

ZEBRAFISH (*DANIO RERIO*)



Fonte: Braunbeck *et al.* (2004)

INTRODUZIONE



Embryo



Larva



Adult

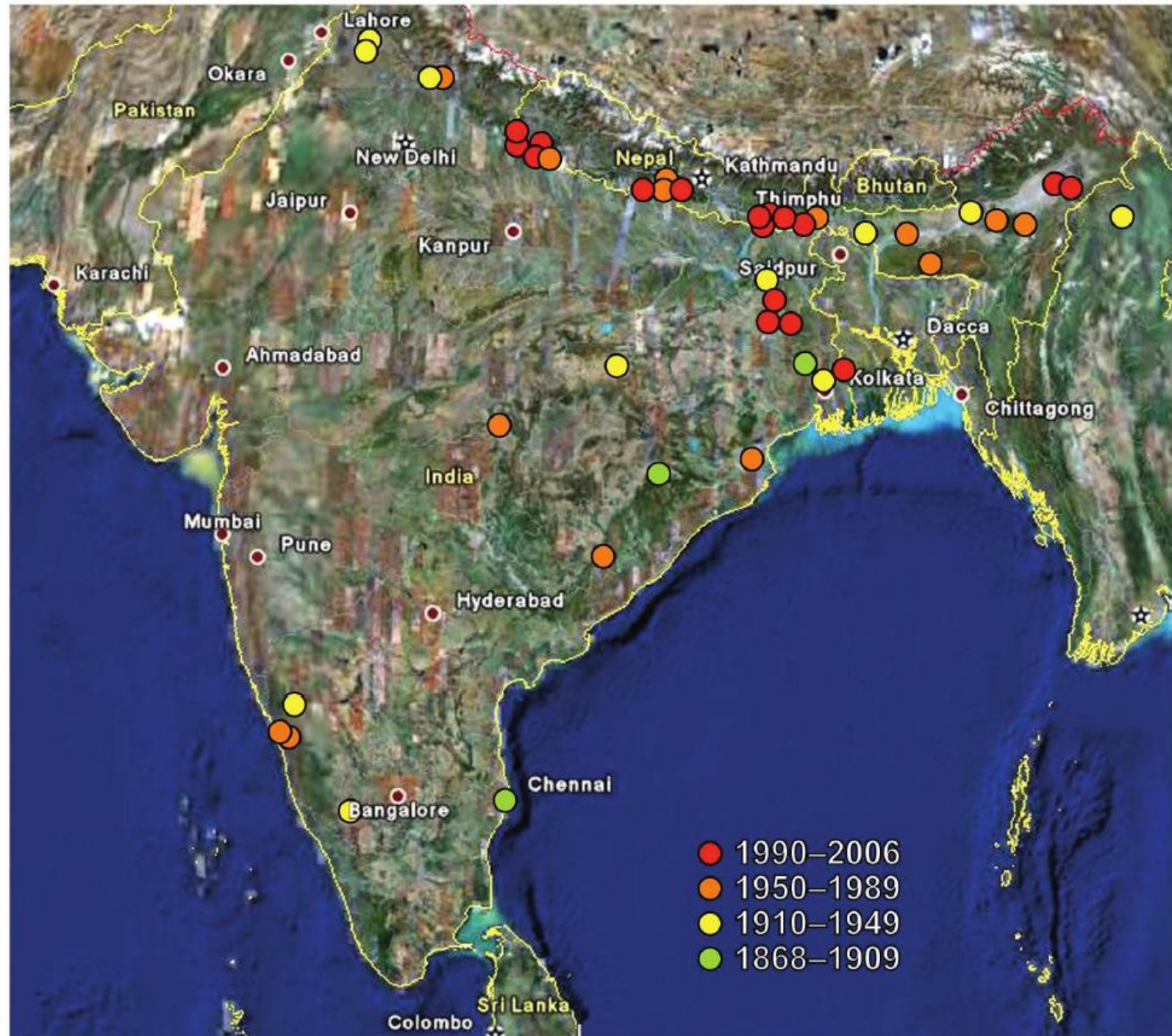


Zebrafish (*Danio rerio*):

- Famiglia *Cyprinidae*
- Acque dolci (Asia)
- Da adulto dimensioni fino a 4-5 centimetri
- Animale a sessi separati con lieve dimorfismo sessuale
- Livrea con dorso olivastro e ventre bianco argenteo, corpo con quattro linee orizzontali blu e quattro linee bianche



Where is zebrafish in the world



Natural habitats of zebrafish



still water (currents, 0 m–sec to 0.1 m–sec) at 27° C to 34° C and pH 7.9–8.2; widths of water bodies ranged from 1 to 12 m, and depths ranged from 16 to 57 cm; water was relatively clear (transparent to 35 cm). 5-6 months for sexual maturation.

Engeszer RE, et al. Zebrafish, 4:21-40, 2007



Zebrafish strain

- ❖ Wild type
- ❖ Mutant
- ❖ Transgenic

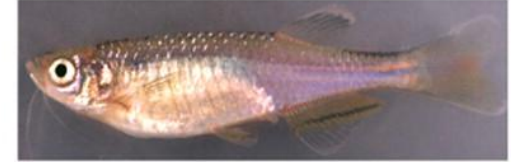
wild-type WT



duchamp/+ DU



D. albolineatus Da



albino A



ednrb1 E



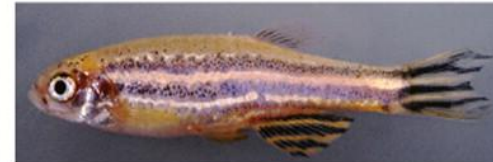
D. choprae Dc



csf1r C



kit K



D. kyathit Dk



csf1r; ednrb CE



mitfa M



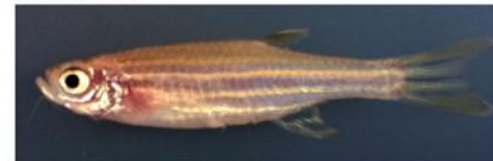
D. nigrofasciatus Dn



csf1r; kit CK



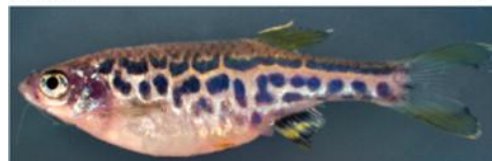
oberon O



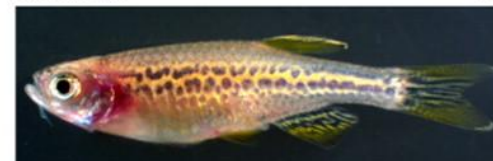
De. shanensis Ds



dali/+ DI



seurat S



Laboratory strains



AB

(pet shop derived)



TÜ



WIK

(Wild type India Kolkata)

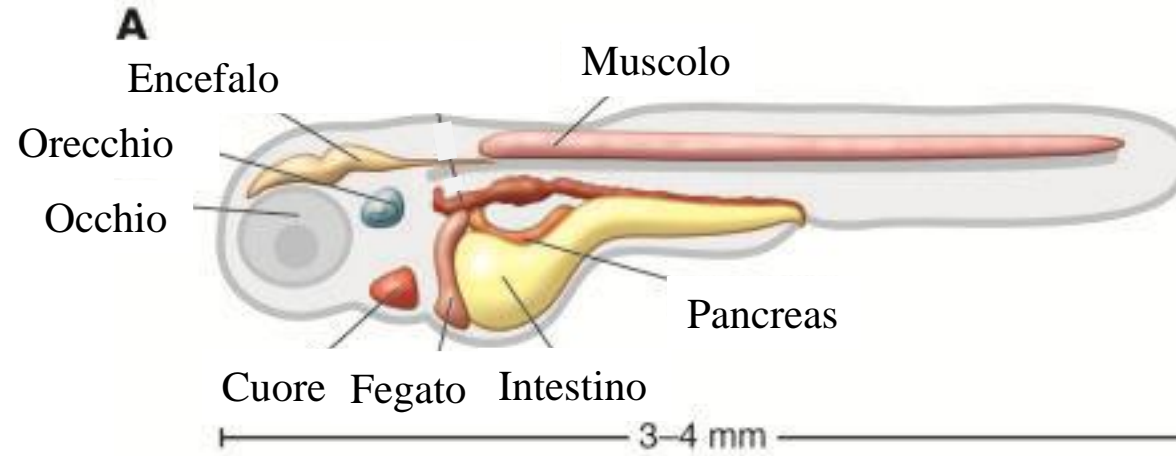
They may look the same, but are genetically different!

