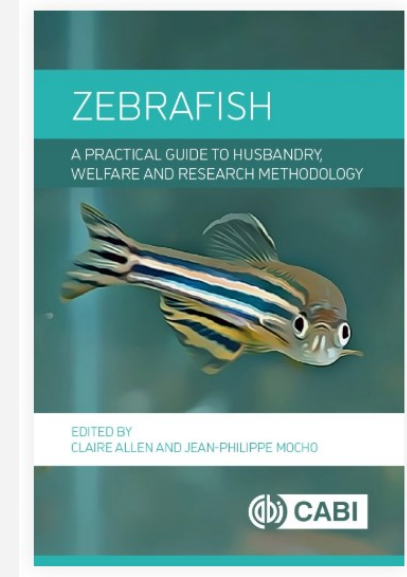


The three EEEs (Environmental Enrichment Endeavours)

C. Allen^{1,2}

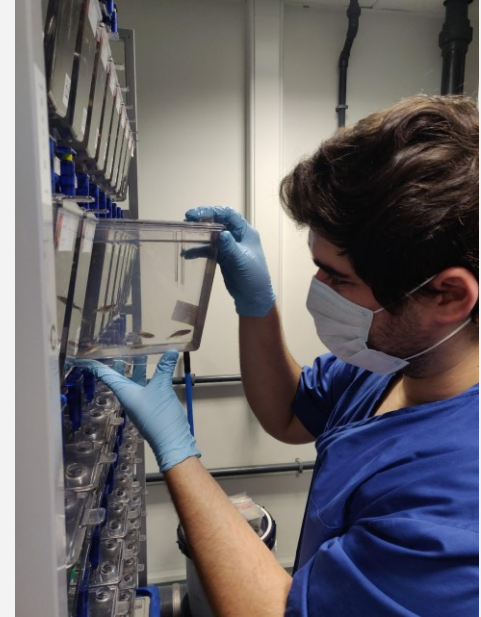
¹The University of Sheffield, Sheffield, United Kingdom,

²The University of Nottingham, Nottingham, United Kingdom.



Introduction to EE

- Moral and legal obligation
- Aim to minimize pain, suffering, distress, and lasting harm
- Meant to provide shelter, stimulation and novel experiences to avoid boredom
- Challenge of technician's daily routine and not interfering in scientific investigation
- Increasing pressure from government bodies and the general public
- Do we offer enough or are we lacking provision?



What is EE?

- Increases complexity of the captive environment
- Enhances welfare
- Promotes natural behaviour
- Decreases stress (TBD)
- Could be social contact, sensory stimulation, tank design, nutrition, physical excursion, water chemistry or novel objects



Why use EE

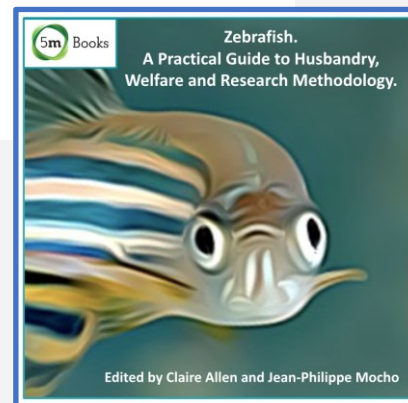
- Brambell's Five Freedoms (1965)
 - Freedom from Hunger and thirst
 - Freedom from Discomfort/provide shelter
 - Freedom from Pain, injury, disease
 - Freedom to Express normal behaviour
 - Freedom from Fear and distress



