PROJECT REPORT

ON

DROSOPHILA CULTURE METHOD AND ITS LIFE CYCLE

B.Sc 6TH SEMESTER ZOOLOGY (HONS)

DEPARTMENT OF **ZOOLOGY**

DUDHNOI COLLEGE, DUDHNOI

Examined

SUBMITTED BY

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ROLL NO: - US-201-097-0017

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CERTIFICATE

THIS IS TO CERTIFY THAT "CHANDAN NATH" STUDENT OF B.Sc 6TH SEMESTER ZOOLOGY (HONS) DUDHNOI COLLEGE HAS SUCCESSFULLY COMPLETED HIS PROJECT WORK ON "CULTURE METHOD OF DROSOPHILA AND ITS LIFE CYCLE" UNDER THE GUIDANCE OF "DR. TAPAN CH. KALITA" (ASSOCIATED PROF.)

DR. TAPAN CH. KALITA
(ASSOCIATE PROF)

ACKNOWLEDGEMENT

I WOULD LIKE TO EXPRESS MY SPECIAL THANKS OF GRATITUDE TO MY TEACHER DR. TAPAN CH. KALITA WHO GAVE ME THE GOLDEN OPPORTUNITY TO DO THIS WONDERFUL PROJECT. THIS PROJECT ALSO HELPED ME IN DOING A LOT OF RESEARCH AND I CAME TO KNOW ABOUT SO MANY NEW THINGS.

I AM EXTREMELY GRATEFUL TO MY FRIENDS WHO GAVE ME VALUABLE SUGGESTIONS AND GUIDANCE FOR MY PROJECT'S COMPLETION. THIS COOPERATION AND HEALTHY CRITICISM CAME IN HANDY AND USEFUL WITH THEM.

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NAME – CHANDAN NATH

SIXTH SEMESTER ZOOLOGY (HONS)

ROLL NO - US-201-097-0017

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INTRODUCTION

Drosophila is a genus of small flies belonging to the family Drosophilidae, whose members are often called 'fruit flies'. Fruit flies are one of the first extensively used model organisms in labo. Morever, it is one of the first model organisms which was brought 'from nature to labs' to understand the genetics of the organisms.

N.M. Stevens was the first scientist who chose Drosophila as a model for his studies in 1906, but it gained more popularity after Morgan's discovery in 1910. Thomas Hunt Morgan's on Drosophila melanogaster led to the discovery of the Sex-linked inheritance and linkage. His theories violated the rules of Mendelian inheritance. Followed by his study A.H. Sturtevant constructed the first genetic map using Drosophila.

Today Drosophila is used in various labs to understand the basic cellular and developmental mechanisms of living organisms that mainly coinside with the humen system. A few examples of studies that include Drosophila are: mutational studies, developmental studies, regenerative biology and drug discovery.

Drosophila melanogaster as a model organism :

The fruitfly Drosophila melanogaster has been widely used as a model organism in biological research, particularly in genetic and developmental studies, since the early 20th century.

Drosophila has a number of qualities that make it desirable for scientific studies, some of its features include –

i) Small size (Adults 3mm and eggs 0.5 mm in length)
ii) Easy to handel.
iii) Sexual demarphism (different males and females).
iv) One female can lay about 100 eggs.
v) Short generation time (9-10 days)
vi) 4 pairs of chromosomes and the whole genome is sequenced.
vii) Low culture and maintenance cost.
(requires maize food, cultured in small bottles and re lener lab space).
viii) They can be easily anesthetized and manipulated individually with unsophisticated equipment.
ix) Virgin fruit flies are physically distinctive from mature adults, making it easy to obtain virgin males and females for genetic crosses.

Culturing Drosophila melanogaster:

Drosophila thrive on fermenting soft fruits. A very suitable culture medium, therefore is crushed banana. It provides all the necessary nutrients for both the larval and adult stages. The banana can be kept along with the flies in sterile pint jars with cotton or foam rubber plugs.

Another standard medium, commonly used by laboratories that raize Drosophila, is a cornmeal molasses - agar mixture. While the batch brews, it flies the scientific hallways with the small of Grandpa's favourite cookies.