WIK was used for mapping mutations from the first mutagenesis screens

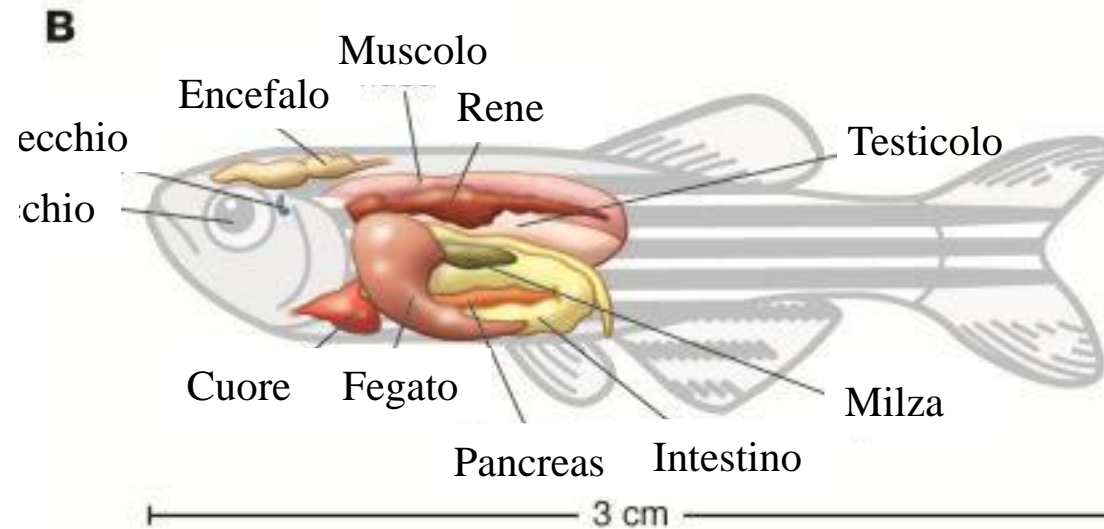
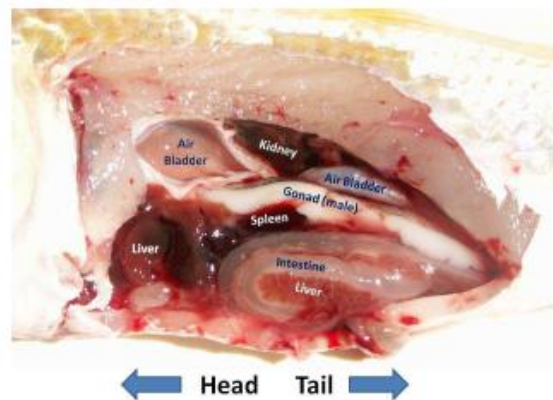
General Features

General Features	Benefits
Appearance <ul style="list-style-type: none">-Dimension ~4 cm-Salient distinguishable features of male and female-Often transparent adult bodies	Large number can be kept easily and cheaply in lab Good model for visualization of cellular activity
Habitat <ul style="list-style-type: none">-Fresh water fish- Tropical fish	Universally available
Feeding <ul style="list-style-type: none">-Omnivorous	Low cost of maintenance
Reproduction <ul style="list-style-type: none">-Female spawns every 2-3 days-Breeds all year round-Several hundreds of eggs produced in single clutch-External fertilization	Large number of offspring- good for batch variation studies Easy availability of eggs

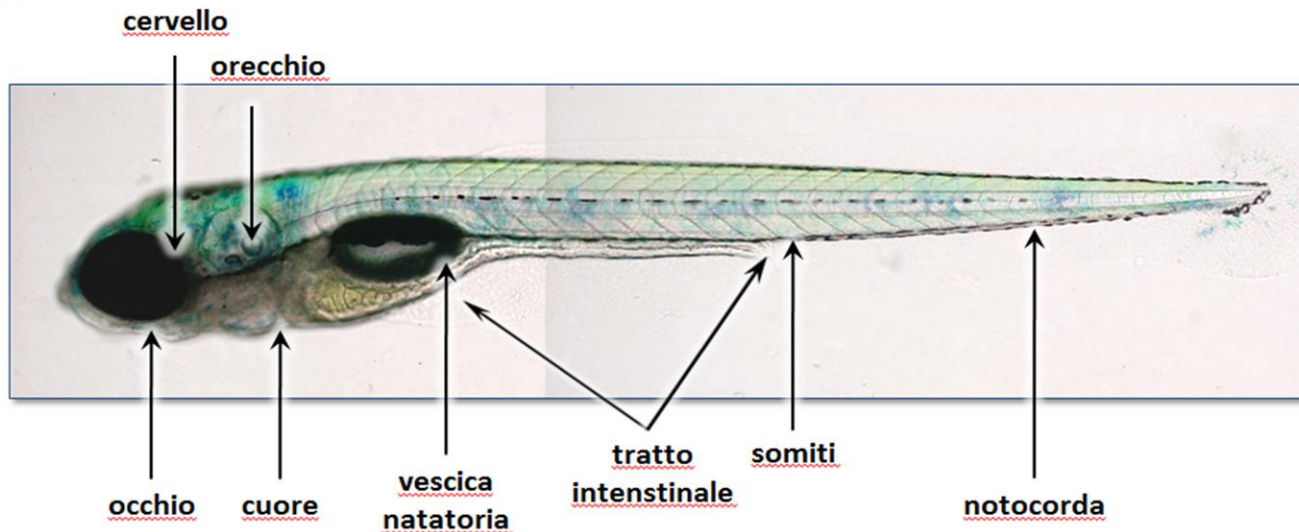
ANATOMIA DELLO ZEBRAFISH



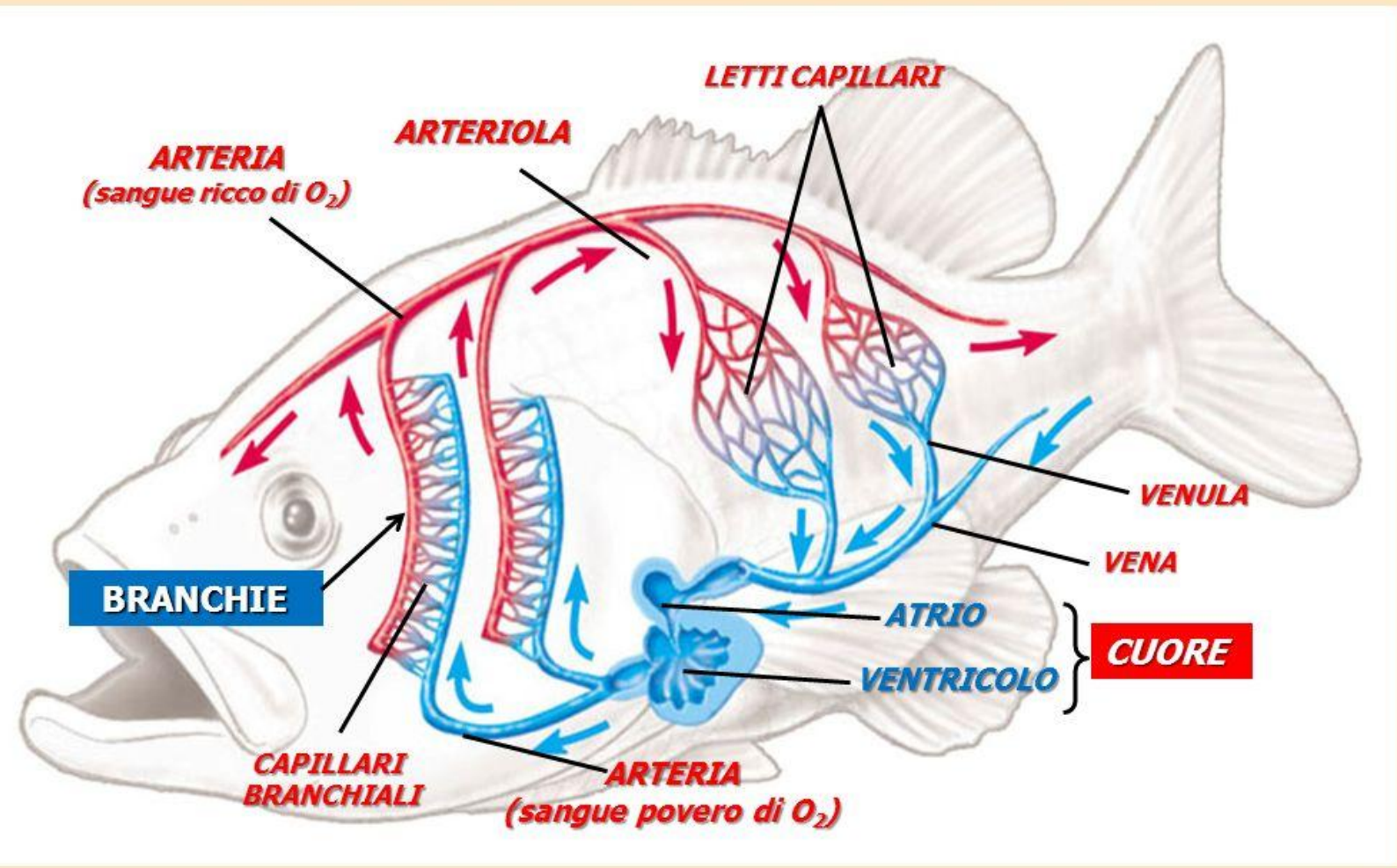
B. CYPRINID FISH:



