebrafish and perch, the caudal mass lifting from the yolk before the cerebellar fold is visible. The opposite order occurs in the smelt.

B:J. aap. Comparing the time of lifting of the caudal mass to the time of formation of the pectoral fins, one finds that lifting is the first to occur in smelt and zebrafish but the last in the perch.

C:F. ppa. Thickening of the olfactory placodes occurs before formation of the cerebellar fold in the smelt and zebrafish, but the converse is seen in the perch.

D:L. aap. The time of formation of the optic

vesicles precedes the time of formation of Kupffer's vesicle in the smelt and zebrafish but follows it in the perch.

F:J. aap. The cerebellar fold is formed before the appearance of the pectoral fins in the smelt and zebrafish. The fins appear first in the perch.

G:I. paa. Somatic pigmentation follows opening of the gut in the smelt but precedes it in the other two species.

G:J. pap. In both the smelt and perch the time of pigmentation follows the time of formation of the pectoral fins. This relationship is reversed in the zebrafish.

H:J. pap. The heart is seen as a straight tube in the smelt and perch after the pectoral fins are observable. In the zebrafish the opposite situation prevails.

H:K. asp. There is a definite heterochrony observable in the development of the straight tube heart and median fins of the smelt and perch, the heart being formed before the fin in the smelt and after it in the perch. In the zebrafish these two points appear to be reached simultaneously.

J:K. apa. In the smelt and perch the pectoral fins are formed before the median fins but in the zebrafish the median fins make their appearance first.

Illustrations of heterochrony between the derivatives of the same and different germ-layers can be made as follows:

2. Between the derivatives of different germ-layers.

Consider the temporal relationship between the figures circled in the columns A and C of Tables 2, 3 and 4. The circled figure in the A variable represents the first observation of a condition with more than 70% of the hatching number of mesoblastic somites, these being of mesodermal origin. The figure circled in the C column represents the first observation of the olfactory pits which develop from ectoderm. In the perch the olfactory pits develop before 70% of the hatching mesoblastic somite number is achieved in contrast to the other two species where the somites develop more rapidly than the olfactory organs.

3. Between derivatives of the same germ-layer.

Observe the sequential relationships existing between the points circled in the C and E columns of the Tables 2, 3 and 4. The figure circled in the C variable represents the first observation of the olfactory pits, these being ectodermal derivatives. The figure indicated in the E column represents the first observation of the otoliths in the ear, which are also of ectodermal origin. In the smelt and zebrafish the olfactory pits form after the otoliths are produced, in the perch the converse occurs.

Rate of somite formation.

In this section the development of one feature, the mesoblastic somites, is considered relative to age (CDH units). Mesoblastic somites were chosen for this consideration because they arise serially and can be counted, thus supplying quantitative data.

1. Absolute rate

The absolute number of somites is plotted against the absolute age (CDH units) in Figure 1. The hand fitted curves indicate that:

a) The absolute age of the embryo at the start of somite formation varies.

b) The initial rate of somite formation is similar in the three species.

c) Somites are formed at different rates as the embryo gets older. An initial period of rapid somite differentiation is seen in the three species, followed by a period of slower differentiation which continues until the final complement has been achieved.

d) The final number of somites is different in all three species.

2. Percentage rate

In Figure 2, the number of somites observed has been expressed as a percentage of the number present at hatching and plotted against the age of the embryo,

expressed as a percentage of the hatching CDH value.

Hand-fitted curves show similarities between the species, with regard to somite formation:

a) The age of the embryo at the onset of somite formation varies between 10 and 21% of the hatching CDH value.

b) In all three species there is an initial period of rapid differentiation during which more than 80% of the hatching number of somites are formed.

c) During the last half of the developmental period the process of the somite formation is slower in all three species.

Visible change of the whole embryo.

Before the rate of change of the embryo can be studied, it is necessary to express the change in numerical terms. An index of visible change was derived as explained in the second chapter. This IVC was then plotted against the percentage age of the embryo and hand fitted curves drawn (Figure 3). The following conclusions can be drawn from the graph:

a) In all three species there is an initial period of rapid change of the embryo.

b) After about half the developmental period has elapsed the rate of visible change slows down and is only gradual from then until hatching.

Changes in the eye relative to age and development of the whole.

We have considered the development of features of different species from the point of view of their relative rates of development. We have also considered the rate of development of one feature (mesoblastic somites) in relation to age. In the last section the rate of change of the whole embryo received attention. Now, similar changes in the same organ, the eye, will be compared in relation to age and total development. The comparison was made as follows:

First of all, similar points were chosen in the development of the eye, these points being:

a) The first observation of the optic anlagen.

b) The first observation of the optic cups.

c) The first observation of the pigmented optic cups.

These points were then plotted within the IVC and percentage hatching CDH axes. In Figure 4, the points can be identified by the key to the right.

Like points were then joined, and a series of triangles produced. The area of each of these triangles is an indication of the dispersion of the like points within the axes.

It can be seen that as development and age progress, the area of the triangles, and thus the dispersion of the points, increases. This means that the

individual ontogenies diverge more and more from each other as development proceeds, not only in structure, as proposed by Von Baer (see 8), but in organizational relationships. This dispersion will have to be established in other species, and with other features, before any generalizations can be made.

(The point 2^{*} in Figure 3 was obtained by processing data given by Hisaoka and Battie (18), as original data were not available.)

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The operation of the system has been explained and the facility with which the coded data can be handled, demonstrated. When compared to extant descriptive systems, both continuous (moving photomicrographs) and discontinuous (written systems), the proposed system is seen to have certain advantages.

Preparation of accounts of development expressed in code does not involve the expense met with in producing moving photomicrographs. If sufficient observations are made the description can be as complete as a photographic record.

Development is more concisely presented in digital form than in writing. The development of each of the three species studied in this paper is described in only one table.

The description produced is unambiguous, the numbers having a standard meaning independent of the observer or the reader. In other words the record is produced and interpreted objectively.

The system facilitates interspecific comparisons of development. Heterochronies are easily illustrated

by manipulation of the figures, in contrast to the difficulties encountered in comparing long written accounts.

Development is reduced to numerical terms thus making possible treatments which are impossible with written accounts. Rates of development of the whole and of parts can be considered relative to time.

The system can be expanded to include any number of features and any number of observations.

There are a number of disadvantages of the system. It must be closely studied and learned before it can be applied with facility. Recognition of like conditions in the development of comparable features is an integral part of the system and it may be difficult to apply the system, in its present form, to deformed individuals. The system also has the disadvantage of all written methods, the reader must interpolate between the points in development described.

The disadvantages of the system are few and seem to be outweighed by the advantages. The data in their coded form can be used for purposes other than those outlined in chapter three. Because of the concise nature of coded presentation it should be easy to produce a key, similar to a botanical 'Flora', for the identification of embryos. A second possible use might be the testing of generalizations made by various authors.

For example, Budd (9) maintains that somatic pigmentation develops 'later' in pelagic than demersal eggs. This could be tested by seeing at what time somatic pigmentation starts, either in terms of age or of IVC, in a large number of species with demersal and pelagic eggs.

The CDH unit served its two purposes. It allowed the sorting of observations to be performed and it served as a basis for comparing rates of development.

The IVC appears to reflect the change that occurs in the embryo as it was designed to do. The index may be criticised on the grounds that it depends too much on the somite number for its magnitude, however it is argued that the somites do comprise a large part of the embryo and changes in their numbers are reflected in the appearance of the whole embryo.

BIBLIOGRAPHY

1. Agassiz, A. (1882).
On the young stages of some osseous fishes.III. American Academy of Arts and Sciences. Proceedings, vol. 17: 271-303, 20 plates.

2. Allen, L. A. and L. M. Mukay (1960).
X Ray effects on embryos of the paradise fish. Growth, vol. 24: 131-168.

3. Balon, E. K. (1960).
Über die Entwicklungsstufen des Lebens der Fische und ihre Terminologie. (Developmental stages of fish life and their terminology.) Zeitschrift für Wissenschaftliche Zoologie. vol. 164, 3-4: 294-314.

4. Battle, H. I. (1940).
The embryology and larval development of the goldfish (Carassius auratus L.) from Lake Erie. Ohio Journal of Science, vol. 40, No. 2: 82-93.

5. Battle, H. I. (1942).
Hepatogenesis of the atlantic salmon (Salmo salar L.) Canadian Journal of Research, vol. 20 (D.20): 79-97.

6. Battle, H. I. and K.K. Hisaoka (1952).
Effects of ethyl carbamate (urethan) on the early development of the teleost (Brachydanio rerio.) Cancer Research, vol. 12: 334-340.

7. Battle, H. I. and W. M. Sprules (1960).
A description of the semi-buoyant eggs and early developmental stages of the goldeye. Hiodon alosoides. (Rafinesque.) Journal of the Fisheries Research Board, Canada, vol. 17 (2): 245-266.

8. de Beer, Sir G. (1958).
Embryos and ancestors. Clarendon Press, pp. 197, p. 3.

9. Budd, P. L. (1940).
Development of the eggs and early larvae of six California fishes. Fish Bulletin No. 56, State of California Department of Natural Resources, Division of Fish and Game, Bureau of Marine Fisheries.

10. Caldwell, D. K. and C. R. Carlin (1962).
A photomicrographic adapter for stereomicroscopes useful in photographing fish larvae. Copeia, 1962, No. 2: 445-446.

11. Garside, E. T. (1959).
Some effects of oxygen in relation to temperature on the development of Lake trout embryos. Canadian Journal of Zoology 37: 689-98.

12. Goff, R. A. (1940).
The effects of increased atmospheric pressure on the developing embryo of the zebrafish. Brachydanio rerio (Hamilton). Kansas Academy of Science, Transactions, vol. 43: 401-410.

13. Hisaoka, K. K. (1955).
The response of the zebrafish embryo to the action of 2-acetylaminoflourene. Anatomical Record, vol. 122: 418.

14. Hisaoka, K. K. (1958).
The effects of 2-acetylaminoflourene on the embryonic development of the zebrafish. I. Morphological studies. Cancer Research, vol. 18: 527-535.

15. Hisaoka, K. K. (1958).
The effects of 2-acetylaminoflourene on the embryonic development of the zebrafish. II. Histochemical studies. Cancer Research, vol. 18: 664-667.

16. Hisaoka, K. K. (1958).
A comparison of the effects of certain teratogenic agents using histochemical methods. Anatomical Record, vol. 131: 566.

17. Hisaoka, K. K. (1958).
Microscopic studies of the teleost chorion. Transactions of the American Microscopical Society, vol. 77: 240-243.

18. Hisaoka, K. K. and H. I. Battle (1958).
The normal developmental stages of the zebrafish, Brachydanio rerio (Hamilton-Buchanan). Journal of Morphology, vol. 102, No. 2: 311-328.

19. Hisaoka, K. K. and C.F. Firlit (1960).
Further studies of the embryonic development of the zebrafish, Brachydanio rerio (Hamilton-Buchanan). Journal of Morphology, vol. 107: 205-225.

20. Hisaoka, K. K. and C. F. Firlit (1962).
The localisation of necleic acids during oögenesis in the zebrafish. The American Journal of Anatomy, vol. 110, No. 3: 203-216.

21. Hisaoka, K. K. and C. F. Firlit (1962).
The embryology of the blue gourami, Trichogaster trichopterus. (Pallas). Journal of Morphology, vol. 111, No. 3: 239-254.

22. Hisaoka, K. K. and C. F. Firlit (1962).
Ovarian cycle and egg production in the zebrafish. Brachydanio rerio (Hamilton-Buchanan). Copeia, 1962, No. 4: 788-792.

23. Hisaoka, K. K. and A. F. Hopper (1953).
The effects of certain nucleic acid inhibitors on the development of the zebrafish. Anatomical Record, vol. 117: 520.

24. Hisaoka, K. K. and A. F. Hopper (1957).
Some effects of barbituric acid, diethylbarbituric acid on the development of the zebrafish. Anatomical Record, vol. 129: 297-308.

25. Hisaoka, K. K., J.N. Ott and A. N. Marchese (1957).
A study of the normal development of the zebrafish by means of time-lapse photography and the phase contrast microscope. Motion picture film, Loyola University, Chicago, U.S.A.

26. Hopper, A. F. (1943).
The early embryology of Platypoecilus maculatus. Copeia 1943, No. 4: 218-224.

27. Hubbs, C. L. (1943).
Terminology of the early stages of fishes. Copeia 1943, No. 4: 260.

28. Jones, R. W. and M. N. Huffman (1957).
Fish embryos as bio-assay material in testing chemicals for effects on cell division and differentiation. Transactions of the American Microscopical Society, vol. 76: 177-183.

29. Kinne, O. and E. A. Kinne (1962).
Rates of development of embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen concentrations. Canadian Journal of Zoology, vol. 40: 231-253.

30. Leach, G. C. (1927).
Artificial propagation of pike-perch, yellow-perch and pikes. Appendix I. Report of the Commissioner of Fisheries, 1927: 1-27.

31. Legault, R. O. (1958).
A technique for controlling the time of daily spawning and collecting of eggs of the zebrafish, Brachydanio rerio (Hamilton-Buchanan). Copeia 1958, No. 4: 328-330.

32. Legault, R. O. (1961).
The early embryology of teleosts with special reference to Osmerus mordax, Brachydanio rerio and Percina caprodes. Ph.D. thesis, University of Michigan.

33. Legault, R. O. (1963).
Replacement of opaque covers on vertical fish incubators. Progressive fish-culturist vol. 25: No. 4: 221-222.

34. Lewis, W. H. (1943).
The role of the superficial gel layer in gastrulation of the zebrafish egg. Anatomical Record, vol. 85: 326.

35. Lewis, W. H. (1944).
The superficial gel layer and its role in development. Biological Bulletin, vol. 87: 154.

36. Lewis, W. H. (1947).
Mechanics of invagination. Anatomical Record, vol. 97: 139-156.

37. Lewis, W. H. and E. C. Roosen-Runge (1942).
The formation of the blastodisc in the egg of the zebrafish. Anatomical Record, vol. 84: 463.

38. Lewis, W. H. and E. C. Roosen-Runge (1944).
Development of the zebrafish egg. Motion picture film. The Wistar Institute, Philadelphia.

39. Mansueti, R. (1955).
Early development stages of the yellow perch studied. Maryland Tidewater News, vol. 12, No. 1

40. Marcotte, A. and Tremblay, J. -L. (1948).
Notes sur la biologie de l'Eperlan (Osmerus mordax, Mitchill) de la province de Quebec. Contributions de la Station biologique du Saint-Laurent, P.Q., Canada.

41. Mather, F. (1876).
Hatching yellow perch.
Forest and Stream, vol. 6: 51.

42. Mather, F. (1892).
Breeding habits of the perch. Transactions of the American Fisheries Society, 1891: 51-55.

43. Muncy, R. J. (1962).
Life history of the yellow perch, Perca flavescens, in estuarine waters of the Severn river. A tributary of Chesapeake Bay, Maryland. Chesapeake Science 1962, No. 3: 143-159.

44. Oppenheimer, J. M. (1937).
The normal stages of Fundulus heteroclitus. Anatomical Record, vol. 68: 1-8.

45. Roosen-Runge, E. C. (1936).
Firchung und Primitiventwicklung von Brachydanio rerio. Anat. Anz. vol. 81: 297-301.

46. Roosen-Runge, E. C. (1938).
On the early development - bipolar differentiation and cleavage of the zebrafish, Brachydanio rerio. Biological Bulletin, 75: 119-133.

47. Roosen-Runge, E. C. (1939).
Karyokinesis during cleavage of the zebrafish, Brachydanio rerio.
Ibid., 77: 79-91.

48. Rugh, R. (1948).
"Experimental Embryology" Burgess Publishing Co., Minneapolis, Minnesota, U.S.A.

49. Runyan, S. (1961).
Early development of the clingfish, Gobiesox strumosus Cope. Cheasapeake Science, vol. 2, Nos. 3 and 4: 113-141.