Tools for culturing Drosophila:

Nowadays, Laboratories use bottles and vials to culture Drosophila. Bottles are used to maintain a large population and culturing vials are used to maintain a small population and make crosses. Generally, glass bottles are preferred but autocleaved plastic bottles can also worked well. Moreover, the size of small vials ranges from 96 mm by 25 mm to large vials. To cover the bottles, plugs are used which can be cotton or foam plugs. However, cotton plugs are mostly preferred.

Methods of collection :

There are several methods in practice to collect fruit from their natural habitat some of the methods are listed below —

1. Installing Trap Bait : For setting-up traps one could use plastic bottles ranging in the volumes of 250 ml to 1L. Fruit slices along with a pinch of yeast could be used as a bait. With the use of blade somewhere in the middle of the bottle a section could be carved out for food access. These traps could be hanged on archard tree at a height 3-4 feet above the ground. The collection area must be damped and moist with minimal human interference. After 1-2 days, trapr must be recovered to collect flies.

. Net swipe : There are many genera that are not attracted to regular traps and need to be captured from their natural food source (wild rotting fruits). Capturing such Drosophila species with the help of nets one more effective. The flies having over the rotten fruits or pils of organic wastes could be captured using this method.

3. Use of aspirator: This method is used when fly's numbers are low and also when investigator is familiar with the species identification and targeting particular species individuals. This method is more appropriate when flies are feeding, mating or resting on petals, Leaves, fruits etc. Flies feeding on mushrooms and flowers are mostly collected using this method. Aspirator is also used to transfer flies from bait bottles or insect net to culture vials.

Some of the common species specific baits are —

* Banana: It is the most commonly used bait. It could be used to collect cosmopolitan Drosophila melanogaster and other commonly found drosophilid species.

* Tomato: Adding yeast to it forms all attractive bait for Drosophila suzukii and Drosophila buskii.

* Orange: It is also an attractive bait for Drosophila suzukii and Drosophila buskii.

* Grapes: With added yeast, grapes act as an attractive bait for species like Drosophila buskii and Drosophila immigrians.

Culture handling and maintenance :

The culture handling and maintenance is required to be learned for making the collected sample to multiply several times and for making isolines and confirming the species level identification.

1. Rearing and culturing Drosophila :

For rearing and culturing the drosophilids, the collected samples need to be transferred from bait bottles to the narrow culture vials. While shifting flies from the bottles to collection tubes, the opening at the bottle must kept under bright light directing the flies towards the light and thus making their transfer easy. For culturing the flies are kept at room temperature (25°c), If room temperature is not adequate, the culture tubes could be kept in the Biological oxygen Demand (BOD) incubator. This would help the culture media to last longer until the drosophilid colony begins to establish.

2. preparing Drosophila media :

The colony used media for various drosophilid stock maintenance include Agar, yeast, Maize flour, Brown sugar, Nepagin and propionic acid.

To prepare fly media, Agar is added to the hot water. Following this, yeast, maize flour and sugar is added. After 20-30 minute of cooking, heat should be turned off. Once media temperature reaches to close to 50°c, nepagin and propionic acid could be added. Throughout the preparation, food needs to be constantly stirred.

Maize powder, brown sugar and dried yeast are used as food. yeast holds special nutritional value for drosophilids. while Nepagin is antifungal in nature and propionic acid is a bactericidal and functions as preservative and increases the shelf life of food.

The food could then immediately be transferred to the stericilized vials or bottles. As soon as the media starts hardening the vials or bottles needs to be covered properly with the help of a cheese cloth. The food could be used after a day. The media tubes or bottles could also be stored in a cool place for 1-2 weeks for future use.-

Names of some of the other food recipies used for drosophila culture are -

i) Cornmeal, Suerose, dextrose, yeast and 2 acid medium.

ii) Cornmeal, molasses and yeast medium.

iii) Cornmeal, dextrose and yeast medium.

