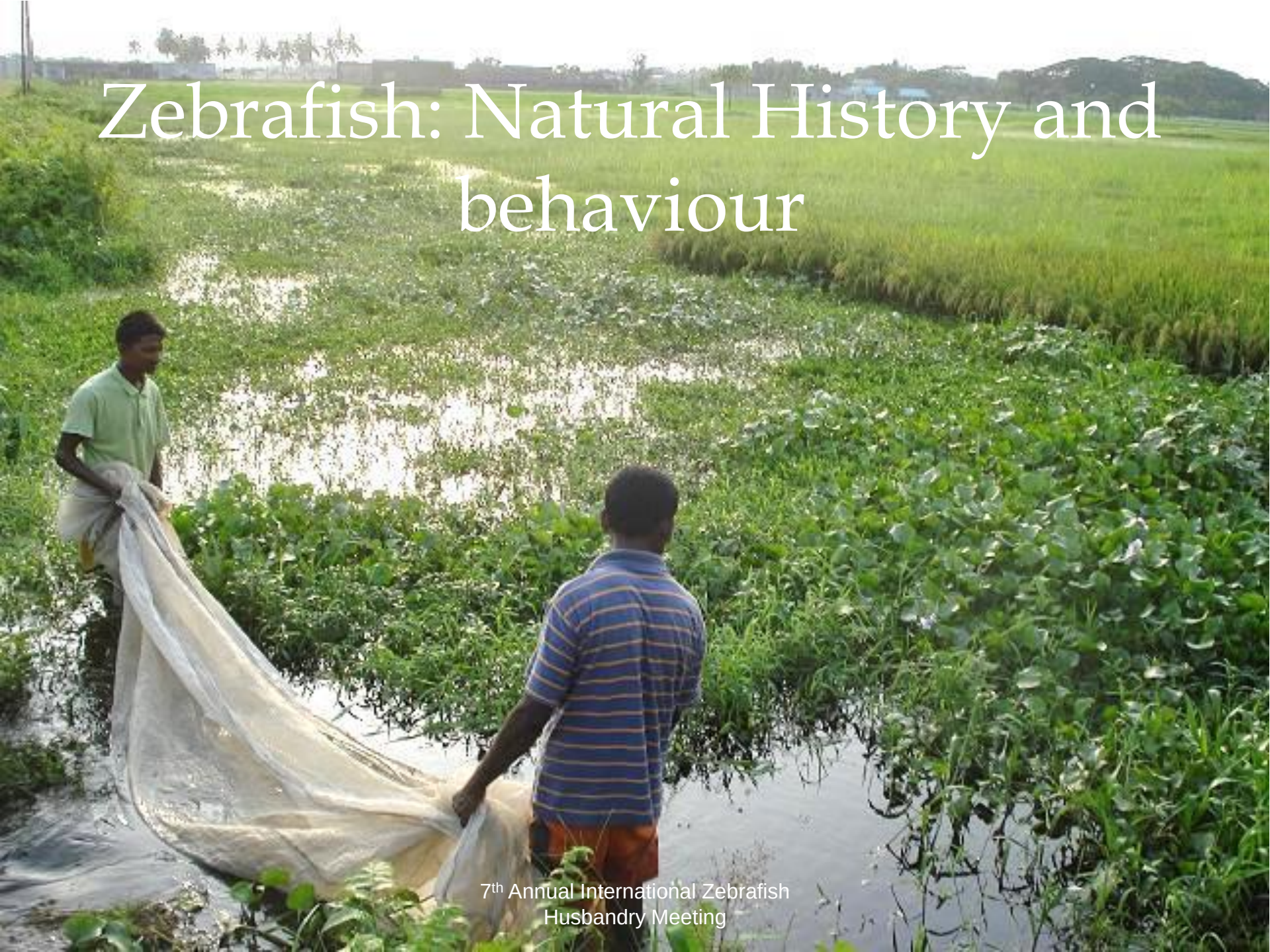
Zebrafish: Natural History and behaviour



7th Annual International Zebrafish Husbandry Meeting

Talk Outline

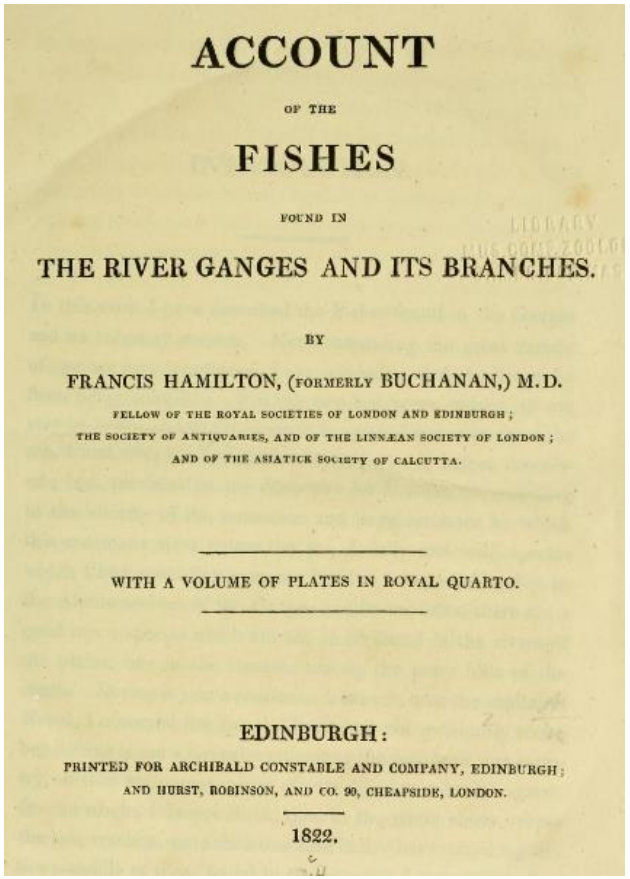
- Taxonomy
- Geographical Range
  & Natural Distribution
- Habitat Type
  - Case Study: Bangladesh
  - Flora & Fauna
- Life History
  - Growth
  - Behaviour
  - Wild vs Lab