50. Ryder, J. A. (1884).
A contribution to the embryology of osseous fishes with special reference to the development of the cod (Gadus morrhua). U.S. Commissioner of Fisheries report. 1882: 455-606, 12 plates.

51. Shaw, E. S. (1955).
The embryology of the sergeant major, Abudefduf saxitilis.
Copeia, 1955, No. 2: 85-89.

52. Tavolga, W. N. and R. Rugh (1947).
Development of the platyfish, Platypoecilus maculatus.
Zoologica, vol.32: 1-15.

53. Wallich, C. (1901).
A method of recording egg development for the use of fish culturists. U.S. Fish Commission Report, 1900: 185-194, 1 plate.

54. Wilson, H. V. P. (1891).
The embryology of the sea bass (Serranus atrarius). U.S. Fish Commission. Bulletin, vol. 9: 209-277, 20 plates 12 figures.

Table 1

Numbers given to developmental conditions of features A to L.

Feature	Number	Developmental condition
A	1 to n	Number of somite pairs fully formed.
B	0	Embryo closely applied to yolk.
	1	Tail bud of embryo lifted from yolk.
C	0	Olfactory placodes not visible.
	1	Olfactory placodes present.
	2	Olfactory pits formed.
D	0	Optic anlagen absent.
	1	Optic anlagen present.
	2	Optic vesicles formed.
	3	Optic cups visible.
	4	Optic cups with isolated pigment cells.
	5	Optic cups with dense pigmentation.
E	0	Otic placodes not visible.
	1	Otic placodes visible.
	2	Otic vesicles containing minute otoliths.
	3	Otic capsules swollen, large otoliths.
F	0	Brain not visible.
	1	Brain solid.
	2	Cerebellar fold visible within brain.
	3	Brain with ventricles and thickenings.
G	0	Somatic pigmentation lacking.
	1	Scattered melanophores present.
	2	Melanophores in dense patches.
	3	Other pigment cells (e.g., Xanthophores).
H	0	Heart primordia not visible.
	1	Straight-tube heart, beating.
	2	Heart bent but with no valves.
	3	Heart with valves and thick walls.
I	0	Gut closed posteriorly.
	1	Gut open posteriorly.
J	0	Pectoral fin buds absent.
	1	Fin buds merely thickenings of [illegible]
	2	Fin buds large, standing away from embryo.
K	0	Median finfold absent.
	1	Finfold present, lacking fin-rays.
	2	Rudiments of caudal fin-rays visible.
L	0	Kupffer's vesicle not seen.
	1	Vesicle visible.
	2	Vesicle obliterated.

Table 2

Developmental data of the smelt.

(For interpretation of numbers in columns A to L, see Table 1).

A	B	C	D	E	F	G	H	I	J	K	L	CDH units at time of observation
0	0	0	1	0	1	0	0	0	0	0	0	600
1	0	0	1	0	1	0	0	0	0	0	0	690
2	0	0	1	0	1	0	0	0	0	0	0	675
4	0	0	2	0	1	0	0	0	0	0	0	810
5	0	0	2	0	1	0	0	0	0	0	0	810
10	0	0	2	0	1	0	0	0	0	0	0	850
12	0	0	2	0	1	0	0	0	0	0	1	900
13	0	0	2	0	1	0	0	0	0	0	1	910
14	0	0	3	0	1	0	0	0	0	0	1	870
19	0	0	3	1	1	0	0	0	0	0	1	810
20	0	0	3	1	1	0	0	0	0	0	1	850
21	0	0	3	1	1	0	0	0	0	0	1	935
22	0	0	3	1	2	0	0	0	0	0	1	1040
24	0	0	3	1	2	0	0	0	0	0	1	1030
25	1	0	3	1	3	0	0	0	0	0	1	1100
31	1	1	3	1	3	0	0	0	0	0	1	1290
32	1	1	3	1	3	0	0	0	0	0	2	1090
37	1	1	3	1	3	0	0	0	1	0	2	2760
40	1	1	3	1	3	0	1	0	1	0	2	1350
44	1	1	4	1	3	0	1	0	1	0	2	1475
45	1	1	4	2	3	0	1	0	1	1	2	1625
46	1	1	5	2	3	0	1	0	1	1	2	1530
51	1	1	5	2	3	0	1	0	1	1	2	1590
64	1	2	5	2	3	0	1	1	1	1	2	2760
64	1	2	5	2	3	0	2	1	1	1	2	3040
64	1	2	5	3	3	0	3	1	1	1	2	2260
64	1	2	5	3	3	0	3	1	2	1	2	3210
66	1	2	5	3	3	2	3	1	2	2	2	3280

Table 3

Developmental data of the zebrafish.

(For interpretation of numbers in columns A to L, see Table 1).

A	B	C	D	E	F	G	H	I	J	K	L	CDH units at time of observation
0	0	0	1	0	1	0	0	0	0	0	0	245
1/2	0	0	1	0	1	0	0	0	0	0	0	322
2	0	0	1	0	1	0	0	0	0	0	0	325
3	0	0	1	0	1	0	0	0	0	0	0	336
4	0	0	2/2	0	1	0	0	0	0	0	0	358
5	0	0	2	0	1	0	0	0	0	0	1/1	320
6	0	0	2	0	1	0	0	0	0	0	1	362
7	0	0	2	0	1	0	0	0	0	0	1	374
8	0	0	2	0	1	0	0	0	0	0	1	330
9	0	0	2	0	1	0	0	0	0	0	1	
10	0	0	2	0	1	0	0	0	0	0	1	
11	1/1	0	2	1/1	1	0	0	0	0	0	1	
12	1	0	2	1	1	0	0	0	0	0	1	
13	0	0	2	1	1	0	0	0	0	0	1	594
14	1	0	2	1	1	0	0	0	0	0	1	594
15	1	0	2	1	1	0	0	0	0	0	1	594
16	1	0	2	1	1	0	0	0	0	0	1	618
17	1	0	2	1	1	0	0	0	0	0	1	618
18	1	0	2	1	1	0	0	0	0	0	1	
19	1	0	2	1	2/2	0	0	0	0	0	1	
20	1	1/1	3	1	2	0	0	0	0	0	2	
22	1	1	3	1	2	0	0	0	0	0	2	
(27)	1	1	3	(2)	3	0	0	0	0	0	2	624
28	1	(2)	4	2	3	0	1/1	0	0	1/1	2	648
29	1	2	5	2	3	1/2	1	0	0	1	2	695
32	1	2	5	3	3	2	2	0	0	1	2	888
33	1	2	5	3	3	2	3	1/1	1/2	2	2	1250
34	1	2	5	3	3	3	3	1	2	2	2	2392

Table 4

Developmental data of the yellow perch.

(For interpretation of numbers in columns A to L, see Table 1).

A	B	C	D	E	F	G	H	I	J	K	L	CDH units at time of observation
0	0	0	1	0	1	0	0	0	0	0	1	820
3	0	0	1	0	1	0	0	0	0	0	1	1680
4	0	0	2	0	1	0	0	0	0	0	1	870
5	0	0	2	0	1	0	0	0	0	0	1	870
7	0	0	2	0	1	0	0	0	0	0	1	1000
10	0	0	3	0	1	0	0	0	0	0	1	1900
13	0	0	3	0	1	0	0	0	0	0	1	1080
15	0	1	3	1	1	0	0	0	0	0	1	1100
16	0	1	3	1	1	0	0	0	0	0	1	1080
17	0	1	3	1	1	0	0	0	1	0	1	1464
18	1	1	3	1	1	0	0	0	1	0	1	1850
22	1	1	3	1	3	0	0	0	1	0	2	1920
24	1	1	3	1	3	0	0	0	1	0	2	2160
27	1	1	3	1	3	0	0	0	1	0	2	1480
29	1	1	3	1	3	0	0	0	1	0	2	1680
30	1	(2)	3	1	3	0	0	0	1	1	2	1838
(31)	1	2	3	(2)	3	1	1	0	1	1	2	2640
c36	1	2	3	2	3	1	2	0	1	1	2	2400
42	1	2	3	2	3	1	2	0	2	1	2	3210
42	1	2	3	2	3	2	3	1	2	1	2	3220
42	1	2	4	3	3	3	3	1	2	2	2	3600
c44	1	2	4	3	3	3	3	1	2	2	2	4000
c44	1	2	5	3	3	3	3	1	2	2	2	5255

(c = circa)

Table 5

Relative time of attainment of previously defined conditions in the development of features A to L.

i. Smelt

	Y series	
		ABCDEFGHIJKL
X series	A	-aaaaaaaaaaa
	B	p-apppaaaaap
	C	pp-pppaaaaap
	D	paa-aaaaaaaa
	E	paap-aaaaaap
	F	paapp-aaaaap
	G	pppppp-ppppp
	H	ppppppa-apap
	I	ppppppap-ppp
	J	pppppppaaa-ap
	K	ppppppapap-p
	L	paapaaaaaaa-

ii. Zebrafish

	Y series	
		ABCDEFGHIJKL
X series	A	-aaaaaaaaaaa
	B	p-apsaaaaaap
	C	pp-pppaaaaap
	D	paa-aaaaaaaa
	E	psap-aaaaaap
	F	ppapp-aaaaap
	G	pppppp-paapp
	H	ppppppa-aasp
	I	pppppppp-spp
	J	ppppppppps-pp
	K	pppppppasaa-p
	L	paapaaaaaaa-

iii. Perch

	Y series	
		ABCDEFGHIJKL
X series	A	-aaaaaaaaaap
	B	p-pppaaaapap
	C	pa-psaaaaaap
	D	paa-aaaaaaap
	E	pasp-aaaaaap
	F	ppppp-aaapap
	G	pppppp-sappp
	H	ppppppps-appp
	I	pppppppp-ppp
	J	papppaaaa-ap
	K	pppppppaaap-p
	L	saaaaaaaaaa-

Explanation of symbols:

a.. Where the condition chosen in the X series A...L appears in development before the stage chosen in the Y series A...L.

p.. Where the condition chosen in the X series A...L appears in development after the stage chosen in the Y series A...L.

s.. Where the conditions in both the X and Y series apparently arise simultaneously.

A to L - the twelve variables considered - vide Table 1.

Table 6

Relative times of attainment of previously defined conditions in the development of features A to L.

	A	B	C	D	E	F	G	H	I	J	K	L
A	---	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aap
B	ppp	---	aap	ppp	psp	paa	aaa	aaa	aaa	aap	aaa	ppp
C	ppp	ppa	---	ppp	pps	ppa	aaa	aaa	aaa	aaa	aaa	ppp
D	ppp	aaa	aaa	---	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aap
E	ppp	asa	aas	ppp	---	aaa	aaa	aaa	aaa	aaa	aaa	ppp
F	ppp	app	aap	ppp	ppp	---	aaa	aaa	aaa	aap	aaa	ppp
G	ppp	ppp	ppp	ppp	ppp	ppp	---	pps	paa	pap	ppp	ppp
H	ppp	ppp	ppp	ppp	ppp	ppp	aas	---	aaa	pap	asp	ppp
I	ppp	ppp	ppp	ppp	ppp	ppp	app	ppp	---	psp	ppp	ppp
J	ppp	ppa	ppp	ppp	ppp	ppa	apa	apa	asa	---	apa	ppp
K	ppp	ppp	ppp	ppp	ppp	ppp	aaa	psa	aaa	pap	---	ppp
L	ppa	aaa	aaa	ppa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	---

Explanations of symbols:

The capital and small letters have the same meaning as in Table 5.

In each trio of letters, the first refers to the smelt, the second to the zebrafish and the third to the perch.

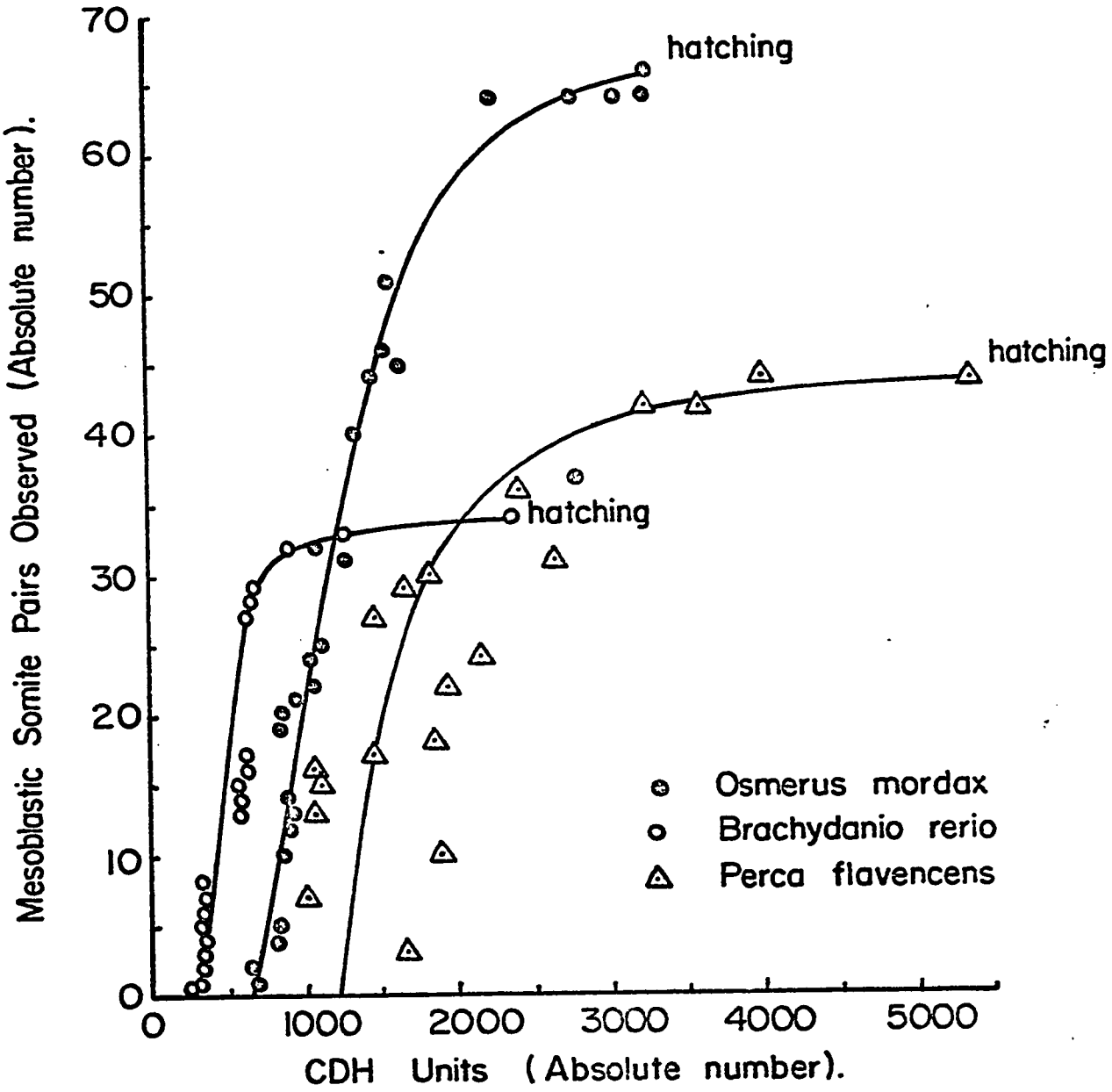


FIGURE I.
Increase in absolute number of observed somite pairs as function of absolute CDH units.

