12th International CULA

April 17th, 2026

The Lecture Starts at 15.05

Stress, Anxiety & Welfare of Laboratory Animals

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Contents

- What is stress?
- How to quantify stress
- Stress and Anxiety
- Stress and Welfare
- Indicators of welfare

WHAT IS STRESS

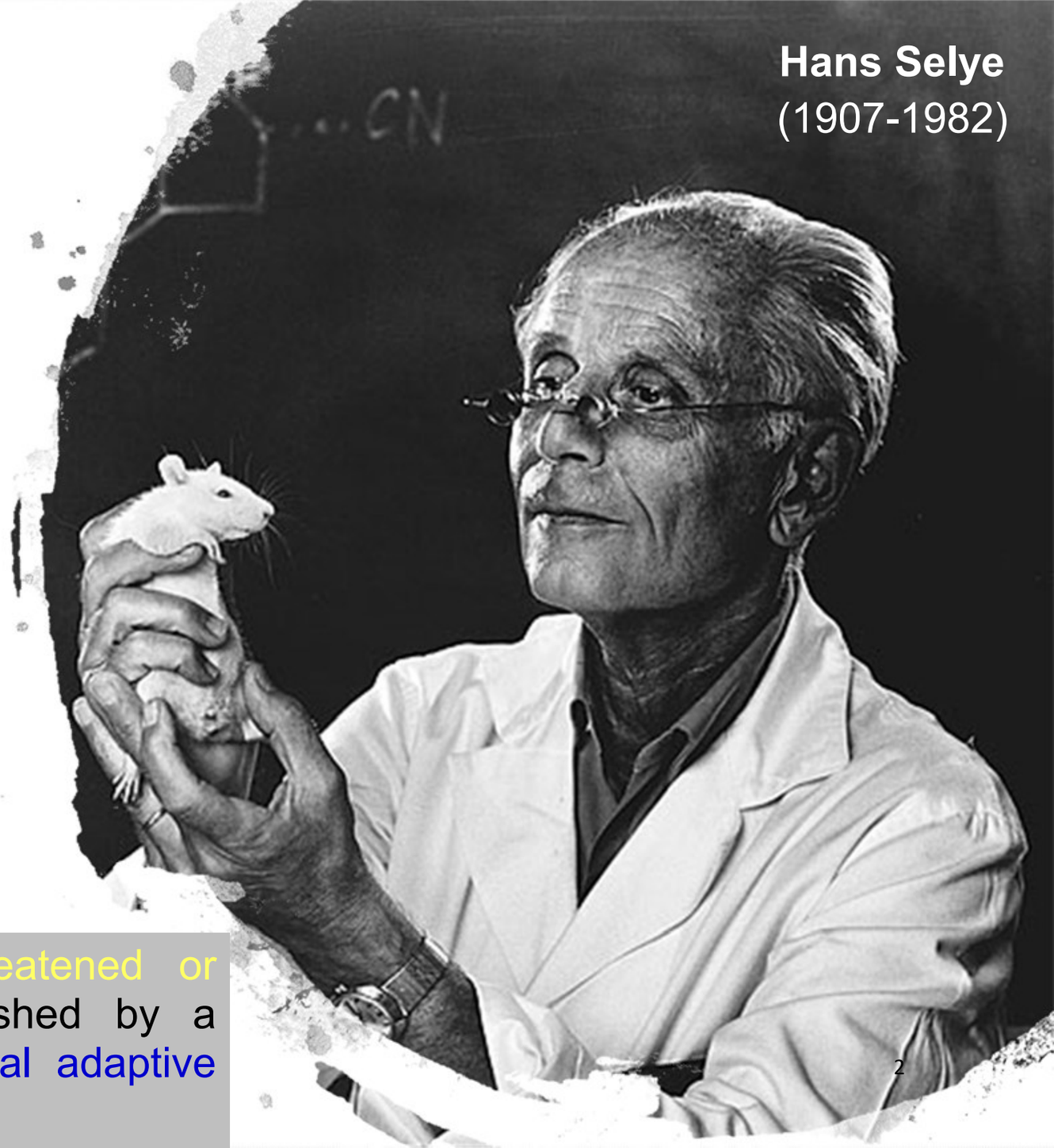
The classical concept of stress

Stressor

"Agent"

Response

A state in which homeostasis is actually threatened or perceived to be so; homeostasis is re-established by a complex repertoire of behavioral and physiological adaptive responses of the organism (Chrousos 2009)



Hans Selye
(1907-1982)

The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 6 FEBRUARY, 1946 NUMBER 2

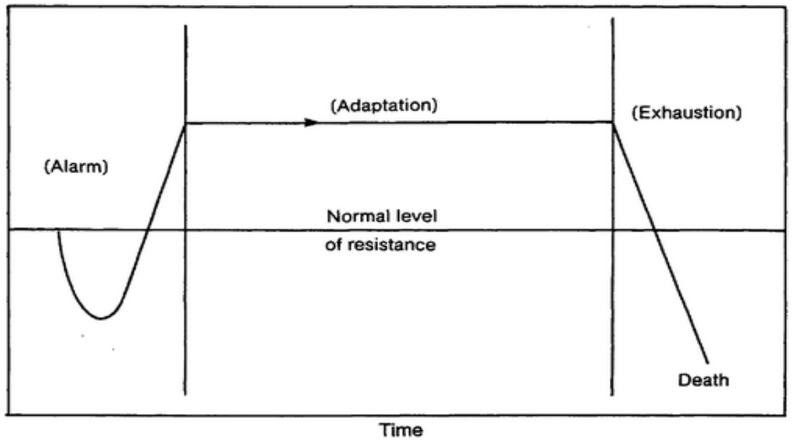
THE GENERAL ADAPTATION SYNDROME AND THE DISEASES OF ADAPTATION[1]

HANS SELYE, M.D.

From the Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada

DEDICATION

Dedicated to the memory of that great Student of homeostasis, whose life (90b) and work (90a) have been the author's greatest inspiration.



1. **Alarm Reaction *(flight or fight response)***
2. **Stage of Resistance *(adaptation response)***
3. **Exhaustion *(mental and physical exhaustion)***