Zebrafish characteristics and housing

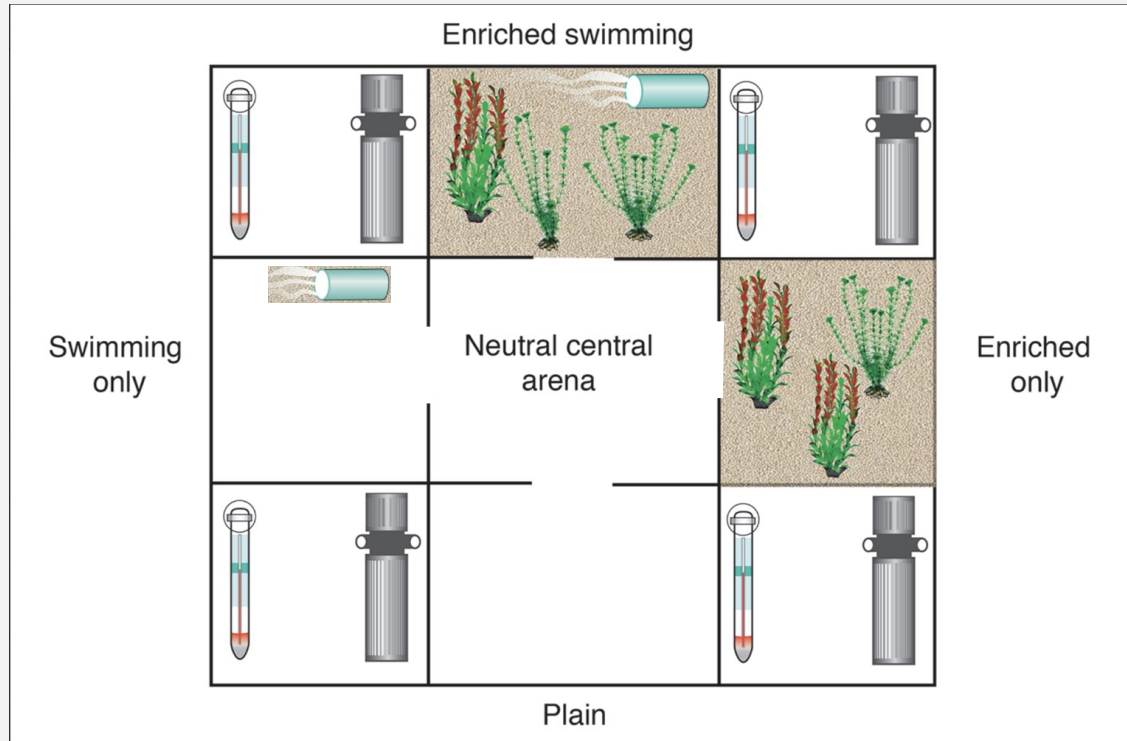


Zebrafish characteristics	Housing recommendations
Highly social species	Group housing. Avoid isolation and housing in pairs but when unavoidable provide enrichment (e.g. plastic plant) and visual and olfactory cues of conspecifics Larval and juvenile fish should be housed separately from adults House in mixed-sex groups to prevent egg over-retention in females Recommended housing density of 5-20 adult fish per litre to reduce stress Females should be allowed to spawn at least once every 2 weeks to prevent becoming egg-bound
Shoal in small, mixed-sex schools	Tank size and stocking density should allow shoaling behaviour
Dominance hierarchies form via aggressive interactions	Avoid holding fish in groups of six or less
Natural environment has abundant aquatic vegetation for spawning and cover from predation	Provide plastic plants and an area or resource (e.g. plastic tubes or large pebbles) for shelter.
Naturally forage on zooplankton and insects	Provide live food at least once a week
Olfaction and vision <u>important</u> sensory systems.	Use of gravel, image of gravel or dark substrate. Fish housed in isolation should have olfactory and visual cues of other fish to reduce stress.
Diurnal	Use of dusk and dawn lighting to avoid sudden bright light. 14:10 light: dark regime.
Preference for structural complexity within tank	Use of substrate (e.g. gravel, pebbles) and plastic plants
Use of whole water column and constant swimming	Tank design should allow swimming both vertically and horizontally. Control water flow based on the age of fish (low to no flow for larval stages and increased rates of flow for adults)

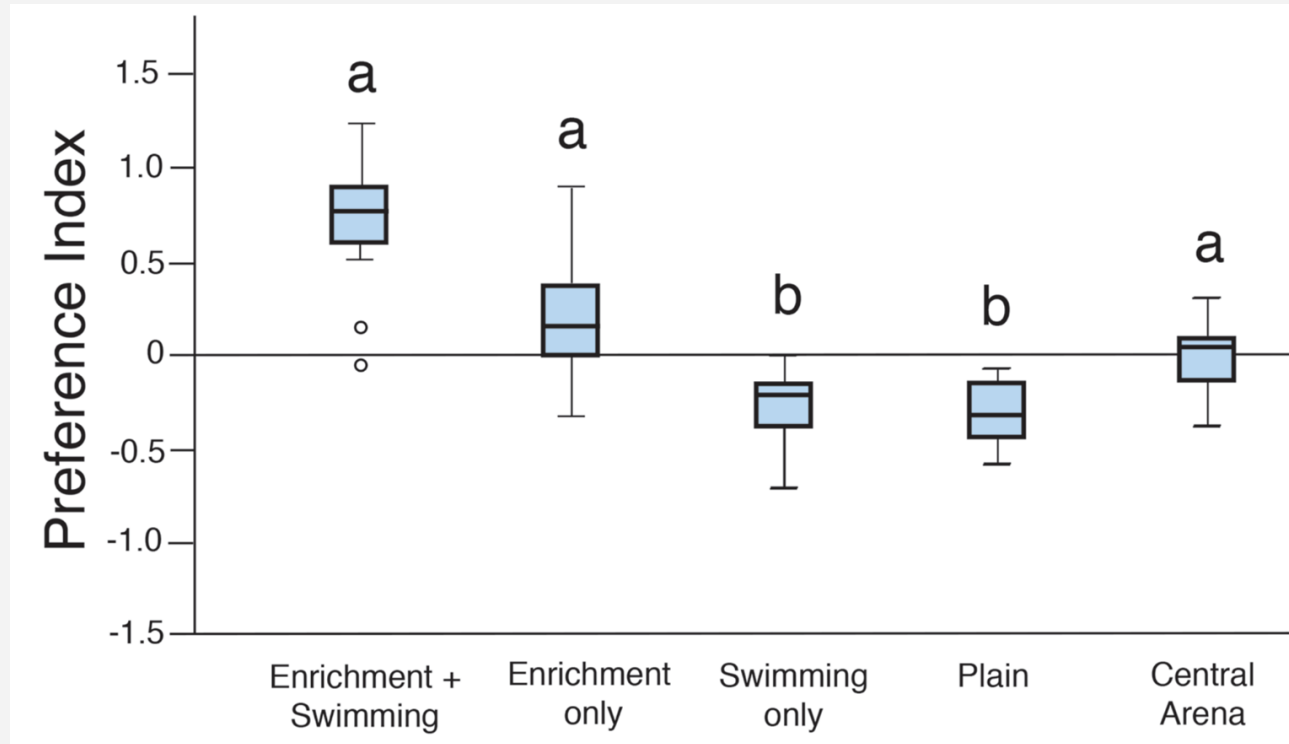


What do zebrafish prefer?

Tank design for place preference testing



What we will be discussing



What EE has been tested so far

What EE has been tested so far

- Social contact and stocking densities
 - Conflict: science requires single housing, which causes depression
 - Solution: breeding tanks or Gene-S
- Sensory stimuli
 - Auditory: classical music exposure
 - Natural noise from system
- Visual
 - Gravel/pebble photos
 - Substrate colour
 - Colour preference
- Nutrition
 - Nutritional content
 - Live vs manufactured feed
 - Frequency/schedule
- Physical excursion
 - Forced vs voluntary exercise
- Novel objects
 - Engage
 - Prevent boredom
 - Exert control over environment
 - Preference testing
 - Cause aggression?