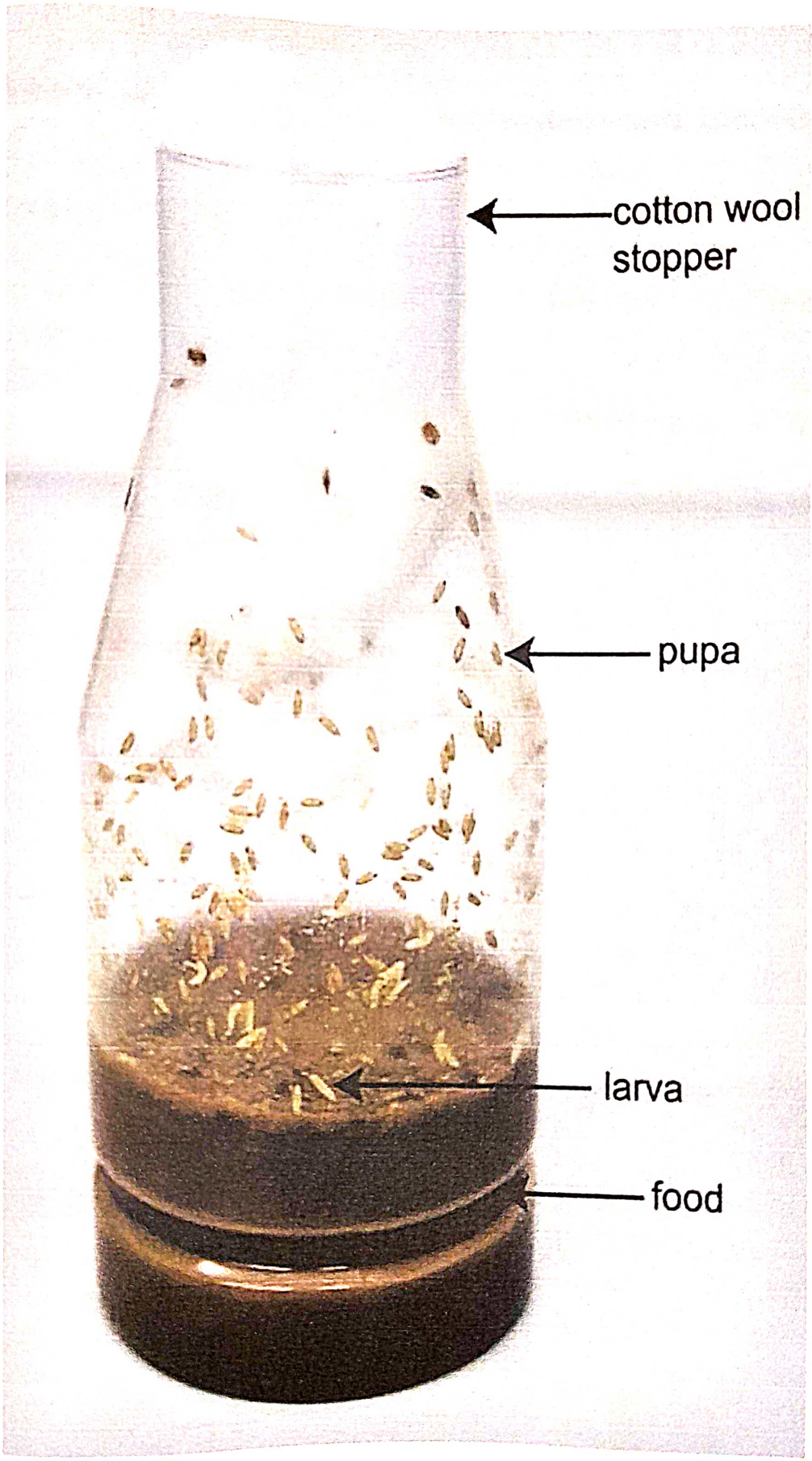


Fig: Drosophila media

General morphological characteristics of Drosophila :

Drosophila are usually small flies, ranging from 1.5 to 7.0 mm in length. Yellowish, golden, brownish or blackish in colour. They possess a number of characteristics, such as red eyes and plumpose. Body is shiny, utter with stripes or stops on the thorax. wings are hyaline or with black patches or marginal areas with dark lines. Abdominal tergite strip patterns (ie. pigmentation) vary species to species. In some species, sexual dimorphism is clear by using patch or pressure of sex combs on legs.

