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Leibniz Institute on Aging –
Fritz Lipmann Institute



Fish Facility



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Zebrafish Welfare Assessment – Why Their Well-being Matters

Dr. Beate Hoppe, Head of Fish Facility
09.04.2025

1960s

George Streisinger
(University of Oregon)
Potential for genetic studies and
developmental biology

1980s-1990s

Large-scale genetic screenings and
mutagenesis studies
Developmental genetics

2000s

Genome sequencing
CRISPR gene editing
Disease modeling (e.g. neurological,
cardiovascular diseases)
Key organism in biomedical research

Today

Cancer research, neuroscience,
toxicology, regenerative medicine,
and drug discovery

Advantages of using zebrafish

- Genetic similarity to humans
(around 70% of human genes)
- Rapid development and high
reproductive rate
- Transparency of embryos
- Multiple genetic tools established
- Ethical and practical advantages
over mammalian models



<https://www.mayo.edu/research/labs/zebrafish-genetics/overview>

1960s–1990s

Little formal attention to welfare, fish traditionally not viewed as sentient animals

Early 2000s

Need for proper husbandry enrichment, and humane handling

Growing recognition of fish welfare

Studies on fish pain perception and stress responses

Milestone 2009

Inclusion in EU Animal Welfare Regulations (Directive 2010/63/EU)

Fish recognized as vertebrate animals requiring welfare protection



2010s

AAALAC, NC3Rs, FELASA, etc. published specific guidelines for zebrafish husbandry, anesthesia, euthanasia, and enrichment

Increased focus on water quality, stocking density, humane endpoints, and pain management

2020s

Further refining housing, enrichment, and pain assessment methods

Welfare discussions now include behavioral indicators of stress, environmental enrichment strategies, and improvements in humane euthanasia methods



Ethical responsibility

- Capability of zebrafish experiencing pain and suffering
- Welfare considerations minimizing harm and damage
- Alignment with the 3Rs Principle

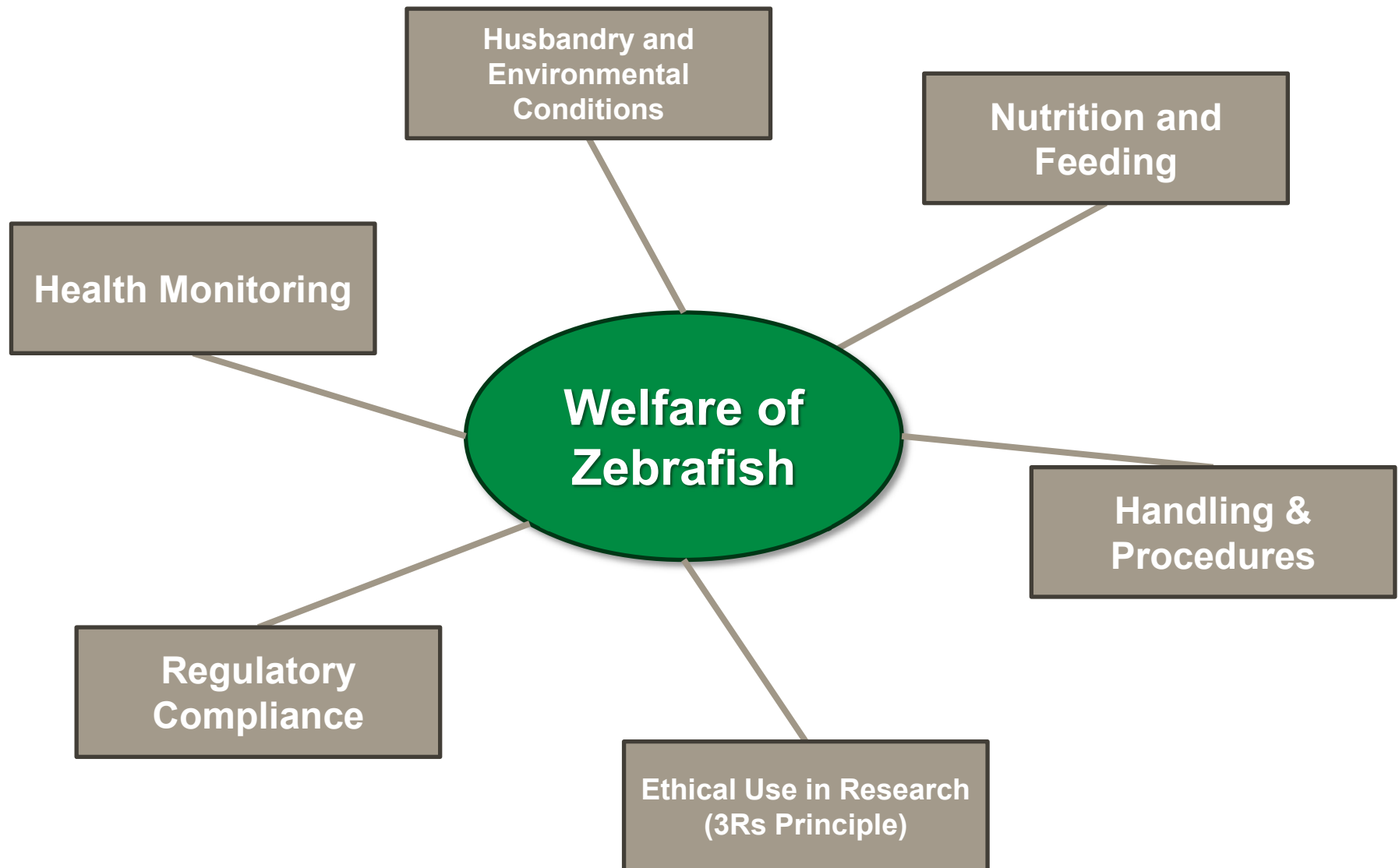
The importance of welfare in research

- Stress and poor husbandry conditions introduce unwanted variables
- No care: negatively affecting research outcomes

Regulatory framework

- International guidelines e.g., EU Directive 2010/63/EU, AAALAC, IACUC standards

Good welfare leads to more reliable and reproducible scientific data



Pain

Standardized Welfare Terms for the Zebrafish Community

Nicola Goodwin^{1,2}, Natasha A. Karp³, Samuel Blackledge¹, Bradley Clark¹, Rosemary Keeble², Ceri Kovacs¹, Katrina N. Murray¹, Michael Price¹, Peter Thompson¹, and James Russell¹

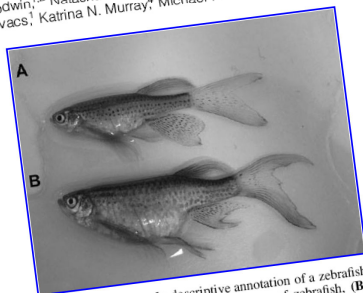


FIG. 1. Example of a descriptive annotation of a zebrafish welfare concern. (A) Normal appearance of zebrafish. (B) female with enlarged abdomen, which would be annotated as *abdomen_general_distended*.

DOI: 10.1089/zeb.2016.1248, 2016

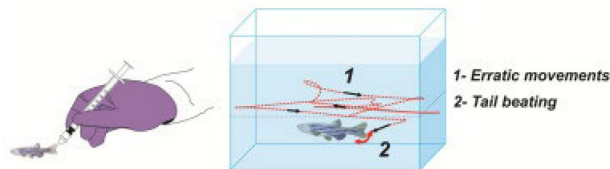
What is known so far?



