Stress in fishes

Definitions
- Over the years a definition of stress has proved difficult to form
- “A shift in normal, homeostatic, physiological processes resulting from the action of any biotic or abiotic force”
- “The response of a cell, or organism, to any demand placed on it such that it causes an extension of a physiological state beyond the normal resting state”

Why should we care about stress?
- Reproduction
- Ionic, osmotic, acid base regulation
- Behavioral responses
- Immunity
- Growth
- Etc.

What do we mean by ‘Stress’

Stressors
- Chemical
- Pollution
- Water quality extremes
- Physical
- Handling
- Capture
- Confinement
- Transport
- Perceived
- Startle response
- Predator detection
Primary Response
- The initial response is reflective of the perception of an altered state and initiates a neuroendocrine response
- A rapid release of 'stress' hormones
- Catecholamines are released from the chromaffin tissue (Kidney)
- Adrenocorticotropic Hormone (ACTH) signals interrenal cells (Kidney) to secrete cortisol
- ACTH can also stimulate adrenaline release
- Cortisol can impact catecholamine storage and release
- There are likely paracrine interactions of these systems

What do we mean by 'Stress'?
Stress in Fishes

- Catecholamines frequently clear from circulation quickly (<30 min)
  - Adrenaline, Noradrenaline
- Cortisol remains elevated for a more extended period.

Cortisol Response

Perception of Stress

HPI Axis

Brain

CRH

Pituitary

ACTH

Interrenal

Cortisol

Tissues

Catabolism

Graph showing plasma cortisol levels over time after handling.
Species Differences
Stress Response to 30-s Handling

Barton (2000)

Stressor
Secondary Responses
Glucose +, Glycogen -
Lactate + (?)
Osmoregulatory disturbance +/-
Reproductive Hormones
Hematological Variables
(Hematocrit, Leukocrit, Hemoglobin)
Immunity (Lysozyme, antibodies)

What do we mean by ‘Stress’
Stress in Fishes

Secondary Response
- The suite of physiological and biochemical responses due predominantly to activities of cortisol of catecholamines
- Why are glucose concentrations used as a secondary response associated with stress?

Why are glucose concentrations used as a secondary response associated with stress?
- Both catecholamines and cortisol have activity on liver tissue
  - Glycogenolysis (catecholamines)
  - Gluconeogenesis (cortisol)
  - Inhibition of glycogen re-synthesis (cortisol)

Why are glucose concentrations used as a secondary response associated with stress?
- Secondary Response
  - Stress is an energy demanding process.
  - Animals need to mobilize energy.
  - Glucose concentrations typically remain elevated for hours after the stressor
  - Elevated glucose appears to be sustained in part due to elevated cortisol concentrations
What about the other secondary indices?

- Hematocrit
- Ions
- Immunity

What do we mean by ‘Stress’

Tertiary Responses
- Growth
- Swimming performance
- Disease Resistance
- Reproduction

Paradigm: Things that are sacrificed as a result of an immediate need to escape chronic stress impacts

Stress in Fishes

- Tertiary Responses
  - Whole animal or population level changes associated with stress
    - If the fish is unable to acclimate or adapt to the stressor, whole animal changes such as decreased reproductive output or decreased growth are observed
    - Goede Index
Stress in Fishes – Awkward Segue

- **Cellular Stress Response**
  - **HSP70**
    - Molecular chaperone
    - Aides in the refolding of proteins
    - Aides in repair and degradation of damaged proteins

Cross protection and HSP70 Induction

- Percent survival in 85 ppt salt after heat shock
- HSP70 measured in gill tissue after heat shock

A Generalized Stress Response

- Disease challenge, confinement, handling, transport, tank color, anesthetics etc can all be viewed as stressors.
- All activate the HPI axis and lead to an increase in primary responses (plasma cortisol) in response to exposure
Generalized Stress Response

- While an elevation in cortisol associated with a challenge is an indication of the stress response caution in interpreting quantitatively is advised.
- Does more cortisol = more stress?

Species Differences Species Differences

Stress Response to 30-s Handling

Does Higher Cortisol in a Stressed State Mean Greater Stress?

