STRESS IN ZEBRAFISH

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A STRESSFUL DEFINITION

"STRESS, IN ADDITION TO BEING ITSELF AND THE RESULT OF ITSELF, IS ALSO THE CAUSE OF ITSELF" (ROBERTS 1950)

- CAN BE USED TO REFER TO:
  - A STIMULUS: MENTAL, PHYSICAL
  - AN INDIVIDUAL'S AWARENESS OF THAT STIMULUS
  - THE PHYSICAL OR BEHAVIORAL RESPONSE TO THAT STIMULUS

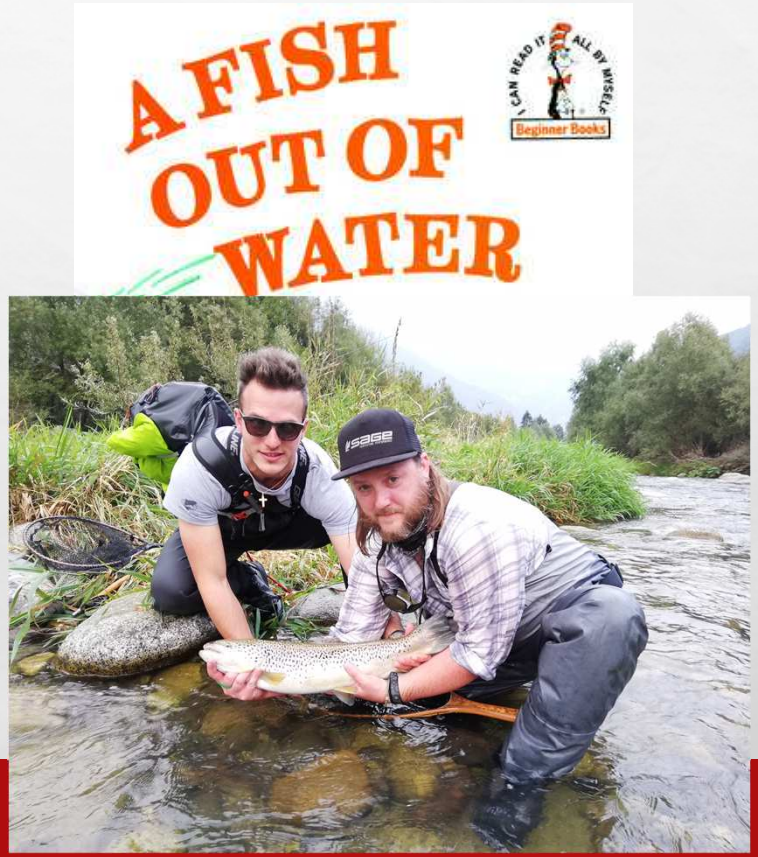
STRESS!!!

"*THE NONSPECIFIC RESPONSE OF THE BODY TO ANY DEMAND FOR CHANGE*"

-HANS SELYE 1936

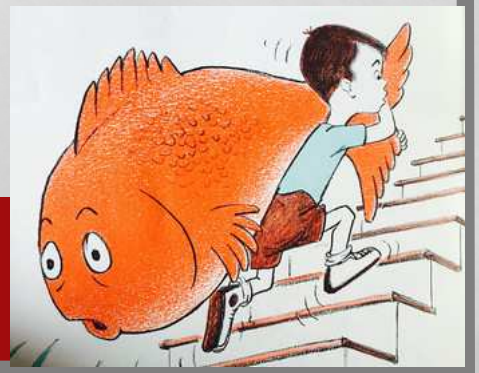
- STRESSOR: A THREAT TO HOMEOSTASIS
- STRESS RESPONSE:
  - REACTION TO A REAL OR PERCEIVED STRESSOR
  - ROLE IS TO RESTORE HOMEOSTASIS
- ACUTE VS CHRONIC

FISH ARE VERY SUSCEPTIBLE TO STRESS



WHAT IS STRESSFUL TO A FISH?

- WATER CONDITIONS
  - CONTAMINANTS
    - AMMONIA
  - RAPID TEMPERATURE CHANGE
  - MALNUTRITION
    - INSUFFICIENT OR OVERABUNDANCE
    - NUTRIENT IMBALANCES
  - OSMOTIC IMBALANCE
- ENVIRONMENTAL
  - VIBRATION
  - LIGHT
- CROWDING
- NETTING
- HARASSMENT FROM OTHER FISH
- NOVELTY