Generally females possess larger body size and have swollen abdomen - than females. In some cases of males, 5th and 6th tergites are pigmented whereas others have wing patches. In a few genera of drosophilid males also possess sex comb on their forelegs. The male genital organs are species specific and differ and species to species, Example, the two sub families viz - drosophilanae and steganinae are differentiated on the basis of the distance between proclimate orbital setae and vestical setae from posterior reclinate setae.

cycle of Drosophila :

The lifecycle of drosophila, from egg fertilization adult life, takes about to days at 25°c. osophila is a model organism particularly used developmental biology because it is a homometa- ous insect, with major morphological differences uring between larvae and adult animal etamorphosis). The drosophila lifecycle is comprised the following developmental 2 stages.

Embryogenesis : It is a ~~fast~~ fast process completed 24 hours after fertilization of the oocyte by the ale sperm. From a one called embryo, a syneytial mbryo is rapidly developed. In the early embryo yncytium, rapid DNA replication and nuclear division ccur, generating upto 5000 nuclei per embryo. Cellularization ccurs after migration of nuclei to the periphery of the yncytium, generating the syncytial blastoderm in a rocess called cleavage. During gastrulation, cells change shape and migrate establishing the mesoderm, endoderm nd ectoderm, which are the layers of the future body plan.

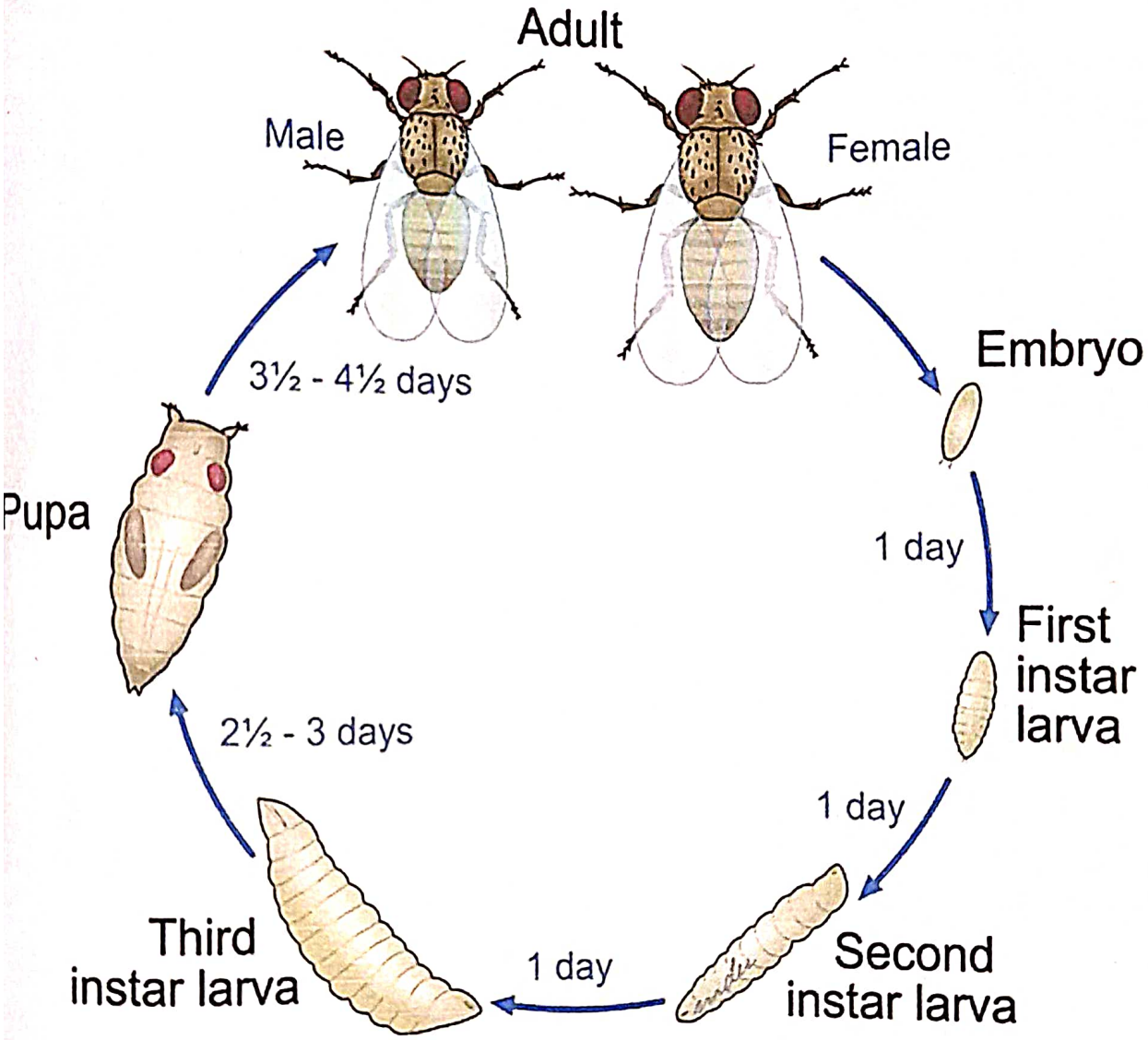
Larval stage : There are three larval stages (3 instares), which take altogether about 4 days. During larval growth, most cell types are already differentiated and functional.

