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# First attempt of captive breeding, embryonic and larval development of *Barilius bendelisis* (Hamilton 1807)

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**ABSTRACT**

The study describes, for the first time, captive breeding, embryonic and larval development of *Barilius bendelisis*. The captive breeding trial was carried out in April, in fish farm of Directorate of Coldwater Fisheries Research (DCFR), Bhimtal, India using differnet doses of ovatide. The most appropriate dose of ovatide was reported to be 0.3 ml/kg body weight to males and 0.6 ml/kg body weight to females. Fertilized eggs measuring 1.4-1.7 mm were spherical, demersal, and transparent with dark yellow coloured yolk. Hatching occurred 140 -160 hours after fertilization at 19.09°C ± 0.395**.** Newly hatched larvae were transparent with yellowish yolk reserve, measuring 5.57 - 6.71 mm in total length and characterized by pigmented eye and yolk sac which completely absorbed in 72 hours.

**Key words:** *Barilius bendelisis*, Captive breeding, Egg ontogeny, Embryonic development, Larval development.

## INTRODUCTION

*Barilius bendelisis* commonly known as Hill trout belongs to family cyprinidae is an upland water fish which dwells in shallow, clear and cold water. The fish is popular ornamental fish in hilly areas and is exported from India under the name of 'Indian hill trout' or 'Hamilton's baril' as ornamental fish (100-210 mm) as well as highly known for its food value by Himalayan population achieved a market price of Rs. 160–200 per kg (Sharma *et al.*, 2015; Mir *et al.*, 2015; Jayalal and Ramachandran, 2012). This fish is commonly found in India, Bangladesh, Nepal (Talwar and Jhingran, 1991), Myanmar, Pakistan, Thailand and Srilanka (Eschmeyer and Fricke, 2011). In India, this species is distributed in Brahmaputra and Ganga drainages along the Himalayan foothills. The fish is characterized by the compressed body, pointed head, blue/black vertical bands on the lateral side of body and origin of dorsal fin inserted behind the mid of the body. Adults of this fish are found in rivers, canals, stream and streamlets along the base of hills (Talwar and Jhingran, 1991) with the rocky bottom (Menon, 1999).

As per IUCN Red list (2016) this fish has been categorized as least concern. However, the population of this species has suffered a drastic reduction in the recent past (Sah *et al.*, 2011). For this reason, to meet the demand and to reduce the pressure on natural resource, the breeding has been considered as an important measure for the conservation of this species. Although the reproductive biology and life history of *Barilius bendelisis* has been described by few authors (Grover, 1971; Dobriyal and Singh, 1987) but to the best of our knowledge, no efforts has been conducted on breeding and early developmental stages of this fish. Therefore, due to importance and the lack of knowledge about the captive breeding and early developmental stages, the present study was undertaken to analyze the captive breeding, embryonic and larval development of *Barilius bendelisis.*

## MATERIALS AND METHODS

**Broodstock collection, maintenance, and captive breeding**: Fishes (n=75) were collected (monthly from October 2013 to November 2014) using a cast net from river Gaula, Kumaon region of central Indian Himalayas (29° 17' 25'' N Latitude, 79° 32' 143'' Longitude). After disinfection, all fishes were reared for one year in a cemented pond(10m x 3m x 1.0 m) of the Fish farm DCFR, Bhimtal. The fishes were fed *ab libitum* with formulated pellets comprising rice bran, soyabean meal and fish meal with crude protein 33%, fat 7.1% and carbohydrate 33%. After proper acclimatization, rearing and maintenance, the healthy and mature breeders (Avg. weight 10.58 g ± 0.9) were selected according to sexual dimorphism and transferred to hatchery in fibre reinforced plastic (FRP) tank of size 200cm X 200cm X 30cm with flow through arrangement of water. The water quality parameters of pond water were measured using multiparameter meter (HANNA HI 9828) (Table 1). GSI was calculated month wise for both the sexes using the formula of De Vlaming *et al.* (1982). GSI= (Gonads weight)/(Total weight of fish ) X 100.