Environmental Enrichment

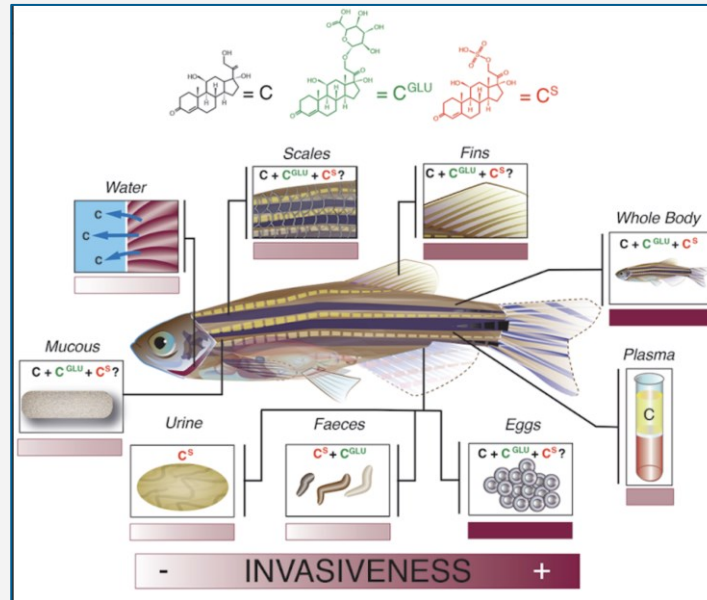
Physical (Structural)	Plastic plants (artificial or live where appropriate)	Provides shelter , hiding spots, and promotes exploration; preferred over barren tanks.
	Shelters (e.g., small tubes, tunnels, or dividers)	Offers refuge, reduces perceived predation risk, and lowers anxiety-like behavior .
	Substrate/Gravel (where appropriate)	Mimics a more natural environment and provides surfaces for foraging/exploration.
Social	Group Housing (appropriate shoal size)	Meets the basic needs of this highly social species ; single-housing should be avoided.
	Visual Barriers/Dividers	Allows for control of social interaction and reduces aggression where necessary.
Dietary	Live Invertebrate Food (e.g., <i>Artemia</i> , <i>Daphnia</i>)	Stimulates natural foraging behavior and provides dietary variety, acting as a form of enrichment.
	Varied Feeding Schedule/Location	Encourages exploratory behavior and prevents predictable monotony.
Occupational	Water Flow/Current	Gives the fish " something to do " (i.e., swimming against the current) and stimulates activity.
	Novel/Moving Objects	Provides a dynamic environment to encourage investigation and activity.
Sensory	Visual Enrichment (e.g., images outside the tank)	Provides visual stimulus and variety beyond the confines of the tank rack.
	Auditory Enrichment (e.g., controlled, non-stressful sounds)	Can provide sensory input, though implementation requires careful control to avoid stress.



Measuring impact of EE

Measuring impact of EE

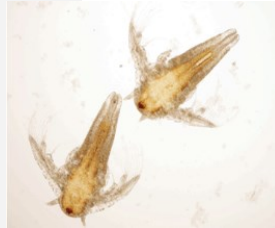
- Cortisol readouts
- Many methods, some invasive



What we recommend

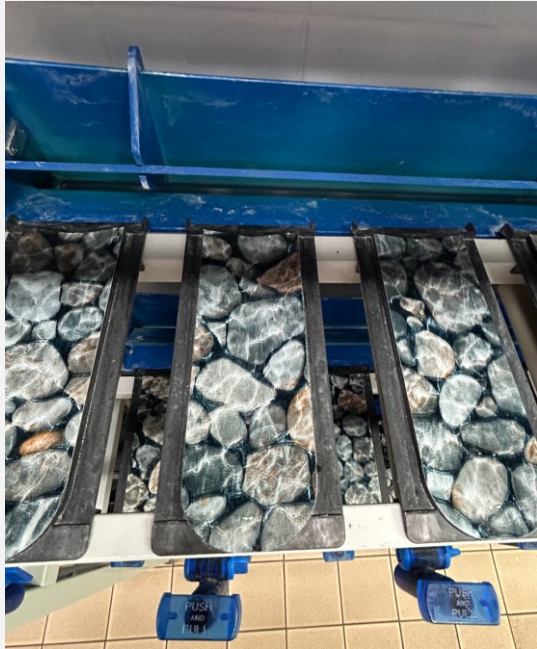
What we recommend (for all research)

- Live feed
- Companionship - breeding
- Gravel/pebble images
- Flow rates
- Lights with dawn/dusk

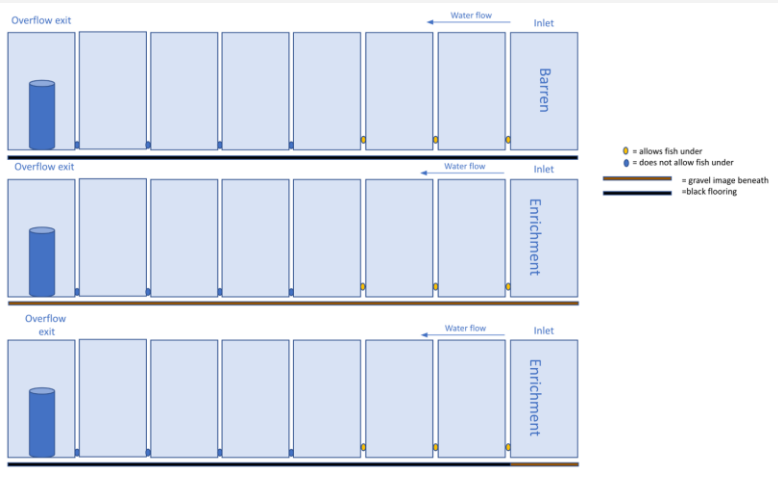


or do we?.....