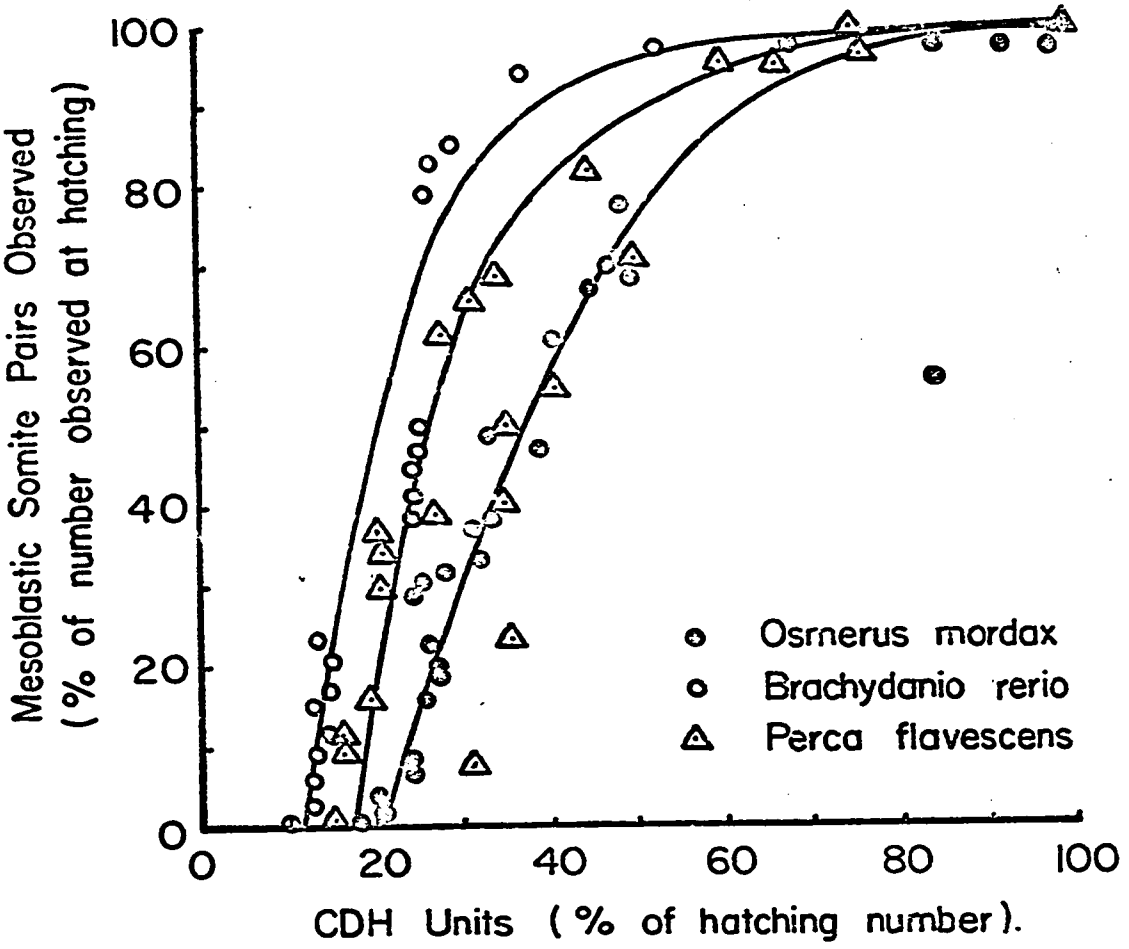


FIGURE 2.
Increase in relative number of observed somite pairs as function of CDH units (% of hatching number of CDH units).

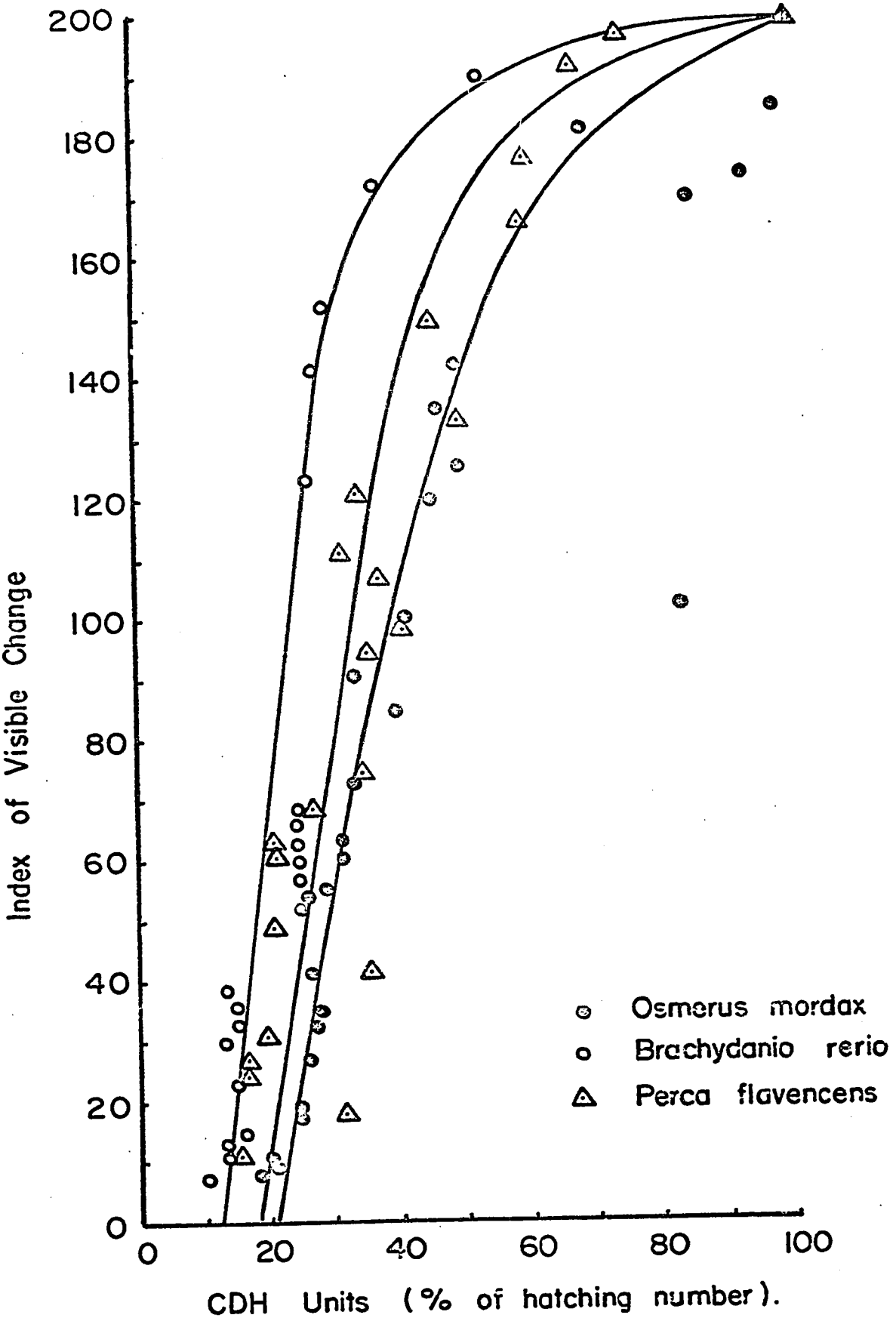


FIGURE 3:
Increase in Index of Visible Change as a function of CDH units (% of hatching number of CDH units.)

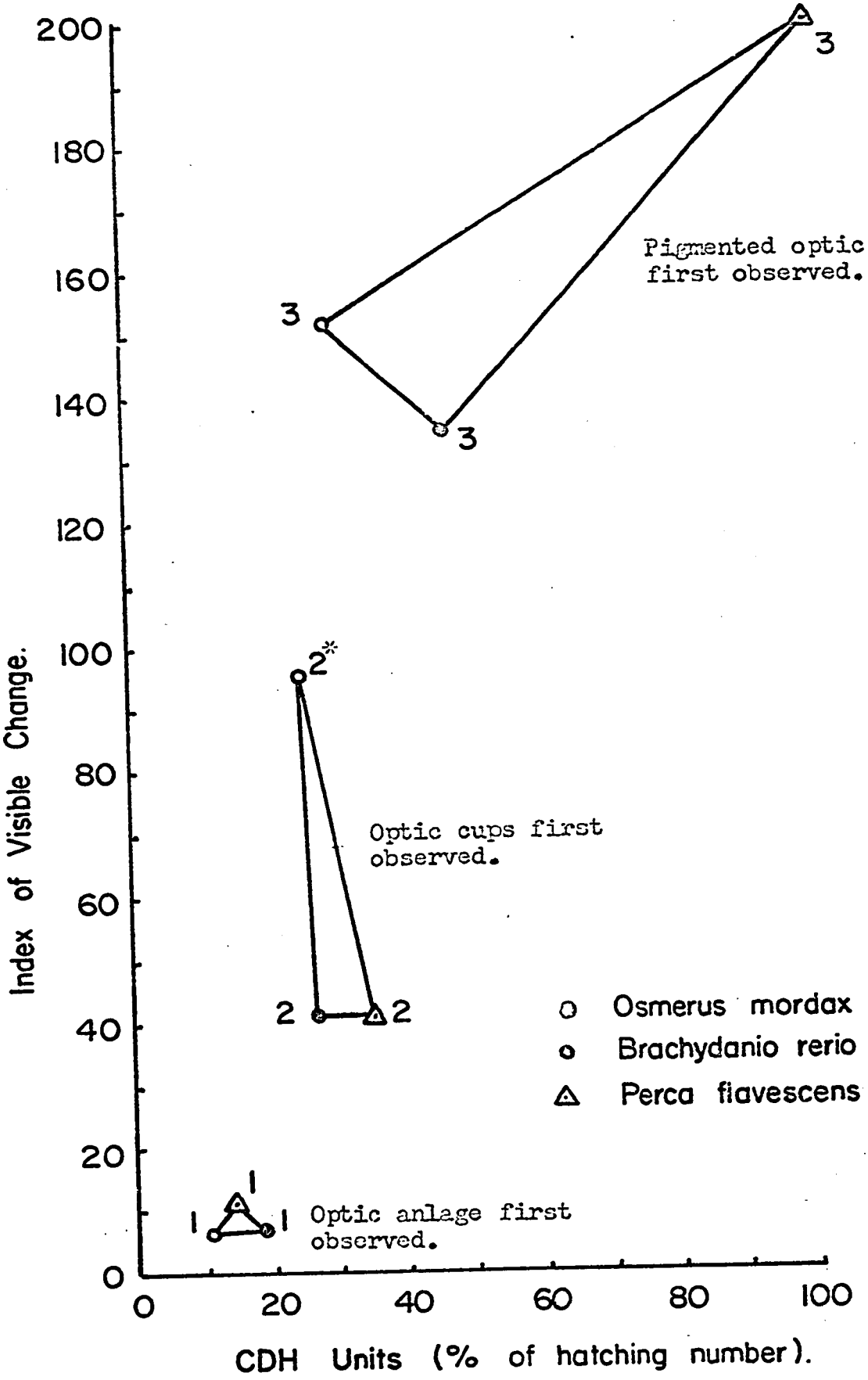


FIGURE 4.
Dispersion of like points of eye development within the CDH and Index of Visible Change axes.

APPENDIX

AN ILLUSTRATED ACCOUNT OF THE POSTGASTRULAR EMBRYOLOGY OF THE SMELT, ZEBRAFISH AND PERCH.

The following descriptions are based on observations of living eggs, made at a magnification not exceeding 50X with a stereobinocular microscope.

The figures enclosed within the first bracket before each paragraph constitute the coded description of the embryo. The second brakcet contains the CDH value of the embryo.

i. Development of the American smelt.
(000101000000) (600 CDH.) Figs. 1 and 2, Plate I.

The keel is visible after about sixty hours of development under the conditions described in the second chapter. At first, the keel appears as a shallow wedge of tissue extending down into the yolk mass. With time the keel becomes deeper and narrower in cross-section. Legault (32), describes the formation of the keel as the anteriad migration of material from the germ ring, in the region of the embryonic shield. The gastropore (blastopore), closes prior to the formation of the first pair of somites. The anterior end of the embryo shows the presence of lateral extensions of the solid central

nervous system. These lateral extensions are the optic anlagen. The embryo is closely applied to the yolk along its length, merging gently with the yolk at its anterior and posterior ends. The yolk contains numerous small, highly refractile oil globules.

(100101000000) (690 CDH.) Fig. 3, Plate I, Fig. 15, Plate III.

The embryo shows the presence of the first pair of somites after about sixty-nine hours. The somite pair is situated about half way between the anterior and posterior limits of the embryo. The anterior and posterior ends of the embryo are seen to slope gently into the yolk. Anteriorly (in dorsal view, Fig. 15, Pl. III) are seen the optic anlagen (arrowed) coming from the brain region. A cavity is still absent from the optic anlage and the brain at this time. The embryo fails to exhibit the structure known as Kupffer's vesicle, but perhaps this is obscured by oil droplets. Kupffer's vesicle represents the remnants of the archenteron according to Wilson (54), who described it in the sea bass. The archenteron was defined by Wilson as being that real or virtual space that exists between the endoderm lamella and the periblast and yolk. The yolk is seen to contain numerous small colourless oil globules, and two or three large yellowish oil globules at this time.

(200101000000) (675 CDH.) (not figured)

Two pairs of mesoblastic somites present. There is no appreciable difference between this stage and the last one described, except for the extra pair of somites.

(400201000000) (810 CDH.) Fig. 4, Plate I, Fig. 16, Plate III.

Four pairs of somites present. The optic anlage are seen to contain a cavity at this time and they are now known as the optic evaginations or vesicles. The somites are seen to be developing about half way along the length of the embryo. In Figure 4, mesoderm is seen continuing posteriorly from the rearmost pair of somites in an undifferentiated condition. The keel can be seen in the photograph of the lateral view as the dark crescent between the embryo and the yolk.

(500201000000) (810 CDH.) (not figured)

Five pairs of somites present. Apart from the increase in the number of somites this condition is similar to that last described except for a slight increase in length of the embryonic axis.

(10,00201000000) (850 CDH.) (not figured)

Ten pairs of somites present. Apart from the increase in number of somites, this condition is similar to that last described.

(12,00201000001) (900 CDH.) Fig. 5, Plate I, Fig. 17, Plate III.

Twelve pairs of somites present. The optic evaginations

are seen to be elongate and laterally compressed in dorsal view (Fig. 17). The embryo is now seen to be distinct from the yolk, but the tail bud shows no lifting posteriorly. Kupffer's vesicle can be seen at the posterior end of the axis (arrowed.)

(13,00201000001) (910 CDH.) (not figured)

Thirteen pairs of somites present. This condition is essentially similar to that just described except for the increase in number of somite pairs.

(14,00301000001) (870 CDH.) Fig. 6, Plate I.

Fourteen pairs of somites present. The extent to which the embryo has encircled the yolk can be seen in Fig. 6. In dorsal view (not shown) the optic vesicles are seen to be invaginating to produce the optic cups. In lateral view these structures are seen to have increased in vertical height. The anterior end of the embryo is showing a more marked junction with the yolk than in the previously described conditions, i.e., the slope of the front of the head is becoming steeper. Kupffer's vesicle is visible posteriorly.

(19,00311000001) (810 CDH.) (not figured)

Nineteen pairs of somites present. The embryo is essentially similar to the last described stage, except for the increase in number of somite pairs, the greater amount of optic invagination, and the presence, mid-way between the eyes and the first pair of somites, of the

otic placodes, i.e. the precursors of the ear. The otic placodes are thickenings of the endoderm that eventually invaginate.

(20,00311000001) (850 CDH.) (not figured)

Twenty pairs of somites present. Apart from the increase in number of somite pairs, this condition is essentially the same as that last described.

(21,00311000001) (935,1040 CDH. Av-987 CDH.)

Apart from the increase in number of the somite pairs, this condition is essentially similar to that last described. Kupffer's vesicle is seen posteriorly.