Distress

Chapter 34 - Nociception-related behavioral phenotypes in adult zebrafish

Fabiano V. Costa^a, Luiz V. Rosa^a, Allan V. Kalueff^b, Denis B. Rosemberg^a



<https://doi.org/10.1016/B978-0-12-820589-1.00034-8>, 2022

Pain management in zebrafish: Report from a FELASA Working Group

Lynne U Sneddon¹, Paul Schroeder², Ana Roque³, Karin Finger-Baier⁴, Angeleen Fleming⁵, Simon Tinman⁶ and Bertrand Collet⁷

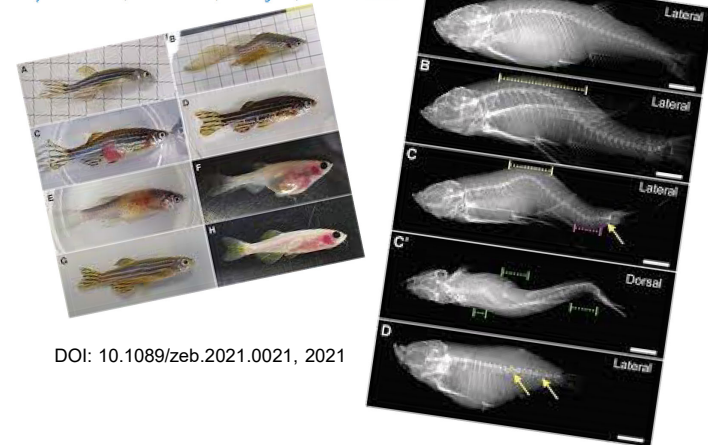
doi: 10.1177/00236772231198733, 2023

Suffering/Harm

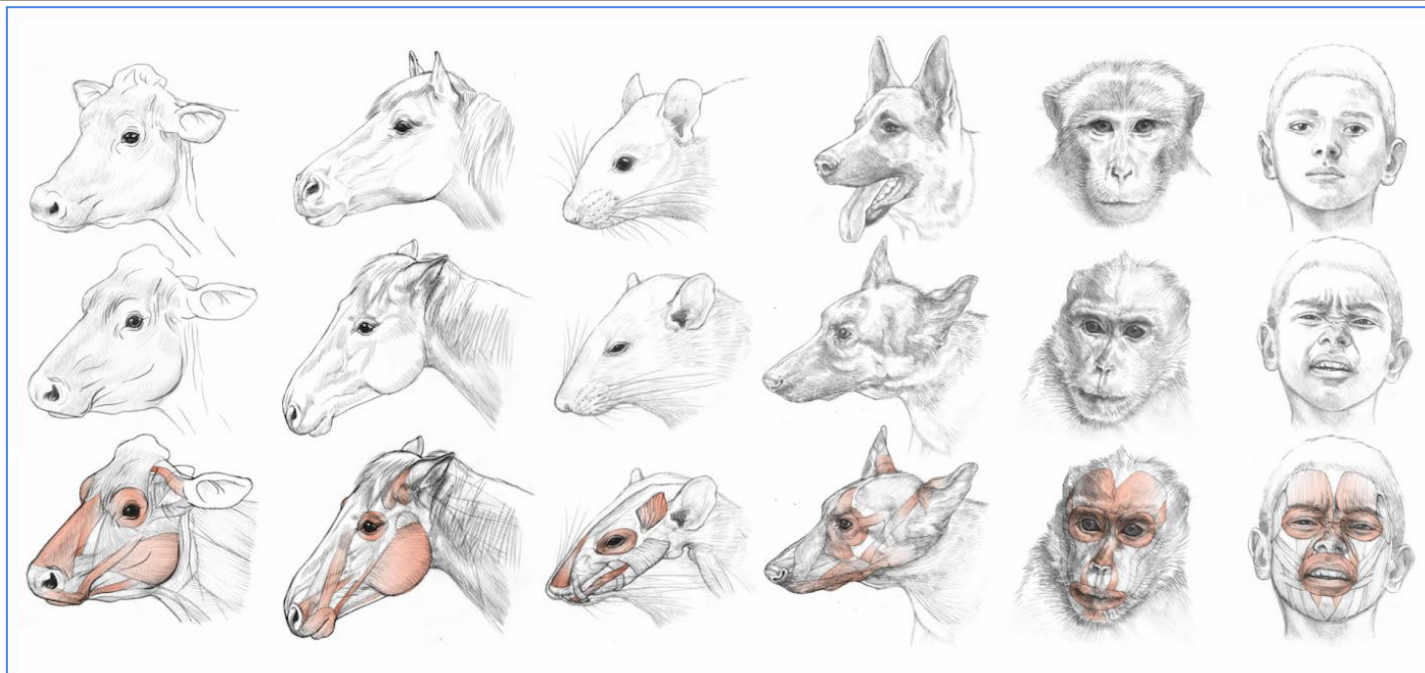
> Zebrafish, 2021 Aug;18(4):282-292. doi: 10.1089/zeb.2021.0021. Epub 2021 Jul 2.

Welfare Assessment of Adult Laboratory Zebrafish: A Practical Guide

Sibylle Sabrautski¹, Manuel Miller¹, Erika Kague², Markus Brielmeier¹

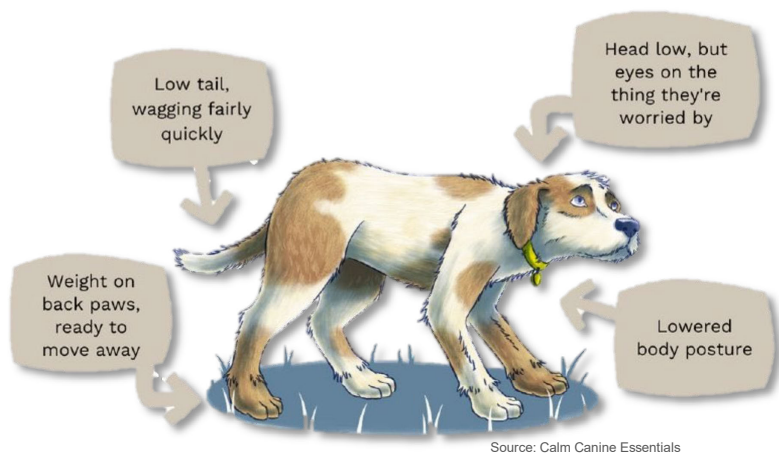


DOI: 10.1089/zeb.2021.0021, 2021



- **Pain**: unpleasant sensory and emotional experience associated with actual or potential tissue damage
- **Nociception**: Series of processes required for an organism to receive a painful stimulus, converting to molecular signal, and recognition of signal to trigger appropriate defensive responses.
- **Pain does not equal nociception.** Nociception is objective, but pain is subjective.