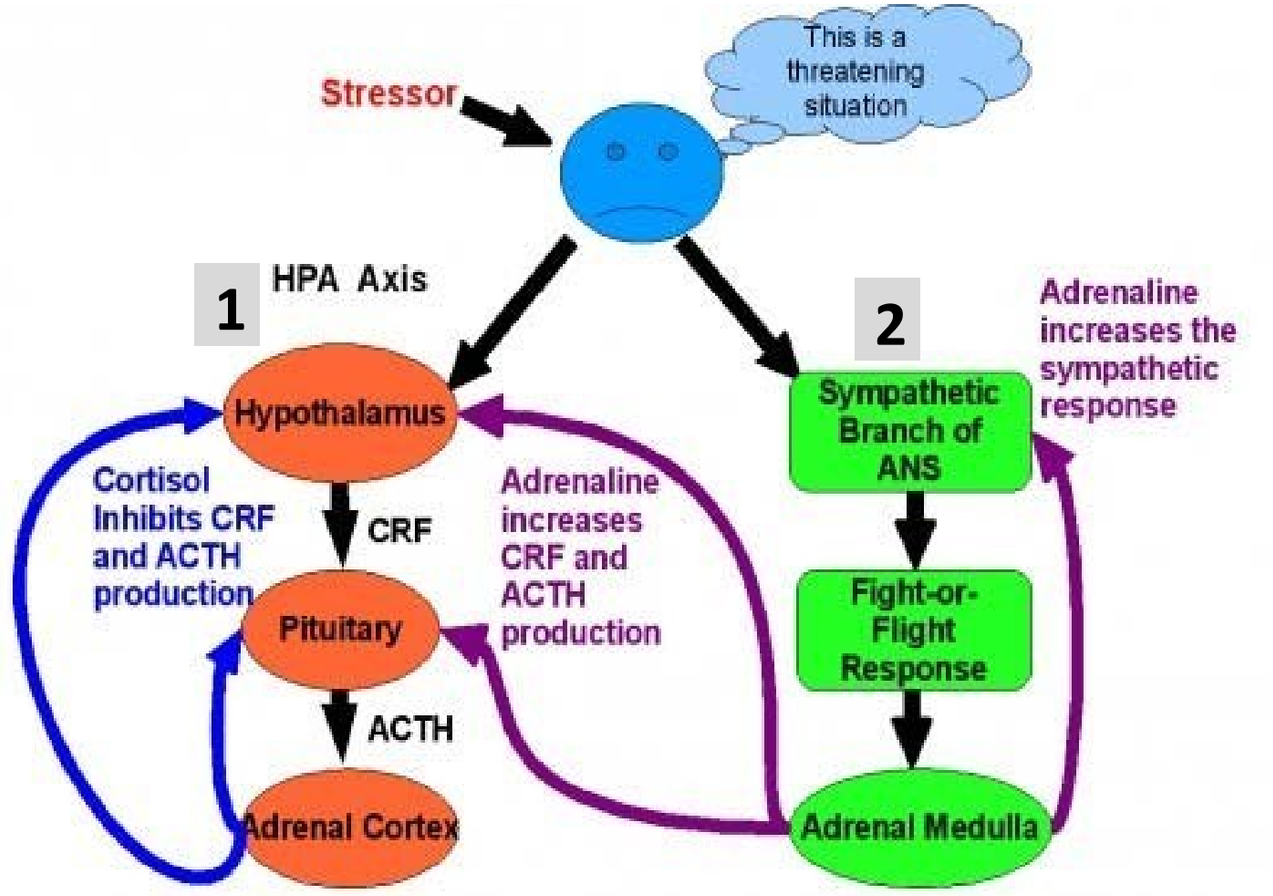
Type of Stress Responses

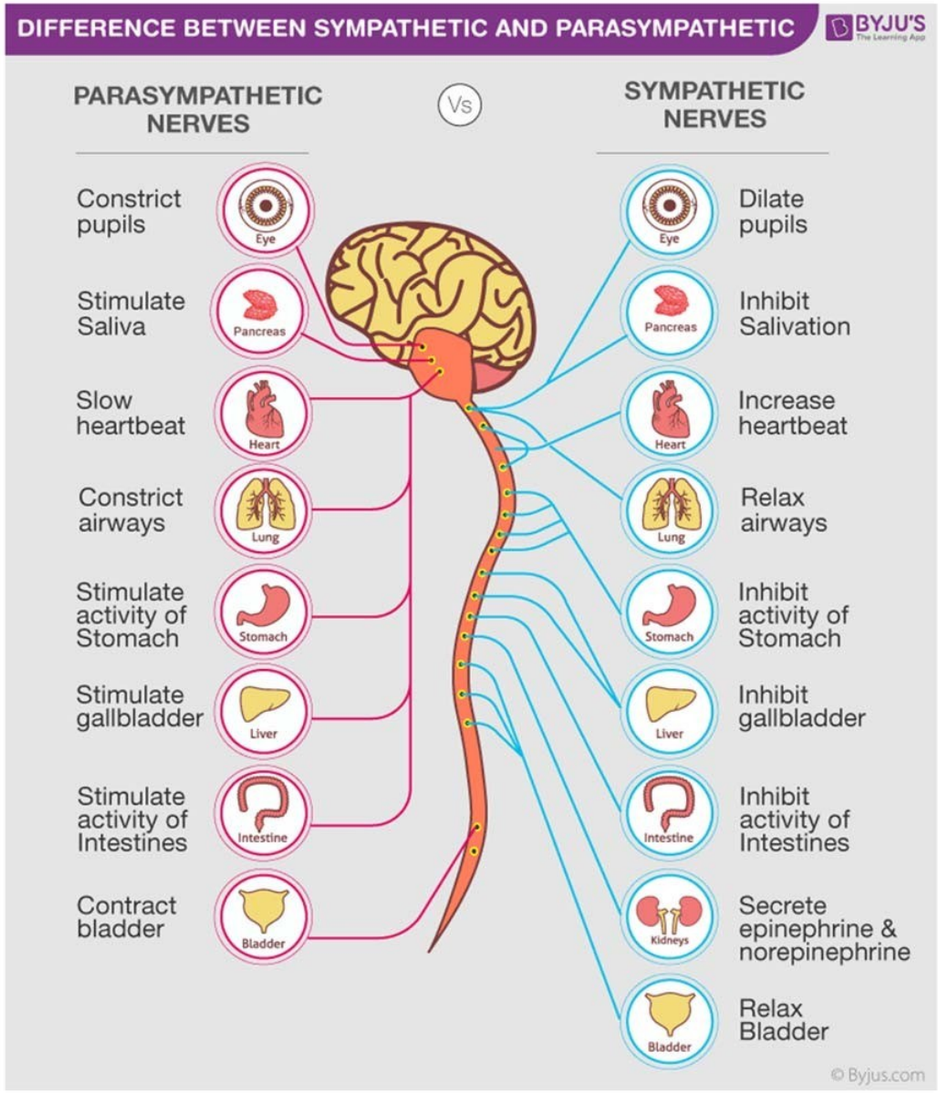
Primary — Secondary — Tertiary

The primary response

Hormonal response

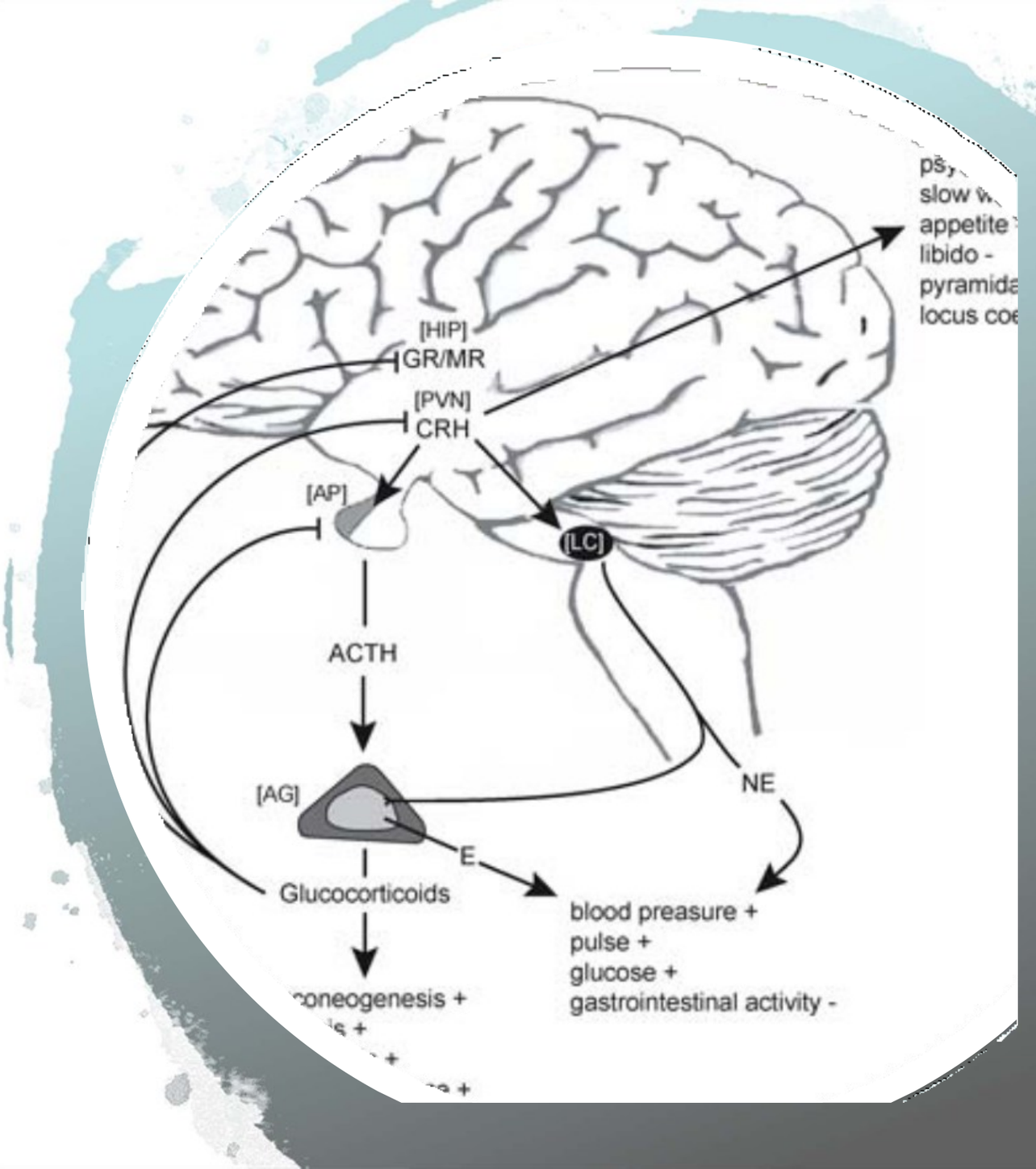
- Cortisol
- Catecholamines (epinephrine / norepinephrine)





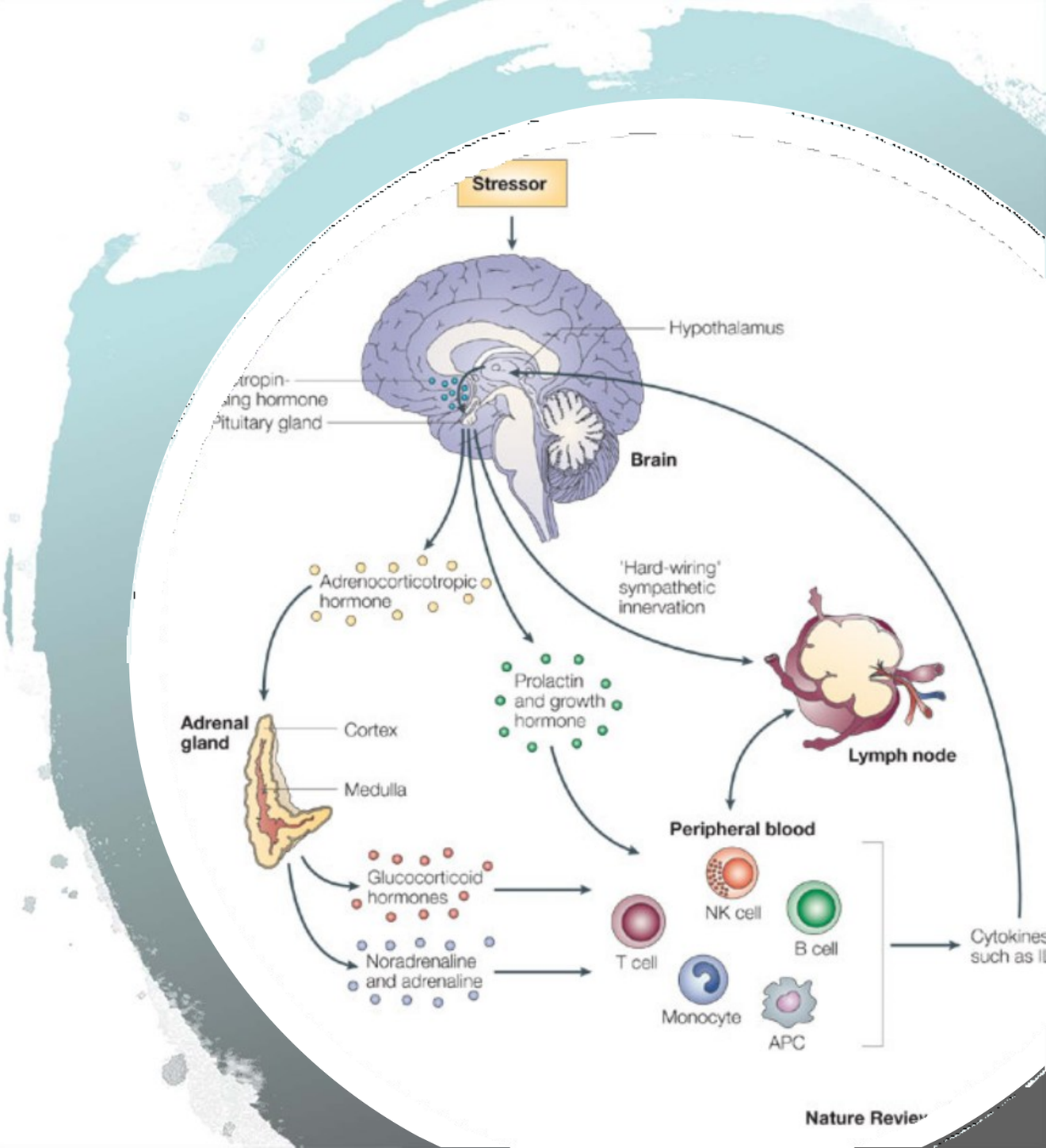
The secondary response

- Metabolism (biochemical profile)
- Water, ionic and salt balance
- Cardio-vascular system
- Immune system

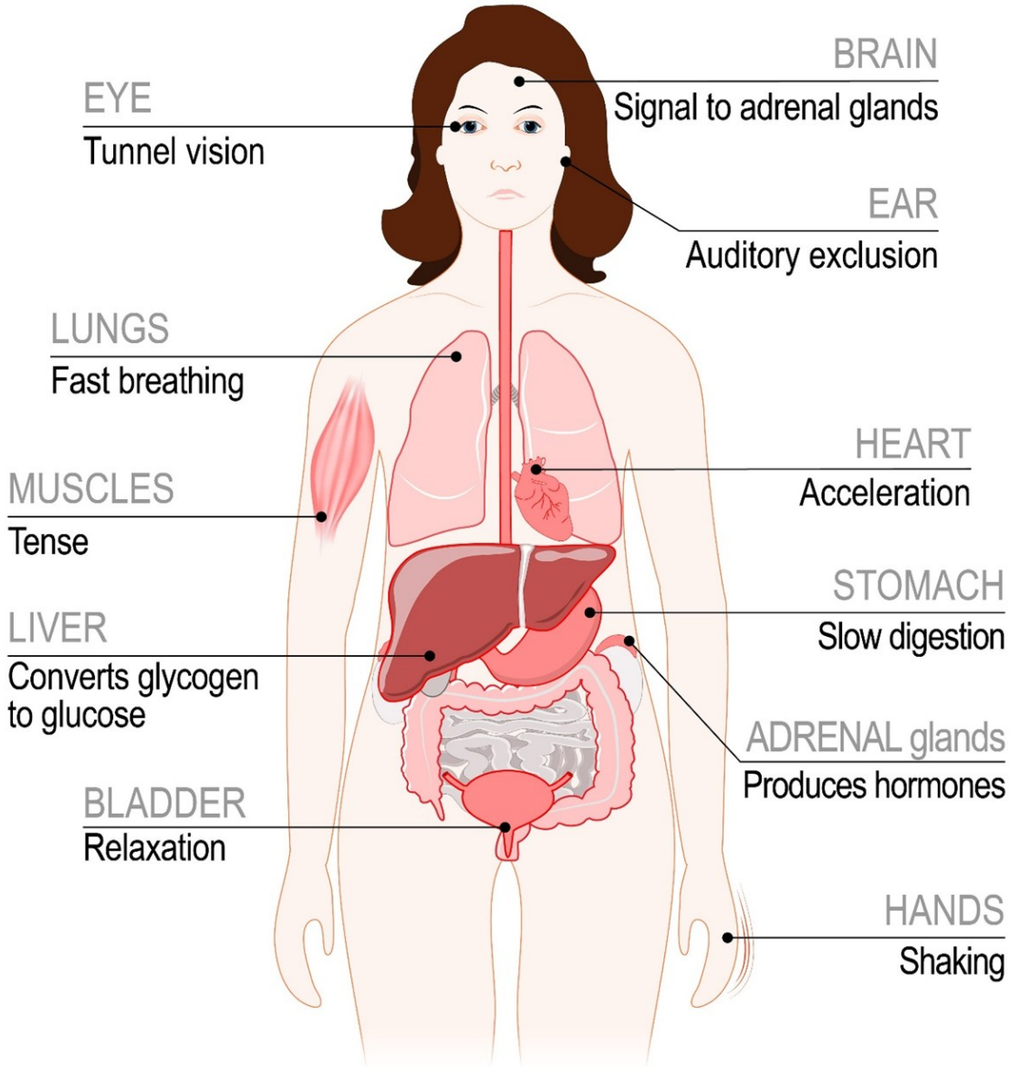


The tertiary response

- Growth
- Reproduction
- Behaviour
- Health



The classic fight-flight response



Welfare

Absence of stress = good welfare?

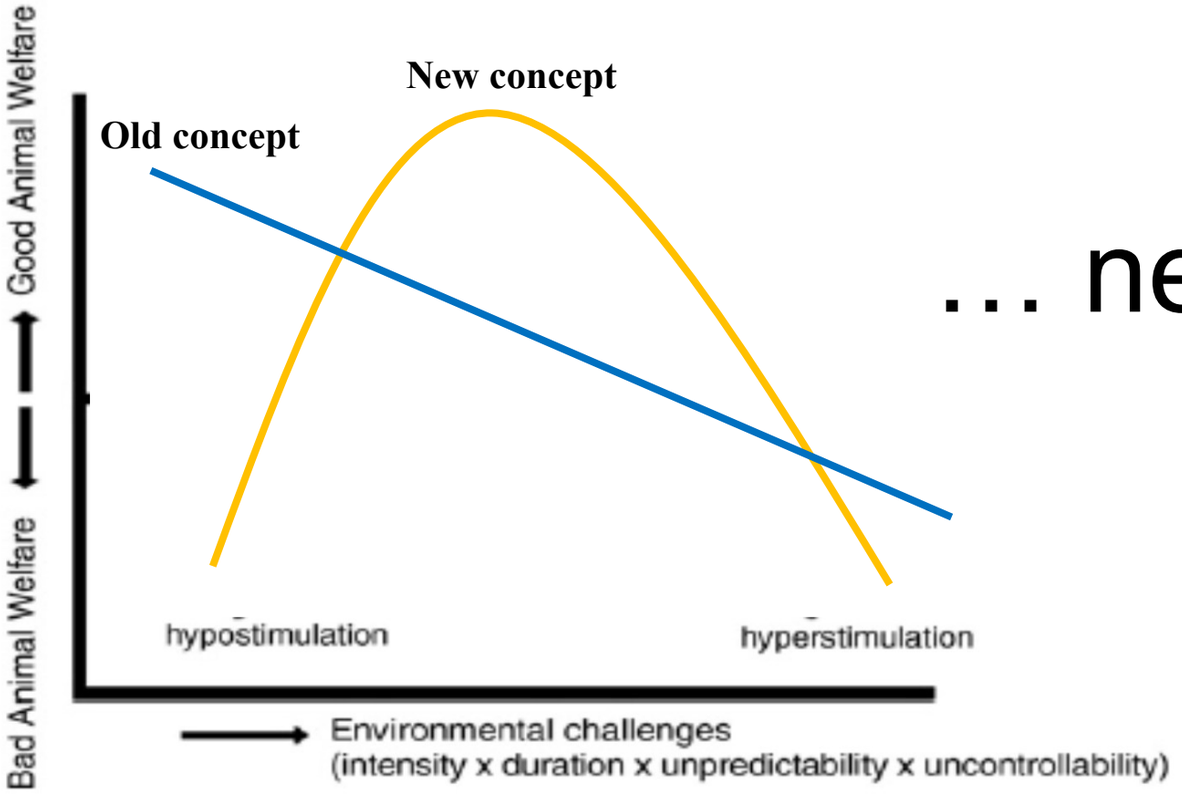


Fig. 1. Animal welfare in relation to environmental challenges as shown by the out-dated concept based homeostasis and the new concept based on the inverted U-curve of (di)stress.

… new ideas…?

Welfare … according to Selye


BRITISH MEDICAL JOURNAL

LONDON SATURDAY JUNE 17 1950

STRESS AND THE GENERAL ADAPTATION SYNDROME*

BY

HANS SELYE, M.D., Ph.D., D.Sc., F.R.S.C.

