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Breeding Behaviour and Embryonic Development of Koi Carp (*Cyprinus carpio*)

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ABSTRACT: Induced breeding experiments of Nikishigoi (koi carp), *Cyprinus carpio* were conducted in mature males and females by administrating a single intramuscular injection of ovaprim at a dosage of 0.3 mL/kg weight. Spawning was observed six hrs after the injection at ambient temperature (26-28^{o}C). The fertilized eggs were adhesive and transparent with diameter ranging between 0.9 mm and 1.10 mm. Incubation period was 73.00hrs. The hatchlings were transparent and measured 2.7-2.9 mm, with a large oval head, a well defined yolk sac and short tail. The yolk got fully absorbed within 3 days and by this time mouth formation was complete and the larvae started exogenous feeding. After 20 days the length of fry ranged between 10 mm and 12 mm and after 35 days length of the fingerlings ranged from 30-35 mm and appeared just like an adult in all respects except sexual maturity.

KEY WORDS: Breeding behaviour, ontogenic events, koicarp.

INTRODUCTION

Koicarp, (Nishikigoi) were developed from colour variants of the common carp *Cyprinus carpio*, in Japan during the Tokugawa period (17-19 century). They are basically delicate but very peaceful towards other occupants and hence well suited to aquarium setup. They grow up to 100 cm length with an elongate body measuring 3 to 4 times less in height than length. In their natural habitat, koicarp live up to 15-24 years (Kuroki, 1981). Males are known to live longer than females. In the present investigation induced breeding techniques, breeding behaviour and ontogenic events of koi carp are discussed since these aspects may be of great help to the farming community venturing into the breeding and culture of ornamental fish species.

MATERIALS AND METHODS

Mature healthy koicarp brooders (200-250 g), two males and one female were selected by sexual dimorphism for breeding experiments (Fig. 1A). Female is usually easier to spot, as the belly of a mature female is generally plump, whereas male remains streamlined and more torpedo shaped. When males are ready for spawning, they develop breeding tubercles on the head and pectoral fins, principally along the bones of the fin rays. These are used during breeding, when the male nudges the female with its head and fins to induce her to spawn. Koi generally prefer to spawn around dawn but they may also spawn throughout the day.

In the present study spawning was induced by intra-peritoneal injections of ovaprim (Fig. 1B) at a dose of 0.3 mL/kg body weight (Haniffa and Sridhar, 2002). The breeding set was released into cement breeding tanks (1mx1mx1m) after the hormonal administration. Aquatic macrophytes like *Hydrilla verticillata* and *Eichhornia crassipes* were introduced into the breeding tank for hiding purposes as well as holding the adhesive eggs (Haniffa and Sridhar, 2002). The breeding behaviour of brooders was observed every hour after the injection.

Spawning activity started immediately after the injection and lasted for about 1-8hrs. The courtship took place at the bottom of the breeding tank. After the first impulse of excitement, the males made advancement towards the female (Fig. 1C). Both the males showed participatory behaviour. Males followed the female touching it frequently. A special behaviour was noticed in males to attract the female for courtship by encircling the female in order to retain her in a given area (Fig. 1D). The excited male came closer to the female, but the female remained quite passive moving gracefully avoiding the approaching male. But the active male chased the female and generally swam underneath her. Often the active male obstructed the path of the female (Fig. 1E) so that it cannot avoid the male and often touched the