The females are usually easier to identify, as belly of a mature female is generally larger, total length of pectoral fin is short, whereas males remains streamlined with

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prominent tubercles' on upper and lower jaw, and total length of pectoral fin is generally larger and goes beyond the origin of pelvic fin. The gravid male and female fishes were administered with different doses of ovatide (a synthetic analog of GnRH and domperidone, Hemmo Pharmaceuticals Pvt. Ltd, Thane-400604) intraperitoneally during evening hours (Table 2) and released in FRP tank in the ratio of 1:2 (Male : Female). Checking of spawning started 10 hour after injection. After no success in first trial (Trial I), successive trial (Trial II, III, IV and V) were conducted with different doses of ovatide (Table 2).

**Checking for ovulation and incubation of egg:** Checking of ovulation started 10-11 hours after hormone administration. Females were tested for ovulation using hand stripping of the abdomen method. Females were considered ovulated by yielding abundant amount of yellowish color eggs. Immediately before stripping of females, males were sacrificed and testes were removed, macerated and squeezed in saline solution (0.9%). Stripped eggs were then immediately fertilized by sperm suspension (0.9% Saline). After few minutes of gentle stirring by feather, the fertilized eggs were washed several times with fresh water to remove excess milt. After that these fertilized eggs were transferred into the FRP troughs and FRP trays for incubation.

**Embryonic and larval development:** The egg hatching and larval rearing up to yolk sac absorption was undertaken up in the same tank that was used for spawning. In the present study, the developmental stages were divided into embryonic and larval development up to yolk sac absorption and were examined randomly under a light microscope with a digital camera, Nikon ECLIPSE E100, (Aral *et al.*, 2011) and measured using ocular micrometer. The embryonic stage was considered when the development occurs inside the egg shell and ends at the hatching. While, the larval phase was recorded as egg hatches and ends when the larvae become capable of exogenous feeding. The water quality of hatchery was measured for temperature, pH, electrical conductivity (EC), total dissolved solids and dissolved oxygen by multiparameter meter (HANNA HI 9828) (Table 3).

## RESULTS AND DISCUSSION

**Gonadal development and captive breeding:** Gonado Somatic Index (GSI) is one of the most important measures in estimation of reproductive period and maturity condition of fish, as, in general, the weight of the gonad increases with maturity (De Vlaming *et al.*, 1982). Several studies have suggested that the seasonal variation in the value of GSI, attributed to corresponding changes in the gonadal development of the species. In the present study, the brooders attained full maturity during May and April with maximum GSI of 13.24 ±0.92 for females and 1.13 ± 0.07 for males, whereas minimum GSI of female was observed in November (4.51 ± 0.60) and for male in July (0.01 ± 0.02) (Fig 1). Similar finding have been reported by Dobriyal and Singh (1987) from the wild stock of Garhwal Himalayas.

To the best of our knowledge, this is first attempt of captive breeding, embryonic and larval development of *Barilius bendelisis*. The most appropriate dose of ovatide was found to be 0.3 ml/kg and 0.6 ml/kg for male and females

