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”**

Stress in fishes

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

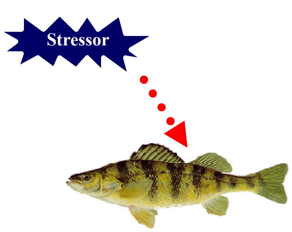
What do we mean by ‘Stress’

Stressors

Chemical
- Pollution
- Water quality extremes

Physical
- Handling
- Capture
- Confinement
- Transport

Perceived
- Startle response
- Predator detection



208 TRANS. AM. FISH. SOC., VOL. 106, NO. 3, 1977

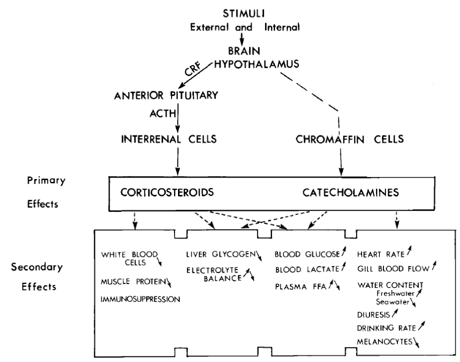
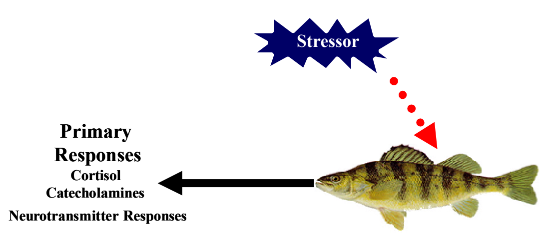


FIGURE 9.—*Scheme incorporating current information on the interrelationship between primary and secondary effects of stress in fish.*

Stress in fishes

- 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

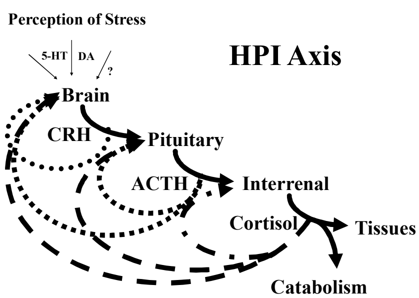
What do we mean by 'Stress'