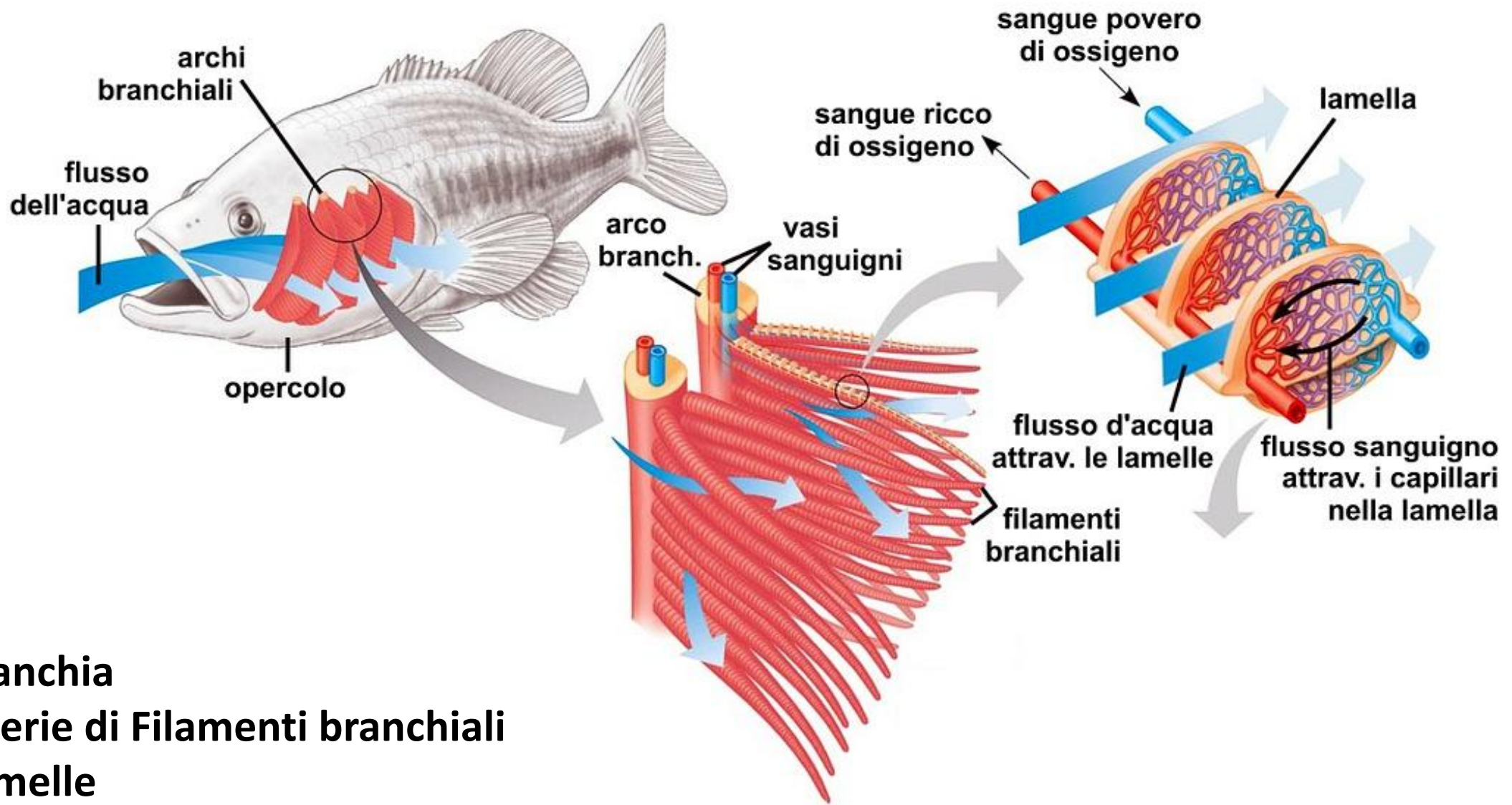
- Sistema circolatorio semplice, a ciclo chiuso
- Sistema digerente semplice (pesce agastrico)
- Sistema respiratorio archi branchiali (4 paia laterali)
- Orecchio interno (otoliti utili per l'orientamento)
- Vescica natatoria, dorsalmente all'intestino, organo idrostatico con miscela di gas (O_2 , CO_2 , N_2) necessario per i movimenti del pesce in acqua