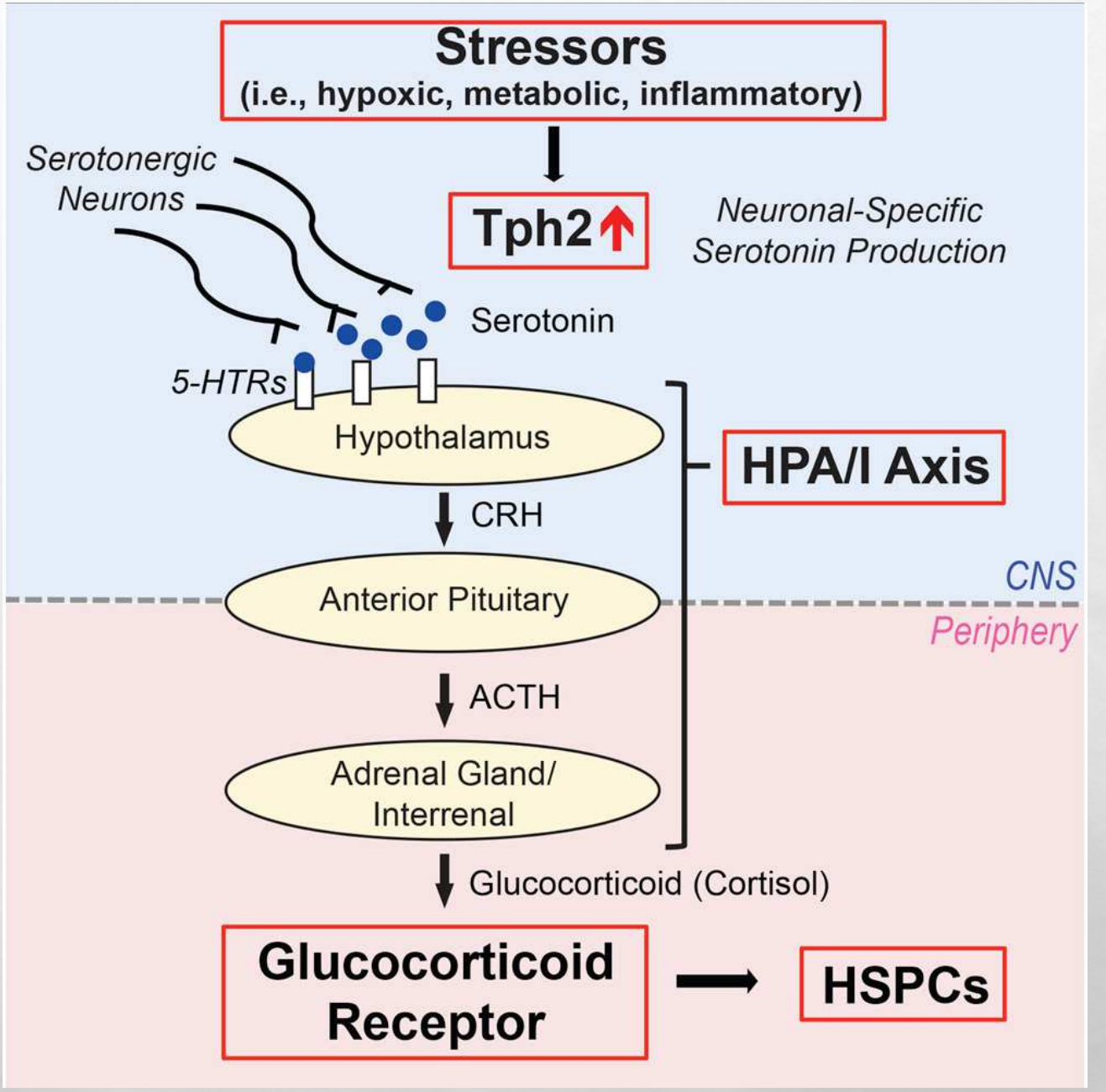


THE GENERAL STRESS RESPONSE

- DEPENDENT UPON STRESSOR
  - GENERAL
  - SPECIFIC
    - E.G. HSP EXPRESSION DUE TO INCREASED TEMPERATURE
- PRIMARY
  - NEUROENDOCRINE RESPONSE: CATECHOLAMINES AND CORTISOL RELEASE
- SECONDARY
  - PHYSIOLOGIC AND METABOLIC RESPONSES
  - HYPERGLYCEMIA, VASODILATION OF ARTERIES IN GILL FILAMENTS, IMMUNE FUNCTION SUPPRESSION
- TERTIARY
  - SYSTEMIC CHANGES
    - MAY RESULT IN ADVERSE EFFECTS ON ANIMAL HEALTH

PRIMARY RESPONSE: THE HPA/I AXIS

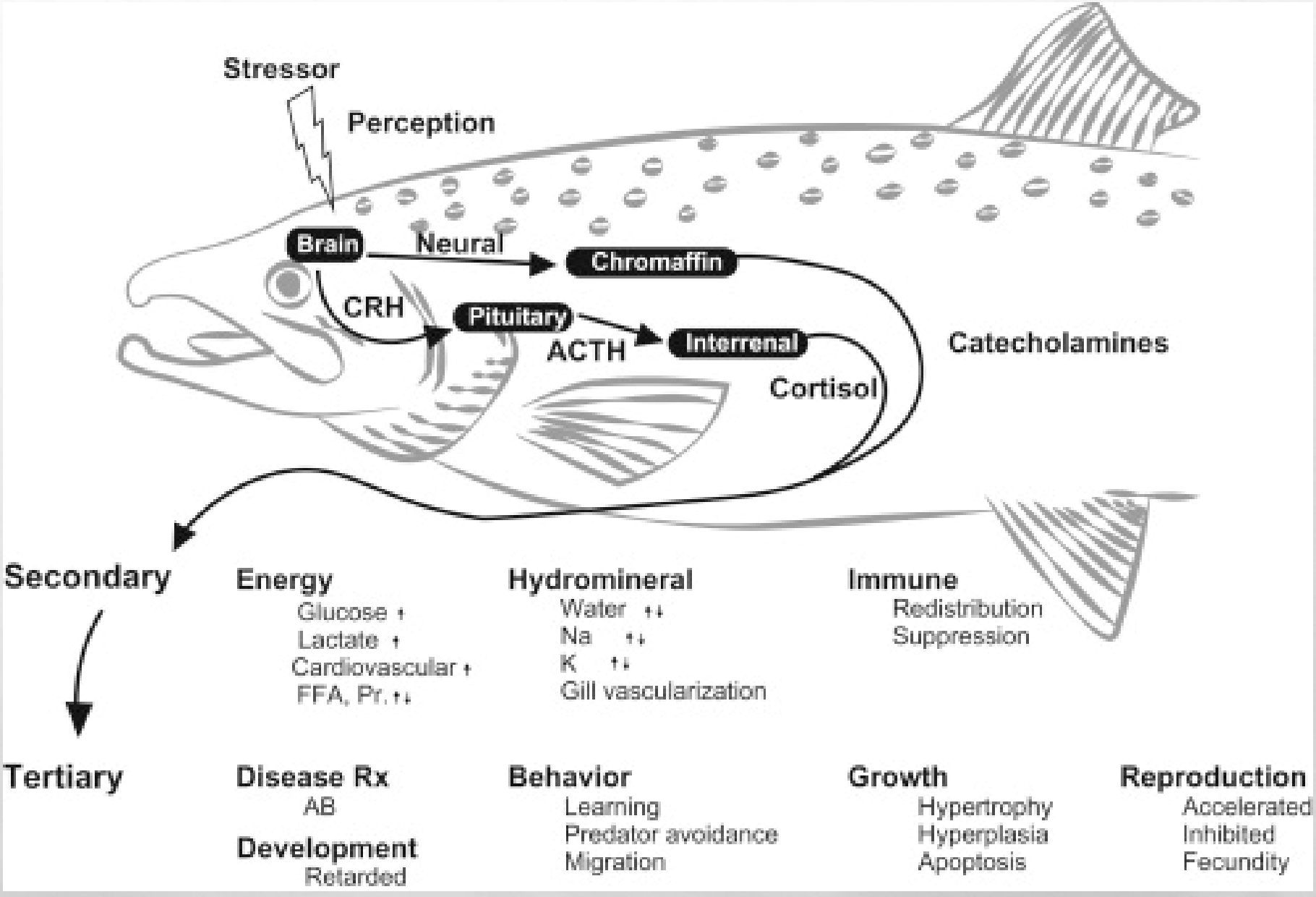
- HYPOTHALAMIC-PITUITARY-ADRENAL/INTERRENAL AXIS
  - HYPOTHALAMUS: AREA OF THE BRAIN THAT SENDS A CHEMICAL MESSAGE TO THE PITUITARY GLAND
  - PITUITARY GLAND: WHEN STIMULATED SENDS A CHEMICAL MESSAGE INTO THE BLOOD TO THE ADRENAL GLANDS
  - ADRENAL GLANDS/INTERRENAL CELLS: RELEASE CORTISOL
- CONSERVED AMONG ALL VERTEBRATES



Cell Stem Cell 2016 19, 370-382DOI: (10.1016/j.stem.2016.06.004)

HORMONES: DRIVING THE RESPONSE

- CORTICOSTEROIDS
  - CORTISOL: PRIMARY NEUROENDOCRINE RESPONSE TO STRESS
- CATECHOLAMINES
  - ADRENALINE AND NORADRENALINE
- MAIN ROLE OF BOTH IS TO MAKE ENERGY AVAILABLE FOR FLIGHT RESPONSE



Carl B. Schreck, Lluis Tort, 1 - The Concept of Stress in Fish, Editor(s): Carl B. Schreck, Lluis Tort, Anthony P. Farrell, Colin J. Brauner, Fish Physiology, Academic Press, Volume 35, 2016, Pages 1-34,

THE STRESS RESPONSE

- NECESSARY MECHANISM TO OVERCOME CHALLENGES AND POSSIBLY RESTORE HOMEOSTASIS
- PRIMARY RESPONSE MAKES ENERGY AVAILABLE FOR NEEDED RESPONSE (E.G. FLEEING A PREDATOR)
- TERTIARY RESPONSES CAN BE MALADAPTIVE

THE STRESS RESPONSE

- LOW LEVELS OF CORTISOL AND CATECHOLAMINES ARE NECESSARY FOR ROUTINE LIFE FUNCTIONS
  - GROWTH
  - THE IMMUNE SYSTEM
  - DEVELOPMENT
  - LEARNING

HOW TO MEASURE PRIMARY/SECONDARY STRESS RESPONSE

- BIOCHEMICAL ASSAYS
  - PLASMA CORTISOL
  - PLASMA CATECHOLAMINES
  - HEAT SHOCK PROTEINS
- IMMUNE FUNCTION
- GENE EXPRESSION PATTERNS (E.G. HSP EXPRESSION)
- MEASUREMENT OF FISH STEROIDS IN WATER

HOW TO MEASURE TERTIARY STRESS RESPONSE

- WHOLE BODY OR ORGAN WEIGHT MEASUREMENTS (E.G. CONDITION FACTOR, GONADOSOMATIC INDEX)
- MACRO/MICROSCOPIC ANATOMY
- CHANGES IN GROWTH
- DISEASE RESISTANCE
- SWIMMING PERFORMANCE
- BEHAVIOR

WHY MEASURE STRESS?