(22,00312000001) (1040 CDH.) Fig. 7, Plate I, Fig. 20, Plate III.

The anterior end of the embryo is seen to be much increased in thickness. The optic cups are seen to be fully formed with the choroid fissure visible in the anteroventral region. The otic placodes are visible. The brain is seen to exhibit the presence of a neurocele, and in addition to this a tranverse fold in the region just between the optic and otic areas. This transverse fold noted is equated with that named by Wilson (54), as the cerebellar fold of Serranus atrarius. Kupffer's vesicle can be clearly seen in the postero-ventral view photographed (Figure 20, Plate III.)

(24,00312000001) (1030 CDH.) (not figured)

Apart from the increase in number of somites, this condition is essentially the same as that last described.

(25,10313000001) (1100 CDH.) (not figured)

Twenty-five pairs of somites present. The optic cups are fully formed but lack pigment. The otic placode is surrounded by an elliptical region which is translucent. This translucent region is thought to appear due to the invagination of the otic placodes to form the otic vesicles. The tail bud is lifting away from the yolk mass at this time. Kupffer's vesicle is clearly seen at the junction of the lifting tail bud and the yolk mass. The cerebellar fold is clearly seen within the brain and the medulla is seen to lie posterior to it. The latter portion of the brain is seen to contain the fourth ventricle, which is diamond shaped in dorsal view.

(31,11313000001) (1290 CDH.) Fig. 8, Plate II.

Thirty-one pairs of somites are present. The olfactory placodes are visible. The photograph of this stage shows the cerebellar fold clearly (arrowed), along with the lifting that has occured posteriorly (arrowed). Kupffer's vesicle is still visible in vivo, but is obscured in the figure by the globules of oil.

(32,11313000002) (1090 CDH.) Fig. 9, Plate II.

Thirty-two pairs of somites are present. Similar to the last described condition except for the increase in number of pairs of somites and the disappearance of Kupffer's vesicle.

(37,11313000102) (2760 CDH. anomalous.) Fig. 10, Plate II.

Thirty-seven pairs of somites are present. Similar to the last described condition except for the presence of pectoral fin buds (arrowed). These are seen as bulges on the sides of the embryo, just dorsal to the yolk.

(40,11313010102) (1350 CDH.) Fig. 11, Plate II, Fig. 18, Plate III.

Forty pairs of somites present. The optic cups still lack pigmentation and otoliths are not yet present in the otic capsules. A dorsal view of the brain is shown in Fig. 18, Plate III. Under the embryo, at the anterior end posterior to the eyes, is a straight tube heart which exhibits peristaltic contraction, in a postermedial direction.

(44,11413010102) (1330, 1620, CDH. Av-1475 CDH.) Figs. 12 & 13, Plate II, Fig. 19, Plate III.

Forty-four pairs of somites present. Essentially the same as the last described condition, except for the additional somite pairs and slight optic pigmentation.

(45,11423010112) (1625 CDH.) Fig. 14, Plate I.

Forty-five pairs of somites are present. The optic [illegible] are slightly pigmented and [illegible] present within the otic vesicle and appear as minute, equal dots. The heart is still a straight tube, beating under the embryo. The embryo makes slight movements within the chorion. The pectoral fin buds are still small. The median fin is present in a rudimentary

fashion but does not extend far forward. Large yellow oil globules are present in the yolk mass.

(46,11523010112) (1530 CDH.) (not figured)

About forty-six pairs of somites are present. The optic cups show pigmentation. The heart is seen to be still in the form of a straight tube. The embryo exhibits much movement within the membrane. This condition is similar to that last described.

(51,11523010112) (1360, 1820 CDH. Av-1590 CDH.) Fig. 21, Plate IV.

More than 70% of the hatching number of somites are present, the actual number of somites observed being fifty-one. The olfactory placodes are present anteriorly, between the eyes, (not visible in figure). The optic cups show pigmentation and the otic capsule contains otoliths that are minute and equal in size. The gut is seen to open posteriorly at a point about two thirds of the distance between the yolk and the end of the tail. The heart is still a straight tube. Both the median and pectoral fins are small. The anterior end of the embryo is seen to be slightly lifted off the yolk mass at this time (arrowed).

(64,12523011112) (2760 CDH.) Fig. 22, Plate IV, Fig. 26, Plate V.

More than sixty-four pairs of somites present, this being more than 70% of the hatching number of somites. The olfactory placodes have invaginated to form

olfactory pits. The otic vesicle has changed from an elliptical to an oval shape. Two otoliths are still minute and equal. Somatic pigmentation is seen to be lacking over the body. The gut is seen to open posteriorly (arrowed, Fig. 22). The median and pectoral fins remain small. The embryo is seen to be lifted slightly at the anterior end. The embryo photographed had a length of 2.7mm.

(64,12523021112) (3040 CDH.) Fig. 27, Plate V.

More than 70% of the hatching number of somites are present. The optic cups are seen to be deeply pigmented. The otic capsule is larger and more elaborate than in previous conditions, the otoliths however remain small. The heart shows some bending. The pectoral fin bud is still small and the median fin extends as far foreward as the level of the pectoral fin bud dorsally. The embryo photographed had a length of 3.45mm.

(64,12533031112) (2260 CDH.) Fig. 23, Plate IV.

More than 70% of the hatching number of somites are present. Anteriorly the head is seen to be lifting from the yolk mass. The otic capsule is seen to be swollen and the otoliths are unequal in size. The heart shows development to the point where individual chambers can be seen. The pectoral fin buds are small, but the median fin is both more extensive and deeper. The embryo photographed had a length of 3.8mm.

(64,12533031212) (3210 CDH.) Fig. 24, Plate IV, Fig. 28, Plate V.

More than 70% of the hatching number of somites are present. The otic capsules are seen to be much swollen and the otoliths occupy the lower portion of the capsule, the dorsal half of which is seen to contain cavities and chamber. The heart is distinctly bent at the posterior end of the ventricle (arrowed Fig. 28). The chamber in which the heart is lying, the pericardium, is seen to be distended. Scattered melanophores are seen over the surface of the yolk mass and pericardium. The pectoral fin buds are larger, and the median fin, as seen in the photograph, deeper. The embryo photographed had a length of 4.0mm.

(66,12533231222) (3280 CDH.) Figs. 25, 29, 30, 31, 32.

There are between sixty-four and sixty-eight somites present when the embryo hatches. The olfactory openings are clearly seen at this time, ventro-medial to the optic cups (arrowed Fig. 30, Plate V). The eyes are large and densely pigmented and can be seen to project from the head in Figs. 30 and 31. These dorsal views reveal that the lens is present within the eye. The otoliths are seen as large irregular concretions. The brain is not too clearly seen at this stage due to the interference of the optic cups and otic capsules with lateral observation. Large melanophores are present over the pericardium, the central part of the trunk and

the yolk. The area of the vent (arrowed) and the ventral part of the tail are seen to be the sites of dense pigmentation, (Fig. 32). The mouth is open as is seen in the dorsal and ventral views of the head. There is no evidence that there is present in the pharynx any supporting tissue. The muscles of the embryo show a yellowish colouration at this time. The pectoral fins are large and the median fins are seen to contain the rudiments of fin rays posteriorly in the caudal region. The notochord can be seen extending as far forward as the otic capsules in Figs. 30 and 31. The embryo photographed measured 5.3mm in length.

ii. Development of the zebrafish.

(000101000000) (245 CDH.) (not figured)

The keel is visible in the zebrafish embryo after about eight or nine hours of development at twenty-seven degrees centigrade. The embryo is seen as a thickening of the blastoderm, closely applied to the surface of the yolk. The anterior end of the axis shows the presence of optic anlagen as lateral extensions of the solid brain anlage mass. The posterior end of the embryo is close to the gastropore (blastopore), which is still open at this time.

(100101000000) (322 CDH.) Fig. 33, Plate VI.

The first pair of mesoblastic somites is seen to lie to each side of the embryonic axis half way along the length of the embryo. The anterior and posterior ends of the

embryo slope gently down to the level of the yolk. The optic anlagencan be seen anteriorly as tear-shaped extensions of the solid nervous system. Both the brain and the optic anlagen lack a cavity at this time. Both lateral and frontal views of the embryo reveal that the keel descends into substance of the yolk in a wedge shape. The mass of material comprising the keel or axial cord, contains the precursor of the nerve cord above and precursor of the notochord below. Hisaoka and Firlit (19). In frontal view (not figured) the lateral mesoderm can be seen, descending ventrolaterally from the somite mesoderm.

(200101000000) (Av. of thirteen observations - 325.5 CDH.) Fig. 34, Plate VI.

Two pairs of somites present. The gastropore is seen to be closed at this time. Apart from the closure of the gastropore and the addition of the second pair of somites this condition is essentially similar to that just described.

(300101000000) (Av. of twelve observations - 336 CDH.) Fig. 35.

Three pairs of somites present. Apart from the increase in number of somites this condition is essentially similar to that last described.

(400201000000) (Av. of eleven observations - 358. 4 CDH.) Fig. 36, Plate VI.

Four pairs of mesoblastic somites present. The optic anlagen have now developed a cavity and are known as

optic vesicles or evaginations. The anterior part of the brain develops a cavity at this time according to Hisaoka and Battle (18). The cavity of the optic vesicle can be faintly discerned in Fig. 36, Plate IV.

(500201000001) (av. of eight observations - 320.6 CDH.) Fig. 37, Plate VI, Fig. 46, Plate VIII.

Five pairs of somites present. Although not seen in vivo, the olfactory placodes are perhaps indicated in the photograph of this stage. From a study of sections of this condition, Hisaoka and Firlit (19), state that the placodes are present at this time. Because they were not actually seen in the developing embryo, the placodes are represented as absent in the digital designation of the stage, in order to make comparisons possible with other species for which information from sections was lacking. Similarly, although Hisaoka and Firlit (19) maintain that the otic placodes are present at this time, they were not observed until later in development, again the feature was considered to be absent for the purposes of this study. The optic anlagen are seen in the dorsal view of Fig. 46, Plate VIII. The lateral photograph of this stage is taken obliquely and reveals two important features of the embryo at this time. First, the optic evaginations can be seen to contain a cavity as mentioned in the last described condition. Secondly, the photograph shows the presence of Kupffer's vesicle that develops posteriorly. The vesicle

is seen as the small highly refractile region at the posterior end of the embryonic axis. The origin of the vesicle is explained in the description of the development of the American smelt.

(600201000001) (Av. of thirteen observations - 362 CDH.) Fig. 38. Plate VI, Fig. 47, Plate VIII.

Six pairs of somites are present. The optic evaginations can be seen in dorsal view in the second of the figures listed above. Apart from the increase in number of somites and a slight increase in length, the embryo is similar to the condition last described.

(700201000001) (Av. of three observations - 374 CDH.) Fig. 39, Plate VII, Fig. 48, Plate VIII.

Seven pairs of somites are present. Apart from a small increase in width of the posterior part of the optic evaginations, as can be seen in the dorsal view of Fig. 47, and the addition of another pair of somites, this condition is similar to the condition last described.

(800201000001) (324, 336, CDH. Av. 330 CDH.) Fig. 40, Plate VII, Fig. 49, Plate VIII.

Eight pairs of somites are present. The optic evaginations are seen to be rounded in dorsal view. The anterior and posterior limits of the embryo are more clearly marked off from the yolk than in previous conditions. This increase in definition of the embryonic mass is particularly true of the anterior end of the embryo which slopes more steeply down to meet the yolk (cf. Figs. 39 and 40). Kupffer's vesicle can be clearly

seen in the posterior part of the embryo.

(900201000001) (Unknown) Fig. 41, Plate VII, Fig. 50, Plate VIII.

Nine pairs of somites are present. The embryo is seen to extend almost all the way around the yolk mass. The optic evaginations are visible in the dorsal view at this stage. Although the embryo is long, there is not, as yet, any lifting of the tail bud away from the yolk (vide Fig. 41).

(10,00201000001) (Unknown) Fig. 42, Plate VII.

Ten pairs of somites are present. The shape of the individual somites when viewed laterally are seen to be in a different condition from that previously seen, in that there is bending of the block of tissue to produce an angle, point-forward. The process of bending of the muscle block continues during development until the adult somite shape is achieved. In the photograph the notochord can be seen running between the somite blocks (arrowed). This stage exhibits the start of the process known as 'lifting', whereby the tail bud lifts away from the yolk mass. The process starts by an increase of the slope of the posterior facet of the embryo. This slope becomes steeper and steeper until the posterior end of the embryo becomes undercut. In the photograph of this condition, the undercutting is just about to start and the angle of the junction, embryo to yolk, is about ninety degrees. Along with the process of lifting,

in the zebrafish, there occurs the process of subdivision of the yolk. The yolk starts off being spherical, however, it eventually becomes subdivided into an anterior globose portion and a posterior cylindrical mass. In this stage, the embryo is departing from its cylindrical shape and assuming a pyriform condition, this condition being intermediate between the divided and undivided states. This last change is not obvious in the photograph.

(11,10211000001) (Unknown) Fig. 43, Plate VII, Fig. 51, Plate VIII.

Eleven pairs of somites present. The processes that were indicated to be occurring in the previous stage are here continued. The lifting is slight, the posterior end of the embryo is seen to be undercut. The yolk mass shows the presence of a furrow (arrowed) just below the posterior end of the embryo; this furrow is the start of the subdivision mentioned in the last stage. The otic placodes are visible half way between the first pair of somites and the optic region. Kupffer's vesicle can be seen in the side view of the embryo in Fig. 43.

(12,10211000001) (Unknown) Fig. 44, Plate VII, Fig. 52, Plate VIII.

Twelve pairs of somites are present. The optic evaginations are seen to be larger in side view, as for that matter is the whole anterior portion of the embryo.

The posterior margin of the head is seen to be delimited from the rest of the embryo by the presence of an angular shape of the dorsal surface. The otic placodes are present. The constriction of the yolk mass is clearly seen in Fig. 44, as in Kupffer's vesicle.

(13,10211000001) (594 CDH.) (not figured)

Thirteen pairs of somites are present. The processes of lifting and subdivision of the yolk continue. Apart from these gradual changes, the embryo is essentially the same as the condition last described, except for the addition of the extra pair of somites.

(14,10211000001) (594 CDH.) (not figured)

Fourteen pairs of somites present. Apart from the increase in number of somites, the embryo is similar to that stage just described.

(15,10211000001) (594 CDH.) (not figured)

Fifteen pairs of somites are present. Apart from the increase in the number of somite pairs, the embryo is similar to that last described. The gradual lifting of the tail and subdivision of the yolk continue.

(16,10211000001) (618 CDH.) Fig. 45, Plate VII, Fig. 53, Plate VIII.

Sixteen pairs of somites are present. The appearance of the embryo can be judged from Fig. 45. This figure shows the complete subdivision of the yolk into the two components previously mentioned. In the dorsal view (Fig. 52) the optic evaginations are clearly seen, this

view also shows the central nervous system, the cavity of which cannot be seen.

(17,10211000001) (618 CDH.) (not figured)

Apart from the addition of the extra pair of somites this condition is essentially the same as that just described.

(18,10211000001) (CDH. Unknown) (not figured)

Apart from the additional pair of somites, this condition is similar to that just described.

(19,10212000001) (CDH. Unknown) (not figured)

Nineteen pairs of somites are present. The contours of the central nervous system are visible through the wall of the anterior end of the embryo, in lateral view. The process of lifting continues.

(20,11312000002) (CDH. Unknown) (not figured)

Twenty pairs of somites are present. The olfactory placodes are visible at this time. Hisaoka and Firlit (19), say that at this time the olfactory placodes undergo thickening. The optic evaginations are seen to have invaginated to form optic cups. The central nervous system is still solid posterior to the otic placodes (Hisaoka and Firlit 19). Kupffer's vesicle is no longer visible.