PESCI: sistema circolatorio chiuso



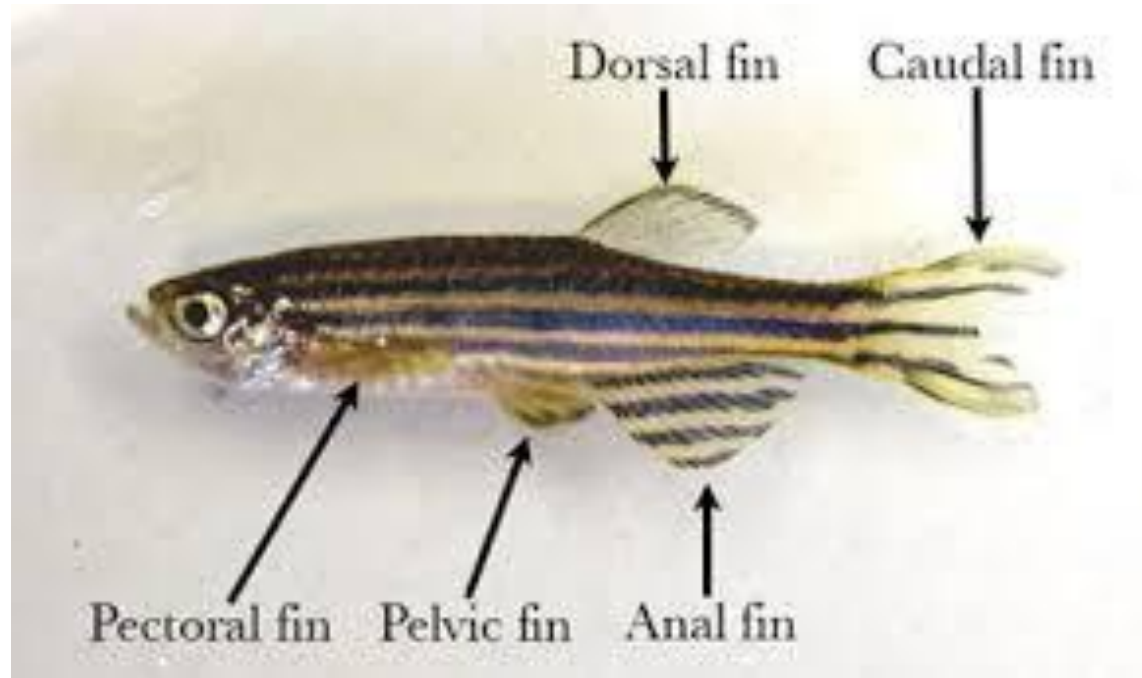




Branchia

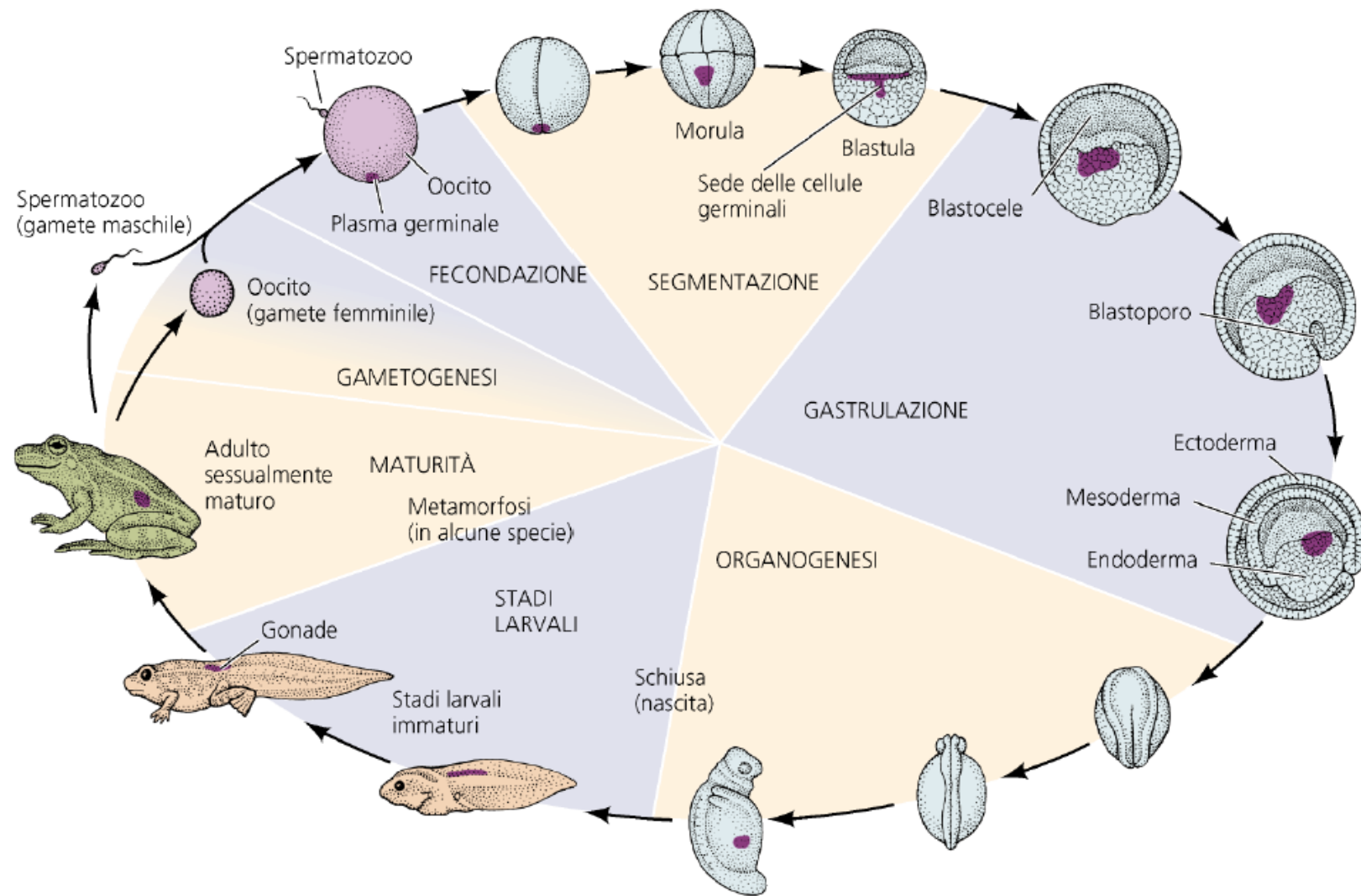
2 serie di Filamenti branchiali

Lamelle

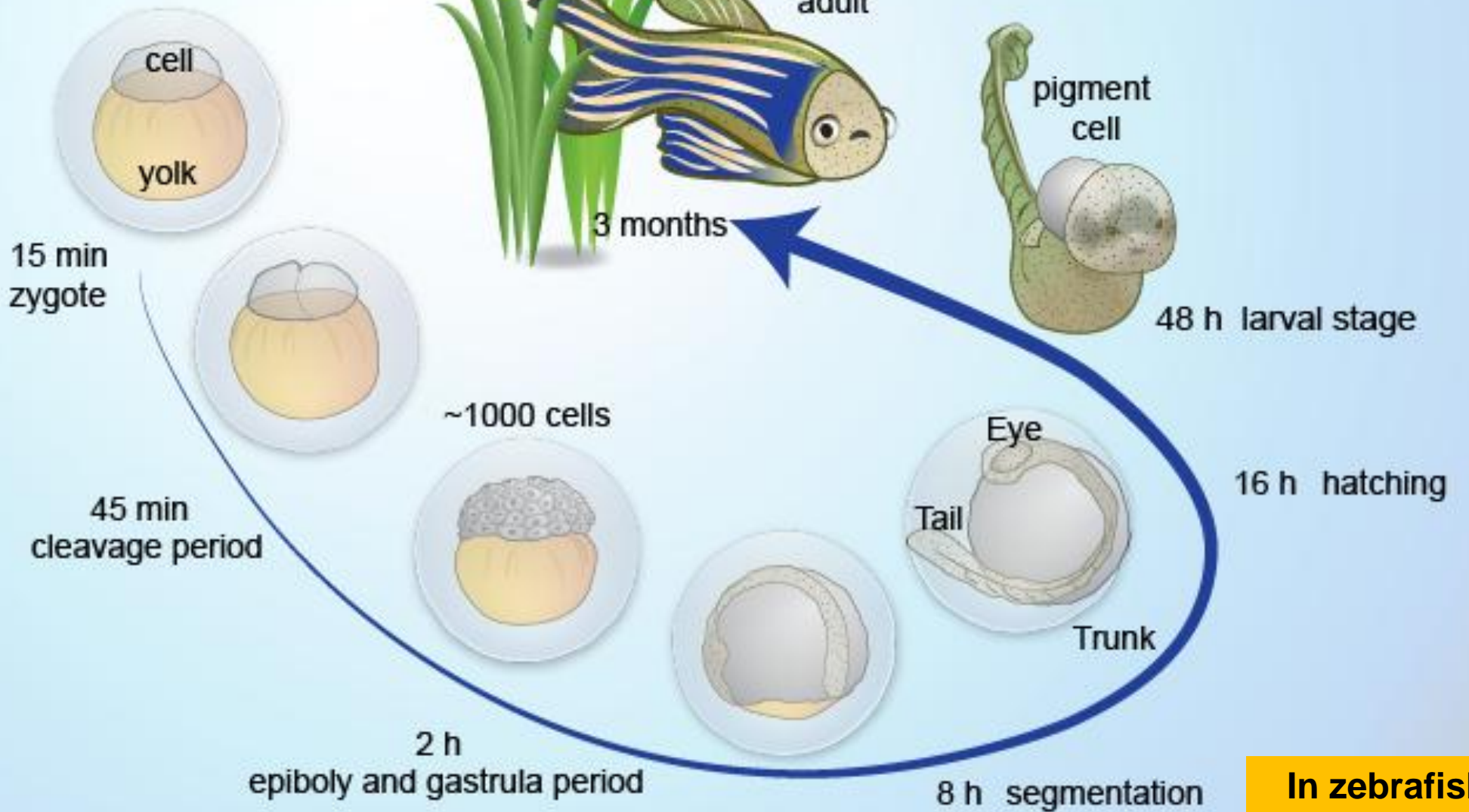


<https://www.jove.com/video/1717/dissezione-di-organi-dal-zebrafish-adulti?language=Italian>

