

Preliminary observations on the circulatory system in eggs and early larvae of some teleostean fishes

K. P. BALAKRISHNAN¹ and C. B. LALITHAMBIKA DEVI²

¹ Oceanographic Laboratory, University of Kerala; Cochin, India
and

² Indian Ocean Biological Centre, National Institute of Oceanography; Cochin, India

Abstract

Observations on the pattern of blood circulation in eggs and early larvae of *Ambassis*, *Mugil*, *Dorosoma* and *Thrissocles* species have been made. In the initial stages, the tubular heart lies on the left side, near the cephalic region. The anterior end of the heart lies near the snout. The posterior end is attached to a point mid-way between the orbits and auditory vesicles; the position of the anterior end is greatly influenced by the size of yolk. Initially, the tubular heart and dorsal aorta lie in a line. As a result of yolk absorption, the anterior end of the tubular heart gradually sinks to a position posterior to the attached end. Liquified yolk is circulated through the heart in early stages; blood corpuscles become apparent only in larvae. The heart-beat, which is initially irregular, gradually becomes regular as development progresses. In the initial stages the blood flows to the caudal and not to the cephalic region. The blood vessels are narrow, and the oval-shaped blood corpuscles must therefore pass through individually, with their long axes parallel to the passage. A definite capillary system is not evident. The blood vessels can dilate and contract when required.

Introduction

The circulatory system in adult fishes is fairly well known, but the circulation pattern in the eggs and early larvae remains obscure, except for the contributions of RYDER (1882), DELSMAN (1924, 1927), SHEARER (1930) and ZIEBA (1956). Hence, it is imperative to study this vital system in detail; preliminary findings are reported.

Materials and methods

Plankton samples were collected from Ernakulam Channel (Cochin Harbour), India, using a net of organdie cloth (mesh size approximately 280 by 220 μ). The eggs were sorted and kept alive in the laboratory; periodic observations on eggs and larvae were performed with a microscope; no chemicals were added to affect the movements of the larvae. Camera lucida drawings were made to show morphological details. Several eggs and larvae of *Ambassis*, *Mugil*, *Dorosoma* and *Thrissocles* species were examined for a period of 4 years, from 1966.

Results

Ambassis species

The eggs of *Ambassis* species are spherical (diameter about 0.60 mm) and contain a single oil globule. Three to 4 h after spawning, blastoderm covers half the yolk and a perivitelline membrane separates the embryo from the egg shell (Fig. 1). Two to 3 h later, tubular heart and eyes begin to develop. A second membrane is found inside the perivitelline membrane. Both membranes lie close together, except near the

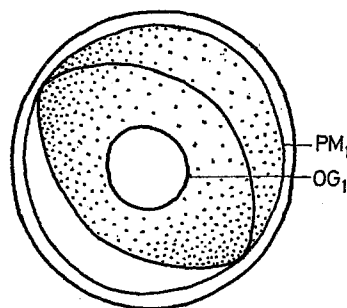


Fig. 1. Eggs of *Ambassis* species (0.60 mm) showing blastoderm covering half the yolk (about 3 h after spawning). OG₁ oil globule (unpigmented); PM₁ perivitelline membrane (outer)

cephalic and caudal ends (Fig. 2). At the cephalic end the membrane leaves a wider space than that at the caudal end. The tubular heart is straight and lies beneath the left eye. It is placed at an angle to the body with the posterior end attached to a point mid-way between orbits and auditory vesicles. The anterior end lies near the snout and communicates with the cephalic space formed by the 2 perivitelline membranes. In later stages, anterior and posterior ends of the tubular heart become parts of the sino-atrial chamber and ventral aorta, respectively. The heart begins to beat at least 3 h before hatching. Initially, pulses are irregular and the heart beat stops occasionally for

about 30 to 40 sec. The eggs hatch within 17 h after spawning.