Therefore, many biological questions can be addressed eady at the larval stage. For instance, larvae ve been important for neuronal studies, including emory formation. The larval central nerval system nsists on only 10000 nervous compared to 250000 in adult fly, providing a simpler model. A molting nsition occurs between the different larval stages.

pupal stage: After encapsulation of the 3rd star larva, pupal stage starts and lasts around days. Many larval structures are lysed and new tructures are formed. New structures are generated om the formation at the imaginal disks, developed om larval undifferentiated cells. Imaginal disks will ive rise to the adult head, legs, wings, thorax nd reproductive apparatus. Some larval structures ike the nervous system or gonads are preserved luring the pupal stage.

Adult life: Adult fly emerges upon eclosion f the pupal case. Lifespan is around 30 days, lthough this is variable according to temperature. he high number of eggs layed pre-female (100 eggs per day), generating a wig progeny after genetic crosses, makes drosophila a good genetic model with very easy growth in laboratory conditions.

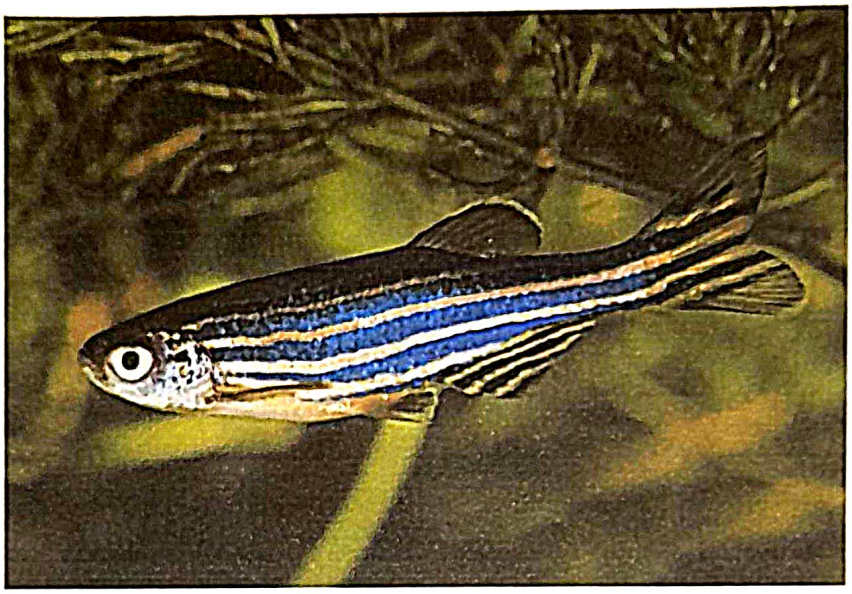
LIFE CYCLE OF DROSOPHILA



Project Report on Zoology (Honours)

Paper: Fish and Fisheries (ZOO-HE- 6026)

B.sc 6th semester (CBCS)