**Table 1:** Physico-chemical characteristics of *B. bendelisis* pond water during November 2013 to October 2014.

| Months | Temperature (°C) | pH | Dissolved oxygen (mg $L^{-1}$) | Alkalinity ( mg $L^{-1}$) | Ammonia ( mg $L^{-1}$) | Nitrite ( mg $L^{-1}$) | Nitrate ( mg $L^{-1}$) |
|---|---|---|---|---|---|---|---|
| **November-13** | 17.5±1.2 | 7..8 ±0.29 | 8.9 ±0.43 | 126±.11.5 | 0.018±0.003 | 0.032±0.005 | 0.126±0.01 |
| **December-13** | 10.3±1.6 | 8.1±0.33 | 8.5±0.38 | 123±14.3 | 0.012±0.004 | 0.029±0.003 | 0.127±0.02 |
| **January-14** | 9.9±1.7 | 7.8±0.42 | 8.2±0.45 | 120±14.3 | 0.015±0.004 | 0.032±0.005 | 0.132±0.01 |
| **February-14** | 11.2±1.9 | 7.6±0.32 | 8.5 ±0.41 | 127±12.5 | 0.017±0.003 | 0.036±0.004 | 0.120±0.01 |
| **March-14** | 17.4±1.2 | 8.1±0.38 | 8.5 ±0.38 | 128±6.22 | 0.019±0.004 | 0.034±0.005 | 0.125±0.02 |
| **April-14** | 21.2±1.3 | 7.9±0.40 | 10.9±0.42 | 112±13.43 | 0.026±0.002 | 0.027±0.005 | 0.133±0.01 |
| **May-14** | 25.3±1.56 | 8.1 ±0.38 | 9.6 ±0.41 | 141±15.5 | 0.028±0.002 | 0.031±0.003 | 0.122±0.02 |
| **June-14** | 25.2±2.45 | 8.0 ±0.41 | 10.1 ±0.39 | 131±4.03 | 0.018±0.005 | 0.043±0.004 | 0.141±0.02 |
| **July-14** | 24.9±1.6 | 7.8±0.36 | 8.5 ±0.36 | 126±3.22 | 0.023±0.004 | 0.063±0.005 | 0.139±0.01 |
| **August-14** | 21.4±1.6 | 7.7 ±0.39 | 8.6 ±0.49 | 115±8.04 | 0.027±0.004 | 0.061±0.005 | 0.145±0.01 |
| **September-14** | 20.6±1.8 | 7.7±0.41 | 9.1 ±0.47 | 123±2.5 | 0.025±0.005 | 0.057±0.004 | 0.154±0.02 |
| **October-14** | 20.8±1.2 | 7.8 ±0.32 | 8.5 ±0.45 | 120±1.45 | 0.017±0.003 | 0.033±0.004 | 0.130±0.01 |

**Table 2:** Breeding Trials of *Barilius bendelisis in captivity.*

| Trial | Male : Female | Average length of fish(cm) | Ovatide dose | | Latency period(h) | Method of breeding | Success of trial | Fertilization rate(%) | Hatching percentage (%) | Incubation period (h) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Male (ml/kg) | Female (ml/kg) | | | | | | |
| I | 1:2 | 11.2 | 0.6 | 0.6 | - | - | - | - | - | - |
| II | 1:2 | 10.5 | 0.3 | 0.6 | 16 | Stripping | Success | 55-60 | 20 | 140-160 |
| III | 1:2 | 11.6 | 0.3 | 0.6 | 16-17 | Stripping | Success | 60-65 | 50-60 | 90-140 |
| IV | 1:2 | 11.75 | 0.3 | 0.3 | 18-19 | Stripping | Success | 20-30 | - | - |
| V | 1:2 | 10.56 | 0.3 | 0.1 | 16-17 | Stripping | - | - | - | - |

**Table 3:** Water Quality Parameters during embryonic and larval development of *Barilius bendelisis.*

| Parameter | Embryonic development | Larval development |
|---|---|---|
| Temperature (°C) | 19.09± 039 (15-21.5) | 21.5 ±.58 (17-24.5) |
| pH | 7.6±0.09 (7.0-8.1) | 7.6±0.01 (7.0-8.1) |
| Dissolved oxygen (mg $l^{-1}$) | 8.8±0.01 (8.5-9.0) | 8.27±0.03 (7.9-8.4) |
| Alkalinity (mg $l^{-1}$) | 110±2.0 | 112±1.9 |
| Ammonia –N (mg $l^{-1}$) | Nil | 0.01±0.0 |
| Nitrite- N(mg $l^{-1}$) | Nil | 0.16±0.00 |
| Nitrate- N(mg $l^{-1}$) | Nil | 0.13±0.00 |
| Water flow at inlet(ml/min) | 2000-2300 | 230-250 |
| Water flow at outlet (ml/min) | 2000-2100 | 200-230 |