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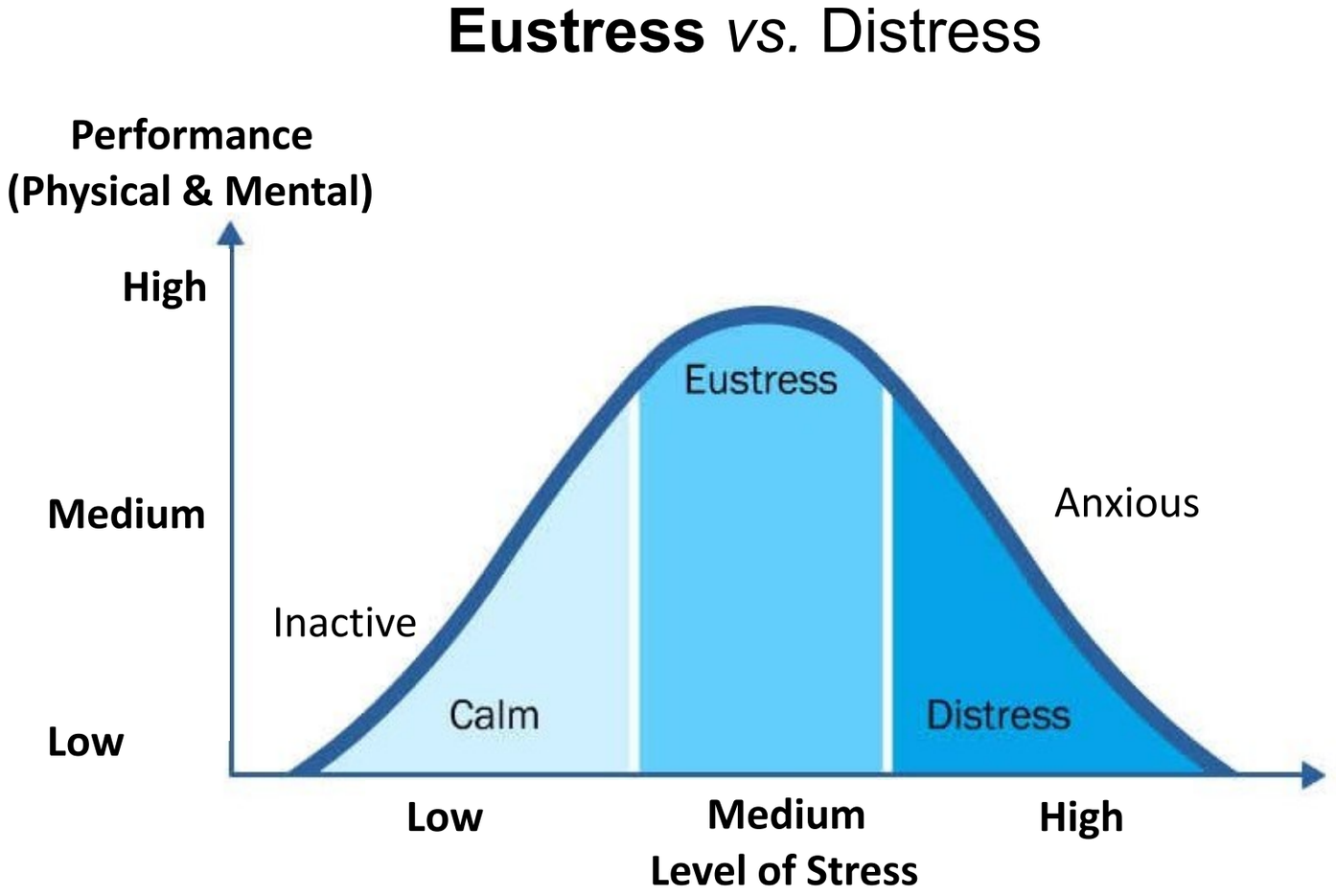
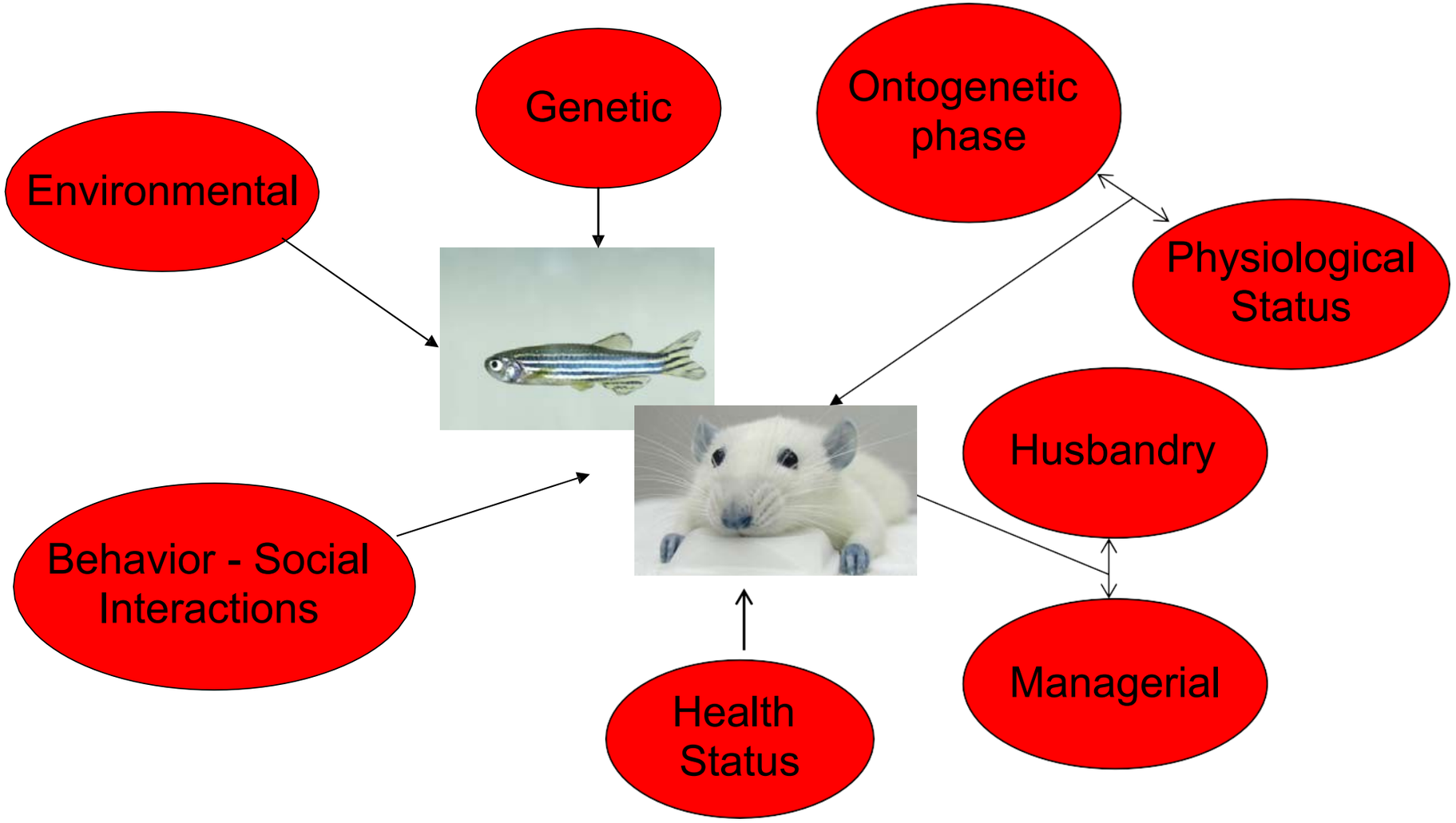
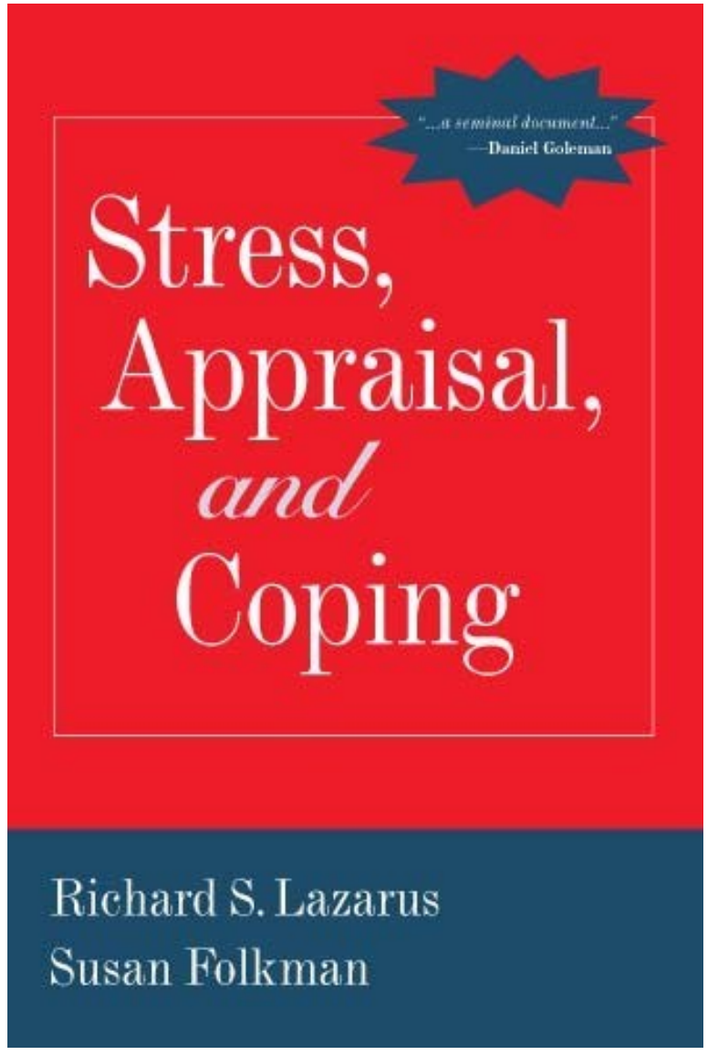


Potential stressors of Life cycle in captivity



The contemporary concept of stress

«…stressful experiences are construed as ***person-environment transactions*** *that are perceived as* ***imbalance*** *between* ***demands and available resources…*** *occurring when pressure exceeds one's perceived* ***ability to cope***



- **Primary appraisal* → ("Am I OK?")**
  Evaluation of the stimuli (***positive***, ***negative*** or ***neutral***)

- **Secondary appraisal → ("What can I do?")**
  Evaluation of the ***controllability*** of the stressor and a person's ***coping resources***

- ***Coping efforts***
  Actual strategies used to mediate primary responses

*"…an automatic, often unconscious, assessment of what is happening and what it may mean for them or those they care about"

FOUNDERS

Richard S. Lazarus

Defined by Lazarus & Folkman in 1977, however was looked into earlier in the 1960s.

Susan Folkman

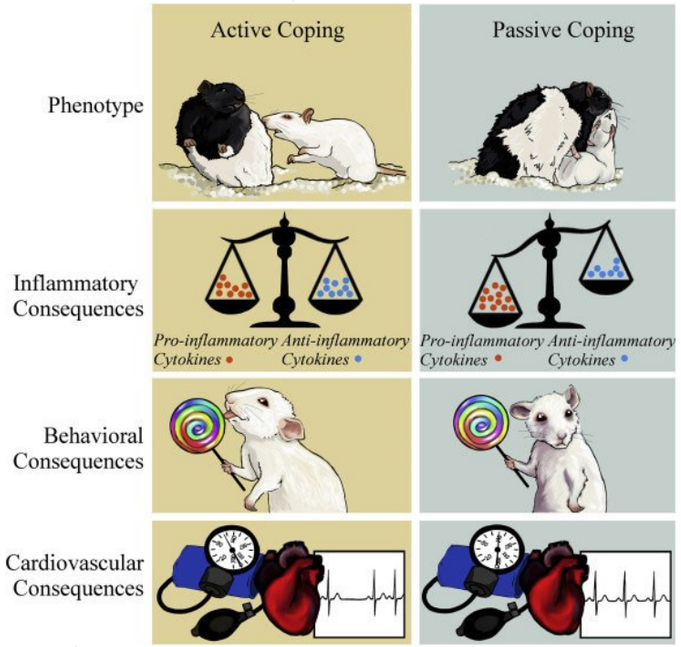
Intra-specific differences

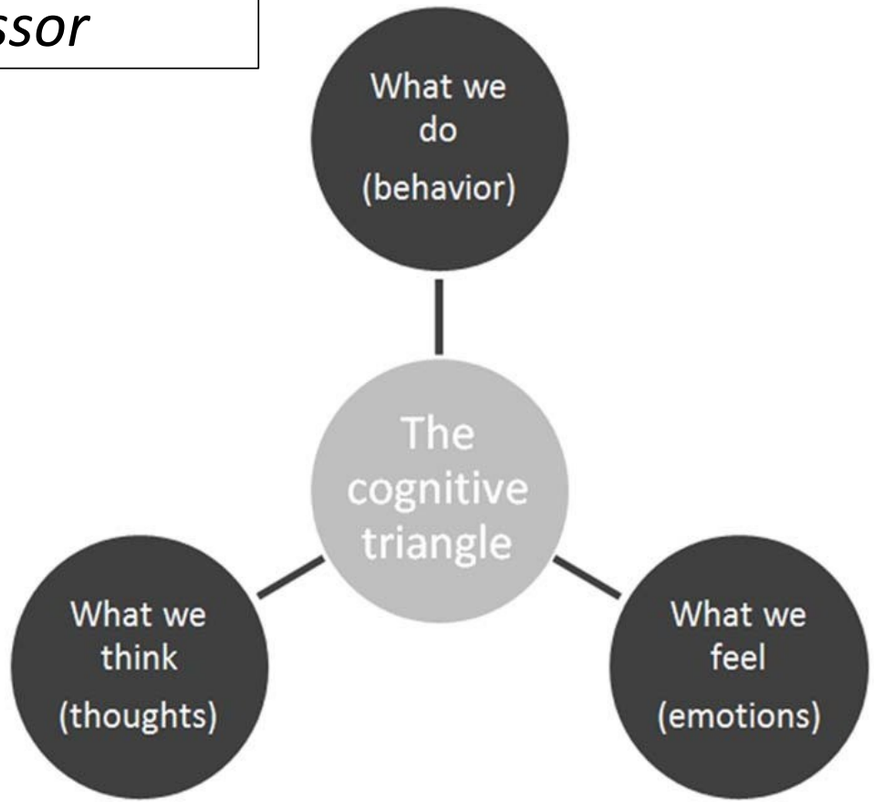
A correlated set of individual behavioural and physiological characteristics that is relatively consistent over time and across situations, and *that can affect a person's emotional or functional reaction to a stressor*