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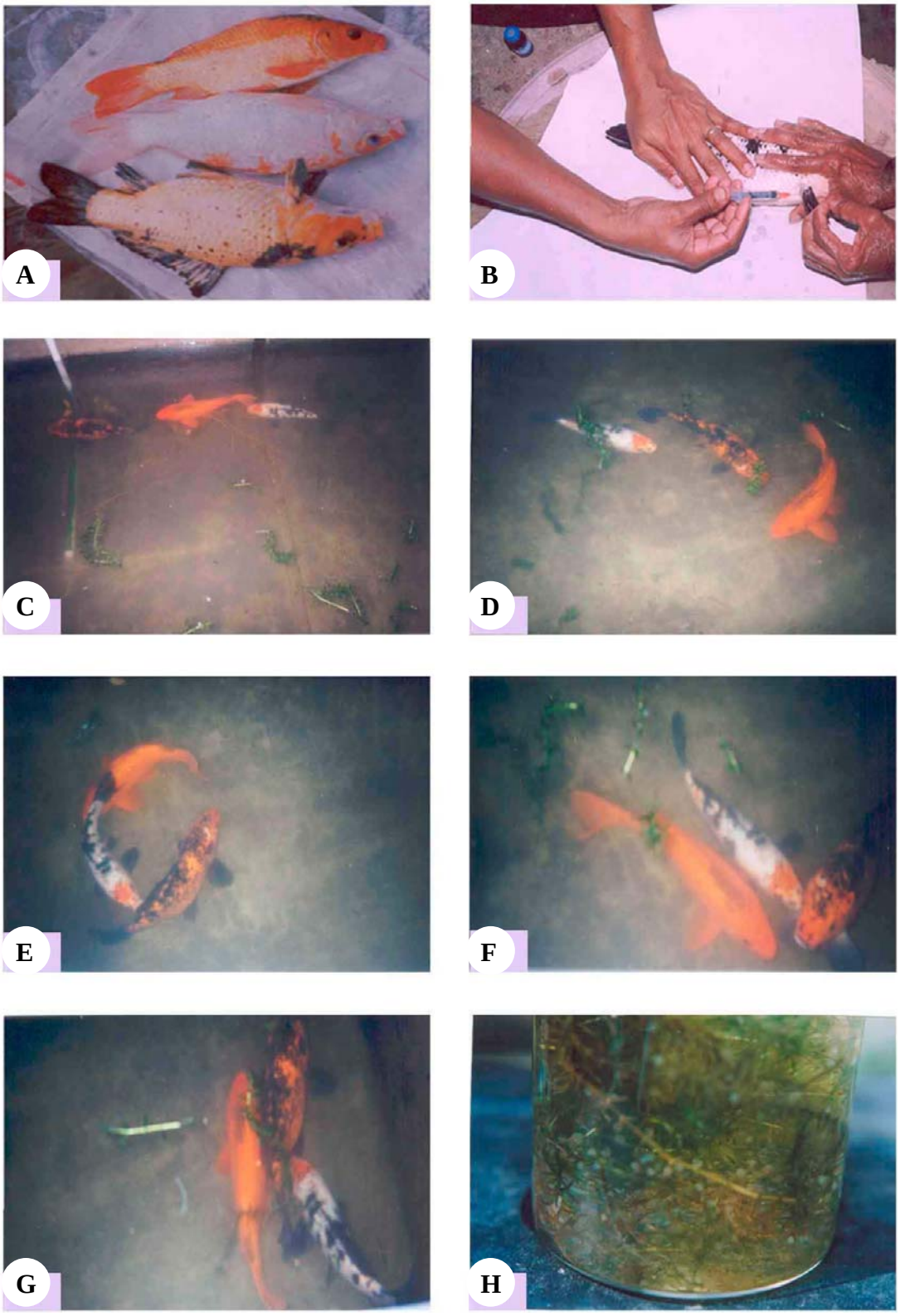

Fig. 1. A: Breeding set. B: Intraperitoneal injection. C & D: Male chasing the female. E: Coutship behaviour. F: Male touching the head of female. G: Male hitting vent of female. H: Adhesive eggs.

vent of the female (Fig. 1F), and frequently touched the head of the female also (Fig. 1G). During mating their dorsal fins were frequently exposed above the water surface and there was much splashing of water and pursuing from one area to another.

During spawning the males were aligned on either side of the female and rubbed their body against the female and released the milt. The adhesive eggs were deposited on submerged vegetation (Fig. 1H) and were fertilized externally. When they have finished spawning, the female hang head down, respiring heavily. After spawning the male and female remained calm in one corner of the tank and did not show any signs of hostility when the eggs were colleted. Koi are not good parents and unless they are separated from the eggs they begin to eat the eggs.

Fertilized eggs were collected with the help of a dropper and were reared at a density of 30/cement tank (3x1x1 m) provided with well-aerated water (D.O: 5.4-5.8 mg/L, pH: 7.2-7.4; temperature: 26 -28 ℃). Larvae were fed with zooplankton (rotifers and moina) two times (at 8.00 and 16.00 hrs) daily a*d libitum* after yolk absorption. Samples of eggs before fertilization and at every 30-min interval were taken for further studies. Descriptions of the developing stages were made by examining live specimens under Nikon Eclipse E 400-UIII microscope and microphotographs of the developmental stages of eggs (Fig. 2) and larvae were taken (Haniffa et al., 2003).