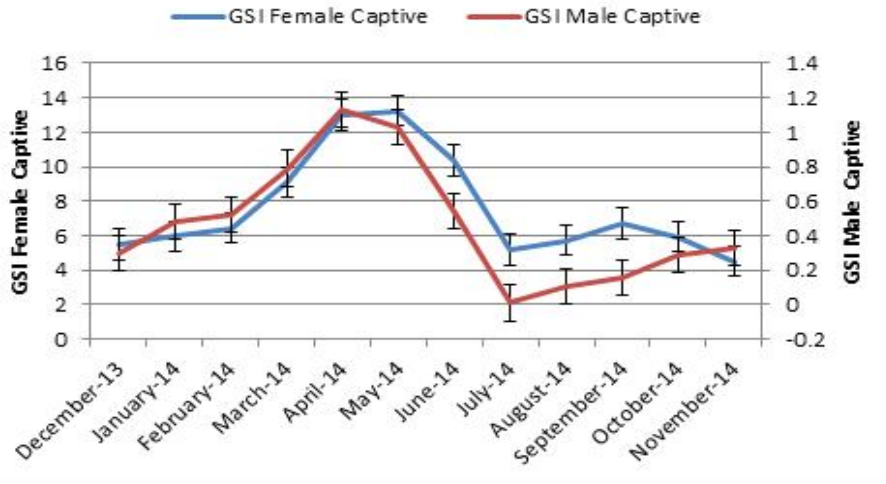


**Fig 1:** Gonado Somatic Index of *Barilius bendelisis*

respectively with moderate fertilization rate (60-65%) and hatching rate (50-60%). Similar results were reported by Najar *et al.* 2012 in snow trout *Schizothorax niger* from Kashmir waters using ovatide. The ovatide dose administered was 0.5 ml/Kg to females and 0.3 ml/Kg to male fishes. The females that given a low dose of ovatide (0.3 ml/kg bodyweight) resulted in lowest fertilization rate. The results suggested that the suboptimal dose of 0.3 ml and 0.1 ml ovatide was not effective for complete ovulation which might be ascribed to the failure of final oocyte maturation of developing oocytes due to insufficient release of gonadotropin (Van der Kraak *et al.*, 1983; Billard *et al.*, 1984). In present study, from a single fish about 60-100 mature eggs were procured that accounted for about 10-13 percent of the ova in an ovary. The fertilization rate of 60-65 % and hatching percentage of 50-60 was achieved, and the larvae hatched out in 140-160 hours at 15–20°C (19.09°C ± 0.395) water temperature.

**Egg morphology, embryonic and larval development:** For the successful egg incubation and larval development egg size and egg quality play a crucial role. Egg size, quality and fecundity of fish rely upon so many extrinsic as well as intrinsic factors like broodstock age, size, maintenance, water and feed quality and geographical location. Besides these, a variety of reproductive strategies of fish can cause a wide range of difference in the fecundity and egg size (Andrade-Talmelli *et al.*, 2001). However, the egg sizes of the particular subfamily are in standard range. In present study, the egg diameter immediately on stripping was 0.9-1.1 mm and after fertilization and water absorption they reached 1.4-1.7mm in diameter that was close to less than 1.5 mm reported by Grover (1971) and for most of the ornamental fish egg (0.8mm) (Watson and chapman, 2002). However, comparing the egg diameter with other members of Cyprinidae, *Barilius* have smaller egg diameter than *Carassius auratus* (Savas *et al.*, 2006) and close to *Barilius canarensis* (Sado and Kimura, 2005), *Puntius conchonius* (Bhattacharya *et al.*, 2005).

Information on early egg and larval ontogeny is of critical importance in order to understand the basic biology, dietary requirement as well as environmental preference of a particular species (Borcato *et al.*,2004). The detailed embryonic and larval development are given in Table 4, Fig 2 and Table 5, Fig 3 respectively. Observation on overall embryonic and larval development stages of *B. bendelisis* in present study are in aggrement with pattern described for other *Barilius* species (Sado and Kimura, 2005). The differnce in incubation period of *B. bendelisis* (140-160 HAF) and *B. canarensis* (45.25 HAF) was observed in present study, as the present study was carried out at comparatively lower