- REDUCING STRESS CAN MAXIMIZE GROWTH AND SURVIVAL
- MINIMIZE THE EFFECTS OF STRESS ON RESEARCH ENDPOINTS
- TO DETERMINE HOW HEALTH, PERFORMANCE, AND WELFARE STATUS OF FISH ARE BEING INFLUENCED BY CAPTIVITY
  - PROVIDE EMPIRICAL EVIDENCE OF THE BENEFITS OF A PARTICULAR TREATMENT TO THE WELFARE OF FISH

FACTORS AFFECTING IMPACTS OF STRESS

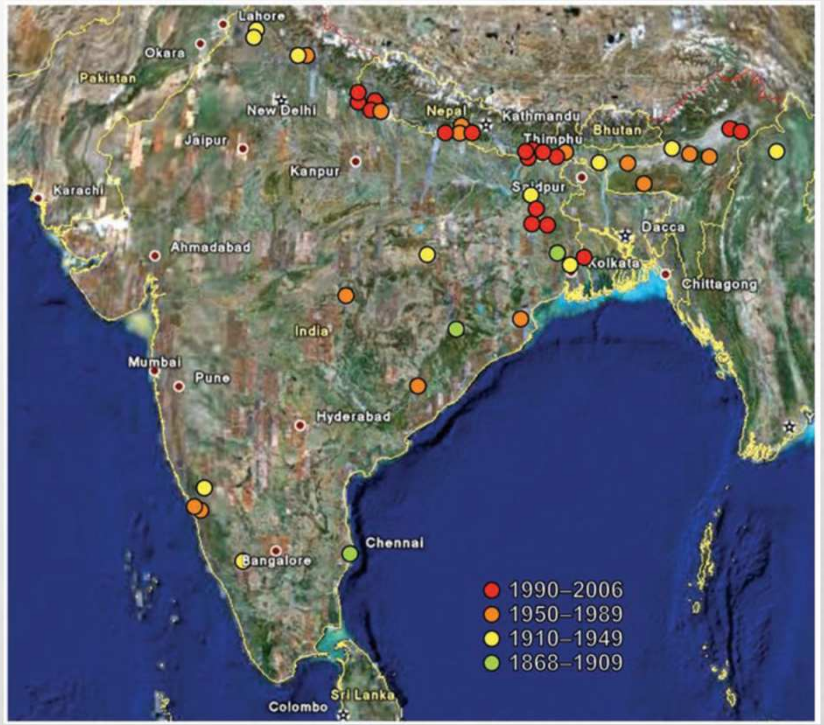
- ENVIRONMENT
  - TEMPERATURE: AFFECTS RATE OF REACTIONS
  - OXYGEN CONCENTRATION
- ENVIRONMENTAL HISTORY
- DEVELOPMENTAL LIFE STAGE
- GENETICS/EPIGENETICS
- SOCIAL HIERARCHY
- NUTRITION STATUS
- PATHOGEN INFECTION

DANIO RERIO

- CAN ADAPT TO A WIDE VARIETY OF ENVIRONMENTAL PARAMETERS
  - CONDUCTIVITY
  - TEMPERATURE
  - DISSOLVED OXYGEN

NATURAL HABITAT OF *D. RERIO*

- CENTERED AROUND THE GANGES AND BRAHMAPUTRA RIVER BASINS
- ZEBRAFISH FOUND IN A BROAD RANGE OF TEMPERATURES IN THESE WATERS
  - UP TO ~40 C!



Engeszer et al 2007. Zebrafish in the wild: a review of natural history and new notes from the field. Zebrafish 4: 21–40.

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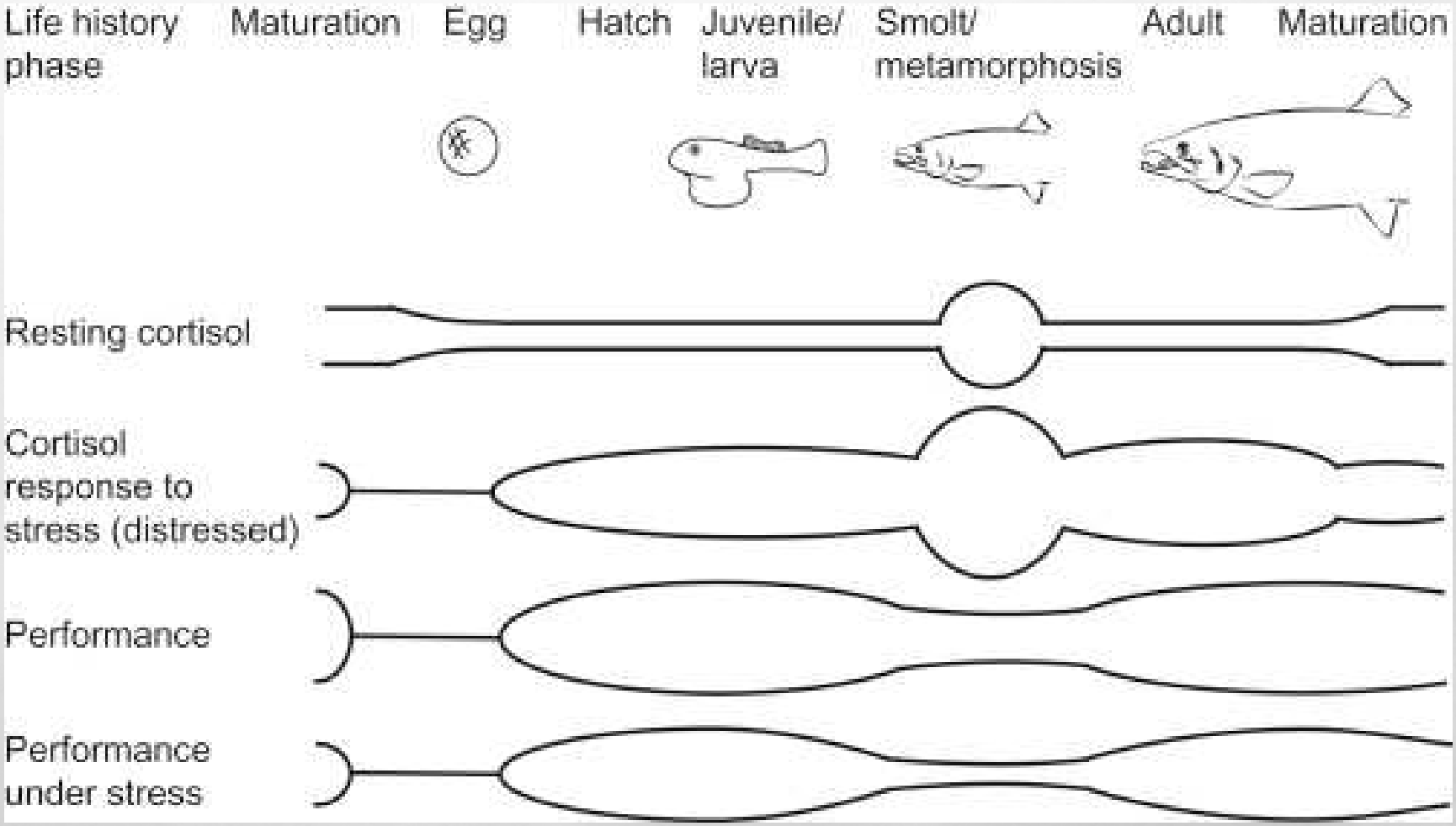
LIFE STAGES AND CORTISOL



