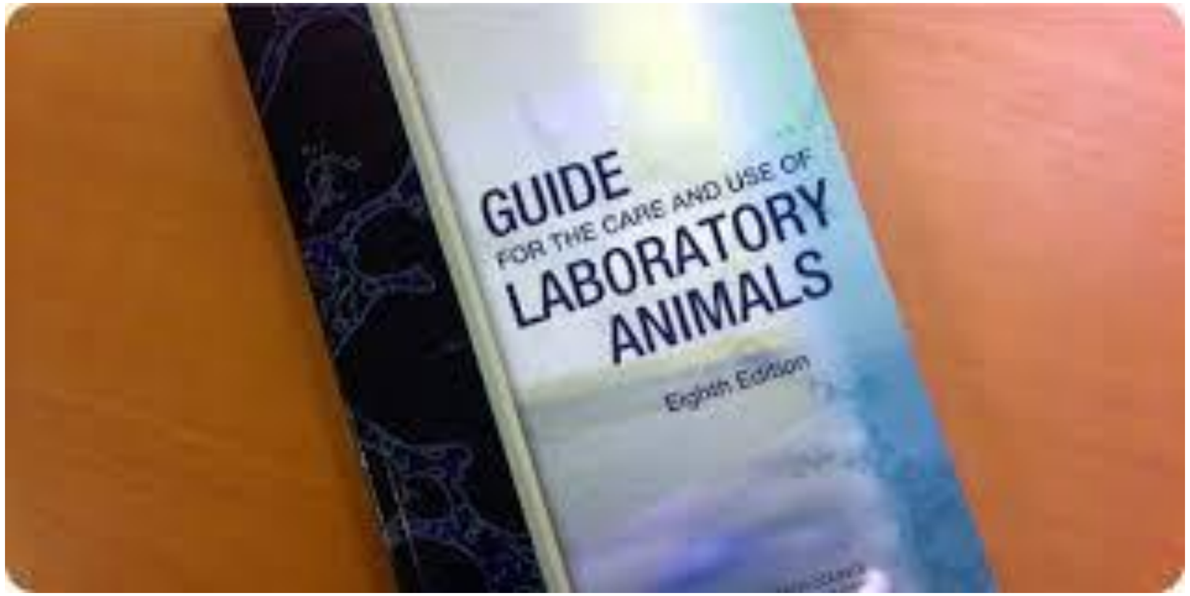
Environmental Enrichment in Zebrafish

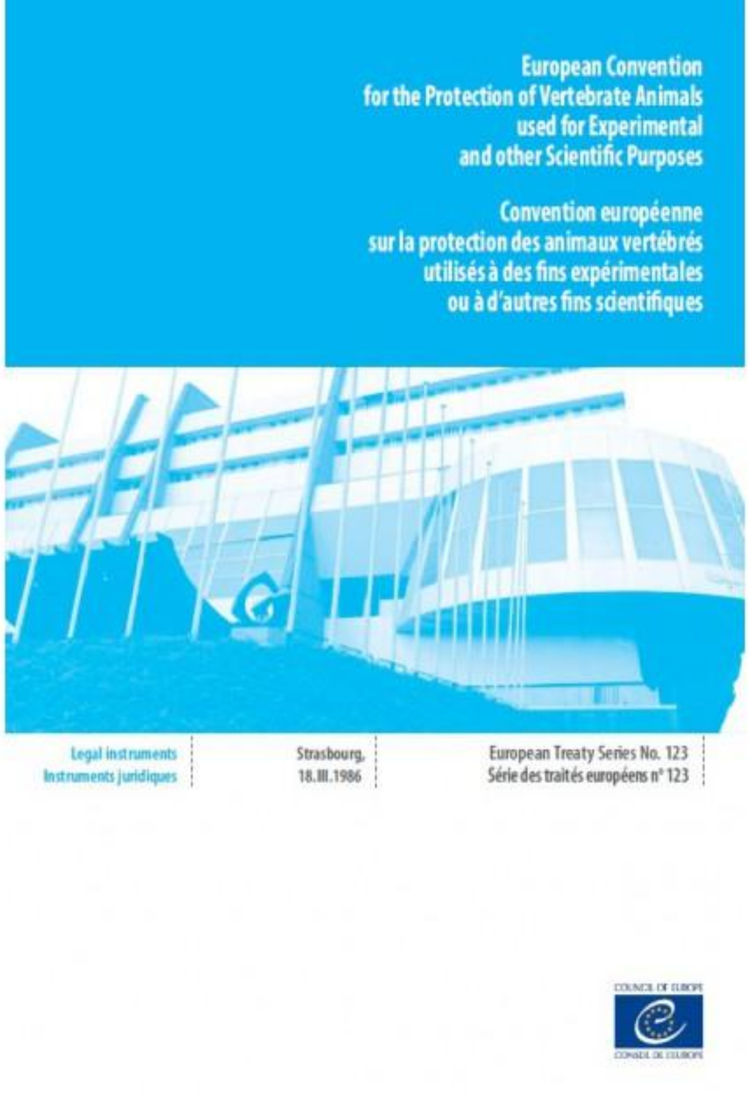


The primary aim of environmental enrichment is to enhance animal well-being *by:*

1) *Sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors*
2) *Promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics*

European Convention # 123

- All animals should be allowed adequate space to express a wide behavioural repertoire
- Animals should be socially housed wherever possible and..
- Provided with an adequately complex environment within the animal enclosure to enable them to carry out a range of normal behaviours.
- Consideration should be given to the potential impact of the type of accommodation, and that of the environmental and social enrichment programmes, on the outcome of scientific studies, in order to avoid the generation of invalid scientific data and consequential animal wastage.



Current Techniques

Table 2 Commonly used techniques of environmental enrichment within the home aquaria (modified after Handley[3])

