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Early ontogeny of walleye, *Stizostedion vitreum*, with steps of saltatory development

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Synopsis

Frequent in vivo observations of arbitrary stages revealed a saltatory pattern of development in the early ontogeny of fluvial spawning walleye. The requirement for an environment rich in dissolved oxygen was indicated by i) spawning site characteristics, ii) the lack of carotenoid pigments, iii) swim-up at hatching, iv) a planktonic (pelagic) existence by means of immobile surface suspension and subsequent surface swimming, and v) a poorly developed temporary embryonic respiratory system. including a subintestinal-vitelline vein, hepatic-vitelline vein and duct of Cuvier. Between the start of hatching and development of the ability to remain planktonic, the temporary embryonic respiratory system was enhanced by an increase in the proportion of the total blood volume passing through the subintestinal-vitelline vein – the largest respiratory surface. Immobile surface suspension was possible due to both the buoyancy of the large oil globule and the forces of surface tension. Also, immobile surface suspension would provide low energy transport from the fluvial spawning grounds to the lacustrine environment where zooplanktonic prey would be relatively more abundant. An intimate relationship between oil globule size (shape) and a dynamic behavioral transition (including the consumption of larger particles, cannibalism, and swimbladder inflation) suggested that energy expenditures occurring during fluvial transport were necessary for appropriate developmental synchrony.

1. Introduction

This study examined the ecological relationships of the walleye (*Stizostedion vitreum vitreum*) during the earliest interval of ontogeny, the interval of primarily endogenous nutrition. These relationships are best investigated by the ecomorphological method which reveals the vital ecological adaptations for respiration and protection from predation (Kryzhanovsky 1949). This hypothesis emphasizes 'two factors [which] play leading roles during embryonic development: predators and availability of oxygen. All other factors are associated with these two and create together an extraordinary variety of adaptations associated with early development. However, the various reproductive strategies and spawning grounds predetermine the respiratory conditions and the potential for defence against predators. Hence, they predetermine to a considerable degree the nature of the adaptations associated with early development. Therefore, the astounding multitude of adaptations associated with development reveal the ecological patterns which reflect the essential relations of fish in nature'.

Some morphological aspects of the early ontogeny of walleye have been described previously (Reighard 1890, Fish 1932, Norden 1961, Olson 1966, Nelson 1968), but without consideration of ecological relationships. Some investigations have, however, considered the effects on eggs, embryos and larvae, of various extrinsic factors such as temperature (Allbaugh & Manz 1964, Anonymous 1967, Smith & Koenst 1975, Koenst & Smith 1976), dissolved oxygen (Van Horn & Balch 1956, Oseid & Smith 1971, Siefert & Spoor 1974), parasites (Newburg 1974) and toxic substances, especially as these relate to wood fiber accumulations on river bottoms (Smith & Kramer 1963, Kramer & Smith 1966, Colby & Smith 1967, Smith & Oseid 1970).

In addition to describing the ecomorphological features of early ontogeny, this study has provided more support for the existence of steps in ontogeny,

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showing that development occurs in a saltatory fashion (Balon 1971, 1975b, 1979a, 1980d). Briefly, steps are characterized as natural intervals of ontogeny during which changes in form and function represent no significant alteration in the animal's environmental relationships. Environmental relationships refer to both the internal environment of the embryo (a factor of greater significance during the cleavage and early embryonic phases) and the external environment (a factor of greater significance during the late embryonic and eleutheroembryonic phases when form acquires visible function). Only certain combinations of synchronous qualitative change will result in the attainment of a threshold, which is an abrupt functional change in ontogeny (Balon 1960a, 1971,

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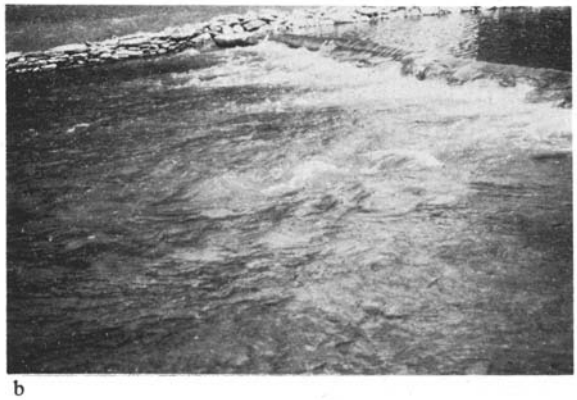

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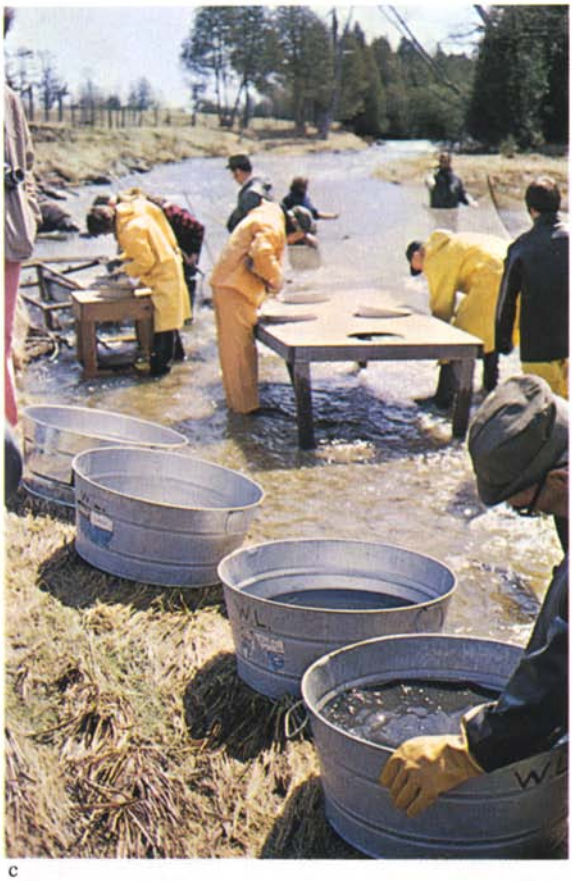

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Fig. 1. Photographs at the Talbot River during spring spawning 1975: a, b – spawning grounds below the dam with spawning walleye and white suckers, c – Ontario Ministry of Natural Resources (OMNR) personnnel capturing (background) and stripping (midground) walleye (*Stizostedion vitreum*) spawners and mudding the inseminated-activated eggs (foreground), d – closer view of OMNR personnel stripping the gametes from ripe walleye. Photo. by E. K. Balon.

1979a) that will result in a new environmental relationship and therefore a new step.

2. Materials and methods

The main spawning site of the Lake Simcoe (Ontario, Canada) walleye is immediately below a dam which separates the Talbot River from the Trent Canal (Fig. 1a, 2). No provision exists for the transport of fish beyond this obstruction and so migrants, halted in their river ascent, spawn mostly over the last 150 m of river below the dam. These spawning grounds, observed from early April to the end of June 1976, always had flowing water over the rock and rubble substrate (Fig. 1b). Throughout the interval of walleye spawning and egg incubation, the water velocity was high, siltation low, and water volume variable, the latter point depending on water level manipulations aimed at controlling the water level of the Trent Canal. In 1976 the mid-April to mid-May temperatures fluctuated between 8.7 and 14.2° C ($\bar{x}$ = 11.6° C). The walleye share these spawning grounds with a large spawning stock of white suckers (*Catostomus commersoni*) that are at least as numerous (and probably more numerous) as the walleye. During 1976, the walleye spawners left the spawning grounds before the end of April while sucker spawners lingered throughout April and all of May. A separate paper will be devoted to the white sucker (McElman & Balon 1980).

For many years the Ontario Ministry of Natural Resources has utilized this walleye stock for hatchery rearing. Adults are stripped and their gametes joined, water activated and mudded at the Talbot River (Fig. 1c, d). The progeny are transported to the White Lake Hatchery (Ontario) where they are jar incubated and pond reared for stocking into various waters.

Adult walleye were captured with dip nets, in a ripe condition, at the spawning grounds of the Talbot River on April 14, 1977. At this time the spawning run was considered to have attained its maximum density of spawners and intensity of spawning.

The three females and six males (Table 1) that provided gametes were transported live from the spawning grounds to holding facilities at the University of Guelph, Guelph, Ontario. Oxygen from a pressurized cylinder was bubbled through the water during transport.

Two separate gamete mixings produced two groups of progeny. The adults (Group I, Table 1) used for one progeny group were held for 22 h, and those of the other (Group II, Table 1) for 36 h, prior to the stripping of gametes.

Each fish was thoroughly dried before the gametes were stripped into a dry bowl. Gametes were mixed, the time recorded as that of insemination, and subsequently stirred with a feather and by rotation of the bowl. The addition of water to the combined gametes was considered the time of activation and the beginning of ontogeny, subsequently used as time zero in age calculations.

Activated eggs were immersed in a dilute solution of fine mud (Leach 1927). This process, referred to as mudding, removes egg membrane adhesiveness, allowing for high density incubation in standard hatchery jars. Though this method exposes the eggs to extremely artificial conditions (external membrane abrasion, high density, constant motion), no acceptable alternative has appeared to resolve this long standing problem of incubating large numbers of eggs that are demersal and adhesive.

Activated eggs were gently mixed with the finger tips or a feather in the mud solution. Consecutive freshwater rinsings over approximately a half hour removed most of the suspended particulate matter. When the rinse water was noticeably free of the suspended matter, the zygotes were added to hatchery jars. The first group of gametes was mistakenly acti-

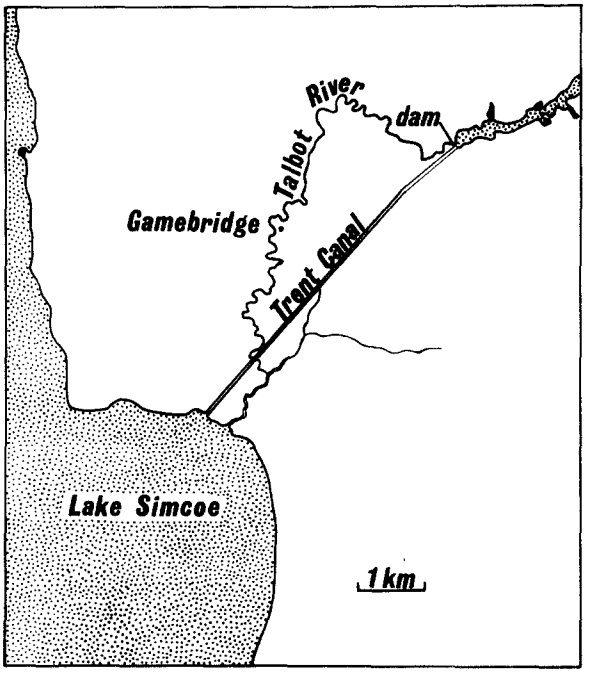

Fig. 2. Map of Talbot River.

Table 1. Mensurable (in mm) and meristic characters of adult (parent) *Stizostedion vitreum*, collected during the 1977 spawning run at the Talbot River.

	Females			Males					
	Group I	Group II		Group I			Group II		
	1	2	3	1	2	3	4	5	6
Weight, g	4692	4796	4480	2120	2990	2837	1886	1803	2998
Total length	725	724	765	602	669	627	537	533	676
Standard length	618	614	644	498	554	532	442	450	572
Body depth (maximum)	140	139	129	101	115	117	105	97	121
Body depth (minimum)	52	52	52	42	47	48	42	40	49
Caudal peduncle, length	132	134	136	115	119	118	96	92	122
Predorsal length	205	199	201	153	179	165	147	143	185
Prepelvic length	215	207	221	166	191	175	149	149	186
Preanal length	436	432	450	332	391	365	313	315	396
Pectoventral length	33	32	28	27	26	24	20	22	28
Ventroanal length	226	252	233	183	207	190	162	163	212
Dorsal fin, basal length D_1	180	164	184	149	152	152	111	126	167
D_2	121	134	151	101	120	118	109	100	131
Dorsal fin depth D_1	109	84	74	74	92	83	68	70	74
Anal fin, basal length	68	83	80	69	73	73	63	61	78
Anal fin, depth	90	97	96	87	91	78	76	69	88
Pectoral fin, length	93	97	102	75	88	80	73	66	92
Pelvic fin, length	94	95	92	82	92	80	75	71	89
Head, length	193	189	192	141	165	159	145	135	166
Head, greatest depth	112	108	115	84	92	90	75	73	95
Head, greatest width	108	107	113	79	90	86	74	75	93
Head, postorbital length	114	109	113	80	94	94	85	77	95
Snout length	56	52	54	40	46	45	39	39	50
Interorbital, least bony width	35	33	36	25	30	30	26	25	30
Orbit length	25	28	28	22	26	23	21	21	23
Upper jaw, length	89	89	86	62	75	68	62	57	73
Lateral line scale count	83	85	88	86	86	84	83	85	82
Rays of dorsal fin D_1	14	14	14	14	14	14	13	14	14
D_2	II 19	I 20	II 19	I 18	II 18	I 19	I 19	I 20	I 20
Rays of anal fin	II 13	II 13	II 12	II 13	II 12	II 12	II 12	II 13	II 13
Rays of pectoral fin	14	14	14	13	14	13	13	13	15
Rays of pelvic fin	15	15	15	15	15	15	15	15	15

vated and mudded simultaneously. This may have affected the fertilization process though observations did not indicate any differences in survival when compared with the second group. Smirnov (1975) noted that in the chum salmon, *Oncorhynchus keta*, the coelomic fluids activate spermatozoa which retain their motility much longer than in water, and also that entry of spermatozoa into the micropylar pore takes place at this time. If this is the case with walleye, it is unlikely that simultaneous water activation and mudding would be detrimental to sperm penetration.

All incubators were contained within systems that employed recirculated water with a constant excess provided by a minimal but continuous addition of fresh, unchlorinated, well water. The well water (for detailed chemical analysis see Hodson & Sprague 1975) was about 5° C lower than the desired temperature of incubation, and so also functioned to cool the systems as required. All water was filtered three times, twice through nylon filtration material for large particulate matter, and once through fine gravel for biological filtration.

An incubation and rearing temperature of 15° C was selected since optimum incubation temperatures (assessed as the highest percent hatch) are in the range of 9 to 15° C (Koenst & Smith 1976). Since Koenst & Smith noted that reduced activation temperatures of 6 to 12° C were advantageous (evaluated as percent hatch) the egg groups were activated at 10.7 and 12.4° C. Oxygen concentrations were considered optimal at 100% saturation since the water turbulence at fluvial spawning sites suggested that oxygen concentrations lower than 100% saturation would be unlikely.

The mean temperature in each of two incubator systems was 14.96 and 15.00° C. The maximum recorded fluctuation of temperatures was 14.0 to 15.8° C and 14.1 to 16.0° C respectively. These temperature regimes were considered equivalent to each other and to a constant temperature of 15° C which was used in the calculation of temperature units (day-degrees). Oxygen concentrations were monitored occasionally and were generally at or just below 100% saturation (Wetzel 1975). During hatching, however, oxygen concentration dropped to about 76% (7.3 ppm).

Light, on a 12L:12D h photoperiod, provided by incandescent bulbs produced light intensities of 2.8 to 22 lux for egg incubation and 11 to 350 lux for embryos after hatching.

The frequency of sampling was predetermined only to the extent that the rate of change in development was expected to be greater earlier. Otherwise the sampling frequency was as often as possible. The identification of thresholds is, after all, dependent on frequent samples.

Within each sampling interval specimens were photographed, drawn, weighed and subsequently preserved in 10% neutral formalin, the time of the latter procedure recorded as the end of each sampling interval. Wherever possible separate samples, not involved in any of the other sampling procedures, were used for live-weight determinations. With the start of cardiac function the rate of heart contractions was recorded with a stopwatch before all other sampling procedures. With the development of motility, light anesthesia with MS 222 rendered specimens immobile. Heart beat rates were always determined from unanesthesized embryos. The circulatory system was notably labile and so it too received a priority of attention at the start of each sampling interval but after heart beat rate was recorded.

Microscopic examinations of specimens used a Wild M4A binocular microscope with transmitted and reflected light. A C&D projection microscope (Scientific Instruments, Hemel Hempstead, England) produced a table top image from which the specimen could be drawn in horizontal view. A Bausch and Lomb projection microscope similarly provided a table top image for drawing though the view was from below the specimen. Camera attachments for both the Wild M4A and C&D Projection Microscope provided photographs of horizontal and vertical views. Future reference to horizontal and vertical top or vertical bottom views always refer to the microscopic view of the egg in its natural (gravity) position and should not be confused with side, dorsal or ventral views which are relative to the embryo.

Neutral-formalin-preserved specimens were cleared in glycerine and stained with Harris hematoxylin (Galat 1972) as required. Cartilage and bone formation was studied after enzyme clearing and staining with alcian blue (cartilage) and alizarin red S (calcified bone) (Dingerkus & Uhler 1977).

Most illustrations represent embryos in vivo and have been produced as composites from drawings and photographs of live material. Detailed drawings of live embryos were by far the more important and substantial contributors to the final product. Photographs rarely provided sufficient detail other than the more external features of morphology. However, as form and visible function became more elaborate, reliance on photographs increased.

Most mensurable characters were derived from drawings and photographs of live material. The emphasis on live material made sampling more than one specimen impossible in most instances. Mensurable values given in the text should, therefore, be considered as characteristic of one size within an undetermined range. The more relevant aspect of mensurable values was the change with time. Since the sequence of ontogeny presented here was determined from a series of different individuals, each examined at a different time (age), it was primarily the identification of trends in morphology and visible function that vindicated the supposition of 'normality' in each embryo examined. The concept of hard and soft selection (Wallace 1970) is, however, very much in conflict with such a supposition. 'Whyte's [1965] argument that many developmental routes from the egg lead not to adults but to embryonic or juvenile death' (Cohen 1977, p. 19) will have to be seriously attended to in future studies.

Ages were determined as the chronological midpoint, to the nearest five minutes, between the start and finish of each sampling interval. Certain of the earlier developments, such as individual cleavages at the beginning of ontogeny, were aged to the moment they were first observed.

Food was supplied for eleutheroembryos soon after the mouth was open and much in advance of the beginning of exogenous feeding. Foods provided were: natural zooplankton (mostly rotifers and cladocerans), Liquifry No. 1 (commercial 'fry' food) and a solution of crushed TetraMin flakes and hard boiled chicken egg yolk.

The terminology used for blood vessels, cartilage and bone was based on presumptive adult structures where such relationships were apparent. Where more than one possibility existed, as was the case with some cartilage, the range of possibilities was noted. Daget (1964), Harder (1975) and Balon et al. (1977a) were generally consulted for the naming of cartilage and bone. The terms artery, vein and capillary have been used generally in accordance with presumptive adult structures and not specifically to measured dimensions (Johansen 1977).

Terminology for intervals of ontogeny followed that proposed by Balon (1971, 1975b, 1980a). The study has included the entire embryonic period which terminates with the start of exogenous feeding. The embryonic period has been divided into cleavage, embryonic and eleutheroembryonic phases, which were further divided into steps described from the sequence of arbitrary stages. One step beyond the embryonic period was described because of the existence of a prolonged interval of mixed nutrition (endogenous and exogenous food sources). The short-form designation for each step follows the system proposed by Balon (1980a) in which capital letters 'C', 'E' and 'F' denote the phases as cleavage, embryonic and eleutheroembryonic (free embryo) respectively, a superscript Arabic numeral following immediately denotes the number of the step within that particular phase, and a subsequent Arabic numeral the number of the step in the total sequence of ontogeny. For example, $E^3 6$ is the third step in the embryonic phase and the sixth in ontogeny. Subheadings utilizing this type of symbol introduce each step and are followed by a brief list of salient developmental events occurring within that step. Following this, the events of ontogeny are described chronologically identifying each stage by age and selectively; in terms of temperature units. Temperature units can be readily calculated for any time by using the 15° C temperature noted previously as the mean for the interval of ontogeny studied.

3. Results

3.1 The embryonic period

The first period in the life history of oviparous fishes starts with egg activation at the time of spawning and ends when the young begin to feed externally. In walleye, the embryonic period ended with the beginning of an interval of mixed nutrition at age 15 days 18 h (236.2 TU). The cleavage phase lasted for 1 day 22 h 10 min (28.8 TU), the embryonic phase for 7 days 1 h 50 min (106.1 TU) and the eleutheroembryonic phase for 6 days 18 h (101.3 TU). Only the cleavage and embryonic phases would occur at the site of spawning since upon hatching eleutheroembryos immediately swim upwards. Once above the shelter of the rock and rubble substrate, they would be swept away by the current.

3.1.1 The cleavage phase

The cleavage phase started when the gamete mixture was immersed in water (activation), and ended with the onset of organogenesis at age 1 day 22 h 10 min. Three steps defined the major events in the cleavage phase of embryonic development.

Step C^1 1. *Formation of the perivitelline space and the blastodisc* (Fig. 3).

The first step of the cleavage phase includes a description of the eggs before, during, and after hydration (water hardening), until the first signs of cleavage at age 2 h 36 min (1.6 TU).

Prior to hydration (Fig. 3a) the eggs were flaccid, delicate, and broke readily with any direct mechanical disturbance. The only visible internal detail was a central, large oil globule and a number of smaller oil globules, which most frequently, though not exclusively, were adjacent the large oil globule. In a mass the eggs were a pale yellow color while individual eggs were colorless.

Egg samples were collected and weighed immediately after stripping each of the three females. Due to the delicate nature of the eggs, no attempt was made to separate the eggs from the coelomic fluids. The eggs were preserved in formalin, later counted, and again weighed. The average wet weight of live and preserved eggs respectively was 3.14 and 2.77 mg (n = 56) for female one (Table 1), 3.91 and 2.98 mg (n = 69) for female two, and 3.54 and 3.00 mg (n = 89) for female three. Wet weights of live eggs between ages 13 h and 7 days 3 h 10 min ranged from 4.6 to 6.0 mg ($\bar{x}$ = 5.4 mg, n = 100). Unactivated, formalin-preserved eggs, from female one had a mean diameter of 1.54 mm (n = 100).

Visual identification of the egg membranes was possible 2 min after the addition of water to inseminated eggs. Reighard (1890) described two membranes, an inner thick zona radiata and an outer thin 'external egg membrane' or chorion. These membranes were evident as a single band surrounding the yolk, not yet separated from the yolk surface. Maximum egg diameters were 1.70 to 1.85 mm. The large oil globules were about 0.90 mm in diameter and the smaller ones as large as 0.45 mm, but most were less than 0.20 mm in diameter. A discontinuous perivitelline space was evident around the yolk circumference 3 min after activation. It became continuous after a total of 12 min. After 5 min of water immersion, bipolar differentiation was indicated by a brownish colored blastodisc. By 21 min the width of a single blastodisc was 1.30 mm. The width did not increase after this within a two hour observation period. Hydration of the eggs was complete 45 min after the eggs were immersed in water. From this time until the end of the cleavage phase eggs were imperfect spheroids with a mean diameter of 2.09 mm.