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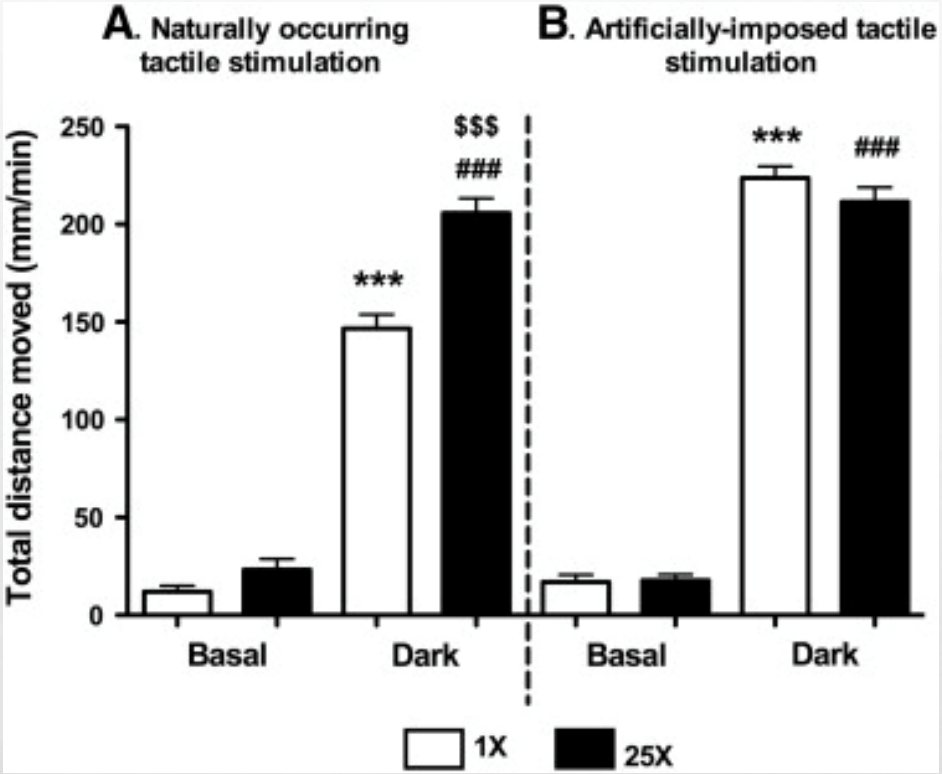
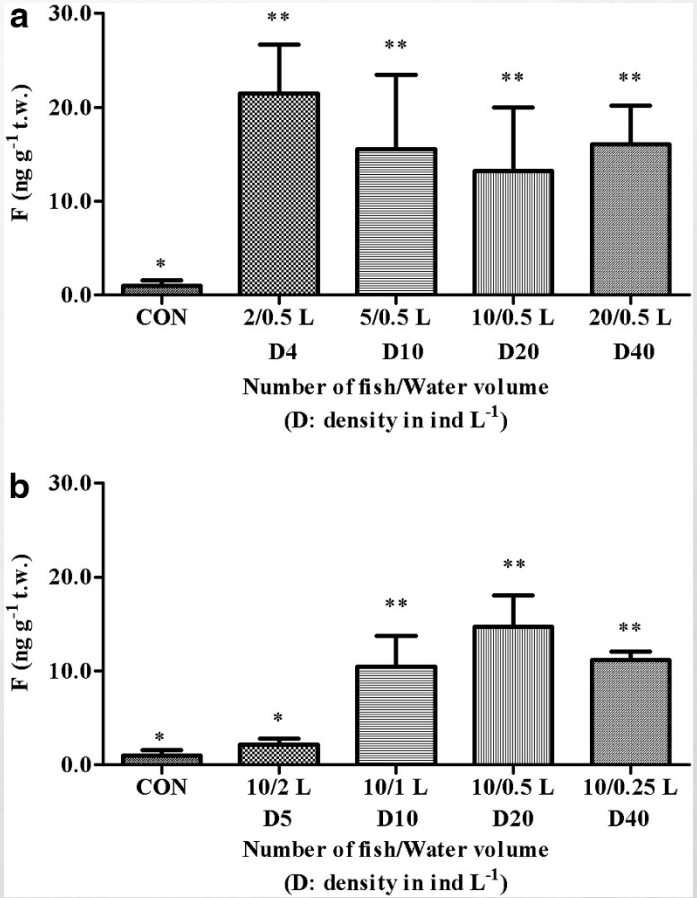
EMBRYO DENSITY



Peter J. Steenbergen, Michael K. Richardson, Danielle L. Champagne, The use of the zebrafish model in stress research, Progress in Neuro-Psychopharmacology and Biological Psychiatry, Volume 35, Issue 6, 2011, Pages 1432-1451,

CROWDING

- ADDITIONAL SEQUELAE
  - POOR WATER QUALITY
  - EXPOSURE TO ORGANIC WASTES
  - CONSPECIFIC AGGRESSION AND PREDATION



Husbandry of Zebrafish, *Danio Rerio*, and the Cortisol Stress Response
Michail Pavlidis, Nikoletta Digka, Antonia Theodoridi, Aurora Campo, Konstantinos Barsakis, Gregoris Skouradakis, Athanasios Samaras, and Alexandra Tsalafouta
Zebrafish 2013 10:4, 524-531