In newly hatched larvae (1.25 mm total length, Fig. 3), there are 10 pre-anal and 15 post-anal myotomes. The yolk extends beyond the level of the snout with the oil globule in front. Small irregular particles are occasionally liberated from the yolk near the middle of the body. Each particle enters the space between the 2 perivitelline membranes, moves for-

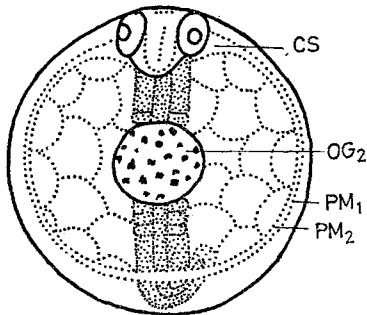


Fig. 2. Egg of *Ambassis* species (about 7 h after spawning) CS cephalic space formed by perivitelline membranes; OG_2 oil globule (pigmented); PM_1 perivitelline membrane (outer); PM_2 perivitelline membrane (inner)

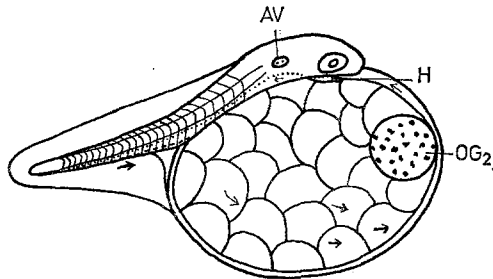


Fig. 3. Newly hatched larva of *Ambassis* species (1.25 mm total length). Arrows indicate the direction of movement of irregular particles. AV auditory vesicle; H tubular heart; OG_2 oil globule (pigmented)

ward to the cephalic end, and then enters the sino-atrial chamber. From the sino-atrial chamber it is pumped out through a vessel lying below the notochord. This vessel is a continuation of the tubular heart, and represents the dorsal aorta, which extends as far as the 25th myotome. Each particle is then returned through a caudal vein, which lies along the ventral edge of the caudal myotomes, to the level of the 5th myotome, where its individual identity is lost. The particles are circulated either singly, or in groups of 2 or 3. Sometimes the liberated particles remain for a short time in the cephalic space formed by the perivitelline membranes, irrespective of the heart-beat. No regularity was observed in the movement of these

particles. The heart-beat stops irregularly for periods ranging from 15 to 20 sec; however, the frequency is less than that of earlier stages. The division of the heart into defined chambers, separated by valves, is not apparent at this stage. The liquified yolk is always transported to the caudal region through the heart, ventral aorta and dorsal aorta. There is no vessel which supplies the liquified yolk to the cephalic region. As the yolk is absorbed, the anterior (sino-atrial) end of the tubular heart is gradually brought to a posterior position, resulting in a flexure near the attached end. This flexure is towards the ventral side.

In 24 h old larvae (1.55 mm total length), 6 to 8 oval, nucleated, blood corpuscles are seen. The blood from the sino-atrial chamber is pumped forward to the end of the ventral aorta; it flows past the flexure through 2 small vessels formed by the division of the ventral aorta. These vessels turn in opposite directions; each forms a loop beneath the orbit and runs posteriorly, to meet the other near the first myotome, and then continues to the dorsal aorta. The dorsal aorta on reaching the last myotome, bends downwards and goes forward as the caudal vein. In the rectal wall, the caudal vein forms a circular loop and proceeds forward as dorsal vein along the dorsal side of the gut wall. This vein runs anterior to a small oval-shaped body (placed between the 2nd and 3rd myotomes), turns obliquely downwards, and enters the right side of the heart through a comparatively larger vessel (Cuvierian duct). The dorsal vein may be the forerunner of the right posterior cardinal vein. Because of the narrowness of the passage, the oval-shaped blood corpuscles move behind each other, with their long axes parallel to the vessel. These corpuscles are circulated either singly or in groups of 2 or 3. At first, despite a heart-beat rate of 120 to 150/min, each corpuscle or batch of corpuscles takes about 10 sec to complete circulation. The blood corpuscles are not pushed out of the heart in regular succession. The corpuscle or batch of corpuscles is pushed out after a lapse of about 10 to 20 sec and, during this period, the other corpuscles remain in the peripheral region of the atrial chamber, from where they are liberated either singly or in small groups. At times, the same corpuscle or group of corpuscles is circulated without disturbing those in the atrial chamber. The chambers of the heart are now differentiated, but valves are not evident in the sino-atrial chamber. Whenever the heart-beat is temporarily stopped, the blood accumulates in the Cuvierian ducts, but when the heart-beat is resumed, contraction waves commence at the sino-atrial end. As there is no efficient valvular mechanism between atrium and sinus venosus, some blood surge and a few retrograded corpuscles are seen in the Cuvierian ducts. It is observed that the larvae remain inactive in-between bursts of activity, when the heart-beat is normal. At the same time, they are found to move about briskly when the heart-beat is temporarily stopped.