- Aversive state, individual is unable to completely adapt to stressors
- Shows maladaptive behaviors
- Results in inappropriate social interaction such as aggression, passivity, or withdrawal

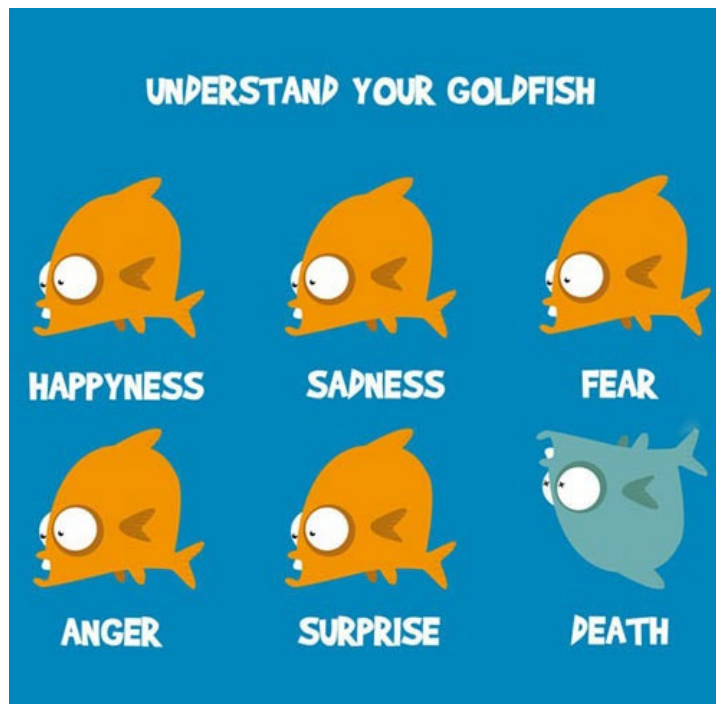


- Lasting or irreversible injuries, impairments, or physiological damage
- Includes organ damage, chronic illness, or permanent behavioral changes due to experimental procedures or poor care

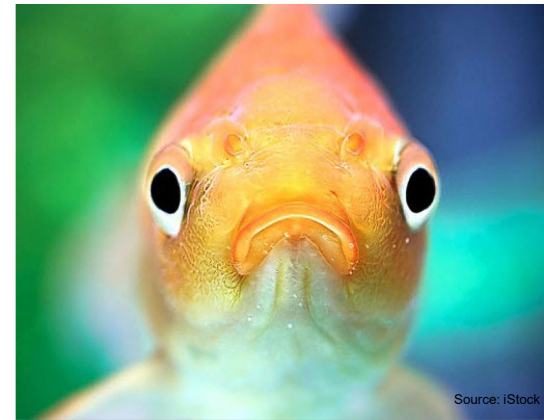


	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye			
Nose bulge • Bulging on the bridge of the nose • Vertical wrinkles on the side of the nose			
Cheek bulge • Bulging of the cheeks			
Ear position • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pointed' shape • Space between the ears increases			
Whisker change • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clump together • Whiskers lose their natural 'downward' curve			

In fish, the expression of pain, distress, suffering, or harm is less pronounced than in mammals



<https://www.iwastesomuchtime.com/68909-2>



What does health inspections mean?

- Based on law (EU-Directive 2010/63)
- Daily visual inspection of **every single** fish

Importance of health monitoring on a daily base

- Reduce suffering, damage or pain of single fish
- Recognize accumulation of certain symptoms
- Remove sick fish from the stock

Importance of health monitoring during experiments

- Reduce suffering to minimal possible level
- Guarantee good scientific practice and reproducibility

→ Reduce pain, distress and suffering to a minimum!
→ Sick and suffering fish do not produce useful data!

Care Sheet for daily use

- Easy to understand
- Cover most of the clinical signs
- Definition of specific criteria (description)
- Due to focus on aging: aging specific criteria

Categories

- Behavior / Body condition / Appearance
- other symptoms (call veterinarian)

Easy and clear

- Short cuts link to clinical signs → Documentation

Color code

- **Green** → everything is fine
- **Yellow** → mild symptoms: further observation needed
→ too many **Yellow** categories lead to **Red**
- **Red** → Severe clinical signs
→ humane endpoints lead to immediate euthanasia



Category		Severity	Clinical signs	Abbr. for documentation	Score
Behavior	Swimming	Physiological	Upright position, balanced, without tilting	SW0	0
		Altered	Sloping position, cannot hold stable	SW2	2
		Strongly altered	Negative or positive buoyancy, tilting	SW3	3
	Feeding	Physiological	Active feeding and hunting	FB0	0
		Reduced	Decreased response to food	young FB2; old FB1	2-(1)
		Stronly reduced	Starvation, completely unreactive towards food	FB3	3
	Social	Physiological	Responsive and purposed swimming	SB0	0
		Reduced	Very delayed reaction or very aggressive	young SB2; old SB1	2-(1)
		Strongly reduced	Apathy, no swimming movements	SB3	3
Body condition		Physiological	Body bigger than head, well-conditioned	BC0	0
		Underconditioned	Head, belly and base of anal fin in a line	young BC1; old BC0	1-(0)
		Severe emaciation	Body narrow than head, concave shape along the belly, juveniles: much smaller than mates	BC3	3
Appearance		Physiological	Clear eyes, scales attached to body, no lesions, intact fins, normal coloration, even skin	[Abbr.]0	0
	Eyes	Moderate damage	One protruding or deformed eye, loss of one eye, enophthalmos	young EY2; old EY1	2-(1)
		Severe damage	Bleeding, exophthalmus, both eyes missing	EY3	3
	Jaw	Moderate deformation	Slight changes, no influence on feeding behavior	JA2	2
		Severe deformationen	Deformed jaw, strongly influenced feeding behavior	JA3	3
	Operculum	Malformations	Missing or deformed gill cover	OP2	2
	Skin	Changed appearance	Small lesion, dark/white discolored foci, pale coloring	SK2	2
		Severe changes	Large discolored patch, hemorrhage, abscess wound with organ exposure, fungi infection	SK3	3
	Circumferential increase	Increased tissue growth	Possible tumor growth, possible spawning problem	CI3	3
	Dropsy	Severe changes in appearance	Protruding scales, massively enlarged abdomen and bulging eyes	DR3	3
	Fin	Mild damage	Slightly injured or partially missing fin	FI2	2
		Severe damage	Missing, severely injured or rotten fins	FI3	3
	Spine	Mild deformation	Moderate skeletal curvature or deformity of the spine	young SP1; old SP0	1-(0)
		Severe deformation	Strong deformity of spine	SP3	3
Other symptoms		Other reasons	Rarely occuring clinical signs, unclear conditions	VET	1-3



DOI: 10.3791/4196

Behavior

- Swimming: upright position, balanced, without tilting
- Feeding: active feeding and hunting
- Social: Responsive and purposed swimming, swarming/schooling

Body Condition

- Body bigger than head, well conditioned

Appearance

- Clear eyes, scales attached to the body, no lesions, intact fins, normal coloration, even skin



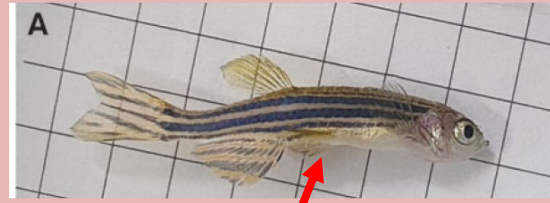
DOI: 10.3791/4196



Category	Severity	Clinical signs	Abbr. for documentation	Score
Body condition	Physiological	Body bigger than head, well-conditioned	BC0	0
	Underconditioned	Head, belly and base of anal fin in a line	young BC1; old BC0	1-(0)
	Severe emaciation	Body narrow than head, concave shape along the belly, juveniles: much smaller than mates	BC3	3



- Head, belly and base of anal fin in a line



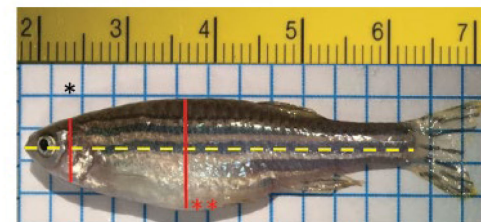
- Body narrow than head, concave shape
- “Skinny” appearance

- Severe emaciation (skinny fish) associated with disease, developmental delay or aging
→ Severe phenotype
- See also “Body condition scoring in adult Zebrafish (*Danio rerio*)”

Body Condition Scoring for Adult Zebrafish (*Danio rerio*)

Tannia S Clark,* Lauren M Pandolfo, Christopher M Marshall, Apratim K Mitra, and Joseph M Schech