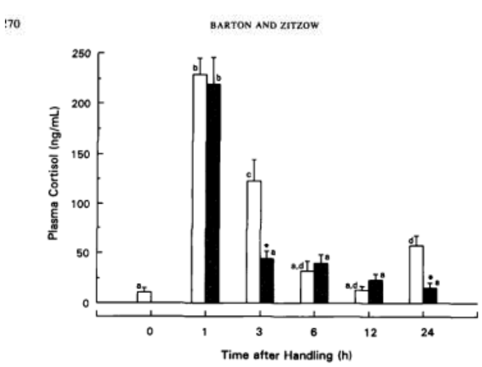


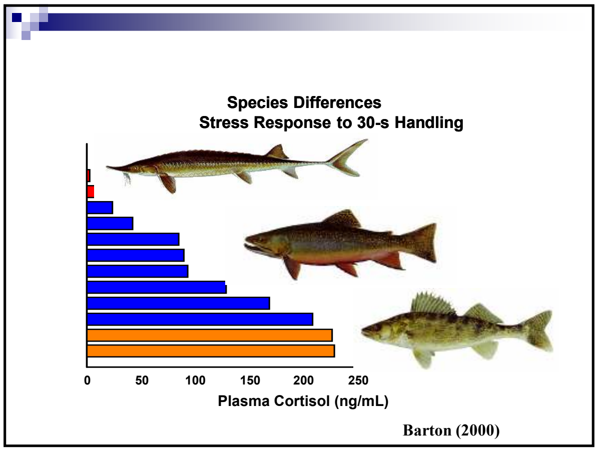
Stress in Fishes

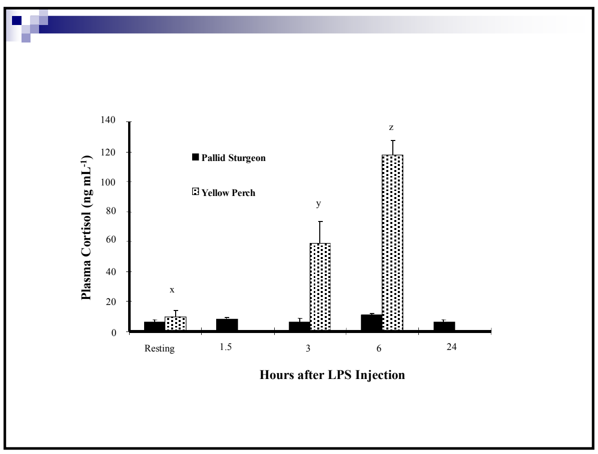
- Catecholamines frequently clear from circulation quickly (<30 min)
  - Adrenaline, Noradrenaline
- Cortisol remains elevated for a more extended period.

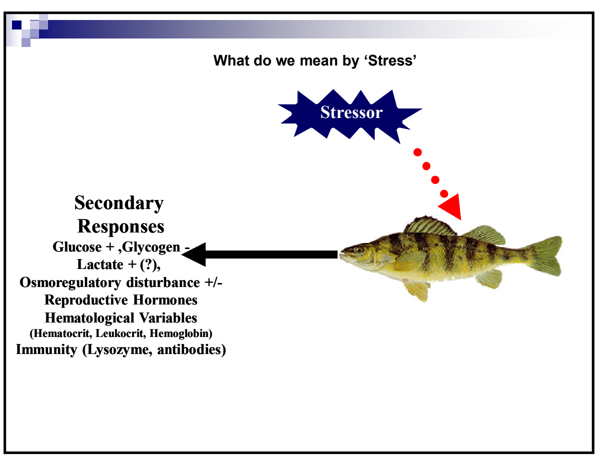
Cortisol Response






Species Differences
Stress Response to 30-s Handling
0
50
100
150
200
250
Plasma Cortisol (ng/mL)
Barton (2000)


Pallid Sturgeon
Yellow Perch
Plasma Cortisol (ng mL-1)
140
120
100
80
60
40
20
0
x
y
z
Resting
1.5
3
6
24
Hours after LPS Injection


What do we mean by 'Stress'
Stressor
Secondary Responses
Glucose + ,Glycogen -
Lactate + (?),
Osmoregulatory disturbance +/-
Reproductive Hormones
Hematological Variables
(Hematocrit, Leukocrit, Hemoglobin)
Immunity (Lysozyme, antibodies)

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)

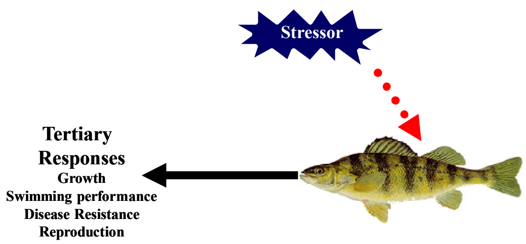
Why are glucose concentrations used as a secondary response associated with stress?

- 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'



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

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Stress in Fishes

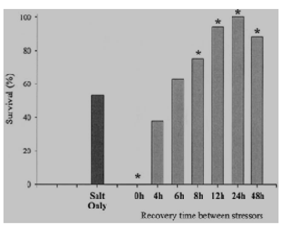
- Tertiary Responses
  - Whole animal or population level changes associated with stress
    - If the fish is unable to acclimate or adapt to the stressor whole animal changes such as decreased reproductive output or decreased growth are observed
    - Goede Index

Stress in Fishes – Awkward Segue

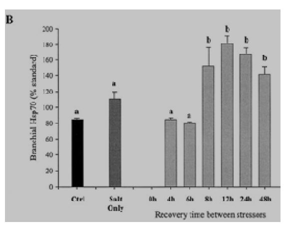
- Cellular Stress Response
  - HSP70
    - Molecular chaperone
    - Aides in the refolding of proteins
    - Aides in repair and degradation of damaged proteins