Coping Styles / Strategies*

**or*

- *Behavioural syndromes,*
- *Behavioural patterns,*
- *Personalities,*
- *Idiosyncrasy,*
- *Temperament…*





Coping Styles / Strategies*

**or*

- *Behavioural syndromes,*
- *Behavioural patterns,*
- *Personalities,*
- *Idiosyncrasy,*
- *Temperament…*

	Hawks	Doves
Coping Style	Proactive	Reactive
Behavioral Strategy	Fight-flight	Freeze-hide
Emotional State	Aggressive & bold	Non-aggressive &cautious
Exploration	Fast & superficial	Cautious & thorough
Behavioral flexibility	Rigid & routine-like	Flexible

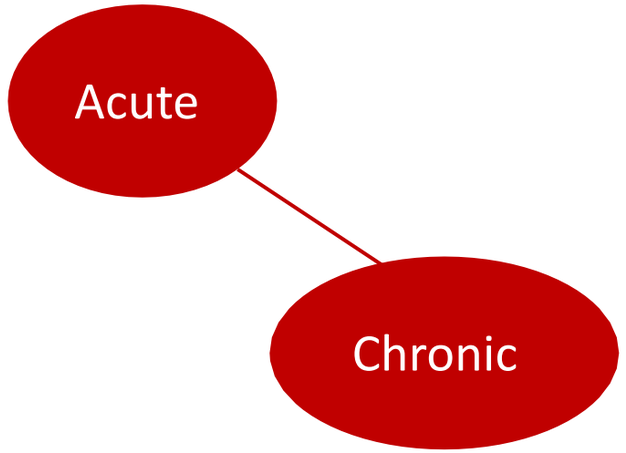
Neuro-endocrine differences	Hawks	Doves
HPG-output (testosterone)	High	Low
HPA-output (cortisol or corticosterone)	Low	High
Hypothalamus (CRF mRNA)	No response	High
Hippocampus (MR mRNA)	No response, except CA1↑	High
Hippocampus (GR mRNA)	No response	No response
Pituitary (ACTH as % of basal)	Low	High
Adrenal cortex sensitivity	Low	High
Neurosympathetic (NE)	High	Low
Adrenomedullary (E+NE)	High	Medium
Parasympathetic	Low	High

Video 1: Fish can be creatures of habit too

https://www.youtube.com/watch?v=ZwW2vJ1we7c

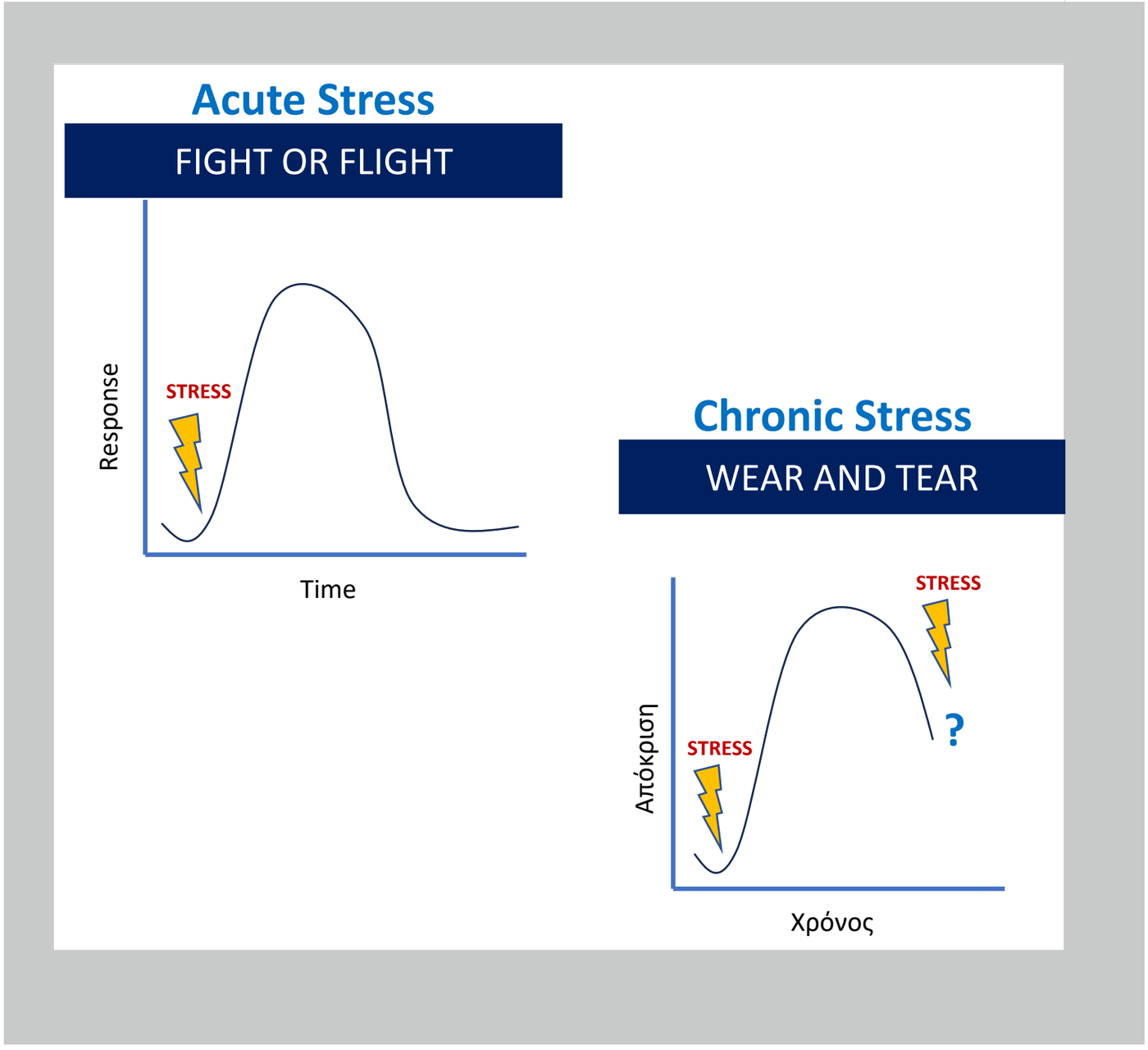
Acute Vs Chronic stress

- Duration of the exposure
- Repetition
- Predictability
- Controllability



	Acute	Chronic
Duration	Short	Long or short but repeated
Intensity	Severe	Severe or mild
Predictability	No	Predictable or unpredictable
Repeatability	No	Yes (potentially)
Controllability	No	Controllable or uncontrollable

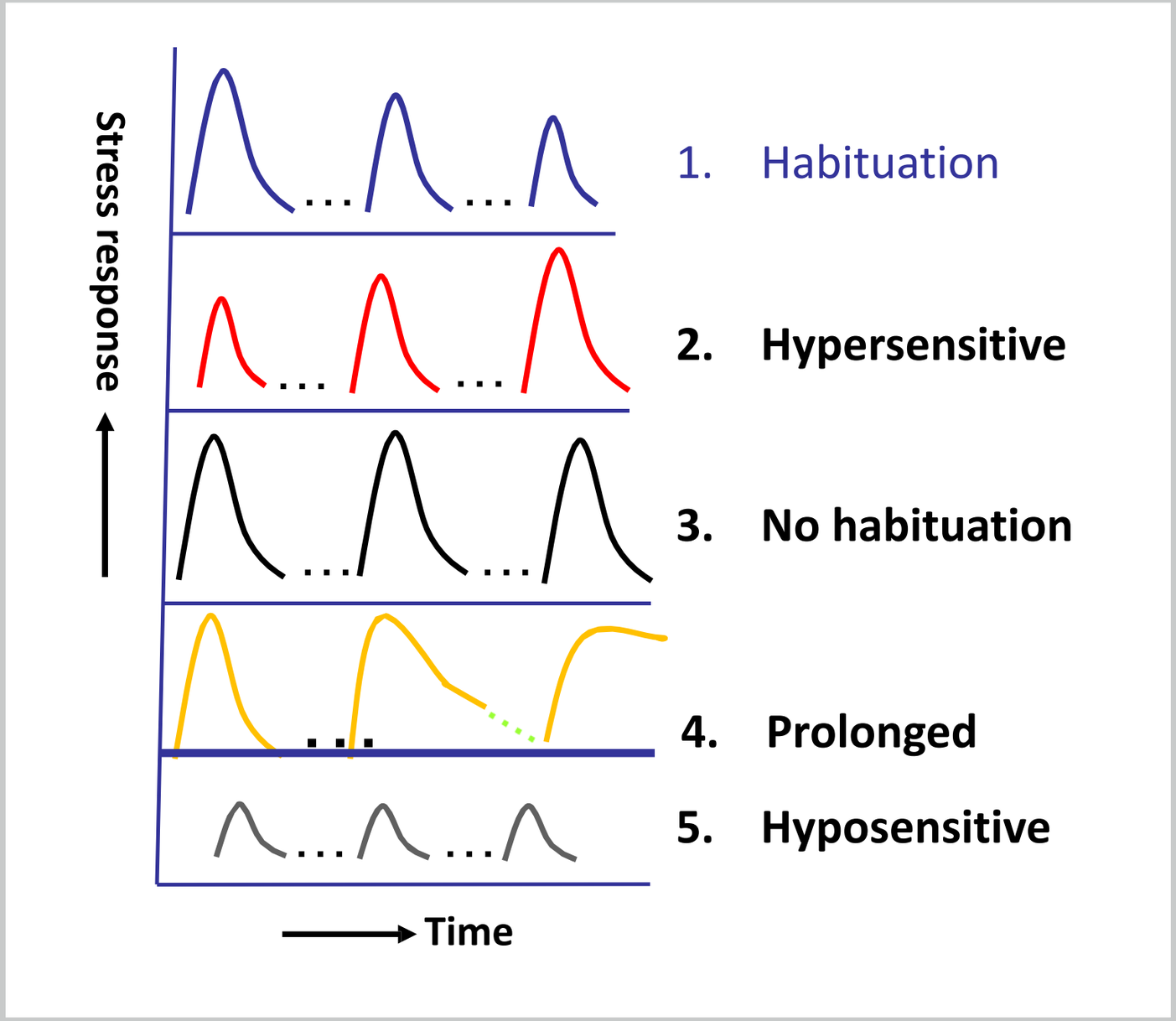
Acute Vs Chronic stress



After repeated exposure to the ***same stressor habituation*** **(1)** of the stress response optimally takes place

However, due ***to different gene x environmental interactions*** the organism may show a

- **Hypersensitive** stress response **(2)**
- **No habituation (3)**
- **Prolonged** stress response **(4)** or
- **Hyponsensitive** response **(5)**



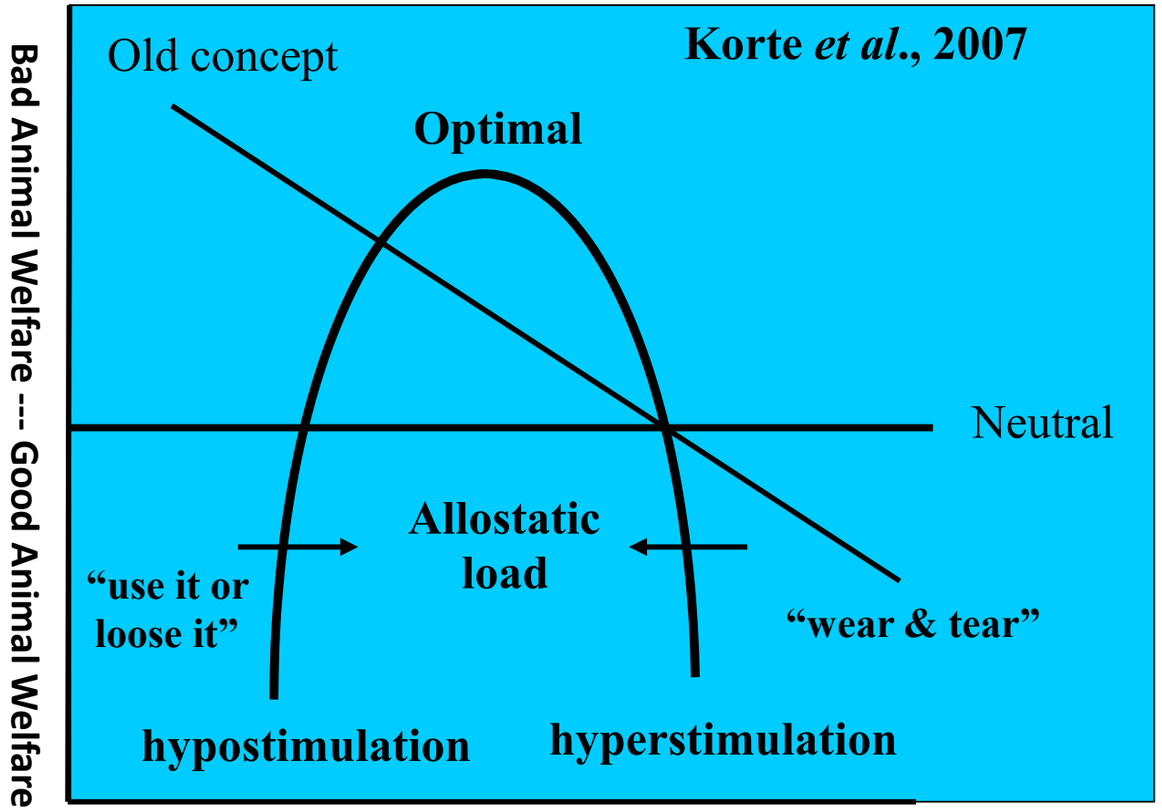
Source: *Korte et al., 2007. Physiology & Behavior 92: 422–428*

…and the Welfare Concept II

Individual Differences

↕

Coping Style



→ **Environmental challenges**
(intensity x duration x unpredictability x uncontrollability)

Source: *S.M. Korte et al., 2007. A new animal welfare concept based on allostasis. Physiology & Behavior, 92: 422-428*

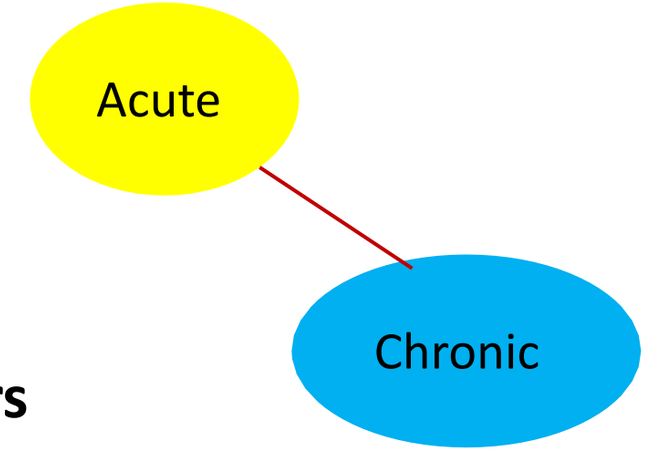
HOW TO QUANTIFY STRESS

Are there any reliable stress assessment indicators – Part I?