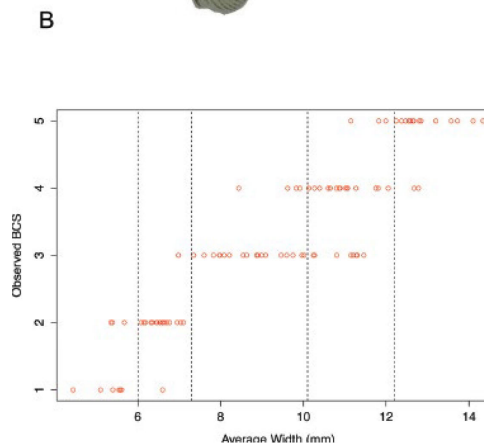
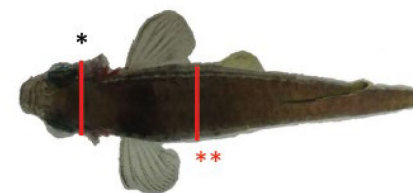
Adult Zebrafish BCS		
	Lateral View	Dorsal View
BCS 1: - Head larger than body (big head) - Lateral- concave ventral surface between head and abdomen (narrow abdomen) - Dorsal- body is more narrow than head and linear Fish is thin (emaciated)		
BCS 2: - Head and body equal size - Lateral- flat ventral surface between head and abdomen - Dorsal- head and width of abdomen are equal Fish is underconditioned		
BCS 3: - Body larger than head - Lateral- slight convex ventral surface - Dorsal- head is slight smaller to a fusiform body Fish is well-conditioned		
BCS 4: - Body significantly larger than head - Lateral- body moderately convex ventral surface - Lateral- Symmetrical ventral surface - Dorsal- head visually smaller to a moderately distended abdomen Fish is over-conditioned		
BCS 5: - Body significantly larger than head - Lateral- body significantly convex ventral surface - Lateral- Symmetrical or asymmetrical ventral surface - Dorsal- head visually smaller to a significantly distended abdomen Fish is obese (large)		



A
Figure 2. Targeted anatomic regions for BCS assessment.

Table 1. Descriptive statistics

	Mean $\pm$ 1 SD	Range
Length (mm)	34.91 $\pm$ 4.28	25.32–45.80
Width (mm)	9.42 $\pm$ 2.55	4.44–14.34
Weight (g)	0.88 $\pm$ 0.45	0.23–2.15
BMI (g/mm ²)	0.67 $\pm$ 0.22	0.27–1.22
Fulton K factor (g/mm ³ $\times$ 10 ⁵)	0.19 $\pm$ 0.05	0.83–2.96



fli Appearance: Circumferential increase vs. Dropsy

Category	Severity	Clinical signs	Abbr. for documentation	Score
◀				
Circumferential increase	Increased tissue growth	Possible tumor growth, possible spawning problem	CI3	3
Dropsy	Severe changes in appearance	Protruding scales, massively enlarged abdomen and bulging eyes	DR3	3



- Circumferential increase
→ Possible tumor at gill cover



- Circumferential increase
→ Possible tumor abdomen



- Circumferential increase
→ Possible spawning problem



- Dropsy
→ Clinical sign
→ Can indicate number diseases: bacterial infections, parasitic infections, or liver dysfunction
→ Danger to hole colony!

- Different origin of clinical signs
- CI and DR are severe abnormalities

Category	Severity	Clinical signs	Abbr. for documentation	Score
Eyes				
	Moderate damage	One protruding or deformed eye, loss of one eye, enophthalmos	young EY2; old EY1	2-(1)
	Severe damage	Bleeding, exophthalmos, both eyes missing	EY3	3



- Missing eye on one side
→ Mild phenotype



- Exophthalmos

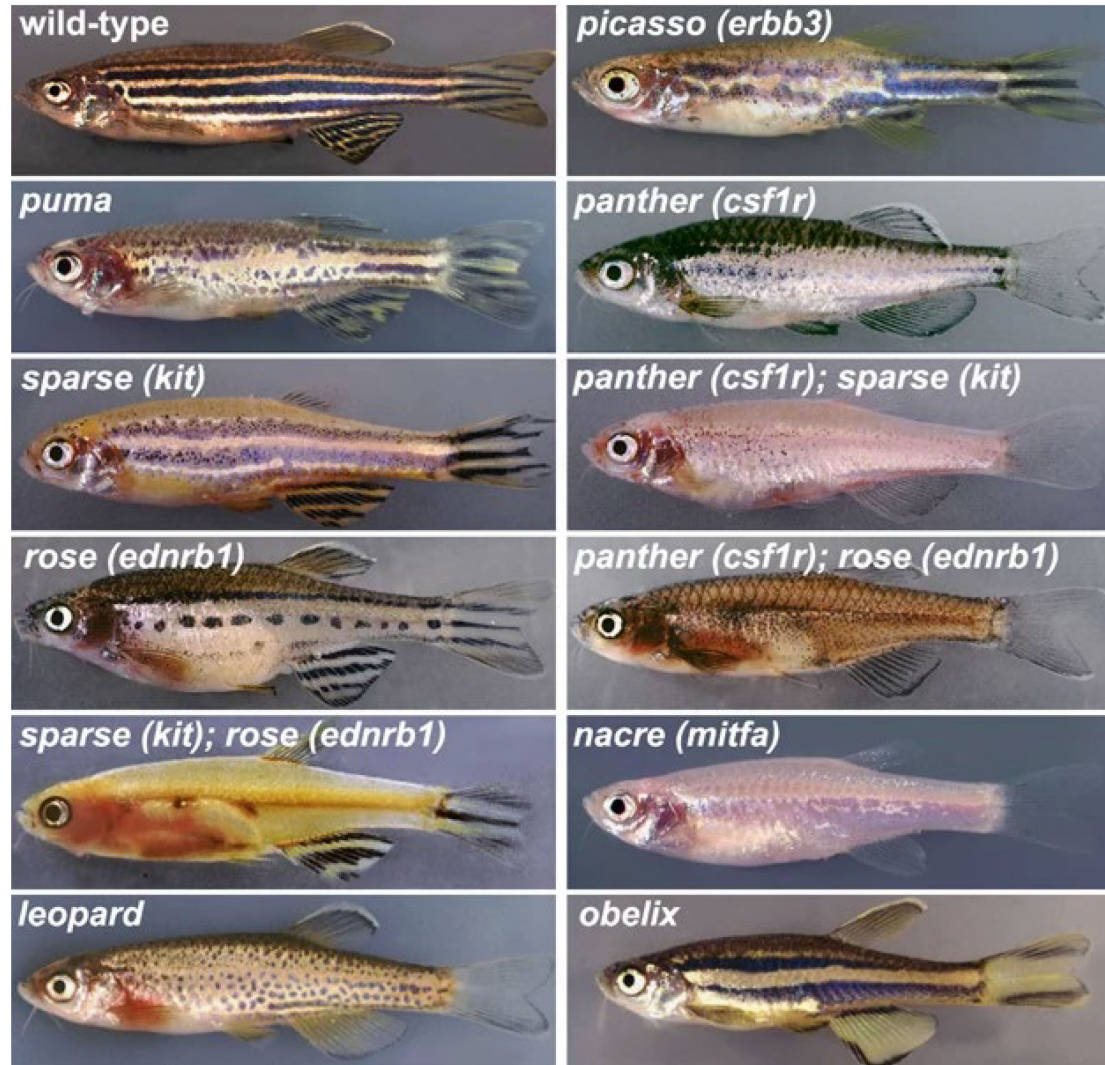
- Bleeding and possible tumor

- Impaired vision influences hunting behavior, orientation, social interaction
→ Total blindness/loss of eyes: severe abnormality
- Exophthalmos, tumors
→ Associated with pain: severe abnormality