Cross protection and HSP70 Induction

Percent survival in 85 ppt salt after heat shock



HSP70 measured in gill tissue after heat shock



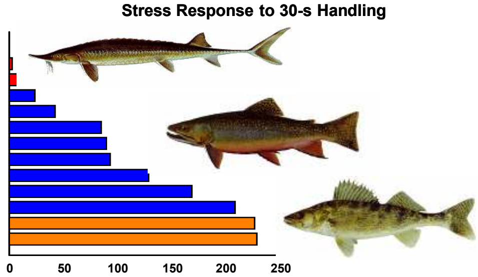
A Generalized Stress Response

- 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
Stress Response to 30-s Handling

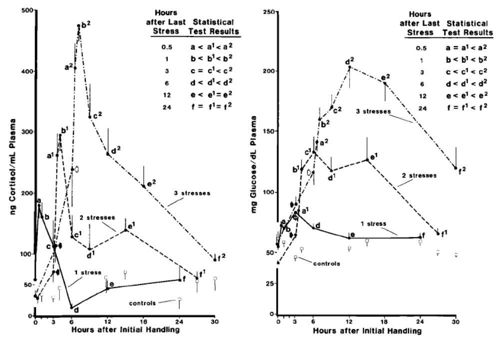


Plasma Cortisol (ng/mL)

Barton (2000)

Does Higher Cortisol in a Stressed State Mean Greater Stress?

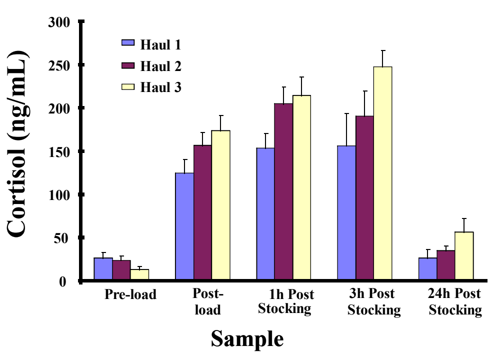
Cumulative Acute Stressors



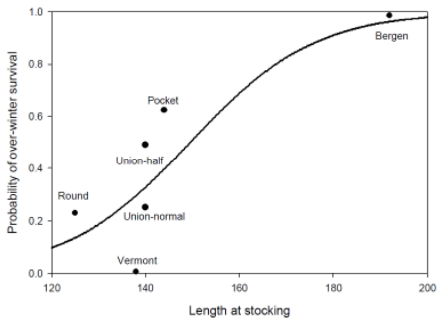
Barton et al 1986

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



Cortisol Response Following LPS Injection
Stizostedion vitreum



Current Thinking on Cortisol and Stress

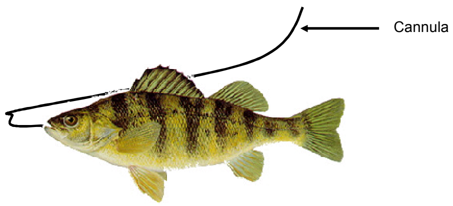
- Stress can be detected by an elevation in plasma cortisol.
- This response can be safely viewed qualitatively (stressed/non-stressed)
- Extreme caution should be taken in making any inference of quantitative differences in magnitude of the response
  - Is 140 ng/mL less stressed than 200 ng/mL?
  - 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)

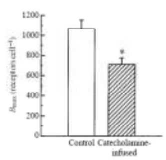
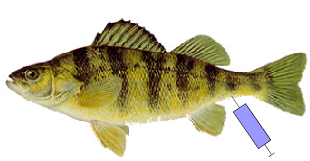


Fig. 2. Receptor densities (B_{max}, number of receptors per cell), determined as the number of isoproterenol-displaceable [^{3}H]CGP binding sites, for red blood cells from trout implanted with catecholamine-loaded mini osmotic pumps (diagonal striped columns) or sham-operated (open columns), 72 h post-surgery. Values are mean ± 1 S.E.M. (N=8). An asterisk indicates a significant difference (P<0.05) from the control.