RESULTS AND DISCUSSION

Fish farmers are much less familiar with the culture of koicarp because of the lack of breeding and feeding techniques and non-availability of seeds (Meehan, 2002). In the present study, spawning was noticed 6hrs after the injection. The fertilized eggs of koi carp were adhesive, demersal and spherical in nature. Since the egg envelope is thick, transparent and sticky, observations on the developmental stages are difficult (Kovac, 2000). Changes in structure emphasize the thresholds between embryonic, larval and post-larval development from the onset of cleavage or epiboly, or at the time of organogenesis, respectively (Kovac, 2000; Carlos et al., 2002). The eggs were deposited singly and were highly adhesive throughout the incubation period. Due to the adhesive nature of the egg, considerable debris adhered to the capsule of the egg. The yellowish white egg capsule was transparent, while the yolk was pale yellow or green and granular. The eggs became translucent as development progressed. The diameter of the fertilized egg capsule ranged between 0.9 mm and 1.10 mm while the yolk sphere ranged from 0.6-0.8 mm. The incubation period of eggs depends largely on water quality parameters such as salinity and temperature (Kuo et al., 1973; Liao, 1975). In the present study the eggs hatched 72hrs after fertilization at a water temperature of 26-28℃.

Although a true metamorphosis is not generally described for fishes, the term hatchling, larvae and post larvae are used to indicate different stages of development from hatchling to fingerling stage (Boglinoe et al., 1992). In the present study the developmental stages of koi carp were divided into six stages. *viz*., embryo, hatchling, larva, post- larva, fry and fingerling (Jhingran and Pullin, 1985) and each stage was characterized by typical anatomical and physiological features. A summary of the important ontogenic events and structure is presented in Tables 1 & 2.

The cleavage was typically meroblastic and the first cleavage occurred (2-celled stage) within 20 min after fertilization followed by the second cleavage, which was completed 30 min after fertilization. The 16-celled stage reached after 39-40 min of fertilization. The yolk invasion started after 3 hrs of fertilization and completed after 5.26 hrs of fertilization. The head and tail ends of the embryo became distinguishable during yolk plug stage. Yolk invasion was over and the blastopore was almost closed. The notochord was clearly seen at 14hrs after fertilization and at that time 22 somites were seen and lens formation was started and heart formation was almost complete. Blood circulation commenced over the yolk into the rudimentary heart lying anterior to the yolksac and 89-93 heartbeats per minute were noticed at this stage. After 24 hrs of fertilization the caudal region was detached from the yolk mass and became free. Two otoliths were seen in the otic vesicle and 130-140 heartbeats per minute were observed. In the final stage of embryonic development, the growing embryo occupied the entire previtelline space; it exhibited frequent twitching movement by lashing the tail against the egg capsule. After a pause of few seconds, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane and the hatchling emerged with tail first.

Hatching occurred 32-34hrs after spawning and the hatchlings were transparent characterized by the presence of an almost round yolk sac. The hatchlings ranged between 2.7 and 2.9 mm in length and tried to hide in any refuge they find. At this stage of development they have no swim bladder, mouth or vent. They breathe by absorbing oxygen through the fine blood capillaries that surround the yolk sac,