Gravel images have been introduced



Setting up a tank design for place preference testing



- 20 wildtype fish added and acclimated to tanks.
- Fish swim underneath 4 tanks
- All glass tanks
- Inlet on right
- Overflow outlet to left
- All 4 tanks are fed
- Gravel base varied



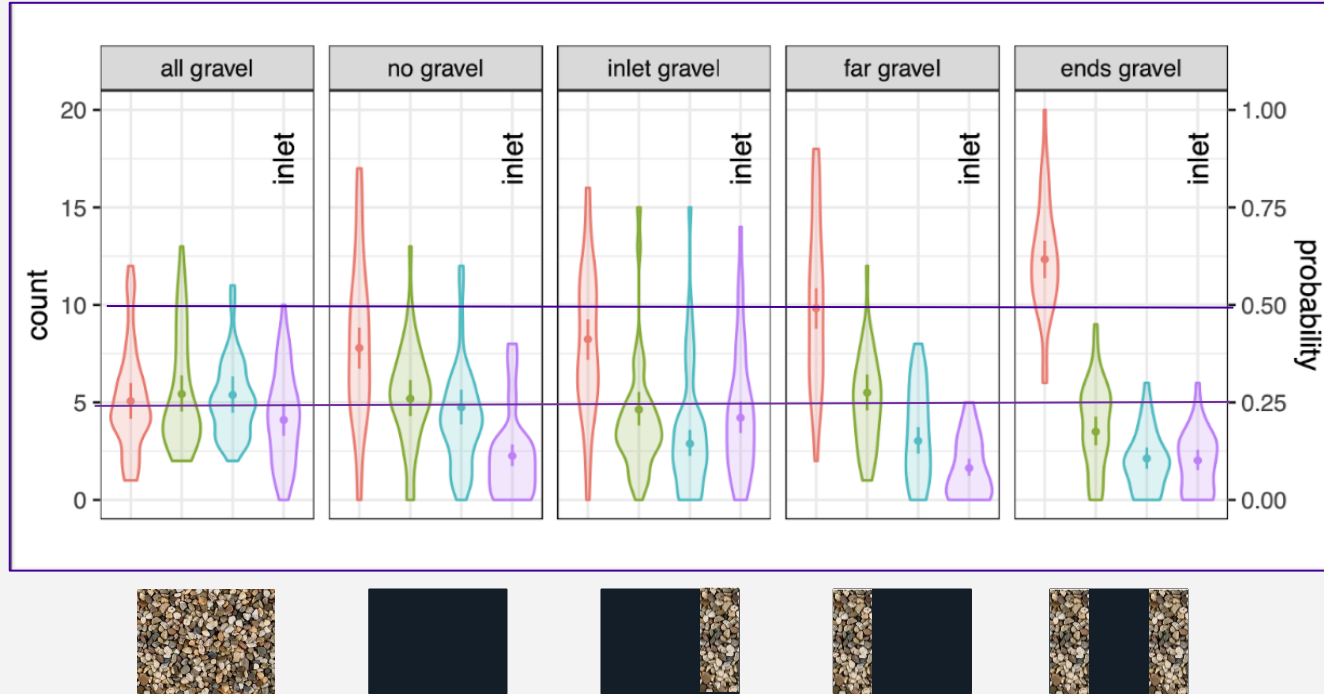
Locations logged am and pm (usually 30 days)



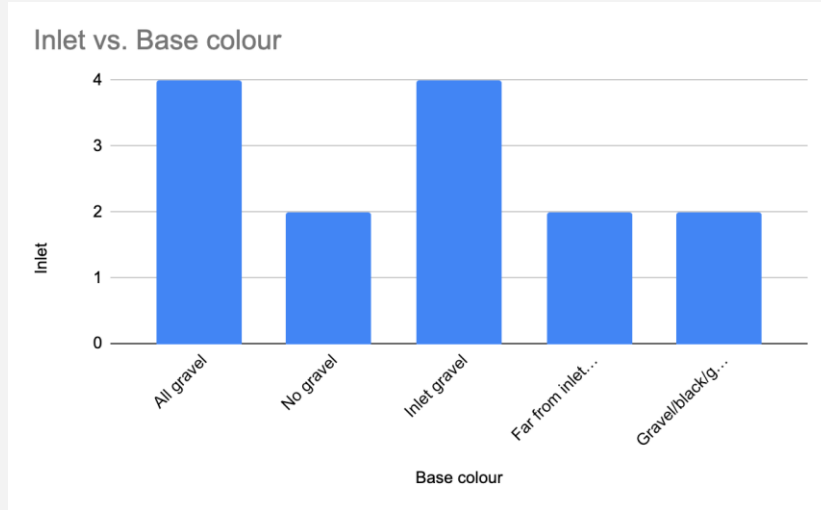
	Inlet					Inlet					Inlet					Inlet					Inlet			
	K125	K126	K127	K128		K115	K116	K117	K118		K015	K016	K017	K018		J414	J413	J412	J411		J314	J313	J312	J311
am	5	4	5	6		10	6	4	0		8	4	4	4		13	2	2	3		13	3	3	2
pm	5	6	3	6		5	6	6	3		11	7	2	0		5	8	6	1		10	8	2	0
am	8	4	3	5		9	5	3	3		2	2	2	14		12	2	2	4		13	4	0	3
pm	4	9	5	2		7	7	4	2		11	3	4	2		11	6	1	2		11	6	1	2
am	2	4	4	10		8	8	4	0		6	4	2	8		10	2	4	4		11	5	2	2
pm	4	4	5	7		10	4	5	1		6	4	7	3		6	8	3	3		7	6	4	3
am	5	3	5	7		5	6	9	0		7	4	4	5		10	7	7	2		17	1	1	1
pm	11	6	3	1		10	3	5	2		5	4	10	1		6	6	4	4		12	1	5	2
am	6	2	7	5		1	13	6	0		6	3	8	3		9	6	5	0		13	3	1	3
pm	7	4	5	4		8	6	3	3		5	7	2	6		11	4	2	3		11	5	2	2
am	9	3	4	4		6	4	3	7		6	3	3	8		8	6	6	0		12	3	1	4
pm	5	7	3	5		10	5	4	1		6	7	5	2		7	4	5	4		10	5	1	4
am	4	4	4	8		4	10	4	2		12	3	0	5		15	2	2	1		11	3	1	5
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pm	4	4	6	6		10	2	3	5		11	5	3	1		17	1	1	16		16	4	0	0
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pm	1	9	10	0		5	5	7	3		10	4	2	4		5	7	5	3		12	6	2	0
am	2	8	8	2		15	3	1	1		4	15	0	1		6	5	4	5		14	5	1	0
pm	4	11	3	2		8	5	5	2		9	2	7	2		6	4	5	5		12	6	2	0
am	1	3	8	8		13	5	0	2		11	6	2	1		11	3	3	3		13	4	2	1
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am	7	4	5	4		16	0	2	2		8	4	4	4		10	8	2	0		12	3	2	3
pm	1	13	5	1		5	8	6	1		7	1	4	8		10	4	6	0		12	5	1	2
am	6	4	6	4		6	4	3	7		11	5	2	2		18	1	0	1		17	3	0	0