Gilmour et al 1994

Evaluating a Cortisol Response



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

The Diagnostic 'Kitchen Sink' of Stress

Table 2
Mean ± S.E. ($n = 9$–12) plasma cortisol and lactate in yearling hybrid and pallid sturgeons after a 30-s aerial-emersion handling stressor

Time (h)	Hybrid sturgeon[a]	Pallid sturgeon[a]
Plasma cortisol ($ng\ ml^{-1}$)		
0	2.19 ± 0.37 a,b	2.29 ± 0.33 a
1	3.17 ± 0.29 b	2.97 ± 0.26 a
3	2.70 ± 0.21 b	2.85 ± 0.42 a
6	2.26 ± 0.25 a,b	2.99 ± 0.23 a
Control-6	1.49 ± 0.16 a	3.15 ± 0.28 a
24	2.07 ± 0.24 a,b	3.42 ± 0.56 a
Control-24	1.58 ± 0.15 a	2.22 ± 0.19 a
Plasma lactate ($mmol\ l^{-1}$)		
0	1.21 ± 0.14 a	0.42 ± 0.10 a
1	1.41 ± 0.09 a	1.27 ± 0.36 a
3	1.32 ± 0.16 a	1.10 ± 0.43 a
6	1.25 ± 0.14 a	0.68 ± 0.10 a
Control-6	1.45 ± 0.17 a	0.91 ± 0.16 a
24	0.65 ± 0.07 b	1.24 ± 0.45 a
Control-24	1.37 ± 0.16 a	0.77 ± 0.19 a

Table 3
Mean ± S.E. ($n = 9$–12) plasma glucose in yearling hybrid sturgeon and plasma chloride in hybrid and pallid sturgeons after: (a) a 30-s aerial-emersion handling stressor, and (b) a 6-h severe confinement (with handling) stressor[a]

Time (h)	Plasma glucose ($mg\ dl^{-1}$)	Plasma chloride ($meq\ l^{-1}$) H	P
30 s handling			
0	59.1 ± 3.44	108 ± 2.77	91.7 ± 0.65
1	51.7 ± 2.82	110 ± 2.01	91.1 ± 3.30
3	60.8 ± 4.00	107 ± 1.29	90.8 ± 2.28
6	59.6 ± 3.34	115 ± 2.04	96.0 ± 1.74
Control-6	61.8 ± 4.54	105 ± 1.89	97.6 ± 1.09
24	59.1 ± 3.18	111 ± 3.20	101 ± 1.81
Control-24	59.7 ± 3.31	111 ± 1.64	97.2 ± 1.04
6 h severe confinement with handling			
0	52.9 ± 3.26	116 ± 2.48	97.2 ± 1.04
1	64.4 ± 3.49	110 ± 2.39	97.3 ± 2.26
2	62.9 ± 3.67	111 ± 1.61	102 ± 1.53
3	63.6 ± 3.82	106 ± 1.72	97.4 ± 1.00
4	62.9 ± 4.86	106 ± 1.46	97.0 ± 0.96
6	50.9 ± 2.85	109 ± 1.51	96.0 ± 1.72
Control-6	48.6 ± 2.37	109 ± 2.51	100 ± 2.75
24	55.6 ± 3.05	106 ± 2.87	98.5 ± 1.64
Control-24	49.3 ± 2.00	110 ± 2.60	101 ± 2.29

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

Evaluations of Stress in Fish

- 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

Evaluations of Stress in Fish

- 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

Evaluations of Stress in Fish

- 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.

Evaluations of Stress in Fish

- 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. 1990; Barton and Iwama 1991; Folmar 1993; Gamperl 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).

