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Features of embryonic and larval development of crucian carp, *Carassius carassius* (L.) with a note on species identification

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Mature and larval crucian carps were captured from natural ponds near Joensuu, Eastern Finland. The spawning fish were stripped and eggs were artificially fertilized. Different stages of embryonic, larval and juvenile development were described from fish kept at 20°C in the laboratory.

In 2–4 days from hatching the larvae started to feed and swim actively. The fins differentiated gradually at the fish lengths of 8.5–15 mm. No difference was noted in the development between larvae taken from the pond and those reared in the laboratory.

1. Introduction

The crucian carp is a cyprinid fish commonly inhabiting shallow, dark-water ponds and other stagnant waters in Finland. It is the only fish species able to survive the long winter anoxia frequent in these habitats (e.g. Blazhka 1960, Holopainen & Hyvärinen 1985).

The spawning of this species takes place at high temperatures (>17°C) and the eggs are laid in 2–3 batches between May and late July (Kryzhanovskii 1949, Astanin & Podgorny 1968, Holopainen & Pitkänen 1985). This species is a fractional spawner: the females release only part of their eggs at each spawning. The eggs attach singly to the vegetation: the species is described as being a phytophil, open substrate spawner. Descriptions of egg incubation times and early development of crucian carp are scarce (Kryzhanovskii 1949, Schäperclaus 1953, Volgin & Dubinina 1977, Koblitskaya 1981, Laurila et al. 1987).

The aim of this paper is to describe each of the early developmental stages of crucian carp from fertilized egg to juvenile fish and to list the features useful in the species identification of the larvae.

2. Material and methods

Field material was collected in the years 1983–84 from two small, natural ponds: Hermanninlampi (HL) and Kivilampi (KL, for descriptions see Holopainen & Pitkänen 1985 and Laurila et al. 1987) near Joensuu in Eastern Finland (62°41′N, 29°41′E). Field observations were made on water temperature, spawning and the hatching of the young.

Eggs and larvae were collected from submerged vegetation by hand nets (mesh size 1 mm). Spawning fish were caught by traps and taken to the laboratory for stripping. The eggs were fertilized by the dry method and placed on plant substrate (juniper twigs) in jars kept at room temperature (20–25°C) and natural light conditions. The water was changed every day. After hatching the larvae were offered *Artemia salina* nauplii and plankton from the ponds *ad libitum*.

The fish were measured with an ocular micrometer in a stereomicroscope and their developmental phase was noted at different lengths. For photographing and drawing they were placed in petri dishes and immobilized with carbon dioxide (larvae) or MS 222 (1:10 000 for juveniles).

A female (14.7 cm) from Hermanninlampi was stripped and spawned eggs from a trap in Varaslampi (female 31 cm) were collected. The eggs of both samples were preserved in 10% formalin and their diameter was measured by an ocular micrometer (×25 magnification) from a sample of regularly shaped eggs.

Gonads and larvae were also fixed in Bouin, handled by the customary wax-embedding method and sliced for histological slides.

3. Results and discussion

3.1. Egg size

The eggs of crucian carp are spherical and yellowish-orange. The diameter of the eggs were 1.37 mm (SD = 0.09, n = 62) in HL but 1.61 mm (SD = 0.10, n = 62) in Varaslampi.

According to Astanin & Podgorny (1968) the mean egg diameter was 0.9 mm but with very large variation (range: 0.2–1.58 mm in the random sample) and with no clear peaks which would indicate separate egg batches of the successive spawnings. Krivoshchekov (1953, cited after Astanin & Podgorny 1968) gives a diameter of 1.05–1.35 mm for mature oocytes (eggs) of crucian carp in West Siberian waters. According to Schäperclaus (1953) the diameter of spawned eggs was 1.45–1.52 mm. The eggs of crucian carp have a water content of 69% and a lipid content of 1.8% (Ginzburg 1968, cited after Balon 1985).

3.2. Embryonic development

After fertilization the eggs swell. The swollen eggs attach readily to plants. Then the blastodisc forms: the cleavage begins approx. 40 minutes after the fertilization at 20°C (Fig. 1a). A small celled morula is formed after 6 h (Fig. 1b) and the blastulation starts 9 h after the fertilization (Fig. 1c). During the first day the germ layers are formed and the embryo outlines appear around the vitelline curvature (Fig. 1d). The head region then starts to differentiate: optic vesicles and the anterior part of the neural tube can be soon distinguished (23 h from fertilization, Fig. 1e).