ZEBRAFISH TAXONOMY

7th Annual International Zebrafish
Husbandry Meeting

Zebrafish Taxonomy

- Francis Hamilton - surgeon stationed in Northern India with the British East India company established to pursue trade
  - described several danio species in the River Ganges catchment
  - including *Danio rerio*
  - Danio derives from the Bengali name ‘dhani’ meaning ‘of the rice field’



ACCOUNT

OF THE

FISHES

FOUND IN

THE RIVER GANGES AND ITS BRANCHES.

BY

FRANCIS HAMILTON, (FORMERLY BUCHANAN,) M.D.

FELLOW OF THE ROYAL SOCIETIES OF LONDON AND EDINBURGH;
THE SOCIETY OF ANTIQUARIES, AND OF THE LINNÆAN SOCIETY OF LONDON;
AND OF THE ASIATICK SOCIETY OF CALCUTTA.

WITH A VOLUME OF PLATES IN ROYAL QUARTO.

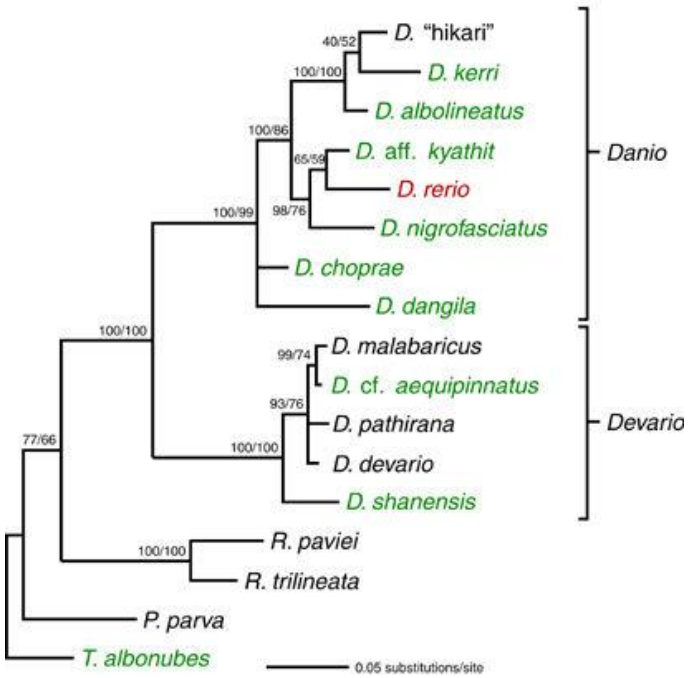
EDINBURGH:

PRINTED FOR ARCHIBALD CONSTABLE AND COMPANY, EDINBURGH;
AND HURST, ROBINSON, AND CO. 90, CHEAPSIDE, LONDON.

1822.

Zebrafish Taxonomy

- 1916 Weber & de Beaufort assigned subgenus *Brachydanio*
  - small, short dorsal fin, incomplete lateral line
  - *Danio* reserved for larger species
  - Laale, HW. 1977. The biology and use of *Brachydanio rerio* in fisheries



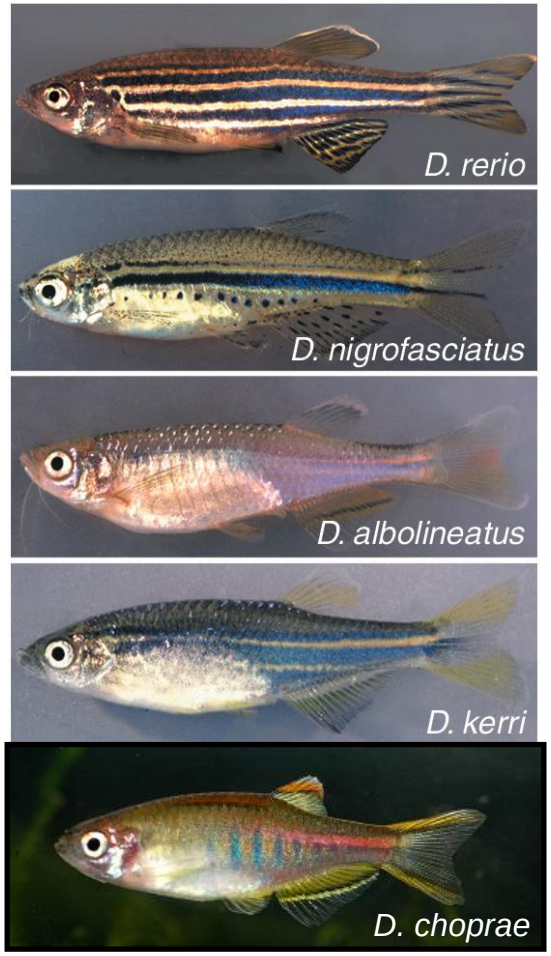
- 1991 Barman synonymised groups
  - diagnostic characters unreliable

- 1993–2005 No. of molecular studies rRNA
  - Proposed 2 subclades - *Devario & Danio*

- 2003 Fang used 38 morphological characteristics
  - Proposed *Devario* (30 Sp.) & *Danio* (9 sp.)
- Ecologically distinct - deeper bodied *Devario* associated with fast flowing streams; slender bodied *Danio* with slow flowing or static water

Zebrafish Taxonomy

- 9 described danio species
  - all relatively small ~ 30mm - 50mm
  - common body shape, fusiform & laterally compressed, terminal oblique mouth pointing upwards
  - distinctive horizontal stripe pattern
  - outlier - moustached danio ~ 150mm
  - giant danio actually a *Devario* sp.