Il ciclo della vita: gli stadi dello sviluppo animale

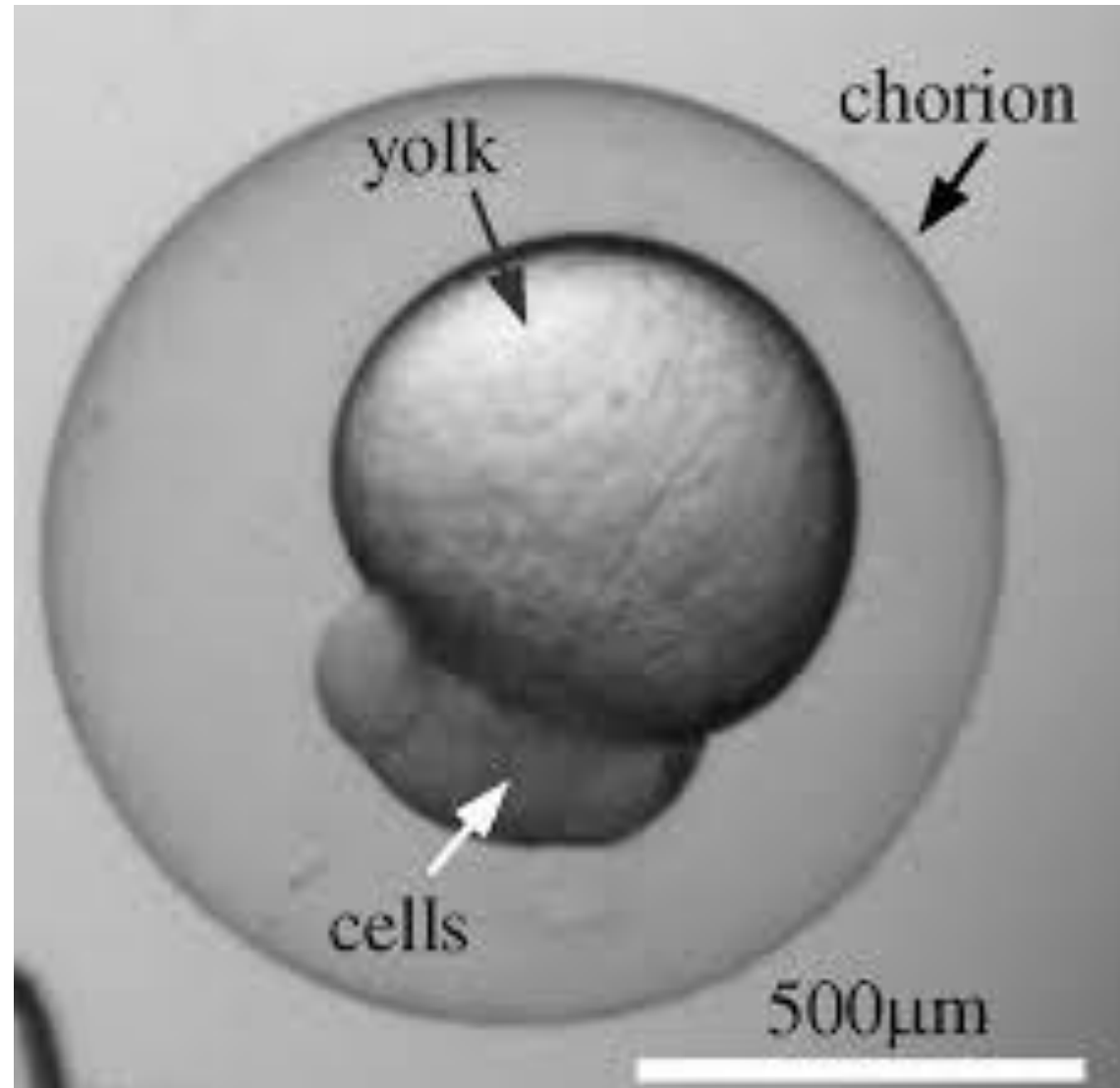


f e r t i l i z a t i o n



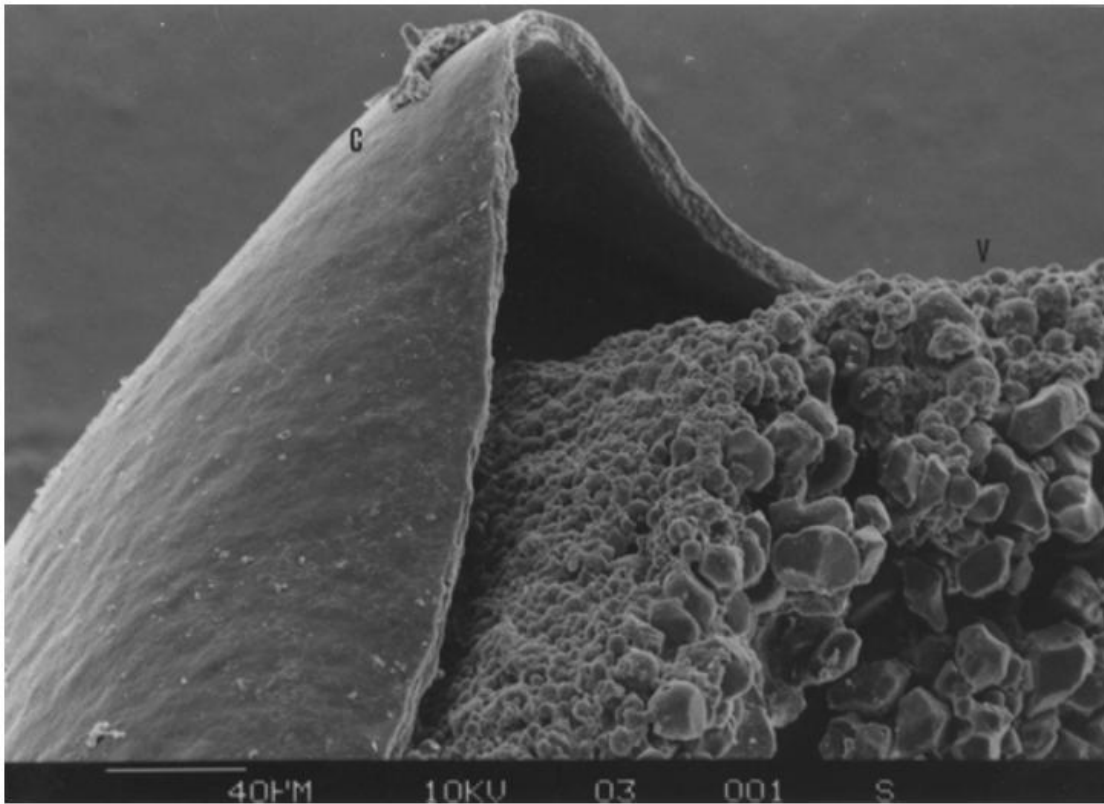
**In zebrafish
l'intero ciclo
dura 90 gg**

LO SVILUPPO EMBRIONALE DELLO ZEBRAFISH

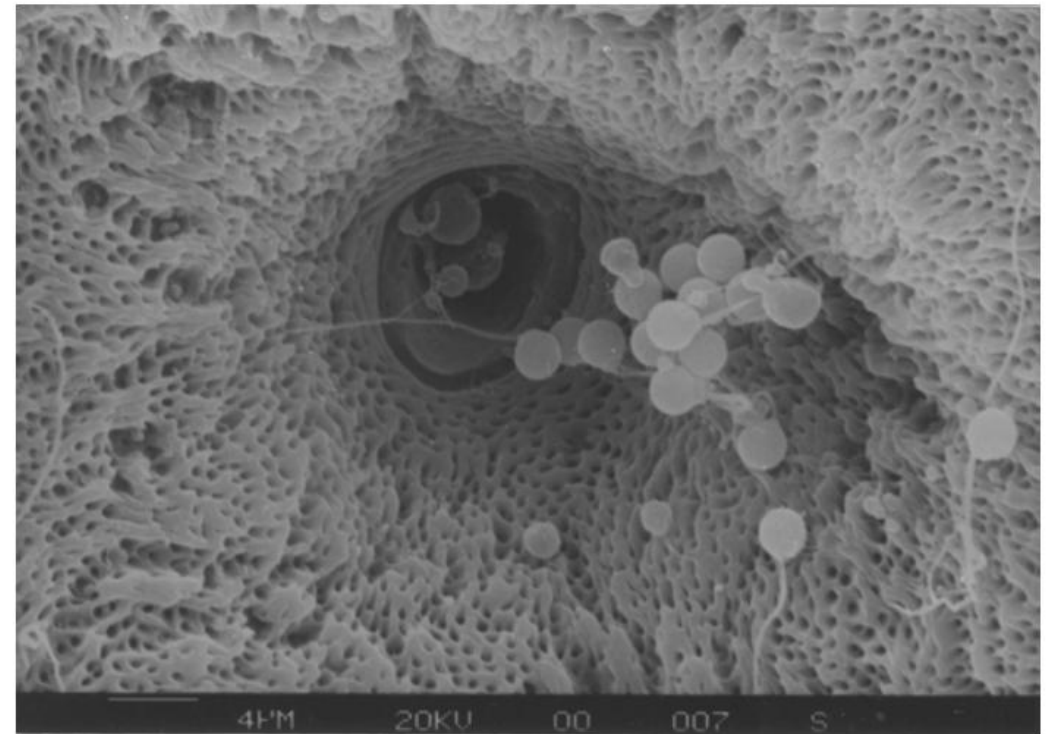


LO SVILUPPO EMBRIONALE DELLO ZEBRAFISH

chorion



chorion



FECONDAZIONE

Dal singolo uovo fecondato (lo **zigote** unicellulare) si origina un n° enorme di diversi tipi cellulari con le più disparate funzioni. Tutte queste cellule contengono il medesimo patrimonio genetico.