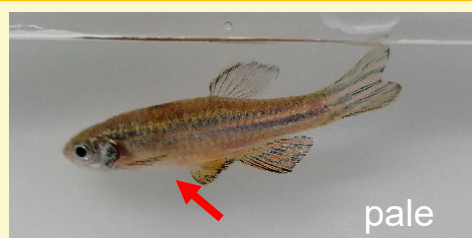
DOI: 10.3791/4196

- What is the normal color of a zebrafish?
- Several genetic modifications lead to change of color/color pattern



<https://doi.org/10.1038/sj.hdy.6800867>

Category		Severity	Clinical signs	Abbr. for documentation	Score
Appearance	Skin	Changed appearance	Small lesion, dark/white discolored foci, pale coloring	SK2	2
		Severe changes	Large discolored patch, hemorrhage, abscess wound with organ exposure, fungi infection	SK3	3



- Loss of color
→ Pale coloring

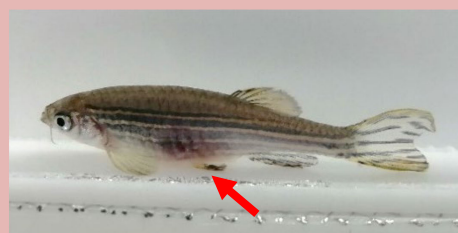
- Changes can indicate signs of diseases, stress, dominance behavior
→ Different severity levels



- Lesion
- Open wound



- Large discolored patch
- Pale coloring

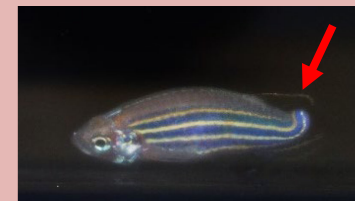


- Lesion
- hemorrhage

Category	Severity	Clinical signs	Abbr. for documentation	Score
Fin	Mild damage	Slightly injured or partially missing fin	FI2	2
	Severe damage	Missing, severely injured or rotten fins	FI3	3



- Fins attached to the body
- Sign of pain
- Further observation needed



- Pale fin

- Rotten fin



- Hyper-coloration at fin
- Possible skin tumor
- Rotten fin

- Changes (abnormal/missing) of fin: mild to severe classification
→ Depending on origin and appearance
- Molecular mechanisms can lead to changes already at larval stages

Category		Severity	Clinical signs	Abbr. for documentation	Score
	Spine	Mild deformation	Moderate skeletal curvature or deformity of the spine	young SP1; old SP0	1-(0)
		Severe deformation	Strong deformity of spine	SP3	3



- Skeletal curvature
- Strong kyphosis
- Impaired swimming



- Skeletal curvature
- Strong scoliosis
- Impaired swimming

- Origins:
 - Diseases (e.g. *Pseudoloma neutrophilia* infection)
 - Aging phenotype
 - Genetic background caused by mutations
 - Nutritional deficiencies

Category	Severity	Clinical signs	Abbr. for documentation	Score
Operculum	Malformations	Missing or deformed gill cover	OP2	2



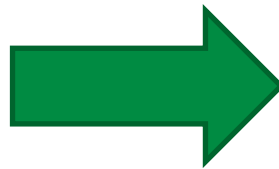
<https://zebrafish.org/documents/faq.php>

- Missing gill cover (operculum)
- Gills directly exposed to water

- Malformations of the operculum common
- Opercular changes:
 - shortening or curling outward
 - curling can be progressive, more pronounced in older fish
- Causes
 - Genetic background
 - Embryonic temperature shock
 - Nutrition or possibly chemical agents

Cave: If both gill covers are missing, monitoring of anesthesia depth difficult

Category		Severity	Clinical signs	Abbr. for documentation	Score
Behavior	Swimming	Physiological	Upright position, balanced, without tilting	SW0	0
		Altered	Sloping position, cannot hold stable	SW2	2
		Strongly altered	Negative or positive buoyancy, tilting	SW3	3
	Feeding	Physiological	Active feeding and hunting	FBO	0
		Reduced	Decreased response to food	young FB2; old FB1	2-(1)
		Strongly reduced	Starvation, completely unreactive towards food	FB3	3
	Social	Physiological	Responsive and purposed swimming	SBO	0
		Reduced	Very delayed reaction or very aggressive	young SB2; old SB1	2-(1)
		Strongly reduced	Apathy, no swimming movements	SB3	3
Body condition	Physiological	Body bigger than head, well-conditioned	BC0	0	
	Underconditioned	Head, belly and base of anal fin in a line	young BC1; old BC0	1-(0)	
	Severe emaciation	Body narrow than head, concave shape along the belly, juveniles: much smaller than mates	BC3	3	
Appearance	Eyes	Physiological	Clear eyes, scales attached to body, no lesions, intact fins, normal coloration, even skin	[Abbr.]0	0
		Moderate damage	One protruding or deformed eye, loss of one eye, enophthalmos	young EY2; old EY1	2-(1)
		Severe damage	Bleeding, exophthalmus, both eyes missing	EY3	3
	Jaw	Moderate deformation	Slight changes, no influence on feeding behavior	JA2	2
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		Changed appearance	Small lesion, dark/white discolored foci, pale coloring	SK2	2
	Skin	Severe changes	Large discolored patch, hemorrhage, abscess wound with organ exposure, fungi infection	SK3	3
		Circumferential increase	Increased tissue growth	Possible tumor growth, possible spawning problem	CI3
	Dropsy	Severe changes in appearance	Protruding scales, massively enlarged abdomen and bulging eyes	DR3	3
		Fin	Mild damage	Slightly injured or partially missing fin	FI2
	Spine	Severe damage	Missing, severely injured or rotten fins	FI3	3
		Mild deformation	Moderate skeletal curvature or deformity of the spine	young SP1; old SP0	1-(0)
		Severe deformation	Strong deformity of spine	SP3	3
	Other symptoms	Other reasons	Rarely occurring clinical signs, unclear conditions	VET	1-3



Implementation of Score Sheet for less paper work



SCORE SHEET

Health issue: ☐

Instructions for abort criteria

1. A fish in a red category must be immediately removed.
2. A fish in a yellow category shall be observed once longer conspicuous, it falls back to the green category (red category), the fish must be euthanized immediately on further measures. The fish must fall into inconspicuous.
3. If a fish reaches a cumulative score of 5 or more it is considered.
4. The following rules apply to old fish: Symptoms in represent normal aging characteristics and do not.
5. In case of uncertainties of the classification or in

Exclusion of the following abnormalities from the score

- Phenotype: Animals with inbreeding phenotypes, with missing gill covers
- Slight spinal curvature
- Slight jaw abnormalities
- Reason: These characteristics do not cause the fish and do not change during life.

- Phenotype: Loss of gill covers
- Reason: Absence of gill cover is not a criterion for to appear intact despite the absence of the gill cover.
- Phenotype: Extended proliferation on the lip
- Reason: Histopathological examinations have shown males and females is considered to be the cause.