(22,11312000002) (CDH. Unknown) (not figured)

Apart from the increase in the number of somites there is no marked difference between this and the previously described condition.

(27,11323000002) (624 CDH.) Fig. 56, Plate IX.

Twenty-seven pairs of somites are present. This number is greater than 70% of the hatching number of somites. The embryo is seen to be much elongated posteriorly with a long tail extending behind the yolk. The olfactory placodes are present anteriorly between the optic cups. The optic cups are seen to lack any pigment and possess an open choroid fissure in their ventral margin. The otic vesicle is seen to have been formed and contains two minute, equal otoliths. The brain is divided into regions and the ventricles can be seen. The cerebellar fold can be discerned with the medulla posterior to it. Somatic pigmentation is lacking over the whole of the embryo. The gut has not broken out posteriorly. The pectoral fin bud is not visible, and the median fin fold is completely lacking. Kupffer's vesicle is not visible. The notochord extends the whole length of the embryo from the level of the otic vesicles to the posterior part of the tail where it continues into the undifferentiated mesoderm. The embryo can move slightly by contracting the somites just posterior to the constriction of the yolk mass. The length of the embryo in the photograph was 2.7mm.

(28,124230100012) (648 CDH.) Fig. 57, Plate IX.

Twenty-eight pairs of somites are present, this number being in excess of 70% of the hatching number of

somites. The embryo is much elongated posteriorly. Anteriorly in the embryo, the olfactory placodes have invaginated to form olfactory pits. There is seen to be very faint pigmentation of the retina. The choroid fissure is closed and the optic cup contains a lens at its mouth. The otic region shows the presence of two minute otoliths. These otoliths, which are equal in size, are contained within an oval vesicle which in turn is surrounded by an elliptical region of translucent material. The brain is clearly seen to contain ventricles, the cerebellar fold can be clearly seen in the photograph of this stage. Posterior to the cerebellar fold is the fourth ventricle, which in dorsal view is diamond-shaped. The posterior part of the ventricle is long and tapering. Under the anterior end of the embryo, slightly to the left of the mid-line, the heart can be seen in the form of a straight tube which pulsates slowly, (about 60 beats per minute at 27°C.). Allen and Mukay (2) report that the heart of the paradise fish also develops to the left of the mid-line. Close observation of the pulsating heart shows that it beats by peristalsis and that the wave of contraction moves from the wide distal portion of the tube, to the narrow proximal portion in a posteromedial direction. There is no circulation over the surface of the yolk or within the tail at this time. Somatic

pigmentation is completely lacking from the embryo. Although the pectoral fin buds are still absent, the median fin is seen to be present in a rudimentary fashion. The latter fin extends forward ventrally only to the level of the posterior limit of the yolk. The notochord is seen running between the somites and containing vacuolated cells. The embryo is sensitive to changes in light intensity and can move around within the membrane with ease. The embryo photographed had a length of 2.7mm.

(29,12523110012) (783, 676, 628 CDH. Av-695 CDH.) Figs. 54, 55, 58, Plate IX.

Twenty-nine pairs of somites are present, this number being in excess of 70% of the hatching number. The most anterior pair of somites is just behind the otic region, on the same tangent to the yolk as the optic cups. Openings to the olfactory pits can be seen at the anterior end of the embryo, medial to the eyes. The optic cups are pigmented and the choroid fissure is closed. Small, equal sized otoliths are seen within the otic capsule which is again surrounded by an area of translucent material. The degree of brain development can be seen in the various photographs. Over the anterior part of the yolk can be seen scattered melanophores. The heart is beating and is still a straight tube. The circulation can be followed in the caudal region of the embryo; the blood flows posteriad in the

dorsal vessel and anteriad in the ventral vessel. From the dorsal and ventral vessels can be seen the small vessels conveying blood to the various tissues above and below the two vessels and returning blood from these areas. The pectoral fin buds are still not present in the embryo. The median fin, while extensive and extending a long way anteriorly, is still rudimentary in that the edges are ragged and ill defined. The embryo is active at this time and can be released with no difficulty, whereupon it can swim with ease.

(32,12533220012) (837, 850, 972 CDH. Av-888 CDH.)
Fig. 59, Plate X.

Thirty-two pairs of somites are present. The embryo is now fish-like with a long caudal region and a small yolk mass. The olfactory pits are seen with their openings near the inner margins of the eyes. In the eye can be seen the lens and there is pigmentation present. The choroid fissure is closed. In the otic capsule can be seen the equal sized, large otoliths. The cerebellar fold is seen and is thick anteroventrally. There are large dendritic melanophores over the surface of the yolk and parts of the embryo. The heart shows the start of the bending process that will convert the juvenile straight tube, to the adult 'S' shape. The circulation is established in the tail and over the surface of the yolk. The gut is still closed posteriorly, the median fin is present, but the pectoral

fin buds are still not visible. The notochord is visible and is seen to contain vacuolated cells. The embryo is sensitive to movement of the water, or changes of light intensity and can move with ease within the membrane.

(33,12533231122) (1250 CDH.) Fig. 60, Plate X.

Thirty-three somites are present. The olfactory region is similar to that described in the last described stage. The optic cups are large and well pigmented, a lens is present and the choroid fissure is closed. The otic capsule shows an increase in complexity, in that it has expanded dorsally and the otoliths are confined to the lower half. The otoliths themselves are large irregularly shaped concretions that are unequal in size. Noticeable within the brain is the fourth ventricle which is diamond shaped, extending posteriorly as far as the otic region. The melanophores are distributed over the anterior part of the head, dorsal to the olfactory organs and between the eyes. Two lines of melanophores extend posteriorly, following the walls of the medulla. These two bands of melanophores join in the region above the posterior end of the fourth ventricle, the area directly over the ventricle being left free of melanophores. Laterally, bands of melanophores follow the gut and extend into the caudal region. There is a band of melanophores running

vertically down the yolk at its anterior end. The heart is seen to beat rapidly and shows much bending and contortion. The returning vessel from the caudal region is poorly differentiated posterior to the vent region, however it is more definite anterior to this opening. The pathway of the blood from the vessels on the yolk can be seen. The gut is seen to have opened posteriorly and the vent so produced causes an interruption in the ventral part of the median fold. The pectoral fin bud is seen in that area of the embryo that lies directly over the yolk mass. In the posterior part of the median fin fold the beginnings of the caudal fin rays can be discerned. The embryo is active and sensitive to change in the environment, upon removal from the membrane, the embryo can swim well. The length of the embryo photographed was 3.6mm.

(34,12533331222) (1872, 2068, 2392 CDH. Av-2110 CDH.) Fig. 61, 62, 63, 64, Plate X.

Prior to hatching the embryo appears as in Fig. 61. Thirty-four pairs of somites are present. The embryo is essentially similar to the condition last described except for increase in pigmentation and somite number and a decrease in size of the yolk mass. In addition to the melanophores being present as in previously described conditions, there are also present choromatophores which give the embryo a yellowish colouration. The otic capsule is large and elaborate as seen in both lateral

and dorsal views. The eyes are seen to move often in a manner that keeps them in parallel at all times. The melanophores are seen in an expanded condition in Fig. 62, and in a contracted condition in Fig. 63.

iii Development of the yellow perch.

(000101000001) (820 CDH.) Figs. 65, 66, 67, Plate XI.

The keel was observed as a shallow wedge, extending below the embryo, in eggs of the first culture, after eighty-two hours of development. The keel continues to develop until after ninety-six hours it is seen as an acute wedge in frontal view Fig. 66. At this time Kupffer's vesicle is observable at the posterior end of the embryonic axis (arrowed, Fig. 67). Anteriorly, the optic anlagen can be seen as lateral extensions of the embryonic axis. The anlagenare broad posteriorly and narrow anteriorly thus when viewed together from the dorsal aspect, they are seen to form a heart shape. Fig. 65 shows a ventral oblique view of the egg with the blastophore (gastropore) still open.

(300101000001) (1680 CDH.) Fig. 68, Plate XI.

Three pairs of somites are present. The embryo is closely applied to the yolk mass. Anteriorly and posteriorly the embryo slopes down to the general level of the yolk. The first pairs of somites are seen to form more posteriorly than in the other two species. Optic anlagen are present anteriorly and may be seen if the

embryo is manipulated until the dorsal surface is exposed. Kupffer's vesicle can be seen posteriorly. The embryo is seen to float with the yolk uppermost - a position that makes observation of the embryo rather difficult. Within the yolk is a large, eccentrically placed oil globule.

(400201000001) (870 CDH.) Fig. 69, Plate XI.

Four pairs of somites are present. The optic anlagen have changed to optic evaginations by the formation of a cavity within the masses of tissue, this cavity is visible in Fig. 69. The anterior end of the embryo is seen to be better defined from the yolk mass, than in previous stages, by the formation of a steep slope on its anterior surface. The embryo is still closely applied to the surface of the yolk in the future caudal region.

(500201000001) (870 CDH.) Fig. 70, Plate XI.

Five pairs of somites are present. The embryo is like that just described, except for the addition of the extra pair of somites. Kupffer's vesicle is present posteriorly.

(700201000001) (1000 CDH.) Fig. 77, Plate XII.

Ten pairs of somites are present. This stage is similar to that last described, except for the addition of the extra pair of somites and the condition of the optic region. The optic evaginations have invaginated to form

the structure known as the optic cup, which shows in the frontal view given in Fig. 77, which also reveals the presence of a cavity in the anterior part of the brain.

(13,00301000001) (1080 CDH.) Fig. 71, Plate XI.

Thirteen pairs of somites are present. Apart from the increase in number of somite pairs there is little to distinguish this condition from that last described. There is a slight increase in the length of the embryonic axis and the optic cups are further invaginated. The posterior end of the caudal region is more marked in this stage than in that previously described.

(15,01311000001) (1100 CDH.) Fig. 72, Plate XII.

Fifteen pairs of somites are present. Two features distinguish this stage from that just described. First, the olfactory placodes are seen anterior to the optic cups, secondly, the otic placodes are visible posterior to the optic cups. Kupffer's vesicle is still seen posteriorly. The oil globule of the yolk has moved anteriorly to lie under the head.

(16,01311000001) (1080 CDH.) (not figured)

Six pairs of somites are present. The embryo is essentially the same in appearance as the condition last described.

(17,01311000101) (1464 CDH.) (not figured)

Seventeen pairs of somites are present. Optic invagination reaches its greatest extent at this time.

Kupffer's vesicle is present. At a point directly over the yolk mass there is an interruption of the line of contact between the embryo and the yolk. This is caused by a thickening of the tissue in this area which will eventually develop into the pectoral fin. The median fin fold is absent.

(18,11311000101) (1850 CDH.) (not figured)

Eighteen pairs of somites are present. This condition resembles that previously described, except for an indication of the start of lifting of the caudal bud from the yolk mass. Kupffer's vesicle is still present posteriorly.

(22,11313000102) (1920 CDH.) Fig. 73, Plate XII, Fig. 78, Plate XII.

Twenty-two pairs of somites are present. Lifting of the caudal mass is seen posteriorly but is not extensive. The olfactory placodes are seen. The optic cups are fully formed but they lack pigmentation. The otic placodes have not yet invaginated. The brain is clearly seen in the lateral view and the cerebellar fold is conspicuous. The hind brain has a segmental appearance, the walls of the medulla being notched on their dorsal surface. In the posterior part of the embryo the mesoderm is observed, extending beyond the somites in an undifferentiated condition. Kupffer's vesicle is not present.

(24,11313000102) (2160 CDH.) Fig. 74, Plate XII.

Twenty-four pairs of somites are present. The otic placodes have invaginated to form vesicles contained within an elliptical region of translucent material. Apart from this change the embryo is similar to the last described condition.

(27,11313000102) (1480 CDH.) (not figured)

Twenty-seven pairs of somites are present. Except for the addition of the extra somite pairs, this condition resembles the last described.

(29,11313000102) (1680 CDH.) Fig. 79, Plate XII.

Twenty-nine pairs of somites are present. Essentially the same as the condition just described, except for the additional number of somite pairs.

(30,12313000112) (2016, 1300, 2200 CDH. Av-1838 CDH.) Fig. 75, Plate XII.

Thirty pairs of somites are present. Olfactory pits are seen, formed by the invagination of the olfactory placodes. The optic cups still lack pigmentation, but show the presence of the lens and the choroid fissure. The choroid fissure is still open. The pectoral fin bud is seen and the median fin fold is present in a rudimentary fashion with the edges ragged and ill-defined. The median fin fold extends forward dorsally and ventrally only to the level of the junction of the tail and the yolk. The embryo can move slightly within the membrane by flexing the somites of the trunk and tail.

(31,12323110112) (2640 CDH.) Fig. 76, Plate XII.

Thirty-one pairs of somites are present. The optic cups as yet lack pigmentation. Within the otic vesicles are seen two minute otoliths which are equal in size. The brain shows the presence of ventricles, particularly noticeable being the fourth ventricle lying behind the cerebellar fold. Anterior to the fold can be seen other cavities within the brain. Under the anterior part of the embryo can be seen a straight tube heart which beats slowly. There are scattered melanophores over the surface of the yolk and parts of the embryo. The pectoral fin buds and median fin fold are still small.

(36,12323120112) (2400 CDH.) Fig. 80, Plate XIII.

About thirty-six pairs of somites are present, this number being in excess of 70% of the hatching number of somites. Anteromedial to the eyes are seen the olfactory organs with their openings to the exterior. The optic cups still lack pigmentation. The otoliths present within the otic vesicles are still minute and equal. Over the surface of the embryo there is present a light distribution of small melanophores. The heart is seen to have a slight kink in it that heralds the greater changes that will occur later in development to produce the adult heart form. Posteriorly, the gut is seen to be still closed. The pectoral fin bud is still

small and the median fin extends on the dorsal surface anteriorly only to a point opposite the posterior limit of the yolk. The oil globule is seen to lie anteriorly within the yolk mass. The embryo is capable of violent movement within the membrane.

(42,12323120212) (3210 CDH.) Fig. 81, Plate XIII.

About forty-two pairs of somites present, this number being in excess of 70% of the hatching number of somite pairs. The olfactory organs are seen as simple pits opening to the exterior. The optic cups still lack pigment. Two small otoliths are present within the otic vesicle. The development of the brain can be seen in the photograph. The somatic pigmentation is in the form of small scattered melanophores over the surface of the trunk, tail and yolk. The heart is beating slowly and is seen to be more contorted than in previous stages. The gut is still closed posteriorly. The pectoral fins are seen to be standing away from the body of the embryo. The median fin is seen to extend anteriorly as far as a point on the dorsal surface of the embryo half way between the level of the pectoral fin bud and the optic region. The single large oil globule is seen to be positioned anteriorly in the yolk mass. The embryo exhibits violent movement within the membrane.

(42,12323231212) (3220 CDH.) Fig. 82, Plate XIII.

More than forty-two pairs of somites are present, this number being in excess of 70% of the total hatching number. This condition is similar to that just described except in the following features. The somite number has increased by one or two pairs. The somatic pigmentation is more intensive and widespread and the heart is more bent upon itself. The gut opens posteriorly and the vent so produced is associated with an interruption of the ventral portion of the median fin fold. The median fin is deeper, however, it does not contain any rudimentary fin-rays. The embryo moves violently within the membrane. The embryo photographed had a length of 3.9mm.

(42,12433331222) (3600 CDH.) Fig. 83, Plate XIII.