Enrichment	Potential advantages	Potential disadvantages
Substrate	• Provides extra biological filtration • Anthropomorphically, it gives fish a natural substrate (but species specific) • Provides material for some species to exhibit spawning behaviours, building nests or territory marking • Different sizes and colours of substrate can offer a choice of background against which some species may prefer to live	• Can cause high organic loading • Time consuming to clean • Hard to remove certain parasitic diseases • Dead fish may be trapped causing ammonia spikes • Eggs are hard to retrieve • Camouflaged fish may be difficult to observe, obscuring early disease symptoms, or mortalities • Increase in surface area may be significant in regulatory testing • May alter water chemistry, e.g. releasing metals and changing hardness
Pipes and other cave- like structures	• Provides cover for small fish • Provides a spawning substrate • Easy to clean • Provides sentinel objects to aid navigation and mark territorial regions • May provide optical barrier to provide a refuge	• May cause aggressive territorial behaviour • May absorb certain test chemicals resulting in reduced test concentrations • Ceramic structures may cause a hazard if they are destabilized by large fish or undermined by excavating species • May obscure the operators view of the fish and delay early observation of disease, etc • Plastic manufacture may include biocides or endocrine disrupting chemicals such as plasticizers and flame retardants, which could leach out
Plants	• Provides cover for some fish • Provides a spawning substrate that may be better than plastic pipes for safeguarding eggs for some species • Plastic plants are easy to clean • Anthropomorphically acceptable as 'natural'	• Fish may become entangled • May obscure the operators view of the fish and delay early observation of disease, etc • Natural plants may be a source of other organisms • Artificial plants may have similar problems to plastic pipes (e.g. leaching of toxic substances)
Recirculation compared with flow through designs	• More consistent water quality for specific species. Potentially slower rate of change of chemistry parameters • Lower water usage • Can sterilize water before reuse • Easy to change water temperature to induce breeding	• Recirculation requires more maintenance effort than flow through • Recirculation systems can be noisy, which may affect fish early life stages, or induce long-term stress • Disease organisms are not easily washed away • Disease treatments difficult to remove • Biological end products such as nitrogenous compounds must be diluted away in the long term
Water movement	• Suitable for specific species • Potentially life stage specific • Pulsing flows provide a more realistic situation and potential respite	• High water usage if on flow through • May be inappropriate for some species and life stages • Additional noise and vibration may add to chronic stress. Recirculating pumps may require maintenance
Artificial light	• Potentially simulate natural light to species relevant levels • Defines night and day and gives fish the required cue by which to set diurnal and seasonal cycles physiologically and behaviourally • Day-length, dawn, dusk and moon light phases are a cue for spawning in many species, and may reduce startle response to lights on/off	• Few home aquaria have timed light phases. When combined with the ambient light of the room they may simulate perpetual long summer day-length • Even fewer home aquaria have phased light to simulate sunrise/sunset and reduce the potential startle response of being plunged into darkness or bright light
Co-specifics	• Housing in groups for appropriate species • Appropriate stocking densities relevant to ontology • Appropriate stocking densities may reduce aggression	• Species specific. Some group sizes of densely shoaling species may be too small to obtain benefit in even the largest aquaria • Group size may be inappropriate to species or life stage, or the scientific intention

Table 2 Continued

Enrichment	Potential advantages	Potential disadvantages
Dither fish	• Small shoals of small species may be used as a companion to larger more 'nervous' or 'skittish' species • The rationale is that the larger species use the smaller more numerous ones as sentinels • Multiple life stages of a single species may be important for social interaction and learning • Dither fish may be housed in adjacent aquaria in full view of the subject fish	• Risk of piscivory • Multi-species aquaria may complicate research or test conditions • Species combinations may not be regionally relevant increasing the risk of disease in naive species • Intra-species interactions may not be benign and behavioural and even chemical signals between the species may not have the intended result
Live food and a varied diet	• Active hunting/foraging instincts invoked • Variation in food target • Potentially improved nutritional balance • Enriched live foods may provide both improved nutritional value and different chemosensory stimulus • Reduce chance of entrainment to a single diet	• Live food must not introduce pathogens • Continuously varying food type can reduce the overall feeding of some species that have evolved to specific prey types and in some cases limited size ranges and generally do not consume an omnivorous diet
Fish–human interaction	• Training of fish to respond to the presence of a human – for example, koi carp coming to the keeper to be 'petted' and stroked. Fish trained in this way may be more easily examined and handled without anaesthesia • Familiarization with nets, etc. to reduce impact of future acute stresses	• May not be desirable if the scientific intention is to observe behaviour • Reinforcement of behaviour with food reward may not be desirable

From: Key issues concerning environmental enrichment laboratory-held fish species (2009)

Minimize Research Variables
How to Evaluate Well-being?

- Several indicators needed for a complete picture of welfare status

- Evaluation of behavior of zebrafish
  - Natural history as initial guide
  - Know species-typical behaviors
  - Know how to evaluate changes

- Physiologic measurements
  - Reflective of psychologically well being
  - Know “standard” measurements
  - Know how to evaluate changes

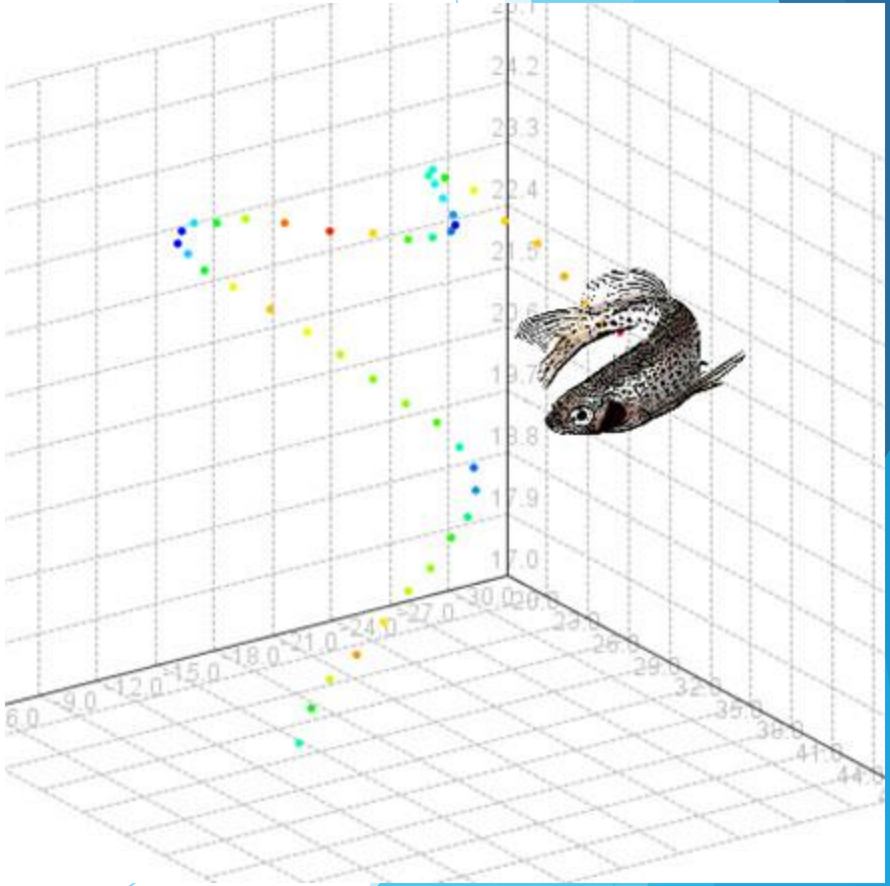
- Implementation based on scientific principles and practical application

Evaluation of Behavior

- Need to assess positive and negative effects of proposed enrichment technique
  - Need to quantify results
  - Assume less anxiety is preferable