Please indicate the number of animals with symptom

Number of animals: *

Behavior - Swimming behavior

- SW0 - 0 - Normal - Full fin movement, Good buoyancy
- SW2 - 2 - Altered - Sloping position, Fish cannot hold
- SW3 - 3 - Strongly altered - Uncoordinated swimmer

Swimming behavior: *

Behavior - Feeding behavior

- FV0 - 0 - Normal - Active during feeding, Active hunt
- FV2 (young fish) / FV1 (old fish) - 2-(1)* - Decreased
- FV3 - 3 - Strongly reduced - Totally unreactive toward

Feeding behavior: *

Behavior - Social behavior
SW0 - 0 - Normal - Zebrafish: Schooling, Coordinated and active swimming / Killfish: Single housing: Swims on the bottom of the tank, Curious when personnel comes close, Reactive to stimuli (e.g. light of the lid), Breeding tank: See single housing, interactive with other fish
SW1 - 1 - Changed - Fish remains longer than usual on the ground, but reacts normal to stimuli
SW2 (Large fish) / SW1 (Small fish) - 2-(1)* - Medium reduced - Delayed reaction to stimuli (food or movements), Very aggressive behavior towards other fish
SW3 - 3 - Strongly reduced - Apathy: Fish lies on the surface of the water, Lies on the bottom of the tank, Shows little or no swimming movements, Constantly attacked by other fish

Social behavior: *

Body condition (Weight)

- BC0 - 0 - Normal - Fish is good in the feed
- BC1 (young fish) / BC0 (old fish) - 1-(0)* - Minor emaciation - 15-30% loss of body weight
- BC3 - 3 - Severe emaciation (Anorexia) - >30% loss of body weight, Body narrower than the head, Fish significantly narrower than siblings

Body condition: *

Appearance

- 0 - Normal - Fish has no injuries or deformations, Eyes are clear and not protruding, Normal gill movement, Scales are attached to the body and completely cover the skin
- AI2 (young fish) / AI1 (old fish) - 2-(1)* - Eyes: Moderate damage - Protruding, deformed eyes, Loss of one eye
- AI3 - 3 - Eyes: Severe damage - Both eyes are missing, eyes have bleedings/ Exophthalmos (proptosis, bulging of the eye anteriorly out of the orbit)

- KI3 - 3 - Jaw: Severe deformation - Deformed jaw strongly influences feeding behavior

- KI2 - 2 - Jaw: Moderate deformation - Deformed jaw strongly influences feeding behavior

- HA2 - 2 - Skin: Changed appearance/Small injuries - Dark coloration, Patches on the skin, Bite wounds or scratches without bleeding

- HA3 - 3 - Skin: Severe injuries - Abscesses (Ulcers), Open wounds with organs exposure

- UV3 - 3 - Circumferential increase - Increase in circumference greater than the abdominal fin, Possible tumor, Possible spawning problems

- BW3 - 3 - Abdominal dropsy (Ascites) - Protruding scales and greatly enlarged abdomen

- FI2 (young fish) / FI1 (old fish) - 2-(1)* - Fin: mild damage - Injured or partially missing fins without restriction of mobility
- FI3 - 3 - Fin: Severe damage - Fins are completely missing, Mobility strongly restricted, Several fins heavily injured/rotten, Fins remain attached to the body (sign of strong pain)

- W1 (young fish) / W0 (old fish) - 1-(0)* - Spine: Mild change - Moderate twisting/alteration of the spine
- W3 - 3 - Spine: Severe change - Remarkable big hump (hunchback), Strong twisting of the spine

Appearance 1: *

Appearance 2: *

Appearance 3: *

Other Symptoms

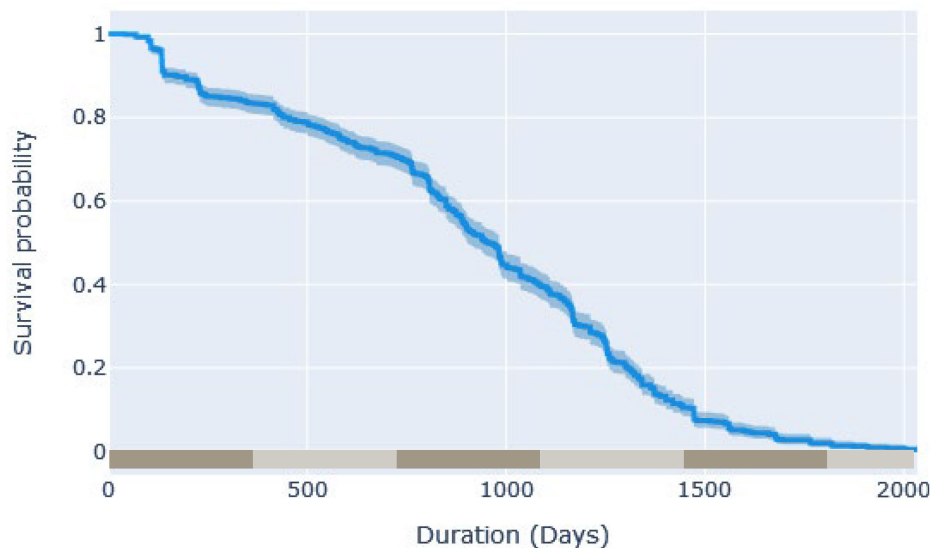
VET - 1-3 - Other reasons after health check - consult the veterinarian

Other symptoms: *

Tags	S	Animal-ID	Strain	DoB	Age	No. of animals
male, sw3		010920/023	ZMZ-1001	09/13/2021	10m 26d	1
au3, ha3, sw3		011593	MZCS-002	11/08/2021	8m 26d	1
gw3, kd2, male		011715/002	GRZ-AD	06/13/2022	2m 24d	1
1 female, 5 male, kd2		011715/006	GRZ-AD	06/13/2022	4m 22d	6
au3, ha3, sw3		011593	MZCS-002	11/08/2021	8m 26d	1
au3, ha3, sw3		011593	MZCS-002	11/08/2021	8m 26d	1

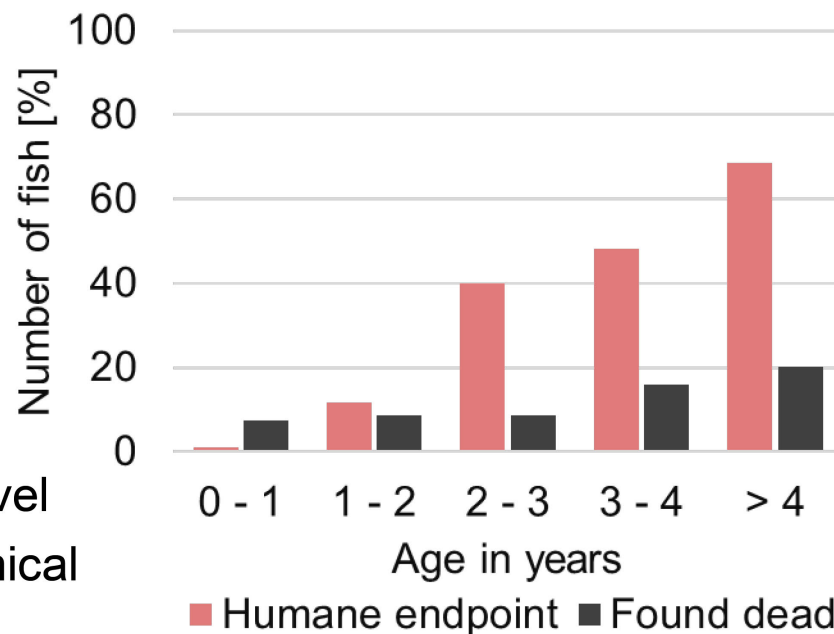
- Animals with scores are labeled in Tick@lab

How does Age effect the Appearance of red Scores?