Topic: Zebra fish

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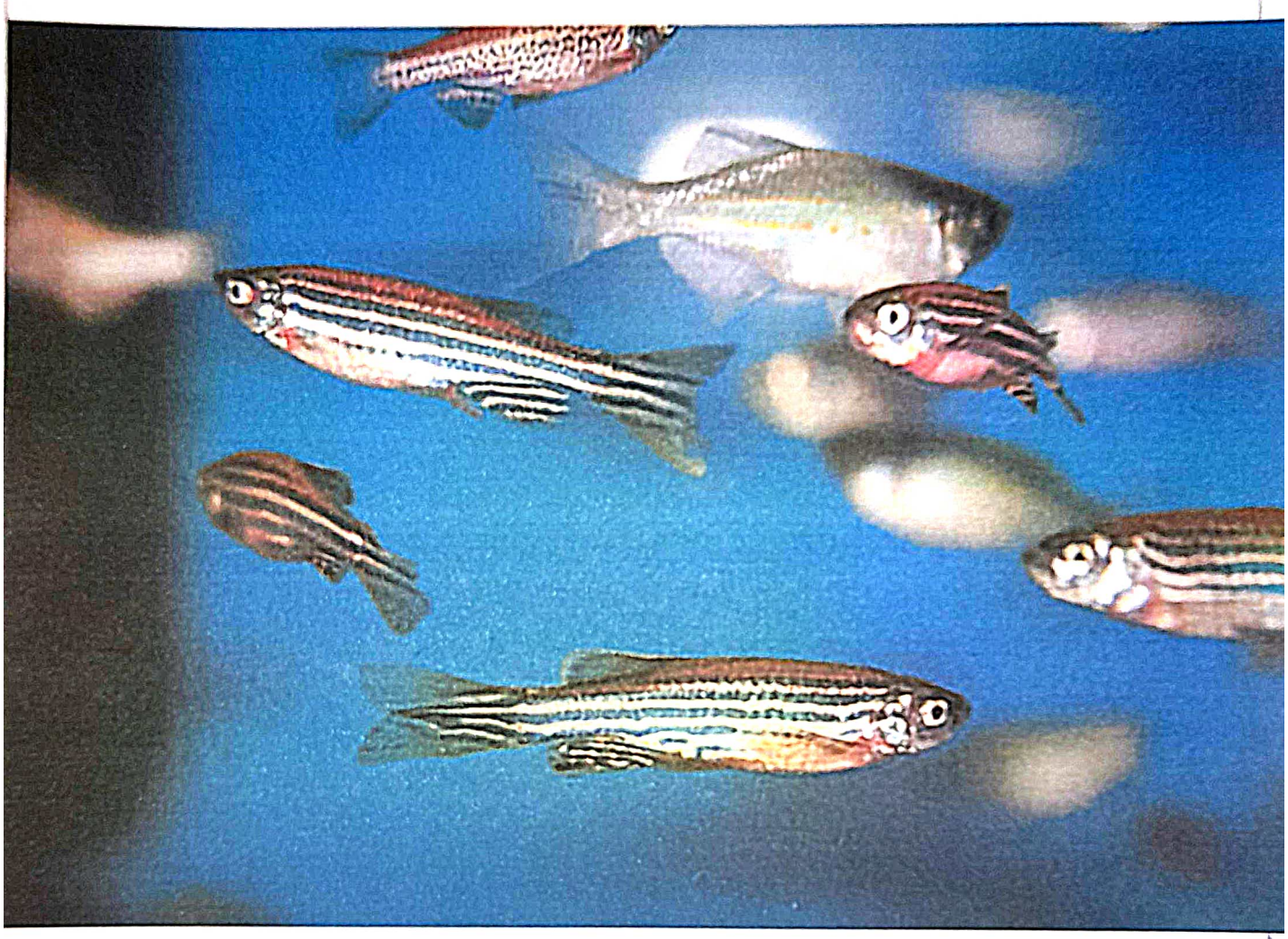
Zebrafish.