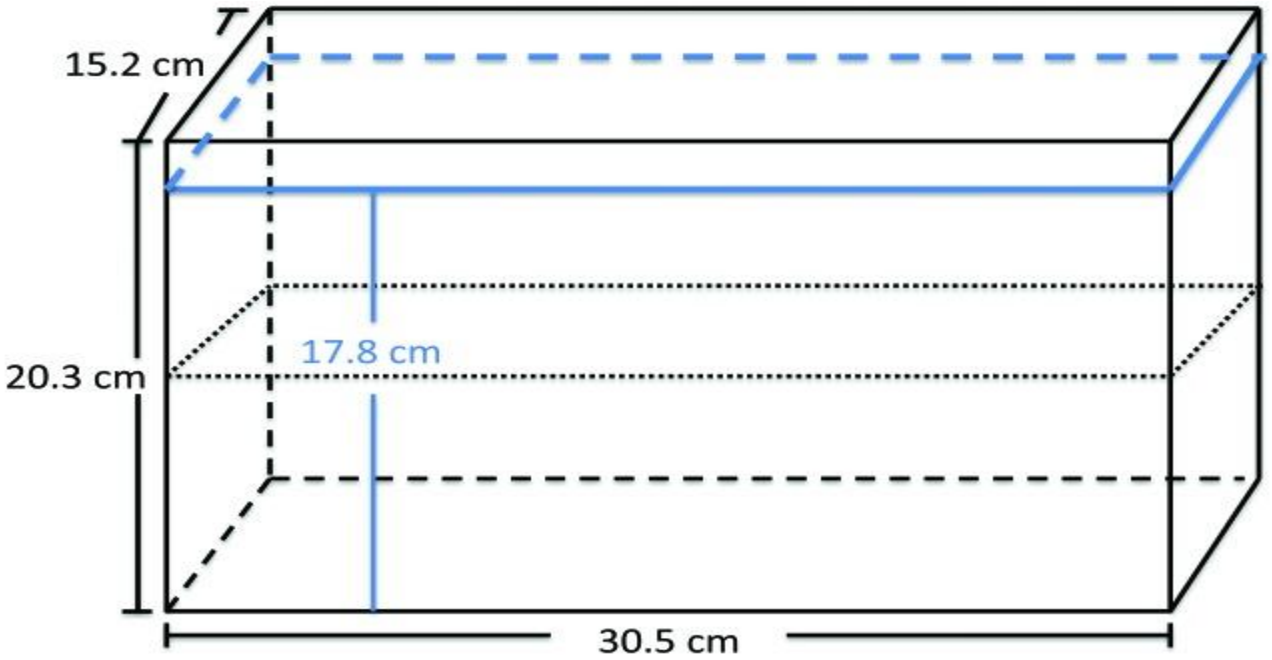
- Changes in behavior
  - Abnormal swimming patterns
  - Charging or fin-nipping
  - Freezing bouts

- Testing Methods
  - Tank-diving assay/Novel tank test
  - Light-Dark test
  - Preference testing
  - Motion detection
    - Muscle activity via telemetry

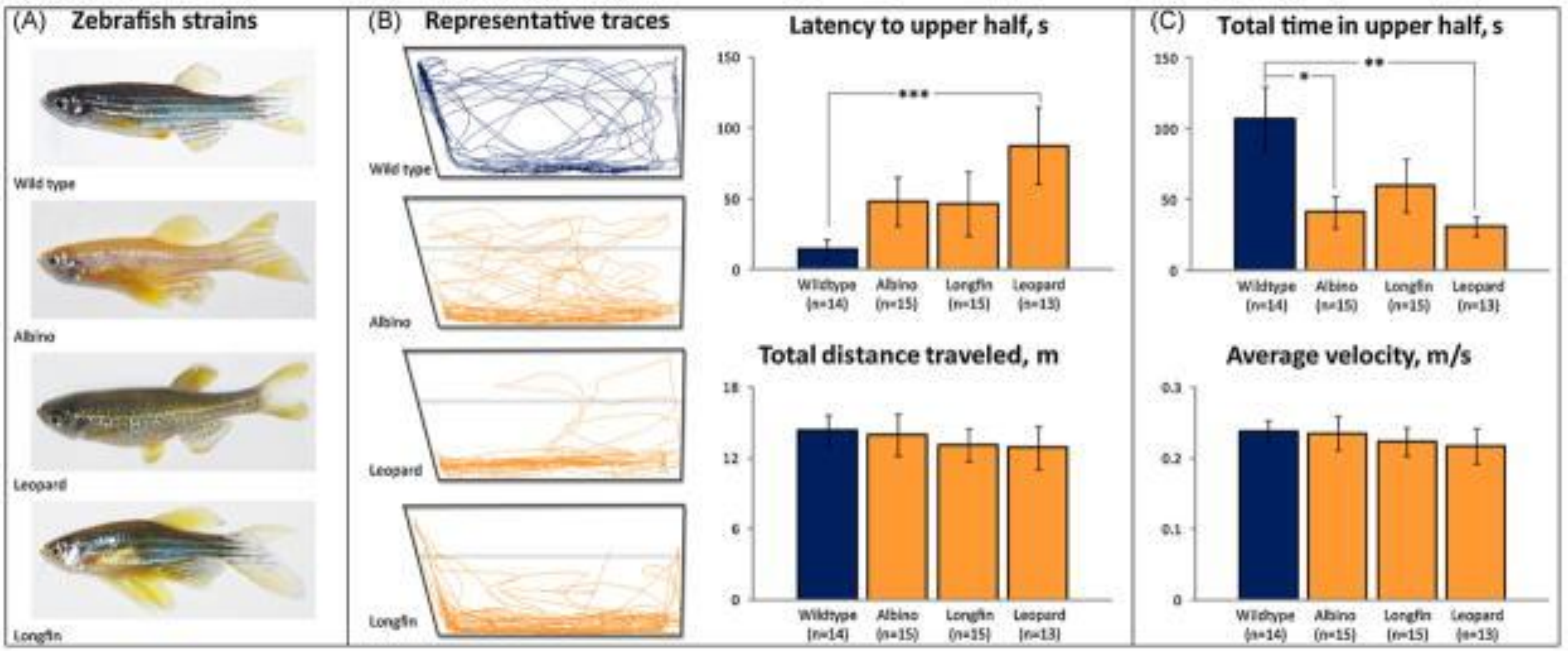


Noldus Tack 3D for fish

Novel Tank Test/ Tank Diving Assay



- Initially linger at sides and bottom; compared to thigmotaxis in rodents
- Prolonged latency to enter upper ½; reduced time in upper ½ and increase of erratic movements and freezing associated with anxiety
- Scored over give period of time