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RAMSEY ET AL 2006

- DIFFERENCES IN FASTED VS FED FISH CORTISOL RESPONSE TO CROWDING

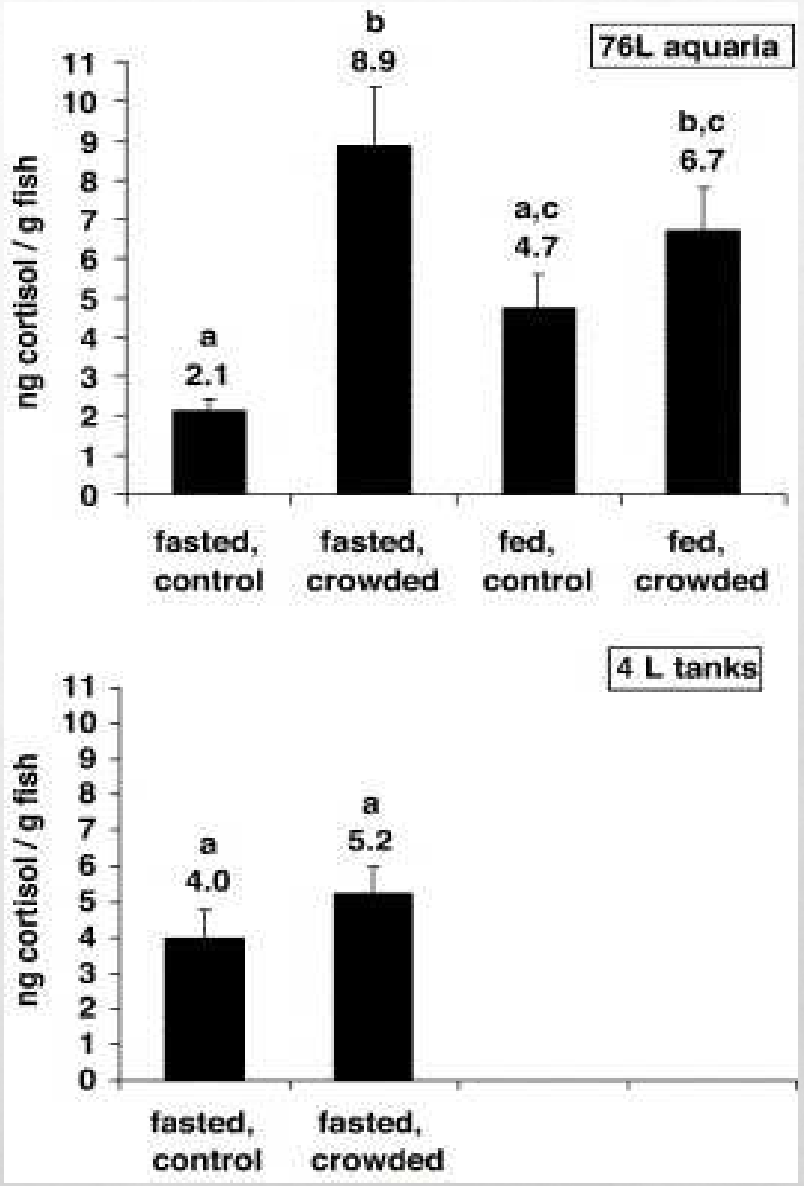


Fig. 2. Experiment 2: Crowding increases cortisol levels in large, but not small tanks. Mean whole-body cortisol (± S.E.M.) (ng/g fish) for 76 L glass aquaria and 4 L plastic tank treatments. Different letters above the standard error bars indicate a significant difference between treatment groups ($p = 0.0002$; $n = 24–25$)

CAPTURE, TRANSPORT, HANDLING

- MAGNITUDE OF STRESS RESPONSE VARIES AMONG GENETIC LINES
- OFTEN CLINICAL EFFECTS ARE NOT APPARENT UNTIL SEVERAL DAYS AFTER EVENT
- POTENTIAL FOR CAPTURE MYOPATHY (RHABDOMYOLYSIS)
- ADDITIONAL SEQUELAE
  - CROWDING
  - HYPOXIA
  - PHYSICAL TRAUMA
  - BAROMETRIC DISTURBANCE

HYPER/HYPOTHERMIA

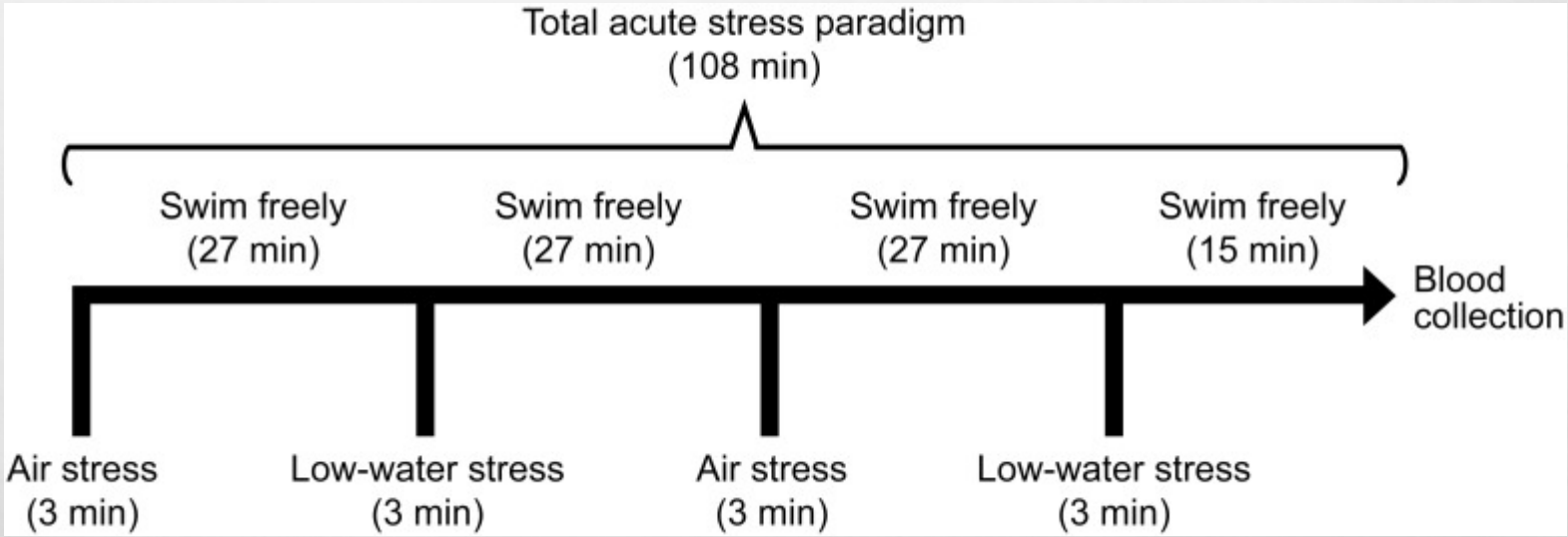
- RAPID FLUCTUATIONS
  - ABSENT OR IMPROPER ACCLIMATION
- INAPPROPRIATE WATER TEMPERATURE
  - BEYOND HIGH OR LOW RANGE OF TOLERANCE
- HAS BEEN USED EXPERIMENTALLY IN PATHOGEN CHALLENGE STUDIES
- TEMPERATURE CAN ALTER IMMUNE FUNCTION
- HYPOXIA DUE TO DECREASED O_2 CAPACITY

HYPOXIA

- ZEBRAFISH CAN ADAPT TO AND SURVIVE HYPOXIC WATER CONDITIONS FOR WEEKS
  - GILL ALTERATIONS
  - BEHAVIORAL PHENOTYPE
- SHORT-TERM AIR EXPOSURE INCREASES CORTISOL LEVELS IN FISH (RAMSAY ET AL 2009)

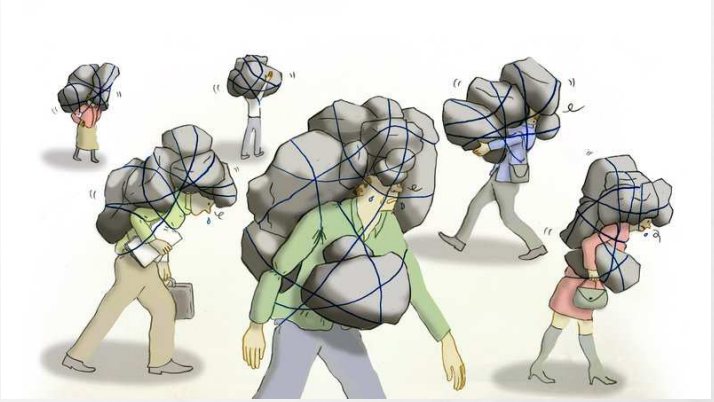
ACUTE STRESS

- **INTENSE**
- **SHORT-LIVED**



Grzelak, A. K., Davis, D. J., Caraker, S. M., Crim, M. J., Spitsbergen, J. M., & Wiedmeyer, C. E. (2017). Stress Leukogram Induced by Acute and Chronic Stress in Zebrafish (*Danio rerio*). *Comparative Medicine, 67*(3), 263–269.