- Molecular
- Cellular

- Physiological
- Metabolic
- Immunological

- Behavioural
- Performance



Common Physiological Indicators

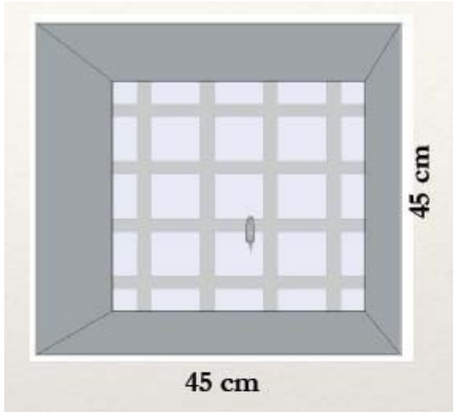
- Cortisol, Corticosterone
  (blood, saliva, faeces, water, urine, hair, scales)
- Glucose, lactate
- Neutrophils (heterophils) to lymphocytes ratio

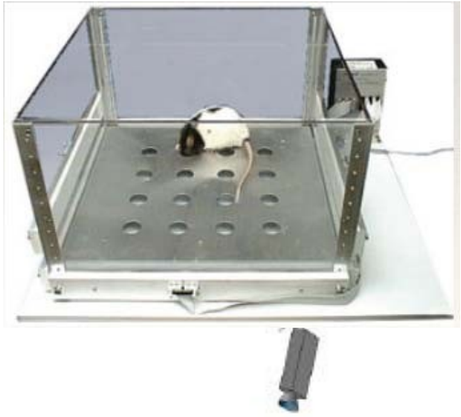
Are there any reliable stress assessment indicators – Part II?

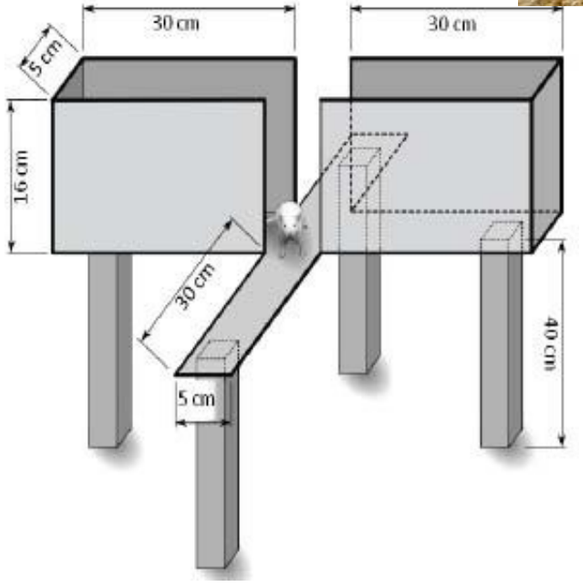
Behavioural Tools

- Open-field
- Elevated plus maze
- Elevated zero maze
- Hole board test
- Dark / Light transition
- Predator
- Social interactions

Source: *Sidiropoulou, 2015*







Behavioural patterns
(Examples; mice & rats)

- ✓**Prefer** closed or protected / **avoid** open places
- ✓**Afraid** of heights
- ✓**Prefer** dark / **avoid** light rooms
- ✓Freezing behaviour, decreased activity, increased thigmotaxis
- ✓Grooming (*normal maintenance behavi*or; *anxiety: inverted U-shaped function*)
- ✓Head-dipping (into holes in the floor) activity

  (*exploratory behaviour/neophilia? escape response?*)

STRESS AND ANXIETY

A conceptual problem?

	Stress	**Anxiety**
Reaction to	Particular problem or situation	Stress / worry
Caused by	Specific, identifiable cause Often goes away once the stressor is resolved	May or may not have a clear or real stressor Often persists even when there's no actual threat
Feeling	Pressure (physical/mental/emotional)	Unease, worry or fear, restlessness
Emotional state	**Present**	**Future**
Symptoms	Mimic those of anxiety	Persistent, excessive fear or worry in situations that are not threatening
Dealing with	More practical manner	Counseling, medication, or psychological treatment

Psychological, physiological & behavioural state induced by a threat to well-being or survival, either actual or potential

Are there any reliable anxiety appraisal indicators / tools?

Stress & Anxiety Indicators Rats & Mice

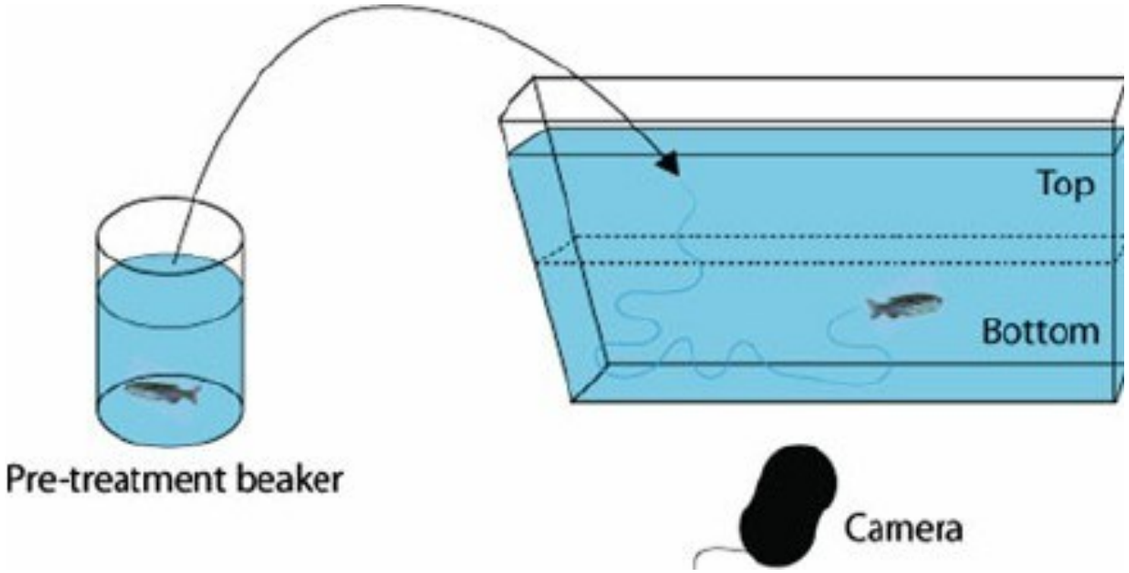
Behavioural	Physiological	Endocrinological
Grooming	Hypothermia	Corticosterone
Appetite	Pulse	Catecholamines
Activity	Respiration (*rapid shallow breathing*)	Thyroxine
Aggression	Weight loss	Prolactin
Vocalization	Blood-cell count	ACTH
Appearance	Blood-cell structure	Glucagon
Posture	Blood flow	Insulin

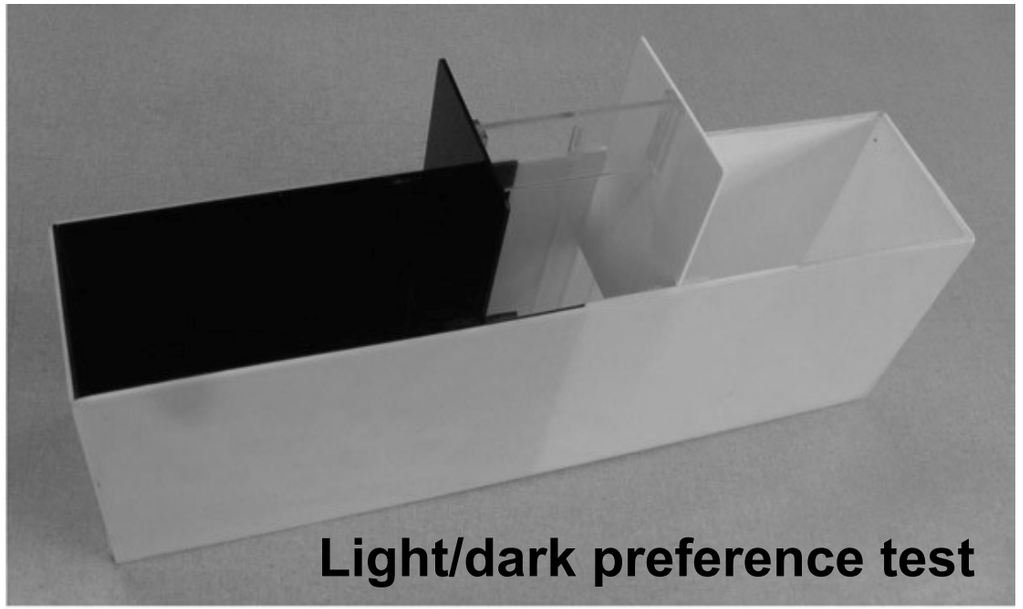
Hunched posture

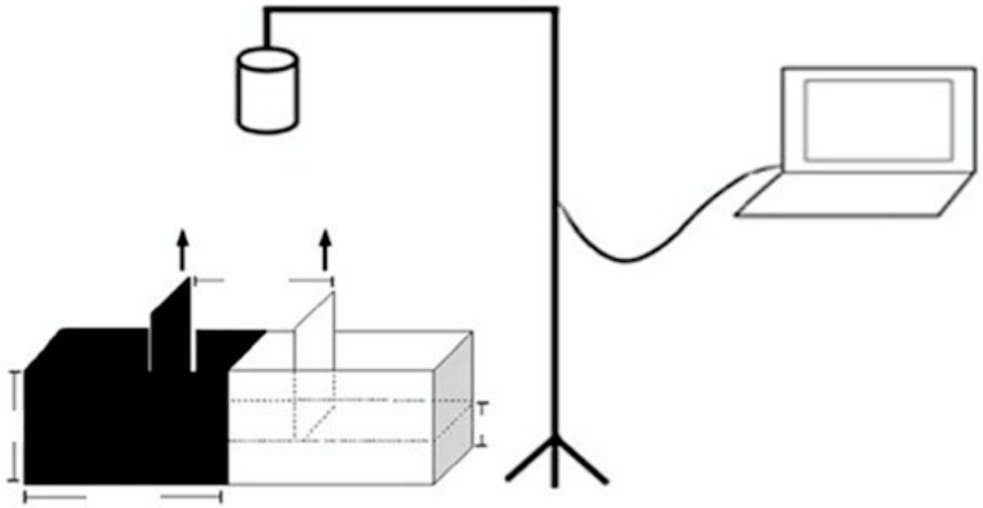
Sources: *National (USA) Research Council, 1992; Carstnes & Moberg, 2000. ILAR Journal*

What about zebrafish?