More than forty-two pairs of somites present, this number being in excess of 70% of the hatching number of somite pairs. The olfactory organs are observed to be surrounded by a circular cushion of tissue in which there are seen to be minute circular pits. The eventual fate or functions of these pits are not known. The optic cups show the first signs of pigmentation, in the form of small separate pigment cells. The otic capsules are seen to be enlarged and different in shape when compared to previous conditions. While previously the vesicles were elliptical, they are now seen to be

squarish with rounded corners. The two equal sized otoliths occupy the lower part of the capsule. The brain is elaborate at this time. The photograph of the lateral aspect of this embryo shows the structure that is taken to be the epiphyseal stalk on the anterodorsal surface of the brain (arrowed). There is a great deal of somatic pigmentation. The heart is completely bent into an 'S' shape. The pectoral fin buds are seen to be large and raised off the surface of the trunk, the median fin contains the rudiments of caudal fin rays posteriorly. Dorsally the median fin fold extends as far foreward as the level of the pectoral fin bud. The length of the embryo photographed was 4.5mm.

(44,12433331222) (circa 4000 CDH.) Fig. 84, Plate XIV.

About forty-four pairs of somites present, this number being in excess of 70% of the total hatching number of somites. This condition is similar to the condition last described except in the following particulars. The otoliths are large irregular concretions but they are still approximately equal in size. There is even more extensive somatic pigmentation than in previous conditions. Anteriorly the cephalic flexure is seen to be unbending, resulting in the freeing of the head from the yolk.

(44,12533331222) (5255 CDH. Av. of 4 observations) Figs. 85, 86, 87, Plate XIV.

The hatching condition has many features in common with

the last described stage, however, the embryo will be fully described. The embryo is large and fragile and the egg membrane ruptures easily if the water is stirred. The olfactory organs open to the exterior and the openings are surrounded by a cushion of tissue containing pits. The optic cups are deeply pigmented and are seen to contain a lens in dorsal view. The otic capsule is large and elaborate. The somatic pigmentation consists of large dendritic melanophores over the whole of the embryo and yolk, particularly that part of the yolk near to the oil globule. In the caudal region the melanophores are seen to have a serial arrangement corresponding to the junctions between adjacent somites. The heart is seen to contain different chambers separated by valves. The different chambers have walls of different thickness. There can be observed waves of contraction passing through the heart from posterior to anterior. The gut opens posteriorly at the vent. The mouth is open at hatching, but the actual time of opening precedes hatching by about fifty hours. The pectoral fins are large at hatching, their extent can be seen in Fig. 86. The median fin which is deep all along its length, contains rudimentary fin rays posteriorly. The median fin extends forward dorsally to a point anterior to the pectoral fins. The anterior end of the embryo shows the presence of much supporting

tissue in the region of the jaws and gills, the latter are seen to be open. The embryo exhibits violent spontaneous movements before hatching. The embryo photographed had a length of 6.65mm.

PLATE I

American smelt

1. 000101000000 Frontal view. 600 CDH. X 35 Keel arrowed.

2. 000101000000 Lateral view. 600 CDH. X 35 Optic anlagen arrowed.

3. 100101000000 Lateral view. 690 CDH. X 35 First somites arrowed.

4. 400201000000 Lateral view 810 CDH. X 35 Undifferentiated mesoblast arrowed.

5. 12,00201000001 Lateral view. 900 CDH. X 35 Kupffer's vesicle arrowed.

6. 14,00301000001 Lateral view. 870 CDH. X 35

7. 22,00312000001 Lateral view. 1040 CDH. X 35 Cerebellar fold and Kupffer's vesicle arrowed.

Plate I

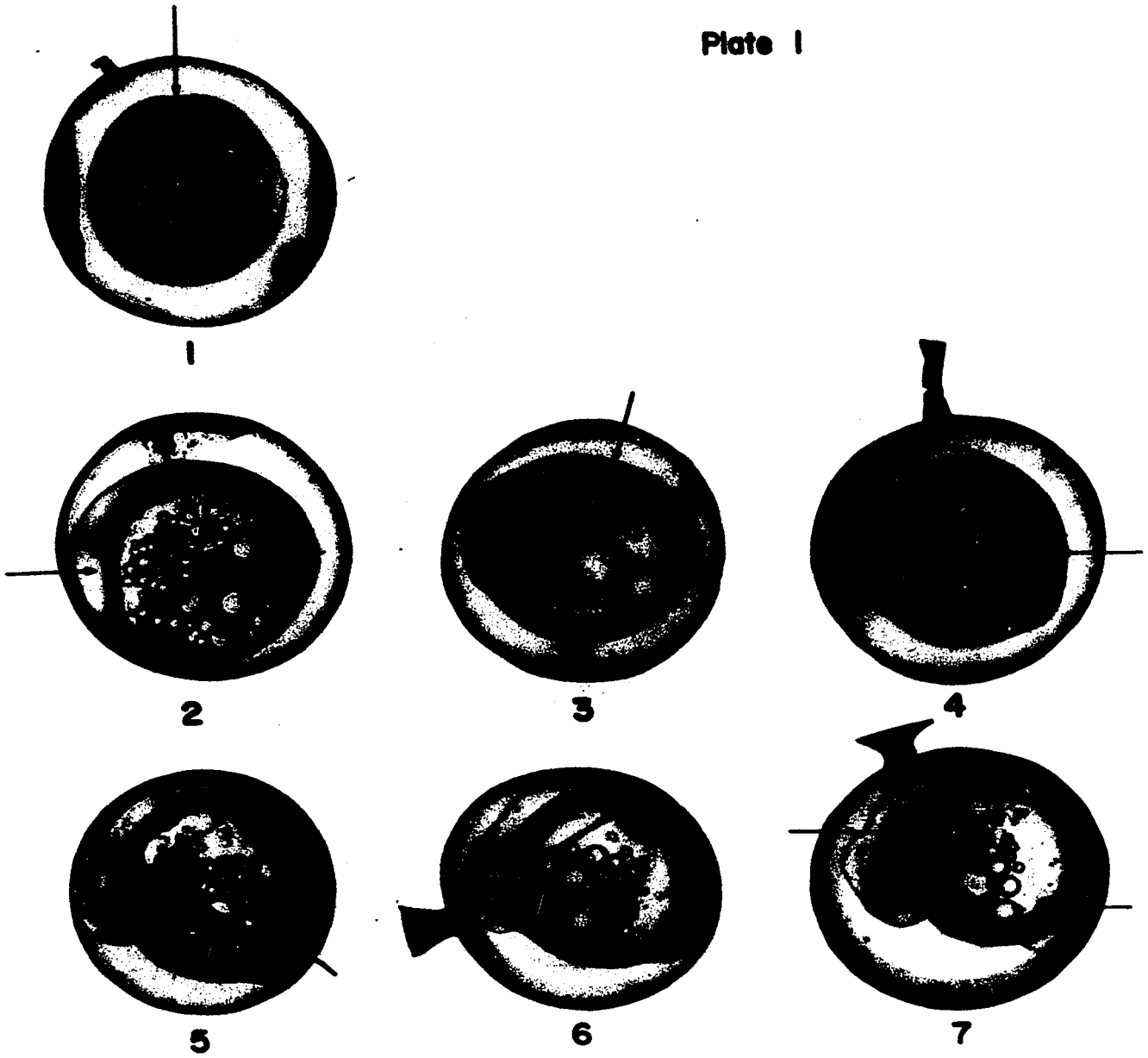


Plate I

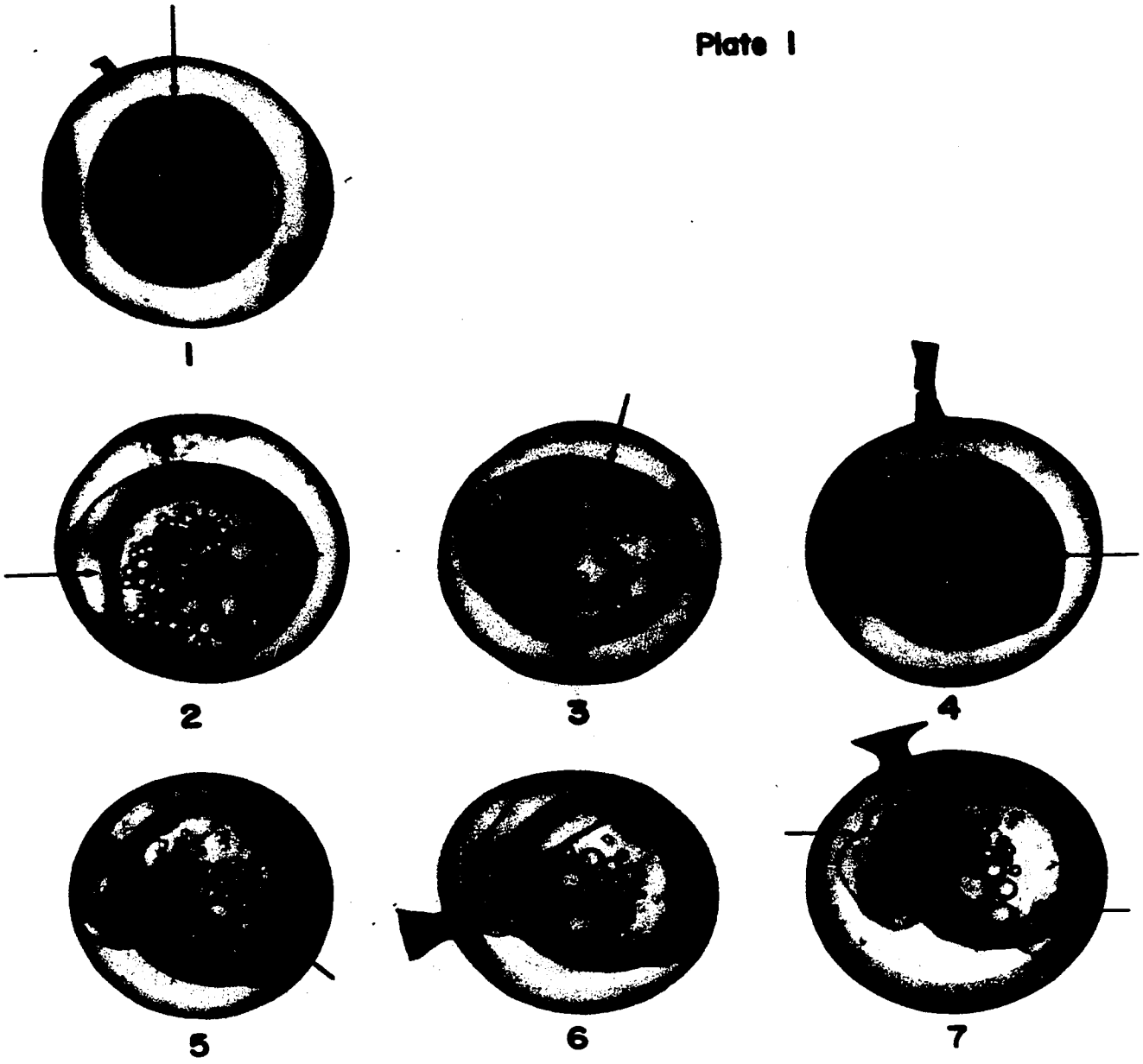
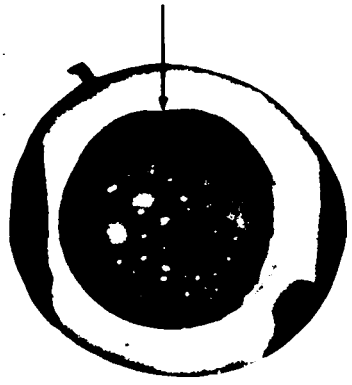


Plate I



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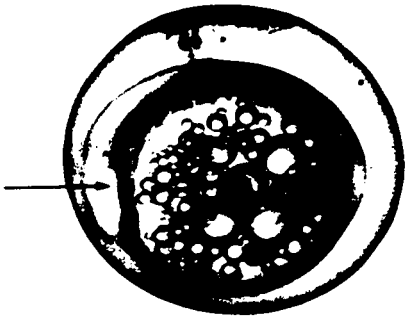

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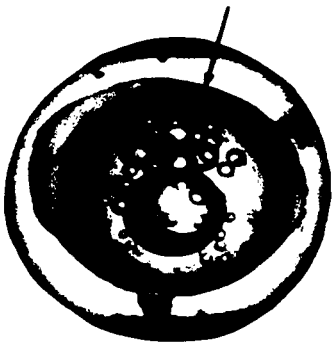

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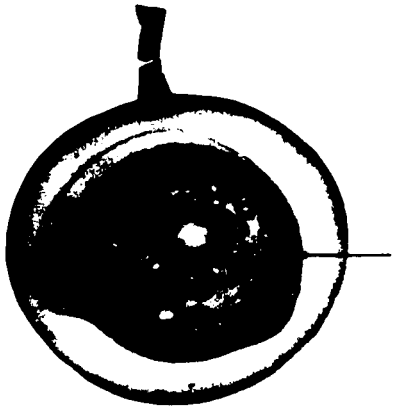

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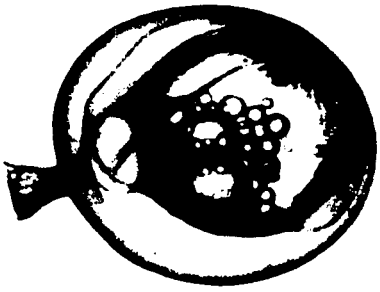

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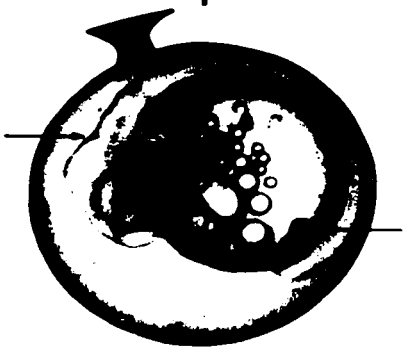

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PLATE II

American smelt - cont.

8. 31,11313000001 1290 CDH. X 35
Lateral view. Cerebellar fold and caudal lifting arrowed.

9. 32,11313000002 1090 CDH. X 35
Lateral view.

10. 37,11313000102 2760 CDH. (anomalous) X 35
Decapsulated. Lateral view.
Pectoral fin buds arrowed.

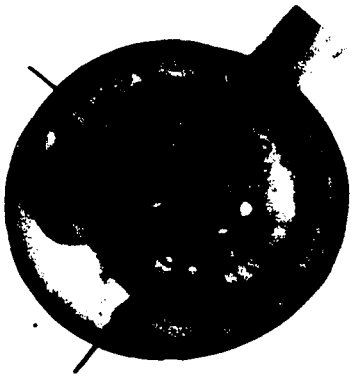
11. 40,11313010102 1350 CDH. X 35
Lateral view.

12. 44,11413010102 1475 CDH. X 35
Lateral view.

13. 44,11413010102 1475 CDH. X 35
Decapsulated. Lateral view.

14. 45,11423010112 1625 CDH. X 35
Oblique view.

Plate II



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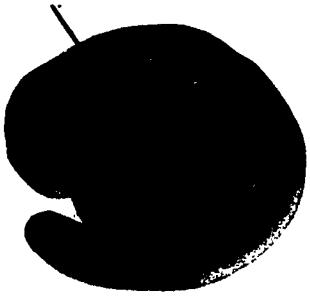

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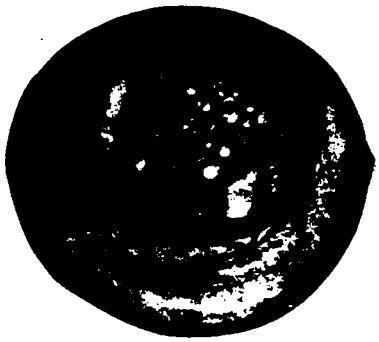

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Plate 11

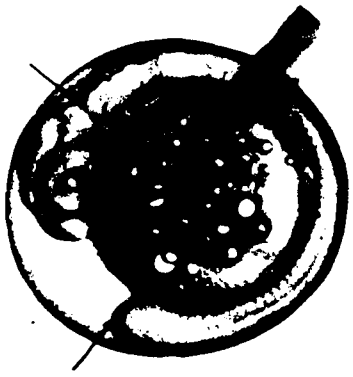

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PLATE III

American smelt - cont.