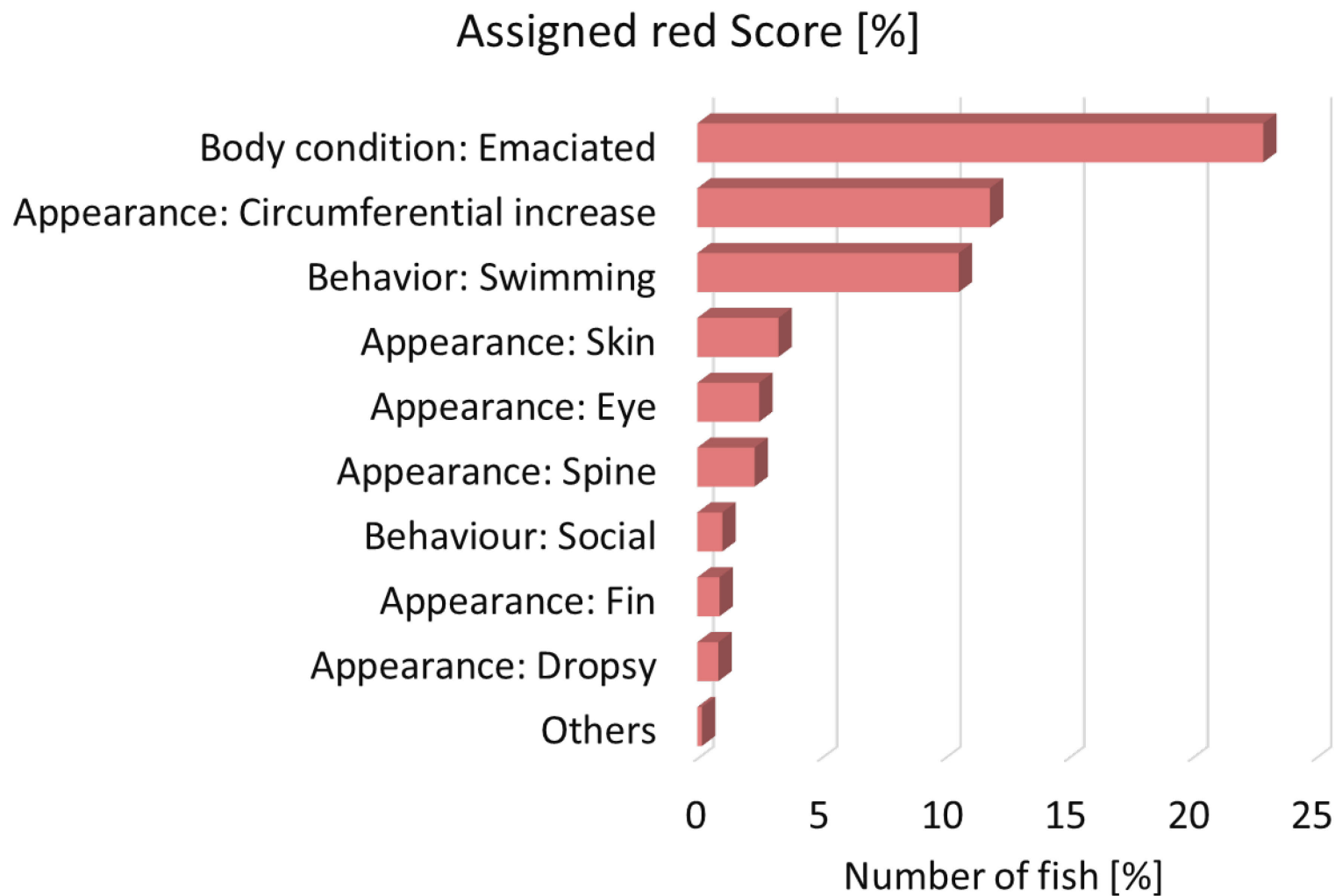


Zebrafish line	WT ABxTü
Number of animals	1043
Median survival	957d (2.6y)
Oldest fish	2034d (5.5y)

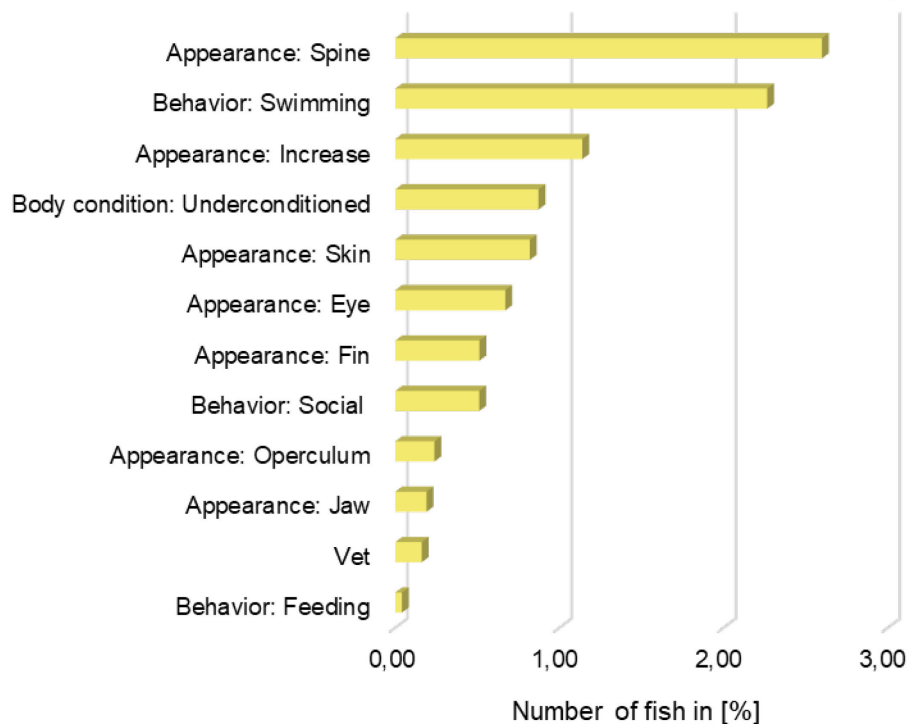
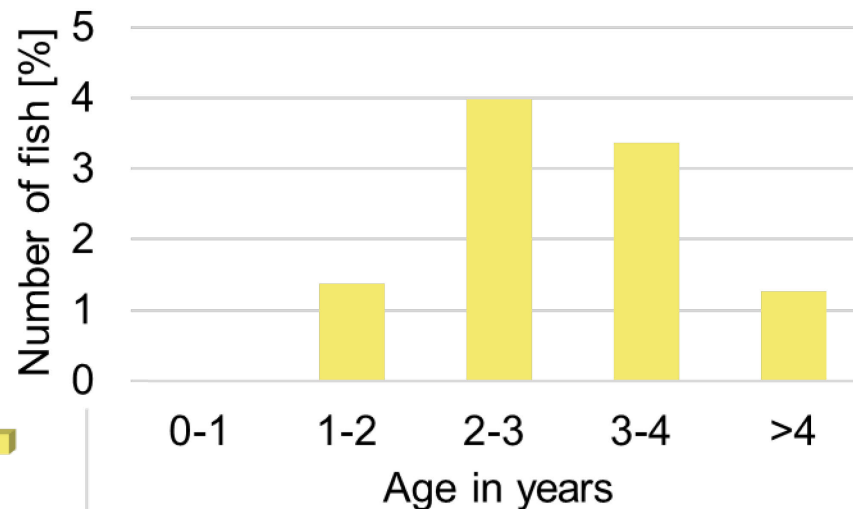
Age groups	Number of fish
0-1	4359
1-2	3694
2-3	2487
3-4	1068
>4	159



- Number of found dead stays at low level
- Aged zebrafish show more severe clinical signs (red category)