The first somites appear during the second day (Fig. 2a). On the third day the eye lenses appear, blood circulation starts (heart beating starts to circulate colourless plasma; the erythroblasts are visible later) and the caudal section of the embryo is detached from the yolk sac (Fig. 1g, 2b).

During the last days preceding the hatching, the embryo is mobile and its pigmentation gradually increases. The embryo of crucian carp has pigment spots before hatching (Fig 2c).

The incubation time depends on temperature being approx. 4 days at 20°C and 2 days at 2–27°C (Laurila et al. 1987).

3.3. The newly hatched free embryo (eleutheroembryo or yolk sac larva)

The development of the yolk sac larvae is usually described giving a certain length range for each developmental phase. A summary of such data on crucian carp is given in Table 1.

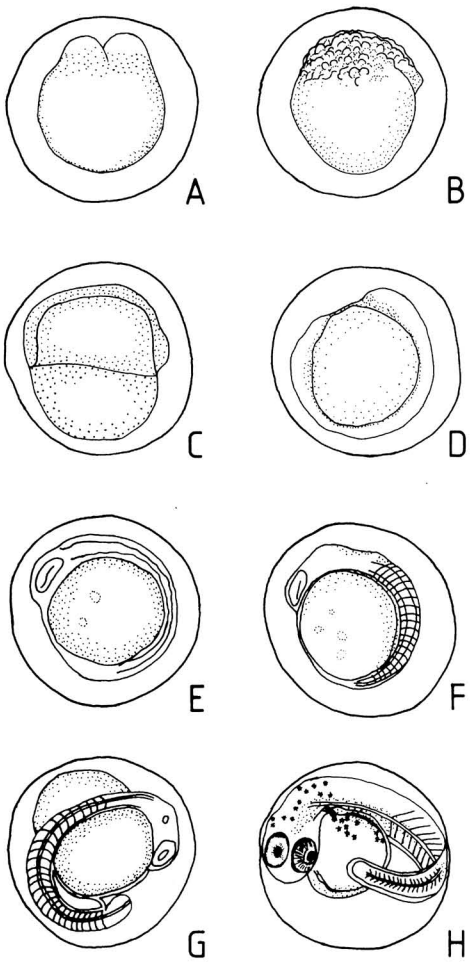

Fig. 1. Eight stages in the development of the fertilized eggs of crucian carp in the laboratory at 20°C. — A: the first cleavage at 40 min; B: high morula at 6 h; C: blastula at 9 h; D: gastrulation; E: 24 h, neural tube formation; F: 36 h, optic vesicles, notochord and somites; G: eye lenses, auditory vesicles and free trunk-tail at 2 d; H: pigmentation at 3 d.

The total length of the larva is 4.5–5.5 mm at hatching (Fig. 2d, 3a). The body is slender and transparent, and the dorsal part has yellow pigmentation. The distance from the tip of the snout to anus is about 66% of the total length. The yolk sac is long with its anterior part enlarged.

The mouth and the digestive tract are not yet developed. Gas exchange takes place in the caudal and yolk sac blood vessels (Meshkov 1951). The pigmentation pattern is typical for cyprinid fishes with dark melanophores forming lines; crucian carp has two lines from head to tail along the ventral and dorsal edges of the body. On the head there are some large melanophores and a paired adhesive gland. Eyes are large and well pigmented. The preanal myotomal count is 22–23 and the postanal 11.

3.4. Larval development

During the first days at room temperature, the embryonic shape of the body and head disappear together with the yolk sac and are replaced with larval organs and functions. The swim bladder fills with gas (Fig. 2e). The jaws and intestine differentiate and the gill filaments form and become functional (Fig. 3b). The yolk sac disappears completely at the length between 6.5–7.2 mm.

The locomotion ability increases gradually while the fins start to develop at 8.5–13 mm. Digestive tract differentiates; intestine folds and digestive organs develop (Fig. 4). Aggregations of mesenchyme tissue appear on the sites of the unpaired fins; first mesenchymal rays appear below the tip of the still horizontal urostyle (Figs. 2f, 3d). Pelvic fins are small lobes in the preanal finfold. Swimbladder has two sections

Table 1. Length range (mm) of crucian carp at various developmental stages.