15. 100101000000 690 CDH. X 40
Dorsal view of head. Optic anlage arrowed.

16. 400201000000 810 CDH. X 40
Dorsal view of head. Holdfast arrowed.

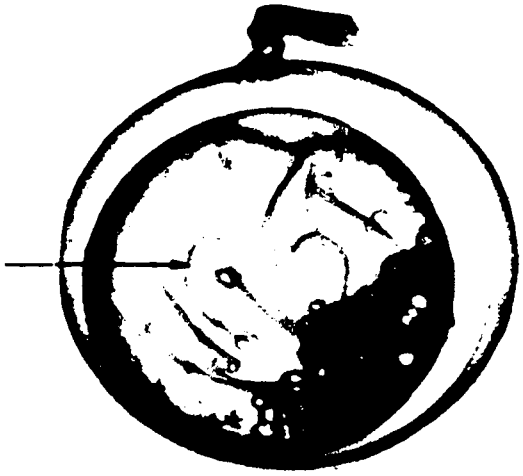
17. 12,00201000001 900 CDH. X 40
Dorsal view of head.

18. 40,11313010102 1350 CDH. X 40
Dorsal view of head.

19. 44,11413010102 1475 CDH. X 40
Dorsal view of head.

20. 22,00312000001 1040 CDH. X 40
Dorsal view of caudal region. Kupffer's vesicle arrowed.

Plate III



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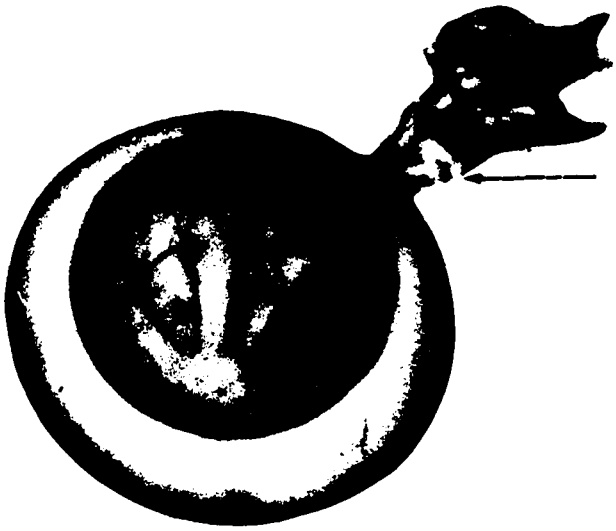

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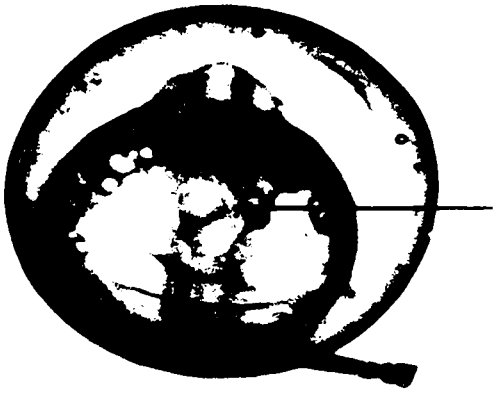

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PLATE IV

American smelt - cont.

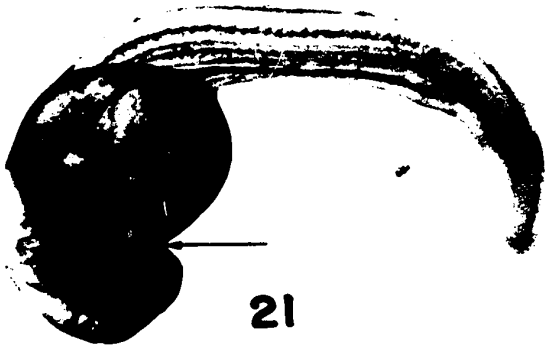
21. 51,11523010112 1590 CDH. X 40
Decapsulated. Lateral view.
Lifting of anterior end arrowed.

22. 64,12523011112 2760 CDH. 2.7mm.
Decapsulated. Lateral view.
Posterior opening of cut arrowed.

23. 64,12533031112 2260 CDH. 3.8mm.
Decapsulated. Lateral view.

24. 64,12533031212 3210 CDH. 4.0mm.
Decapsulated. Lateral view.

Plate IV



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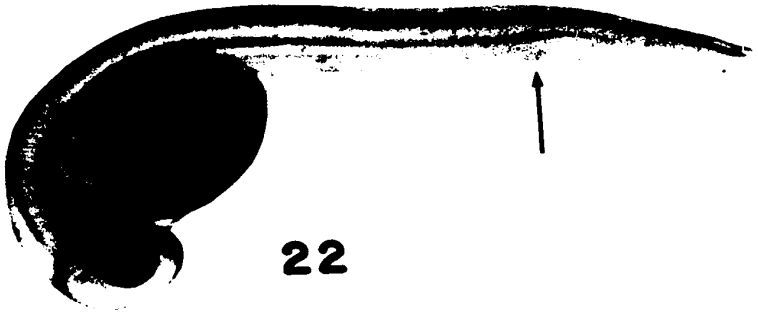

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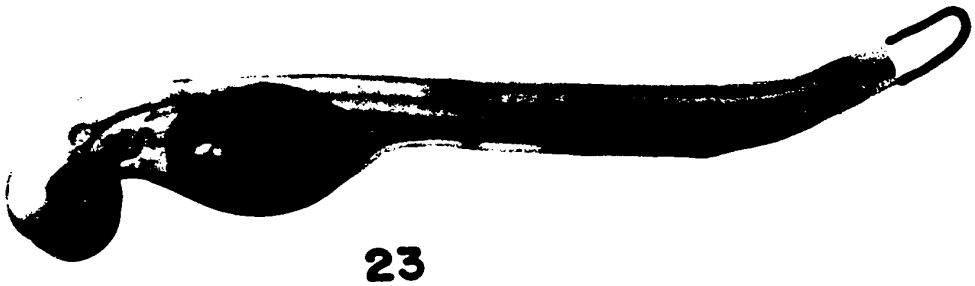

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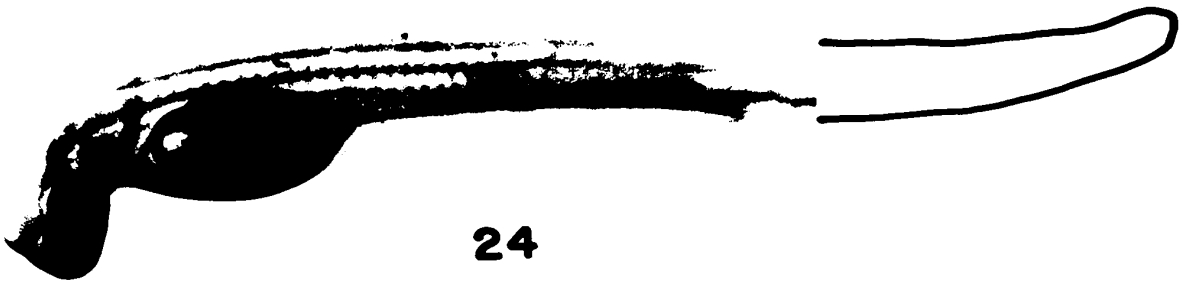

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PLATE V

American smelt - cont.

25. 66,12533231222 3280 CDH. 5.3mm. Hatching condition. Lateral view.

26. 64,12523011112 2760 CDH. 2.7mm. Decapsulated. Lateral view.

27. 64,12523021112 3040 CDH. 3.45mm. Decapsulated. Lateral view.

28. 64,12533031212 3210 CDH. 4.0mm. Decapsulated. Lateral view. Heart arrowed.

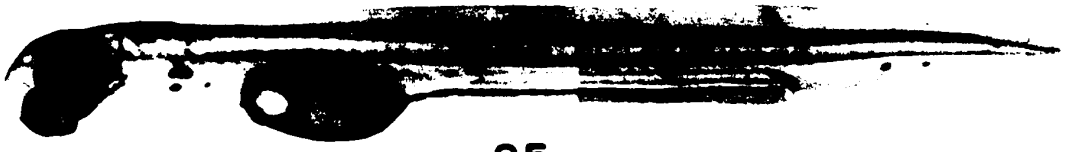
29. 66,12533231222 3280 CDH. 5.3mm. Hatching condition. Lateral view of anterior end.

30. 66,12533231222 3280 CDH. 5.3mm. Hatching condition. Ventral view of head. Olfactory openings arrowed.

31. 66,12533231222 3280 CDH. 5.3mm. Hatching condition. Dorsal view of head. Anterior end of notochord arrowed.

32. 66,12533231222 3280 CDH. 5.3mm. Hatching condition. Lateral view of tail. Pigmented region in region of gut opening arrowed. Note rudimentary rays in caudal fin.

Plate V



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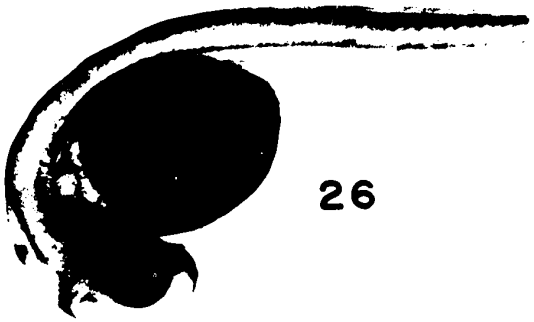

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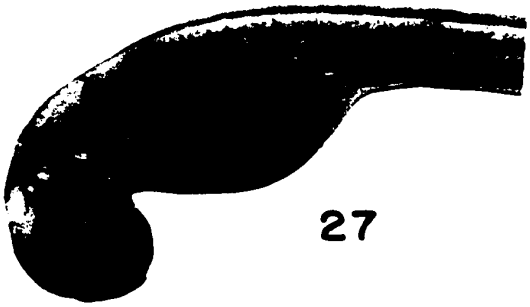

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PLATE VI

Zebrafish

33. 100101000000 Lateral view. pair arrowed. 322 CDH. X 50 First somite

34. 200101000000 Lateral view. 325.5 CDH. X 50

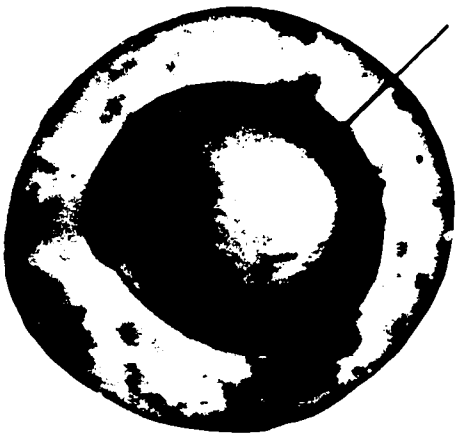
35. 300101000000 Lateral view. 336 CDH. X 50

36. 400201000000 Lateral view. 358.4 CDH. X 50

37. 500201000001 Lateral view. 320.6 CDH. X 50 Kupffer's vesicle arrowed.

38. 600201000001 Lateral view. 362 CDH. X 50

Plate VI



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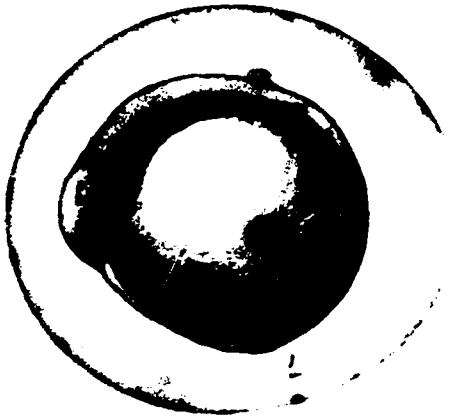

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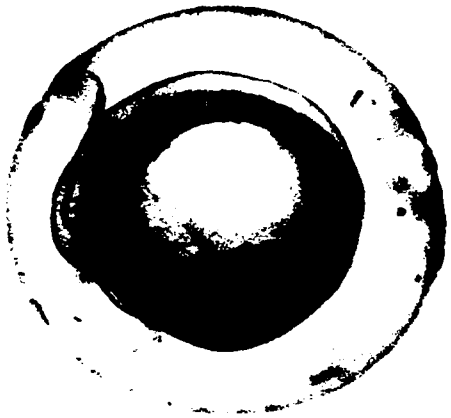

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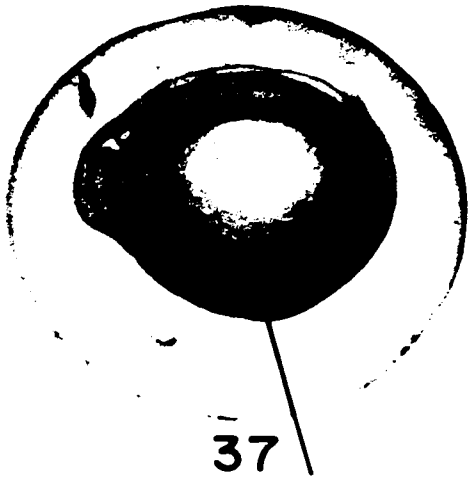

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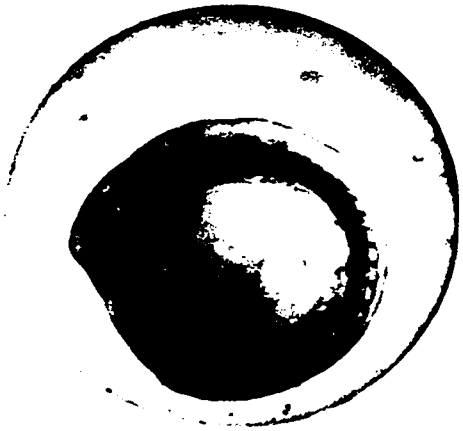

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PLATE VII

Zebrafish - cont.

39. 700201000001 374 CDH. X 35
Lateral view.

40. 800201000001 330 CDH. X 35
Lateral view.

41. 900201000001 CDH. unknown. X 35
Lateral view. Undifferentiated mesoderm arrowed.

42. 10,00201000001 CDH. unknown. X 35
Lateral view. Notochord arrowed.

43. 11,10211000001 CDH. unknown. X 35
Lateral view. Start of furrow of yolk arrowed.

44. 12,10211000001 CDH. unknown. X 35
Lateral view.

45. 16,10211000001 618 CDH. X 35
Lateral view.

Plate VII

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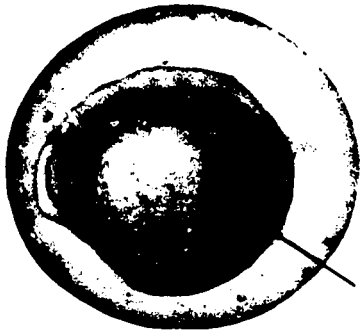

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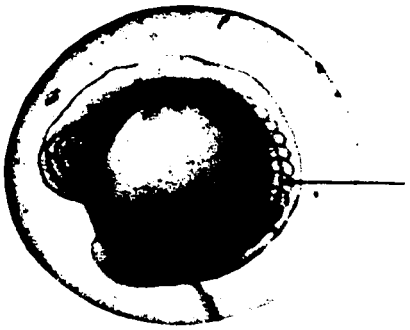

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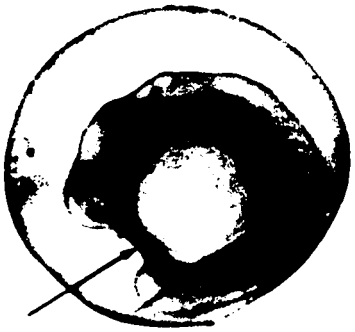

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PLATE VIII

Zebrafish - cont.