From 45 min until the first signs of cleavage there were no obvious morphological changes. The maxi-

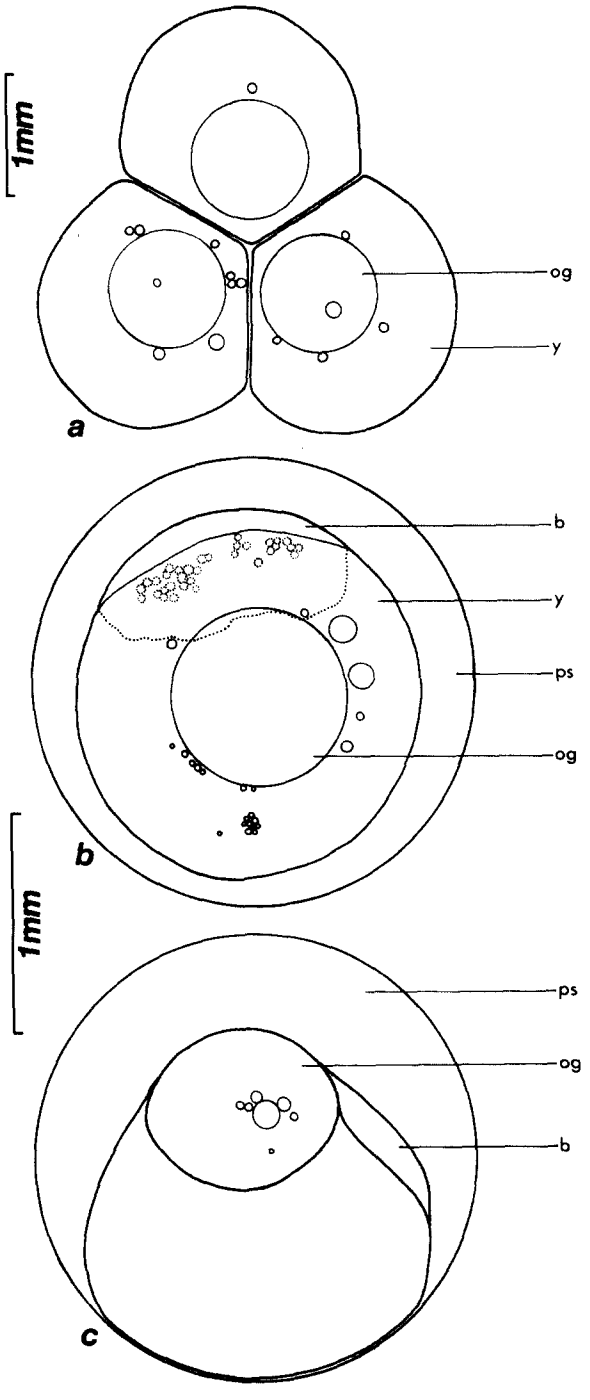

Fig. 3. Formation of the perivitelline space and blastodisc in *Sitzostedion vitreum* eggs during step C^1 1: a – eggs before immersion in water, vertical view, b, c – two hours after immersion in water, vertical (b) and horizontal (c) views (b = blastodisc, og = oil globule, ps = perivitelline space, y = yolk).

mum yolk diameter parallel to and below the base of the blastodisc was 1.60 mm. The diameter perpendicular to this inclusive of the blastodisc was also 1.60 mm. The yolk surface below the blastodisc was irregularly flattened. The external junction of the blastodisc cytoplasm and the yolk cytoplasmic layer was smooth, giving the egg a round shape (Fig. 3b).

The yolk, oil and cytoplasm viewed from the side were bell-shaped (Fig. 3c), probably because of the buoyancy of the oil globule. Though floating in the yolk its upward position would be limited by the maximum extension of the enveloping cytoplasm. Throughout most of the cleavage phase, large oil globules consistently measured about 0.75 mm high and 0.90 mm wide. The force of its own buoyancy against the extended enveloping cytoplasm would probably cause a fluid sphere to become distorted in this manner. Until the embryo developed motility, the buoyancy of the large oil globule maintained it on top of the yolk.

In horizontal view the blastodisc overlapped the lateral margin of the oil globule at about the same position as did the yolk on the opposite side. The lateral position of the blastodisc brought it and the zona radiata into close proximity. They possibly touched on the lower edge of the blastodisc.

Step $C^2 2$. *Cleavage of the blastodisc* (Fig. 4, 5, 6). The second step of the cleavage phase lasted for about a day. It began with the first signs of cleavage at age 2 h 36 min (1.6 TU) and ended at age 1 day 2 h 15 min (16.4 TU) with the start of epiboly.

The earliest indication of cleavage was an increase in cytoplasmic density along the plane of the first

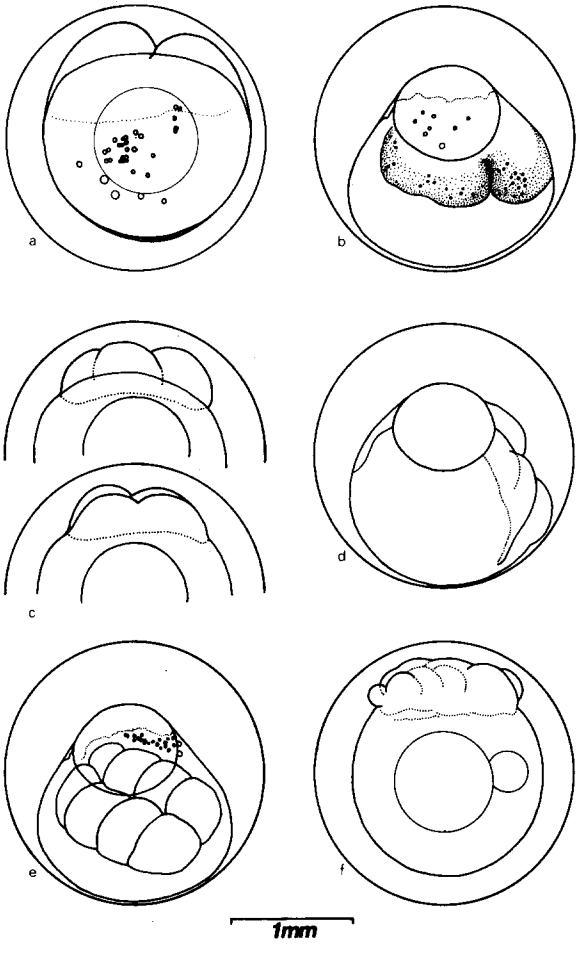

Fig. 4. Early cleavage of the blastodisc in *Stizostedion vitreum* eggs during step $C^2 2$: a – two blastomere stage at age 3 h 47 min, vertical view from above, b – two blastomere stage at age 4 h 7 min, horizontal view, c – four blastomere stage at age 4 h 27 min showing different alignments of cleavage furrows 1 and 2 in different eggs, vertical view, d – four blastomere stage at age 4 h 44 min, horizontal view, e – eight blastomere stage at age 5 h 5 min, horizontal view, f – 8 to 16 blastomeres at age 6 h 41 min, vertical view.

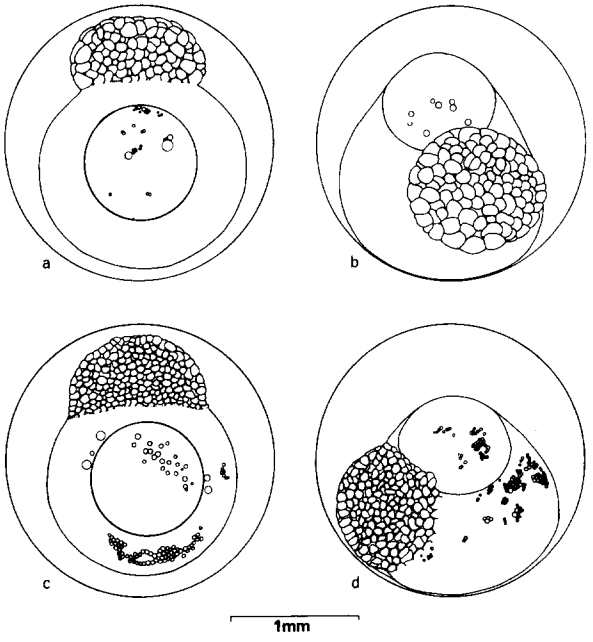

Fig. 5. Late cleavage of the blastodisc in *Stizostedion vitreum* eggs during step $C^2 2$: a, b – age 9 h 20 min, vertical (a) and horizontal (b) views, c, d – age 13 h, vertical (c) and horizontal (d) views.

cleavage furrow. The initially shallow furrow became increasingly deep, finally producing two equal blastomeres (Fig. 4a, b). The cleavage furrow attained a maximum depth of 0.15 mm. The basal width of the two blastomeres was 1.45 mm.

Individual variation was evident by the first cleavage. Eggs with different numbers of blastomeres could be found at any single time and the plane of the first cleavage furrow, commonly perpendicular to the horizontal, was on one occasion in the horizontal plane. Eggs at the four blastomere stage (Fig. 4c, d) indicated that the first cleavage furrow also occurred midway between the forementioned planes.

The second cleavage began 3 h 39 min (2.2 TU) after activation, and by age 4 h 27 min (2.7 TU), the majority of eggs had divided into four equal blastomeres. The basal width of the blastodisc, viewed vertically from above, was 1.20 mm, 17% less than two blastomere stage.

At age 5 h (3.1 TU) the third cleavage produced two rows of four equal blastomeres (Fig. 4e). The basal width of the blastomeres was unchanged from that of the four blastomere stage. A maximum of eight blastomeres was observed up to and including 6 h 10 min after activation.

Cleavage four (Fig. 4f) at age 6 h 41 min (4.2 TU) displayed asynchronous division of blastomeres which were unequal in size and intermediate in number between eight and sixteen. The loss of synchrony was most evident in the form of small rounded blastomeres protruding from the lateral periphery of the mass of larger blastomeres.

Eight hours after activation, there was a double layer of blastomeres. Those on the surface appeared to be bound entirely by cell membranes. The exterior surface of the blastodisc was smoother having lost the pebbled appearance of previous cleavage stages. A view from the side, looking directly at the top of the blastodisc, revealed its perimeter to be roughly circular. The base width of the blastodisc was reduced to 0.80 mm and the maximum width above the base was 0.95 mm. At age 9 h 20 min (Fig. 5a, b) the morphology of the egg was unchanged except for a further reduction in blastomere size and increase in their number.

One hour later, at age 10 h 20 min, the widest portion of the blastodisc was its base, a shape maintained until 13 h after activation (Fig. 5c, d). The blastodisc basal width was 1.05 to 1.15 mm during this interval. The lack of a discrete cell wall between the bottom of the marginal cells and the yolk cytoplasmic layer indicated they were still united. Variation was noted at this time by differences in the cell size of different eggs.

Thirteen hours and 50 min (8.6 TU) after activation (Fig. 6a) the marginal cells of the blastodisc enveloping layer were separated from the cytoplasm be-

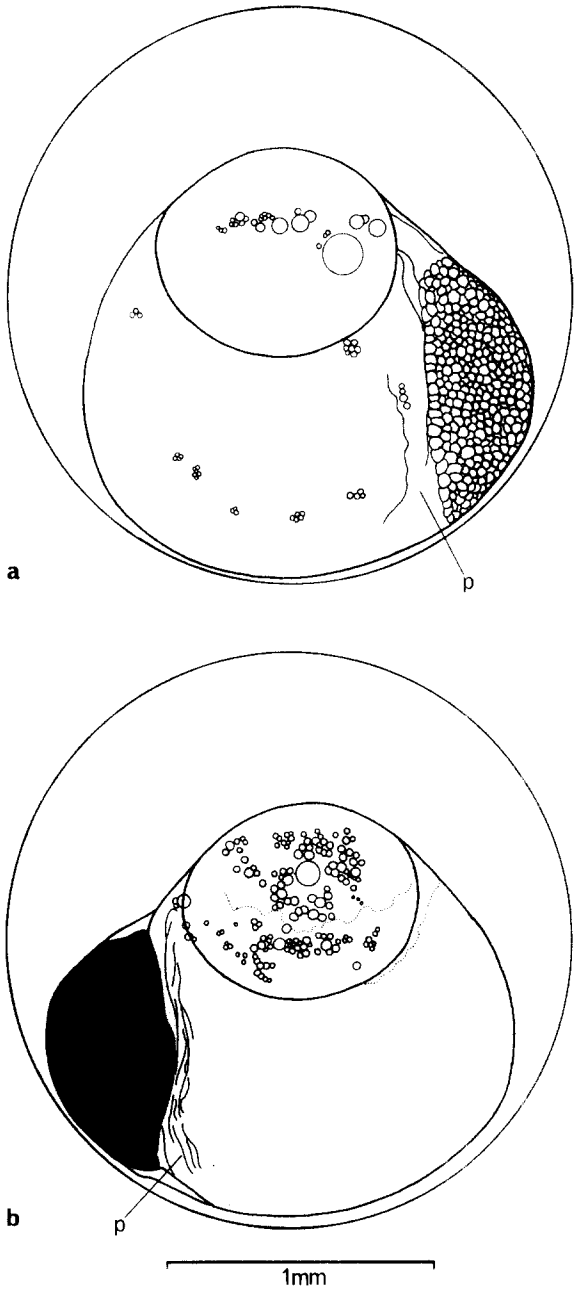

Fig. 6. The blastula of *Stizostedion vitreum* during step $C^2 2$: a – age 13 h 50 min, horizontal view, b – age 20 h 40 min, horizontal view (p = periblast).

low. At the same time the periblast was discernable around the margin of the blastodisc. Throughout the remainder of this step the blastodisc was dome shaped and more compressed than previously. Cells diminished in size (Fig. 6b) becoming individually indistinguishable and the previously translucent cell mass became dark and opaque. By age 18 h 15 min the periblast attained its maximum observed width of 0.20 mm and its mottled surface was distinct from the relatively smooth yolk cytoplasmic layer. A yolk-sac cavity was never visible in the live blastula.

Brief consideration must be given to the possible designation of the blastula as a separate step. Smirnov (1975) and Peňáz (1975) considered the blastula to be a separate step. Pavlov & Soin (1976) included the blastula in the preceding cleavage step while Balon (1980a) placed it in the subsequent step with epiboly and gastrulation. These works dealt with members of Salmonidae, namely *Oncorhynchus keta, Thymallus thymallus, Salmo mykiss* and *Salvelinus* (*Cristivomer*) *namaycush* respectively. The problem is not one of species specific developmental patterns but rather of the subjective assessment of essential criteria required for separate step designation. Although in terms of visible gross morphology this interval appeared quiescent, changes in the morphology and behavior of cells and of noncellular cytoplasm have been described (Trinkaus 1966, 1969, 1973a, b, Lentz & Trinkaus 1967, Trinkaus & Lentz 1967, Ballard 1973). A number of these events (for example, formation of a syncytial periblast, deep cell movements) may involve developmental thresholds, however, it appears more likely they are lesser thresholds directed toward the threshold of the next step, that being organized mass cell movements and aggregations.

Step $C^3$3. *Epiboly and gastrulation* (Fig. 7, 8).
The last step of the cleavage phase started with epi-

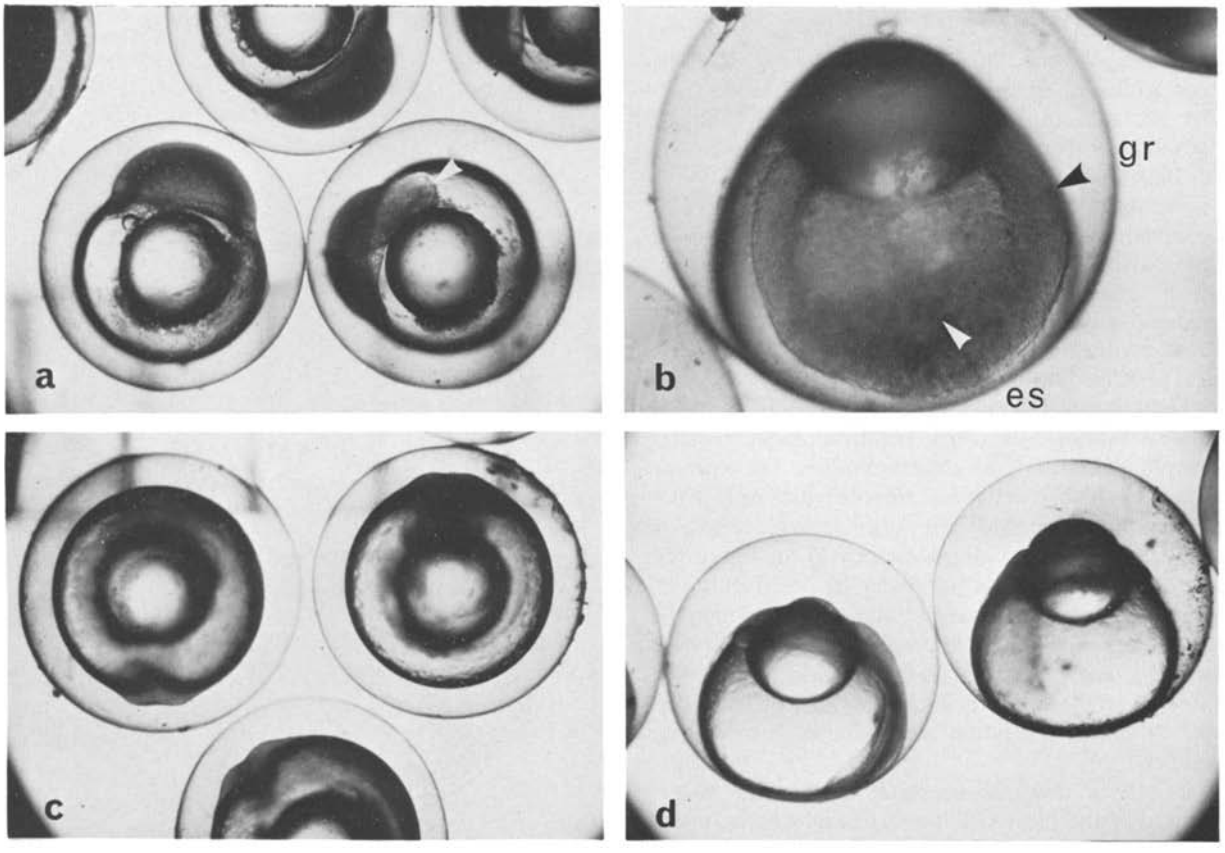

Fig. 7. Photomicrographs of epiboly and gastrulation in *Stizostedion vitreum* eggs: a – early epiboly at age 1 day 2 h 15 min, morphology of left egg conforms with usual teleost epiboly, right egg shows apparent cellular mass (arrow) projecting from marginal cells of the enveloping layer, b – early gastrulation at age 1 day 4 h 50 min showing germ ring (gr) and embyonic shield (es), c – late epiboly and gastrulation at age 1 day 17 h, vertical view from above shows a neural groove, d – same as (c) but in horizontal view showing that only the upper part of the large oil globule protrudes beyond the germ ring.

boly at age 1 day 2 h 15 min (16.4 TU) and ended with the onset of organogenesis at age 1 day 22 h 10 min (28.8 TU).

Epiboly was indicated by several changes in blastodisc morphology (Fig. 7a). The width of the blastodisc at the margin and the distance between the margin and the apex of the blastodisc both increased. The blastodisc no longer sat atop the yolk but rather had thinned and was wrapped around it. The uniformity of light transmission indicated equal density of the blastoderm in all areas. In some eggs, what appeared to be a cellular mass projected over the yolk surface interrupting the circular symmetry of the blastodisc margin (Fig. 7a). It was seen only in the first sample of this step at age 1 day 2 h 15 min.

At age 1 day 4 h 50 min (18 TU) the germ ring and embryonic shield were evident as thicker areas in the blastoderm (Fig. 7b). The germ ring was 0.20 mm wide and the embryonic shield, a slightly wider portion of it, was forming on the side opposite the oil globule in closest proximity to the zona radiata (compared to any other area of the blastoderm).

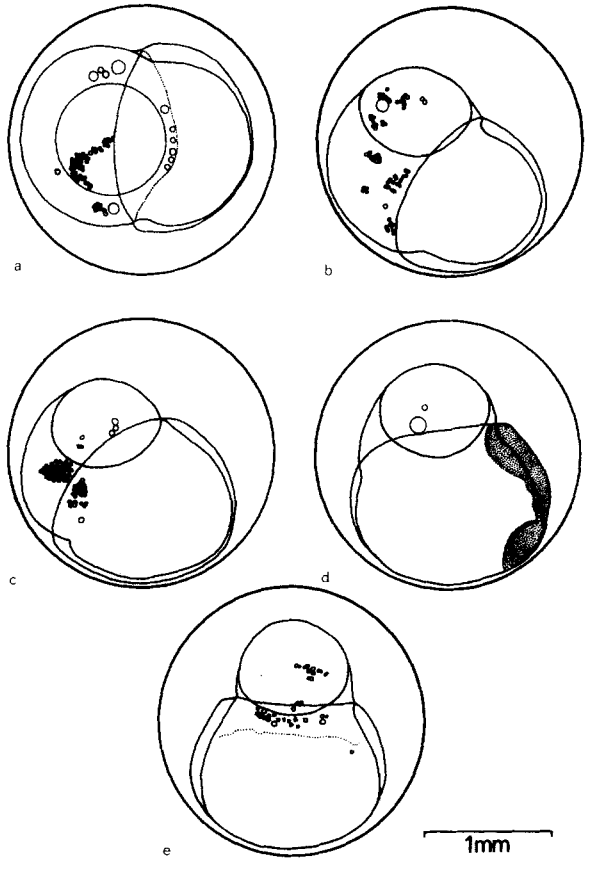

Fig. 8. Epiboly and gastrulation in *Stizostedion vitreum* eggs during step C^3 3: a, b – mid epiboly at age 1 day 7 h 10 min, vertical (a) and horizontal (b) views, c, d, e – varying degrees of epiboly at age 1 day 11 h 45 min, horizontal views.