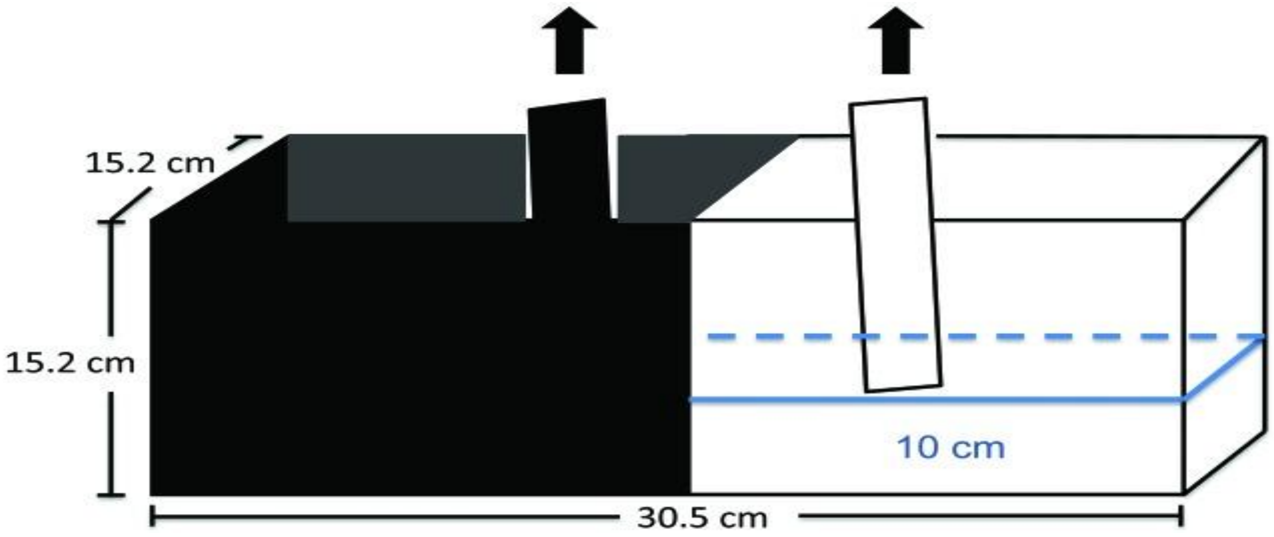


Strain differences in zebrafish novel tank diving test behavior. Different strains of zebrafish used in this study: (A) display specific patters of their exploratory behavior, as illustrated by representative phenotypic variations in swimming behavior for 3 min, (B) and behavioral endpoints and (C) analyzed using video-tracking software (CleverSys Inc.). *P < 0.05, **P < 0.01, ***P < 0.005 vs. wild type strain, *U*-test.

From: Egan, R.J. *et al*. 2009

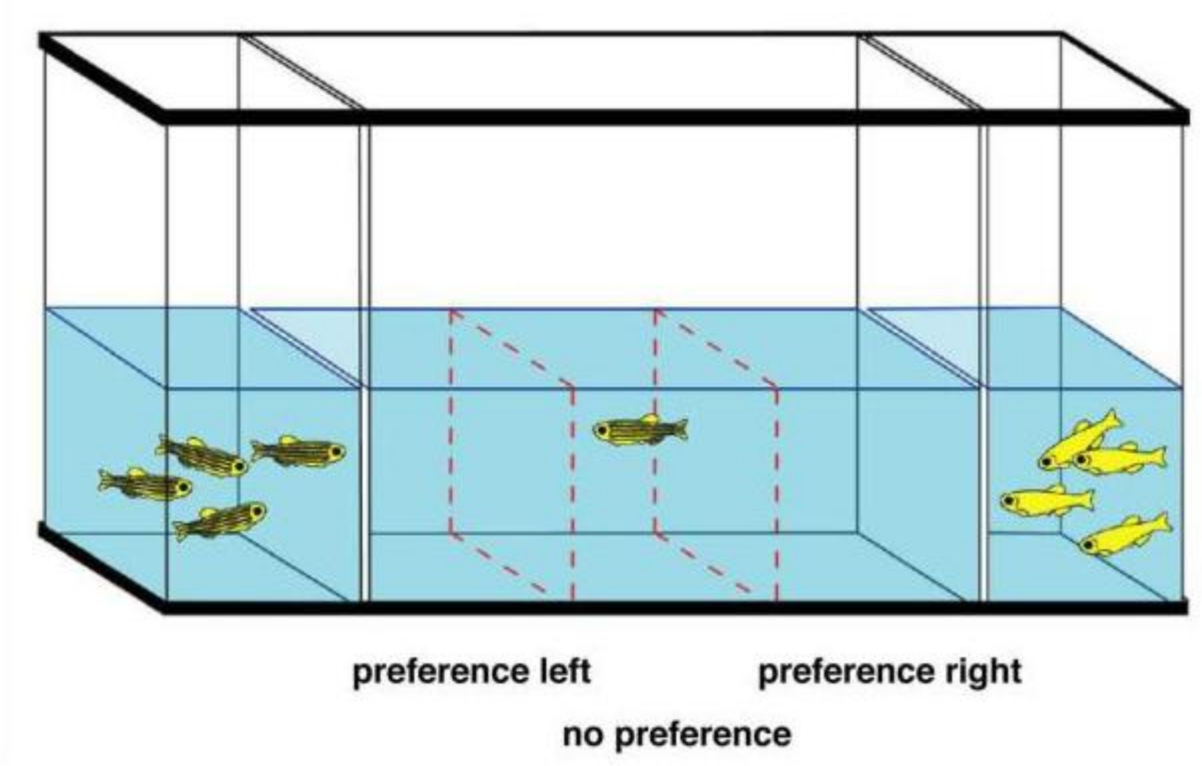
Light-Dark Test

scototaxis protocol



- Zebrafish typically are scototaxic
- Greater time in dark associated with increased tendency toward anxiety-like behaviors
- # of transitions to white compartment, total time in white compartment, number of shuttling events (< 1 s into white)

Preference Testing



Engeszer et al, 2007

Evaluation of Physiologic Measurements

- Health
  - General body and fin condition
  - Lifespan
  - Resistance to disease

- Normal appetite, growth rate
  - BCS: Length-weight data
  - Indirect measurement of food conversion

Kou, Ikuyo et. al., 2013

- Breeding performance
  - Production of eggs/embryos
  - Survival of eggs/embryos
- Biological Measurements
  - Cortisol concentrations in water or feces
  - May not be specific to poor welfare
  - Lack precision of direct measurements on individuals

GOOD NEWS!

BAD NEWS!

- Several published studies
- Not always consistent results
  - Many variables to consider
  - Strain differences
  - Husbandry differences
- More work to be done
- Let's look at a few……..