Negli embrioni di zebrafish la prima divisione comincia 40 minuti dopo la fecondazione

Fertilization

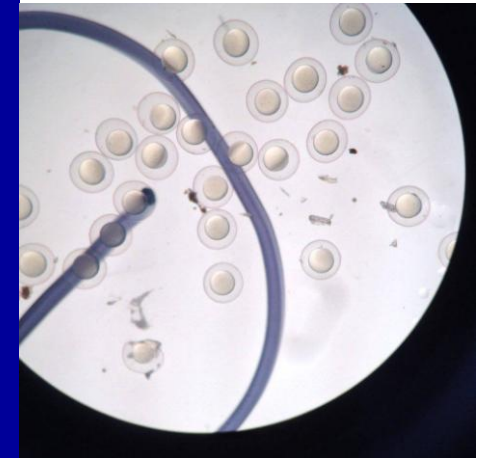
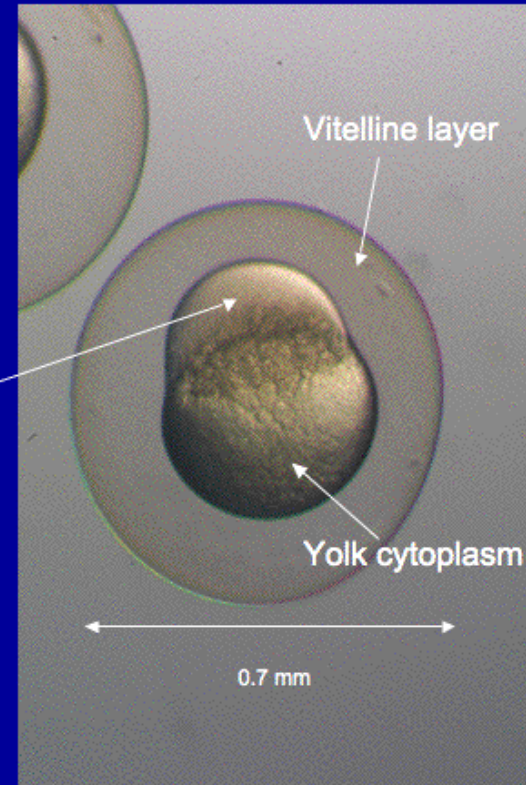
1 cell stage zygote

Blastodisk

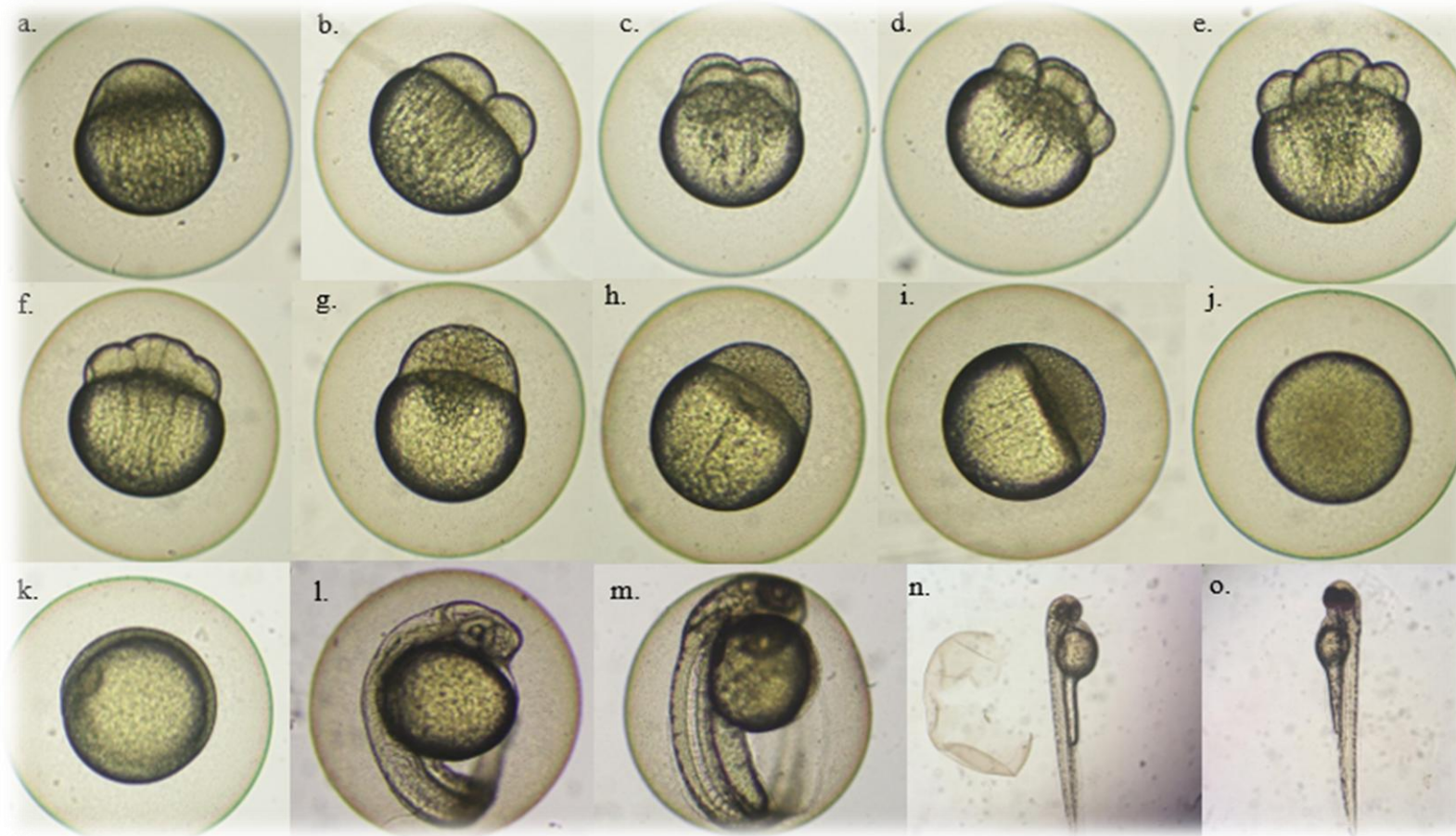
Vitelline layer

Yolk cytoplasm

0.7 mm



LO SVILUPPO EMBRIONALE DELLO ZEBRAFISH

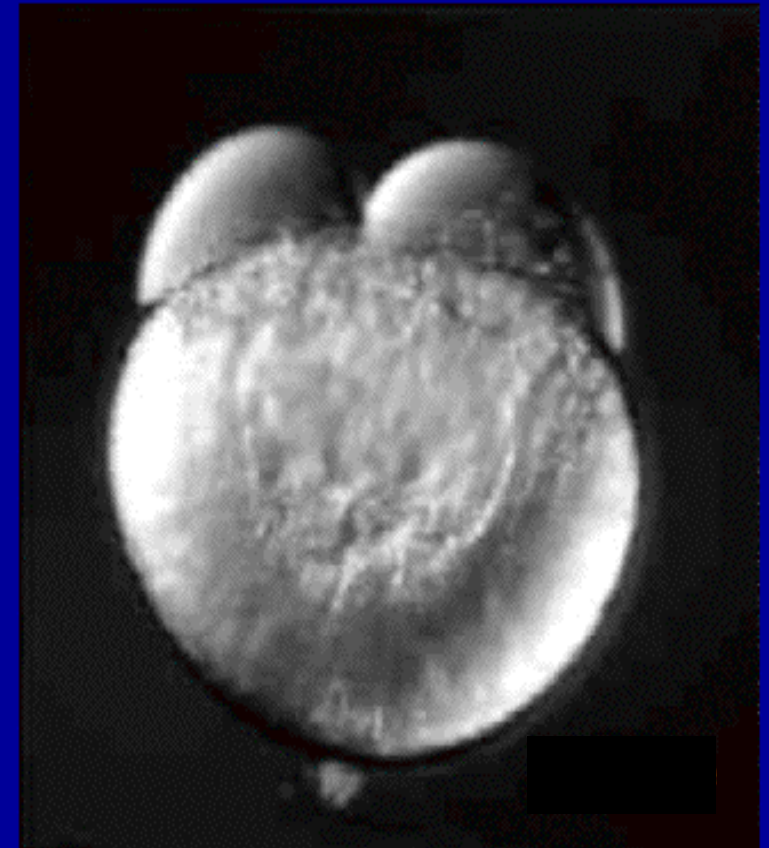


- a) 1-cell stage (0.2 hpf)
- b) 2-cell stage (0.75 hpf)
- c) 4-cell stage
- d) 8-cell stage (1.5 hpf)
- e) 32-cell stage (1.75 hpf)
- f) 64-cell stage (2 hpf)
- g) High stage (3 hpf)
- h) Oblong stage (3.5 hpf)
- i) Sphere stage (4 hpf)
- j) Germ ring stage (5.5 hpf)
- k) 70% epiboly stage (7.5 hpf)
- l) Prim-5 stage (24 hpf)
- m) Long-pec stage (48 hpf)
- n) Protruding-mouth stage (72 hpf)
- o) Early larval period (96 hpf)