By age 1 day 7 h 10 min the blastoderm had enveloped about 50% of the surface area of the yolk (Fig. 8a, b) and the direction of epiboly was in the horizontal plane. By age 1 day 11 h 45 min (Fig. 8c, d, e), in some eggs only the large oil globule protruded out from the circular margin of the germ ring (Fig. 8e). The relative position of the embryonic shield had changed due to a 90° shift in egg orientation (compare Fig. 8b and e). Epibolic movement was in the vertical plane in an upwards direction with the embryonic shield, now 1.45 mm long, on the side of the egg with only the primordial head region touching the zona radiata. At this time some eggs showed as little as 75% yolk engulfment (Fig. 8c, d). In these eggs the direction of epibolic movement was intermediate at about a 45° angle to the horizontal plane. The first and second halves of yolk epiboly were approximately equal in rate at 5 h and 4½ h respectively. Closure of the germ ring at the top of the oil globule was not complete for another 10 h 30 min.

By age 1 day 17 h 50% or less of the oil globule was exposed (Fig. 7d). The germ ring caused a lateral compression of the oil globule such that where previously it was 0.75 mm high and 0.90 mm wide (horizontal view), now it was 0.80 mm by 0.85 mm. Germ ring width was the maximum observed at 0.30 mm. The embryonic shield was laterally compressed into a neural plate, concave externally, convex internally (Fig. 7c), and notably elevated above the rest of the yolk blastoderm. The neural plate was 1.75 mm long and its anterior end was in contact with the zona radiata. A terminal node was visible at the germ ring margin in some eggs. The blastoderm was thinner and a yolksac cavity was now visible between the blastoderm and the yolk, below the germ ring on the side opposite the neural plate.

3.1.2 The embryonic phase

This phase of ontogeny began with organogenesis at age 1 day 22 h 10 min (28.8 TU), encompassed intense morphogenesis, growth and the development of visible function (muscular contraction, blood circulation, sensory perception) and ended with hatching at age 9 days (135 TU). The development of sensorimotor control (swimming) and sensory perception

(positive phototaxis) can probably be isolated as extremely important events because of their importance in the subsequent phase, that being transport from the fluvial to the lacustrine environment. These features are further appreciated in view of the apparent poorly developed respiratory adaptations which also appear during this phase. Without these features eleutheroembryos would probably perish in the inhospitable fluvial pools where reduced water velocities and subsequent siltation would spell their demise. Six steps defined the major events in the embryonic phase of the embryonic period.

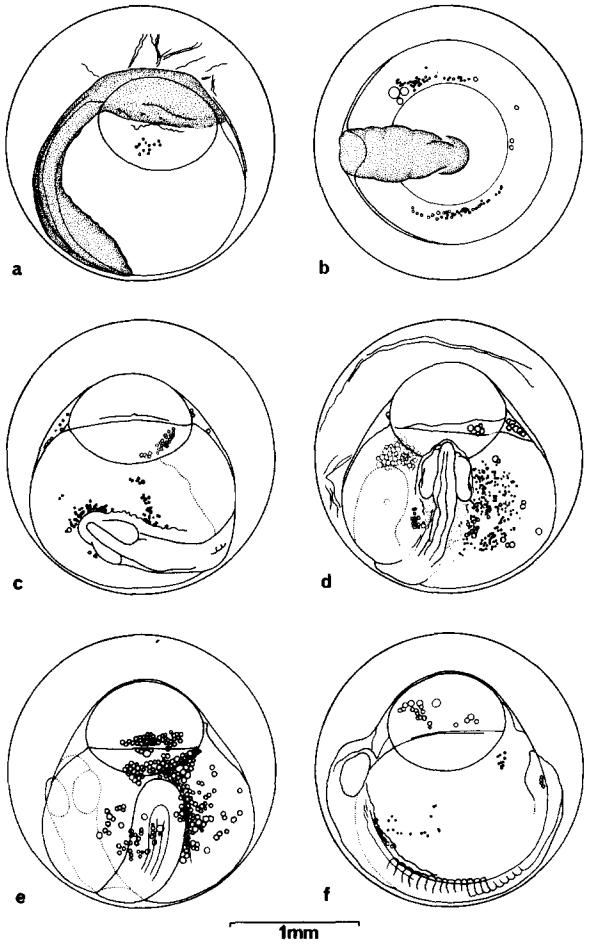

Fig. 9. Head and trunk development in *Stizostedion vitreum* embryos during step E¹4: a, b – embryo at age 1 day 22 h 10 min illustrated in horizontal, left side view (a) and vertical from below in dorsal view (b) of head region, c – age 2 days 3 h 15 min, embryo shows lateral translocation, dorsal view of head, d, e – age 2 days 16 h 10 min, dorsal view of head (d) and trunk-tail anlage (e), f – age 2 days 17 h 25 min, right side view.

Step E¹4. *Head and trunk formation, epiboly complete, body translocation* (Fig. 9, 10, 11).

The first step of the embryonic phase began at age 1 day 22 h 10 min (28.8 TU) and by its end at age

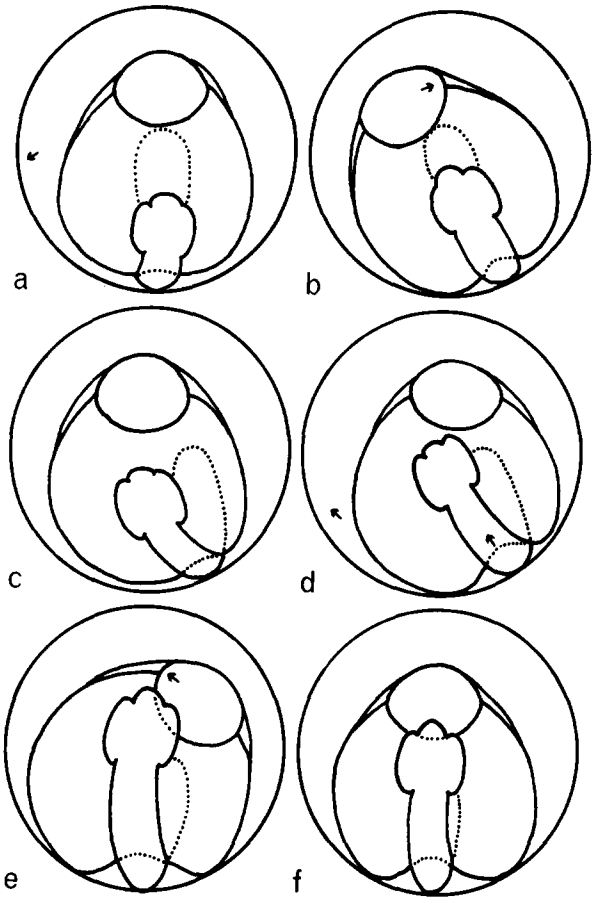

Fig. 10. Morphological changes hypothesized to cause lateral translocation of the body of *Stizostedion vitreum* during step E¹4: a – elevation of the body tissues, b – embryo falls to one side due to an inability to balance on the elevated body (this assumes the weight of the lateral yolk is sufficient to overcome the buoyancy potential of the oil to hold the embryo upright), c – the oil globule having been shifted to one side, moves back to the uppermost position of the egg thereby losing the previous bilateral symmetry (this assumes the oil globule is free to move between the blastoderm and the yolk, a hypothesis which is supported by the clearly defined yolksac cavity which surrounds the oil globule), d – the embryo's body creates an increased groove-like depression in the yolk, which in turn causes dorso-lateral bulges (relative to the embryo body) in the yolk, e – with the dorsal surface of the body and the dorso-lateral bulges of the yolk providing support to the embryo, the embryo body again lies at the bottom of the yolk causing displacement of the oil globule to one side once again, f – the oil globule moves back to the uppermost position restoring bilateral symmetry.

2 days 22 h (43.7 TU), a well formed head and trunk had developed. Changes in relative body position occurred throughout this step.

The appearance of primordial optic vesicles and slight constrictions in the brain (Fig. 9b) separated this step from that preceding it and also the embryonic phase form the cleavage phase. The germ ring had just closed around the top of the oil globule forming a trunk-tail mound which enveloped the upper half of the oil globule and was flat on the upper external surface (Fig. 9a). The embryo measured 2.95 mm from the most posterior position of the trunk-tail mound to the anterior tip of the head. Further lateral compression of the embryonic shield produced a more definitive axial body form, slightly elevated above the yolk blastoderm. The yolksac cavity was enlarged and in some embryos, its extension to the upper surface of the oil globule indicated that the trunk-tail mound had already begun its translocation process away from the oil globule. Throughout this step the embryo body moved (relative to the oil globule) such that by the end of this step it had undergone a 90° anterior translocation (compare Fig. 9a, f).

Translocation was not a simple anterior shift since throughout this step the position of the embryo's body showed lateral translocation accompanying the anterior shift (Fig. 9c, d, e). The net result of translocation was to move the trunk-tail mound down and away from the oil globule, and the head up and closer to the oil globule. Meanwhile asymmetry of the lateral yolk blastoderm developed as a result of lateral translocation. Translocation was pronounced only in this step and by its end the embryonic body defined a line of bisection. Lateral translocation may have been related to the gradual elevation of the embryonic body and subsequent movements of the large oil globule. Figure 10 presents a series of hypothetical changes that would explain the observed lateral translocation.

The trunk-tail mound had moved off the top of the large oil globule by age 2 days 1 h 30 min. Lateral translocation was observed in most eggs. The highest concentration of small oil globules, around and below the posterior half of the body and especially in the region of the trunk-tail mound, appeared to have resulted from their adhesion and movement with first, the germ ring, and then the trunk-tail mound. This concentration was evident only during the initial development of the trunk-tail anlage and was therefore confined to this step (Fig. 9e). Large vesicular structures below the trunk-tail mound were interpreted as a diffuse form of Kupffer's vesicle. Figure 11 shows these large vesicles and two later stages of Kupffer's vesicle. There were six somite pairs in the mid-body region. A notochord was visible but not yet vacuolated. The optic vesicles were more expanded and

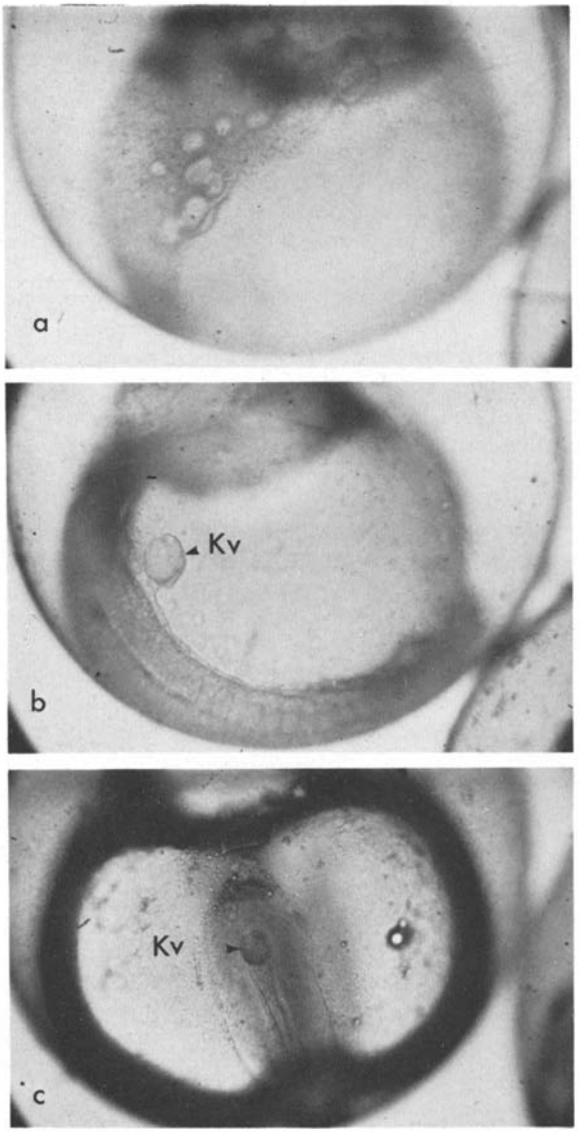

Fig. 11. Photomicrographs of vesicular structures in *Stizostedion vitreum* embryos during step $E^1 4$: a – large vesicles beneath the trunk-tail anlage at age 2 days 1 h 30 min, horizontal view through yolk to lower surface of trunk-tail anlage, b – side view of Kupffer's vesicle (Kv) at age 2 days 5 h 40 min, horizontal view of left side, c – dorsal view of trunk-tail anlage showing Kupffer's vesicle at age 2 days 16 h 55 min, horizontal view.

their interior front halves continuous with the brain in a 0.30 mm long embryo of age 2 days 3 h 15 min (Fig. 9c).

By age 2 days 6 h the trunk-tail anlage did not overlay even the lateral margin of the large oil globule. Enhanced visibility revealed that the clear yolk-sac cavity surrounded the mid-periphery of the oil globule. Kupffer's vesicle was visible below the trunk-tail anlage (Fig. 11b). The head was elevated 0.25 mm above the yolk blastoderm and had a maximum width of 0.50 mm at the optic vesicles. Vesicular structures, apparent on the ventral body surface near the anterior somites (which totalled 9 to 11), increased in number and area of coverage throughout this step and subsequent steps, and eventually formed the hepatic anlage.

Over the next 16 h morphological changes were primarily elaborations of features previously described. By age 2 days 10 h the head was 0.30 mm above the yolk blastoderm and three slight constrictions of the hindbrain were the first indication of subdivision into encephalomeres. Optic stalks developed between the brain and each optic vesicle. Somite pairs totalled 10 to 13.

By age 2 days 16 h 10 min the optic vesicles had invaginated producing optic cups (Fig. 9d). The optic cups were still 0.30 mm long and 0.15 mm wide but they appeared to have no connection with the brain due to the expansion of fore- and midbrain regions. There were still numerous small oil globules beneath and around the trunk-tail anlage, however posterior to it and adjacent the large oil globule, their concentration had increased (Fig. 9e). The relative positions of the head and trunk-tail anlage in proximity to the large oil globule had reversed (Fig. 9d, e). The trunk-tail was below the lower margin of the oil globule and the head above.

By age 2 days 17 h 15 min the embryo body (Fig. 9f) had reestablished its bilateral symmetry with respect to the yolk and the large oil globule. The embryo was 3.20 mm in total length, an increase of 0.25 mm (13%) since the onset of organogenesis. There were 17 to 19 pairs of somites. The high concentration of small oil globules had disappeared from around the trunk-tail anlage, which was elevated 0.10 mm. The recession of yolk from the lower half of the large oil globule was the first indication of yolk diminishment. A vertical view of the embryo revealed that the yolk length (relative to the body axis) was 80% of the yolk width. Yolk recession produced a larger yolksac cavity around the oil globule. Kupffer's vesicle was still apparent at the posterior extremity of the notochord (Fig. 11c), however it was not seen again after this observation.

Step $E^2 5$. *Formation of the eye lenses, olfactory placodes, auditory placodes and trunk-tail bud* (Fig. 12, 13).

The sudden appearance of sensory organs in the head, and the formation of an elevated trunk-tail bud marked the start of this step at age 2 days 22 h (43.7 TU).

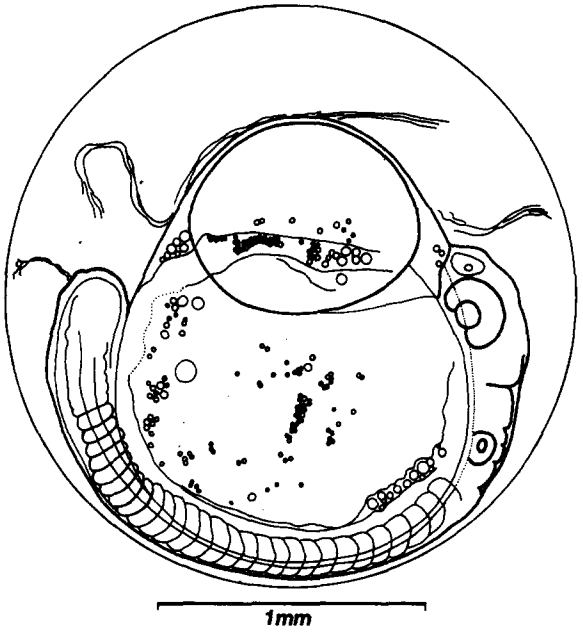

Fig. 12. Embryo of *Stizostedion vitreum* at age 3 days 50 min (step $E^2 5$), left side view.

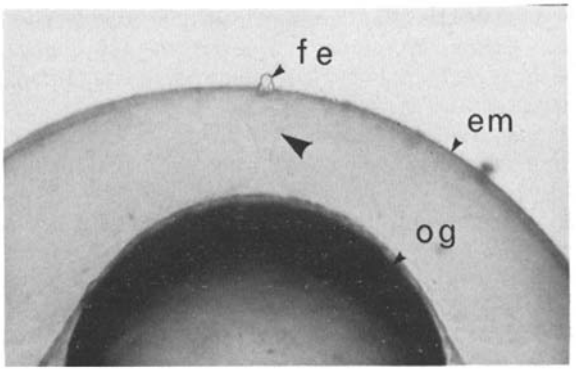

Fig. 13. Photomicrograph of an unidentified structure in the perivitelline fluid (arrow) of a *Stizostedion vitreum* embryo at age 3 days 4 h 50 min (em = egg membranes, fe = fluid expulsion, og = oil globule).

It ended almost nine hours later at age 3 days 6 h 40 min (49.2 TU).

Immediate changes in the head included the development of lenses in the optic cups, olfactory placodes and auditory placodes, the latter structure forming 0.35 mm posterior to the eye. The trunk-tail bud was elevated 0.25 mm and was slightly undercut by its junction with the yolk blastoderm. The dorsal median finfold was apparent anterior to the tip of the trunk-tail bud and posterior to the somites, which now totalled 20 to 24 pairs. Vesicular structures and cellular protrusions were on the ventral body surface between the first and eighth somites. Relative to the embryo's body, the yolksac cavity was largest laterally and smallest on the anterior and posterior sides of the large oil globule. Total length was constant throughout this step at 3.2 mm.

At age 3 days 50 min the eye lens was spherical and what were previously the olfactory and auditory placodes, were now vesicles. The cerebellum was evident due to its lateral expansion and posterior to it the hindbrain was split by a midline cleft. Ventricular inflation of the hindbrain was evident by the transparent appearance of its dorsal surface. There were 29 to 31 pairs of somites and all except those most recently formed in the posterior displayed a slight 'V' shape. The trunk-tail bud had increased its overgrowth of the yolk blastoderm junction and a median finfold was apparent on the dorsal, posterior and ventral surfaces (Fig. 12).

The only notable observation for the remainder of this step pertained to the lack of homogeneity in the perivitelline fluid. Throughout development within the egg membranes, fluid threads of an apparently different viscosity were evident in the perivitelline fluid (for example, see Fig. 9a, d, 12). They often appeared to be continuous with a thin layer of similar viscosity adjacent any part of the embryo. These 'viscous threads' also appear in the Eurasian pike-perch *Stizostedion lucioperca*, as illustrated by Kryzhanovsky et al. (1953). In one specimen (age 3 days 4 h 50 min) there was a definite shape that was not threadlike, but which still appeared as a difference in viscosity (Fig. 13). It arose from the large oil globule blastoderm surface, passed through the perivitelline fluid, made contact with the egg membranes, and produced a fluid expulsion through the zona radiata and chorion to the outside of these membranes.

[1] 'trunk-tail portion' refers to that part of the embryo's body occurring posterior to the yolk.

Step $E^3$6. *Formation of melanophores, contraction of the primordial skeletal and cardiac musculature, beginning of rapid increase in total length, and the appearance of otoliths* (Fig. 14, 15).

The third step of the embryonic phase began at age 3 days 6 h 40 min (49.2 TU) when melanophores and body motion were first observed, and ended at age 4 days 10 h (66.2 TU) when blood circulation began.

At the beginning of this step fine stellate melanophores were distributed evenly throughout the ectoderm overlying the yolk and large oil globule. After one hour of observation, motion was seen in the head region of a single embryo. Figure 14 depicts the earliest motions observed over a 12 minute interval showing their sites, sequence and direction of motion. Motions were short contractions in both time and distance with no apparent pause between contraction and relaxation. There were 35 somite pairs and about 7 to 10 of these occurred in the trunk-tail portion[1] which was now between 0.7 and 0.8 mm long. The embryo was still 3.2 mm in total length but the distance between the posterior margin of the eye and the anterior margin of the auditory vesicle was reduced, being slightly less than 0.30 mm.

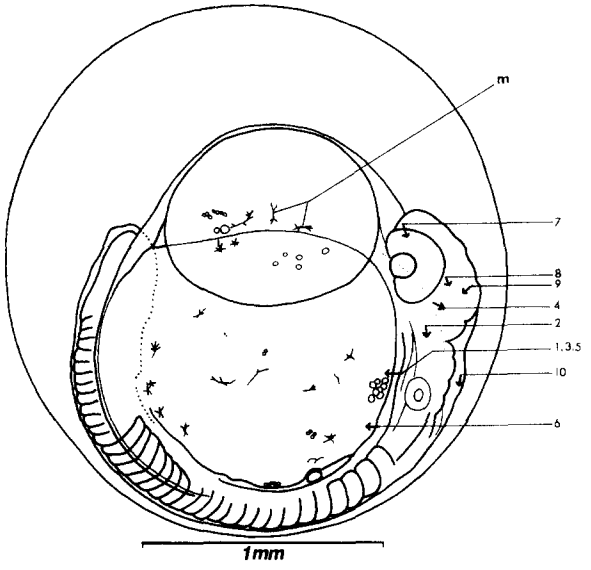

Fig. 14. Early movements in a *Stizostedion vitreum* embryo at age 3 days 6 h 40 min (step $E^3$6). Left side view with anterior body slightly dorsad and posterior body slightly ventrad; arrows indicate direction and centre of motion, numbers indicate sequence of motion. Motions 1, 3, 5 and 6 consisted of pressing the head region into the yolk. Motion 4 turned the head slightly to the left while remaining motions appeared more like epidermal twitches (m = melanophores).

By age 3 days 11 h 10 min (52 TU) the newly visible heart was beating at a rate of 57 to 62 times per minute with a mean of 58 min^{-1} (n = 5)[2]. The large dorsal aorta was visible in the anterior body. A broad area on the mid ventro-posterior yolk surface behind the large oil globule had a roughened appearance. This was the primordial subintestinal-vitelline vein. Melanophores were larger with increased numbers of radiating arms and melanin concentration. The entire body moved during contractions of the axial musculature and the greatest amplitude of motion occurred in the trunk-tail portion. The yolksac cavity was diminished laterally and posteriorly while the anterior portion, which the head partially overlay, was considerably enlarged. The trunk-tail portion was 0.7 to 1.0 mm long and contained 9 to 14 of the total of 38 pairs of somites in 3.4 mm long embryos. Over the next 18 hours total length increased 1.0 mm indicating an interval of rapid increase in total length (Fig. 31). Changes other than length increment and the elaboration of features previously described were minimal for the remainder of the step.