Stage	Kryzhanovskii 1949	Schäperclaus 1953	Volgin & Dubinina 1977	Koblitskaya 1981	Present data at 20–25°C
Hatching	5.0–5.2	4.2–4.9	3.8–5.2	–	4.5–5.5
Loss of the yolk sac	6.0	–	–	6.0–7.0	6.5–7.2
First caudal fin rays	9.0–9.5	–	–	7.5–8.0	8.5–10.0
Clear unpaired fins	10.5–11.0	–	–	11.0–12.0	11.0–13.0
Loss of preanal finfold	14.0–14.5	–	–	–	12.0

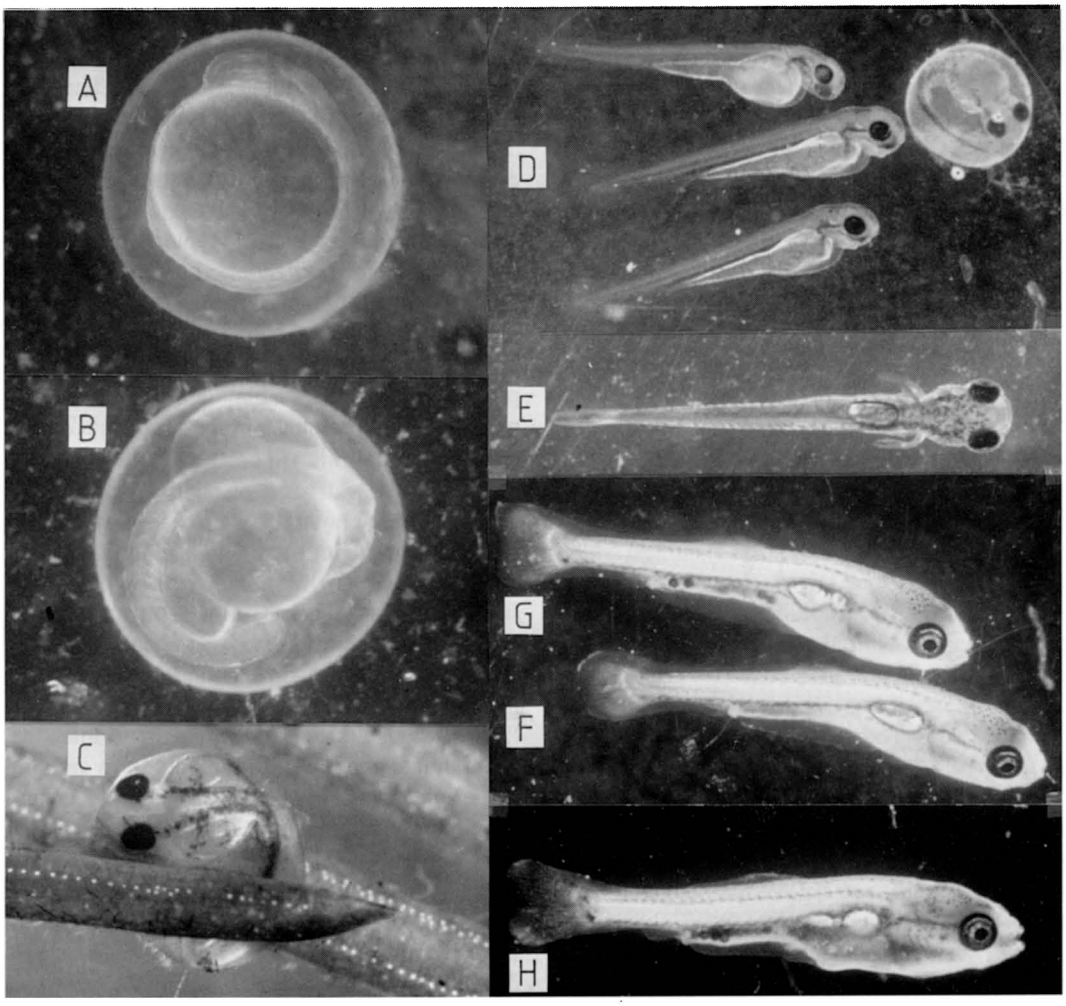

Fig. 2. Photomicrographs of various developmental stages. — A: 20 h, first somites; B: 29 h, free trunk-tail; C: embryo before hatching, fixed to a juniper needle; D: newly hatched free embryos; E: 24 h, dorsal view; F: 9.5 mm; G: 10 mm, the swim bladder divides, dark spot at the base of caudal fin; H: 12.5 mm, *Artemia* cysts in the gut.

