References used according to the letters given at the bottom of the slides


C: Farrell, AP (1991) From Hagfish to Tuna: A Perspective on Cardiac Function. Fish Physiological Zoology, 64, (5), 1137-1164; URL: http://www.jstor.org/stable/30156237


I: An Overview of the Histological Study of Marine Finfish; http://research.myfwc.com/features


O: de Jesus, EG, Toledo, JD, Simpas, MS (1998) Thyroid hormones promote early metamorphosis in grouper (Epinephelus coioides). General and Comparative Endocrinology, 112: 10-16.
Fish physiology

Horst Kaiser

Ichthyology II, 2012
Respiration
Life in water (1/2)

- Water is 840 times more dense and 60 times more viscous than air.
- Oxygen:
  - Air: 210 ml/L $O_2$ at 21% partial pressure
  - Water: up 15 mg/L $O_2$ (dep. on temperature)
  - Sea water holds 18% less $O_2$ than freshwater
- Oxygen consumption in fish
  - 17 mg/kg/h @10°C
  - 100-500 mg/kg/h @ 30°C
- More than 40 genera of fishes breath oxygen using other methods than their gills.
Life in water (2/2)

• Energy demand to accomplish respiration:
  – approx. 50% of total demand but can be up to 90%
• Blood volume: 2-4 mL / 100 g; (nucleated RBC)
• Gill surface area: 150–300 mm² / g tissue
• Systolic blood pressure: about 44 mm Hg
• Gill irrigation: 5-20 L H₂O / kg BM / h
• Opercular beat counts: 40-60 / minute
Henry’s law

\[ \text{VO}_2 = \alpha \text{PO}_2 \]

\( \text{VO}_2 = \text{O}_2 \text{ concentration in ml/L (or mg/L)} \)

\( \alpha = \text{solubility coefficient: the volume of O}_2 \text{ dissolved in water: ml O}_2/\text{L/atm} \)

\( \text{PO}_2 = \text{PO}_2 \text{ partial pressure (atm)} \)
### Metabolism of trout vs turtle

<table>
<thead>
<tr>
<th></th>
<th>Trout</th>
<th>Turtle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen requirement</td>
<td>~ 5 ml / min / kg</td>
<td>~ 5 ml / min / kg</td>
</tr>
<tr>
<td>Ventilation volume</td>
<td>600 ml H₂O / min / kg</td>
<td>50 ml air / min / kg</td>
</tr>
<tr>
<td>Routine costs for ventilation</td>
<td>10 %</td>
<td>2 %</td>
</tr>
</tbody>
</table>

ref. D
Gills and kidney: Osmoregulatory organs
PAIRED HOLOBRANCHS

tongue

Gill rakers

Four paired holobranchs
FIG. 1. Schematic of the teleost fish gill. See text for details. [From Campbell and Reece (81).]
Countercurrent exchange

The **difference** in oxygen partial pressure $PO_2$ between water and plasma strongly influences the uptake of oxygen at the gill lamella / water interface!
Hagfish gill pouch

tentacles  
gill slits (twelve pairs)  
mucous glands  

Excurrent duct  
Afferent Branchial Artery  
Efferent Branchial Artery  
Lamellae  
Incurrent duct
Atlantic mackerel, *Scomber scombrus*

Horse mackerel, *Trachurus trachurus*

Skipjack tuna, *Katsuwonus pelamis*

Oyster toadfish, *Opsanus tau*

Eel, *Anguilla japonica*

Angler, *Lophius piscatorius*
Unit gill area: mm²/g body mass

- *Patagonotothen tessellata*
- *Gobionotothen gibberifrons*
- *Notothenia rossi*
- *Opsanus tau*
- *Lophius piscatorius*
- *Anguilla anguilla*
- *Katsuwonus pelamis*
- *Trachurus trachurus*
- *Scomber scombrus*
Number of gill lamellae / mm filament length

- *Patagonotothen tesselata*
- *Gobionotothen gibberifrons*
- *Notothenia rossi*
- *Opsanus tau*
- *Lophius piscatorius*
- *Anguilla anguilla*
- *Katsuwonus pelamis*
- *Trachurus trachurus*
- *Scomber scombrus*
Oxygen consumption in grunter

Model: $R = a \cdot W^b$

$R = 0.56 \cdot W^{0.679}$

Data are from John Radull’s thesis
Oxygen consumption in grunter

Model: $R = a \cdot W^b$

$R = 0.67 \cdot W^{-0.042}$

Data are from John Radull’s thesis
Possible explanations for metabolic rate changes with size

• Developmental changes of relative weights of different organs
  – Liver and gills weigh relatively less
  – Swimming musculature becomes more developed
• Metabolic intensities of different tissues may decline with increasing size (age)
• Differences between species
• Possible effect of test temperature
• Possible interactions between temperature and species
• Salinity?
Metabolic rate in fish

- Standard metabolic rate (SMR)
- Routine metabolic rate (RMR)
- Active metabolic rate (AMR)
- Metabolic scope (MS): $MS = SMR - AMR$
Oxygen consumption (mg.g⁻¹.h⁻¹)

AMR

SMR

Hour of day

ref. F
Intermittent respirometry

- Syringe
- Mechanical stirrer
- Oxygen probe
- Water flow
- Glass flask

Water flow

ref. G
Physiology of the stress response

An interesting and important relationship between the stress response and respiration
The stress response

- **Primary stress response**: Perception of a stressor and initiation of physiological responses.

- **Secondary stress response**: Biochemical and physiological changes (i.e., release of stress hormones).