CHRONIC STRESS



- GENERALLY LESS INTENSE
- OCCURS DURING A PROLONGED PERIOD

	Morning	Evening
Day 1	Chasing (8 min)	Tank changes (x6)
Day 2	Over-crowding (60 min)	Dorsal body exposure (2 min)
Day 3	Social isolation (30 min)	Tank changes (x6)
Day 4	Dorsal body exposure (2 min)	Chasing (8 min)
Day 5	Tank changes (x6)	Dorsal body exposure (2 min)

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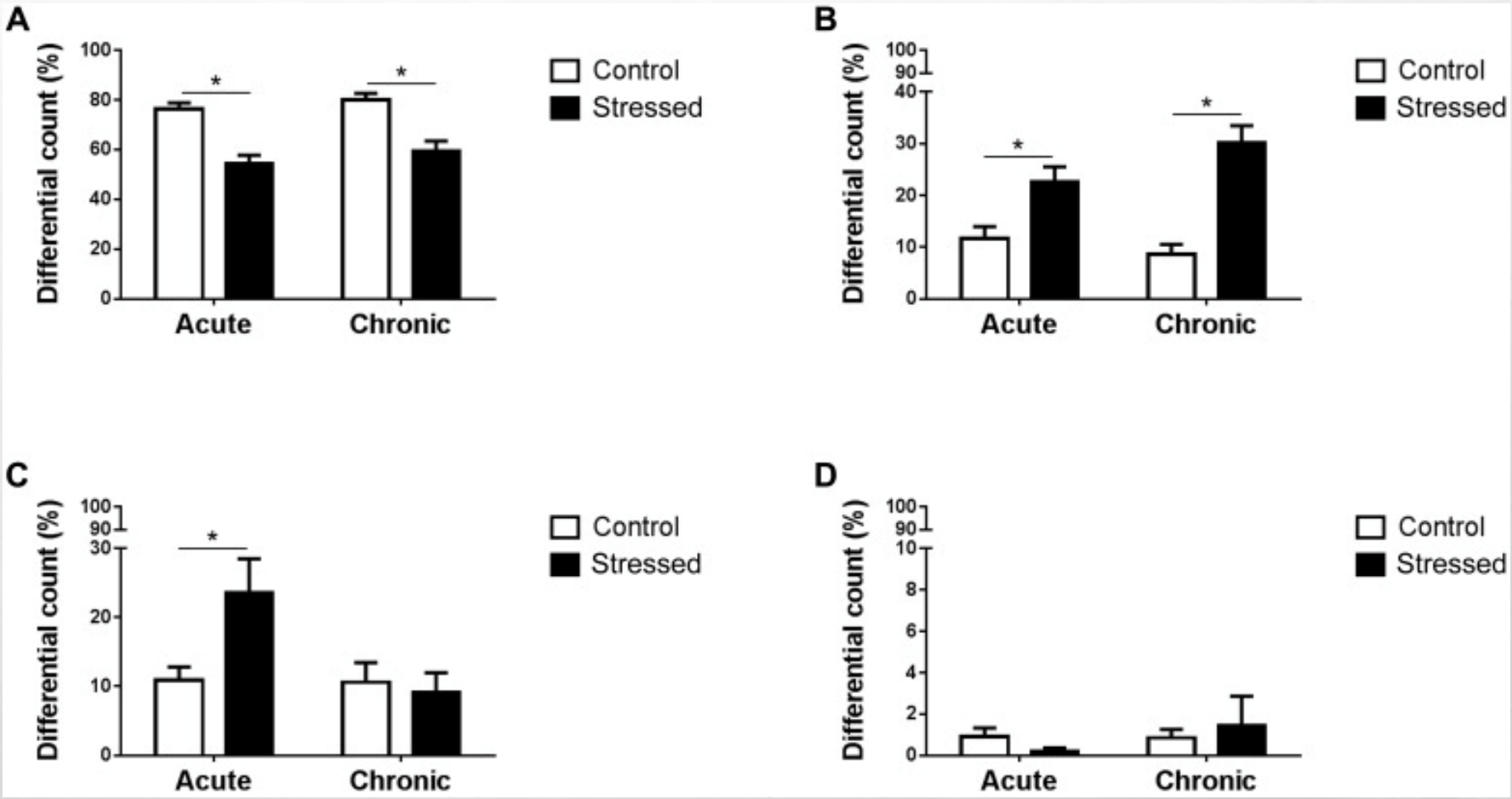

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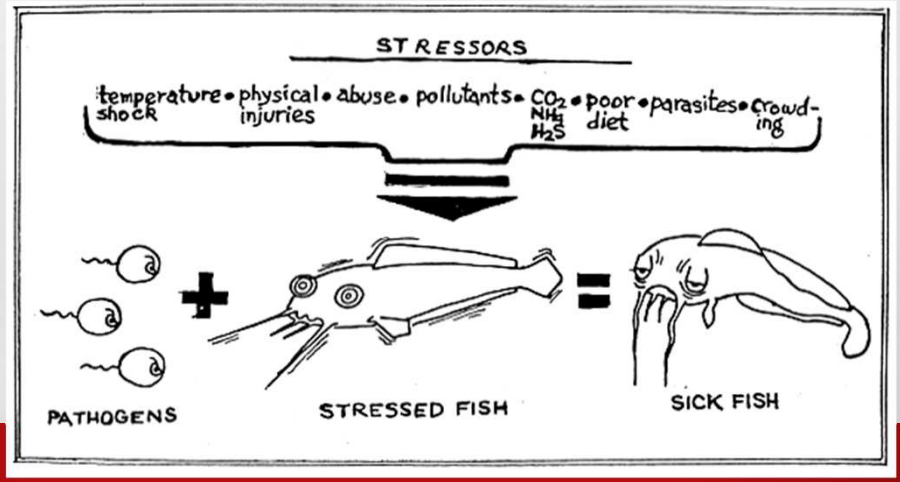
RESULTS OF STRESS

- MORTALITY
- SUSCEPTIBILITY TO OPPORTUNISTIC PATHOGENS
- LOW FECUNDITY



Fedoruk, A N. A management perspective on stress and infectious diseases in Clarias farming. 1981

BEHAVIORAL RESPONSES TO STRESS

- AVOIDANCE
- THIGMOTAXIS
- ALARM MOVEMENT
  - FREEZING
  - DARTING
- ALTERED SHOALING BEHAVIOR

WHERE CAN YOU SEE THE EFFECTS OF STRESS?