By age 3 days 16 h 35 min, the notochord was 0.05 mm wide and vacuolated. The cerebellum was more distinct, segregated by large constrictions anteriorly and posteriorly, and considerably elevated above the hindbrain.

The rate of heart contractions increased to 88 or 89 min^{-1} at age 4 days 3 h 15 min (n = 5). Two small otoliths were discernable in the auditory vesicles. Small and inconspicuous melanophores were visible on the body, but only posterior to the yolk. Total length was 4.0 mm, somites totalled 45 pairs and the trunk-tail portion was 1.0 to 1.2 mm long with 18 to 23 pairs of somites.

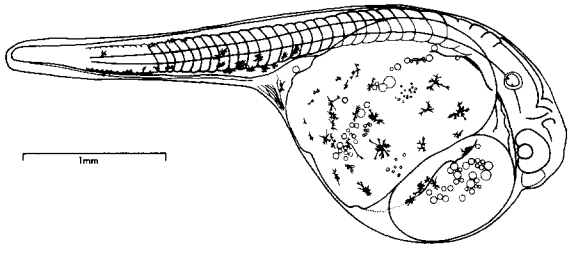

Fig. 15. Embryo of *Stizostedion vitreum* at age 4 days 5 h 15 min (late step $E^3 6$), right side view after excision from egg membranes.

[2] 'n' values are always the number of different specimens examined for heart beat rates.

Several hours later (age 4 days 5 h 15 min) ten embryos displayed a wider range of heart contractions (74 to 97 min^{-1}), but the mean, 87 min^{-1}, was approximately the same. The head now overlapped the large oil globule in such a manner as to place the heart immediately over the large clear yolksac cavity (pericardial cavity), previously described as expanded in this area. The auditory vesicles were now more irregularly circular than oval in shape. The distance between them and the eye was 0.25 mm. In one of the ten embryos examined, several moving blood elements were observed in the subintestinal-vitelline vein which was now a large discrete channel running between the presumptive rectal area and the large oil globule. No further blood elements were observed in motion, even after excision from the egg membranes. The indication was however, that blood circulation was imminent. Prior to excision, the large oil globule measured 0.70 mm high by 0.80 mm wide (side view). After excision (Fig. 15) it measured 0.60 mm high by 0.90 mm wide. Excision also resulted in straightening of the body with the exception of the head, the axes of each meeting at about a 90° angle. The embryo measured 4.4 mm in total length and 1.9 mm in trunk-tail length.

Step $E^4 7$. *Development of blood flow in a simple subintestinal-vitelline circulatory system, increased motility, eye pigmentation, completion of somite segmentation, rectal anlage, hatching gland cells* (Fig. 16).

The fourth step in the embryonic phase began at age 4 days 10 h (66.2 TU) with the observation of blood elements in circulation, and ended after 24 hours when blood flow had increased and branches of the main system were forming.

Throughout the first 16 hours of this step color-

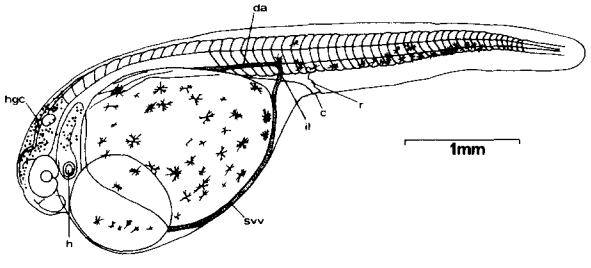

Fig. 16. Early blood circulation in a *Stizostedion vitreum* embryo at age 5 days 2 h (step $E^4 7$), left side view after excision from egg membranes (c = cloaca, da = dorsal aorta, h = heart, hgc = hatching gland cells, il = intestinal loop, r = rectal anlage, svv = subintestinal-vitelline vein).

less blood elements were circulating through a simple system (Fig. 16) that included in sequential order: a two chambered heart, sited as having a central constriction at age 4 days 16 h 10 min; a dorsal aorta, from the heart to the posterior limit of the yolk; a short intestinal loop; a subintestinal-vitelline vein to the oil globule periphery; and last, a diffuse and apparently random course across the external surface of the oil globule, back to the heart. Also, the number of circulating blood elements increased from single cells seen occasionally to single cells seen frequently. At age 4 days 10 h the heart contraction rate was unchanged from the previous step (76 to 94 min^{-1}, $\bar{x}$ = 86 min^{-1}, n = 3), but by age 4 days 16 h 10 min, three embryos displayed rates of 115, 120, and 125 min^{-1}. Embryos at age 5 days showed little difference in rate (118 to 128 min^{-1}, $\bar{x}$ = 123 min^{-1}, n = 4) but they did show a static group of blood elements on the right ventrolateral side of the large oil globule.

At age 4 days 10 h embryo movement was sufficient to change orientation within the egg membranes. A thickening of tissues in the ventral finfold immediately posterior to the yolk indicated development of the primordial rectal region. On the ventral body surface centered below somites four, five and six, a small bulge, indicative of the hepatic anlage, had replaced the previously described vesicular structures. Somite pairs increased to 49 with 32 of these occurring posterior to the yolk.

At age 4 days 16 h 10 min the eyes were slightly pigmented. Melanophores in the yolk ectoderm had expanded. Large dark cells in the head region were later identified as hatching gland cells based on their subsequent elaboration and sudden disappearance at hatching. The entire embryonic ectoderm, inclusive of the head, body, finfolds and yolk, was covered with a second type of large cell which was not dark in color. Their function was not determined and they declined later in development.

At age 5 days, eye pigmentation was still slight, but visible through the external membranes. Of the total of 52 pairs of somites 34 were posterior to the yolk. This was the greatest number of somites observed. The embryo was 5.1 mm in total length, with 2.5 mm posterior to the yolk.

During the last eight hours of this step axial blood flow was extended in a posterior direction in the caudal artery, 1.7 mm beyond the yolk. It then reversed direction in the caudal vein and, on contacting the yolk formed the subintestinal-vitelline vein. The blood was colorless but the number of circulating elements was substantially increased.

At age 5 days 2 h the rate of heart contraction had decreased. Two of seven embryos examined displayed less eye pigmentation and a heart contraction rate of 88 min^{-1}. The five remaining had eyes that were more pigmented and a mean rate of 107 min^{-1} (range 105 to 111 min^{-1}). Embryos excised from the egg membranes displayed increased activity, indicating the ability to perceive and respond to external stimulation.

Certain mensurable and meristic parameters were previously based on the posterior extremity of the yolk. The development of a discrete rectal anlage and cloacal aperture represented a new reference point of greater stability. Somite counts and measurements, previously taken posterior to the yolk, were replaced by 'postanal' counts and measurements. For comparison both were examined for the embryo at age 5 days 2 h. Total length was 5.5 mm and somites totalled 52 pairs. The embryo was 2.8 mm long posterior to the yolk and 2.3 mm in postanal dimension. There were 34 somite pairs posterior to the yolk and 29 in the postanal part (Fig. 16).

By age 5 days 8 h, the embryo's perception of tactile stimulation was evidenced by the vigorous movement that resulted from depression of the egg membranes. A single embryo examined for rate of heart contraction produced a value of 130 min^{-1} from both in and out of the egg membranes. Hatching gland cells were clearly evident on all exposed surfaces of the head to just behind the auditory vesicle and their opacity obscured the inner detail of the head.

Step E^5 8. *Increase in heart contraction rate, haemoglobin synthesis, blood flow to the head, liver and myomeres, ability to swim forward and upward, start of hatching* (Fig. 17, 18).

The fifth step of the embryonic phase started at age 5 days 9 h 55 min (81.2 TU) and lasted for 1 day 18 h. Major changes occurred in the circulatory system. Development of the ability to swim, and to direct this behavior into surface orientation, existed prior to the observed hatching interval. Embryos started to hatch late in this step.

Throughout step E^5 8, heart contraction rates were elevated above those of the preceding and following steps. By the end of step E^4 7 the highest rate recorded was 130 min^{-1} at age 5 days 8 h. At age 5 days 9 h 55 min the mean rate of contraction was 146 min^{-1} (range 140 to 158 min^{-1}, n = 4). The highest mean rate attained was 164 min^{-1} (range 162 to 167 min^{-1}, n = 3) at age 6 days 18 h 10 min. Increases in the rate of heart contraction were paral-

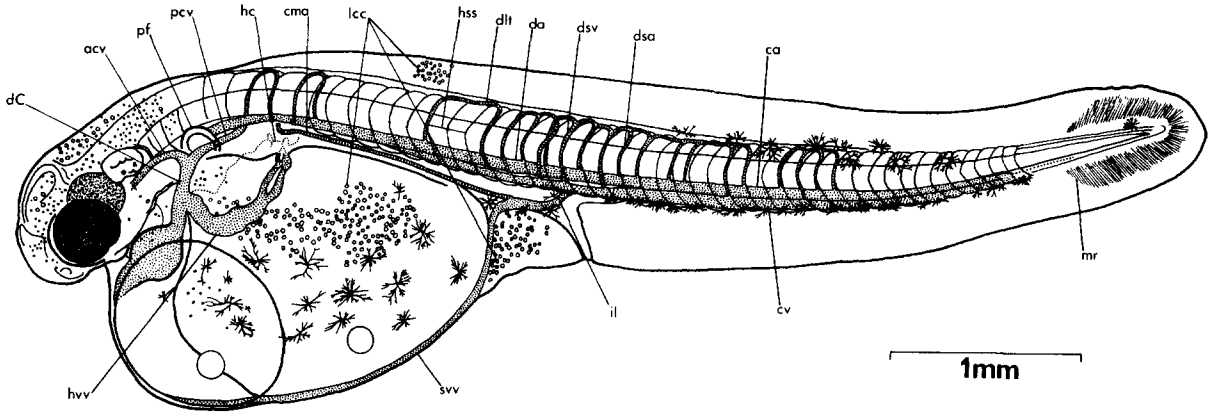

Fig. 17. Embryo of *Stizostedion vitreum* at age 6 days 18 h 10 min (step E[5] 8), left side view after excision from egg membranes (acv = anterior cardinal vein, ca = caudal artery, cma = coeliac-mesenteric artery, cv = caudal vein, da = dorsal aorta, dC = duct of Cuvier, dlt = dorsal longitudinal trunk of the segmental vessels, dsa = dorsal segmental artery, dsv = dorsal segmental vein, hc = hepatic capillaries, hss = horizontal skeletogenous septum, hvv = hepatic-vitelline vein, il = intestinal loop of the caudal vein, lcc = large clear cells, mr = mesenchyme rays, pcv = posterior cardinal vein, pf = pectoral fin bud, svv = subintestinal-vitelline vein).

leled by increases in blood flow (more blood elements mobilized) and new blood vessels became functional carrying blood to areas previously without circulation.

At age 5 days 9 h 55 min the caudal vein was the first branched blood vessel, now producing the posterior cardinal veins as well as the subintestinal-vitelline vein. Blood flowed over the external surface of the large oil globule in a single broad channel. Lateral striations in the somites indicated differentiation into muscle fibres and therefore myomeres. A small pectoral fin bud overlay myomeres four and five. Hatching gland cells extended as far back as the most anterior myomere. Throughout this step the auditory vesicle (now triangular with a flattened top and broad ventral base) changed its peripheral shape and internal contours. Four nonfunctional branchial arteries were visible between the posterior margin of the eye and the ventral margin of the auditory vesicle. Egg membranes were slightly brownish in color and movement within was usually a sideways flexure, though one or several complete rotations were also observed, indicating an ability to move in a forward direction. Total length was 5.5 mm and there were 50 pairs of myomeres. Postanal length was 2.5 mm and postanal myomeres numbered 28.

At age 5 days 18 h 10 min (86.4 TU) major changes in the circulatory system included: a red color in the blood, indicative of haemoglobin; blood flow in the head including, a small vessel around the eye and anterior cardinal veins; blood flow through the hepatic anlage that produced the hepatic-vitelline vein on the left anterior dorso-lateral surface of the yolk; and, fusion of the hepatic-vitelline vein and the duct of Cuvier to produce a short broad vessel leading to the atrium, also on the left side of the embryo. The dorso-lateral yolk surface was devoid of melanophores. Hatching gland cells were evident in the anterior dorso-lateral yolk ectoderm. In preserved specimens the horizontal skeletogenous septa were evident in the preanal myomeres. Total length was 5.7 mm and postanal length 2.7 mm.

At age 5 days 21 h (88.1 TU), egg turgor pressure was noted as reduced during embryo excision. On release, embryo motion was erratic but it did produce forward propulsion. Embryos were oriented dorsal side down during swimming and resting, evidently unable to overcome the buoyancy of the large oil globule. Embryos placed in 5.0 cm of water immediately began swimming upward and succeeded in reaching the surface where they either continued swimming, apparently attempting to maintain the surface position, or ceased swimming, falling head first to the bottom. The path of ascent was in spirals as in *S. lucioperca* (Kryzhanovsky et al. 1953).

By age 6 days 3 h 10 min, individual blood elements were moving forward and backward in a 0.30 mm extension of the caudal artery and vein. The subintestinal-vitelline vein went around the right side of the rectum which was quite robust in appearance. The urinary bladder was adjacent and posterior to the lower half of the rectum. The eyes were black but not yet opaque. Ectoderm overlying the auditory vesicle contained hatching gland cells. The vesicle was now

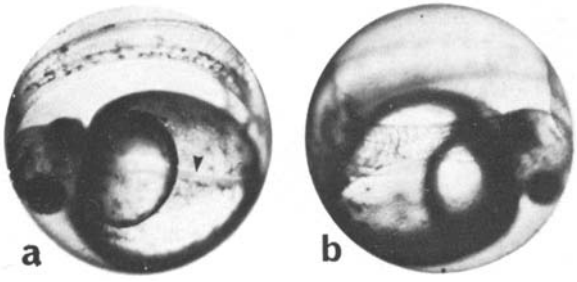

Fig. 18. Photomicrographs of *Stizostedion vitreum* embryos at age 6 days 18 h 10 min (step $E^5 8$) showing relative embryo size to egg volume just before the start of hatching: a – ventral view of yolk shows subintestinal-vitelline vein (arrow), b – right dorso-lateral view.

only 0.13 mm posterior to the eye. The pectoral fin base, parallel to the body axis, was 0.18 mm at its widest. The maximum length of the left pectoral fin, measured perpendicularly from the base to the most distal point of the fin, was 0.11 mm. Melanophores were more branched and concentrated on the lateral and ventral yolk surfaces and along the ventral surface of the caudal vein. There were fine rays of mesenchyme in the median finfold of the caudal fin.

At age 6 days 10 h 40 min, blood flow to the segmental arteries and veins was sporadically evident as single elements moving dorsally out of the dorsal aorta and caudal artery. The anterior and posterior extremities of these observations correspond to myomeres seven and twenty-eight respectively. The blood elements did not usually complete a circuit, but rather moved up and down with heart contractions. The dorsal aorta and the caudal artery and vein were 0.06 mm wide. A capillary network in the hepatic anlage coalesced to form the hepatic-vitelline vein which was somewhat larger than previously observed. The duct of Cuvier also exhibited an increase in size (greater flow). Melanophores were again increased along the ventral surface of the caudal vein and intestinal loop. The branches of a few melanophores extended into the dorsal and ventral median finfold in the postanal segment. The myomere count was reduced to 48 pairs with 27 of these being postanal.

Embryos at age 6 days 18 h 10 min (Fig. 17) displayed an increased flow of blood in the segmental vessels. The segmental arteries outnumbered the segmental veins by a ratio of 15:9 since two or more arteries would join to form the dorsal longitudinal trunk which would then form a single segmental vein. Blood flow in the left posterior cardinal vein had increased and its fusion with the anterior cardinal vein formed a broad duct of Cuvier. A new blood vessel in the head appeared on the posterior side of the auditory vesicle, turned ventrally, and joined with the anterior cardinal vein. The capillary network of the hepatic anlage formed a wide lacuna of the hepatic-vitelline vein. The coeliac-mesenteric artery arose from the dorsal aorta above the hepatic anlage, ran posteriorly and diagonally across the left side of the poorly visible undifferentiated gut (primordial intestine), and joined the subintestinal-vitelline vein. The caudal artery and vein were still being extended at their posterior extremity as evidenced by the back and forth movement of blood elements in this region. The head had started to lift away from the large oil globule in its gradual process of straightening. Mouth formation was apparent by the vaguely discernable separation of the mandibular and maxillary regions. The internal morphology of the auditory vesicle showed new contours above the otoliths. Hatching gland cells had attained their maximum concentration and distribution. In a preserved specimen the horizontal skeletogenous septa were evident in the anterior 40 of 48 myomeres. There were 26 postanal myomeres. Melanophores formed a continuous series on the ventrum of postanal myomeres. Total length was 6.4 mm and postanal length 3.3 mm. Figure 18 shows the embryo's size relative to the egg volume.

The first observation of an embryo hatching occurred at age 6 days 18 h 30 min (101.6 TU). At age 6 days 20 h 30 min the number of segmental vessels had increased, but circulation was otherwise similar to that for embryos at age 6 days 18 h 10 min. The most overt difference was the lighter color of the head; presumably resulting from the release of chorionase, the proteolytic enzymes from the hatching gland cells (Yamamoto 1975). The yolk had narrowed dorso-ventrally and lengthened anterior to posterior, being 18% longer than in the previous unhatched embryo. The eyes were entirely black and totally opaque. Several melanophores were evident on the body anterior to the anus. Total length was 6.7 mm and myomeres totalled 49 pairs. Postanal length was 3.5 mm and contained 27 pairs of myomeres.

The most important change was comparatively inconspicuous but indicated the transition to another step. Blood flow had decreased between the caudal vein and the posterior cardinal veins in contrast with the immediately preceding observation of an unhatched embryo. The result of this alteration was an increase in blood flow through the subintestinal-vitelline vein. The trend to maximal subintestinal-vitelline

blood flow was the basis for the next step's designation.

Step $E^6 9$. *Maximized vitelline circulation, positive phototaxis, completion of the first half of hatching, cartilage formation in the head* (Fig. 19, 20). The last step of the embryonic phase began at age 7 days 3 h 10 min (107 TU) and ended 9 days (135 TU) after activation.

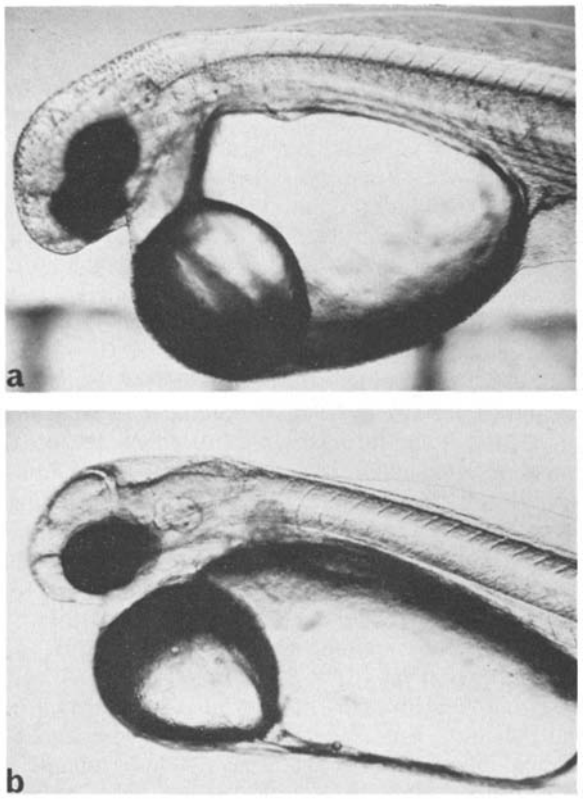

Fig. 19. Photomicrographs of *Stizostedion vitreum* embryos at age 7 days 3 h 10 min (start of step $E^6 9$), left side view after excision from egg membranes: a – bulbous yolk with hatching gland cells showing, b – elongate yolk with inconspicuous hatching gland cells.

A reduced rate of heart contraction was one feature that separated this step from that preceding it. Nine hours earlier the rate was 164 min^{-1}. At the beginning of step $E^6 9$ the rate was 120 to 133 min^{-1} ($\bar{x}$ = 125 min^{-1}, n = 5). By age 7 days 18 h it had dropped to a range of 91 to 113 min^{-1} ($\bar{x}$ = 106 min^{-1}, n = 4). It rose again at the end of this step with a single recorded rate of 128 min^{-1} at age 8 days 11 h 10 min.

Concurrently, blood flow from the caudal vein to posterior cardinal veins ceased. No exception to this alteration was observed in step $E^6 9$. All blood that previously flowed from the caudal vein to the posterior cardinal veins was diverted to the subintestinal-vitelline vein. Proportionately more blood was therefore exposed to the yolk morphogenetic substances and to external dissolved oxygen. Only the hepatic-vitelline vein and the duct of Cuvier shared this characteristic of proximity to both the external environment (perivitelline fluid) and the yolk.

At age 7 days 3 h 10 min, the posterior cardinal vein was still carrying blood in an anterior direction, but only blood received from the segmental veins of the anterior 18 myomeres. The reduced flow was particularly evident at the junction with the anterior cardinal vein, which now provided a heavy flow and by far the greatest contribution to the duct of Cuvier. All blood flowing through the segmental veins posterior to myomere 18 was received by the caudal vein. The posterior extremity of the axial blood flow was at the last myomere. A new blood vessel, between the cerebellum and the midbrain, was visible only in its dorsal position since its origin and destination were

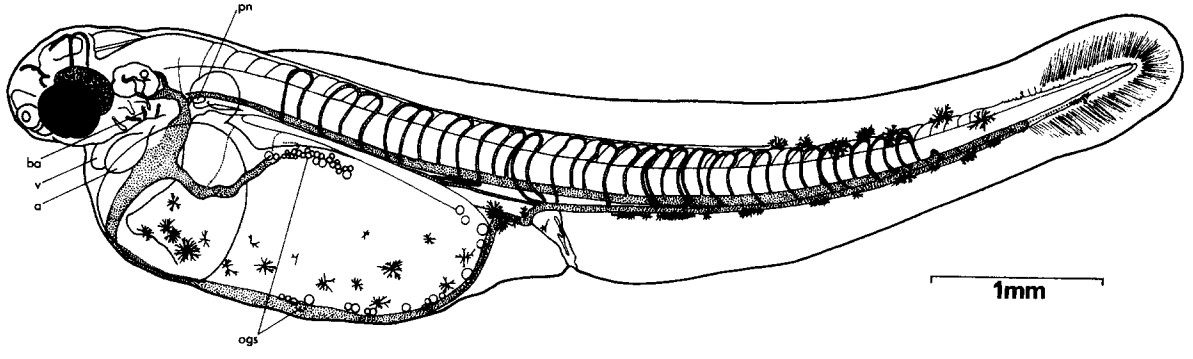

Fig. 20. Embryo of *Stizostedion vitreum* at age 8 days 1 h 35 min (step $E^6 9$) immediately after hatching, left side view, slightly dorsad (a = atrium, ba = branchial artery, ogs = small oil globules, pn = ponephros, v = ventricle).

behind the opaque eyes. Below the auditory vesicle the mandibular and hyoid segments were separately identifiable. Of 45 pairs of myomeres, 25 were postanal in two embryos examined. Both were 6.8 mm in total length and 3.5 mm in postanal length. The pectoral fin bud was still widest at its base (0.20 to 0.24 mm) and was 0.14 to 0.16 mm long. Although both embryos (Fig. 19) were excised from the egg membranes, they displayed the same differences observed in hatched and unhatched embryos at the end of the previous step. The elongated yolk (23% longer) was again associated with inconspicuous hatching gland cells and a comparatively greater lifting of the head, and therefore straightening of the head-trunk section. Movement within the egg membranes included vigorous rotations and a quivering motion which may have been the same as the 'trembling movements' noted to occur at hatching in *S. lucioperca* (Belyy 1972).