= 10 + fish
 = 6-9 fish
 = 1-5 fish
 = 0 fish

Initial Location Findings



Inlet Location Findings



Inlet tank shows “little/no preference” if barren areas or unenriched tanks

Gravel and “inlet only gravel” have the same occupancy - significantly increased compared to other tanks - all inlets not preferred

These fish maybe the subordinate animals - less favourable tank, but do have options and space.

Gravel vs flow of inlet water

Gravel across the 4 tanks sees fish using the whole space with even distribution

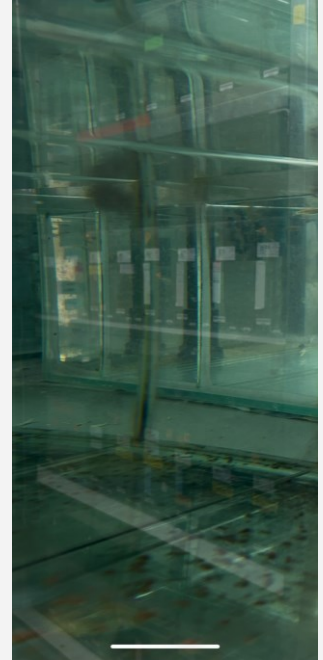
The inlet is always the least favourite tank (was the flow too high?)

When the furthest tank has gravel, inlet gravel is not preferred

Gravel slightly pulls the fish away from a barren outlet

Without gravel the fish go into the outlet end and not the inlet

Strong preference for the exit?



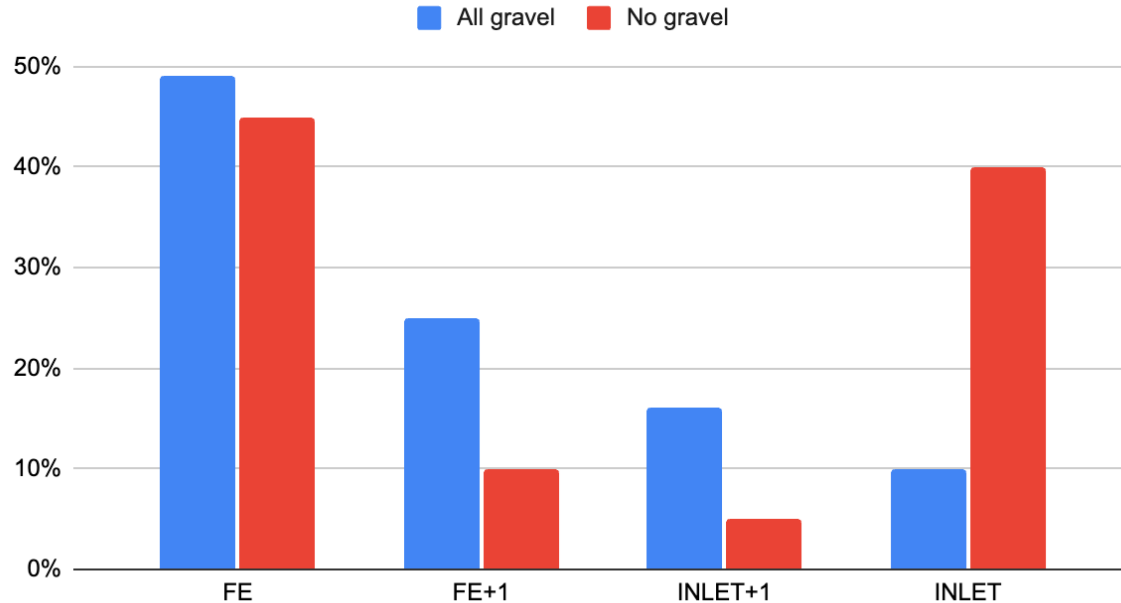
Further investigations

Tank scenario	AM tanks with barren areas	AM. enriched (all gravel)	PM with barren and gravel options	PM all gravel or no knowledge of gravel
high flow	2-5 FE	1 INLET+1	3-5 FE	1-2 FE+1
low flow	2-5 FE	1 INLET+1	3-5 FE	1-2 FE+1
blocked/low flow	all FE	acclimitisation shifts fish	3-5 FE	1-2 FE+1
blocked/high flow	2-5 FE	1 INLET+1	3-5 FE	1-2 FE+1

- Month long countings - same fish
- Enrichment moves fish towards inlet- particularly in the AM
- AM is different to PM
- PM “all barren” mirrors enriched
- Combinations of enriched with barren areas sends the majority to the outlet in both AM and PM