In 27 h old larvae, the dorsal aorta gives out a branch, at the level of the 12th myotome, which joins the caudal vein. From the circular loop formed by the caudal vein, besides the right posterior cardinal vein, a ventral vein (precursor of the left posterior cardinal vein) is also found to proceed ventrally to the middle level of the gut (position of 6th myotome), where it crosses the gut wall, obliquely, to the dorsal side, reaching behind the oval shaped body; it then runs obliquely to the ventral side and joins the left Cuvierian duct. By this time the number of corpuscles has increased and counting becomes difficult. A good percentage of blood is now returned through the ventral vein. The sino-atrial contraction becomes stronger than before, forces out all the blood, and the corpuscles no longer remain in the atrial chamber. The heart is still tubular in shape, and beats intermittently. Retrogressive corpuscles are seen in the Cuvierian ducts, as before.

Cuvierian duct and from there to the anterior and posterior cardinal veins; the extent of this activity could be assessed from the movement of the retrogressive blood corpuscles in this region.

Sometimes the retrogressive corpuscles could be traced in the ventral vein (left posterior cardinal vein) up to the circular loop in the rectal wall. In most cases, the blood corpuscles move forward with increasing momentum and push the retrograded corpuscles forward; this restores normal circulation. The anterior cardinal veins are cleared first. Sometimes, the retrograded corpuscles do not join the corpuscles moving forward. The resistance developed due to the meeting of 2 blood streams causes the ventral (left posterior cardinal) vein to dilate, and the retrogressive corpuscles are detained; they die and disintegrate.

Occasionally, 1 or 2 corpuscles passing through the caudal artery (the portion of dorsal aorta beyond the

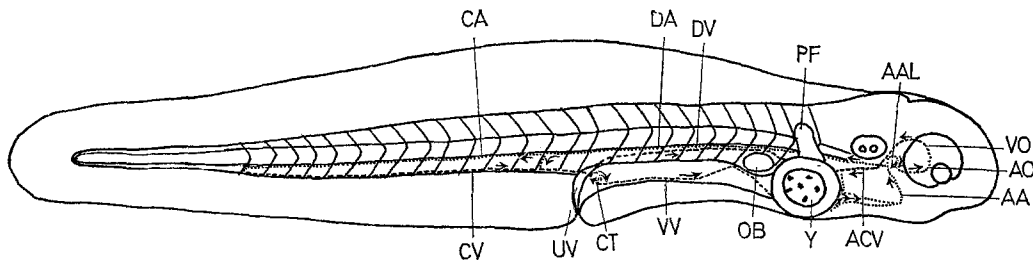


Fig. 4. Larva of *Ambassis* species (about 36 h after hatching; 1.65 mm total length). Arrows indicate direction of flow of blood corpuscles. AA aortic arch; AAL loop formed by aortic arch; ACV anterior cardinal vein; AO artery supplying orbit; CA caudal artery; CT circular loop of blood vessel in the rectal wall; CV caudal vein; DA dorsal aorta; DV dorsal vein (Right posterior cardinal); PF pectoral fin bud; OB small ovalshaped body; UV urinary vesicle; VO vein from orbits; VV ventral vein (Left posterior cardinal); Y remnants of yolk and oil globule

In 36 h old larvae, more than 90% of the yolk is absorbed (Fig. 4), and the mouth begins to differentiate. The loop of the artery beneath the orbit becomes wider and, before it runs down to join the dorsal aorta, a small branch, from its inner corner, takes blood more than half way around each orbit. The blood is returned to the Cuvierian duct by a vein (forerunner of the anterior cardinal vein) lying alongside the pharynx. From the origin of the dorsal aorta, a small artery takes blood to the small oval-shaped body, and is returned to the dorsal vein (right posterior cardinal) before it joins the right Cuvierian duct.