The zebrafish (Danio rerio) is a freshwater fish belonging to the minnow family (Cyprinidae) of the order Cypriniforms. Native to South Asia, It is a popular aquarium fish, frequently sold under the trade name zebra danio. It is also found in private ponds.

The zebrafish is an important and widely used vertebrate model organism in scientific research, for example in drug development, in particular pre-clinical development. It is also noted notable for its regenerative abilities, and has been modified by researchers to produce many transgenic strains.

Reproduction.

The approximate generation time for Danio rerio is three months. A male must be present for ovulation and spawning to occur. Zebrafish are asynchronous spawners and under optimal conditions (such as food availability and favorable water parameters) can spawn successfully frequently, even on a daily basis. Female are able to spawn at intervals of two to three days, laying hundreds of eggs in each clutch. Upon release, embryonic development begins; in absence of sperm, growth stops after the first few cell divisions. Fertilized eggs almost immediately become transparent, a characteristic that makes D. rerio

a convenient research model species. Sex determination of common laboratory strains was shown to be a complex genetic trait, rather than to follow a simple ZW or XY system.

The zebra embryo develops rapidly with precursors to all major organs appearing within 36 hours of fertilization. The embryo begins as a yolk with a single enormous cell on top and continues dividing until there are thousands of small cells. The cells then migrate down the sides of the yolk and begin forming a head and tail. The tail then grows and separates from the body (24h panel). The Yolk shrinks over time because the first few days (72 h pane). After a few months, the adults reaches reproductive maturity.

Feeding

Zebrafish are omnivorous, primarily eating zooplankton, phytoplankton, insects and insects larvae, although they can eat a variety of other foods, such as worms and small crustaceans. If their preferred food sources are not readily

available.

In research, adult Zebrafish are often fed with brine or shrimp or paramecia.

Scientific research.

D. rerio is a common and useful scientific model organism for studies of vertebrate development and gene function. Its use as a laboratory animal was pioneered by the American molecular biologist George Streisinger and his colleagues at the University of Oregon in the 1970s and 1980s; Streisinger's zebrafish clones were among the earliest successful vertebrate clones created. Its importance has been consolidated by successful large-scale forward genetic screens. The fish has a dedicated online database of genetic, genomic and developmental information, the Zebrafish Information Network (ZFIN). The Zebrafish International Resource Centre (ZIRC) is a genetic resource repository with ~~29,0~~ 29,250 alleles available for distribution to the research community. D. rerio is also one of the few fish species to have sent into space.

Model Characteristics :

As a model biological system, the Zebrafish possesses numerous advantages for scientists. Its genome has been fully sequenced, and it has well-understood, easily observable and testable developmental behaviours. Its embryonic development is very rapid, and its embryos are relatively large, robust, and transparent, and able to develop outside their mother. Furthermore, well-characterized mutant strains are readily available.

Other advantages include the species nearly constant size during early ~~embryo~~ development, which enables simple staining to be used, and the fact that its two-celled embryo can be fused into a single cell to create a homozygous embryo. The Zebra fish is also demonstrably similar to ~~mme~~ mammalian models and humans in toxicity testing, and exhibits a diurnal sleep cycle with similarities to mammalian sleep behavior.

Regeneration:

Zebrafish have the ability to regenerate their heart and lateral line hair cells during their larval stages. In 2011, the British Heart Foundation ran an advertising campaign publicising its intention to study the applicability of this ability to humans, stating that it aimed to raise £50 million £/in research funding.

Zebrafish have also been found to regenerate photoreceptor cells and retinal neurons following injury, which has been shown to be mediated by the dedifferentiation and proliferation of muller ~~cit~~ glia. Researchers frequently amputate the dorsal and ventral tail fins and analyze their regrowth to test for mutations.

Drug discovery and development.

The Zebrafish and Zebrafish larva is a suitable model organism for drug discovery and development. As a vertebrate with 70% genetic homology with humans, it can be predictive of human health and disease, while its small size and fast development facilities experiments on a larger and quicker scale than with more traditional in vivo studies, including the development of higher-throughput, automated investigative tools. As demonstrated through ongoing research program, the be Zebrafish model enables researchers not only to identify genes that might underline humans disease, but also to develop novel therapeutic agents in drug discovery programmes.