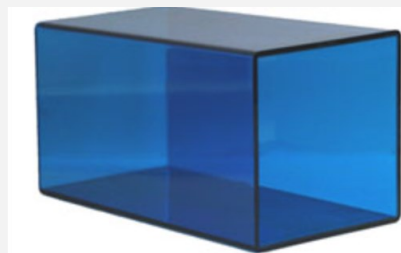
Further investigations - Mass breeding results Blocked/high flow

All gravel and No gravel

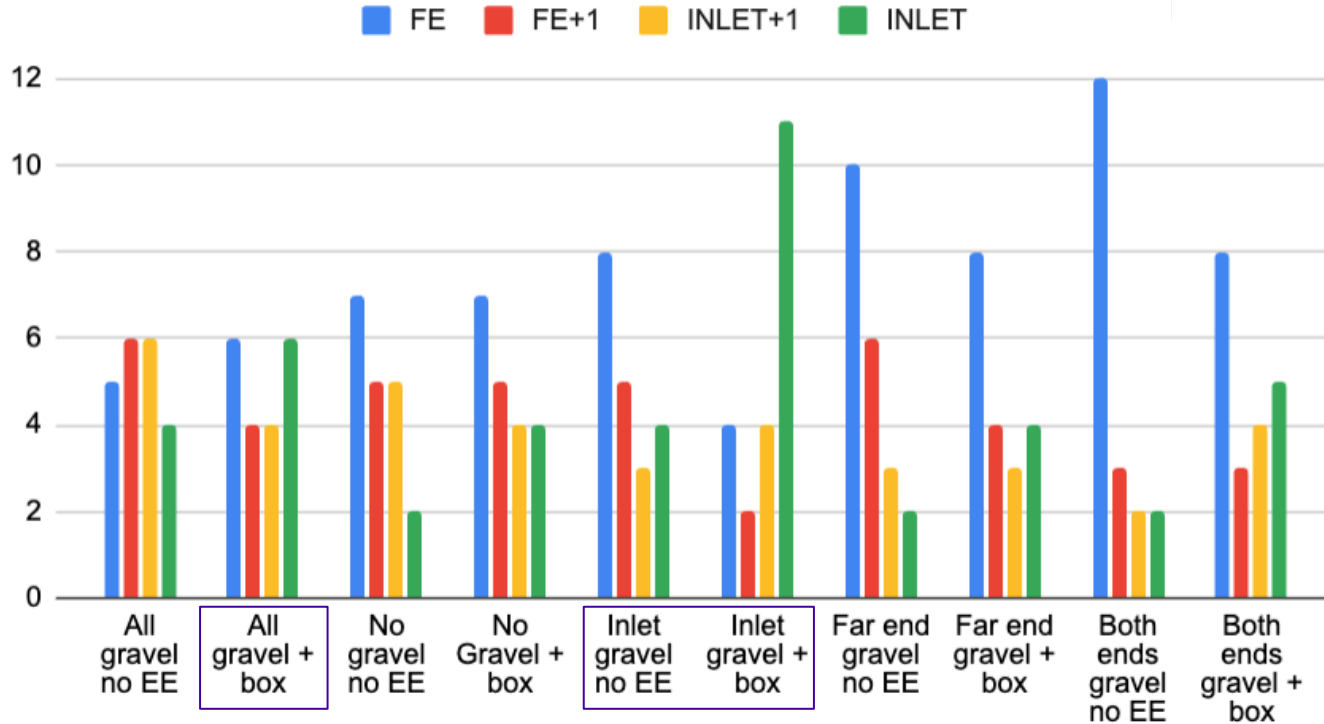
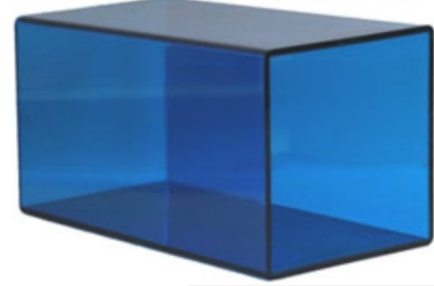


Preliminary results
Follow occupancy
patterns
Far end favoured
Unenriched like ends