46. 500201000001 Dorsal view. Optic anlagen arrowed. 320.6 CDH. X 35

47. 600201000001 Dorsal view. 362 CDH. X 35

48. 700201000001 Dorsal view. 374 CDH. X 35

49. 800201000001 Dorsal view. 330 CDH. X 35

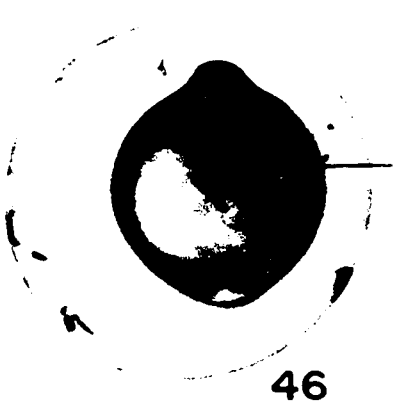
50. 900201000001 Dorsal view. CDH. unknown. X 35

51. 11,10211000001 Dorsal view. CDH. unknown. X 35

52. 12,10211000001 Dorsal view. CDH. unknown. X 35

53. 16,10211000001 Dorsal view. 618 CDH. X 35

Plate VIII



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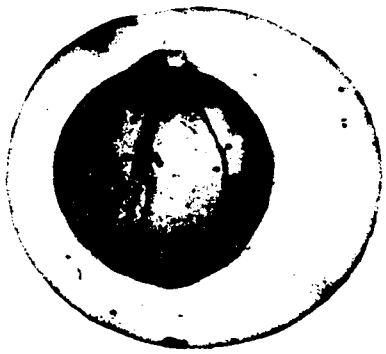

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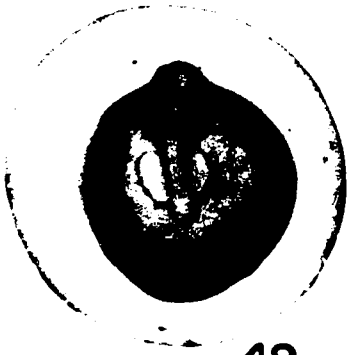

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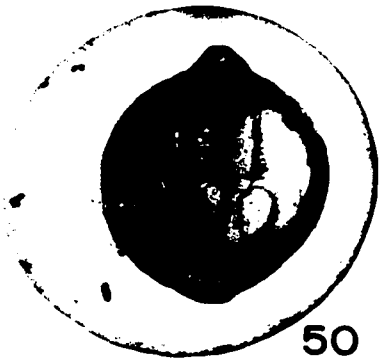

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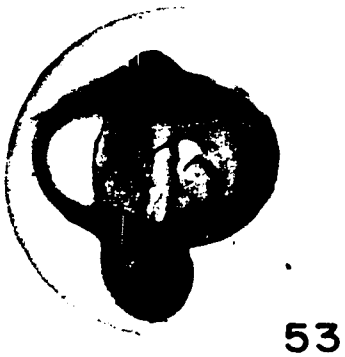

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PLATE IX

Zebrafish - cont.

54. 29,12523110012 695 CDH. X 50
Dorsal view.

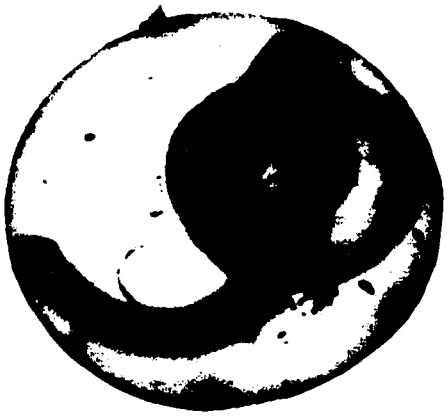
55. 29,12523110012 695 CDH. X 50
Frontal view.

56. 27,11323000002 624 CDH. 2.7mm.
Decapsulated. Lateral view.

57. 28,12423010012 648 CDH. 2.7mm.
Decapsulated. Lateral view.

58. 29,12523110012 695 CDH. X 35
Decapsulated. Lateral view.

Plate IX



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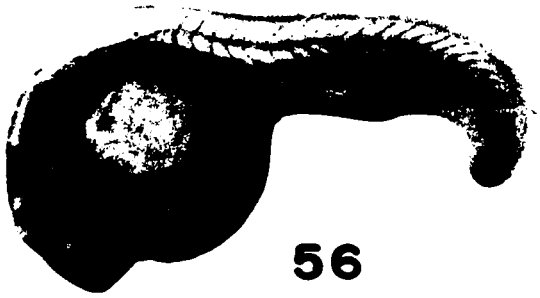

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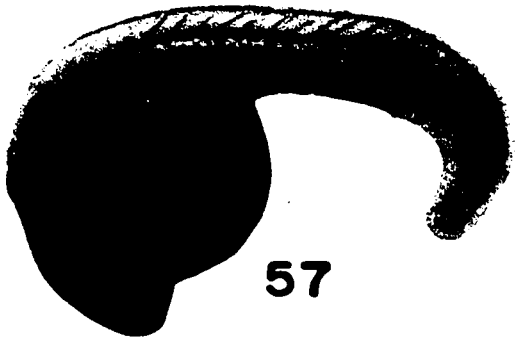

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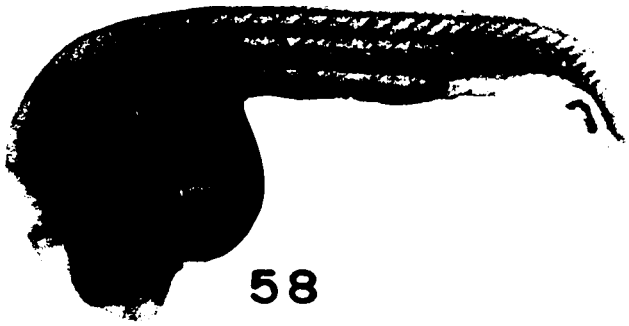

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PLATE X

Zebrafish - cont.

59. 32,12533220012 880 CDH. X 30
Decapsulated. Lateral view.

60. 33,12533231122 1250 CDH. 3.6mm.
Decapsulated. Lateral view.

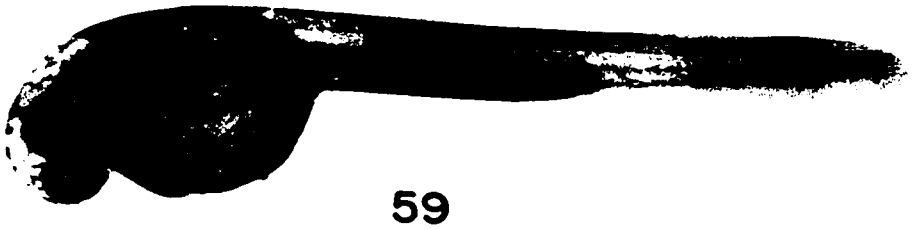
61. 34,12533331222 2110 CDH. X 30
Hatching condition. Lateral view.

62. 34,12533331222 2110 CDH. X 30
Hatching condition. Dorsal view.
Melanophores expanded.

63. 34,12533331222 2110 CDH. X 30
Hatching condition. Dorsal view.
Melanophores contracted.

64. 34,12533331222 2110 CDH. X 30
Hatching condition. Oblique ventral
view - melanophores expanded.

Plate X



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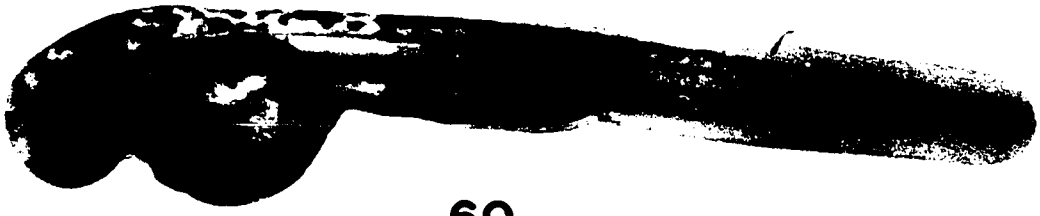

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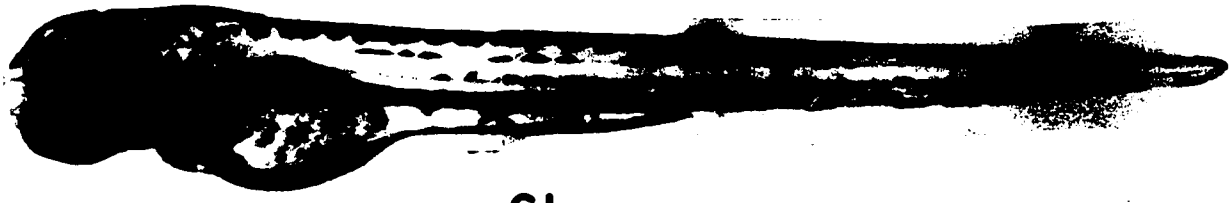

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PLATE XI

Yellow perch.

65. 000101000001 820 CDH. X 30
Membrane not shown. Blastopore arrowed.

66. 000101000001 820 CDH. X 30
Membrane not shown. Frontal view - keel arrowed.

67. 000101000001 820 CDH. X 30
Membrane not shown. Lateral view. Kupffer's vesicle arrowed.

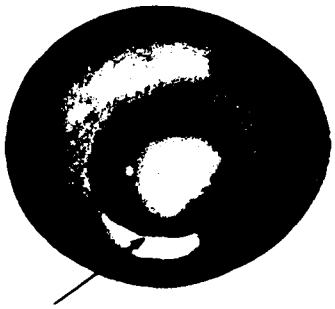
68. 300101000001 1680 CDH. X 30
Membrane not shown. Lateral view. Region of first somite formation arrowed.

69. 400201000001 870 CDH. X 30
Membrane not shown. Lateral view.

70. 500201000001 870 CDH. X 30
Membrane not shown. Lateral view.

71. 13,00301000001 1080 CDH. X 30
Membrane not shown. Lateral view.

Plate XI



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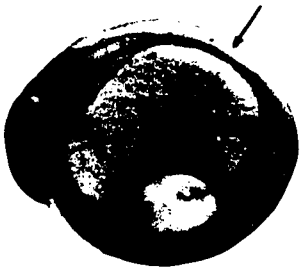

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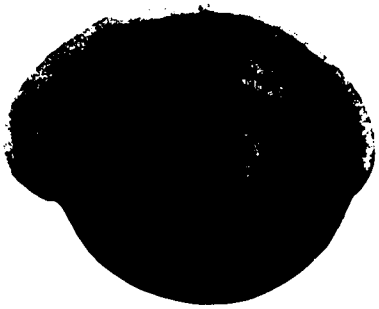

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PLATE XII

Yellow perch - cont.

72. 15,01311000001 1100 CDH. X 40
Membrane not shown. Lateral view.

73. 22,11313000102 1920 CDH. X 40
Membrane not shown. Lateral view.

74. 24,11313000102 2160 CDH. X 40
Membrane not shown. Lateral view.

75. 30,12313000112 1838 CDH. X 40
Decapsulated. Lateral view.

76. 31,12323110[illegible] 2640 CDH. X 40
Decapsulated. Lateral view.

77. 700201000001 1000 CDH. X 40
Membrane not shown. Frontal view.

78. 22,11313000102 1920 CDH. X 40
Membrane not shown. Frontal view.

79. 29,11313000102 1680 CDH. X 40
Membrane not shown. Frontal view.

Plate XII

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PLATE XIII

Yellow perch - cont.

80. 36,12323120112 2400 CDH. X 35
Decapsulated. Lateral view.

81. 42,12323120212 3210 CDH. X 35
Decapsulated. Lateral view.

82. 42,12323231212 3220 CDH. 3.9mm.
Decapsulated. Lateral view.

83. 42,12433331222 3600 CDH. 4.5mm.
Epiphyseal stalk arrowed.

Plate XIII

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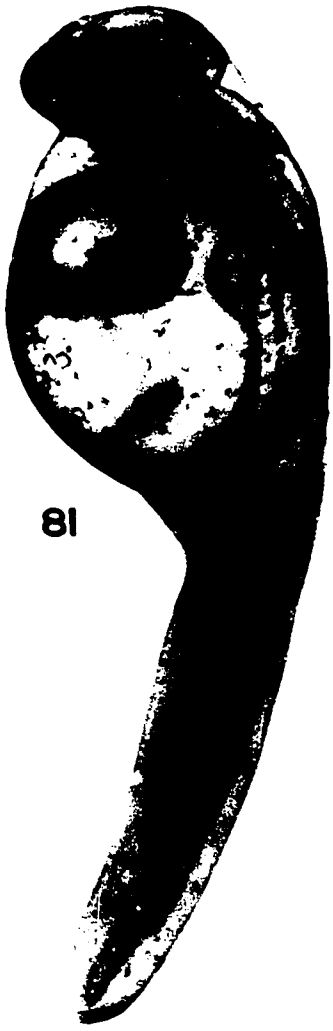

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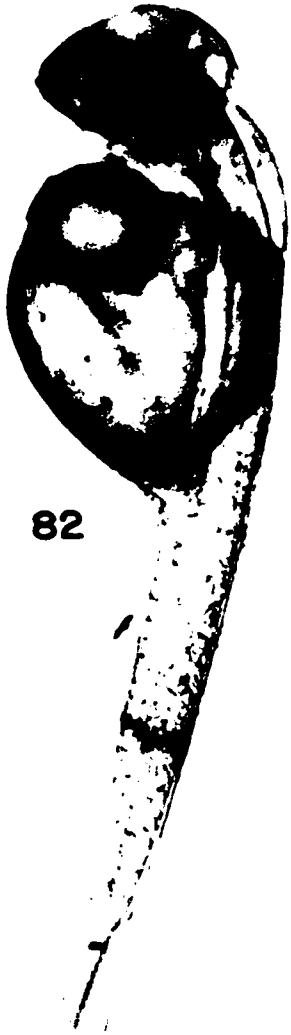

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PLATE XIV

Yellow perch - cont.

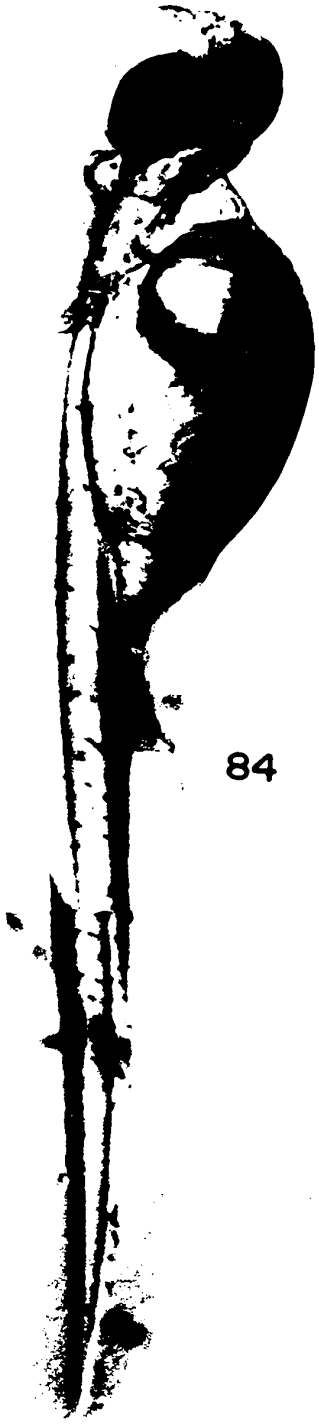
84. 44,12433331222 4000 CDH. X 30
Decapsulated. Lateral view.

85. 44,12533331222 5255 CDH. 6mm.
Hatching condition. Lateral view.

86. 44,12533331222 5255 CDH. 6mm.
Hatching condition. Enlarged lateral view of anterior end. Pectoral fin and heart arrowed.

87. 44,12533331222 5255 CDH. 6mm.
Hatching condition. Dorsal view.
Notochord arrowed.

Plate XIV



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