Hatched embryos were still rare by age 7 days 10 h. The most apparent difference was the increased distribution of segmental vessels, which as yet did not provide all myomeres with blood. The dorsal longitudinal trunk of the segmental vessels was longer in the anterior half of the body where it spanned a maximum of nine myomeres from the first segmental artery to the first segmental vein. In the postanal segment the maximum span was three myomeres.

All myomeres, excluding the first three and last two, received blood from the segmental vessels by age 7 days 18 h (116.2 TU). The posterior extremity of axial blood flow surpassed the last myomere by 0.5 mm. The segmental vein between myomeres 15 and 16 produced two branches at its most ventral position. The one that carried blood to the posterior was joined by other segmental veins, the confluence joining the intestinal loop of the caudal vein where it bent around the gut. The other branch provided an anterior flow that was also joined by other segmental veins. This confluence formed the minimal flow of a posterior cardinal vein. The hepatic-vitelline vein was an expanded network of blood vessels which joined the duct of Cuvier and the subintestinal-vitelline vein mixing in a large lacuna-like area. A new blood vessel was evident in the dorsal anterior part of the brain. The pectoral fin showed a slight diagonal inclination in a ventro-posterior direction. Numerous small oil globules were concentrated at the lateral junction of the yolk and the large oil globule and ventral to the hepatic anlage. Differentiation of the straight gut was indicated by discrete walls in the regions of the posterior intestine and the rectum. Two bands of cartilage, each representing the fusion of trabecules, polar cartilages and the paranotochord, were visible after alcian blue staining (elements shown in Figure 21b for an older embryo). They did not extend as far as the anterior margin of the eyes. A slight indication of cartilage was evident in elements of the branchiocranium. In a preserved specimen, the total myomere count was 46 pairs and the postanal count 25. Total length was 6.8 mm, no change from an embryo 15 hours earlier.

At age 8 days 1 h 35 min, a hatching embryo (Fig. 20) was placed in a circular glass container that was 20 cm in diameter, contained 2.5 cm of water, a 60 watt incandescent light 25 cm above the bottom, and with half of the area shaded from direct light. Even with the membranes enveloping the anterior half of the embryo it succeeded in swimming upwards to the surface, as was also observed in *S. lucioperca* (Belyy 1972). Fifteen minutes passed before the membranes were discarded. An extreme positive phototaxis was demonstrated by a strong aversion to the shaded area. In ten consecutive intervals of activity the durations were 4.3 to 40.9 sec ($\bar{x}$ = 15.6 sec). Ten consecutive intervals of inactivity were 12.4 to 59.4 sec in duration ($\bar{x}$ = 36.2 sec). Intervals of activity began with first motion on the bottom, included swimming towards and usually reaching the surface, and ended after a passive descent when contact was made with the bottom. Belyy (1972) also timed this activity in *S. lucioperca* but subdivided it into an ascending part, with an average duration of 7 sec, and a descending part, with an average duration of 8.6 sec. Water depth and 'n' values were not reported, however the average total time of 15.6 sec was a striking parallel.

In the same specimen, blood flowed through the third of four branchial arteries. The development of a canal in the auditory vesicle indicated differentiation into a simple labyrinth. The liver showed a definite increase in size after a long interval of little change. There were still numerous small oil globules concentrated below it and along the length of the dorsal side of the subintestinal-vitelline vein. The posterior side of the pectoral fin was further ventrally inclined. Its greatest width (0.29 mm) occurred above the base and maximum length was 0.22 mm. Oval shaped structures on the ventral body-surface behind the pectoral fin, were the first indication of the pronephros. There were 45 pairs of myomeres in a formalin-preserved specimen and 24 were postanal. The embryo was 7.0 mm in total length and 3.8 mm in postanal length.

By age 8 days 11 h 10 min the fused paranotochord, polar cartilage and trabecule components extended from the posterior margin of the auditory capsules to a distance half way between the anterior margin of the eyes and the anterior tip of the head. Cartilage lined the ventral surface of the auditory capsules and also formed a ring around each eye. Meckel's cartilage had developed in the lower jaw which extended to the anterior margin of the eyes. In the upper jaw a fine uncalcified strand, the percursor of the maxillary, had no cartilaginous intermediate since its only positive reaction to stain was with alizarin later in development. On the side of the head between the posterior extremity of the lower jaw and the ventral surface of the auditory capsule, two independent nonarticulating cartilaginous components were early forms of the palatoquadrate and the hyomandibular. In the branchiocranium cartilaginous components were the anterior copule, one pair of ceratohyals, and four pairs of ceratobranchials. There were 48 pairs of myomeres in a formalin-preserved specimen and most displayed a well defined horizontal skeletogenous septum. There were 26 pairs of postanal myomeres. The total length of the preserved specimen was 7.3 mm.

3.1.3 The eleutheroembryonic phase

By definition, the eleutheroembryonic phase refers to the interval during which the young fish exists beyond the confines of the egg membranes while still feeding mainly on the yolk. Under the conditions of this study the hatching process for walleye lasted for an interval of 4½ days, between ages 6 days 18 h 30 min (101.6 TU) and 11 days 6 h 50 min (169.2 TU). The eleutheroembryonic phase was designated as commencing at age 9 days (135 TU) based on a number of observations. First, this time represented an actual step boundary. Before age 9 days the subintestinal-vitelline circulation was maximized as described above. After age 9 days it was reduced by the reestablishment of flow from the caudal vein to the posterior cardinal veins. Also at age 9 days, eleutheroembryos developed an ability to remain in motionless suspension immediately below the water surface. The eleutheroembryo would be in an environment of maximal oxygen concentration with a minimal expenditure of energy, and with continuous and essentially effortless transportation from the fluvial to the lacustrine environment. Finally, this time corresponded with an interval of intensive hatching with 40 to 60% hatched by 9 days of age.

The eleutheroembryonic phase lasted for 6¾ days. It consisted of two steps with the first being about 4 days duration. The start of external feeding at age 15 days 18 h (236.2 TU) ended this phase with the beginning of an interval of mixed nutrition.

Step $F^1$10. *Surface suspension, reduced vitelline circulation, pectoral fins moving, mouth open, blood circulation to the gills, end of hatching interval, end of rapid increase in total length* (Fig. 21, 22, 23).

During the first step of the eleutheroembryonic phase, the appearance of functional blood vessels in the gills and digestive tract signified changes directed toward gill respiration and feeding. On the other hand, blood flow to the subintestinal-vitelline vein was reduced by the reestablishment of flow from the caudal vein to the posterior cardinal veins. The step ended when blood flow from the caudal vein to the subintestinal-vitelline vein was discontinued at age 13 days 1 h 15 min (195.8 TU). Structural changes occurred in the head (especially the mouth) and also

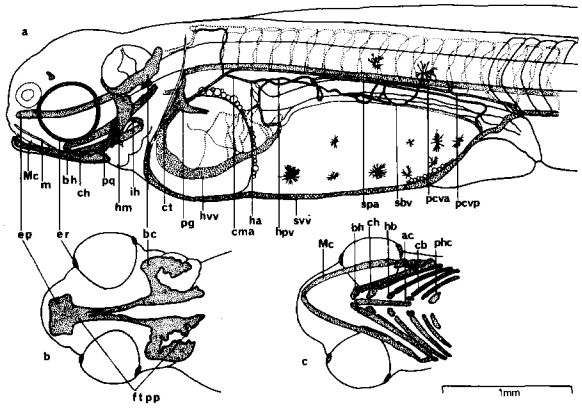

Fig. 21. Eleutheroembryo of *Stizostedion vitreum* showing cartilage at age 10 days 18 h and preanal blood circulation at age 10 days 23 h (step $F^1$10): a – left lateral view, b – dorsal view, c – ventral view of branchiocranium (ac = anterior copule, bc = basicapsular commissure, bh = basihyal, cb = ceratobranchials, ch = ceratohyal, cma = coeliac-mesenteric artery, ct = cleithrum, ep = ethmoidian plate, er = eye ring, ftpp = fused trabecules, polar cartilage and paranotochord, ha = hepatic artery, hb = hypobranchial, hm = hyomandibular, hpv = hepatic portal vein, hvv = hepatic-vitelline vein, ih = interhyal, m = maxillary, Mc = Meckel's cartilage, pcva = posterior cardinal vein with anterior directed blood flow, pcvp = posterior cardinal vein with posterior directed blood flow, phc = pharyngeal cartilage, pg = pectoral girdle (cartilage component), pq = palatoquadrate, sbv = subintestinal vein, spa = supraintestinal artery, svv = subintestinal-vitelline vein).

through the length of the digestive tract. Eleutheroembryos retained their positive phototaxis and surface-suspension-ability throughout this step. The process of hatching was completed during this step. Total length was 7.4 mm at age 9 days and 9.4 mm at age 12 days 18 h. In the same interval postanal length increased from 3.9 to 4.9 mm. In preserved specimens there were 49 to 51 pairs of myomeres and 28 to 30 pairs were postanal.

Though mortalities occurred throughout walleye embryonic ontogeny, they increased in this step between the ages of 9 days 12 h (142.5 TU) and 10 days 20 h (162.5 TU). This interval corresponded with an observed decrease in specimen vitality which was manifested by a rapid failure of the circulatory system during the observation procedures. Specimens between and including 8 days 11 h and 10 days 18 h displayed this decrease in vitality.

Eleutheroembryos that were found hatched by age 8 days 14 h were transferred from the high flow of the hatchery jar to a rearing box where the flow was imperceptible. Ten hours later they were observed hanging in a motionless state from the water surface. Agitation of the water caused them to fall to the bottom suggesting that their suspension ability was not due to any persistent change in buoyancy. The body hung in a slightly diagonal position with the tip of the head just below the water surface and the ventral side of the body uppermost, probably due to the buoyancy of the large oil globule. The lower jaw extended beyond the eye but was short of the anterior extremity of the upper jaw by 0.13 mm. The posterior edge of the operculum was half way between the posterior margin of the eye and the pectoral fin. The dorsal ends of the gill arches extended beyond the operculum and their cartilaginous supports (ceratobranchials) were visible in live eleutheroembryos. Other changes included: an expanded gut throughout its length, a lumen in the posterior intestine that contained a green-yellow substance (bile, Pelluet 1944), and segmentation of the liver into lobules. The larger clear cells previously observed throughout the ectoderm were substantially reduced in their concentration.

By age 9 days 16 h 30 min (145.3 TU), the mouth was moving, but not open, and the pectoral fins moved slightly and displayed a cartilaginous girdle at their base. The girdle was 'L' shaped with the longest extension parallel to the body axis and the shorter one oriented perpendicularly upward. Where the two extensions met there was a central foramen. The minimum presumptive potential of the girdle was likely that of the scapula and coracoid. The subintestinal-vitelline vein had lost some of its blood flow from the caudal vein due to the reestablishment of flow through the posterior cardinal veins. More than half of the eleutheroembryos examined during step F^1 10 exhibited this bifurcating flow from the caudal vein. The remaining group was split evenly into those with flow to only the subintestinal-vitelline vein and those with flow to only the posterior cardinal veins. There was no apparent relationship between the course of blood flow and the variable rate of heart contractions (107 to 162 min^{-1}), which was possibly related to variability in the time required to obtain this measurement. The increased activity of eleutheroembryos, the rhythmic opening of the mouth, and the thickening of tissues over the pericardial cavity contributed to make this measurement increasingly difficult to obtain. The hyomandibular cartilage overlapped the anterior basicapsular commissure of the ventro-anterior margin of the auditory capsule. The anterior extremity of the trabecules was laterally expanded forming the ethmoidian plate. Two pharyngeal teeth were visible but only after the specimen was cleared. The lateral body surface over the posterior half of the yolk displayed a new distribution of melanophores.

At age 9 days 23 h 15 min the eleutheroembryo examined was known to have hatched four hours earlier. The caudal artery and vein produced a loop at their posterior extremity which consisted of a single crossing of their paths so that for a short distance their dorso-ventral positions were reversed. A new blood vessel arose from behind the auditory capsule carrying blood posteriorly along the dorsal surface of the notochord. Its junction was with the first segmental vein. Numerous small oil globules were still adjacent the lateral and dorsal surfaces of the subintestinal-vitelline vein and also at the junction of the yolk and the large oil globule. The lumen of the posterior intestine still contained bile. The pectoral fin was 0.27 mm in base width, 0.47 mm in maximum width, 0.38 mm in maximum height, and inclined at about a 45° angle to the body axis. The forerunner of the cleithrum was a fine uncalcified strand anterior to, and in contact with, the cartilaginous component of the pectoral girdle. It later retained alizarin and had no cartilaginous intermediate. The peduncular median finfold had narrowed and the caudal finfold had broadened.

The mouth was open by age 10 days 5 h 45 min (153.6 TU). The pectoral fin base was almost at a right angle to the body axis.

The upper and lower jaws were equally anterior in position by age 10 days 18 h (Fig. 21). Several teeth were visible in both. In the branchiocranium, the interhyals, basihyals, hypobranchials and pharyngeals were visible after staining with alcian blue. A small triangular-shaped piece of cartilage was visible above the eye in the constriction between the fore- and midbrain regions. The ventral half of the anterior margin of the auditory capsule was covered with cartilage.

The last incidence of high mortality during the hatching interval was recorded at age 10 days 20 h. At this time eleutheroembryos were mostly at the surface in a definite concentration nearest the light source. This phototaxis was most apparent in a large aquarium where the available space for distribution was large in comparison to the observed concentration of eleutheroembryos near the light source.

Four branchial arteries displayed blood flow by age 10 days 23 h (164.4 TU). Blood flow to the newly visible pseudobranch in the pseudobranchial artery was approximately equivalent to that of any single branchial artery. Very small filaments projected posteriorly from the ceratobranchials. Figure 21a illustrates the circulatory system anterior to the anus and posterior to the head. The coeliac-mesenteric artery bifurcated to form the hepatic artery and the supraintestinal artery. The hepatic portal vein was newly apparent and contributed the blood supply of the liver. The liver capillaries formed a diffuse plexus on the left side of the yolk-oil globule surface and collected in the broad hepatic-vitelline vein. The supraintestinal artery ran in a posterior direction along the dorsal side of the intestine, which by this time displayed a lumen throughout its length. At the posterior end of the intestine the supraintestinal artery entered the newly formed subintestinal vein, immediately anterior to the point where the confluence of the two joined the subintestinal-vitelline vein at the posterior extremity of the yolk. Blood flow to the digestive tract was present sporadically and generally minimal when it existed. This may have been a function of the deteriorated oxygen conditions likely to be prevalent during the observation procedures. The response to reduced environmental oxygen concentration may be a reduction in flow to non-essential areas and an increase to essential areas. The digestive tract would be a non-essential area and the respiratory vessels, such as the subintestinal-vitelline vein and hepatic-vitelline vein, essential areas. A ventral bend in the gut at the liver was the earliest indication of a stomach and weak peristaltic undulations were visible above the anus. There were melanophores on the top of the head between the eyes and at the posterior extremity of Meckel's cartilage.

By age 11 days 6 h 50 min (169.2 TU) hatching was almost complete. Posterior to the large oil globule, the entire length of the digestive tract had developed small transverse folds that were most pronounced posterior to the yolk. Melanophores blackened the dorsal and ventral surfaces of the intestine where the intestinal loop of the caudal vein crossed. Small oil globules were still concentrated around the remaining junction of the yolk and the large oil globule. Positive phototaxis was still evident. A group of 14 eleutheroembryos, given a choice of illuminated and unilluminated areas, were all congregated in the illuminated area within 20 sec. Reversal of the areas caused the eleutheroembryos to reverse their location. They did however venture into the unilluminated area on occasion and would subsequently leave it, but not always with the greatest of haste.

By age 11 days 18 h 5 min (176.3 TU) peristaltic undulations occurred through the entire intestine. Several segments of the dorsal longitudinal trunk formed loops where they bent ventrally to become the segmental veins. These loops never extended into the dorsal median finfold. The pectoral fin had a maximum width of 0.80 mm, a maximum length of 0.64 mm and a base width of 0.29 mm. The distal edge was not smoothly rounded as before but rather formed a blunt point at the middle. Calcification was indicated by the retention of alizarin stain in the maxillary, cleithrum, and the distal tips of teeth in the upper jaw, lower jaw and pharyngeal cartilage. Subsequent development produced a pattern in calcification, however there were exceptions where no calcification was evident in much older eleutheroembryos and larvae. Cartilaginous epibranchials were apparent but poorly defined. The posterior copule was apparent between the last pair of ceratobranchials. A small piece of cartilage projected dorsally inward from the posterior of Meckel's cartilage and posterior to this, the palatoquadrate articulated with Meckel's cartilage. Viewing the auditory capsule laterally revealed cartilage on the anterior, ventral, and posterior margins. The ethmoidian plate was expanded laterally. The rate of increase in total length decreased from this time on (Fig. 31).

The transverse folds of the digestive tract were more pronounced by age 12 days 6 h 30 min. A slight, left, lateral bend in the stomach region produced an optical cross section revealing an enlarged lumen. The mouth was opening and closing rhythmically at a rate of 35 min^{-1}.

At age 12 days 8 h 15 min, eleutheroembryos were still in motionless suspension at the water surface. The lower jaw extended slightly beyond the upper jaw and was the point of contact with the surface. Numerous small protuberances extended from the epidermis at the anterior tip of the lower jaw and also from the ventral projection caused by the posterior extremity of Meckel's cartilage. Their positions on surfaces that could potentially come into contact with various substrates suggested that they possibly functioned as adhesive glands. In the upper jaw, the premaxillary was vaguely apparent as a clear uncalcified strand (Fig. 22). It resembled the maxillary before calcification. The paired pharyngeal components were well defined and each contained three teeth. There was a foramen in the dorsal portion of the hyomandibular. Epibranchials were better defined. There was a small piece of cartilage on the dorsal margin of the auditory capsule that was not in contact with any other cartilage of the auditory capsule. In the pectoral fin blood was flowing in the subclavian vein and distal to it, rays of mesenchyme were convergent toward the projecting mid periphery of the fin.

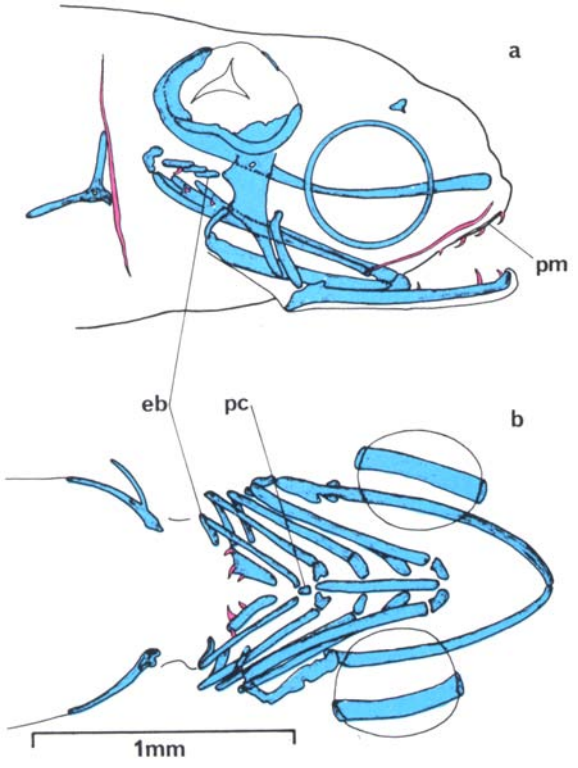

Fig. 22. Skeletal structures in *Stizostedion vitreum* at age 12 days 8 h 15 min (step F' 10). Blue areas indicate cartilage (retention of alcian blue stain), red areas indicate calcified bone (retention of alizarin red): a – right side view, b – ventral view of branchiocranium (eb = epibranchial, pc = posterior copule, pm = premaxillary).

At age 12 days 18 h (191.2 TU) (Fig. 23), a new blood vessel, identified as the profundal caudal vein, arose ventrally from the caudal artery 0.5 mm posterior to the anus, crossed the plane of the caudal vein running ventral and parallel to it and joined with the subintestinal-vitelline vein on the ventro-anterior side of the intestinal-rectal junction. The last observation of blood flow from the caudal vein to the subintestinal-vitelline vein occurred at this time. The pseudobranchial artery was now smaller than any single branchial artery, in contrast with earlier observations where they were approximately equal in size and flow. The gill filaments were a maximum of 0.06 mm long but did not yet receive blood. Rhythmic mouth movements increased to 51 min^{-1}. Eleutheroembryos displayed a broad gaping of the jaws that appeared to produce maximum distension. The width of the intestine and the depth of the transverse folds were increased. The lumen of the stomach had also increased

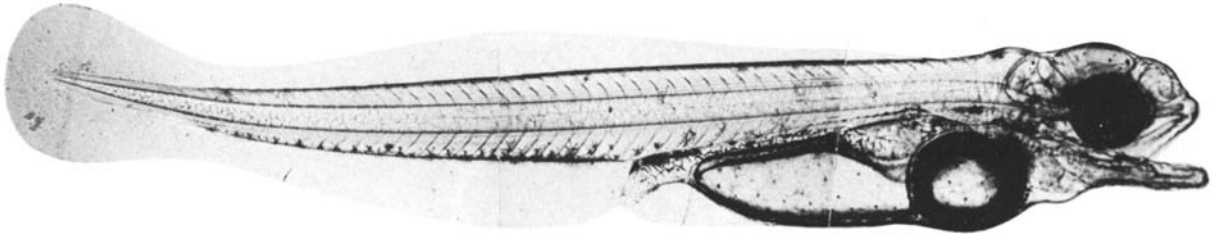

Fig. 23. Photomicrograph of *Stizostedion vitreum* eleutheroembryo at age 12 days 18 h, right side view.

in size and its left ventro-lateral bend was more pronounced. The liver extended ventrally about halfway across the left side of the large oil globule. Melanophores in the yolk epidermis were closer together due to the reduction in yolk volume. In lateral view the large oil globule was still rounded and measured 0.76 by 0.83 mm. The earliest measurements of the large oil globule produced dimensions of 0.75 by 0.90 mm showing that its volume had not undergone a similar decrease to that of the yolk. The large clear cells, once concentrated in all epidermal surfaces, were almost totally absent.