Cumulative Acute Stressors
Can you mitigate impacts of hauling stressors

- Salt
- Density
- Ram ventilation
- Cribs
- Current research..activation of the cellular stress response
- Others
  - Voodoo charm bracelets
  - Standing on one foot with one eye closed when interpreting the data
Current Thinking on Cortisol and Stress

- Stress can be detected by an elevation in plasma cortisol.
- This response can be safely viewed qualitatively (stressed/non-stressed)
- Extreme caution should be taken in making any inference of quantitative differences in magnitude of the response
  - Is 140 ng/mL less stressed than 200 ng/mL?
  - No clear answer to this question

Mode of Action

- Hormones activity is dependent upon receptors and signaling pathways and these are not constant in number
  - Catecholamine,
    - nongenomic receptors (fast acting)
  - Cortisol,
    - classical genomic receptors (not so fast acting)
    - non-genomic receptors (fast acting)

Fasting acting receptors are extremely important in the cardiovascular, respiratory, and metabolic changes associated with acute stress
Evaluating a Catecholamine Response

The Catecholamine Response is Rapid & Sampling Requires No Handling

Negative Feedback (Receptor Down Regulation)

Evaluating a Cortisol Response

The Cortisol response is slower. Getting samples within 2-3 minutes of capture may suffice. Lethal anesthesia is routinely used

Gilmour et al 1994
The Diagnostic ‘Kitchen Sink’ of Stress

### Important but less frequently used evaluations of stress in fish

- **Heat Shock Proteins**
  - **Indicator of cellular stress**
  - Advantages: A very sensitive indicator of cellular responses to acute and chronic stressors
  - Disadvantages: Linkages between various stressors and HSP responses & and relationships between neuro-endocrine stress axis and HSP responses are not entirely understood.

- **Neurotransmitters**
  - Indicates central nervous system responses to stressors
  - Advantages: May explain the underlying changes in peripheral endocrine responses and certain behaviors associated with stress.
  - Disadvantages: Rapid sampling and freezing of samples required. Proper interpretation likely requires analysis of preparations from specific (very small) regions of the brain.

### Evaluations of Stress in Fish

- **Plasma Catecholamines**
  - Rapid primary endocrine response to stress & functionally associated with oxygen delivery and energy mobilization
  - Advantages: Very responsive to acute stressors
  - Disadvantages: Requires cannulation to obtain samples form unstressed fish because of the rapidity of the response
Evaluations of Stress in Fish

- The common tool box

Plasma Cortisol

- Primary endocrine response to stress & used commonly as an indicator of stress with multiple roles (metabolism, osmoregulation, immunoregulation)
- **Advantages:** Predictable indicator of response to acute stress and useful in part because of the delay between stressor and manifesting a stress response
- **Disadvantages:** Influenced by genetic, developmental, environmental factors and the response may become desensitized in chronically stressful conditions

Plasma Glucose

- Metabolic response to stress due in large part by energy mobilization associated with cortisol and catecholamines
- **Advantages:** A useful measure that is very easy to determine either by commercial diagnostic kits or portable meters
- **Advantages:** Readings can be influenced by a variety of non-stress factors. Species, rearing history, temperature, diet
Evaluations of Stress in Fish

- **Plasma Lactate**
  - Metabolic response to intense muscular activity
  - Advantages: Very easy assay to perform and increasing availability of diagnostic meters.
  - Disadvantages: Still not clear if it is a ‘good’ indicator of stress as it has more to do with activity than neuro-endocrine signaling.

- **Tissue Glycogen**
  - Indicates energy reserves stored in liver and muscle for metabolism
  - Advantages: Depletion indicates mobilization of energy possibly due to stress
  - Disadvantages: Prior animal history is required since values may be influenced by recent feeding.

- **Plasma Chloride, Plasma Sodium, Osmolality**
  - Change indicative of osmoregulatory disturbance
  - Advantages: Clinical meters available. Standardized challenge approaches to salmonids have been developed in the case of sodium
  - Disadvantages: A variety of species are not all that responsive. In the case of osmolality the specific ion imbalance is never known.
Evaluations of Stress in Fish

- Plasma Protein
  - Change might be indicative of water imbalance and perhaps an osmoregulatory disturbance.
  - Advantages: Very easy assay approaches
  - Disadvantages: Not all that sensitive of a measurement

Evaluations of Stress in Fish

- Hematocrit
  - A measurement of packed cell volume in the blood
  - Advantages: Very easy to perform
  - Disadvantages: Not a very sensitive approach. Difficulty in interpreting differences (More cells or changes in cell size)