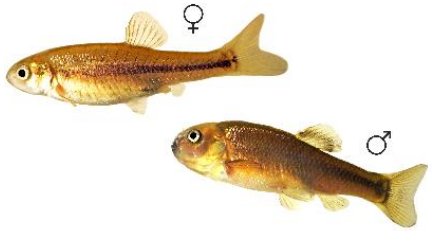


7th Annual International Zebrafish Husbandry Meeting

Adapted from: Parichy, D.M. Current Biology, 2003.

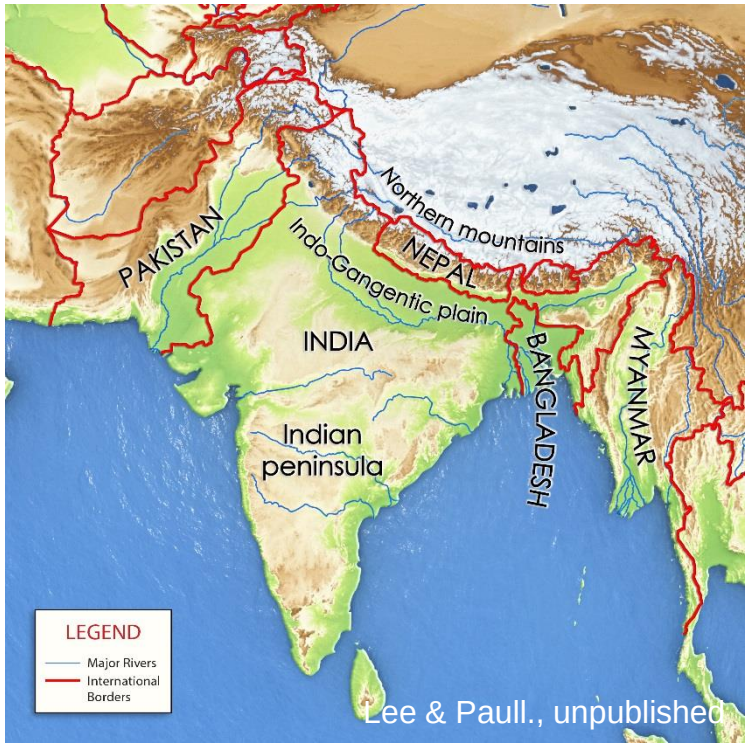
Zebrafish Taxonomy

- Members of the family Cyprinidae (carps and true minnows) - most specious vertebrate family >3000 sp.
- Excellent model species for ecotoxicology…..



Geographical Range & Natural Distribution

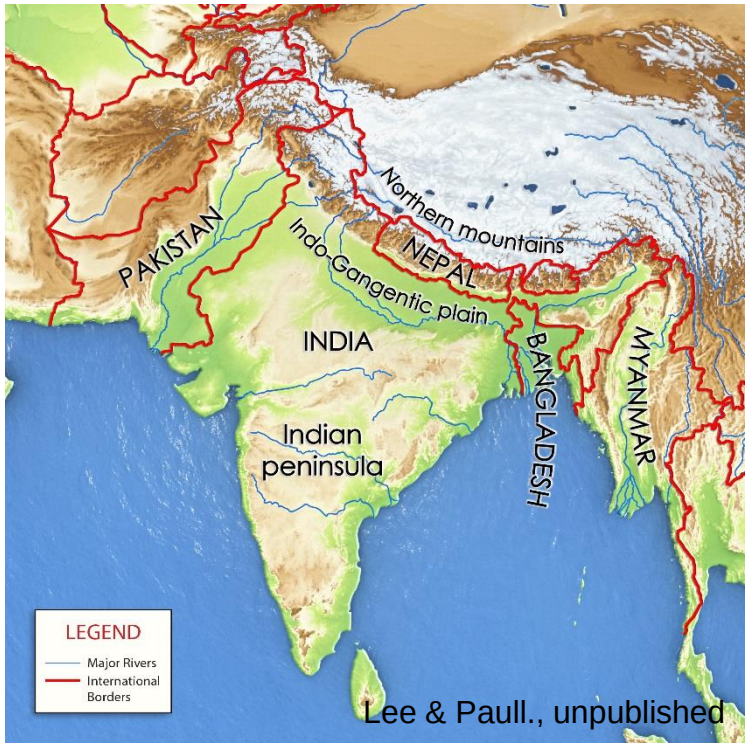
Geographical Range & Natural Distribution

