

# Colour widow tetra: A new and highly preferred aquarium fish in West Bengal

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*Different varieties of colour widow tetra.*

Known as 'glow widow tetra', 'glow skirt tetra', 'colour widow tetra' in the ornamental fish industry, a genetically modified variety of the black widow tetra *Gymnocorymbus ternetzi* is the most favoured by aquarium fish hobbyists in West Bengal. Stunning and sparkling red, blue, yellow, green, pink, orange, and purple bodied colour variations of this tetra have been developed through gene transfer or transgenic technology. The fish glow when kept under blue LED lights, hence the name 'glowfish'.

Maintenance of these brightly coloured and reasonably priced fish in small and medium-sized aquariums at home, the office, hotels, and other settings has become popular. A peaceful, schooling fish, the glow widow tetra is attractive and adds colour to the aquarium. Many hobbyists keep at least four different colour varieties together. From around 15 mm in length, colour widow tetra takes 30-45 days to reach 50-60 mm. In a large-sized aquarium, it can be kept with other small community-friendly fishes such as zebrafish and barbs but is

not compatible with larger cichlids or figger fish. Other tetras such as neons and emperors, and dwarf gourami are suitable tank mates.

Colour widow tetra are essentially the same as the unmodified black widow in terms of behaviour, feeding habit, body shape, and size; the only difference is the colour, the natural type being dark bodied with black stripes. Black widow tetras are native to the rivers of Brazil, Bolivia and Paraguay in South America.

## The science behind production of transgenic colour widow tetra

Aquaculture biotechnologists have created transgenic tetras of different colours by inserting genes that code for fluorescent proteins into normal black widow tetras. The genes were sourced from a variety of marine invertebrates:

- In February 2012, Yorktown Technologies based at Texas, USA brought a green coloured black widow tetra to market for the first time incorporating a fluorescent green protein gene from the jellyfish *Aequorea victoria* found off the west coast of North America. To produce this transgenic and stable green fish, jellyfish green fluorescent protein gene was ligated to two promoter sequences used, namely *krt8* and *mylz2*.
- Yellow, pink, blue and purple widow tetras contain fluorescent protein genes isolated from corals. Additional red and green fluorescent protein genes have also been isolated from corals.
- The red widow tetra contains a fluorescent protein encoding gene isolated from an Indo-Pacific sea anemone *Discosoma* sp.<sup>2</sup>
- Orange and yellow-orange widow tetra contain a modified jellyfish fluorescent protein gene, i.e., a variant of jellyfish gene giving rise to a different colour.
- In 2020 Yorktown Technologies made fluorescent widow tetras available in six colour varieties. The colours stay with fish all throughout their life, they are born with it and the genes are passed onto next generation.

In gene transfer research conducted with black *G. ternetzi*, foreign genes of economic importance were introduced at early stages of embryonic development, integrating them into the genome. Subsequently, expression of the target genes occurred (facilitated by appropriate promoter/regulatory gene sequences made to combine with coding/structural gene of choice, i.e., fluorescent colour-encoding genes), which are passed on to offspring as a hereditary trait.

In a study at the National University of Singapore during 2007-2008, a muscle-specific promoter sequence of zebrafish *mylz2* was used to create red widow tetra. The gene construct *rfp* (red fluorescent protein gene, a reporter gene) - *mylz2* was prepared, and DNA solution microinjected into the eggs of black *G. ternetzi* within 50 minutes of fertilisation at the 1-8 cell stage. Finally, the expression of red fluorescent protein gene occurred, bright red colour was prominently observed in adult fish<sup>1</sup>.



Hapa nets containing different aquarium fishes including colour widow tetra.



Different body colours of colour widow tetra in a farm.

## Breeding and seed production process

Around twelve private aquarium fish breeders-cum-farmers in Howrah District are doing controlled breeding, seed production and farming of coloured widow tetras. Water sources used include rainwater, filtered tap water, or deep tube wells. Fish are bred in small, rectangular cemented tanks with a 15 cm depth. Large green nylon spawning mops are placed on the tank bottom at one corner to imitate small aquatic plants occurring in nature, which assists in inducing spawning in confinement. The fertilised eggs are demersal and non-sticky.