Zebrafish Behavior- Social Isolation

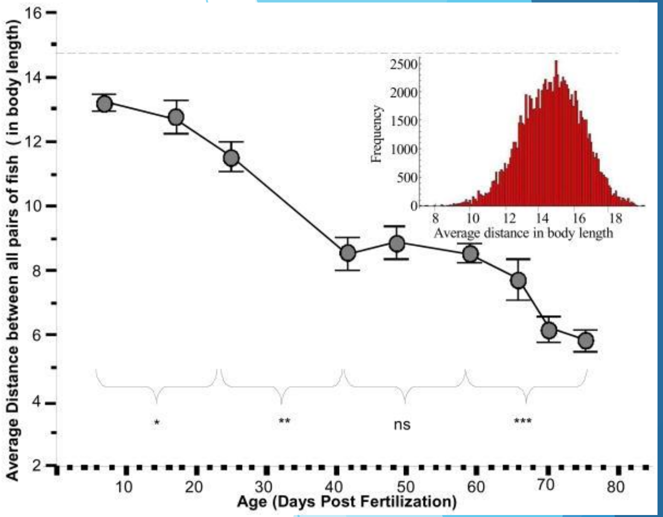
- **Natural Behavior is to Avoid Social Isolation**
  - If reared in isolation will quickly form shoals without discrimination

- Social isolation decreases neurogenesis (sensory regions)
- Timing Matters
  - **Social novelty decrease neurogenesis (stressful?)**
  - Social enrichment does not consistently rescue deficits in cell proliferation following isolation

Lindsey et al., 2014 Giacomini, et. al., 2015 Shams et. al., 2015

Social Shoaling Preferences

- Males prefer female shoals compared to males but no preference for shoal size
- Females prefer larger shoal regardless of sex
- Prefer to shoal with well-fed conspecifics
- Prefer more active shoals (influenced by temperature)
- Size of tank doesn’t impact between fish distance in shoals
  - Stress results in tighter shoals
  - Age influence



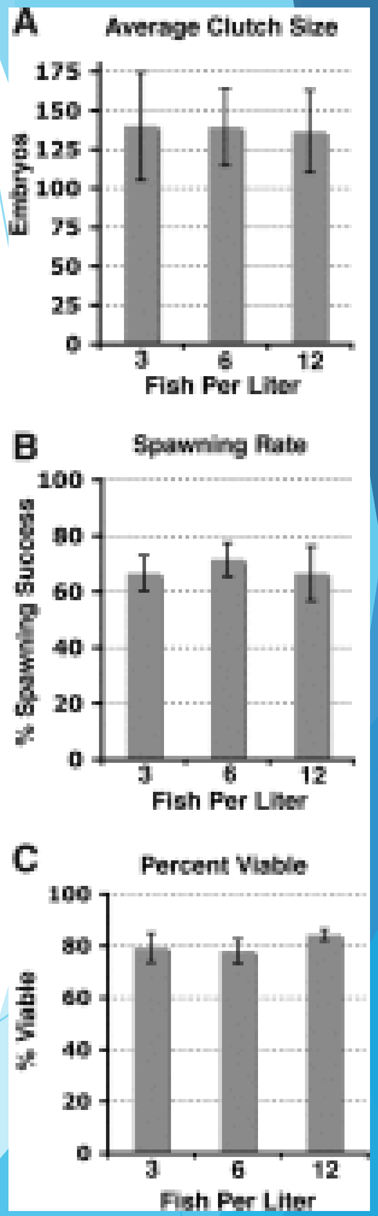
Social Environment

- Prefer presence of other fish
  - Group housing considered standard
- Isolation for disease, genotyping, behavioral testing
  - Ability to view other fish
  - Dither fish: placement of well acclimatized fish around or in the tanks of “anxious” fish
- Last fish in stock
  - Consider “mixed” housing
- How many fish?
- Tank hierarchy?

Social Density Effect on Reproductive Performance

- Data collected from 8 different facilities
- Evaluated pair spawning after housing at densities of 3, 6, or 12 fish/L
- Viable and non-viable embryos counted a 1 dpf
- No significant differences in average clutch size*, spawning success*, or percent viable embryos
    - Some had better success at lower densities, some at higher

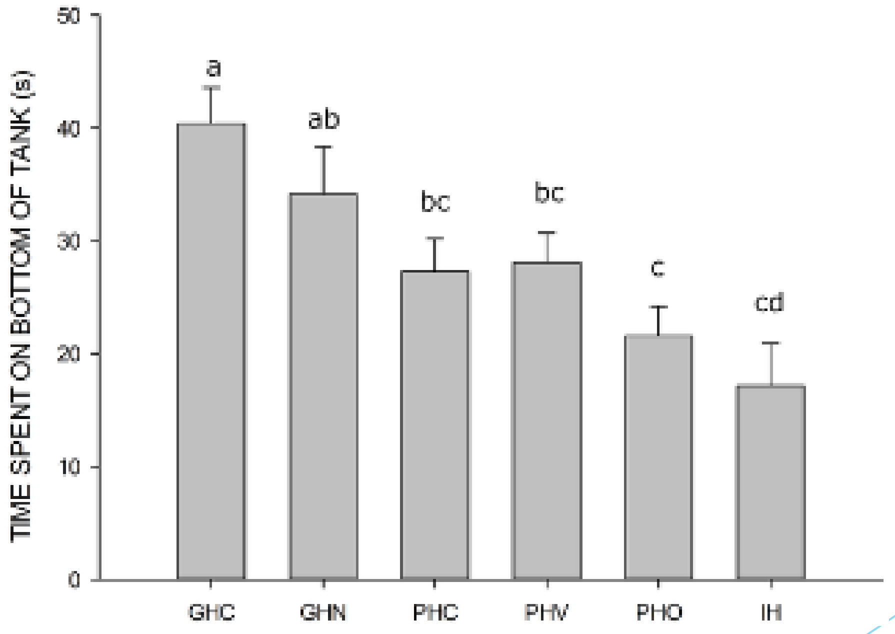
- **Variable across facilities
    - Husbandry not standardized
    - Feed (type and amount), breeding management strategies, age of fish
    - Water quality considerations



Castranova, et. al 2011.