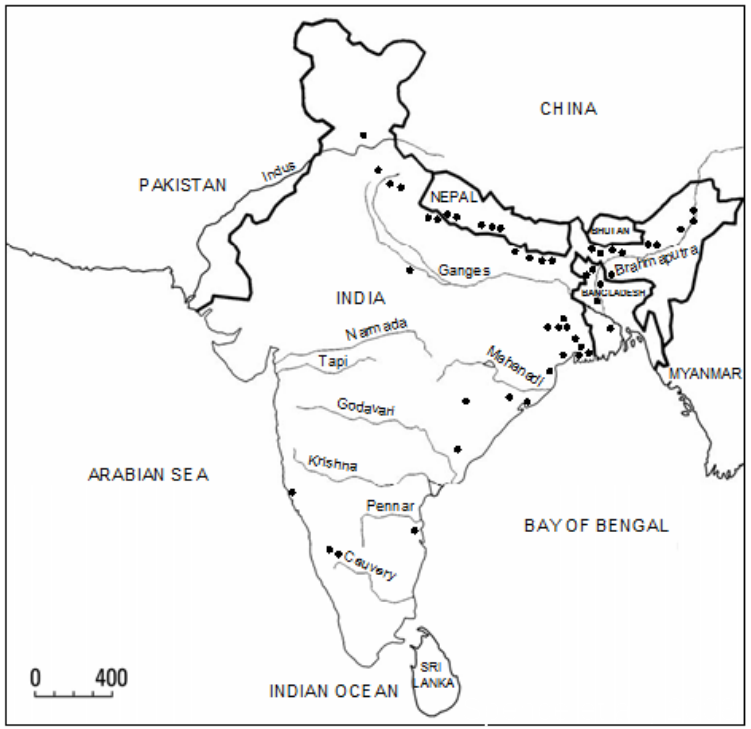


Range of zebrafish extends across much of India, Bangladesh and Nepal, from the Pakistan border in the west to the Myanmar border in the east, and from the foothills of the Himalaya in the north to the paddy fields of Karnaka in the south (Engeszer et al., 2007)

- Ability to expand its range constrained by physical boundaries – mountain ranges to the north, Indian ocean to the south

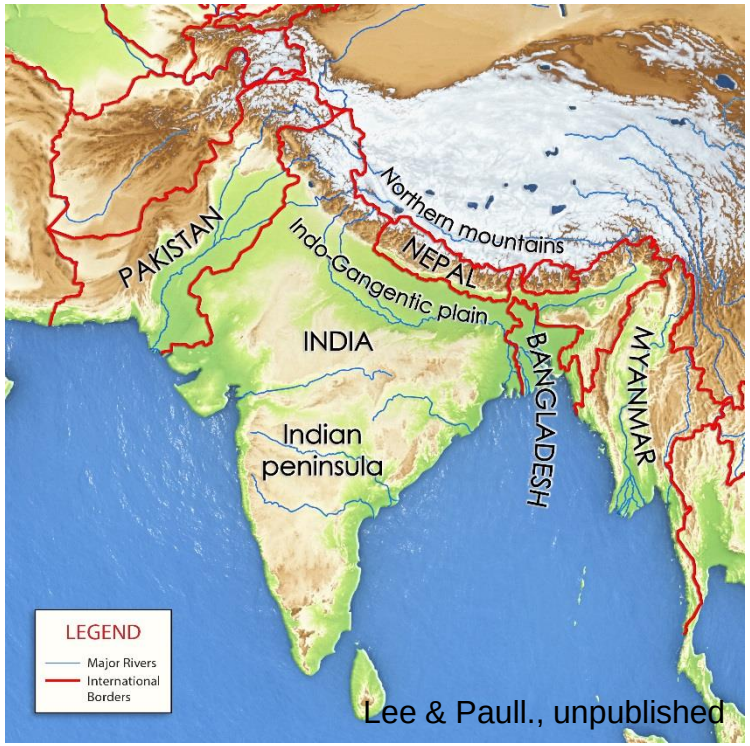
Geographical Range & Natural Distribution

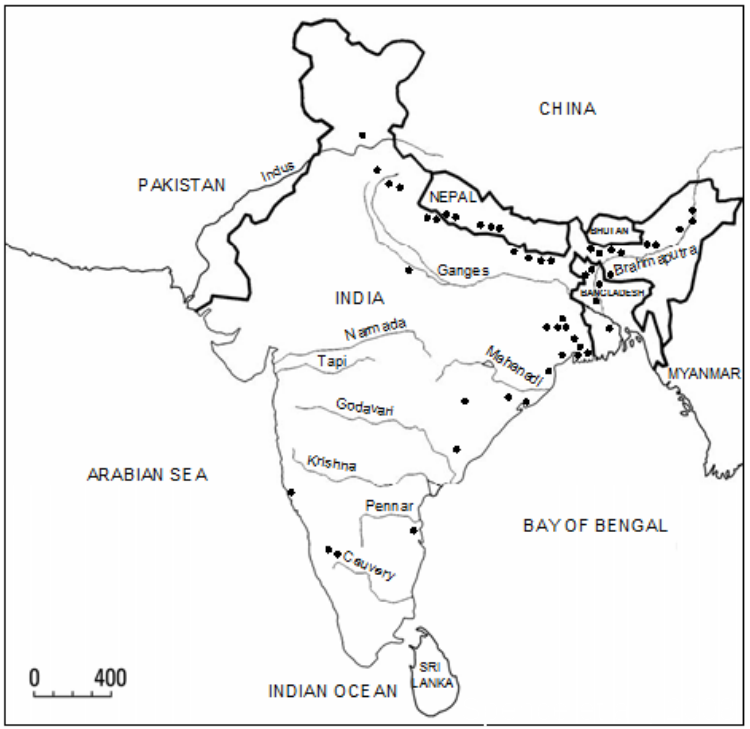




- Zebrafish distribution is not even across its range
- Distribution is focused in the Himalaya drainage system of Nepal & the floodplains of the Ganges & Brahmaputra river basins in north-eastern India & Bangladesh

Geographical Range & Natural Distribution





- Abiotic factors - e.g. altitude, temperature, water chemistry/flow
- Biotic factors – e.g. predation, competition, suitable prey/food
- Dynamic process – no systematic field studies for zebrafish