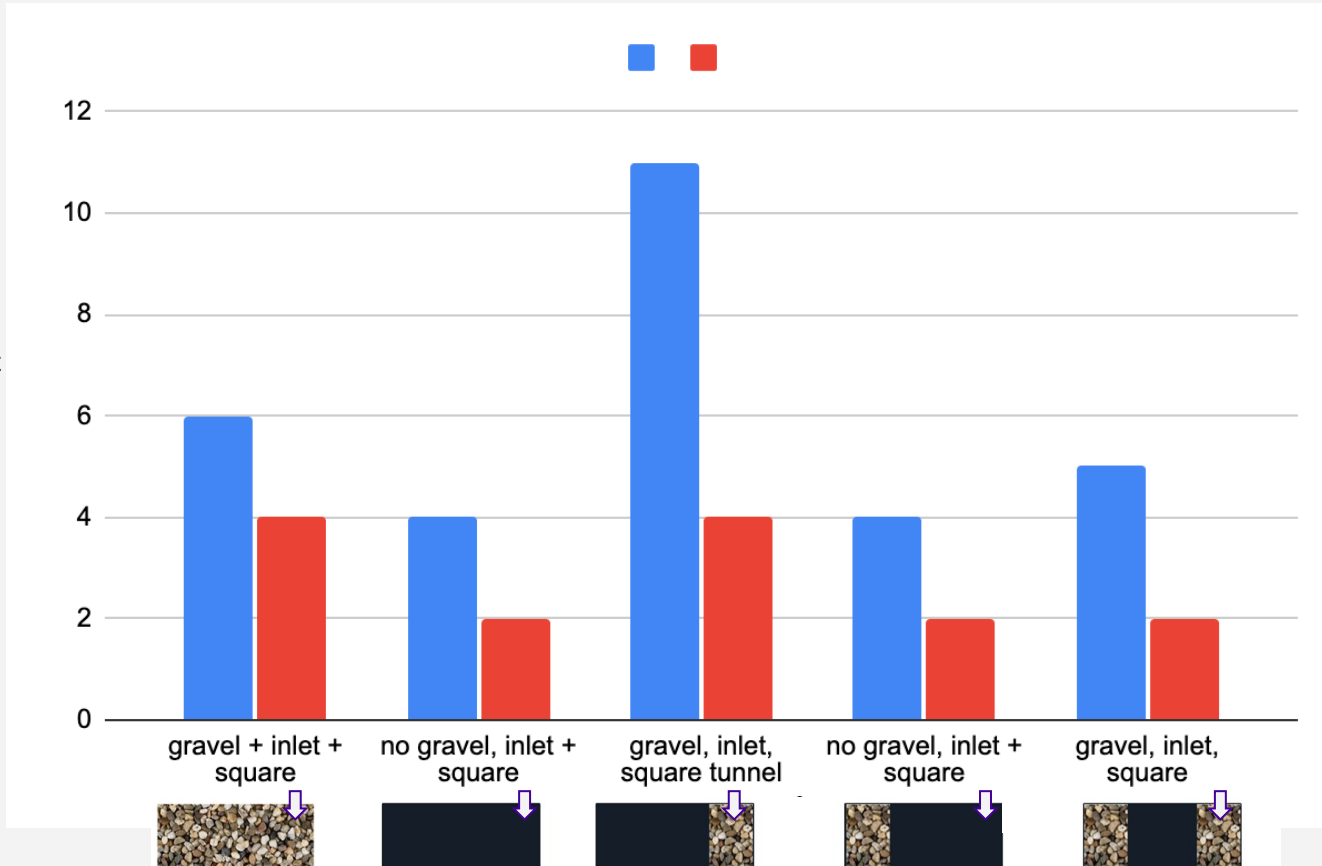
Physical enrichment tests



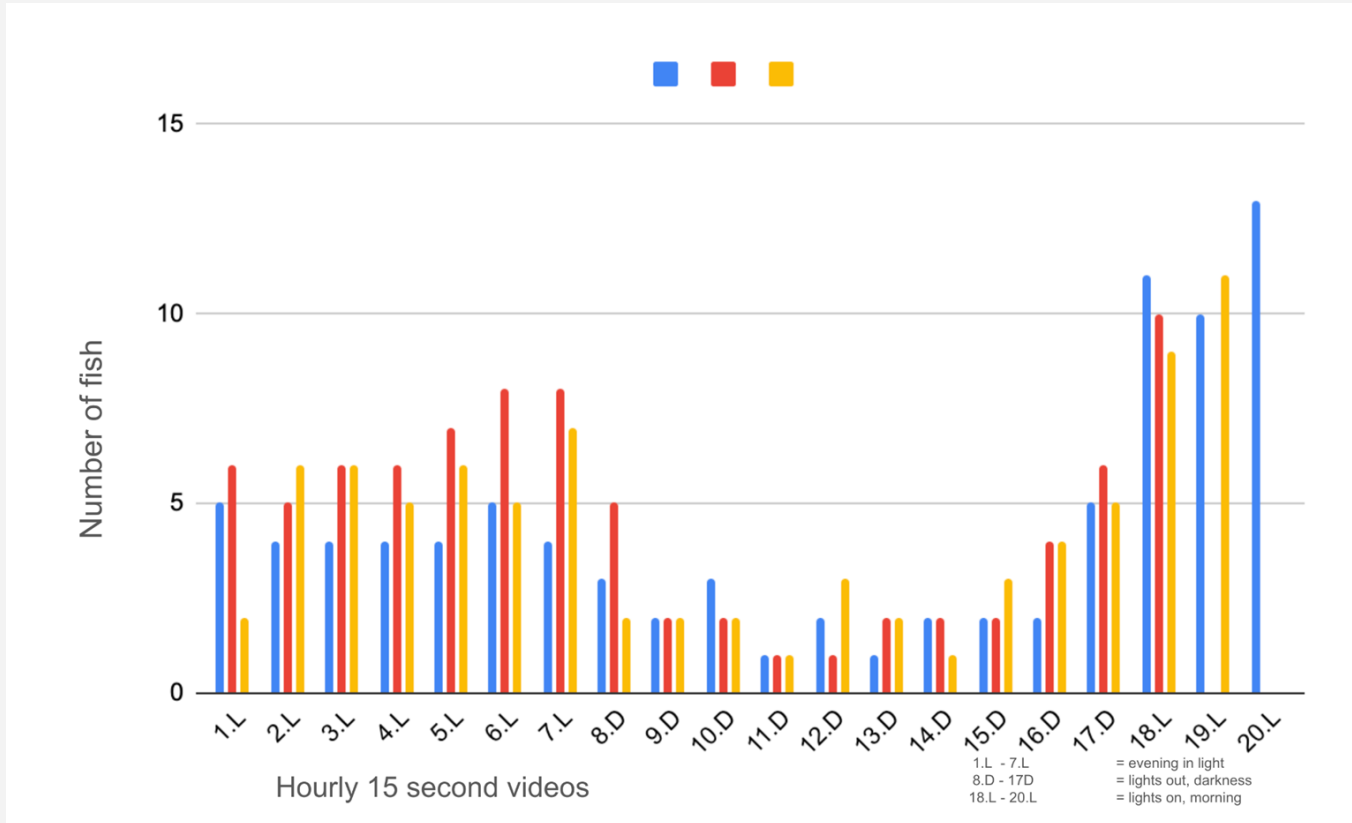
Enrichment in inlet tanks



Inlet with/without square

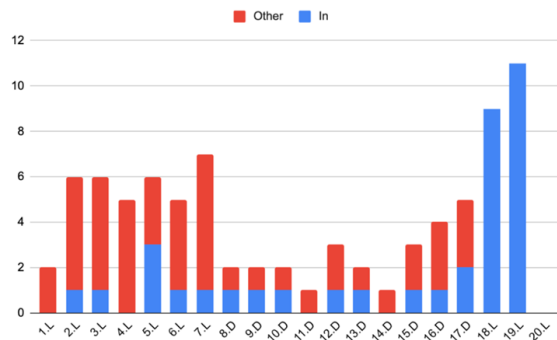
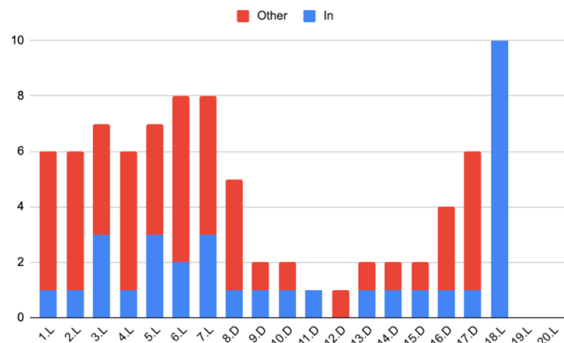
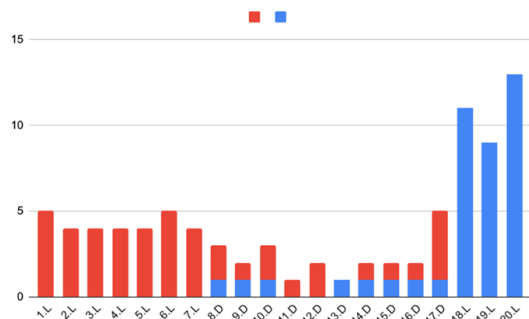


Situation at night - inlet, gravel and square



In the square or in the area around

In/other



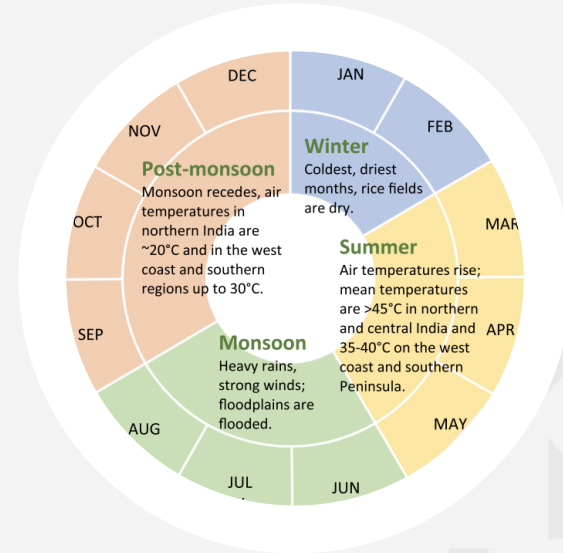
Conclusions

- Fish like space and are seen to move between tanks often
- They are a very active species but not by exercising in direct flow
- Rarely see all fish in one tank or no fish in one tank
- **Strong preference for gravel demonstrated**
- Preference does not seem to be influenced by “exit route”
- Flow is tolerated more with increased enrichment
- Different behaviours are seen at night with “guarding/dominant fish” controlling it