In 60 h old larvae, the artery which takes blood to the oval-shaped body continues to run along the dorsal side of the gut wall beneath the dorsal vein, and joins the circular loop formed by the caudal vein in the rectal wall. This artery may be the precursor of the coeliac artery. At this stage also, the heart-beat was observed to stop irregularly, but for a lesser duration (about 5 to 10 sec only). When the heart-beat is resumed, part of the blood is retrograded into the

12th myotome) may slip into the space between adjacent myotomes and find their way across to join the caudal vein. A few more corpuscles may pass through this newly formed passage, but it does not become a regular path. Some of these corpuscles may not be able to join the caudal vein, and consequently die and disintegrate. However, a good percentage of them succeed in joining their partners.

In 72 h old larvae, a branch arising from the rear end of the coeliac artery runs across the gut wall and joins the ventral (left posterior cardinal) vein, in front of the circular loop. By now the yolk is used up and the eyes become black. A gill slit is differentiated. A small artery arising from each branch of the ventral aorta runs to the gill slit and joins the dorsal aorta. This represents the formation of afferent and efferent arteries.

In 110 h old larvae, a second branch arises from the coeliac artery in front of the first one, and joins the ventral vein (left posterior cardinal vein). Another small artery arises from the anterior end of the coeliac

artery in front of the oval-shaped body. It runs obliquely down, crosses the ventral (left posterior cardinal) vein, and forms a loop in a clockwise direction; it then joins the descending oblique section of the ventral (left posterior cardinal) vein. The liver is differentiated, and the ventral vein passes through the liver before it joins the left Cuvierian duct.

In 140 h old larvae, the ventral vein in the liver divides into 3 branches and emerges out as a single vein which joins the Cuvierian duct. A third branch arises from the coeliac artery in front of the second one and joins the ventral vein. The number of gill-slits increases. The heart beats at the rate of 120 to 150 beats/min but not intermittently as before. The anterior region of the larvae becomes less transparent. This condition, together with the brisk movement of the larvae, makes it difficult for the pattern of circulation to be followed clearly in advanced stages of growth.

The speed of blood flow, as evident from the movement of the blood corpuscles, varies in different vessels as well as in the same vessel at different stages of development. In larvae up to 36 h old, the blood flows mainly through the caudal region and, after this period, through the trunk region. After 72 h, most of the blood flows through the coeliac artery and the supply through other channels is reduced; by this time, the intestine becomes a functional organ.

Larvae from fresh collections, resembling 96 h old larvae, had food particles (diatoms) in the gut. These food particles are pushed back and forth several times by the peristaltic action of the gut wall, and become reduced in size. The intestine is then distended with fluid matter. Following peristaltic action of the gut wall, the blood flow in the coeliac artery stops, and the blood corpuscles begin to accumulate for about 10 to 20 sec. At the same time, the blood flow through other vessels is incessant. The phenomenon of piling up of blood corpuscles in the coeliac artery is more frequent after strong peristaltic action of the gut wall, particularly when the intestine is distended with food. This does not occur when the intestine is empty, or in larvae younger than 72 h. The sphincter muscles present in the intestine at the level of the 3rd and 8th myotomes, prevent food particles from being thrown out during the peristaltic action.

Mugil species

The disposition of the circulatory system is similar to that of *Ambassis* species, except for the relative position and length of the blood vessels, which varies with the number of pre-anal myotomes.

Dorosoma species

The eggs are larger (0.77 mm diameter) than those of *Ambassis* and *Mugil* and contain a number of oil globules of varying sizes. The yolk is vacuolated and clear. The space between perivitelline membrane and the egg shell is wider than in *Ambassis* species, but the general disposition of the early circulatory system is similar to that of *Ambassis* species. In newly hatched larvae (2.0 mm total length) there are 34 pre-anal and 7 post-anal myotomes. The straight, tubular heart lies almost parallel to the cephalic region. Six pairs of semi-circular swellings, spaced at regular intervals, occur in the heart. The movement of the irregular yolk particles are more clearly seen than in *Ambassis* species. The heart beats and stops irregularly, as in the case of *Ambassis* species. In 3 h old larvae (Fig. 5, 2.16 mm total length), 2 to 3 oval nucleated blood corpuscles leave the ventral aorta and enter the