<https://www.youtube.com/watch?v=bEgygtbEo2A>

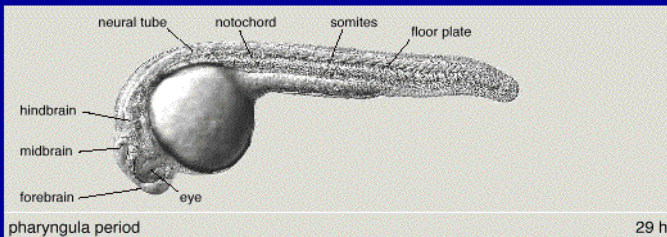
Developmental Timetable

Zygote	0-0.75 hr
Cleavage	0.75-2.25 hr
Blastula	2.25-5.25 hr
Gastrula	5.25-10 hr
Segmentation	10-24 hr
Pharyngula	24-48 hr
Hatchling	48-72 hr
Larval Fish	72 hr

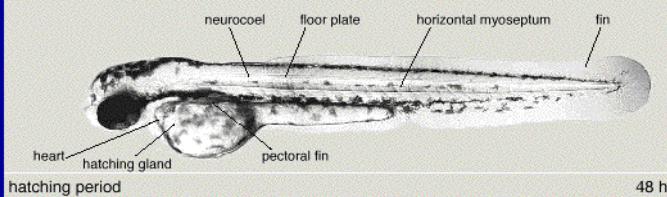


17 Hours of Development

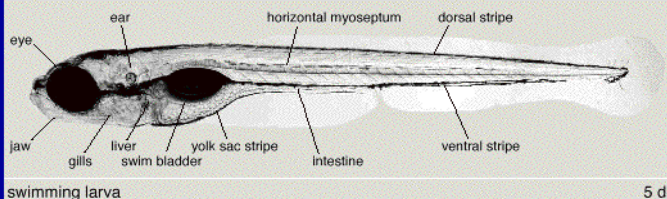
Pharyngula



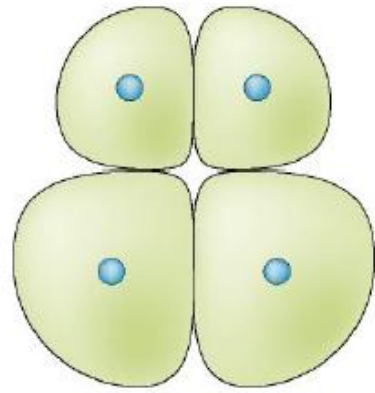
Hatchling



Larva

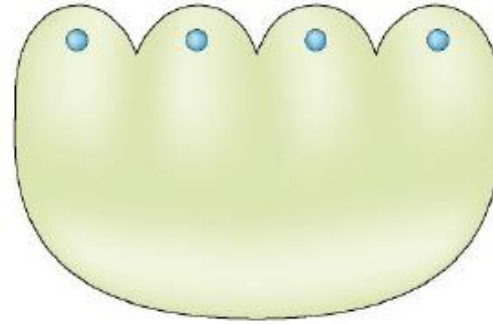


Segmentazione



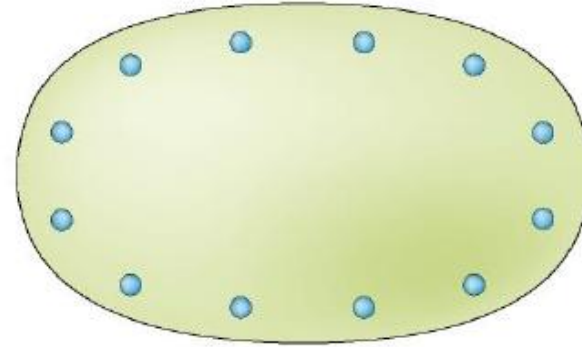
(a) Oloblastica

Zebrafish



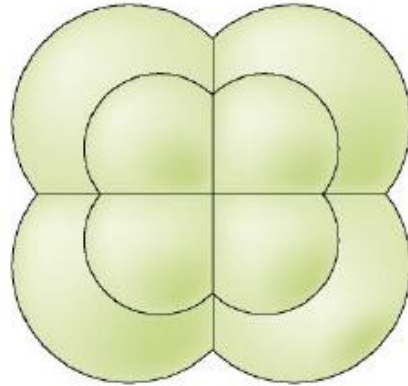
(b) Meroblastica

Drosophila movie18.3



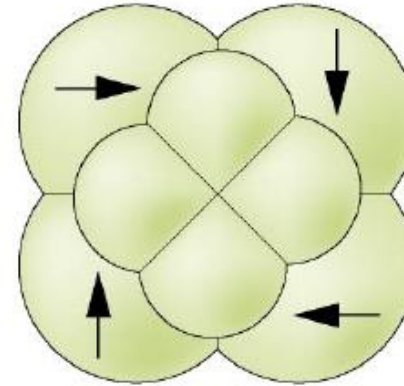
(c) Superficiale

Xenopus



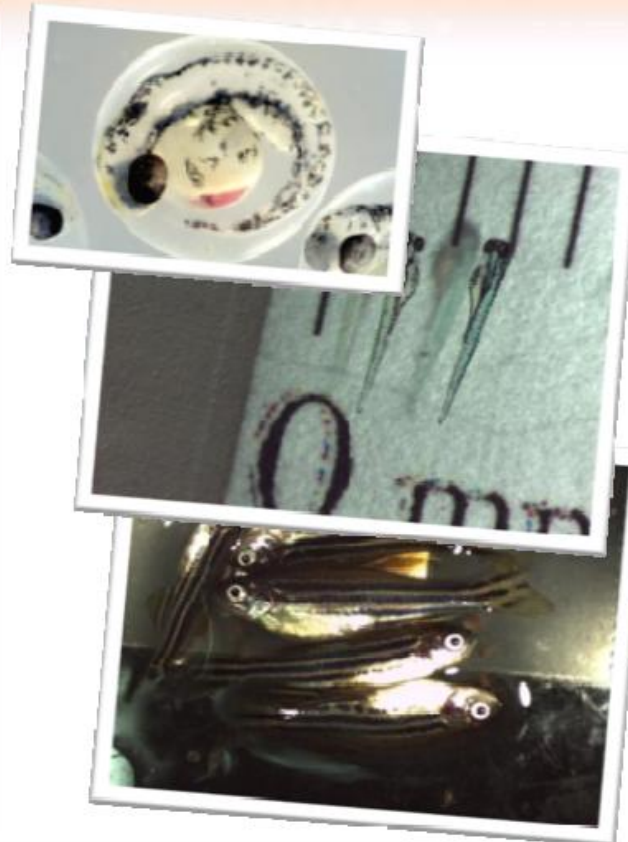
(d) Radiale
echinodermi

Discoidale



(e) Spirale
molluschi

fry phase	Embryos (0 to 72 h.p.f.)
	Early larvae (72 h.p.f. to 13 d.p.f.)
	Mid larvae (14 d.p.f. to 29 d.p.f.)
juvenile phase	(30 d.p.f. to 90 d.p.f.)
adult phase	(> 90 d.p.f. up to 2 years)