Novel Tank test





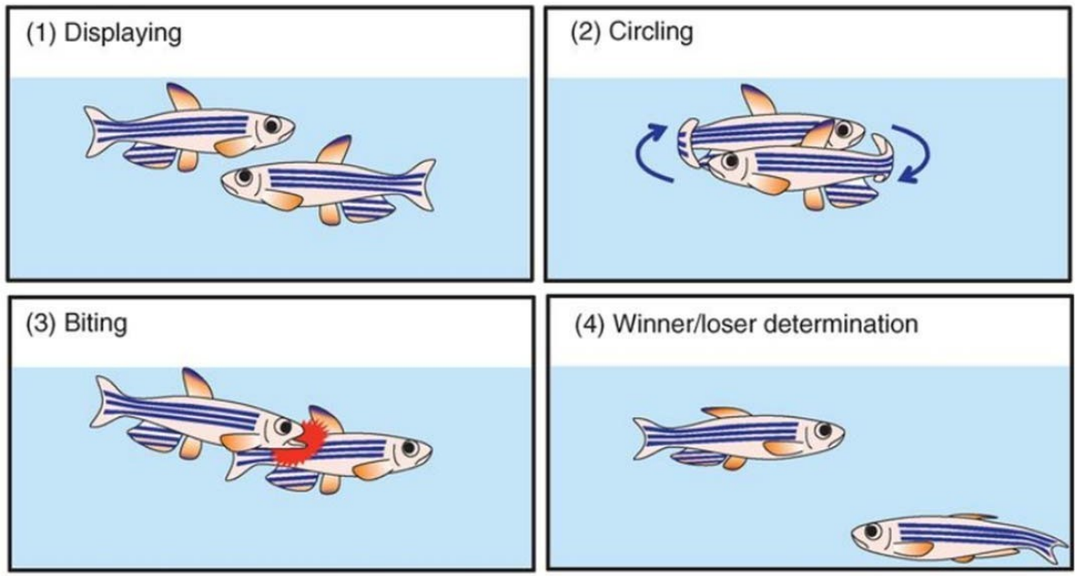


Source: *A. Theodoridi, ACES, 2021*

Exploratory behaviour test

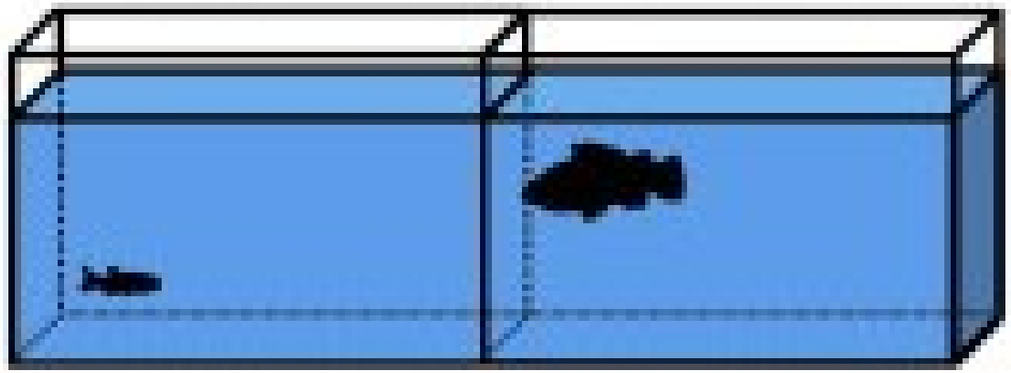
T-maze

Unconditioned social interaction test / Paired aggression test



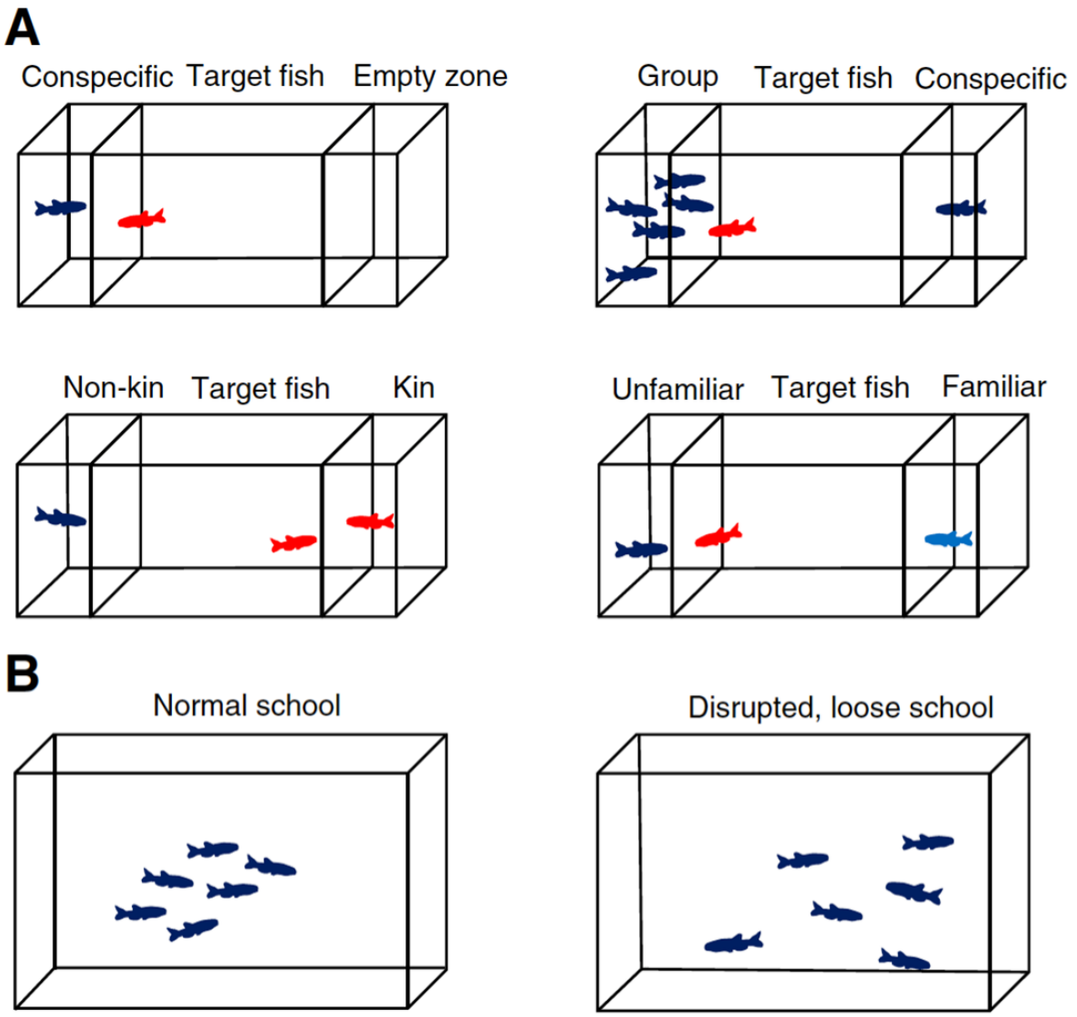
Source: *A. Theodoridi, ACES, 2021*

Predator avoidance test



Source: *A. Theodoridi, ACES, 2021*

Social behavior assessment test



10.1016/j.pnpbp.2013.11.014

Video 2: Effect of predator (Leaf fish) exposure on zebrafish stress and anxiety-like behavior (ZENEREI Institute (ZNRC-Japan) www.kaluefflab.com)

https://www.youtube.com/watch?v=bKIEww8RG5Y

https://www.youtube.com/watch?v=qAa0SxLaob4

STRESS AND WELFARE

Old goodies: The 5 Freedoms

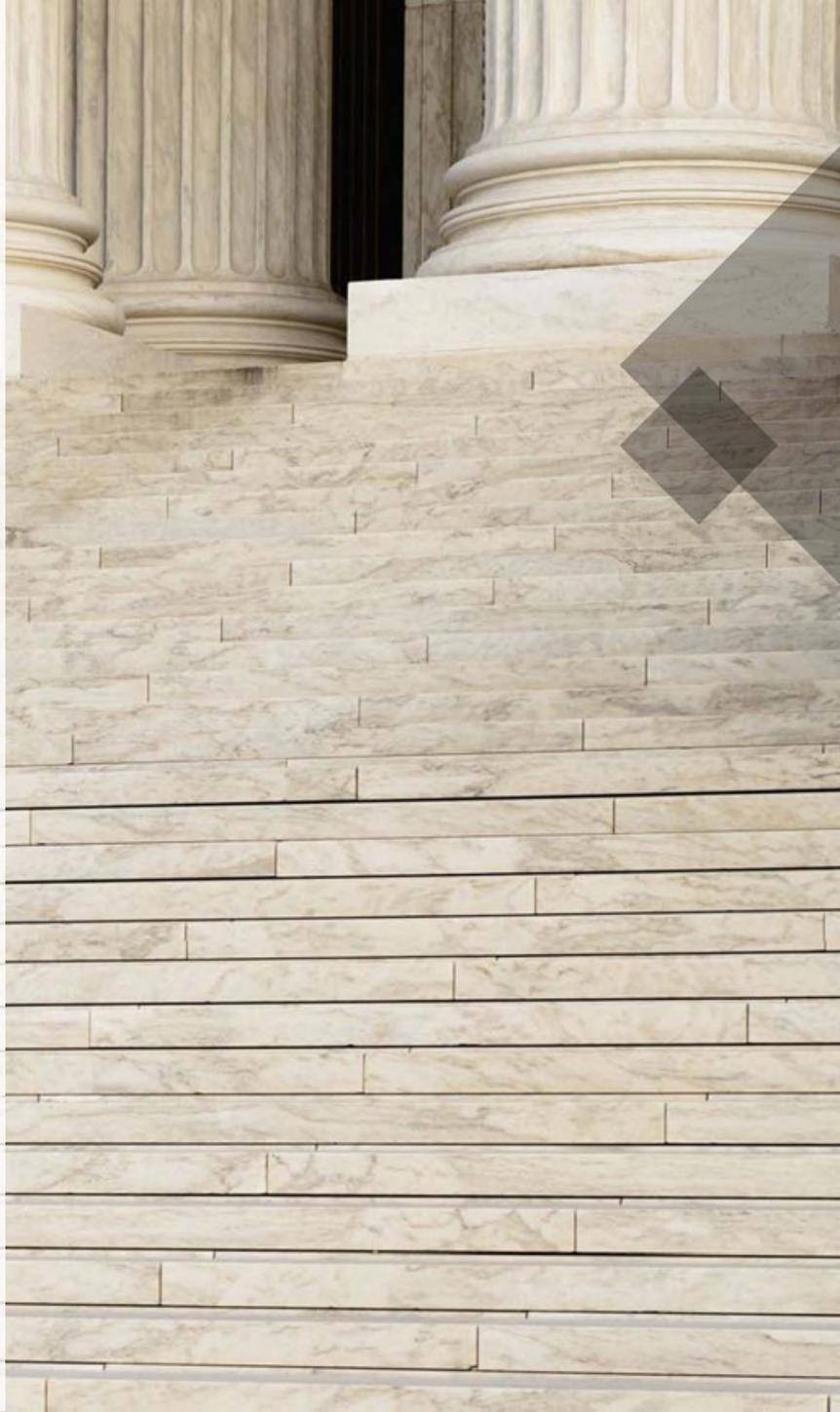
1. Freedom from thirst, hunger and malnutrition

2. Provision of appropriate comfort and shelter

3. Prevention, rapid diagnosis and treatment of injury, disease or infestation with parasites

4. Freedom from distress

5. Ability to display normal patterns of behaviour



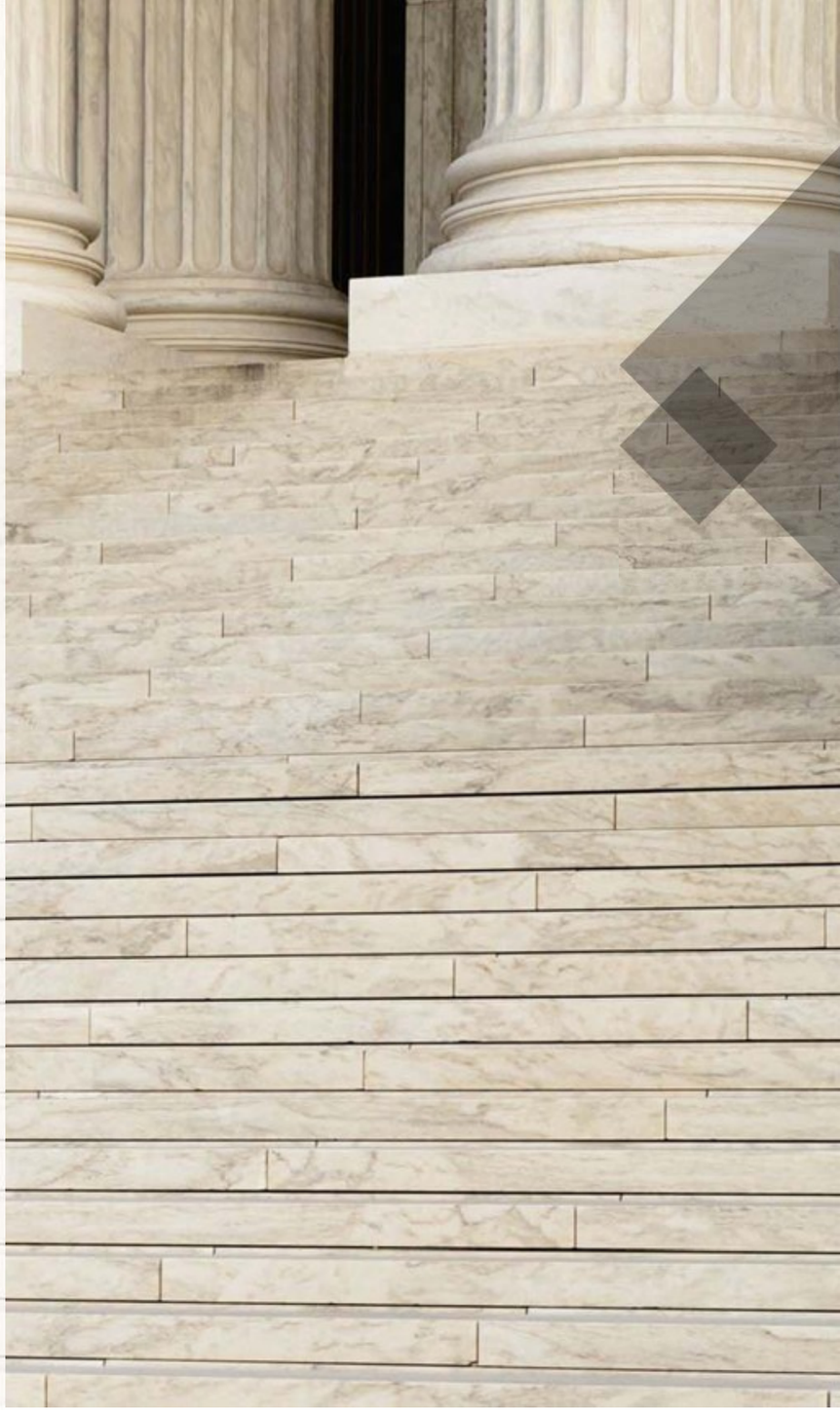
From Science to … Philosophy

The concept will always have
ethical, scientific & empirical dimensions

A) Function-based definitions

B) Feelings-based definitions

C) Nature-based definitions



Approach	Function-based	Feelings-based	Natural based
Theoretical background	Its state as regards its attempts to cope with its environment	Feeling-based; subjective; mental state of the animal in mind	Each species of animal has an inherent biological nature that it must express
Working approach	Animals suffer if they cannot maintain homeostasis	Animals suffer if they are exposed to negative experiences such as pain or fear	Animals suffer if they cannot express the full repertoire of behaviour that they show in the wild
Good welfare	Good health / proper function of biological systems	The animal should have access to positive experiences	The animal is able to lead a natural life & express its natural behaviour
Welfare assesment	***Physiology*** *(stress hormones, health, growth, reproduction…)*	***Emotional*** (secondary level ethological & physiological)	***Ethological***