(Fig. 2g). At 11–13 mm the posterior end of the notochord curves upwards and the caudal lobe becomes homocercal. There are 9 rays in the dorsal fin and 6 in the anal fin (Figs. 2h, 3d).

At the length of 12–15 mm the number of rays is 16–19 in the dorsal fin and 6 in the anal fin. The trunk is less than 60% of the total length. No finfold is left and the pelvic fins are rayed at the length of 14–21 mm, when the first scales appear (Fig. 3e). Squamation is complete at 30 mm length.

3.5. Juvenile phase

In the ponds the fish reach the juvenile phase in July–August depending on the spawning time. The juvenile resembles adult crucian carp, but it

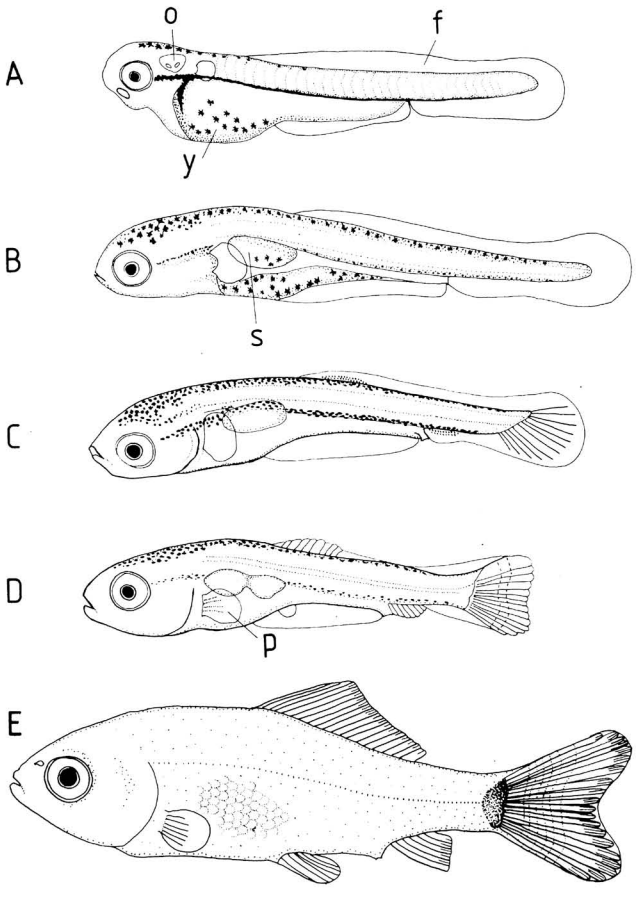

Fig. 3. Development of free embryo to a juvenile in crucian carp. — A: 5.5 mm, a newly hatched free embryo; B: 6.5 mm, jaws and intestine forms; C: 9 mm, the caudal fin-rays form; D: 11.5 mm, the unpaired fins are formed; E: 17 mm, first scales appear. f = finfold, o = otoliths, y = yolk sac, s = swim bladder, p = pectoral fin.

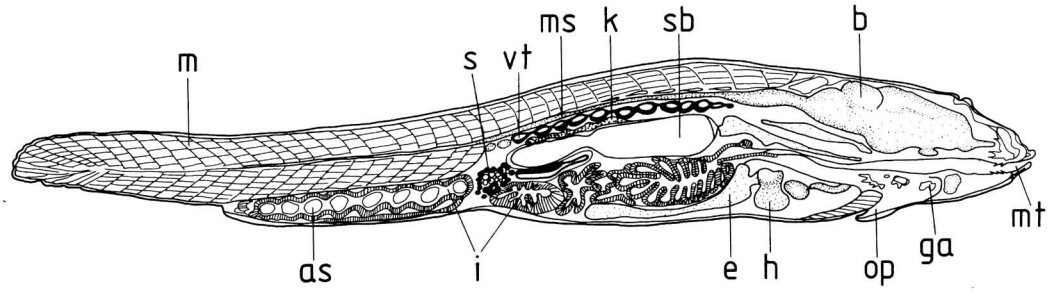

Fig. 4. A drawing of a median section of a 12 mm larva to show the main organs. mt = mouth, ga = gill arch, op = operculum, b = brain, h = heart, l = liver, sb = swim bladder, k = kidney, ms = spinal cord, vt = vertebra, i = intestine, as = *Artemia salina* cyst, m = myotome.