Step F^2 11. *Transfer to gill respiration, termination of surface suspension, increase in active swimming, rapid yolk depletion* (Fig. 24).

The events of the last step of the eleutheroembryonic phase indicated that the primary site of apparent respiratory function was shifting to the gills. The flow of blood from the caudal vein to the subintestinal-vitelline vein ended, but only after an alternative, though substantially reduced flow, had been added to the subintestinal-vitelline circulation from the profundal caudal vein. This was supplemented by blood from the subintestinal vein. The combination of these relatively small blood vessels did not provide an equivalent flow to that lost by the termination of flow from the caudal vein. The gill filaments were later vascularized and the gill arches contained both afferent and efferent branchial arteries. The hepatic-vitelline vein no longer formed a surface plexus, however, its structure and position as a single broad vessel on the lower left surface of the large oil globule suggested it still contributed to respiration. Heart beat rates were 120 to 150 min^{-1} and again there was no apparent correlation with any particular aspect of development. Other changes included: the rapid depletion of yolk, continued enlargement of the stomach and intestine, appearance of the gall bladder and swimbladder (though the latter was not inflated), and an increase in the numbers and distribution of melanophores on the head and anterior body. Surface suspension ended and the eleutheroembryos indulged more in active swimming. The smallest eleutheroembryo, observed at the beginning of this step, was 8.7 mm in total length. The largest was 9.6 mm, only 0.2 mm greater than the largest observed at the end of the preceding step. This step ended at age 15 days 18 h (236.2 TU) when small particles were observed in the digestive tract.

Step F^2 11 began at age 13 days 1 h 15 min (195.8 TU) when the flow of blood from the caudal vein to the subintestinal-vitelline vein ended without subsequent exception. All blood from the caudal vein, henceforth returned to the anterior via the paired posterior cardinal veins. The swimbladder was not visible but melanophores that characteristically covered its dorsal surface could be seen. Melanophore coverage had increased on the dorsum of the head and anterior body. In the epidermis of the yolk they were most concentrated along the mid ventral line, less concentrated laterally, and further reduced dorso-laterally. The liver extended ventrally across most of the left side of the large oil globule. Eleutheroembryos tended to be distributed around the sides of aquaria.

By age 13 days 6 h 25 min the nostrils and the anterior tip of the lower jaw received blood circulation. It was apparent that the eleutheroembryo observed had only recently opened its mouth since strands of tissue still joined the upper and lower jaws. The gaping motion of the jaws, noted above to extend the jaws to their apparent maximum distension, may represent a behavioral requirement for complete separation of the jaws. A single tooth in the anterior of the upper jaw had erupted through the epidermis. The calcified maxillary had broadened at its posterior end. The small triangular piece of cartilage previously noted above the eyes in the dorso-lateral constriction

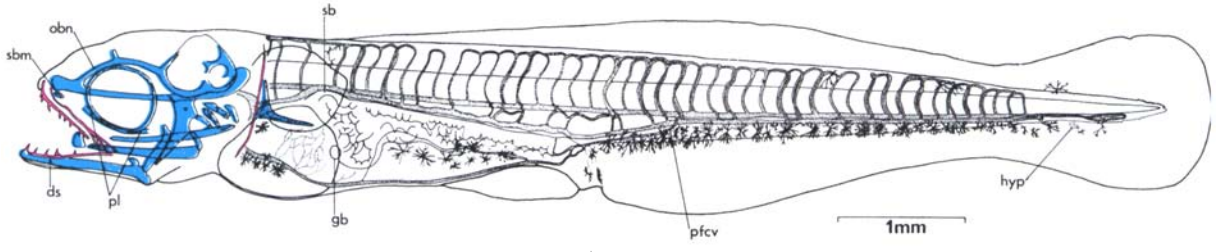

Fig. 24. Cartilage, bone and blood circulation in a *Stizostedion vitreum* eleutheroembryo at age 15 days 6 h (step F^2 11), left side view (ds = dentosplenial, gb = gall bladder, hyp = site of hypural formation (thickening), obn = orbitonasal, pfcv = profundal caudal vein, pl = palatine, sb = swimbladder, sbm = submaxillary).

between the fore- and midbrain regions, was joined to the anterior basicapsular commissure by a new band of cartilage. Posterior to the ethmoidian plate but anterior to the eye, there was a short dorsal extension of cartilage that would later unite with the triangular piece above. This was the orbitonasal cartilage. Below the eye and inner to the upper jaw a band of cartilage was newly visible representing some or all of the palatine and the ethmo- and rostropalatine apophyses.

In the gills, the filaments were a maximum length of 0.14 mm by the age of 13 days 18 h 30 min. The course of blood consisted of the afferent and efferent branchial arteries with flow between in the filamental arteries. The pseudobranchial artery formed a circular loop at the pseudobranch. Dorsal to the stomach, the coeliac-mesenteric artery produced the hepatic artery and the supraintestinal artery. The supraintestinal artery ran along the dorsal side of the anterior two thirds of the intestine. Small blood vessels arising from it passed ventrally around the intestine and were collected on the ventral side by the subintestinal vein. At the posterior tip of the yolk the subintestinal vein was joined by the profundal caudal vein. The confluence of the two formed the subintestinal-vitelline vein, finally joining with the hepatic-vitelline vein on the left ventro-lateral side of the large oil globule. The stomach was expanded and almost as ventral in position as the large oil globule. Only a thin layer of yolk separated the stomach from the dermal tissue. In the upper jaw there were five teeth on each side and in the lower jaw there were four.

Eleutheroembryos were seldom seen in motionless suspension by age 14 days 6 h 35 min (214.1 TU). The swimbladder, dorsal to the stomach, was oval in shape, displayed no apparent circulation, was covered dorsally with melanophores, and was not inflated. There was a thickening of cells ventral to the notochord in the caudal fin where the first cartilaginous hypural would later develop.

By age 14 days 18 h the larger gill filaments, no longer uniform cylindrical projections, had lateral expansions that indicated the beginning of lamellar-development. The gall bladder was a yellow-green oval area at the left mid-posterior margin of the large oil globule.

At age 14 days 23 h 50 min the caudal extremity of axial blood vessels displayed two bypasses between the caudal artery and vein. One occurred before these two vessels reversed their dorso-ventral position and the other after (as seen later, Fig. 26). In the space centered above the anus and between the axial arterial and venous flows, anterior directed blood flow joined adjacent segmental veins below the dorsal aorta. The remaining yolk was wedge shaped and its greatest depth (0.32 mm), immediately posterior to the stomach, was only slightly greater than the width of the intestine.

By age 15 days 6 h (228.8 TU) the premaxillary and dentosplenial had started to calcify (Fig. 24). The distal tips of all teeth showed retention of alizarin stain. Each side of the upper and lower jaws contained seven and five teeth respectively. There was a total of 12 pharyngeal teeth and at least half of these did not emerge from the visible pharyngeal cartilage. Cartilage above the eyes formed a pair of arches (one on each side of the head) that connected the posterior ethmoidian plate and the anterior basicapsular commissure. The pieces that formed first, now in the middle of each arch, extended dorsally inward and would eventually unite to form a continuous cartilaginous bridge over the dorsal fore-midbrain constriction. Lateral to both sides of the ethmoidian plate weakly staining components were the submaxillary cartilages.

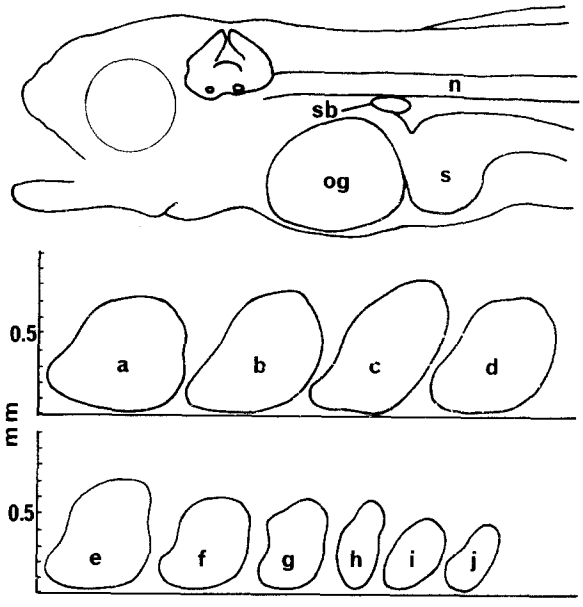

Fig. 25. Changes in shape and size of the large oil globule of *Stizostedion vitreum* larvae during the interval of mixed nutrition (step PP' 12). Left side view with large oil globules oriented with notochord parallel to the horizontal axis: a – age 15 days 18 h, b – age 16 days 18 h 5 min, c – age 17 days 6 h 45 min, d – age 18 days 6 h 35 min, e – age 20 days 18 h, f – age 21 days 12 h 55 min, g – age 22 days 5 h, h – age 23 days 6 h, i – age 24 days 5 h, j – age 25 days 5 h (n = notochord, og = oil globule, s = stomach, sb = swimbladder).

An approximate 50% mortality occurred between the ages of 14 days 6 h 35 min and 15 days 18 h. Also during this interval there was an increase in what appeared to be a fungus infection. The fungus generally appeared first at the anterior tip of the lower jaw and subsequently spread over the rest of the head. Not all eleutheroembryos that were found dead or dying displayed this infection, however those infected were in the majority.

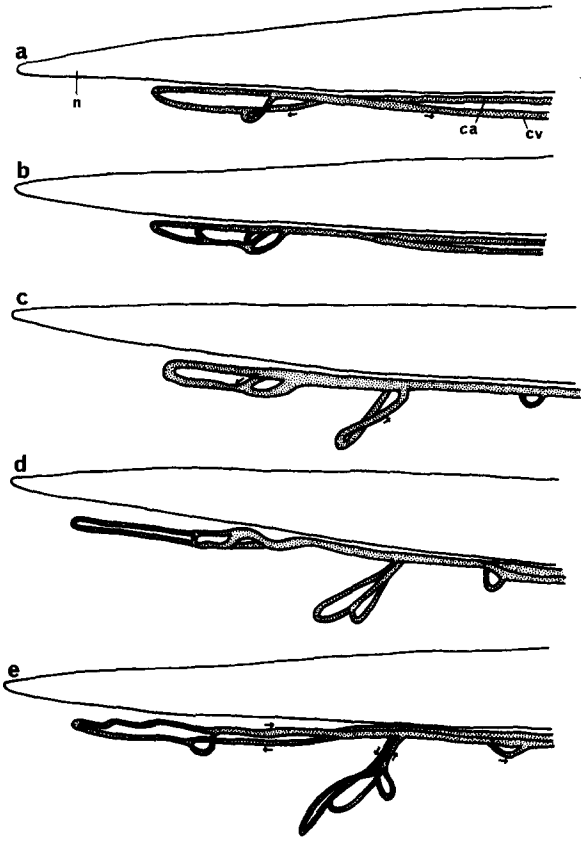

Fig. 26. Caudal blood vessels at the posterior end of the notochord in *Stizostedion vitreum* larvae, right side views: a – age 15 days 18 h, b – age 16 days 6 h 5 min, c – age 17 days 18 h 55 min, d – age 19 days 20 h 55 min, e – age 24 days 5 h (ca = caudal artery, cv = caudal vein, n = notochord).

3.2 The early larval period – an interval of mixed nutrition

Step PP[1] 12. *Beginning of mixed nutrition, rapid oil globule absorption* (Fig. 25, 26, 27, 28, 29, 30, 31).

The first step of the larval period (and the first of the protopterygiolarval phase (PP), Balon 1975b) was characterized by exogenous feeding concurrent with endogenous nutrient-utilization. The yolk disappeared and the oil globule's size decreased rapidly (Fig. 25). As the volume of endogenous nutrients diminished, the resultant increase in available coelomic space was taken up by further expansion of the stomach and intestine. The gill filaments increased in size and the larger filaments displayed as many as three lamellae. Smaller filaments at the ends of the gill arches had no lamellae. Blood vessels in the filaments maintained their superficial position by following the contours of the lamellae. New blood vessels appeared in the axial musculature and ventral to the notochord in the caudal fin. Heart beat rate varied from 107 to 154 min^{-1} ($\bar{x}$ = 142 min^{-1}, n = 16). There were new cartilage formations in the head, body, and caudal fin. Throughout this step there was no apparent increase in total length which ranged between 9.0 and 9.7 mm ($\bar{x}$ = 9.3 mm, n = 10).

This step began at age 15 days 18 h (236.2 TU) when food particles were first observed in the digestive tract. Larvae reared under laboratory conditions at 15°C failed to undergo a dynamic behavioral transition that was observed in a large group of larvae

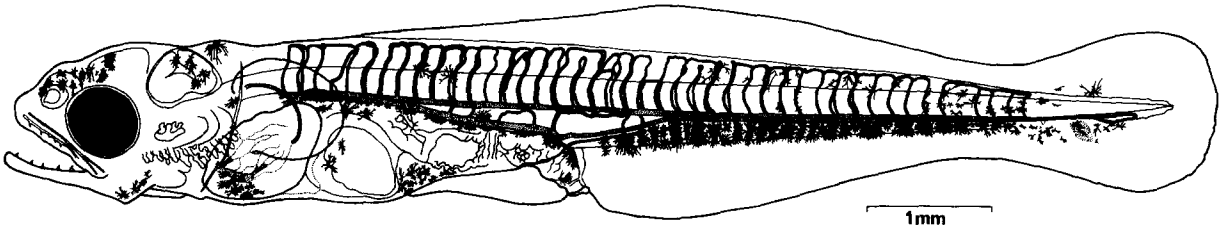

Fig. 27. Larva of *Stizostedion vitreum* at age 17 days 6 h 45 min (step PP[1] 12), left side view.

obtained from a hatchery[3]. This behavioral transition marked the end of step PP[1] 12. The hatchery group were reared under environmental conditions different from those for the laboratory reared group and so the equivalent time of step termination for development at 15° C was estimated by comparing their thermal histories and morphologies. The comparisons produced a range of step termination ages from 22 days 5 h (333.1 TU) to 24 days 14 h (368.7 TU).

The food particles consumed during this step had a maximum diameter of 0.03 mm and were spherical to ovoid in shape. These particles were probably from the commercial aquarium fish food preparation Liquifry No. 1 (Liquifry Company Ltd., England) that had been frequently added to the water during the three days prior to the observation of ingested particles. Liquifry No. 1 particles were also spherical to ovoid in shape with a maximum diameter of 0.05 mm.

At age 16 days 6 h 5 min a new pattern of blood flow existed in the caudal loop (Fig. 26b). The oval shaped swimbladder was enlarged measuring 0.27 by 0.18 mm. The stomach was in contact with the ventral dermal layers and was 0.64 mm wide, its lumen only slightly smaller than the large oil globule. Two pairs of small cartilaginous projections on the dorsolateral surface of the anterior notochord immediately posterior to the cleithrum, were the lateral portions of neural arches. New cartilage on the margins of the auditory capsules included some on the outer ventroanterior quarter and also the inner posterior third. There was a small piece of cartilage in the mid-dorsal region of the fore-midbrain constriction, however, it was not yet continuous with the opposing lateral projections.

By age 16 days 18 h 5 min the stomach lumen was of equal size to the remnants of the oil globule. The intestine filled the ventral space that was previously occupied by yolk. In live larvae only a small amount of yolk was visible behind the stomach. Although the yolk was either not visible or poorly visible from this time on, its presence was established in some preserved larvae up to age 24 days 5 h (Fig. 28). A sphincter appeared at the front end of the posterior third of the intestine. The difference between this area and that anterior to it became more notable later in this step as the stomach and anterior two thirds of the intestine continued to expand. Their walls thinned becoming more transparent, while the posterior end of the intestine remained a thick walled structure with large folds and considerable melanophore coverage. Several dorsal segmental arteries and veins, located above the stomach, displayed a new vessel con-

[3] A large group of walleye eleutheroembryos were obtained from the White Lake Hatchery (MNR), Ontario. Their thermal history was recorded as minimum and maximum daily temperatures.

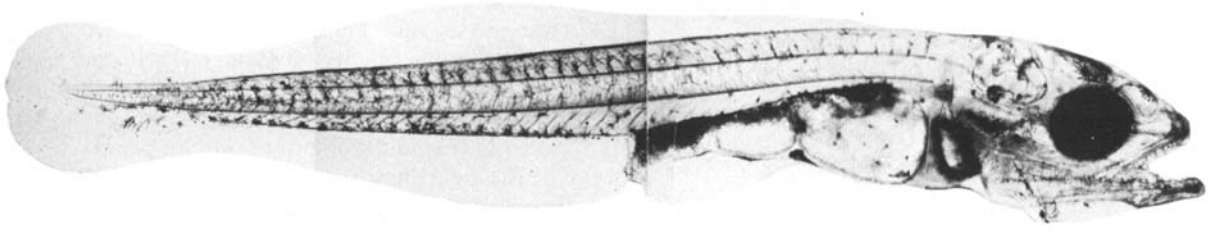

Fig. 28. Photomicrograph of *Stizostedion vitreum* larva at age 24 days 5 h, right side view.

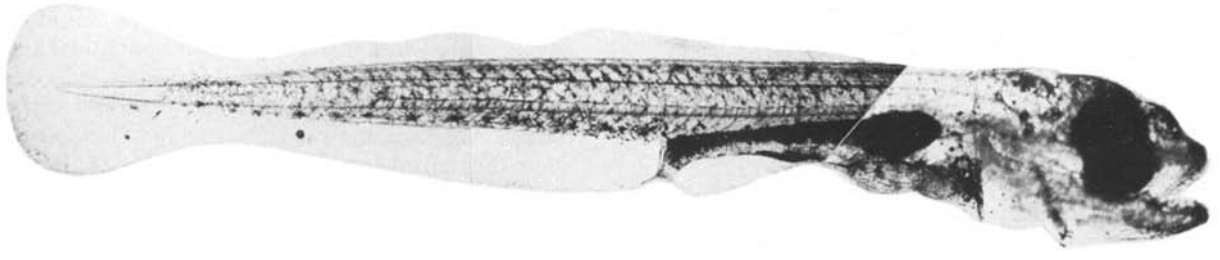

Fig. 29. Photomicrograph of hatchery reared larva of *Stizostedion vitreum* at age 38 days, right side view.

necting the two on the dorsal side of the notochord. Six larvae were observed in a container where only 20% of the area was illuminated. No fewer than four were in the illuminated area throughout a five minute interval indicating the persistence of positive phototaxis.

At age 17 days 6 h 45 min (Fig. 27) larvae were first observed to defecate. Throughout this step the fecal matter was clear or slightly brown, came out in strands of viscous fluids (gelatin-like), and sometimes contained solid particles enclosed in the fluid matter. From this time on the contents of the digestive tract were rapidly defecated after the start of observations. Several dorsal intersegmental arteries and veins were observed running diagonally across the myomeres. The first cartilaginous hypural was indicated by the slight retention of alcian blue stain. The cartilaginious bridge in the fore-midbrain constriction was complete, however its absence in subsequent specimens revealed that some larvae had not developed it by this time.

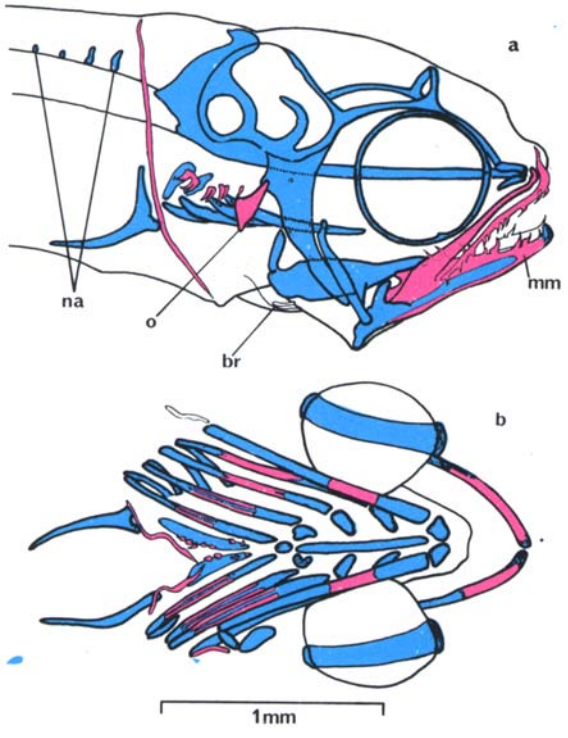

Fig. 30. Cartilage and bone formation in a laboratory reared *Stizostedion vitreum* larva at age 29 days 8 h. Blue areas are chondrified, red areas calcified: a – right side view, b – ventral view (br = branchiostegal ray, mm = mentomekelian, na = neural arch lateral components, o = opercle).

At age 17 days 18 h 55 min a new vascular loop extended ventrally into the caudal fin beneath the area described above as thickened and covered with melanophores (Fig. 26c). The dorsal surface of the digestive tract posterior to the large oil globule was blackened with melanophores.

At age 19 days 20 h 55 min two hypurals were evident. The anterior one was the least developed. There were four pairs of lateral neural arch components with the most posterior pair being only slightly chondrified. What was previously a single vascular loop ventral to the axial vessels in the caudal fin now consisted of two branches (Fig. 26d).

For the rest of this step the visible changes in morphology involved primarily further reduction of the oil globule and changes in its spatial relationships. At age 21 days 12 h 55 min the oil globule was reduced to such an extent that its dorsal surface was clearly withdrawn from the vicinity of the esophagus and the swimbladder. At age 22 days 5 h the swimbladder displayed small blood vessels on its ventral surface and was 0.42 mm long with a maximum height of 0.23 mm. By age 23 days 6 h it was 0.53 mm by 0.27 mm and the oil globule's shape and size (Fig. 25h) were about the same as in hatchery reared larvae with filled swimbladders. In larvae from the hatchery reared group there was a relationship between oil globule shape and size (Fig. 25), and swimbladder inflation. The expansion of the stomach and intestine was probably responsible for earlier changes in oil globule shape in so much as their expansion continuously filled the space created by oil absorption and therefore maintained an anterior directed force on the oil globule. This force applied to the posterior margin of the oil globule would explain the lesser dorso-ventral diminishment as compared to that in the anterior-posterior plane.