WELFARE INDICATORS

WELFARE INDICATORS

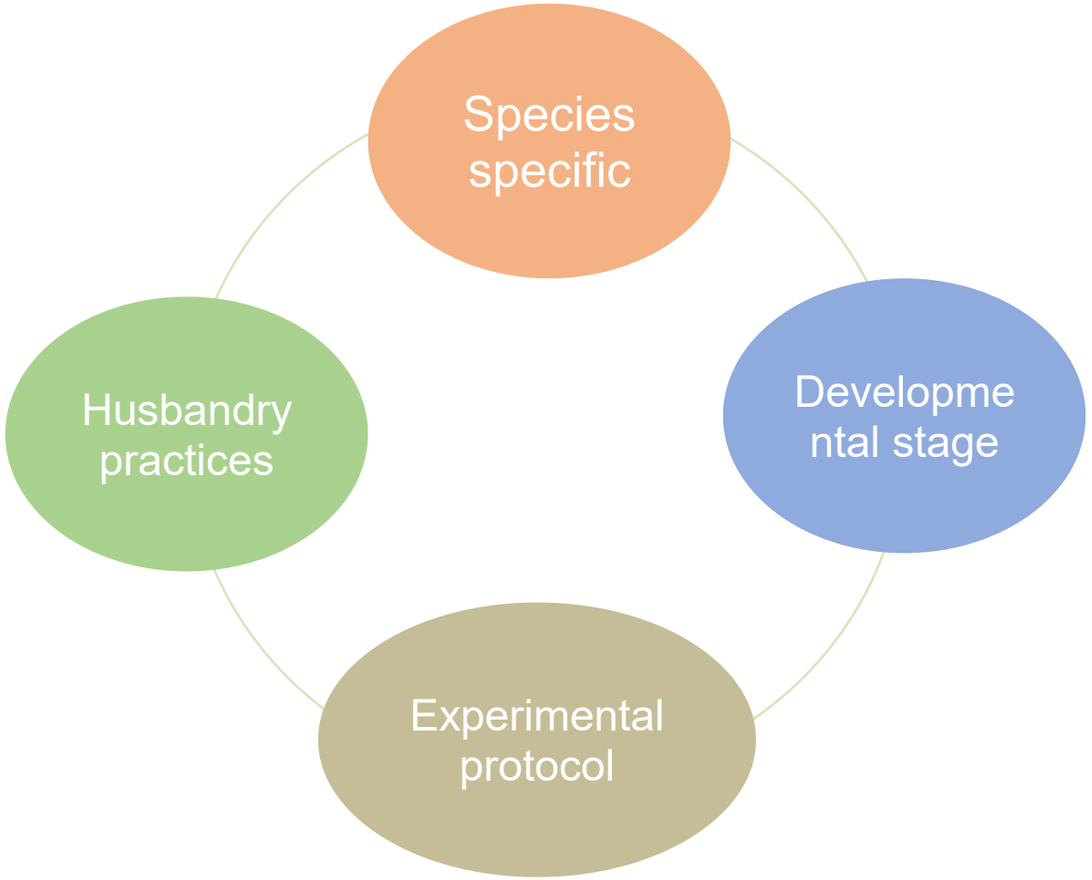
- Measurable
- Practical
- Universal or Species-specific

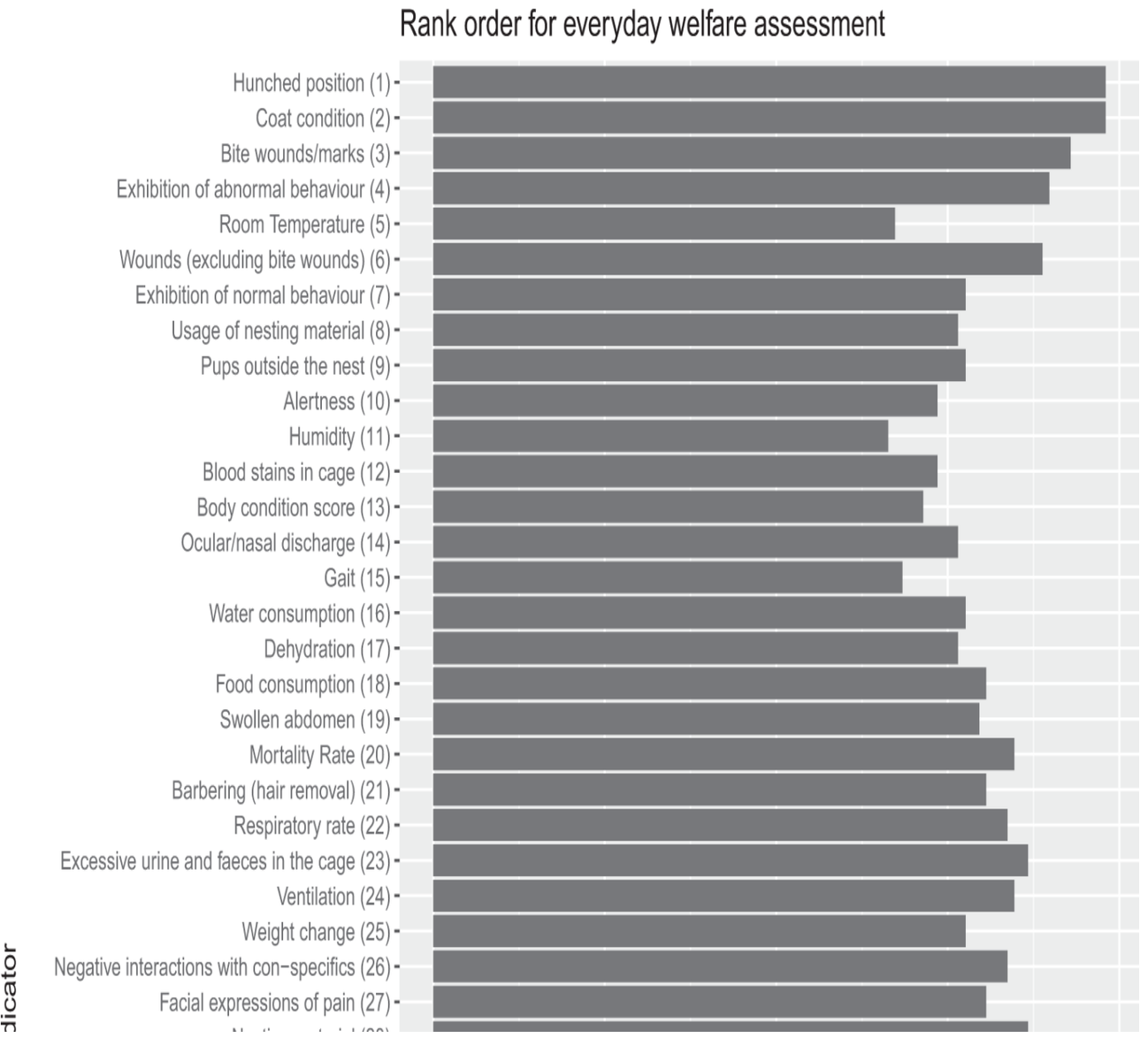
Type of WI

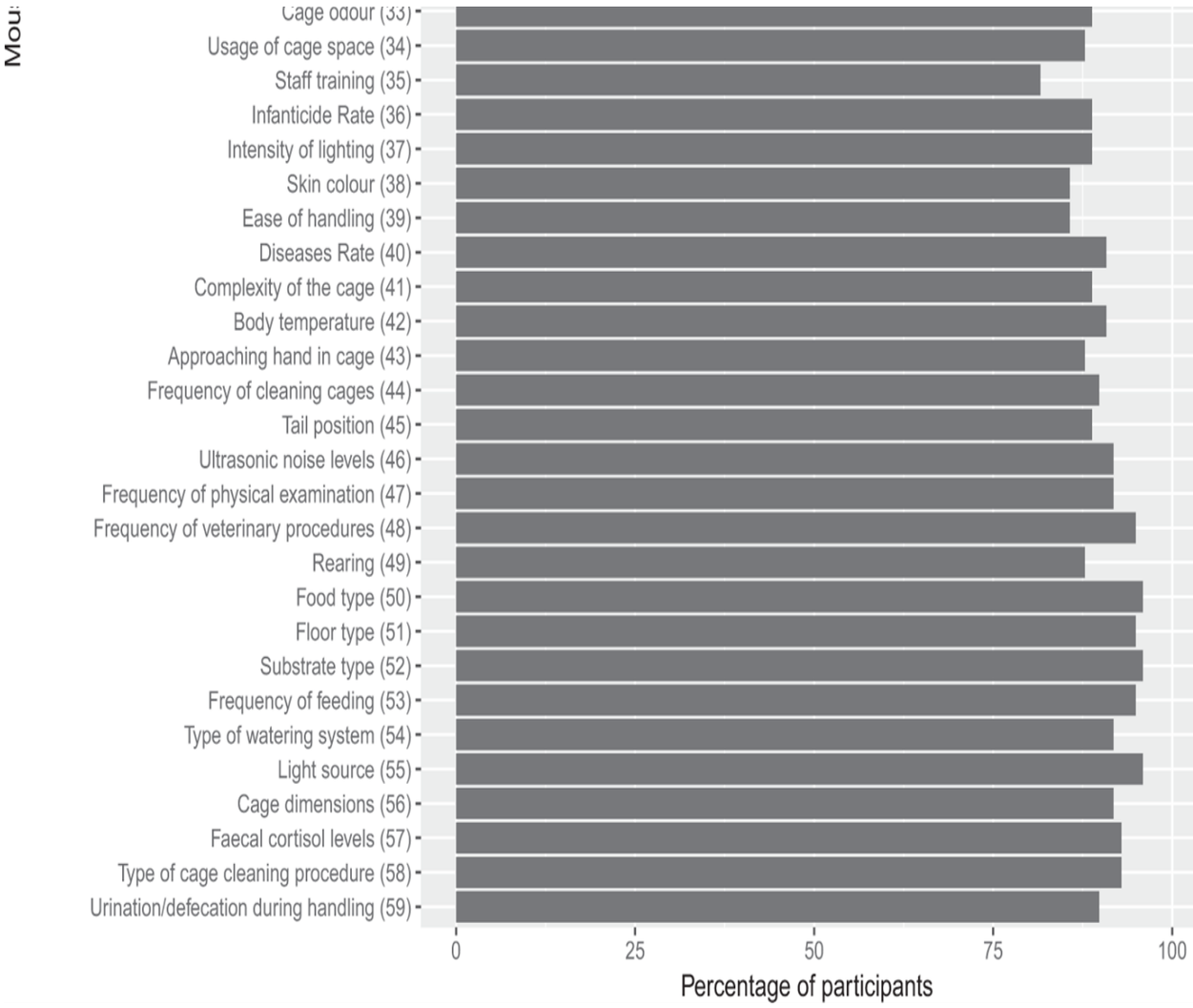
Indirect (environmental)	**Biological**
Temperature	Health
Humidity	Feeding
Housing density	Behavior

Application site

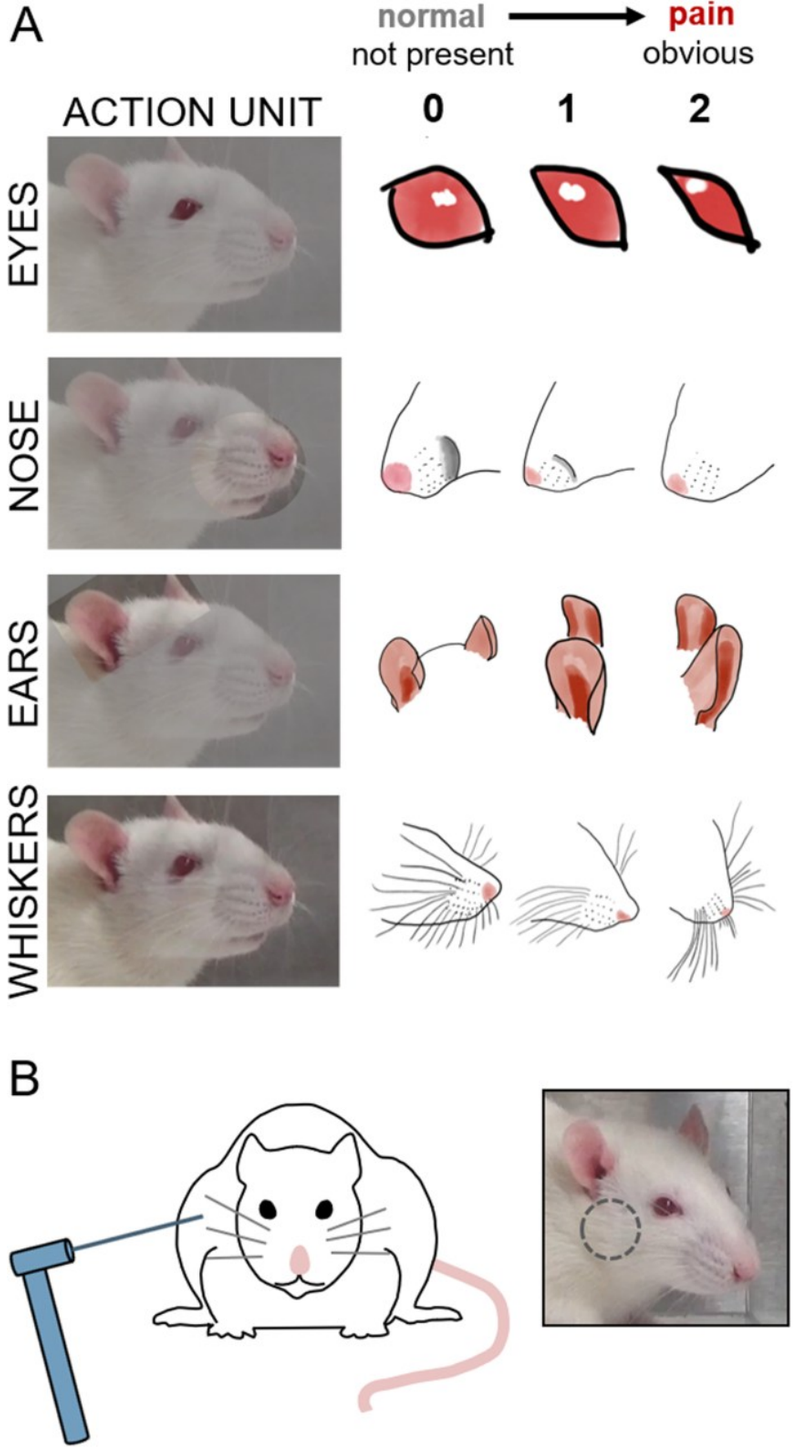
Animal house	**Laboratory**
Temperature	Health
Feeding	Physiological markers
Behavior	Body condition