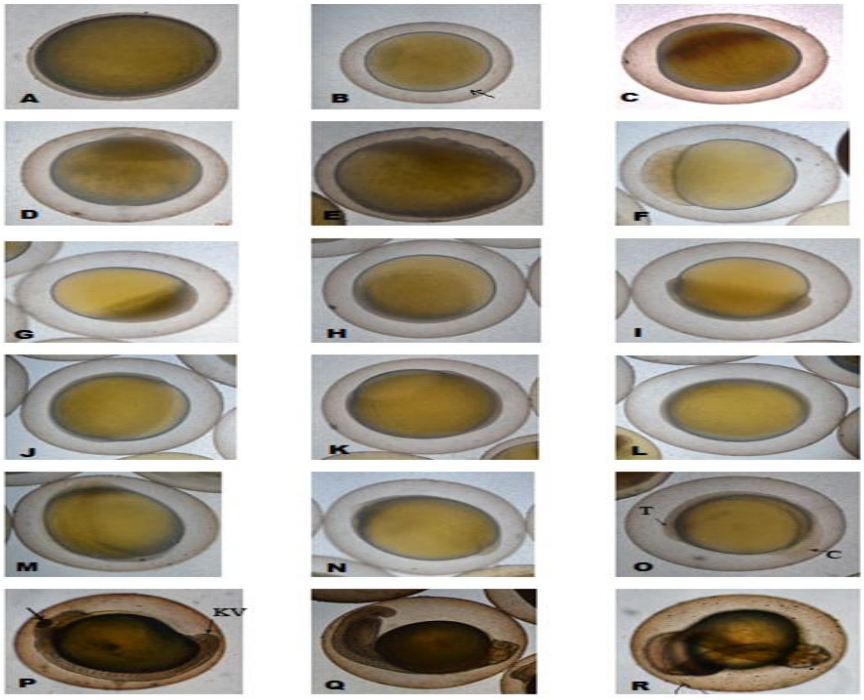


**Fig 2**: Embryonic development of *Barilius bendelisis*. A-F cleavage stage; A, Fertillized egg; B, chorion uplifted egg; C, cytoplasm movement towards animal pole; D, 2 celled stage; E, 8 celled stage; F,32 celled stage; G, Blastula stage, Dome shaped stage; H- L Gastrula stage; H. 30% epiboly; I, 50% epiboly; J-K, 75% epiboly; L,90% epiboly; M-Q segmentation period; M, notochord seen; N , Bud stage; R, Hatching period. Arrow indicate perivetilline space, T,-Tail region, C- Cephalic region, KV, Kupffer vesicle

**Table 4:** Embryonic development of *Barilius bendelisis.*

| Stages | | Hours after Fertilization | Main Event |
|---|---|---|---|
| Zygote period | Fertilization | 0.00 | Water hardening stage, eggs are demersal, spherical with dark yellow coloured yolk |
| Cleavage period | 2-celled stage | 2:30 | First cleavage at animal pole divide the blastodisc into two equal blastomere |
| | 4-celled stage | 3:15 | Second cleavage dividing the blastodisc into 4 blastomere |
| | 8-celled stage | 4:15 | Third cleavage |
| | 16-celled stage | 5:16 | Fourth cleavage |
| | 32 celled stage | 6:33 | Fifth cleavage, 32 equal size blastomere were seen |
| Blastula Period | Oblong transition | 9:01 | Blastula acquires a smoothly outlined ellipsoidal shape. |
| | Oblong to sphere | 11:18 | Border between the blastodisc and the yolk was considerably flattened. |
| | Dome shaped | 13:34 | Blastoderm formed a dome-like shape because of the bulging up of the yolkcell towards the animal pole and the epiboly began. |
| Gastrulation Period | 30% Epiboly | 17:14 | Blastomere cells begins to spread over the yolk and germ ring epilobed 1/3 of the yolk |
| | 50% Epiboly, | 20:30 | germ ring epilobed 1/2 of the yolk |
| | 75% Epiboly | 24:56 | 75% of the yolk is covered by blastomere |
| | 80%Epiboly | 29:03 | 80% of the yolk is covered by blastomere |
| | Bud stage | 31:34 | Epiboly comes to a close as the blastoderm completely covers the yolk plug. Early polster seen. Tail bud appeared. |
| Segemntation period | Anterior and Posterior axis distinguishable | 34:40 | Embryo increased in size, anterior and posterior axis distinguishable as broader cephalic region with distinct forehead and tail |
| | Heart beat stage | 43:12 | optic lens starts differentiating , v shaped myomere and somites were visible, heart beat @ 70-80 beats /minutes |
| | | 49:56 | 22-25 somites, lens formed in eye |
| | | 97:30 | Lens fully formed, pectoral fin bud seen |
| Hatching Period | Hatching started | 120:24 | Few larvae hatched but died soon |
| | Hatching completed | 160 | Larvae emerged from the shell by breaking its side and tail emerges first |