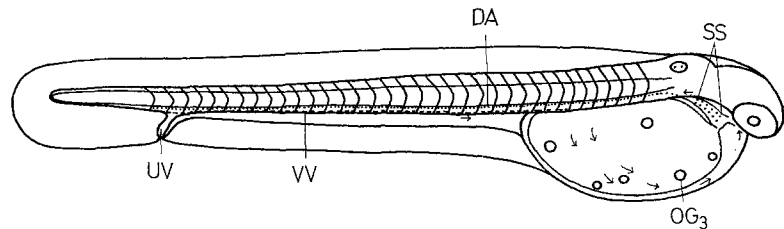


Fig. 5. Larva of *Dorosoma* species (2.00 mm total length; about 4 h after hatching). Arrows indicate the direction of movements of irregular particles and blood corpuscles. OG₃ oil globules; SS paired semicircular swellings of the heart. (Other lettering as in Fig. 4)

dorsal aorta. These corpuscles are carried up to the 35th myotome, and brought to the level of the 7th myotome by the ventral vein, and then enter the heart through the space between the 2 perivitelline membranes. At this stage, the ventral aorta is not divided, and the sino-atrial chamber is situated anterior to the ventral aorta. The heart and the dorsal aorta, therefore, lie on the same level.

The larvae are very delicate and die in small quantities of water; further observations were thus impeded.

Thrissocles species

Circulation of irregular yolk particles are clearly seen at the time of hatching. The disposition of the heart is the same as in *Dorosoma* species. The heart-beat is intermittent, as in the other species. Six pairs of semicircular swellings are also found in the heart as in *Dorosoma* species.

The larvae of *Thrissocles* species also die in small quantities of water; further observations were, therefore, not possible.

Discussion

In the initial stages, the tubular heart is straight and lies at an angle towards the left side, beneath the cephalic region. The end which later becomes part of the ventral aorta is attached mid-way between orbits and auditory vesicles, and continues as the dorsal aorta. The anterior end of the tubular heart, which later becomes the sino-atrial portion, lies anterior to the ventral aorta; this position is greatly influenced by the size of the yolk. As the yolk becomes absorbed, however, the sino-atrial end gradually moves to a position posterior to the ventral aorta, resulting in a flexure of the ventral aorta near the point of attachment.

Movement of irregular yolk particles indicate that the yolk is liquified and transported to the caudal region. These particles are different from blood corpuscles. Blood corpuscles occur only in larvae. RYDER (1882) and DELSMAN (1924, 1927) have, however, reported blood flow in the ventral and lateral vessels and in the Cuvierian ducts, in eggs of *Belone longirostris*, *Hemiramphus* and *Trichiurus* species. According to DELSMAN, the rudiment of the circular blood vessel which occurs in the eggs of *Trichiurus* species corresponds to the lateral vessel of *Hemiramphus* species, and represents the Cuvierian ducts.

In early stages, the ventral aorta is not divided into aortic arches; it continues directly to the dorsal aorta. The tubular heart, ventral aorta and dorsal aorta, therefore, lie in a line. The dorsal aorta continues directly to the caudal vein, and a capillary system is, therefore, not evident. The blood vessels are so narrow, that the oval-shaped blood corpuscles can pass through only one behind the other, with their long axes parallel to the passage.

Retention of blood corpuscles in the atrial chamber in very early larvae indicates that the contraction of the atrial chamber is incomplete. The presence of a retrograde blood flow occurring in early larval stages and its absence in advanced stages suggest that the valves between atrium and sinus venosus are inefficient.

The dilatation found in the ventral (left posterior cardinal) vein to detain the resisting retrogressive corpuscles show that the blood vessels can expand. The accumulation of blood corpuscles in the coeliac artery during strong peristaltic action of the distended intestine indicates that the vessels can contract. These observations suggest that the blood vessels have soft walls which facilitate dilatation or contraction.

The passing of blood corpuscles from the caudal artery through the space between adjacent caudal myotomes suggests the possibility of such spaces being converted into blood vessels.

Variation in the speed of blood-flow through different vessels at different stages of development may be an adaptation to meet the metabolic require-

ment of the larvae. In the initial stages, more blood is supplied to the caudal region than to the cephalic portion. Consequently, the posterior cardinal veins develop earlier than the anterior ones. As the yolk is absorbed, the mouth and intestine develop and, subsequently, become functional. At this stage more blood is found to flow through the anterior region.

In very early stages of the larvae, metabolic waste products are eliminated through the body surface. The larvae may suspend all movements to perform this function efficiently (SCHUMANN, 1965; BALAKRISHNAN, 1969).