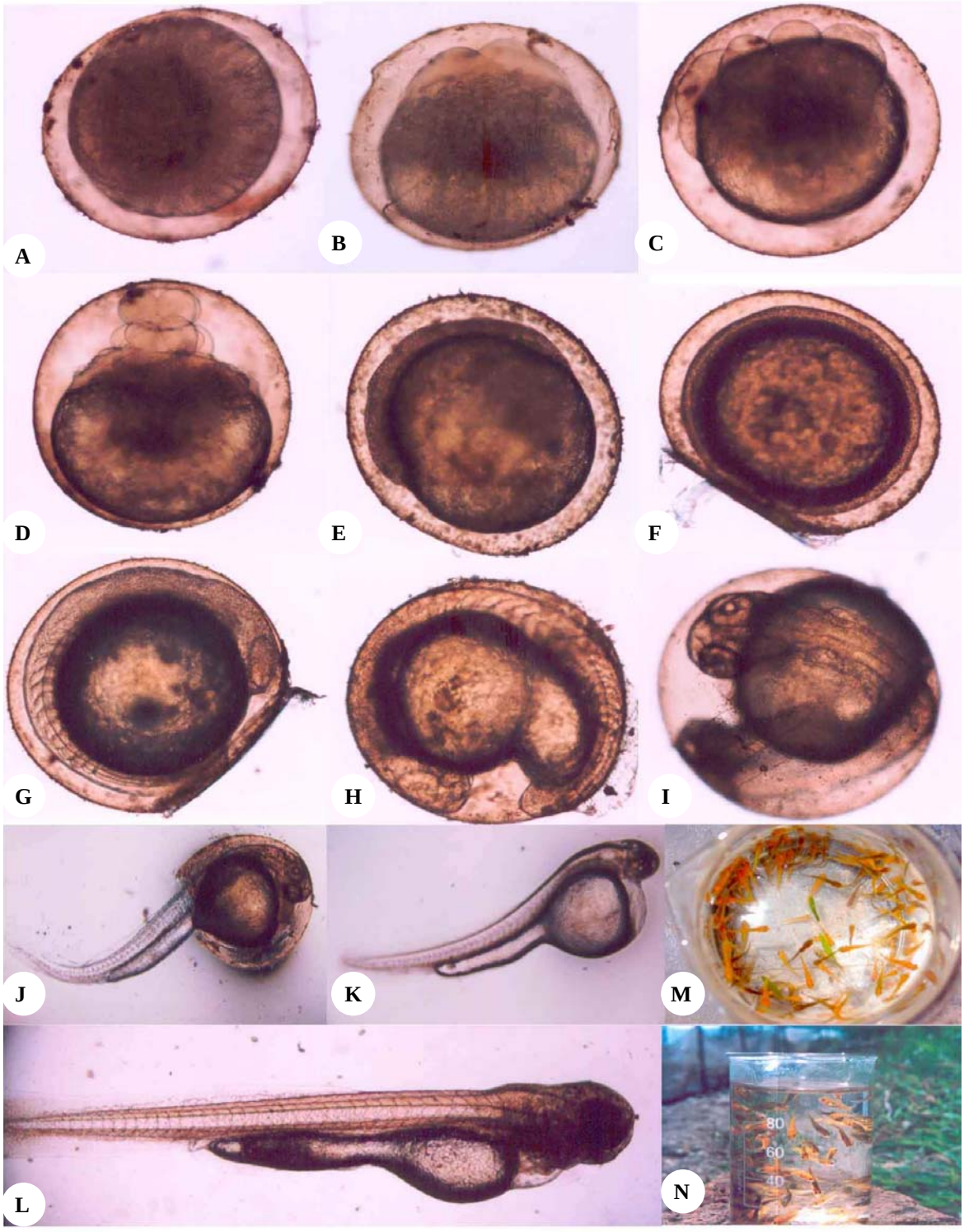

Fig. 2. A: Fertilized egg. B: Formation of two blastomeres. C: Eight cell stage. D: Morula stage. E: Eight hours old embryo. F: Eighteen hours old embryo. G: Twenty hours old embryo. H: Thirty hours old embryo. I: Thirty six hours old embryo. J: Seventy one hours old embryo. K: Just hatched larva. L: One day old larva. M: Fifteen days old fry. N: Thirty five days old fingerlings.

Table 1. Embryonic development of koicarp.

Time after fertilization (hrs).	*Progress in embryonic development*
0.40	Blastodic formation
1.00	2 celled stage
1.30	4 celled stage
1.50	8 celled stage
2.20	16 celled stage, two tires
4.40	Morula
7.30	Germinal ring formed
11.40	Half, yolk invasion completed.
18.00	Yolk invasion completed.
24.00	Cephalic region broadened with distinct fore brain
30.30	14 somites; cephalic region broadened
36.25	16-18 somites; optic lens starts differentiating
42.20	embryonic fin fold formed; gut differentiation started
49.00	22-25 somites, lens formed in the eye; heart formed; blood circulation commenced, embryo showed slight movements; 89-93 heartbeats / minute.
63.15	The embryo encircles the whole of the yolk; vigorous twitching movements seen.
68.20	Lens fully formed, pectoral fin buds seen; 130-140 heart beats / minute; 2 otoliths in otic vesicle.
71.20	Hatching started.
73.30	Hatching completed.

Table 2. Larval Development of Koi carp.