**Fig 3**: Larval development of *Barilius bendelisis.* A, Newly hatched Larvae; B, 12 hours old Larvae; C, 1 day old larvae; D, 2 day old larvae; E, 3 day old larvae. OT-otic vesicle with two otoliths,Y-Yolk, M- V shaped Myomere, P- pectoral fin bud, H-Heart,N- Nostrils, A- rudimentary anus. F-G, Five day old Larvae; H-I, Nine day old Larvae; J-K, 16 day old larvae, L- 30 Day old larvae. A- Air bladder, B- black spot at tail

**Table 5:** Larval development stages of *Barilius bendelisis.*

| Days after Hatching | Main Events |
|---|---|
| Newly hatched larvae | Newly hatched larvae measured 5.6 ± 0.56 (4.75-6.71) mm in total length, Pigemntation were absent except eye, V-shaped myomeres were present. Larvae were devoid of mouth and anus. |
| 1 Day old Larvae | The larvae measured 6.07mm ± .31 (5.60-6.71 mm) in total length. Buccal invagination appeared, alimentary canal started developing. Rudimentary gill opening was differentiated. Heart beat gradually increases to 116-120 beats per minutes..Larvae showed darty movement.. A nostril starts forming. |
| 2 Days old Larvae | Larvae measured 6.5mm ± 0.38 (5.80-7.09 mm) in total length. Yolk sac was almost absorbed and few larvae started exogenous feeding. Eye started horizontal movement. Spleen and liver started developing. Nostrils were prominent. Single row little pigmentation over the cephalic region was noticed. |
| 3 Days old Larvae | Total length of larvae measures 7.3 mm ± 0.16 (7.10-7.67 mm. All the larvae started exogenous feeding and yolk reserved was fully absorbed. Functional mouth was observed. Melanophores had spread all over the body but pigment mostly concentrated on head and dorso-caudal side of body. The digestive system is well developed and urogenital orifice become functional. Air bladder becomes visible. |
| 5 Days old Larvae | Larvae measured 8.22 mm ± 1.06 (7.15-9.27 mm) in total length. Notochord flexion started. Although, black pigments were dominating but yellow pigments were also visible. Dorsal fin fold notched anterior to anus. Larvae found to be in shoal. |
| 9 Days old Larvae | Total length of larvae measure 10.11 mm ± .52 (9.2-11.02 mm).Dorsal and anal fins were clearly demarcated and started separating from the caudal fin and gill structures were clearly visible from outside. |
| 15 Days old Larvae | Larvae of total length measured 12.0 mm ± 0.5 (11.09-13.0 mm) At the base of caudal fin black spot was noticed. Air bladder with two chambers were observed. Dorsal and anal fins begins to develop but were devoid of fin ray. |
| 21 Days old Larvae | The larvae ranged from 12.7 mm ± 1.19 (11.0-15.0 mm) in total length were observed. Muscular base beneath the pectoral fin was noticed. |
| 30 Days old Larvae | Total length of larvae ranged from 14.1 mm ± 1.01 (12.5-16.0 mm). Scales started their appearance in the pectoral region. |
| 45 Days old Larvae | Total length of larvae measured from 18.6 mm ± 0.88 (17.0-20.0 mm) |
| 60 Days old Larvae | Larvae measured 20.66 mm ± 2.90 (16.0-26.0 mm) in total length. It completely resembles adult |

temperature (15-24°C) then *B. canarensis* (26.8°–27.4°C) (Sado and Kimura, 2005).

*Barilius bendelisis* is an altricial species as it hatched with undifferentiated developmental stages. The size of newly hatched larvae showed a wide variation among the cyprinid. They are larger than the several other cyprinids as in *Barilius canarensis* it is 5 mm (Sado and Kimura, 2005). In contrast they are smaller to larvae of *Tor ambroides* 7.44 mm (Azuadi *et al.,* 2013) It depends upon various factors like species variation, temperature variations, egg size and morphology, fertilization rate and incubation period and procedures (Jonsson and Svavarsson, 2000).

Results indicated that *B. bendelisis* mature well under the captive condition, and fish have been reported to breed for the first time in captivity using ovatide. Over findings may provide the basis for further studies to develop better larval culture methodologies for higher success rate in the larval culture.

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