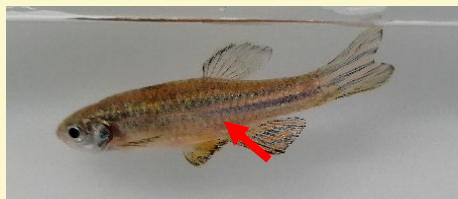
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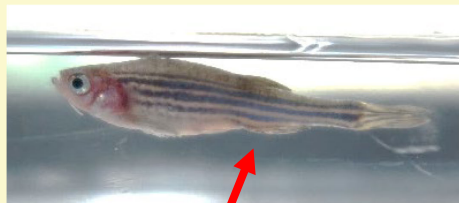
- Most yellow scores seen at age of 2-3 years
- Score “spine” most common
 - Also associated with aging



- Under-conditioned



- Loss of color



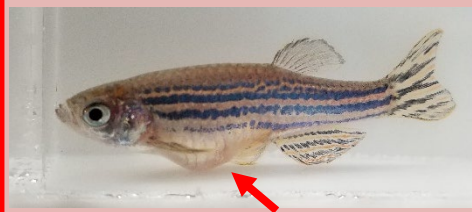
- Clamp of fins



- Missing eye



- Strong kyphosis
- Influenced swimming behaviour



- Circumferential increase



- Dropsy
- Scales detached from the body



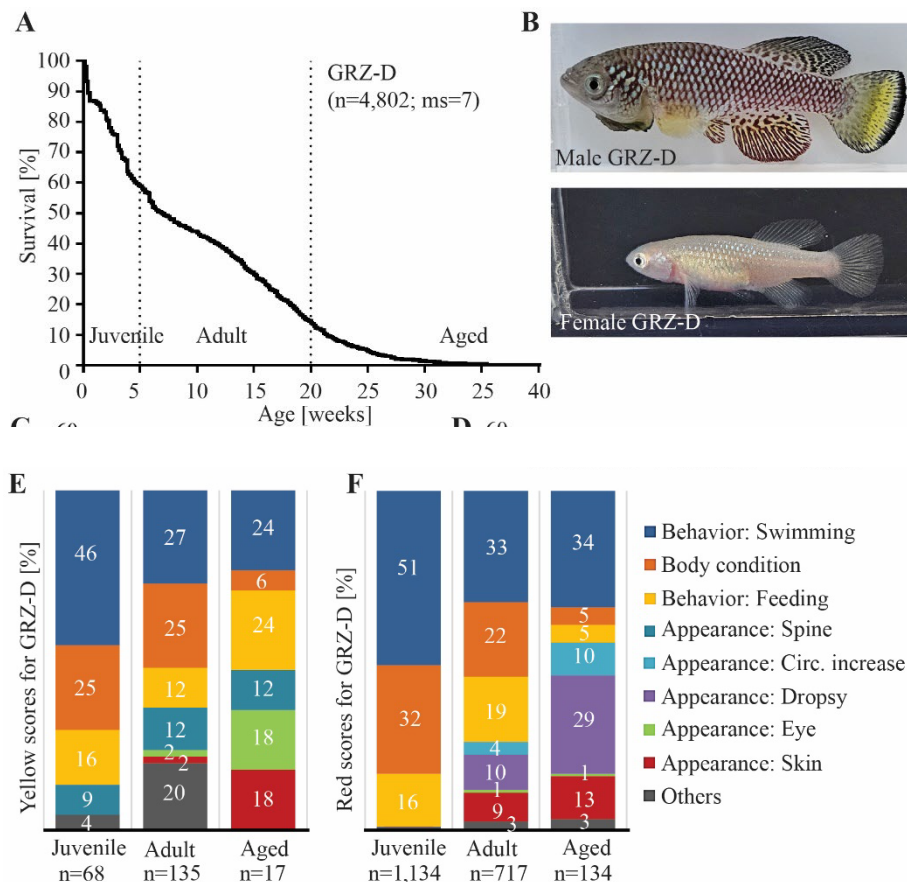
- Rotten fin
- Hyperpigmentation

- Published in JAALAS
- DOI: 10.30802/AALAS-JAALAS-23-000067.



Comprehensive Colony Health Management and Emerging Pathogens of the Annual Killifish Species *Nothobranchius furzeri*

Uta Naumann,¹ Jennifer L Brazzell,² Marcus J Crim,² and Beate Hoppe^{1,*}

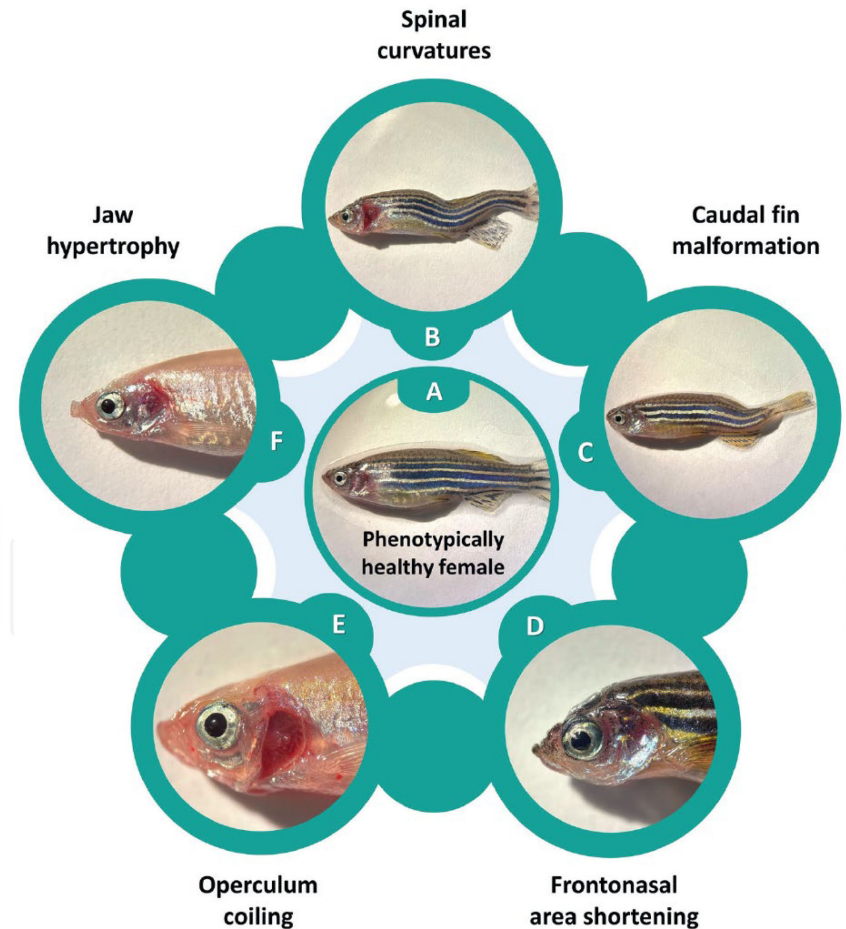


Chapter

Colony Management at Zebrafish Facilities

Inês Gonçalves and Joana Monteiro

- Morphological abnormalities tend to become evident after six to eight generations
 - inbreeding depression
- More frequent and severe developmental defects (e.g. small head, underdeveloped lower jaw and caudal fin malformation)
- Decrease in fertility, growth and survival
- Gender biases (typically more males)



1. Good animal welfare is crucial for high-quality science
2. Assessing care sheets help to improve welfare in zebrafish
 - Standardizing observations of health, behavior, and physical condition
 - Identifying early signs of distress or disease, enabling timely intervention
 - Providing objective, trackable data for continuous welfare assessment
3. The research community has a duty to increase knowledge about fish health, pain and welfare