Physiological parameter	Resting	Poststress
plasma epinephrine (nmoles/L)	1–6	5–200
plasma norepinephrine (nmoles/L)	1–14	10–100
plasma cortisol (ng/mL)	2–50	30–300
plasma glucose (mg/dL)	50–150	100–250
plasma lactate (mg/dL)	20–30	40–80
plasma chloride (meq/L)	100–130	≈10% ↑ or ↓ [a]
plasma sodium (meq/L)	140–170	≈10% ↑ or ↓ [a]
plasma potassium (meq/L)	2–6	≈10% ↑ or ↓ [a]
plasma osmolality (mOsm/kg)	290–320	≈10% ↑ or ↓ [a]
hemoglobin (g/dL)	5–9	< 4
hematocrit (% packed cell volume)	25–40	40–50+

[a] Blood ions and other features related to hydromineral 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

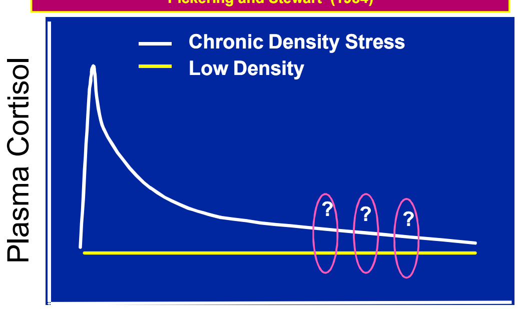
Physiology Models

- **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 Model ??

Pickering and Stewart (1984)

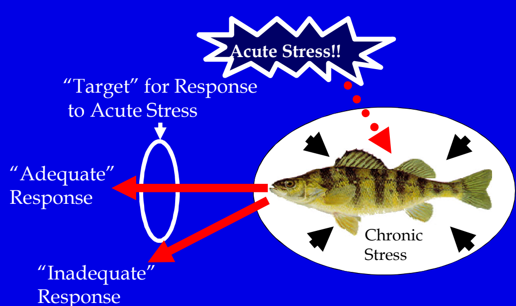


Proposed Consequences -- Allostatic Load Model

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



Allostatic Load

- 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

Agonistic Behaviors

Exposure to Pathogens

Etc.

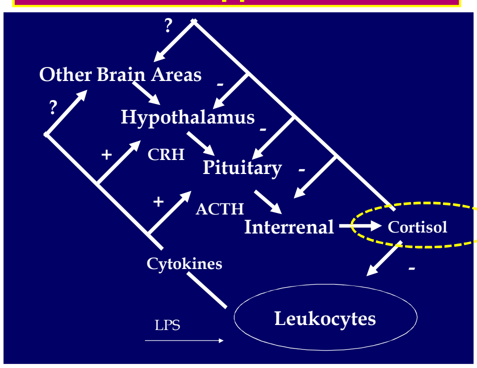
Predators

Environmental Cues

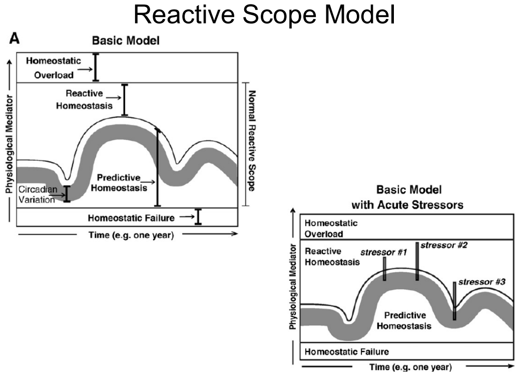
Energy Utilization

An "appropriate" stress response necessary

What Happens If….