- Zebrafish anticipate lights coming on and like breeding in the square blue rat box

Discussion

- Optimal animal care = reproducible data
- Cannot replace natural experiences
- Fecundity and survival may not indicate optimal well-being
- Proponents say complex environments yield better results
- Adverse effects must be managed/observable*
- GAA lines must remain genetically pure*
- Consider acclimitisation
- Consider subsequent indifference

Future developments



Sounds of India

Seasonality
Auditory
Space to swim - Exercise
Holiday tanks
Physical enrichment

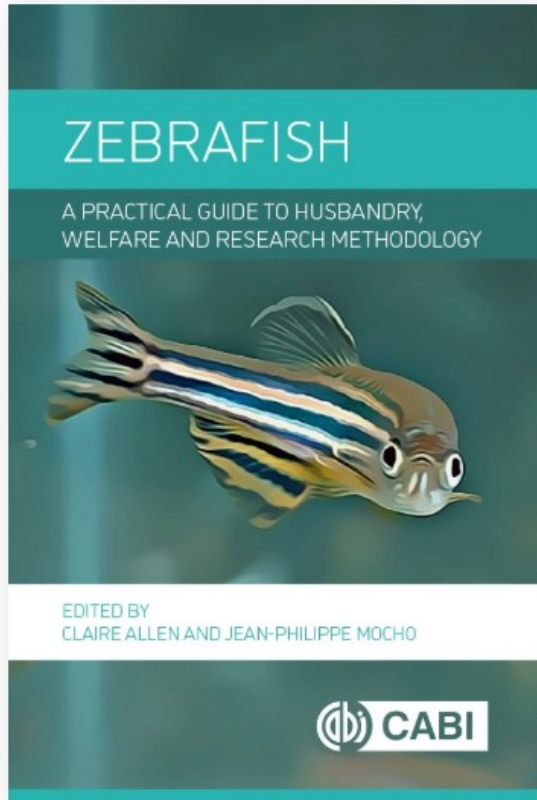
Paull *et al.*, 2023



University of
Sheffield



University of
Nottingham
UK | CHINA | MALAYSIA



Thanks to my colleagues @ both
Sheffield and Nottingham
for their tireless focus on zebrafish care

Special thanks to the authors of the EE
chapter

Mollie Millington, Lynne Sneddon and Christian Lawrence