has thinner body, lighter coloration and a dark spot at the base of the caudal fin.

3.6. Identification of larvae and juveniles

The characteristic body size and shape at each stage of development are affected by e.g. temperature and food to some extent, but primarily are genetically determined (Kendall 1984). Larvae reared in captivity tend to be shorter and fatter (Blaxter 1988). In our experiment no significant difference in pigmentation and development was noted between laboratory reared larvae and those captured from the pond at lengths below 20 mm (Laurila et al. 1987).

European cyprinid larvae differ from the other North European freshwater species firstly by the shape of the body: height comprise over 15%, and the trunk approx. 60% of the total length. The linear pigmentation pattern (pigment lines) is characteristic, too. Cyprinid species closely resemble each other at larval stages and are difficult to identify. The crucian carp has a low myotomal count (10–11 preanal and 22–23 postanal myotomes) compared with other cyprinid larvae, which have usually over 13 postanal myotomes (Koblitskaya 1981). The pigmentation is also specific with only two rows of melanophores (a strong ventral line from eye to tail and another in the dorsal contour of the body), while most other cyprinid larvae have 3 pigment lines (Kryzhanovskii 1949, Koblitskaya 1981).

For the identification of free embryos and early larvae (<13 mm) the myotomal counts and the pigmentation pattern are feasible. The pigmentation pattern is a constant feature in contrast to the shape of the individual melanophores. In high light intensity environment the melanophores are shown to become concentrated whereas in darkness they are large and spidery (Mooij 1989).

After the development of the unpaired fins (>13 mm) the number of their fin rays can be used for identification: the count is 16–19 in the dorsal fin and 6 in the anal. Typical at this stage is the dark spot on the base of the tail.

3.7. Larval ecology and ethology

The newly hatched free embryos are passive: they lie on the bottom of the aquarium, for example. In the second day after hatching they try to swim upwards, towards the light (positive phototaxis, Disler 1971) and attach to the plants. The locomotion and orientation are aided by the pectoral fins and the undifferentiated finfold. The pectoral fins appear as small round lobes, consequently they do not help in holding the position or balance.

From the end of May newly hatched fish were caught by hand net in the ponds among the floating and submerged vegetation of the central pond areas. The larvae were taken to the laboratory aquarium, where they were hanging on plant surfaces by their head gland or swimming with jerky zigzag movements. A few days (3 days at 20°C) after hatching the fish swim to the surface to fill the swim bladder and then switch to exogenous feeding when yolk reserves are reduced. The transformation period to exogenous feeding is considered to be a critical period in fish development and especially sensitive to environmental conditions (e.g. food availability, temperature, predation, cannibalism; e.g. Blaxter 1988). Crucian carp reach this stage in a short time, compared to other cyprinids. E.g. bream fills its swim bladder 12–24 hours after hatching but does not start feeding before 7–8 days later in the laboratory, at least (Kennedy & Fitzmaurice 1968).

Crucian carp larvae feed on small Cladocera, Copepoda and larval Chironomidae (Penttinen & Holopainen 1990). In the pond, the larvae were often found hiding in the mud and vegetation of the littoral areas, where temperatures may rise over 30°C at times. The larvae stay always close to the vegetation and none could be caught by hand net in the offshore area. In late summer the juveniles equally stayed close to the shore vegetation and hardly any were found in the open (pelagial) areas of central ponds.

No schooling has been noted in the laboratory, but the larvae appear to form groups of 20–50 individuals at fish lengths between 7 and 20 mm.

Further studies are obviously needed on factors affecting embryonic and larval development in their natural environments. The identification, ecology and ethology of many freshwater fish species at larval stages is a rather neglected area as well.

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