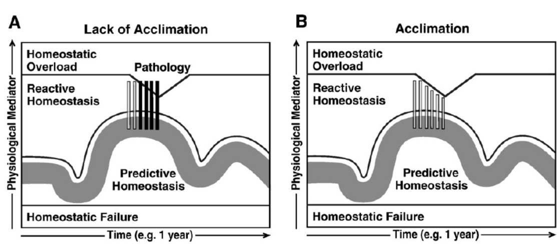
Reactive Scope Model

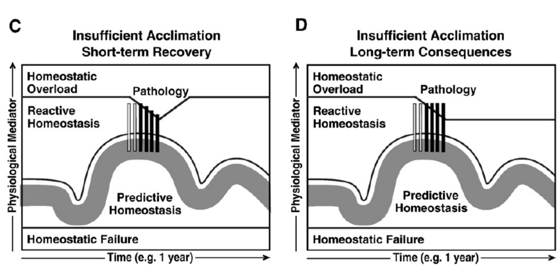


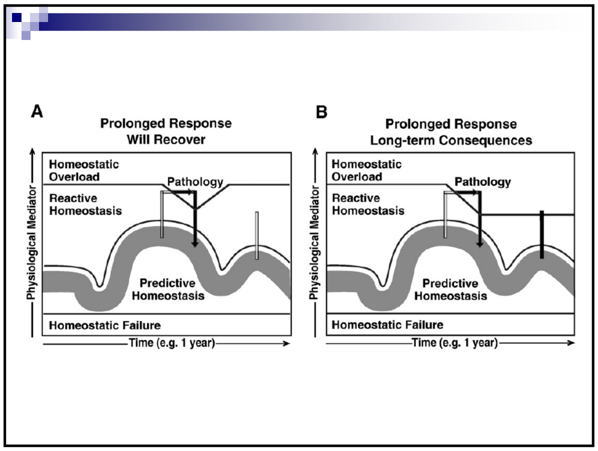
Reactive Scope Model

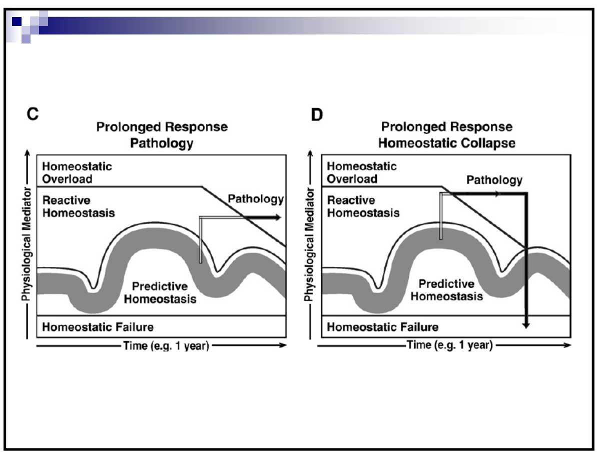
Table 1

Physiological system	Physiological mediator	Predictive homeostasis range	Reactive homeostasis range	Homeostatic overload range	Homeostatic failure range
Immune	Prostaglandin T-cell activation Antibody titers Cytokines	Seasonal ability to fight infection	Mobilization of immune system	Autoimmune Immunosuppression	Immune failure
HPA	Glucocorticoids ACTH	Seasonal life-history needs a. Energetic needs b. Behavioral needs c. Preparative needs	Inhibit immune system Energy mobilization Change behavior Inhibit reproduction Inhibit growth	Immunosuppression Diabetes Muscle breakdown Reproductive suppression Decreased survival	Energy dysregulation Water balance failure Catecholamine insufficiency Decreased survival
Cardiovascular (catecholamines)	Heart rate Heart rate variability Blood pressure	Life-history energy needs	Fight-or-flight Energy mobilization	Hypertension Myocardial infarction Muscle breakdown	Hypotension Lethargy Decreased survival
Behavior	Foraging/feeding Locomotion Migration Conspecific aggression	Life-history changes: a. Energy needs b. Energy availability c. Predator presence d. Mate access	Fleeing behavior Freezing behavior Increase/decrease foraging Increase food intake Increase vigilance Conspecific fighting	Tonic immobility Obesity Anxiety Fear Violence	
Central nervous system	Neurogenesis Dendritic arborization Neurotransmitter concentrations Cytokines	Life-history changes in neural networks Learning and memory	Increase neurotransmission (titers or receptors) Increase learning and memory	Neuronal atrophy/death Depression Decrease learning and memory	Post traumatic stress disorder






A
Prolonged Response
Will Recover
B
Prolonged Response
Long-term Consequences
Homeostatic Overload
Pathology
Reactive Homeostasis
Predictive Homeostasis
Homeostatic Failure
Physiological Mediator
Time (e.g. 1 year)


C
Prolonged Response
Pathology
D
Prolonged Response
Homeostatic Collapse
Homeostatic Overload
Pathology
Reactive Homeostasis
Predictive Homeostasis
Homeostatic Failure
Physiological Mediator
Time (e.g. 1 year)