Geographical Range & Natural Distribution

- Zebrafish temperature tolerances in the wild are not well known
  - Field studies have recorded 12°C in Arunchal Pradesh to 39°C in Orissa
  - Laboratory studies suggest a lower lethal temperature of 6.2 ± 0.28°C & an upper lethal temperature of 41.7 ± 0.35°C
- Summer Temperatures (mean daytime):
  - Northern & central areas experience temperatures in excess of 45°C
  - West coast & southern parts of the peninsula generally 5–10°C cooler
  - Temperatures in the far northern mountains rarely exceed 25°C.
  - In Bangladesh, temperatures range from 38 to 41°C
- Winter Temperatures (mean daytime):
  - Northern parts of India & Bangladesh ~ 16°C to 20°C
  - West coast & southern India ~ 30°C, falling to 20°C at night
  - Night time temps. fall below freezing in some north and western regions

Geographical Range & Natural Distribution

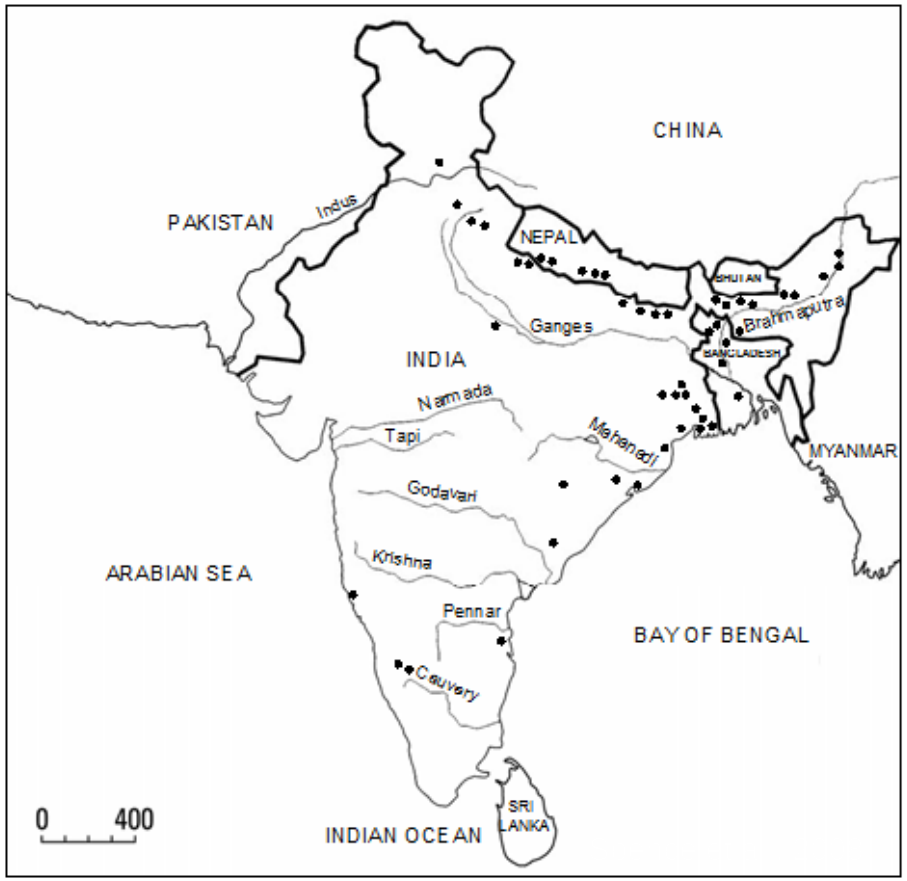

2017 - 4 meters of rain fell in 3 days


2018 – Kerala worst floods in 100 years

- Monsoon climate - important driver in zebrafish distribution
- Indian sub-continent – 4 seasons: Winter/Dry (Jan-Feb); Summer/Pre Monsoon (Mar-May); Monsoon (June-Oct); Post Monsoon (Nov-Dec)
- Monsoon results in wide fluctuations in water flow, vegetation, turbidity & temperature