Evaluations of Stress in Fish

- Leukocrit
  - An indication of the fraction of white blood cells in the blood
  - Advantages: A very easy index to measure
  - Disadvantages: Not very sensitive and different stressors lead to varying results +/-
Evaluations of Stress in Fish

- Hemoglobin
  - An indication of the oxygen binding capacity of the blood
  - Advantages: Very easy to measure
  - Disadvantages: Not a very sensitive indicator to stress

Table 2. Ranges of typical resting and stress-elevated values for primary and secondary physiological parameters used as indicators of stress in fish (compiled from Wedemeyer et al., 1999; Barton and Iwama, 1993; Folmar, 1993; Campbel et al., 1994; and authors’ unpublished data). However, considerable variation among these values and many exceptions outside of these ranges exist depending on species, genetic background, rearing history, and environmental conditions (see text and cited reviews).

<table>
<thead>
<tr>
<th>Physiological parameter</th>
<th>Resting</th>
<th>Poststress</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma epinephrine (nmol/L)</td>
<td>1–4</td>
<td>5–200</td>
</tr>
<tr>
<td>plasma norepinephrine (nmol/L)</td>
<td>1–14</td>
<td>10–100</td>
</tr>
<tr>
<td>plasma cortisol (ng/mL)</td>
<td>2–10</td>
<td>30–500</td>
</tr>
<tr>
<td>plasma glucose (mg/dL)</td>
<td>60–150</td>
<td>100–250</td>
</tr>
<tr>
<td>plasma lactate (mg/dL)</td>
<td>20–40</td>
<td>40–80</td>
</tr>
<tr>
<td>plasma chloride (mEq/L)</td>
<td>100–130</td>
<td>10% ↑ or ↓</td>
</tr>
<tr>
<td>plasma sodium (mEq/L)</td>
<td>140–170</td>
<td>10% ↑ or ↓</td>
</tr>
<tr>
<td>plasma potassium (mEq/L)</td>
<td>2–4</td>
<td>10% ↑ or ↓</td>
</tr>
<tr>
<td>plasma osmolality (mOsm/kg)</td>
<td>290–320</td>
<td>10% ↑ or ↓</td>
</tr>
<tr>
<td>hemoglobin (g/dL)</td>
<td>5–9</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>hematocrit (% packed cell volume)</td>
<td>25–40</td>
<td>40–50+</td>
</tr>
</tbody>
</table>

* Blood size and other features related to hemodynamics status will fluctuate upward or downward depending on whether fish is marine or freshwater species, respectively.

So what? How do we interpret these data?
All Models are Oversimplifications...but

- **Homeostasis**
- **Claude Bernard -- 1860s**
  - milieu extérieur in which the organism is situated
  - milieu intérieur in which the tissue elements live.
  - The premise emphasizes maintenance of milieu intérieur within a range of set points
  - Most of our plasma indices reflect narrow resting ranges

Fish Physiology

- Is there anything wrong with this notion?
  - 
  - 
  - 
  - 

Predictive Models Surrounding Stress

- **1932 Cannon**
  - Fight or Flight
- **1936 Selye**
  - General Adaptation Syndrome
- **1977 Mazeaud et al.**
  - Primary, Secondary, Tertiary
Predictive Models Surrounding Stress

- "However, a definition of stress that fits into every disciplines conceptual framework is not on elusive, it may be impossible" Barton 1997

- Useful definitions and models allow for wide ranging discussion. e.g ecology, deep space travel

- The latest iterations of these include the concepts of allostasis and reactive scope model

Physiology Models

- **Homeostasis** implies that an organism remains within a certain range of physiological parameters to maintain stable function.

- **Allostasis** implies that an organism constantly varies and adjusts physiological parameters to maintain stable function.

Lumpers and splitters exist in every discipline
Allostasis: “Maintaining stability (or homeostasis) through change” Sterling and Eyer 1988

Allostatic Load: “Wear and tear that the body experiences due to repeated cycles of allostasis” McEwen 1998

Proposed Consequences -- Allostatic Load Model

What do ‘appropriate’ and ‘inappropriate’ really mean?

What is the stress response involved with?
What is stress response involved with?
- A shift in normal, homeostatic, physiological processes resulting from the action of any biotic or abiotic force

Every Thing
Social Interactions Predators
Agonistic Behaviors Environmental Cues
Exposure to Pathogens Energy Utilization
Etc.
An “appropriate” stress response necessary

What Happens If…?

Reactive Scope Model