Larval age (days)	Length (mm)	Characters
	Just hatched (Hatchling)	Transparent with heavy yolk
1	2.7-2.9	Transparent; fin fold originates and heavy ovoid yolk sac.
3	5.0-5.4	Yolk sac absorbed; started exogenous feeding; eye pigmented.
6	5.8-6.0	Yellow pigments on the body and a silvery band on the lateral sides
15	6.8-7.0	Fin rays differentiated; pigmentation gets dark
20	12-14	Pigmentation started; various colour combinations noticed.
35	24-32	Fin rays complete; started feeding artificial feed, colouration more conspicuous.

which are still attached to the gut. The head of the hatchling was noticed above the yolk sac and the brain was clearly visible. After 6-8 hrs of hatching the fin folds were seen continuously around the tail. After 2 days the hatchlings showed free movement and after 3 days of hatching the larvae started exogenous feeding and are fed with finely sifted zooplankton (Jameson and Santhanam, 1996) in spite of the presence of little yolk sac as Balon (1985) defined this stage as eleutembryonic.

Autonomous feeding and morphological changes characterized the larval stage. A relatively broad space appeared between the head and anterior margin of the yolk sac. The yolk sac was elongate, oval in thorasic region and became cylindrical in abdomen and occupied about 1/3 of the body from the anterior end. At this stage the outline of the brain in the cranial cavity could clearly be seen under a microscope. When 8hrs old the vent and gill rudiments were formed. Gut was straight to slightly curved in anterior portion. Air bladder was shallow, behind pectoral region, which develop into two chambers in the post larval stage. Pigmentation was more pronounced throughout the head and body. The larvae attained free movement with the help of fins when 8 hrs old. The larval development is summarized in Table 2.

Hubbs (1943) defined post-larva as the stage that began immediately after absorption of the yolk sac that last as long as the structure and form are unlike that of fry. The pectoral fin was differentiated and was in the form of a flap just behind the operculum; at this time sidewise movement of the larvae commenced. Two chambered air bladder was seen at this stage. After 7 days the colour of the post-larvae was lemon yellow and they attained 4.0-4.5 mm length. By this time the yolk was completely absorbed and the larvae began wandering in search of food.

Metamorphosis from post larva to fry stage took place after 15 days. Most of the fry were lemon yellow in colour whereas some of them showed black and orange colouration. At this stage the length of the fry ranged between 7-8 mm and they gradually resembled the adults in external features. The koi fry have only one fin, encircling the posterior end of the body. As they grow they developed paired fins, mouth and other organs. The young koi swim up to the surface and take two or three gulps of air, which they force into their swim bladder.

After 35 days of hatching the fingerling stage was noticed. At this stage the fingerlings ranged between 15-17 mm in length, with 8 branched rays in the dorsal fin and 7-9 in the caudal fin. Fins were well

developed with 17-18 pectoral fin rays, 17-21 pelvic fin rays and 5-6 anal fin rays. They are entirely covered by scales and appeared just like an adult. Mouth is terminal with well-developed jaws and teeth. Changes in the pattern of the entire structure of an organ in relation to the environment are decisive for evaluating the developmental patterns of species (Balon, 1999).

CONCLUSION

The high fecundity, short embryonic period and fast development of the fish suggest that koi are suitable species for commercial culture. The breeding technology discussed in the present paper may be taken as the base material for breeding this wonderful fish.

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錦鯉的繁殖行為及胚胎發育

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摘　　要

錦鯉 (*Cyprinus carpio*) 人工繁殖是將成熟的雌魚以肌肉注射的方式注射一劑 ovaprim (每公斤魚體重注射 0.3 mL)。在 26-28℃的溫度下，注射六小時後即會產卵。受精後的卵呈透明狀具黏性，直徑在 0.9 mm 到 1.10 mm 之間。73 小時後仔魚即會孵化，魚苗體長 2.7-2.9 mm，身體呈透明狀，頭卵圓型，卵囊相當完整，尾巴很短。卵黃在三天內會被吸收，在這期間，仔魚口部發育完全後會開始覓食。20 天後，仔魚可長到 10 mm 到 12 mm，35 天後，稚魚體長 30-35 mm，此時的錦鯉除了尚未性成熟外，樣子已和成魚相似。

關鍵詞：繁殖行為、個體發生、錦鯉。

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