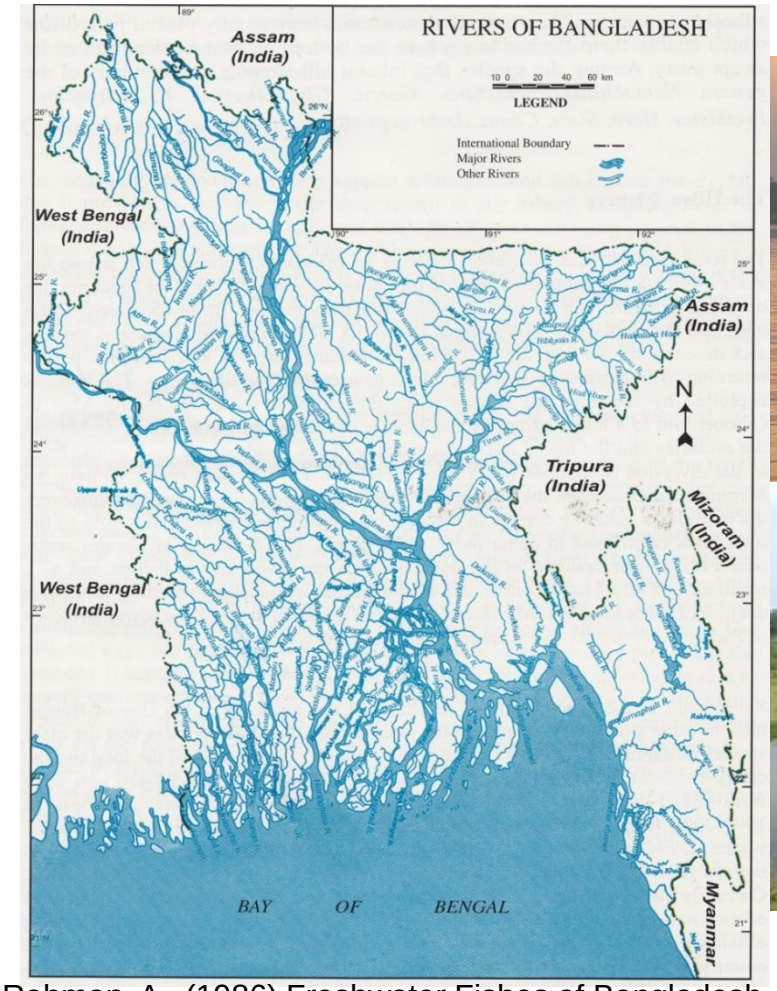
Habitat Type

Habitat Type – Case Study Bangladesh

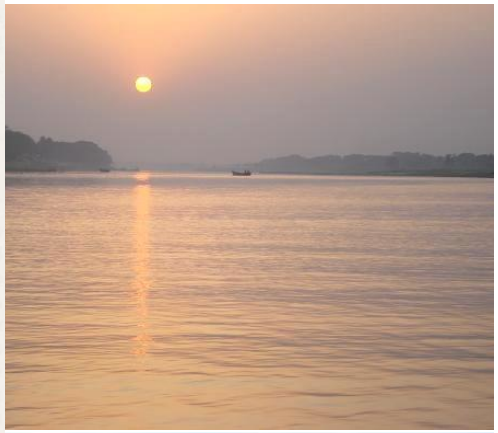


- Paull, G.C. (2008) Improving the welfare of laboratory-kept fish: https://wcmt.org.uk/users/gregorypaull2008

Habitat Type – Case Study Bangladesh



Rahman, A . (1986) Freshwater Fishes of Bangladesh



Rivers & streams cover some 5.8% total area of the country

3 major rivers - Brahmaputra, Jamuna, Meghna

Monsoon Climate – Annual flooding June to October inundates about 70% of the total land surface

Up to 4 metres of rain annually – 80 % during monsoon

Habitat Type – Case Study Bangladesh

- Monsoon climate- creates diverse wetland habitats
- Rivers, streams, lakes, ponds, ditches, floodplains, rice paddies, mangrove swamps
  - 'haors', 'baors', 'beels', 'jheels'
- Supports ~ 260 freshwater fish species, 63 prawn species and an elaborate food web

Habitat Type - Rice paddies are home to zebrafish

Danio derives from the Bengali name ‘dhani’ meaning ‘of the rice field’

Habitat Type - Ponds & ditches are home to zebrafish

Cover 3,000 – 4,000 km^2 - similar area to rivers & streams

Habitat Type - Streams are home to zebrafish

- Several studies have also reported zebrafish in relatively fast moving streams or tributaries flowing up to 18 cm/s or 0.65 km/h (Suriyampola et al., 2015)

Habitat Type - Subsistence fish farms are home to zebrafish

Butter fish (*Ompok sp.*)

largest zebrafish at these sites

Habitat Type - No zebrafish in the main river channels

Habitat Type - No zebrafish on commercial farms

Habitat Type - Not able to sample floodplains in April

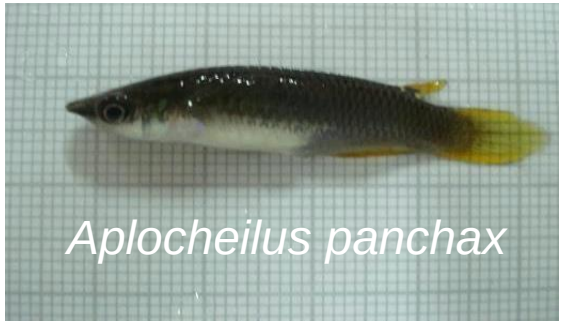
- Dry season – nutrients accumulate from animal droppings & rotting vegetation
- Wet season - nutrients rapidly enter into solution, combine with river-borne silt stimulating growth of flora & fauna
- Highly productive habitat – feeding, breeding & nursery for many fish
- Seasonally flooded floodplains ~ 58,000 km^2 out of 78,000 km^2 of wetlands

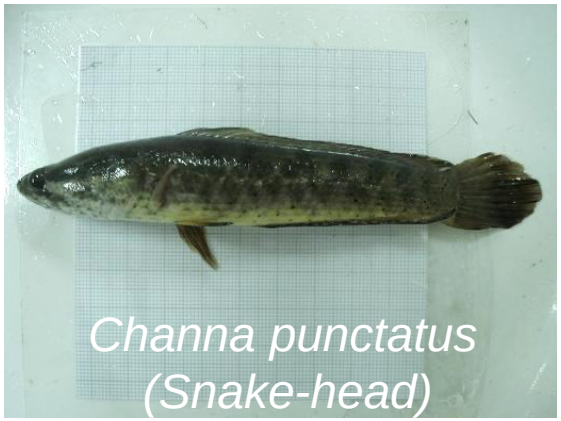
Habitat Type – Flora & Fauna

Flora - Identified over 20 aquatic plant species

- *Eichhornia crassipes, Pistia stratiotes, Lemna sp., Salvinia natans*
- Terrestrial & semi aquatic vegetation

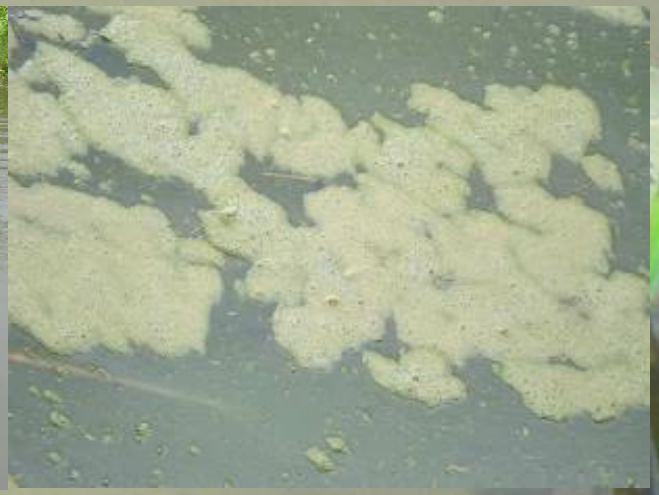
Fauna - fish species living with zebrafish