- GILLS
- LIVER
- SKIN
- REPRODUCTIVE SYSTEM
- NERVOUS SYSTEM
- CARDIOVASCULAR SYSTEM
- SYSTEMIC/MULTIORGAN

GILLS

- RELATIVELY FRAGILE STRUCTURES
- CONTINUALLY EXPOSED TO EXTERNAL ENVIRONMENT
  - HYPOXIA
  - HYPO/HYPERTHERMIA
  - OSMOTIC STRESS
  - CHEMICALS

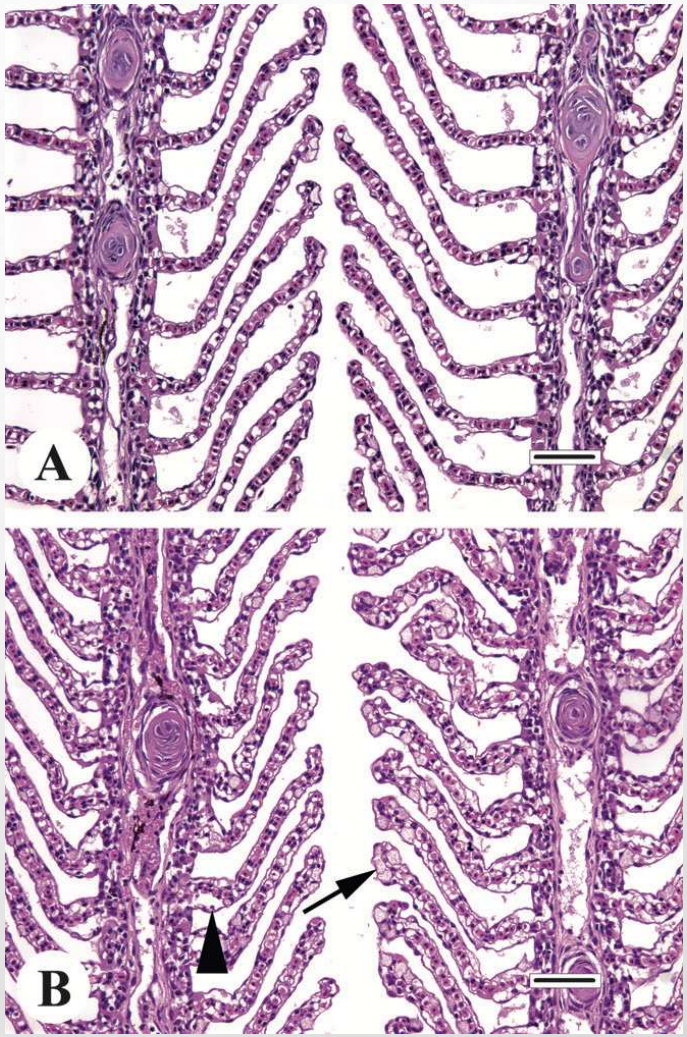
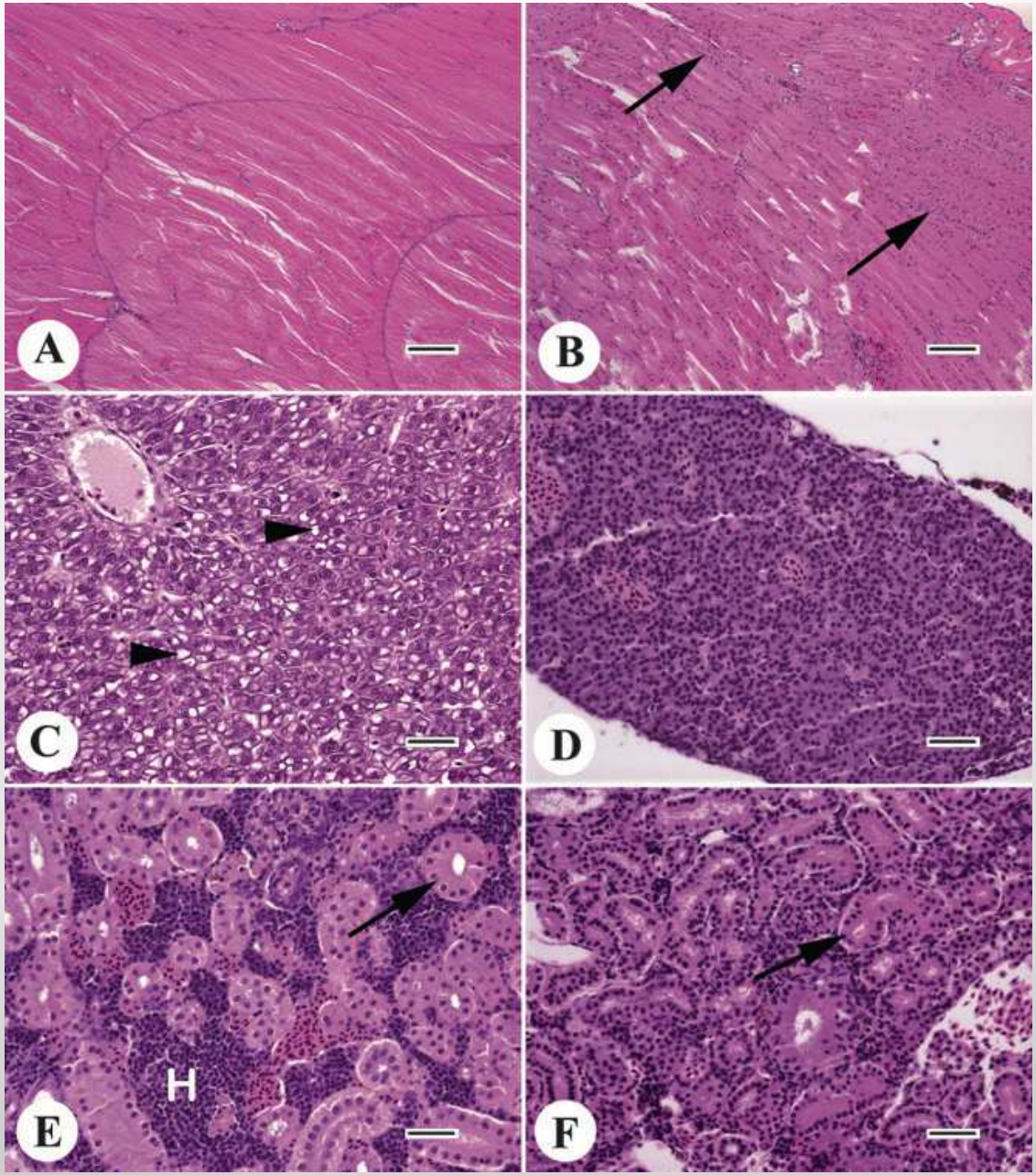


Figure 1 Nonspecific stress response in the gills of adult Atlantic salmon (*Salmo salar* L.). (A) Normal gill (two adjacent filaments). (B) Findings associated with several types of stressors; the most prominent changes are mucus cell hyperplasia (arrow) and epithelial lifting (arrowhead). Bar = 50 microns.