Males and females are kept separately for ten days or more prior to scheduled date of breeding and their release into breeding tank causes a quick release of eggs. In a farm, females of any 3-4 colours out of all are often gravid and ready to spawn. Tank water temperature must be adjusted. Females are released first into the spawning tank and allowed to acclimatise to the new environment. Around 30 minutes later males are released into the tank, maintaining a 1:2 ratio of females to males.

Females lay eggs in the early morning onwards of the next day. The spawning process continues for 4-5 hours. All the females lay eggs at once, producing good numbers of fertilised eggs with a high fertilisation rate. Females lay eggs only in presence of males. Brood fish are carefully removed, and aeration maintained. The fertilised eggs, around poppy seed in size, lie underneath the mop on the tank floor. Fertilised eggs have a faint golden colour and are transparent. A few



Male brooders of red widow tetra in large glass tank.



Five body colours of adult colour widow tetra.

drops of anti-fungal medicine are added to keep eggs safe and free from fungal contamination. The upper portion of tank is covered with a nylon mosquito net to prevent entry of mosquitoes. If the tank is kept outdoors, it must be protected from rainwater.

Coloured widow tetra larvae hatch within 48-72 hours. Proper-sized brooders must be at least seven months in age and can be bought from breeders in West Bengal at INR 45/ piece. Adult/marketable sized fish are one and a half to two months in age, and highly preferred by aquarium fish keepers and hobbyists. In concrete rearing tanks, an aquarium fish farmer-cum-breeder may have 450-500 pieces in stock, which are 25-26 days old. During breeding, 3-4 gravid females may be paired with 7-8 males in medium-sized 20-25 cm diameter plastic circular trays. Females are fat with a rounded body, greater in size than males, with round anal fins and a prominent notch in the shoulder region. Males are shorter, having a lengthier slim body and a pointed anal fin, and shoulder notch isn't distinguishable. The body colours of females fade slightly

as they attain gravid condition, while the colours of brooder males remain bright. Both are 36 mm and above in length at 5-6 months and above in age.

It is better to keep males and females segregated for about a month before arranging breeding. Live tubifex worms are fed for 2-3 days in a week, and dry pelleted feed every morning. Mosquito larvae or pupae are fed for another 2-3 days in week; bloodworms may also be used alternatively. Zooplankton *Daphnia* sp. is fed to brooders one the remaining 2 days. All live food is provided during evening hours. This schedule continues for 30 days. Farmers-cum-breeders have around 70% hatching success, out of which, 50-55% larvae survive and reach adult stage.

Breeding tank water should be iron- and chlorine-free. Rock salt is added @ 5 g/25 litres water and allowed to settle, with the upper portion of water used for breeding after decanting it. Water is left in the breeding tank undisturbed for three days with the addition of a commercially available anti-chlorine treatment. Thereafter methylene blue may be



*Green widow tetra broodstock.*

added. Submerged weed *Hydrilla* sp. can be kept in the tank. Coloured widow tetra is bred alternatively in 18-20 litre plastic buckets. A heater used in these tanks during winter months and dosage of methylene blue increased. Each female lays 200-250 eggs in a single spawning. Any colour combination of male and female fishes (in appropriate breeding condition) may be selected and used. From the seventh day onwards, hatchlings are fed boiled egg yolk suspension as a dense liquid in very small droppings, using a 50 ml syringe. There is no need to feed in the first 2-3 days after birth.

In South 24 Parganas, two or three farmers are breeding coloured widow tetras. Grown adults are sold to owners of aquarium shops @ INR 20-22/piece. Blue fish are the most preferred colour variety, sold to shop owners @ INR 25/ piece, and retailed at @ INR 30/piece. In large aquarium tanks of 120-180 cm in length the fish grow to 48-60 mm but do not grow conspicuously in smaller aquariums of 60 cm. In mid-2020, for the first time, coloured widow tetra were imported to Kolkata city from Thailand and Singapore in oxygen-packed condition. These fish were sold @ INR 150-200/piece in the beginning. Later, the wholesale price fell to INR 80-82/piece and with retail to hobbyists @ INR 90-100/ piece. From March-April 2021 onwards, seed production and farming have been conducted locally in Howrah and South 24 Parganas districts in private ornamental fish farms.