Fauna…..plus many invertebrates

Water Transparency



Site	Habitat Description	Area (m^2)	Water Depth (cm)	Secchi Depth (cm)	Vegetative surface cover (%)	Temp (°C) (April)	pH	Conductivity (µS)	Zebrafish Caught	Zebrafish fork length (mm) range & (mean)
1	Disused pond (still)	140	66	52	90	28	7.65	371	37 (93 *C. lalia*)	22-38 (31)
2	Drainage ditch (still)	1000	50	48	40	28.5	7.73	534	20 (47 *Esomus*)	28-35.5 (32.1)
3	Edge of paddy field (slow moving)	>1000	16	16	90	29	6.99	242	25 (22 panchax)	20.5-27 (23.3)
4	Stream between paddy fields (slow moving)	1 – 2 meters wide – 100's meters long	22	22	75	28	7.06	490	29 (12 panchax)	15-27 (20.9)
5	Large pond – rice and fish production (still)	>3000	35	24	<1	30.5	6.85	317	30 (279 *Oryzias*)	16-29.5 (21.1)
6	Large pond – rice and fish production (still)	>2000	50	8	<1	27	7.46	298	104 (41 *Esomus*)	20.5-30.5 (27.4)
7	Paddy field (still)	~4000	30	30	90	30.5	6.94	152	49 (>500 *Esomus*)	17-32 (24.4)
8	Small pond – subsistence aquaculture (still)	625	107	7	<1	30.5	7.53	221	38	29-40 (33.3)

The distribution and habitat preferences of the zebrafish in Bangladesh: Journal of Fish Biology (2006) 69, 1435-1448

TABLE II. Environmental variables for each sampling site: Khulna District: 1, ditch; 2, large artificial lake; 3, seasonally isolated arm of a canal; 4, pond treated with lime and rotenone; 5, semi-natural pond; 6, natural ponds; 7, village pond; 8, Bhatiaghata River; 9, isolated river channel; 10, artificial pond; 11, isolated river channel; 12, irrigation channel; 13, isolated river channel. Mymensingh District: 14, cultivated pond; 15, cultivated pond; 16, isolated pond; 17, isolated pond; 18, isolated pond; 19, Brahmaputra River; 20, Dhuno River; 21, old fish farm; 22, semi-natural pond; 23, ditch; 24, small semi-natural pond; 25, channel; 26, creek

Site	*Danio rerio* $\log_{10}$ abundance	Locality	Flow	Depth (cm)	Secchi depth (cm)	Substratum	Vegetation	Temperature (° C)	pH	Ammonia or ammonium (mg l^{-1})	Salinity	Connection with paddy	Predators
1	1	Khulna	Still	80	51	Mud		20	8	0·01	0		Yes
2	0	Khulna	Still	100	92	Mud	Yes	22	8	0·01	0		Yes
3	0	Khulna	Still	80	16	Mud		18·5	8	0·1	0·4		
4	0	Khulna	Still	73	37	Mud		20	7·4	0·1	0·2		Yes
5	0	Khulna	Still	50	50	Mud	Yes	17·5	7·6	0·1	0·2		
6	0	Khulna	Still	35	35	Mud	Yes	20	8	0·1	0	Yes	Yes
7	0	Khulna	Still	85	37	Mud		18	7·4	0·1	0·8		
8	0	Khulna	Running	125	75	Mud		21·5	8	0·1	0·6		Yes
9	1	Khulna	Still	50	19	Mud	Yes	20	8	0·1	0		
10	0	Khulna	Still	71	64	Mud	Yes	20·5	8	0·1	0		
11	0	Khulna	Still	73	52	Mud	Yes	20·3	8	1	0·4		
12	0	Khulna	Still	50	44	Mud		19	8	0·1	0·4		
13	0	Khulna	Still	76	23	Mud		22	8	0·1	0·2		
14	0	Mymensingh	Still	30	16	Mud	Yes	20	7·6	0·01	0·6		
15	3	Mymensingh	Still	15	15	Mud	Yes	20·5	8	0·01	0·6	Yes	
16	2	Mymensingh	Still	40	15	Mud		19·5	8	0·01	0·6		
17	2	Mymensingh	Still	103	30	Mud		16·5	8	0·01	0·6		
18	0	Mymensingh	Still	25	32	Mud		19	8	0·01	0·6		
19	0	Mymensingh	Running	200	197	Sandy Mud	Yes	21	8	0·01	0·4		Yes
20	0	Mymensingh	Running	130	46	Sandy Mud	Yes	21	7·6	0·01	0·4		Yes
21	0	Mymensingh	Still	80	12	Mud		22	8	0·01	0·4		
22	1	Mymensingh	Still	96	31	Mud	Yes	21	8	0·01	0·4		
23	2	Mymensingh	Still	50	50	Mud	Yes	23	8	0·01	0·4	Yes	
24	2	Mymensingh	Still	65	15	Mud	Yes	33	8	0·01	0		
25	2	Mymensingh	Still	75	15	Mud	Yes	33	8	0·01	0	Yes	
26	0	Mymensingh	Running	120	15	Mud	Yes	30	8	0·01	0	Yes	Yes