It is not at present possible to give a satisfactory explanation for the intermittent heart-beat and for the brisk movements during the suspension of heart-beats in early larval forms. The paired semicircular swellings found in the heart may function as pace makers.

Summary

1. In early teleostean embryos the tubular heart is straight and situated towards the left body side, with its sino-atrial end near the snout. The position of the sino-atrial end is greatly influenced by the size of the yolk. The heart begins beating at least 3 h before hatching; heart-beats are, initially, irregular, ranging from 120 to 150 beats/min. Occasionally, the heart stops beating for about 30 to 40 sec.

2. Circulation of liquified yolk could be traced by movement of the irregular yolk particles. These particles are different from blood corpuscles.

3. In *Ambassis* species, 6 to 8 oval-shaped, nucleated corpuscles are present in 24 h old larvae. As the yolk becomes absorbed, the anteriorly situated sino-atrial end is gradually brought posterior to the ventral aorta. The anterior end of the ventral aorta divides into 2 aortic arches, which then unite to form the dorsal aorta. The right posterior cardinal vein is developed. Division of the heart into various chambers is not apparent.

4. In 27 h old larvae, the left posterior cardinal vein is developed.

5. In 36 h old larvae, the mouth begins to appear and the anterior cardinal veins develop.

6. In 60 h old larvae, the coeliac artery becomes differentiated from the anterior end of the dorsal aorta. It runs along the dorsal side of the gut wall, beneath the right posterior cardinal vein. The heart-beat is still irregular, but stops are less frequent. The spaces between the caudal myotomes could temporarily function as blood vessels.

7. In 72 h old larvae, gill slits are differentiated and formation of afferent and efferent arteries occur.

8. In 110 h old larvae, the liver is developed and the posterior cardinal vein on the left side passes through the liver.

9. A definite capillary system is not observed in early larvae.

10. The pattern of circulation is similar in early larvae of *Mugil*, *Dorosoma* and *Thrissocles* species, except in some minor details. In *Dorosoma* species the blood corpuscles are found in 3 h old larvae. The dorsal aorta extends to the 35th (first post-anal) myotome.

11. The larvae of *Dorosoma* and *Thrissocles* species are sensitive to small quantities of water, in which they die.

12. The speed of the blood flow varies in different vessels, as witnessed by the movement of the blood corpuscles. Initially, the blood flows only to the caudal region. Hence, the posterior cardinal vein develops earlier than the anterior one.

Acknowledgements. The authors are indebted to Mr. D. J. TRANTER, C.S.I.R.O. Cronulla (Australia), Professor G. M. HUGHES, University of Bristol (England), Dr. K. JOHANSEN, University of Washington (USA), Dr. N. BALAKRISHNAN NAIR, University of Kerala (India), and to Dr. S. Z. QASIM, National Institute of Oceanography (India) for their valuable comments and criticism, and also to Dr. N. K. PANIKKAR,

Director, National Institute of Oceanography (India) for his interest and encouragement in this work.

Literature cited

- BALAKRISHNAN, K. P.: Eggs and early larvae of *Thrissocles* species (Engraulidae: Pisces) Mar. Biol. **2**, 224—227 (1969).
 DELSMAN, H. C.: Fish eggs and larvae from Java Sea. 3. A pelagic scombriscoid egg. Treubia **5**, 408—418 (1924).
 — Fish eggs and larvae from Java Sea. 11. The Genus *Trichiurus*. Treubia **9**, 338—351 (1927).
 RYDER, J. A.: The development of silver gar (*Belone longirostris*). Bull. U.S. Fish Commn **1**, (1882).
 SCHUMANN, G. O.: Some aspects of behaviour in clupeid larvae. Rep. Calif. coop. oceanic Fish. Invest. **10**, 71—78 (1965).
 SHEARER, E. M.: Studies on the embryology of circulation in fishes. Am. J. Anat. **46**, 393—459 (1930).
 ZIEBA, J.: The blood vessels of the yolk sac of the pike (*Esox lucius* L.). [In Polish]. Biul. Zakł. Biol. Stawów **3**, 5—18 (1956).

First author's address: K. P. BALAKRISHNAN
 Oceanographic Laboratory
 University of Kerala
 Cochin, India

Date of final manuscript acceptance: May 16, 1970. Communicated by N. K. PANIKKAR, Panaji