The transition to a new step occurred when larvae became increasingly predacious, chasing, biting and sometimes swallowing various moving objects. The objects pursued and bitten or swallowed were small air bubbles, unidentified natural zooplankton, and other walleye larvae. The results of this behavioral change were: the inflation of the swimbladder, the consumption of larger food particles, and a reduction in the number of larvae through extensive cannibalism. Although the predator and prey larvae were approximately the same size, the predator not only survived this event, but also derived nutrients from it. The head, which was disproportionately large in comparison to the rest of the body (Fig. 29), was in some instances almost totally severed from the body of the

prey, trailing behind the predator and attached by only a fine strand of tissue. Typically, when the predator became aware of the prey, it ceased moving, bent the posterior body to one side, then lunged and pursued. The pursuit was often terminated prior to capture and swimming at the surface was continued. When conspecifics were the prey, ingestion was tail first. Specimens preserved during this transition interval typically displayed the loss of portions of the median finfold and the pectoral fins, indicating that the predators (other larvae) bit out pieces without necessarily eating the entire body.

As noted above, this behavioral transition was witnessed in larvae incubated elsewhere under different environmental conditions. The problem was to correlate the observed transition in hatchery reared fish, with a time coordinate for larvae reared under laboratory conditions at 15° C. The comparisons included: total length, temperature units, oil globule shape and size, and the extent of cartilage and bone formation.

The hatchery reared group measured 8.9 to 9,8 mm ($\bar{x}$ = 9.4 mm, n = 18 formalin-preserved specimens). This was the approximate range in total length for all laboratory reared specimens from the beginning of step $F^2 11$ at age 13 days 1 h 15 min to the last observation of a laboratory reared specimen at age 29 days 8 h. Total length was therefore of no use in the determination of a time coordinate for development at 15° C.

After age 34 days 9 h the hatchery group was starting to undergo the behavioral transition. This time represented 369 TU and equalled 24 days 14 h of development at 15° C.

Endogenous nutrient reserves would not usually represent an appropriate comparative feature for the estimation of equivalent developmental stages. Variables that influence energy expenditure without altering the rate of development would create differences in the amount of endogenous nutrients at equivalent developmental stages. Under the artificial conditions

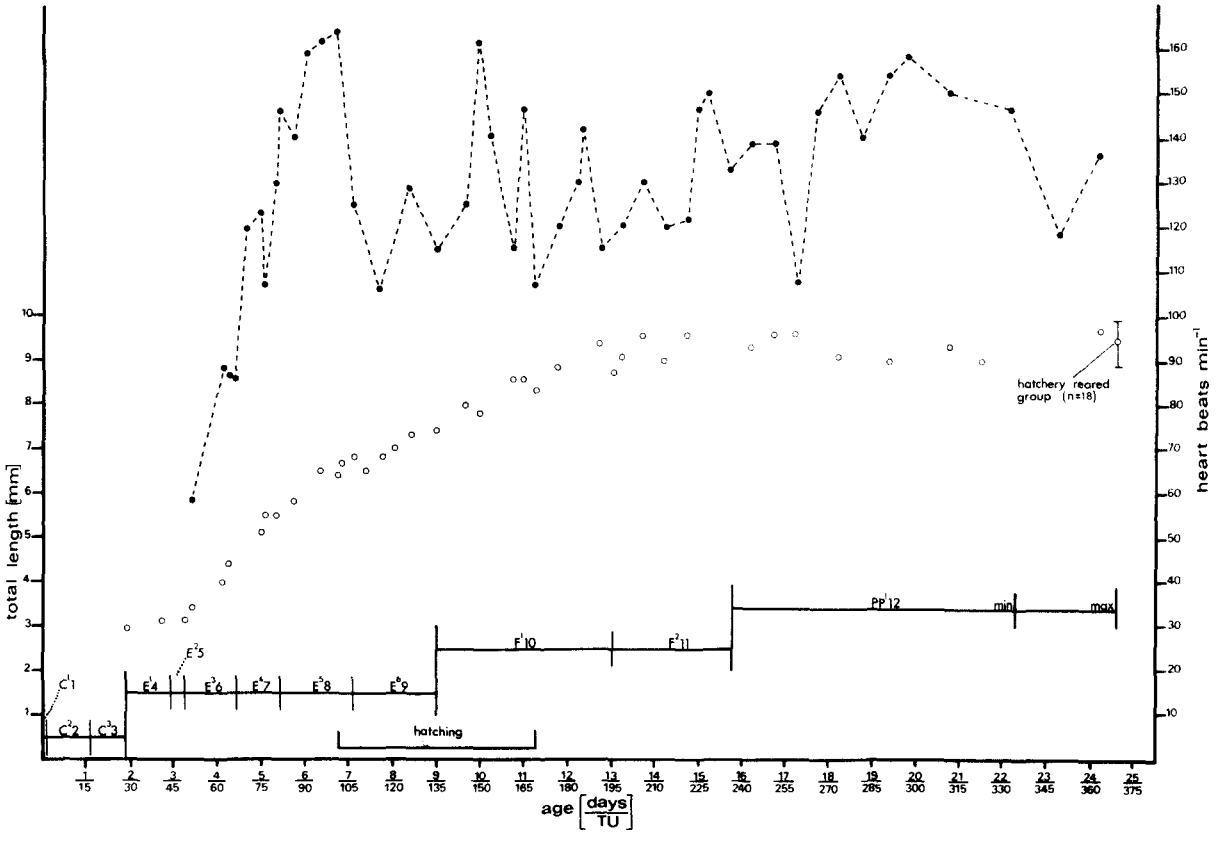

Fig. 31. Total length increments (o) and heart beat rates (•) by steps, days and temperature units (TU) for *Stizostedion vitreum*.

of either the hatchery or laboratory, the density of conspecifics may be such a variable. Higher or lower densities may stimulate more or less activity and therefore alter the rate of utilization of endogenous nutrients. In walleye however, the oil globule appeared to have a buoyancy function, and therefore may exhibit a relationship between its shape and size and the filling of the swimbladder. Also, the pattern of oil absorption appeared to suggest a strategy of oil preservation. First, major oil depletion did not begin until after most of the yolk was consumed and second, at about the same time as the oil globule started to change shape and reduce in size, exogenous feeding commenced. Since one of the apparent results of the behavioral transition was the filling of the swimbladder, the shape and size of the oil globule was considered as a potential indicator of the behavioral transition. Belyy (1972) also noted the oil globule of *S. lucioperca* was almost its original size until the end of the yolk absorption, that it performed a hydrostatic function prior to swimbladder inflation, that feeding on small immobile particles (rotifer eggs) started when oil was still present, and that the number of food items in the larval gut increased as the oil diminished. Larvae older than 23 days 6 h (Fig. 25h) displayed oil globules that were sufficiently diminished to be considered within the range of those observed in hatchery reared larvae with inflated swimbladders. The fact that larvae were highly surface oriented demonstrates the potential importance of the buoyancy function. If the relationship between oil globule shape and size, swimbladder inflation, and the change in behavior does in fact exist, then other morphological features such as cartilage and bone formation, would be expected to differ in their relative state of development, as a direct result of factors causing differences in the rate of oil utilization. In this event, the objective of the comparison between laboratory and hatchery reared larvae would not be to find comparable morphologies, but rather to demonstrate the existing range of morphologies associated with the transition after rearing under different environmental conditions. For this reason Figure 30 presents the maximal cartilage and bone formation observed in a laboratory reared larva that still contained oil. It was 29 days 8 h old and contained only a very small oil globule. It shows that the subsequent step (not described here) involved the start of chondral bone formation. Portions of the mentomekelian, ceratohyals and ceratobranchials were the earliest to calcify.

The extent of calcification was consistent within larvae from the hatchery. Calcified elements included the premaxillary, maxillary, dentosplenial, cleithrum, distal tips of all teeth, and the opercle at the upper posterior projection of the hyomandibular. The first branchiostegal ray was visible as a short thin uncalcified strand at the ventro-posterior extremity of the ceratohyal. This description applied to laboratory reared larvae by the age of 22 days 5 h.

The hatchery reared larvae exhibited considerable variation in cartilaginous meristic characters. There were from one to four pairs of lateral neural arch components, hypurals were absent to a total of three, and lateral postanal hemal arch components were as yet unformed. In the laboratory reared larva of age 22 days 5 h, there were four pairs of lateral neural arch components, two hypurals, and 12 pairs of lateral postanal hemal arch components (observed for the first time). The problem of determining comparable morphologies was therefore further confused by the existence of heterochrony in the meristics of cartilaginous components.

In summary, temperature units produced a comparable age of 24 days 14 h, oil globule shape and size a minimal age of 23 days 6 h, and comparative cartilage and bone formation an age of 22 days 5 h. In accordance with these times the mixed feeding interval (step PP^1 12) was from 6 days 11 h (96.9 TU) to 8 days 20 h (132.5 TU) in duration.

4. Discussion

Since all parent fish were obtained from a river spawning site located at an impassable dam, ecomorphological features will be considered in the context of the general features of this environment. It cannot be assumed that all aspects of ontogeny are the same for progeny developing at different locations (different environmental parameters) and from different spawning stocks, especially as in this case, where spawners are known to home to lake or river spawning grounds, and therefore to represent discrete stocks (Stoudt 1939, Stoudt & Eddy 1939, Eschmeyer 1950, Smith et al. 1952, Eschmeyer & Crowe 1955, Rawson 1957, Olson & Scidmore 1962, Crowe et al. 1963, Priegel 1968, Baker & Scholl 1971, Van Vooren 1978).

Fluvial spawning in the early spring is advantageous in as much as the increased water volume of the spring runoff may expand the river margins producing a larger spawning area than would exist immediately before and after these conditions. The cool temperature and high turbulence of the water at the

spawning site guarantee high dissolved oxygen concentrations. The large discharge minimizes siltation and possibly maximizes the probability of successful transport of eleutheroembryos from the fluvial to lacustrine environments. The time taken to traverse this distance may be an important factor in the determination of year-class abundance. The general characteristics of the spawning sites selected, the lack of carotenoid pigments, swim-up at hatch, surface suspension thereafter, and a poorly developed respiratory circulatory system are indicators that walleye embryonic ontogeny takes place in oxygen rich environments. Since the egg has a density greater than freshwater and an adhesive chorion, incubation within the egg membranes will therefore occur at the site chosen by the parents.

During the embryonic phase there was an evident correlation between heart contraction rates, the formation of blood circulation, and increases in both total length and motility. From the time the heart was first observed to contract (near the start of step $E^3 6$), until blood circulation reached most areas of the embryo (near the end of step $E^5 8$, Fig. 17), heart beat rates generally increased (Fig. 31). There were concurrent increases in i) the number of circulating blood elements (from the start of step $E^4 7$), ii) the rate of increase in total length (Fig. 31) and iii) the motility of embryos.

At the beginning of step $E^6 9$ a sudden large reduction in the rate of heart beats occurred (Fig. 31) as the subintestinal-vitelline circulation was increased by the complete cessation of blood flow from the caudal vein to the posterior cardinal veins. The cause of this diversion was not determined, however, it appeared possible that a reduced heart beat rate, and resulting reduced vascular pressures, may lead to blood flow via a 'path of least resistance', as would be offered by a relatively larger subintestinal-vitelline vein. This was likely since it was noted during in vivo observations that a reduced heart beat rate was inevitably accompanied by a reduction or cessation of blood flow to the smaller vessels, especially those in the caudal extremity or those that had only recently appeared.

A reduction in heart beat rate appears paradoxical when considered as an adaptation for enhancing gaseous exchange and/or the acquisition of nutrients. Though the converse (an increase in heart beat rate) may accomplish the same net result, it would also involve greater energy expenditures in heart muscle contractions. The observed strategy may be more efficient since it would expose proportionately more blood elements to external dissolved oxygen for a greater time, while an elevated heart beat rate would expose a lesser proportion of blood elements for a lesser time but more frequently (assuming increases in heart beat rate necessarily increase the rate of flow of blood elements).

None of these speculations can be verified without measurement of the appropriate physiological parameters, however it was apparent that the observed pattern of circulation made optimal use of a minimal embryonic respiratory system, which at this time consisted of the subintestinal-vitelline vein, hepatic-vitelline vein, and duct of Cuvier. With this system, blood coming from the head and anterior myomeres in the anterior and posterior cardinal veins respectively would be briefly exposed to external dissolved oxygen in the short duct of Cuvier. Blood from the liver would be exposed in the hepatic-vitelline vein and blood from the posterior (postanal) trunk in the large subintestinal-vitelline vein. The result is a system in which there existed no apparent course of blood that would not provide each element with superficial exposure on each circuit. Also, the largest proportion of the total blood volume was exposed in the largest (by surface area) respiratory vessel; that being the subintestinal-vitelline vein. Surface diffusion as well should be considered as a potential contributor to respiratory function.

The ecological significance of this adaptation appears to involve the ability and tendency to swim-up (and therefore a change of environments), timing of the ability to remain in motionless suspension at the surface, and the interval of hatching. Maximized subintestinal-vitelline circulation exists mainly in the interval between the start of hatching and the beginning of the ability to remain suspended at the surface. Embryos hatched in this interval would swim up and therefore be swept away from the spawning grounds, but would not as yet be capable of remaining at the surface where high oxygen concentrations would be assured. This behavior would remove embryos from the vicinity of hatching enzymes released by other hatching embryos, but may result in the embryo being deposited in fluvial pools where reduced water velocities may produce adverse conditions (for example, siltation and low oxygen concentrations at night caused by vegetation). This change of environments, which may include a reduction in oxygen availability, would explain the existence of such a respiratory adaption prior to the development of the ability to escape (suspension at the surface) from such conditions. Also, the embryo's initial inability to overcome the buoyancy of the large oil globule re-

sults in a resting position with the ventral side up. This position will expose the subintestinal-vitelline vein, hepatic-vitelline vein and duct of Cuvier to water rather than to the bottom of the fluvial pool, as would occur if the embryo was oriented with either the left side or ventral side down. Whether embryos survive or succumb, should they be exposed to such conditions, would probably be determined at least in part by the extent to which they had developed the combination of affinity for light, ability to swim, and maximized subintestinal-vitelline circulation. Embryos hatching prior to the development of maximized subintestinal-vitelline circulation would not possess as high a probability of survival as those hatching after that development. Since hatching gland cells were evident as early as age 4 days 16 h 10 min, it might be surmised that hatching could occur that early and that the negative effects of such a premature hatch may not be manifested until development of the ability to swim up. The 1 day 6 h interval between the development of the ability to swim up (age 5 days 21 h) and the development of the maximized subintestinal-vitelline circulation (age 7 days 3 h 10 min) might therefore be considered as an interval in which there are no apparent ecomorphological features (other than the poorly developed swimming ability) that could compensate for this potentially adverse change of environments. High mortalities (= critical interval) associated with this time of ontogeny support the foregoing conclusions.

Surface suspension, besides maintaining the eleutheroembryo in an environment of maximum dissolved oxygen, would also provide transport at a low energy cost from the fluvial to the lacustrine environment where appropriate food resources would exist in greater abundance. Notably the surface suspension ability was lost when the surface was agitated. The buoyancy provided by the large oil globule was therefore supplementary to the forces of the surface tension, both of which contributed to maintain the eleutheroembryo at the surface. In the context of a fluvial system, the loss of the surface position (falling to bottom) upon agitation of the water suggests that too steep a gradient below the spawning area could inhibit the rate of transport just as too gentle a gradient would with its lesser water velocity. Priegel (1970) found that high mortalities of eleutheroembryos occurred at low-head dams during times of high discharge. Eleutheroembryos were either killed immediately after going over the dam or were trapped in eddies below the dam. Frequent turbulence would cause the eleutheroembryo to lose its surface suspension, subsequently falling to a lower stratum and requiring vertical swimming to regain its surface position. The result would be an increased rate of utilization of the yolk morphogenetic substances. The question is, what amount or portion of the energy budget could be afforded to this behavior before depletions of the yolk morphogenetic substances would affect morphogenesis, or even possibly, as will be considered further, what amount is necessary for appropriate synchrony in subsequent transitions that may be related to the quantity of morphogenetic substances?

Besides being an interval of transport, the eleutheroembryonic phase was predominated by morphological and functional changes directed toward gill respiration and exogenous feeding. The transition to gill respiration provides an excellent example of a major functional change in ontogeny, and how such changes occur in a saltatory manner following a combination of abrupt qualitative changes. When viewed as a series however, these qualitative changes appear as a more gradual process of smaller shifts in the proportion of contribution to total respiratory function, provided by the various respiratory surfaces (Table 2).

The proportion of contribution (always meaning that of an individual morphologically distinct respiratory surface, as it contributes to the sum total of respiration) can be altered positively by an addition (for example a greater proportion of total blood volume passing through the subintestinal-vitelline vein) which may at times be generated by a subtraction (for example the termination of blood flow from the caudal vein to the posterior cardinal veins). The proportion of contribution can also be altered negatively by subtraction, as occurred at the start of the eleutheroembryonic phase when the subintestinal-vitelline vein lost some of its blood flow to the reestablished flow from the caudal vein to posterior cardinal veins. Positive alterations in the proportion of contribution to total respiration may occur during the formation of the temporary embryonic respiratory system and later the gills. Negative alterations would occur in the temporary embryonic respiratory system as it is replaced by gill respiration. Additions and subtractions are not only relative changes in the volume of blood exposed to external oxygen. Additions may involve numerous factors that influence respiratory function such as the synthesis of haemoglobin, the formation of blood vessels that expand a respiratory surface, and the differentiation and mobilization of more blood elements. More differentiated and mobilized blood elements will produce both absolute and rela-

Table 2. Morphological and functional changes involved in the respiration of walleye embryos and eleutheroembryos.

Time	Addition	Subtraction	Proposed relative change in proportion of contribution to total respiration
6 days 18 h	more blood to subintestinal-vitelline vein	reduction of flow caudal to to posterior cardinal veines	– positive alteration in subintestinal-vitelline vein – positive alteration at duct of Cuvier, fewer blood elements competing for brief superficial exposure in short duct of Cuvier
7 days 3 h	maximized subintestinal-vitelline flow	complete stoppage of flow from caudal to posterior cardinal veins	– positive alteration in subintestinal-vitelline vein persists for all of step $E^6 9$
8 days 2 h	branchial arteries begin to carry blood		– positive but minimal alteration as start of gill respiration
9 days 16 h	reestablished flow from caudal to posterior cardinal veins	reduced subintestinal-vitelline flow	– negative alteration in subintestinal-vitelline vein
10 days 5 h	mouth open		– positive alterarion to gill respiration by moving water around the poorly formed gills
10 days 23 h	pseudobranchial and all branchial arteries with blood flow		– positive alteration to gill respiration
12 days 18 h	blood from caudal artery to subintestinal-vitelline vein via the profundal caudal vein		– positive alteration to subintestinal-vitelline vein buffers effect of subsequent subtraction
13 days 1 h		blood flow from caudal to subintestinal-vitelline vein stops	– negative alteration, net change of less blood to subintestinal-vitelline vein
13 days 18 h	afferent and efferent branchial and filamental arteries		– positive alteration to gill respiration

tive increases when assessed as a function of the capacity of the system. Unfortunately many of the possible factors could not be readily assessed individually, let alone in conjunction with all others. Although addition and positive alteration are often synonymous, as are subtraction and negative alteration, the terms addition and subtraction refer to observations of morphological change and are differentiated from positive and negative alterations which are hypothetical net results that have not been confirmed by quantitative measurements.

One question that arises from this view is how do we quantitatively evaluate function in such a dynamic system? In this study only the observed qualitative morphological changes (Table 2) have been used to estimate relative (positive or negative) changes in the proportion of contribution to total respiration for steps $E^6 9$, $F^1 10$ and $F^2 11$.

The events leading up to step $F^2 11$ were particularly demonstrative of the theory of saltation and were manifested by individual additions and subtractions. The primary change interpreted as the determinant of a threshold (step boundary) was the total and final subtraction of blood flow from the caudal vein to the subintestinal-vitelline vein. This major subtraction from the subintestinal-vitelline vein was buffered however by an addition of blood from the caudal artery via the profundal caudal vein, observed for the first time only seven hours earlier, as well as by the evidently sporadic addition from the subintestinal vein; their confluence providing the only sources of blood to the subintestinal-vitelline vein for the remainder of the embryonic period. While these changes occurred in the subintestinal-vitelline flow, the hepatic-vitelline vein still existed as a potential respiratory surface on the left ventro-lateral periphery

of the large oil globule (Fig. 24). The gills had undergone several evident positive alterations in contribution to total respiration (see Table 2), one of which was provided by the opening of the mouth which would result in water inspiration and passage over the gills. The negative alteration of the subintestinal-vitelline vein would result in the other respiratory structures assuming responsibility for a greater proportion of total respiration without having undergone any immediately evident positive alterations themselves. However, about 17 hours later the gills had developed blood flow in the filaments. It may have been that prior developments in other respiratory surfaces (especially the gills) were adequate in compensating for this negative alteration, but on the other hand, this time may represent a potential 'critical period' in the ontogeny of the walleye.

As stated above, the proposed importance of transport to the lacustrine environment during the eleutheroembryonic phase is based on the relative abundance of food (plankton) there as compared to the fluvial system. Under the conditions of this study at a mean temperature of 15° C, the elapsed time and temperature units to first feeding (and therefore potential durations of transport) were 9 days or 135 TU from the start of hatch, 6 days 18 h or 101.3 TU from the start of surface suspension and 4 days 11 h or 66.9 TU from the end of the hatching interval. The problem of reaching the lacustrine system might be expected to intensify (perhaps exponentially) throughout the hatching interval. As hatching progresses, a lesser amount of time exists to traverse the same distance. Increases in water temperature and decreases in discharge cause both an increase in the rate of development and a decrease in the rate of transport. Although the observed hatching interval spanned only 4½ days, on the spawning grounds the interval of hatch for all eggs spawned would span a much greater length of time depending on the duration of spawning and the ensuing incubation temperatures. Spawning durations have been reported to occur over a range of four to 34 days (Colby et al. 1979) with an average of about two weeks. A difference of two weeks between the start and completion of hatch could surely encompass substantial changes in temperature, and especially discharge as the spring runoff subsides.