January

August

Summary of zebrafish habitat - Case Study Bangladesh

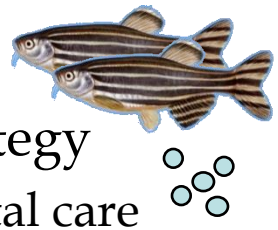
- Shallow, slow-moving or standing water bodies which may have seasonal connections to the main rivers as well as man-made lakes, ponds, & irrigation channels, constructed for fish & rice cultivation
- Most fish associated with vegetation
- Zebrafish habitat highly transitory & local distribution likely intrinsically linked to patterns of rainfall
  - Systematic field studies needed

ZEBRAFISH LIFE HISTORY

Zebrafish Life History

- Small - 25-35mm as adults, many predators - low in the food chain

- Grow and reproduce quickly
  - commonly regarded as an annual species



- Asynchronous batch spawners, "drop and go"strategy
  - large numbers of offspring produced with no parental care
  - R-strategy enables rapid colonisation of new habitats

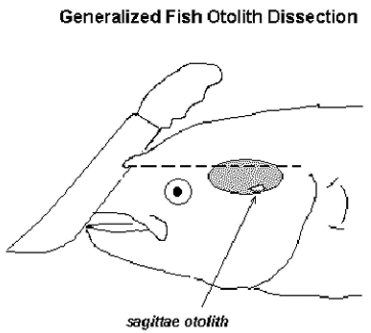
- Embryos hatch as relatively undeveloped larvae after 2-3 days
  - limited yolk reserve, begin exogenous feeding 4-6 days
  - grow up in shallow, heavily vegetated zones, rich in zooplankton
  - undergo juvenile hermaphroditism – begin life as females!

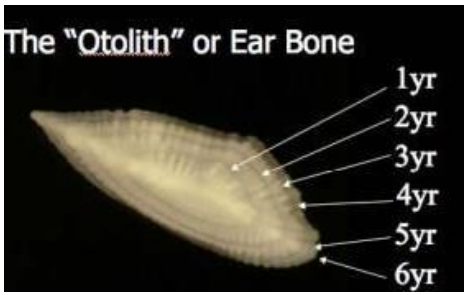
Zebrafish Life History - growth

- Wild fish collected from a single site over a 12 month period reached 25mm & sexual maturity
    - Max size recorded - 35mm
- F1 wild fish in captivity mature at 23mm ~ 10 months of age (Spence et al, 2006)
- Maturation size is comparable to domesticated strains 23.1mm (males), 24.9mm (females) (Eaton and Farley, 1974b)
- Time to maturation significantly slower in wild fish vs in domesticated strains ~ 75 days

Zebrafish Life History – growth

- Interestingly we collected fish up to 40mm in length
  - possibly larger than might be expected from an annual fish
- Fish containing mature ova collected in January
  - outside the typical spawning period
- Individuals survive to a 2nd season,
  - different cohorts?
  - generally regarded as an annual species
- Otolith study required to establish age class structure of different populations

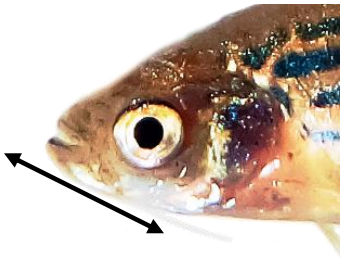




Zebrafish Life History - diet

- Gut contents revealed adult zebrafish to be omnivorous:
  - adults primarily feed on zooplankton, insects, some plant and algal materials – (Spence et al, 2008)

- Dietary composition varied between months
  - monsoon period – increase in terrestrial invertebrates (fly/dipteran larvae)
  - selectivity or seasonal availability?
- High proportion of planktonic & terrestrial items indicates zebrafish feed chiefly in the water column & at the surface
  - NB - terminal oblique mouth pointing upwards



Zebrafish Life History – behaviour

- Shoaling species
  - shoaling behaviour in adults observed in the wild
  - all sites contain fish of mixed sex & size
  - early reports suggest small shoal sizes of 5-20 (Pritchard et al., 2001)
- Potential for larger shoals where;
  - fish become confined to small water bodies (dry season)
  - presence of perennial ponds
  - where artificially rich sources of food occur
  - spawning
  - recent supporting evidence of shoals >300 strong
- Pers.comm: Fredrik Jutfelt reported groups of 5-10; dozens too hundreds and a few cases many thousands

https://vimeo.com/200660695

Zebrafish Life History – behaviour

- Much of our evidence for shoaling preference is still from laboratory based studies:
  - shoaling behaviour in zebrafish commences soon after hatching
  - zebrafish reared in isolation quickly shoal when placed together
  - cross-reared individuals (e.g. wild-type and nacre strains) preferred the colour pattern with which they had been raised
  - other studies have suggested that stripes appear a key shoaling cue
- Olfactory cues used for species & kin recognition in zebrafish
  - juveniles preferred unfamiliar family to non-kin; familiar to unfamiliar kin
  - individual recognition may therefore be as important as phenotype matching for shoaling preference

Zebrafish Life History – behaviour

- Suggested that siblings exist as juveniles but then move apart as adults – avoid inbreeding depression?
- Other factors affecting shoaling preference in zebrafish include: activity levels, nutritional/health status, sex
- NOT RANDOM – Whatever the mechanisms being played out, clear preference for shoaling & even structure within those shoals
    - shoaling preference likely involves both genetic & learned components

Summary

- Native habitat of zebrafish is extremely rich – far cry from our laboratory tanks

Environmental requirements → Water quality management
Prey selection & diet → Feeding practices
Social Behaviour → Appropriate housing
<u>Reproductive biology</u> → <u>Spawning techniques</u>