A farmer in South 24 Parganas District explained that this fish can be bred three times during April (onset of summer) to September every year. A total of 50 males and females were introduced into a glass tank of 1.8 m x 0.9 m, whereas 15 of each sex were placed in multiple 1.2 m x 0.45 m breeding tanks. He observed fertilised eggs within 18-24 hours of release of brooders. Once fertilised eggs are produced the speed of two diffuser aerators is reduced. Whether in large



*Square cement tanks for colour widow tetra and other aquarium fishes.*

glass tanks or rectangular cement cisterns, male and female broodstock or near-brooder stage are not kept in same place to avoid unwanted and untimely spawning. Presently some aquarium fish shop owners in Kolkata buy colour widow tetra @ INR 15-16/piece (24-36 mm), maintain them for a brief period with proper water quality and feeding management and sell onwards @ INR 25/piece. Sometimes shopkeepers in Kolkata buy fishes of 12-15 mm in length from Eastern India's renowned Galiff Street aquarium fish wholesale market @ INR 4-6/piece, maintain in their set-up and sell grown-up fishes @ INR 30/piece.

## Rate of sale of colour widow tetra

In some well-established ornamental fish farms located in Howrah Sadar Sub-Division and some under Howrah Municipal Corporation of Howrah District near to Kolkata, colour widow tetra is now bred. Seed is produced on a medium- to large scale, with good quality and shining colours, and sold to buyers in and outside West Bengal, mostly aquarium shop owners. Breeding and seed production continues from March till early-November every year. From a farm, 15–18-day-old seeds are sold at varying prices depending on the colour:

- Purple: INR 3/piece
- Yellow: INR 1.50-2.00/piece
- Red: INR 3.00-3.50.

There is demand for younger 10-day-old seeds also, with price lessened by INR 0.50-0.75/piece. The 15-18 days-old seed have to be fed *Daphnia* for the next 25-30 days. On the 45th day, aquarium shop owners can sell fishes to hobbyists. In aquarium conditions fishes which are 20-24 mm in size may be fed *Daphnia* for the first month; thereafter small amounts of tubifex worms and a little *Daphnia*. Subsequently, dry fine pelleted feed or flake feed should be used for the fishes. Red widow tetra, 24-25 mm in length and 35-40 days old, are sold @ INR 7/piece. Three-week-old small-sized seed of the same fish are sold @ INR 5/piece.

At another farm, the owner maintains semi-adult fishes in small ponds (160-200 m<sup>2</sup>) with concrete sides and bottom. After harvesting, those kept in rectangular cement tanks for another ten days are sold to general customers (after 'setting' and 'seasoning', in words of farm owner). Fishes harvested from such larger open areas are not directly packed and transported, otherwise it will cause mortality. Here, the retail price of red widow medium size is INR 15/piece. Four month old red widow broodstock are also maintained. Medium to large colour widow of 28-30 days old are sold @ INR 15-16/piece in winter months and INR 8-9/piece in summer. Normal black widow tetra sold @ INR 4/piece.

From a third farm, small-sized red widow (15-20 mm) are sold at a wholesale rate of INR 6-7/piece. From fourth farm, breeder-size (maximum size) red widow tetra sold @ INR 100/piece, purple widow INR 60/piece, yellow @ INR 50, green @ INR 60-80. Smaller-sized green widow sold @ INR 8/piece (two colours of green available, rare green and common green); smaller-sized yellow @ INR 10 and purple @ INR 15/piece, red @ INR 28/piece.



Some red widow tetra maintained in cement tank.

From a fifth farm, five colours available for brooder size widow tetra, blue is rare. Fry are available for sale @ INR 2/piece for green, INR 2.50 for yellow. Fry of other colour fishes all have separate rates. Here, medium-sized colour widow tetra are made available for sale in October and thereafter. This farm owner does not breed colour widow tetra any more as great numbers of sub-adult fishes are now available in market. He sells brooder size fish @ INR 25-30/piece (48-60 mm; red, green, yellow, purple, blue) and smaller ones (24 mm) for INR 7-10/piece.