During the interval of mixed nutrition (step PP[1] 12) the particle size (0.03 mm) consumed was small. Small immobile food particles, in the form of diatoms, were ingested by walleye larvae in Lake Erie (Hohn 1966). Other investigators have noted the first food taken to be larger zooplankters such as rotifers and nauplii (Smith & Moyle 1943) and copepods and cladocerans (Houde 1967, Priegel 1970). In this investigation, the consumption of larger particles was associated with cannibalism and for this reason may be the more critical time by which the larvae must reach the lacustrine environment. Aggregations of walleye larvae existing at this time are therefore likely to utilize each other in the absence of other available food resources of appropriate size, density and possibly quality (Houde 1967, Priegel 1970).

Although the more intensive feeding behavior that included cannibalism was not observed in laboratory reared larvae, the time at which it would occur at 15° C was estimated by comparison with hatchery reared fish that did undergo this change in feeding at 22 days 5 h or 330 TU based on comparative cartilage and bone formation, at 23 days 6 h based on oil globule size, and at 24 days 14 h based on a calculation of 369 TU. These ages represented durations of 15 days 11 h (231.9 TU) to 17 days 20 h (267.5 TU) from the start of hatch, 13 days 5 h (198.1 TU) to 15 days 14 h (233.7 TU) from the start of surface suspension and 10 days 22 h (163.7 TU) to 13 days 7 h (199.4 TU) from the end of hatch. Compared to the start of mixed nutrition these potential durations of transport represent increases of 72 to 98% from the start of hatch, 96 to 131% from the start of surface suspension and 145 to 198% from the end of hatch.

The change in the size of food particles consumed appeared to be associated with the size and shape of the oil globule (Fig. 25). Such a relationship would be logical since the larger particles included air bubbles which contribute to the inflation of the swimbladder. Since the oil globule and swimbladder are both hydrostatic organs there would likely be an optimal time for swimbladder inflation based on oil globule volume. If swimbladder inflation occurred too soon the result would be an interval of excess buoyancy and too late an interval of too little buoyancy. As previously suggested, expansion of the digestive tract may explain the observed changes in oil globule shape as it diminished in volume. The oil globule maintained its dorso-ventral dimension while diminishing in its anterior-posterior dimension. The dorsal side of the oil globule therefore applied a force to the ventral side of the esophagus, possibly obstructing the passage of particles greater than a certain size. If this hypothesis is correct then variable energy expenditures experienced in fluvial transport may cause the duration of the interval of mixed nutrition to vary.

By the time feeding was first observed at age

15 days 18 h, larvae appeared to have all the requirements necessary for external feeding: swimming ability, cartilage and bone formations in the head, teeth, a complete digestive tract with stomach and intestine exhibiting peristalsis, a large liver and a gall bladder with bile. It appeared probable that the start of the interval of mixed nutrition was in fact the minimal time (extent of other morphological development) at which the observed transition to the consumption of larger particles could have occurred had the oil globule been sufficiently diminished. Even the swimbladder was at least visible as early as age 14 days 6 h 35 min, a day and a half prior to first feeding. This may explain discrepancies in the initial food-particle-size relationship with larval total length. In Oneida Lake, New York, Houde (1967) found larvae as small as 7 mm consuming the relatively large copepods and to a lesser extent cladocerans. Hohn (1966), on the other hand, noted copepods and cladocerans occurring in the diet of Lake Erie walleye only after they had attained total lengths of 10 mm or more. Before this, from about 7 mm total length, four species of diatoms were predominant in the diet. Unfortunately, Houde (1967) did not sample the lake for the abundance of smaller particles (phytoplankton) and Hohn (1966) did not sample for the abundance of larger particles (zooplankton).

One of the possibly abnormal conditions arising from laboratory or hatchery rearing of walleye, may be a reduction in the energy expenditures which would occur during fluvial transport. Assuming that for most aspects of ontogeny there exists an optimal time and sequence for the development of related features, factors increasing the duration of utilization of yolk morphogenetic substances that do not affect the rate of morphogenesis (for example activity as opposed to differences in temperature or oxygen) may in this case, where feeding and swimbladder inflation are potentially related, cause asynchrony in important events. Subsequent mortalities may not be evident until the complete utilization of endogenous nutrient reserves and then be simply attributed to failures at the time of transitions in feeding, as frequently done when considering the causes of 'critical periods' in fish (Marr 1956, Balon 1960b, May 1974) in spite of the many other possibilities (Dekhnik et al. 1970, Vladimirov 1970, Dekhnik & Sinyukova 1976) that might exist. If these considerations are valid, it is evident why lacustrine, or fluvial but proximal to lacustrine spawning may contribute less to the recruitment of a year-class, than would fluvial spawning, when interposed between it and the lacustrine system are appropriate conditions for the optimal rate of utilization of morphogenetic substances. These considerations would also explain why laboratory reared larvae, held from hatching onward under conditions that would be similar to the lacustrine environment, failed to inflate their swimbladders, consume larger particles, partake of cannibalism and survive. The very existence of such a large oil globule may imply its use as an energy source for the activity necessary for the eleutheroembryo to reach a site where appropriate food resources exist. Kryzhanovsky (1960) noted the importance of the oil globule's function as a hydrostatic organ but also suggested that its usual lack of protein components meant that the young fish lived at the expense of the oil but without growth and development.

Balon's (1975a) reproductive guilds were based in part on the substrate selected by the spawners. When considering the common characteristics of walleye spawning, substrate appears in at least one instance to be secondary to the more general characteristics of '... moving water for aeration and clean substrate on which to deposit the eggs' (Machniak 1975). In this regard various rock substrates in the fluvial and wave washed lacustrine habitats are equivalent to the 'grass-sedge mats' of spawning marshes used by walleye in Wisconsin, in as much as they are 'free from organic materials and subject to a free flow of water at all times' (Priegel 1970). However, Priegel (1970) also noted complete failure on the marshes during years of low water levels. Low oxygen concentrations at night were apparently involved. Also, Eschmeyer (1950), Johnson (1961) and Priegel (1970) noted substantial mortalities evident as windrows of young walleye along lake shore lines. Baker & Scholl (1971) noted that shallow offshore walleye spawning reefs in western Lake Erie experienced strong wave effects during storms. Boulders 70 cm in diameter were moved from the reef. Busch et al. (1975) made similar observations but also noted that walleye eggs in the cracks and crevices of the reef had disappeared after the storm and were probably deposited in the mud below and around the reef. The relatively 'constant' fluvial habitat (as opposed to the lotic habitat of a temporary flood marsh) of the river bed is probably, for this short duration, more stable since a complete stoppage of flow is unlikely, though water levels may vary. Even with reduced flows, spawning grounds located at impassable falls and dams, will recieve water rich in dissolved oxygen. Daily fluctuations in oxygen concentration, such as occurred in the lotic marsh during years of lesser water volume (Priegel 1970),

would not be experienced in the absence of vegetation.

Stizostedion probably evolved from marine ancestors (Balon et al. 1977b). In sea water, the large oil globule was probably sufficient to assure neutral or positive buoyancy in an oxygen rich medium. Later, the same oil globule probably assisted planktonic drifting of the eleutheroembryos. Extensive development of embryonic respiratory structures was therefore unnecessary. Upon invasion of freshwater, however, the zygotes of walleye were negatively buoyant. Consequently, only a certain combination of oxygen rich spawning grounds and lacustrine habitats rich in planktonic prey (within drifting distance), were capable of assuring success in reproduction. The evolution of subtle adaptations in early ontogeny was therefore necessary to support the invasion of freshwater.

A better understanding of ecological relationships and their practical implications (critical period, impact of waterworks, hatchery manipulations, etc.), obviously result from the study of ecomorphological principles of saltatory development. Intricate environmental relationships expressed as heterochronous shifts in form and function (Balon 1979b) are also explained by these principles.

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References cited

Allbaugh, C. A. & J. V. Manz. 1964. Preliminary study of the effects of temperature fluctuations on developing walleye eggs and fry. Prog. Fish-Cult. 26: 175–180.

Anonymous. 1967. Temperature for hatching walleye eggs. Prog. Fish-Cult. 29: 20.

Baker, C. T. & R. L. Scholl. 1971. Walleye spawning area study in western Lake Erie. Ohio Dept. Nat. Res., Div. of Wildl., Fed. Aid Proj. F-35-R-10, Job 1. 24 pp.

Ballard, W. W. 1973. Normal embryonic stages for salmonid fishes, based on *Salmo gairdneri* Richardson and *Salvelinus fontinalis* (Mitchill). J. Exp. Zool. 184: 7-25.

Balon, E. K. 1960a. Über die Entwicklungsstufen des Lebens der Fische und ihre Terminologie. Zeitschrift für wissenschaftliche Zoologie 164: 294–314.

Balon, E. K. 1960b. Die Entwicklung der Fische bei ungünstigen Nahrungsbedingungen. Acta Hydrobiologica 2: 125–131.

Balon, E. K. 1971. The intervals of early fish development and their terminology (A review and proposals). Věst. Čs. spol. zool. 35: 1–8.

Balon, E. K. 1975a. Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 32: 821–864.

Balon, E. K. 1975b. Terminology of intervals in fish development. J. Fish. Res. Board Can. 32: 1663–1670.

Balon, E. K. 1979a. The theory of saltation and its application in the ontogeny of fishes: steps and thresholds. Env. Biol. Fish. 4: 97–101.

Balon, E. K. 1979b. The juvenilization process in phylogeny and the altricial to precocial forms in the ontogeny of fishes. Env. Biol. Fish. 4: 193–198.

Balon, E. K. 1980a. Early ontogeny of the lake charr, *Salvelinus (Cristivomer) namaycush*. pp. 485–562. In: E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*, Dr. W. Junk Publishers, The Hague.

Balon, E. K. 1980b. Early ontogeny of the North American landlocked arctic charr – sunapee, *Salvelinus (Salvelinus) alpinus oquassa*. pp. 563–606 In: E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*, Dr. W. Junk Publishers, The Hague.

Balon, E. K. 1980c. Early ontogeny of the brook charr, *Salvelinus (Baione) fontinalis*. pp. 631–666. In: E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*, Dr. W. Junk Publishers, The Hague.

Balon, E. K. 1980d. Comparative ontogeny of charrs. pp. 703–720. In E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*, Dr. W. Junk Publishers, The Hague.

Balon, E. K., D. L. G. Noakes & F. Cichocki. 1977a. Ichthyology. Supplements to lectures and laboratory manual. Department of Zoology, College of Biological Science, Univ. of Guelph. 195 pp.

Balon, E. K., W. T. Momot & H. A. Regier. 1977b. Reproductive guilds of percids: results of the paleogeographical history and ecological succession. J. Fish. Res. Board Can. 34: 1910–1921.

Belyy, N. D. 1972. Downstream migration of the pike-perch *Lucioperca lucioperca* (L.) and its food in the early development stages in the lower reaches of the Dnieper. J. Ichthyol. 12: 465–472.

Busch, W. N., R. L. Scholl & W. L. Hartman. 1975. Environmental factors affecting the strength of walleye (*Stizoste-*

dion vitreum vitreum) year-classes in western Lake Erie 1960–70. J. Fish. Res. Board Can. 32: 1733–1743.
Cohen, J. 1977. Reproduction. Butterworths, London. 356 pp.
Colby, P. J., R. E. McNicol & R. A. Ryder. 1979. Synopsis of biological data on the walleye *Stizostedion vitreum vitreum*. FAO Fish. Synop., Rome (in print).
Colby, P. J., & L. L. Smith, Jr. 1967. Survival of walleye eggs and fry on paper fiber sludge deposits in Rainy River, Minnesota. Trans. Amer. Fish. Soc. 96: 278–296.
Crowe, W. R., E. Karvelis & L. S. Joeris. 1963. The movement, heterogeneity and rate of exploitation of walleyes in Northern Green Bay, Lake Michigan, as determined by tagging. Internat. Comm. N.W. Atl. Fish., Spec. Publ. 4: 38–41.
Daget, J. 1964. Memoires du Museum National D'histoire Naturelle. Le crane des teleosteens. Serie A, Zoologie Tome Fasicule 2. Editions du Museum, Paris. 340 pp.
Dekhnik, T. V., L. A. Duka & V. I. Sinyukova. 1970. Food supply and the causes of mortality among the larvae of some common Black Sea fishes. J. Ichthyol. 10: 304–310.
Dekhnik, T. V. & V. I. Sinyukova. 1976. Survival of marine fish larvae in relation to food availability. J. Ichthyol. 16: 294–302.
Dingerkus, G. & L. D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology 52: 229–232.
Eschmeyer, P. H. 1950. The life history of the walleye, *Stizostedion vitreum* (Mitchill) in Michigan. Mich. Dept. Conserv., Inst. Fish. Res. Bull. 3: 1–99.
Eschmeyer, P. H. & W. R. Crowe. 1955. The movement and recovery of tagged walleyes in Michigan, 1929–1953. Mich. Dept. Cons., Inst. Fish. Res., Misc. Publ. 8: 1–32.
Fish, M. P. 1932. Contributions to the early life histories of sixty-two species of fishes from Lake Erie and its tributary waters. Bull. U.S. Bur. Fish. 10: 293–398.
Galat, D. L. 1972. Preparing teleost embryos for study. Prog. Fish-Cult. 34: 43–48.
Harder, W. 1975. Anatomy of fishes. Part 2: Figures and plates. E. Schweizerbart'sche Verlagsbuchhandlung (Nagele u. Obermiller), Stuttgart. 132 pp.
Hodson, P. V. & J. B. Sprague. 1975. Temperature-induced changes in acute toxicity of zinc to Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 32: 1–10.
Hohn, M. H. 1966. Analysis of plankton ingested by *Stizostedion vitreum vitreum* (Mitchill) fry and concurrent vertical plankton tows from south-western Lake Erie, May 1961 and May 1962. The Ohio J. of Sci. 66: 193–197.
Houde, E. D. 1967. Food of pelagic young of the walleye, *Stizostedion vitreum vitreum*, in Oneida Lake, New York. Trans. Amer. Fish. Soc. 96: 17–24.
Johansen, K. 1977. Respiration and circulation. pp. 306–391. In: A. G. Kluge (ed.) Chordate Structure and Function, MacMillan Publ., New York.
Johnson, F. H. 1961. Walleye egg survival during incubation on several types of bottom in Lake Winnibigoshish, Minnesota, and connecting waters. Trans. Amer. Fish. Soc. 90: 312–322.
Koenst, W. M. & L. L. Smith Jr. 1976. Thermal requirements of the early life history stages of walleye, *Stizostedion vitreum vitreum*, and sauger, *Stizostedion canadense*. J. Fish. Res. Board Can. 33: 1130–1138.
Kramer, R. H. & L. L. Smith, Jr. 1966. Survival of walleye eggs in suspended wood fibers. Prog. Fish-Cult. 28: 79–82.
Kryzhanovsky, S. G. 1949. Eco-morphological principles of development among carps, loaches and catfishes. Tr. Inst. Morph. Zhiv. Severtsova 1: 5–332. (In Russian). (Part 2, Ecological groups of fishes and patterns of their distribution, pp. 237–331, Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2945, 1974).
Kryzhanovsky, S. G. 1960. On the significance of fat inclusions in fish eggs. Zool. zh. 39: 111–123. (In Russian).
Kryzhanovsky, S. G., N. N. Disler & E. N. Smirnova. 1953. Eco-morphological principles of development in percids. Trudy Inst. Morph. Zhiv. Severtsova 10: 3–138. (In Russian).
Leach, G. C. 1927. Artificial propagation of pike perch, yellow perch, and pikes. U.S. Bur. Fish. Ann. Rept. (App. 1): 1–27.
Lentz, T. L. & J. P. Trinkaus. 1967. A fine structural study of cytodifferentiation during cleavage, blastula, and gastrula stages of *Fundulus heteroclitus*. J. Cell Biol. 32: 121–138.
Machniak, K. 1975. The effects of hydroelectric development on the biology of northern fishes (reproduction and population dynamics). III. Yellow walleye *Stizostedion vitreum vitreum* (Mitchill). A review and bibliography. Fish. Mar. Serv. Res. Dev. Tech. Rep. 529: 1–68.
Marr, J. C. 1956. The 'critical period' in the early life history of marine fishes. J. Conseil. Intern. Exploration Mer. 21: 160–170.
May, R. C. 1974. Larval mortality in marine fishes and the critical period concept. pp. 3–19. In: J. H. S. Blaxter (ed.) The early life history of fish, Springer-Verlag, New York.
McElman, J. F. & E. K. Balon. 1980. Early ontogeny of white sucker, *Catostomus commersoni*, with steps of saltatory development. Env. Biol. Fish. 5 (in print).
Nelson, W. R. 1968. Embryo and larval characteristics of sauger, walleye, and their reciprocal hybrids. Trans. Amer. Fish. Soc. 97: 167–174.
Newburg, H. J. 1974. Planarians as a mortality factor on spawned fish eggs. Prog. Fish-Cult. 36: 227–230.
Norden, C. R. 1961. The identification of larval yellow perch, *Perca flavescens* and walleye, *Stizostedion vitreum*. Copeia 1961: 282–288.
Olson, D. E. 1966. Physical characteristics of fertilized and unfertilized walleye eggs during early stages of development. Minn. Dept. Conserv., Minn. Fish. Invest. 4: 31–38.
Olson, D. E. & W. J. Scidmore. 1962. Homing behavior of spawning walleyes. Trans. Amer. Fish. Soc. 91: 355–361.
Oseid, D. M., & L. L. Smith, Jr. 1971. Survival and hatching of walleye eggs at various dissolved oxygen levels. Prog. Fish-Cult. 33: 81–85.
Pavlov, D. A. S. G. Soin. 1976. The reproductive ecology and development of the freshwater 'Kamachatka trout', *Salmo mykiss*. J. Ichthyol. 16: 284–294.
Pelluet, D. 1944. Criteria for the recognition of developmental stages in the salmon (*Salmo salar*). J. Morph. 74: 395–407.
Peňáz, M. 1975. Early development of the grayling *Thymallus thymallus* (Linnaeus, 1758). Acta Sc. Nat. Brno 9: 1–35 + 8 pl.
Priegel, G. R. 1968. The movement rate of exploitation and homing behavior of walleyes in Lake Winnebago and connecting waters, Wisconsin, as determined by tagging. Wisc. Acad. Sci., Arts, Lett. 56: 207–223.
Priegel, G. R. 1970. Reproduction and early life history of

the walleye in the Lake Winnebago region. Wisc. Dept. Natur. Res., Tech. Bull. 45: 1–105.
Rawson, D. S. 1957. The life history and ecology of the yellow walleye, *Stizostedion vitreum*, in Lac la Ronge, Saskatchewan. Trans. Amer. Fish. Soc. 86: 15–37.
Reighard, J. 1890. Development of the wall-eyed pike (*Stizostedion vitreum vitreum*). A popular introduction to the development of bony fishes. Appendix, 9th Bienn. Rept., Mich. St. Bd. Fish Comm. (1888–1890): 93–158.
Siefert, R. E. & W. A. Spoor. 1974. Effects of reduced oxygen on embryos and larvae of the white sucker, coho salmon, brook trout, and walleye. pp. 487–495. In: J. H. S. Blaxter (ed.) The early life history of fish, Springer-Verlag, New York.
Smirnov, A. I. 1975. Biology, reproduction and development of Pacific salmons. Izd. Moskovskogo Univ., Moskva. 335 pp. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 3861, 1976).
Smith, L. L. & J. B. Moyle. 1943. Factors influencing production of yellow pike-perch, *Stizostedion vitreum vitreum*, in Minnesota rearing ponds. Trans. Amer. Fish. Soc. 73: 243–261.
Smith, L. L., Jr. & W. M. Koenst. 1975. Temperature effects on eggs and fry of percoid fishes. Ecological Research Series, Rep. No. EPA-660/3-75-017. 91 pp.
Smith, L. L., Jr. & R. H. Kramer. 1963. Survival of walleye eggs in relation to wood fibers and *Sphaerotilus natans* in the Rainy River, Minnesota. Trans. Amer. Fish. Soc. 92: 220–234.
Smith, L. L., Jr., L. W. Laurits, W. Krefting & R. L. Butler. 1952. Movements of marked walleyes *Stizostedion vitreum vitreum* (Mitchill) in the fishery of the Red Lakes, Minnesota. Trans. Amer. Fish. Soc. 81: 179–196.
Smith, L. L., Jr. & D. M: Oseid. 1970. Toxic effects of hydrogen sulfide to juvenile fish and fish eggs. Engineering Extension Series, Purdue Univ. 137: 739–744.
Stoudt, J. H. 1939. A study of the migration of the walleyed pike (*Stizostedion vitreum*) in water of the Chippewa National Forest, Minnesota. Trans. Amer. Fish. Soc. 68: 163–169.
Stoudt, J. H. & S. Eddy. 1939. Walleye pike tagging study, 1937; 1938, Chippewa National Forest. Trans. 4th N. Am. Wildl. Conf.: 305–310.
Trinkaus, J. P. 1966. Morphogenetic cell movements. pp. 125–176. In: M. Locke (ed.) Major problems of developmental biology (25th Symp. Soc. Develop. Biol.), Academic Press, New York.
Trinkaus, J. P. 1969. Cells into organs: The forces that shape the embryo. Prentice-Hall, Inc., Englewood Cliffs. 215 pp.
Trinkaus, J. P. 1973a. Surface activity and locomotion of *Fundulus* deep cells during blastula and gastrula stages. Dev. Biol. 30: 68–103.
Trinkaus, J. P. 1973b. Modes of cell locomotion in vivo. Locomotion of tissue cells. Ciba Foundation Symposium 14 (new series): 233–249.
Trinkaus, J. P. & T. L. Lentz. 1967. Surface specialization of *Fundulus* cells and their relation to cell movements during gastrulation. J. Cell. Biol. 32: 139–153.
Van Horn, W. M. & R. Balch. 1956. The reaction of walleyed pike eggs to reduced dissolved oxygen concentrations. Purdue Univ. Eng. Ext. Dept., Ser. Bull. 91: 319–341.
Van Vooren, A. R. 1978. Characteristics of walleye spawning stocks. Ohio Dept. Nat. Res., Div. of Wildl., Fed. Aid Proj. F-35-R-16, Study 4. 13 pp.
Vladimirov, V. I. 1970. Ontogenetic qualitative differences as one factor in the dynamics of a fish population (research tasks). Hydrobiol. J. 6: 7–18.
Wallace, B. 1970. Genetic load, its biological and conceptual aspects. Prentice-hall, Englewood Cliffs. 116 pp.
Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Philadelphia. 743 pp.
Whyte, L. 1965. Internal factors in evolution. Tavistock Publications, London. 81 pp.
Yamamoto, T. 1975. Medaka (killifish) biology and strains. Keigaku Publishing Company, Tokyo, Japan. 365 pp.