Source: Campos-Luna et al., 2019; doi:10.1038/s41598-019-45810-y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normal
not present
pain
obvious
ACTION UNIT
0
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2
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EARS
WHISKERS
B

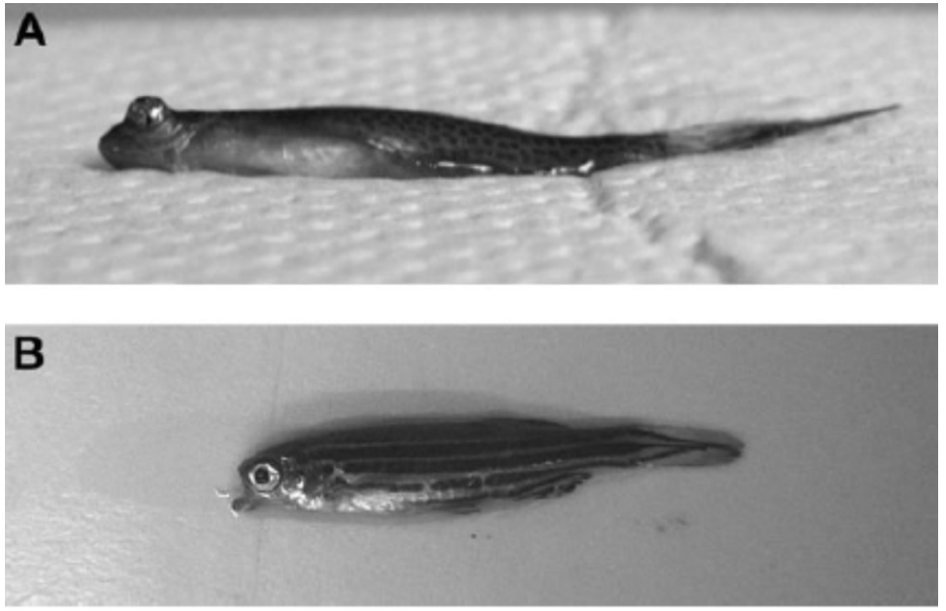
ZEBRAFISH
Volume 13, Supplement 1, 2016
Mary Ann Liebert, Inc.
DOI: 10.1089/zeb.2016.1248

Standardized Welfare Terms for the Zebrafish Community

Nicola Goodwin,[1,2] Natasha A. Karp,[3] Samuel Blackledge,[1] Bradley Clark,[1] Rosemary Keeble,[2] Ceri Kovacs,[1] Katrina N. Murray,[4] Michael Price,[1] Peter Thompson,[1] and James Bussell[1]

TABLE 1. EXAMPLES OF THE STANDARDIZED WELFARE TERMS

Parameter	*Sub parameter*	*Welfare indicators*	*Indicator sub category*	*Synonym*	*Definition*
Appearance	General	Loss of scales			Scales detached from body
Appearance	General	Lesion all over		Wound	
			Open-Abrasion		Damage to the skin consisting of loss of the epidermis and portions of the dermis but not the complete thickness of the skin.
			Open-Incision		A wound created by a sharp object. Edges are smooth and trauma to surrounding tissue is minimal.
			Open-Laceration		An irregular wound created by tearing of tissue. Damage to both superficial and underlying tissue is variable.
			Open-Puncture		A penetrating wound caused by pointed object.
			Closed-Contusion		Damage of the skin and/or underlying structures without breaking the skin for example, bruising, crush.
Appearance	General	Skin ulcers			Nonhealing erosions of skin.
Appearance	General	Multiple masses under skin		Swellings, raised areas, lumps	Abnormal appearance of masses of all descriptions (hard, soft, different shapes, etc.)
Appearance	General	Raised scales		Protruding scales	Scales protruding outward from body.
Appearance	General	Obese		Large, fat	Extremely fat, grossly overweight.
Appearance	General	Weight loss			Reduction in body weight compared to controls.
Appearance	General	Weight gain			Increase in body weight compared to controls.
Appearance	General	Thin		Emaciated, skinny	Lean or slender in form



Journal of the American Association for Laboratory Animal Science
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Vol 57, No 6
November 2018
Pages 698–702

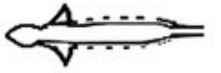
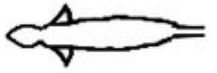
Body Condition Scoring for Adult Zebrafish (*Danio rerio*)

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

Body condition scoring (BCS) is a simple, rapid, noninvasive tool used to assess body condition in animals. In this study, we developed and validated a diagram-based BCS for adult zebrafish (*Danio rerio*), a popular research model. After receiving 20 min of hands-on training regarding the scoring system, 5 people each rated 95 adult zebrafish. The fish then were euthanized and measured to establish body condition indices (BMI and the Fulton K factor). Both condition indices were highly correlated with fish width. Using correlation data and observed trends in fish width, we established expected BCS definitions. We validated the BCS definitions in 2 ways. First, we calculated the Pearson correlation coefficient between the average observed BCS and expected BCS; this statistic revealed very strong correlation between observed and expected BCS. In addition, we assessed the predictive power of BCS by using multinomial logistic regression and then applied the fitted model to evaluate the accuracy of the predictions (BCS compared with BMI, 85%; BCS compared with K factor, 61%). Finally, to determine the robustness of BCS to variation among raters, we calculated the intraclass correlation coefficient and demonstrated high interrater reliability. In conclusion, adult zebrafish BCS can be used to quickly identify animals with different body condition indices (thin to obese). In addition, the diagram-based chart is easy to use and implement accurately, with minimal training.

Abbreviation: BCS, body condition score

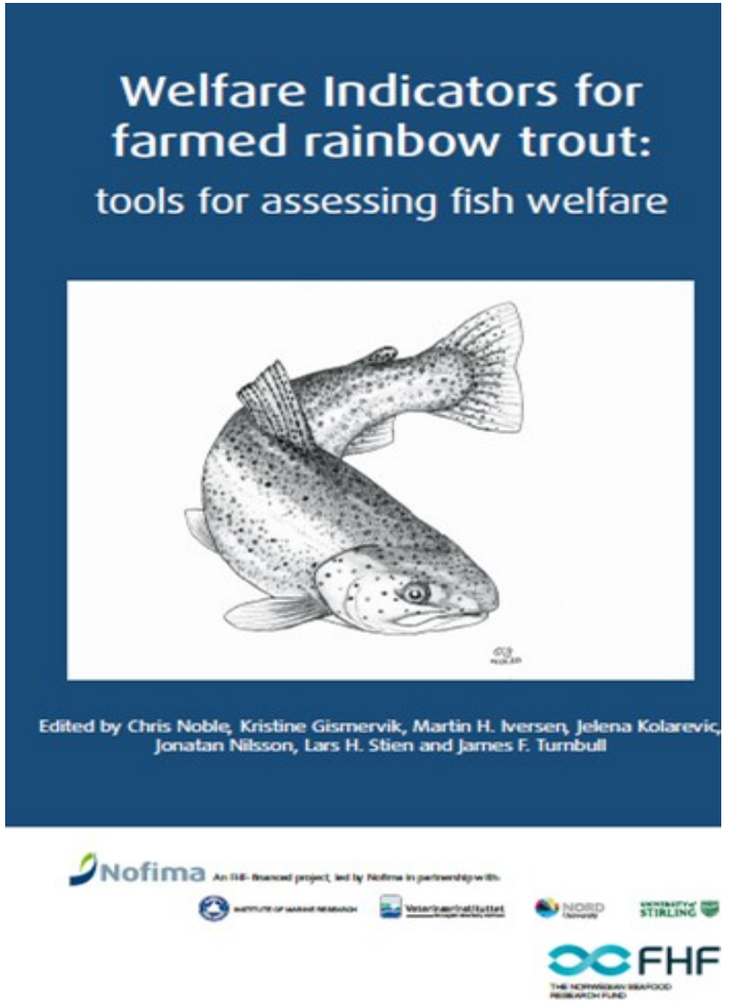
DOI: 10.30802/AALAS-JAALAS-18-000045

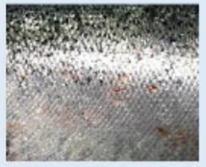
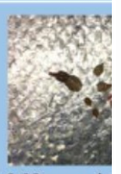
Adult Zebrafish BCS		
	Lateral View	Dorsal View
BCS 1: • Head larger than body (big head) • Lateral- concave ventral surface between head and abdomen (narrow abdomen) • Dorsal- body is more narrow than head and linear • **Fish is thin (emaciated)**		
BCS 2: • Head and body equal size • Lateral- flat 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)**		

Salmon, 2018

Sea bass & sea bream, 2019

Trout, 2020

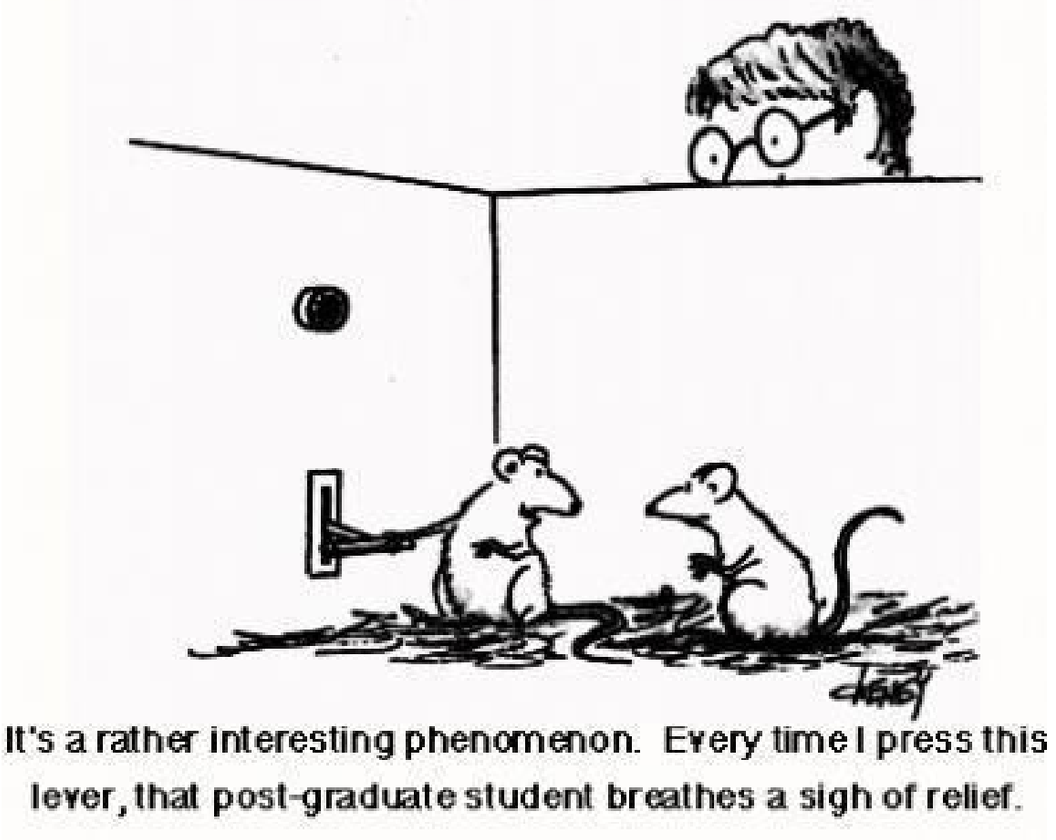


	1	2	3
Emaciation	 Potentially emaciated	 Emaciated	 Extremely
Vertebral deformity	 Signs of deformed spine	 Clearly visible spinal deformity (e.g. short tail)	 Extreme
Skin haemorrhages	 Minor haemorrhaging, often on the belly of the fish	 Large area of haemorrhaging, often coupled with scale loss	 Significant bl with severe scal and skin
Lesions / wounds [1]	 One small wound (< 10 pence piece)[1], subcutaneous tissue intact (no muscle visible)	 Several small wounds	 Large, severe w often exposed pie
Scale loss	 Loss of individual scales	 Small areas of scale loss (< 10% of the fish)	 Large areas (≥ 10% of
Sea lice infection	 Light infection	 0.05 - 0.08 pre-adult or adult lice cm^{-2} of fish skin	 ≥0.08 pre-adu cm^{-2} of

	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent on one of the gills (gill exposed)	 Both opercula absent (both gills exposed)
Snout damage	 Minor wound on snout (either jaw)	 Moderate wound and broken skin on snout	 Large deep and extensive wound. Can cover the whole head
Upper jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards
Lower jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards

THANK YOU

And you thought there was stress in *your* life !



It's a rather interesting phenomenon. Every time I press this lever, that post-graduate student breathes a sigh of relief.