Social housing conditions effect physiological and behavioral stress response

- Evaluated tank-diving and basal cortisol levels in response to ethanol and water changes in various housing conditions
  - Tank-diving response mediated by housing conditions prior to testing
  - Ethanol had anxiolytic effect with individual housed only
  - Water changes (olfactory contact) effected tank-diving of group-housed only (2 fish/L)

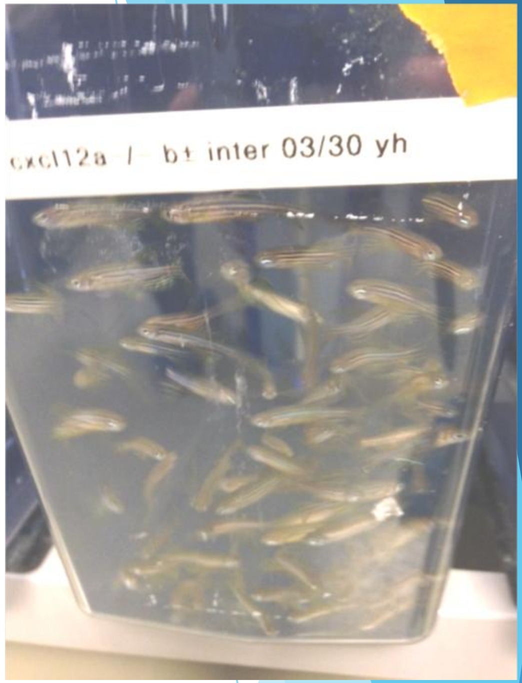


Parker, et. Al. 2012

Social Interactions

- Group-housed fish establish dominant-subordinate relationships
  - Not associated with sex or body size
  - Subordinate fish have reduced food intake, greater anxiety-associated behavior
- Low density pairing have increase aggressive behavior

- Density within tank
  - Lower densities decrease biologic load (lower stress from water quality, less food competition, etc.)
  - Higher densities may alleviate stress-related behavioral responses



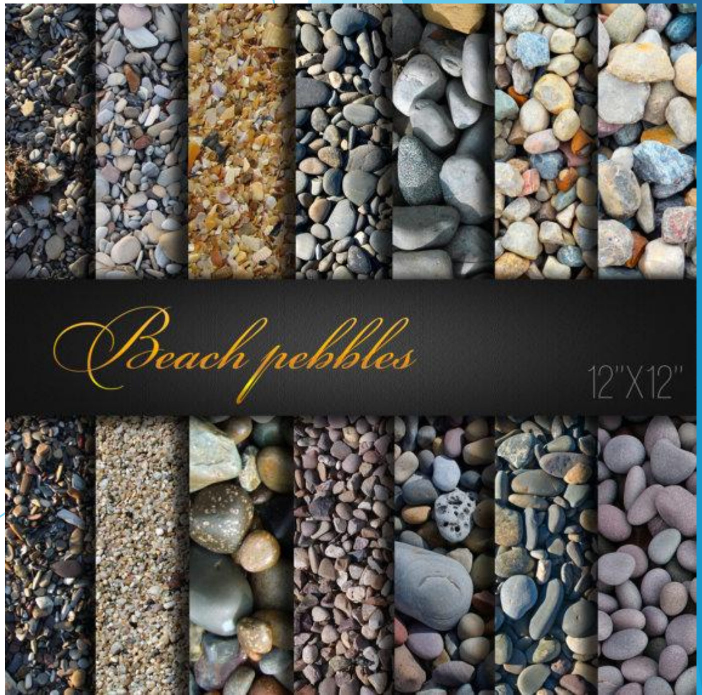
Social Housing Considerations

- Aggression levels influenced by habitat complexity, housing density, and food availability
- Group sizes interactions
- Timing of group creation
- All factors together influence water quality, fish to fish interaction and overall welfare

ONE SIZE DOES NOT FIT ALL!

External Environment and Influence on Welfare

- Visible structures outside of the tank
  - Pebbles or grass under tanks
  - Color of the tank
- Lighting
  - Startle response with sudden light changes
  - Influence reproduction



In-Tank Environment and Influence on Welfare

- Structures within the tank
  - Plants, shelter
  - Breeding inserts/slopes
  - Dividers
  - Substrate

- Type of food

- Flow rates
  - Water Quality
  - Behavior

Lawrence, 2012

Physical Enrichment Considerations

- Increased surface area for microbial growth in the tank
  - Can impact water chemistry

- Surface for chemical binding
- Release of chemical components (i.e. phthalates)
  - Direct or indirect biologic effects on pharmacokinetics, neoplasias, etc.
  - Disinfection and resulting exposure to disinfectants
- Harboring of pathogens (i.e. parasites)
  - Requires adequate disinfection

Physical Enrichment Considerations, contd.

- Skin damage
  - Secondary infections
  - Immunosuppressed fish?

- Choke hazard?

- May impact behavior
  - Swimming patterns
  - Interactions with other fish

Physical Enrichment Considerations

- Live feed
  - Promotes wider range of behaviors
    - Pro-active foraging and capture of food and predator/prey interactions.
    - Pelleted diet elicits “capture” response and later “foraging” behavior
    - Irregular supply, less quality control, pathogen risk?
  - Feed variety used in several mammalian species as enrichment
    - Intermittent treat

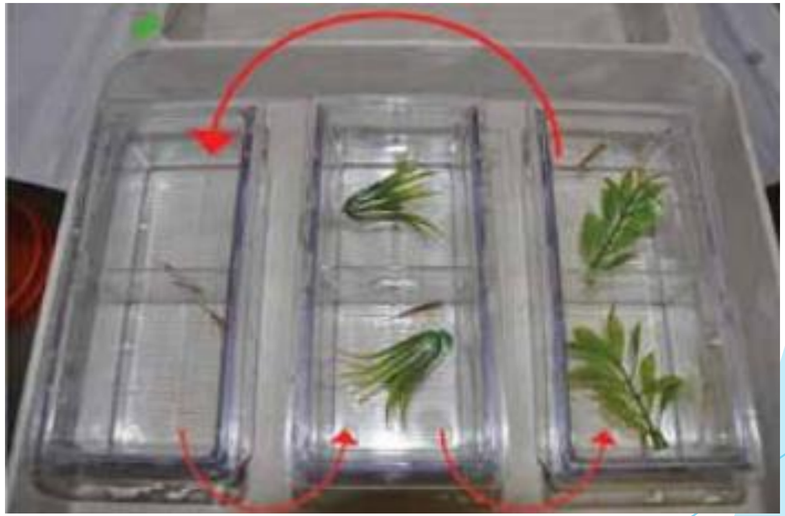
Stewart, 2004)

Journal of the American Association for Laboratory Animal Science
Vol 55, No 3
May 2016
Pages 291–294

Effects of Environmental Enrichment on the Fertility and Fecundity of Zebrafish (*Danio rerio*)

Lemnique N Wafer,[1,*] V Behrana Jensen,[2] Jesse C Whitney,[1] Thomas H Gomez,[1] Rene Flores,[1] and Bradford S Goodwin[1]

Question: Evaluation of structural enrichment on egg count



- Breeding pairs tests once /week in a different condition
- Repeated 3 times
- Structures bleached between each session

Evaluation of Enrichment on Egg Production & Fry counts

- # of eggs produced throughout experiment:

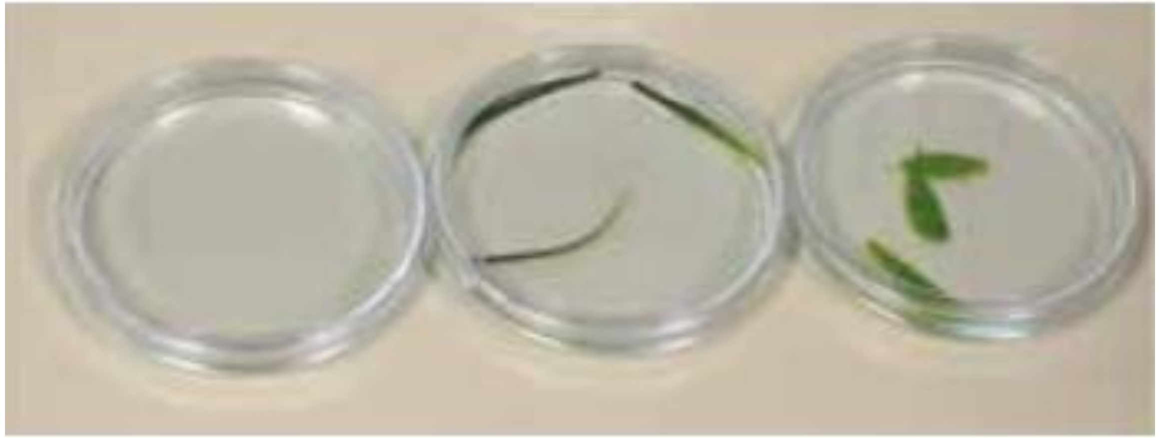
  Grass>Leaf>Barren

- # of successful spawning events throughout experiment:
  - NSD: 58.6% for all groups

- # of 6-dpf fry varied as function of age AND enrichment condition
  - Grass preferred when pair 110-160dpf
  - Leaf preferred when pair 173-180dpf

Evaluation of Enrichment on Survivability of Fry

- Eggs incubated in various conditions for 6 days
- Previously used plants may harbor nitrifying bacteria
  - Natural bioflter in Petri dish
  - Impact survival as a result of water quality differences?



No, however…

Increased survival 0-30 dpf

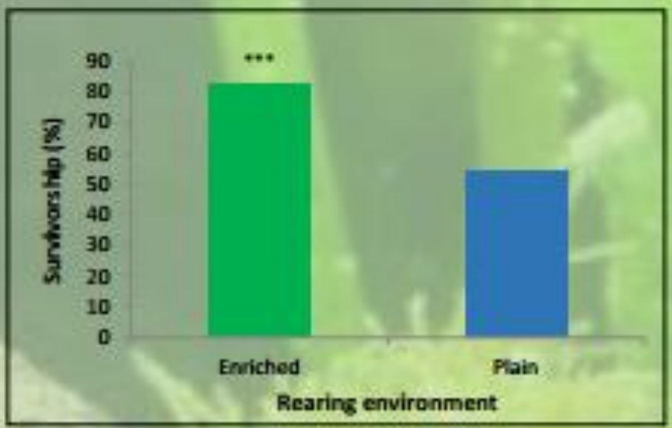


Figure 3. Percentage of larvae surviving from age 0–30 dpf in enriched and plain tanks.

Summary

- Greater fertility and fecundity with plant enrichment in breeding tank
- Suggestive of increased survival rates for larval survival 6-30dpf
- May be variable across different strains, facilities, etc.

Lee (2015)

What’s the relationship between social & physical enrichment strategies?

Effects of Habitat Complexity on Pair-Housed Zebrafish

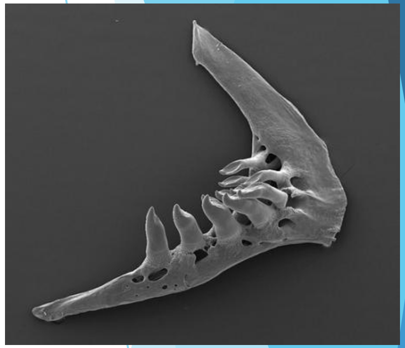
Victoria A Keck,[1,*] Dale S Edgerton,[2] Susan Hajizadeh,[2] Larry L Swift,[1] William D Dupont,[3] Christian Lawrence,[4] and Kelli L Boyd[1]

- Individually housed male fish (control; low basal cortisol)
- Single male-female pair in barren tank
- Single male-female pair with plastic plant

- Terminal cortisol levels evaluated at 1 day, 5 days, 10 days

Results

- Housing impacts physiological parameters
  - Cortisol level increased in single-housed fish over time
  - Subordinate fish typically had higher cortisol level
- Housing impacts behavioral parameters
- Housing zebrafish as pairs for >5 days resulted in welfare concern
  - Death and wounding in single male-female pair in barren tank by day 5 (25% morbidity/mortality)
  - Not associated with cortisol levels
- Novel element (plant) may require up to 10 days to adapt
  - Aggression still seen in pairs with plants*
  - Perhaps less death since plant provided shelter



The Behavioral Effects of Single Housing and Environmental Enrichment on Adult Zebrafish (*Danio rerio*)

Chereen Collymore,[1,†] Ravi J Tolwani,[2] and Skye Rasmussen[2,*]

- 4 housing conditions
  - Single-housed barren (1 fish/2.5L)
  - Single-housed enriched
  - Group-housed barren (5 fish/2.5L)
  - Group-housed enriched
- 5 mo. old fish housed in each condition for 3 weeks prior to testing by novel-tank, light-dark behavior, and place-preference tests. N=30/condition

Summary of Results

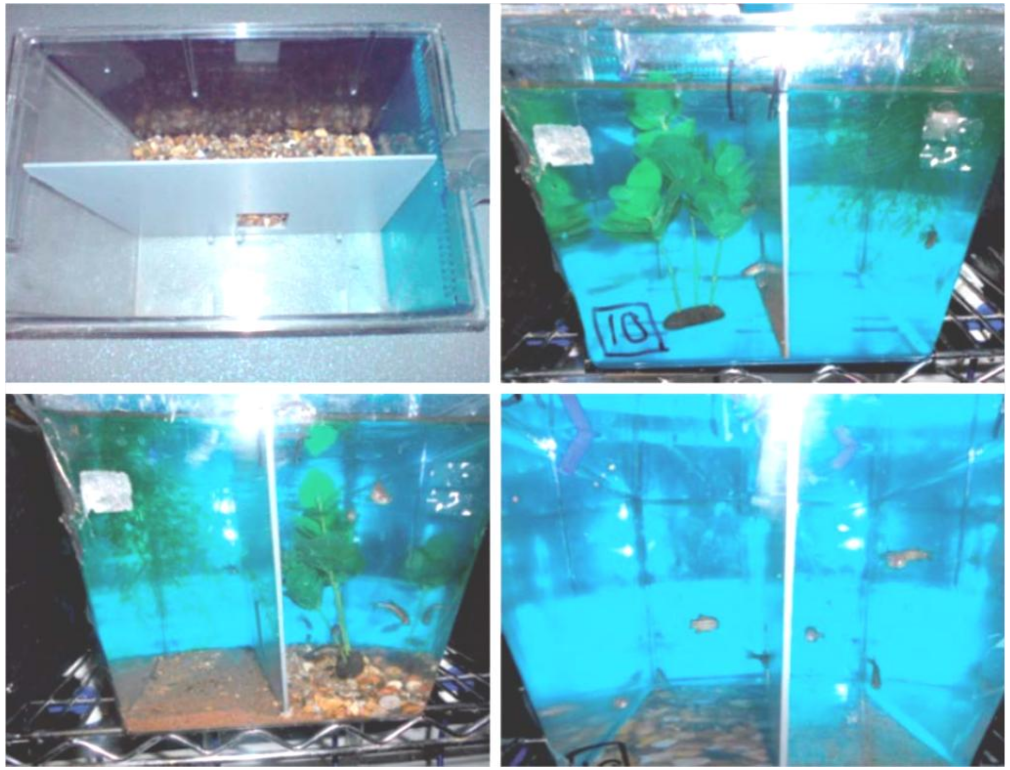
- Fish housed singly in barren environment
  - Alterations in anxiety-like behavior
  - Altered behavior in preference tests ((less frequent crossing to plant; less exploration)