Fasi dello sviluppo
Classificare in modo univoco

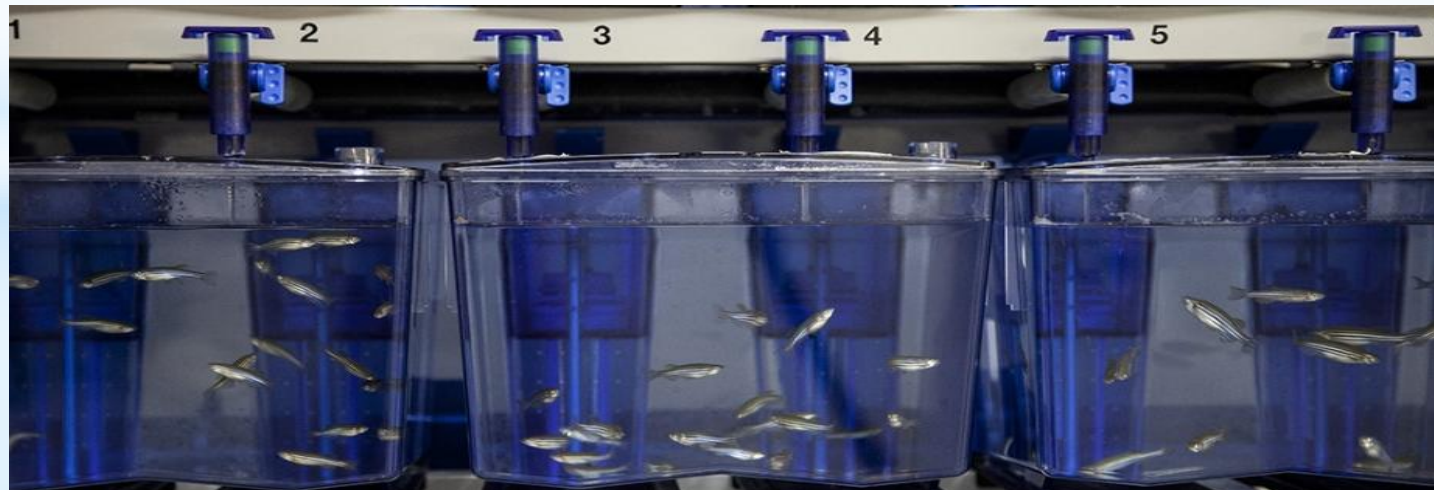
**A partire dal 5° giorno dopo la schiusa
 è in grado di alimentarsi autonomamente**

* GESTIONE E MANAGEMENT IN FACILITY

In Italia Dir. 2010/63/UE “sulla protezione degli animali utilizzati a fini scientifici” con **D.lgs 4 marzo 2014, n.26** (elenco animali art. 10 comma 1)

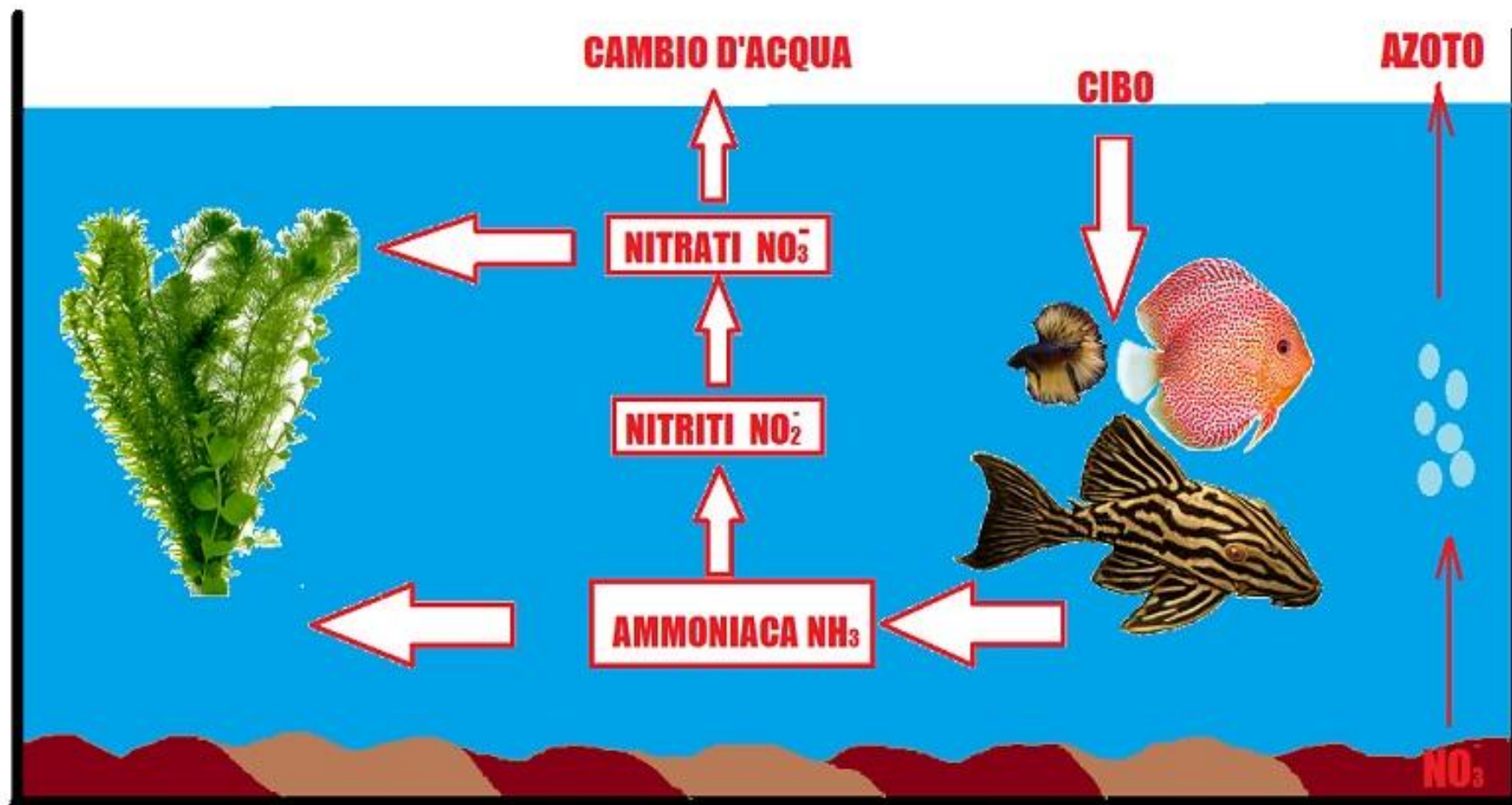
Allegato III «cura e management degli animali»

- colonie da 10-15 pesci in vaschette per assicurare il benessere animale
- sistema a gocciolamento per ricambio giornaliero continuo e lento dell'acqua





Il ciclo dell'azoto



* GESTIONE E MANAGEMENT IN FACILITY



PARAMETRI OTTIMALI

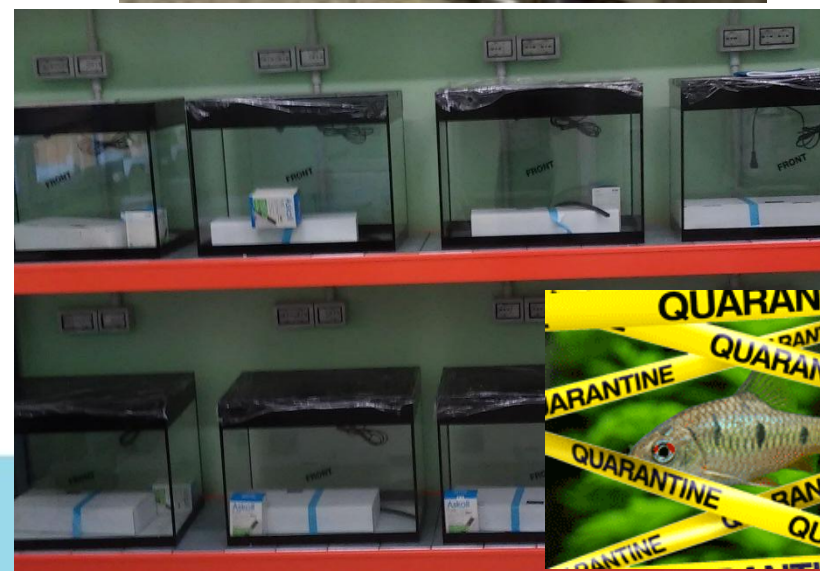
Temperatura H ₂ O	28±1°C
Ossigeno disciolto	~ 7.8 mg/L a 28 °C
Scorie azotate	NH ₃ e NO ₂ = 0 ppm, NO ₃ =10 ppm
pH	tra 7.3 ed 8
Salinità	0,35 a 0,7 ppt di sali
Fosfati	0,02 e 0,50 mg/l
Fotoperiodo	12/12 o 14/10 luce/buio

sistema di filtri (meccanico, a carbone, microbiologico) e raggi per assicurare una buona qualità delle acque

(Zebtec, Tecniplast, Italy) sistema “stand alone”



The habitat in the lab...





Lab habitat: physico-chemical properties

Found in two recent studies:

Temperature: 12.3 - 38.6 °C

pH: 5.9 - 9.8

Conductivity: 10 - 271 μS

In the lab:

26-29 °C

6.5-8.0

250-600 μS



Quite tolerant – in line to what was observed in the laboratory

Targeted values: $\text{NO}_3^- < 2.5 \text{ mg/l}$, $\text{NO}_2^- < 0.025 \text{ mg/l}$



Water Quality

Temperature

pH

Ammonia (NH_3
/ NH_4)

Nitrite (NO_2)

Nitrate (NO_3)

Dissolved Oxygen
(DO)

Conductivity