Brooder widow maintained in a sixth farm are not sold; the smallest sized sellable fry sold @ INR 1.00-1.50/piece (depending on size). In a seventh farm, light green/yellowish green widow sold @ INR 10-12/piece (wholesale rate), standard size. At an eighth farm, 24 mm-sized colour widow tetra sold @ retail price INR 5.50/piece (purple, green, yellow, red). At a ninth farm, brooders are maintained belonging to same four colours; 25-day-old fry sold @ INR 1/piece. At a tenth farm, 10-12 mm-sized seeds sold @ INR 1/piece; INR 10/piece is wholesale price for 'medium-large' size (36-48 mm) fishes. From a wholesaler and exporter shop at CTI (Central Training Institute), Dasnagar in the same district, fishes sold at wholesale rate; standard size @ INR 6.50/piece and large/breeding size @ INR 25-30/piece. Mainly, five colours are available in stock, namely red, yellow, purple, green, and blue. Blue is only seldom available, INR 15/piece at standard size, with the same sized purple widow reaching INR 12/piece.

## Live food culture for colour widow tetra

### Tubifex worms

In nature, tubifex occurs in places with heavy organic content. Aquarium fish breeders-cum-growers can culture tubifex, which involves the following steps: Spread mud enriched with sand (50%), silt, clay and organic manure spread over the floor of a small rectangular concrete tank; this is base media. Maintain a low water level in the tank and inoculate with tubifex @ 50 g/m<sup>2</sup>. After 2 hours, feeds such as dairy sludge, rice mill sludge, or manure can be given @ 40 g for every 10 g of tubifex inoculated on a daily basis. Maintain a continuous water flow @ 1.2l/minute over culture media. After



Colour widow tetra in a large hand net on farm.

10-14 days, tubifex can be harvested by draining. Part by part, the entire content of base media can be screened over a fine-meshed nylon net or white cloth and washed with water. The tubifex biomass remains within the net/cloth and can be collected and put into freshwater (Courtesy: Kalyani Regional Centre of ICAR-CIFA, West Bengal).

### **Daphnia**

Culture of the zooplankton *Daphnia* sp. can be done as follows: In small rectangular or circular cement tank, 15 g cow dung, 10 g mustard oil cake and 1 g bakers yeast are added to every 50 litres of water and exposed to sunlight. After a period the water will turn green as a phytoplankton bloom develops, which is food for *Daphnia*. Add a small number (25-80) *Daphnia* for every litre of water, and add the mixture to a tank at intervals of around a week. After three weeks a good population of *Daphnia* will grow, which can be harvested with a fine mesh net and fed to fish in the rearing tanks. Baby *Daphnia* can grow to maturity and breed in around eight days.

### **End note**

In comparison to 2021, hobbyists in West Bengal have had to pay a lower price to buy this GMO fish because of high availability in the market. The sale price of adult colour widow tetra at some wholesale shops in Kolkata is now INR 4.50-5.00/ piece. Coloured widow tetras are now a preferred variety with higher popularity than traditional molly, goldfish, platy, guppy, and swordtail. Aquarium fish growers opined that it has led the market down for other familiar exotic aquarium fishes. If a hobbyist wishes to buy one or two dozen colour widow tetra

from a large aquarium shop, he will get it at retail price. But if bought in quantity of 50 or more, the customer will get it at a reduced wholesale rate. One can get the fish at a wholesale rate if placing an order for 500-2,000 of the same from fish farms-cum-breeding units and in quantity of 2,000 or more from aquarium fish businesses at wholesale delivery shops at CTI Dasnagar. Many self-employed youths and elderly persons in the Howrah Sadar Sub-Division and Howrah Municipal Corporation areas do breeding, seed production and propagation of different exotic freshwater aquarium fishes on 80-320 m<sup>2</sup> of land at home as a profession on a commercial scale.

One of the reasons for genetic manipulation of species used in aquaculture is to produce new expensive variants of ornamental species with novel shapes and/or colours<sup>2</sup>. It must be remembered that since multi-coloured widow tetra and fluorescent 'glofish' zebrafish are genetically modified, like other exotic species, they must never be released by hobbyists into ponds or natural water bodies.

### **References**

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