- All fish preferred to associated with conspecifics except single-housed with enrichment (no preference)
  - Associated with novelty?
  - Enrichment can influence behavioral preferences

- Single-housed fish appear to benefit from artificial plant

Impact of Social Grouping, Dominance, & Gender on Preference

Clockwise from top left: 1. Experimental tank with gravel substrate versus a barren cue. 2. Both plant types as competing cues. 3. Gravel substrate image versus barren. 4. Substrate/plant combinations in direct comparison with sand and floating plant on the left side and gravel and submerged plant on the right.



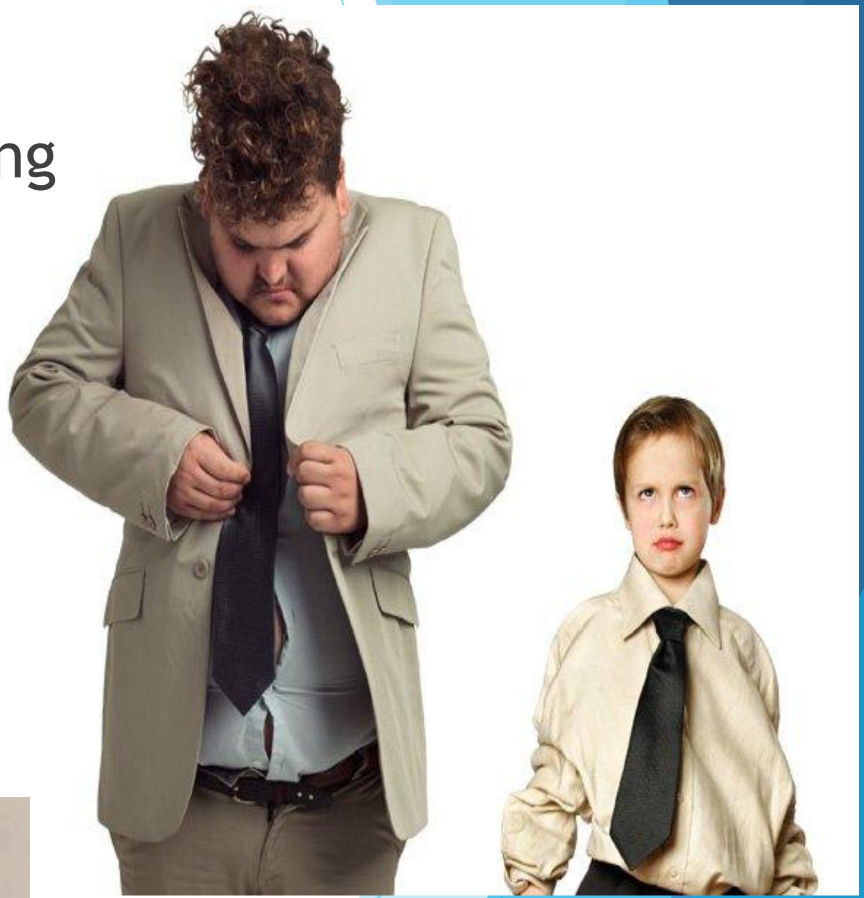
Schroeder et al, 2016

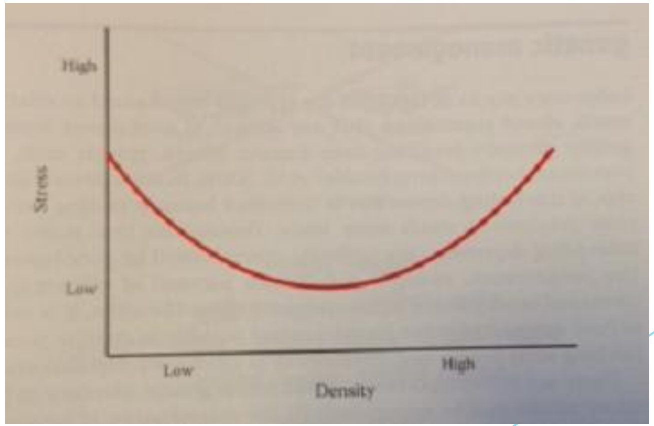
Summary of Results

- Pairs
  - Dominant preferred substrate (excluded subordinate)
  - Dominance relationship changes behavior
- Groups
  - Preference for all substrate and plant enrichments over barren
  - Preference for gravel over sand (regardless of sex)*
  - Males preferred floating plant; females had no preference
- Zebrafish preferred enrichment over barren
- Preference influenced by social conditions and sex
- Airstone was avoided

What is our current level of knowledge?

- Housing conditions impact behavior and cortisol levels
- Zebrafish prefer social housing (>2) to individual housing
- No ideal housing density
- Adding habitat complexity to zebrafish housing may reduce aggression and may improve reproduction
- Zebrafish may prefer complex environments to barren environments
  - Influenced by social housing, especially in pairs
- Avoid turbulent flow
- Don’t forget about the lighting





What about cortisol measurements….

- Aggressive tank situation was not associated with elevated cortisol levels

- Fish isolated during development or in adulthood do not have elevated cortisol levels compared with group raised fish

- More closely correlated with changes in social context
  - Higher cortisol associated with more subordinate social status
  - Isolated fish have reduced cortisol response to acute stressor
    - Possibly due to lack of cues from others in the group

Consider…

- Preference does not guarantee improved welfare
  - May not enhance well-being

- Cautions with physical enrichment:
  - Observe behaviors
  - Impact on water quality
  - Requires SOP for disinfection
  - Influence of novelty?

- Influence on Research
  - Compromised research results require repeat testing
  - Scientifically valid, high-quality studies needed

DO NO HARM!

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