Claudia Harper, Jeffrey C. Wolf; Morphologic Effects of the Stress Response in Fish, *ILAR Journal*, Volume 50, Issue 4, 1 January 2009, Pages 387–396

MALNUTRITION

- INSUFFICIENCY OR OVERABUNDANCE OF NUTRIENTS
- RELATIVE NUTRIENT IMBALANCE
- DIFFICULT TO SEPARATE STRESS RESPONSE FROM DIRECT EFFECTS
- FOOD DEPRIVATION CAN LEAD TO REDUCED STRESS RESISTANCE



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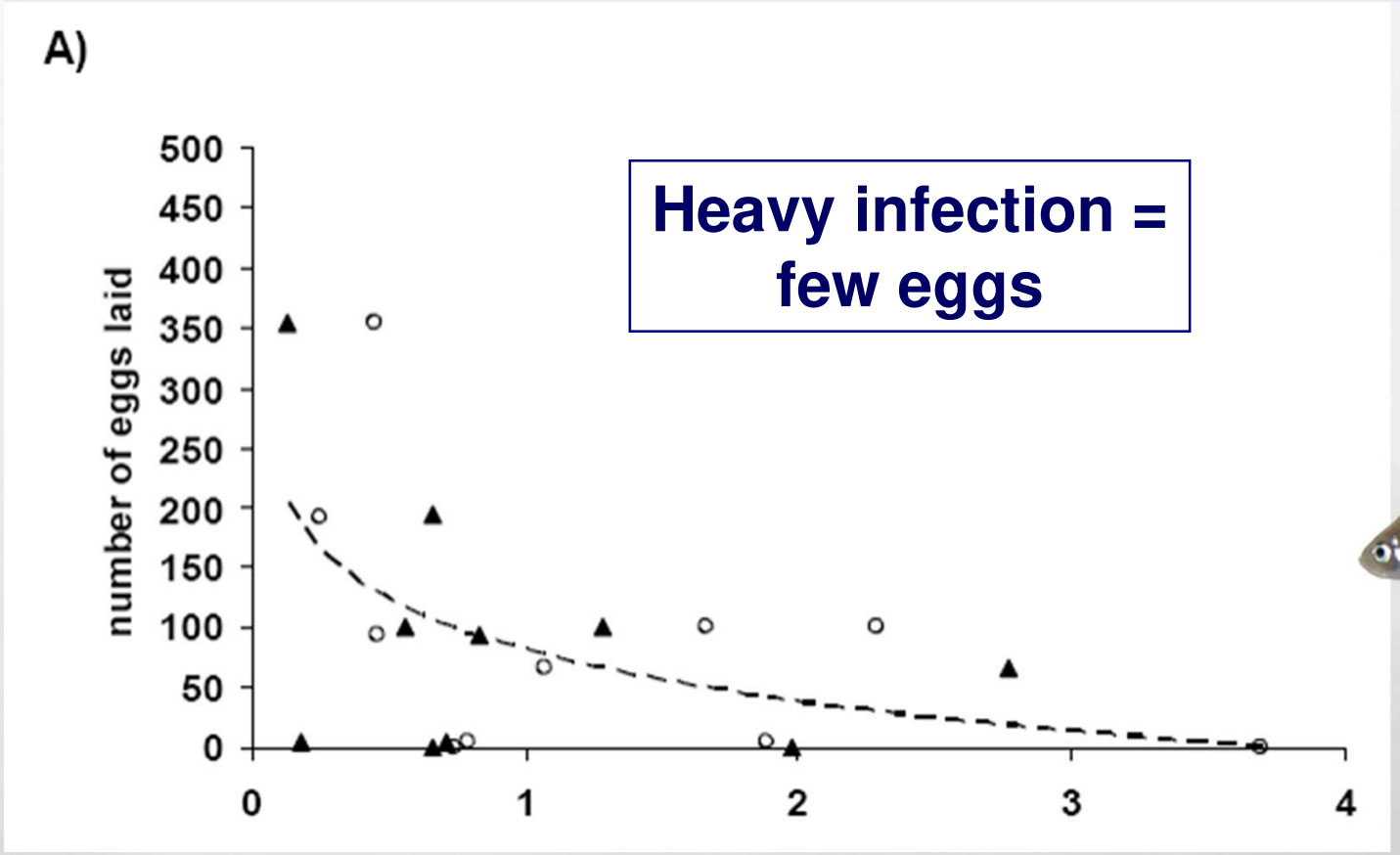
Fecundity: Eggs laid vs. parasite area
Stressed Fish



Danio rerio Experimental Exposure, Stress and Fecundity – AB experiment. Number of eggs laid vs. parasite (xenoma) area (%) at wk 20 post-exposure to *Pseudoloma*. A) Among the *Pseudoloma*-infected-stressed fish the relationship was described by the following equation: eggs laid = 80 – 61 ln (xenoma area); $R^2 = 0.25$; p=0.025

Stress-induced analgesia

Stress, fear, anxiety blunts the response of larval zebrafish to noxious substances

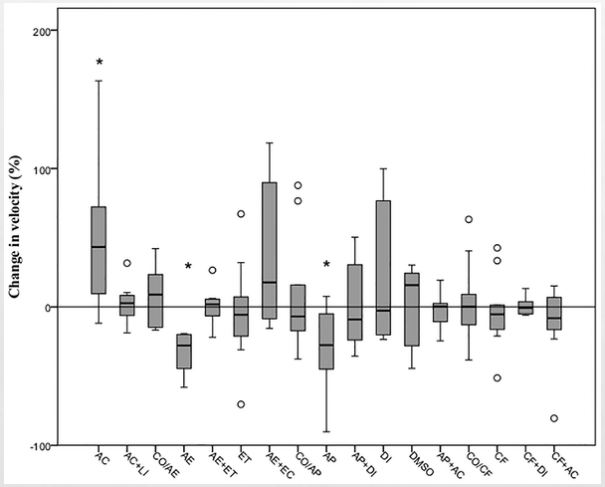


FIG 4. CHANGE IN VELOCITY (%) SHOWN BY 5DPF ZEBRAFISH EXPOSED TO DIFFERENT TREATMENTS.

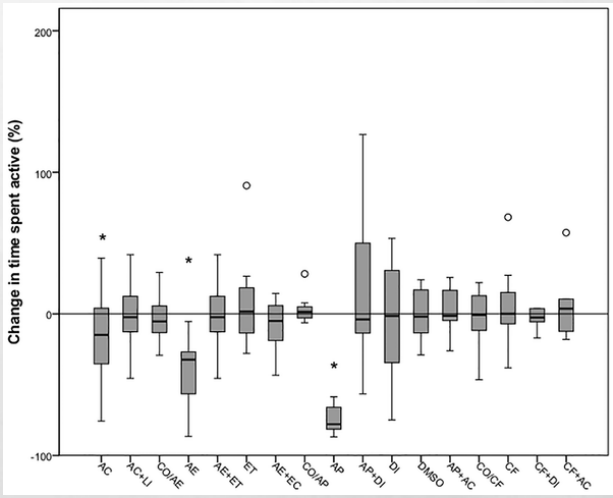


Fig 5. Change in time spent active (%) shown by 5dpf zebrafish exposed to different treatments.

Lopez-Luna J, Al-Jubouri Q, Al-Nuaimy W, Sneddon LU (2017) Impact of stress, fear and anxiety on the nociceptive responses of larval zebrafish. PLOS ONE 12(8): e0181010. https://doi.org/10.1371/journal.pone.0181010
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0181010

PLOS ONE

HOW TO COMPLETELY ELIMINATE STRESS IN FISH

HOW TO MINIMIZE STRESS IN FISH

- OPTIMIZE MANAGEMENT/HUSBANDRY PRACTICES
- OBSERVE YOUR FISH