- **Tertiary stress response**: Changes in metabolic rate. This may be followed by various other responses.
Endocrinology of the stress response

Brain / hypothalamus

- Sympathetic nerves
  - Chromaffin cells
    - Catecholamines

- Hypothalamic factors
  - Pituitary gland
    - ACTH (Adrenocorticotropic hormone)
      - Inter-renal cells
        - Cortisol
Generalised change in adrenaline and cortisol concentration in fish plasma following a stressor
The main effects of elevated cortisol levels

- Initiates a switch from anabolism to catabolism
- Affects osmoregulation
- Very immunosuppressive
- Important effects on reproductive processes
The effect of handling stress on metabolic rate changes in spotted grunter

Ref.: Radull et al. 2000
Respiration

Gas exchange
Respiration

Ventilation

O2 diffusion

O2 dissolves into plasma

O2 binds to haemoglobin in RBCs

O2 diffuses across capillary walls & cytoplasm to mitochondria

Once O2 has been offloaded CO2 diffuses into the RBC

CO2 is transported to gills and excreted
Fish have nucleated red blood cells!

Some species have more than 1 type of Hb

Each protein molecule has 4 globin subunits each with one haem group to bind O\(_2\)

Hb exists in 2 states, a tense (T) state with low affinity to O\(_2\), and a relaxed state (R) with high affinity.

A shift from T -> R increases O\(_2\) binding capacity

The four units cooperate to increase O\(_2\)-uptake

What changes the state from T to R?
Blood and serum

Capillary glass tube filled with blood

Spin tube at 13 g

Plasma / Serum

Red blood cells / packed cell volume

Small volume of lymph
Oxygen carrying capacity depends on ...

- Number of red blood cells
- Concentration of haemoglobin within the red blood cells
- Oxygen binding properties of haemoglobin
- Partial pressure of oxygen
Where do red blood cells come from?
Spinal cord
Haemopoietic granular cell precursors
Haemopoietic sinus
Dorsal aorta
Bone marrow
Vertebral Bone
Haemopoietic foramen
Dorsal aorta
Haemopoietic sinus
Haemodinamamic organ in dorsal aorta
Kidney

HAEMOPOIETIC ORGAN IN RAINBOW TROUT
Carbon dioxide transport – the chemistry of CO$_2$ in water

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \]

Carbon dioxide
Carbonic acid
Bicarbonate ion
Carbonic anhydrase speeds up carbon dioxide dissociation in the cell

Extracellular

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+
\]

Carbonic anhydrase

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+
\]

Red blood cell
At the tissues

Carbonic anhydrase

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+
\]

Fast reaction

Hemoglobin changes state and releases \( \text{O}_2 \)

Drop in pH!

HCO\(_3^-\) transport to plasma in exchange for Cl\(^-\)

\( \text{O}_2 \) diffuses to plasma and tissues
At the gills

Carbonic anhydrase

HCO$_3^-$ + H$^+$ $\rightarrow$ H$_2$CO$_3$ $\rightarrow$ CO$_2$ + H$_2$O

- HCO$_3^-$ transport to blood cell in exchange for Cl$^-$
- Fast reaction
- Release of CO$_2$ increases pH
- CO$_2$ diffusion to plasma and then to the water

Hemoglobin changes state and is ready to pick up O$_2$ at the gills.
Gill interface

$\text{O}_2 \quad \text{CO}_2$

Blood stream

$\text{CO}_2 \quad \text{pH} \quad \text{pH} \quad \text{O}_2$

to organs

Increased Bohr and root effect

$\text{O}_2 \quad \text{CO}_2$

Hyper-capnia

Hyper-lacticaemia

Excess $\text{CO}_2$ through respiration, and in water

Reduced opercular movement

$\rightarrow$ impaired $\text{CO}_2$ excretion

Drop in blood pH (lactic acid, stress, excessive swimming)
Partial pressure of oxygen ($P_{O_2}$)

Percent saturation of Hb with $O_2$

Bohr effect

- Higher pH (gills)
- Lower pH (tissues)

Exponential increase due to Hb subunit cooperation

Dissociation curves parallel

Reduced affinity under acidic conditions

ref. B
Bohr effect – species differences

Sessile species with high Hb-affinity to oxygen
Can cope with low-oxygen conditions

Active species with low Hb-affinity to oxygen require high oxygen levels to survive.

Reduced affinity under acidic conditions

ref. B
Percentage oxygen in blood

Red blood cells / ml (*1000)

Toadfish
Goosefish
Puffer fish
Sea robin
Sculpin
Mackerel

ref. E
Sea robin (*Prionotus* spp)

Toadfish (*Opsanus* spp)

Mackerel (*Scomber scombrus*)

**Root effect**
Why is the Root effect unique to fish?

- Oxygen supply to the retina
- Gas bladder function
Release of gas from the gas bladder

• Physostomous fishes
  – Pneumatic duct to the gut in most but not all species

• Physoclistous fishes
  – Closed gas bladder
  – Release gas into the blood (specialised oval area)
  – Excess gas is carried to the gills
The rete counter-current system

Example: gas bladder rete in eel
- Cross section: 5 mm$^2$
- Volume: 21 mm$^3$
- Surface area: 30 cm$^2$
- Capillaries: 20,000 to 40,000
- Artery diameter: 9 - 10 µm
- Venous capillary: 11 – 13 µm
- Diffusion distance (capillaries): 1 µm
- Capillary length: 4 mm
- Holes in capillary: 20 – 80 nm
- Hole diaphragm: 5 nm

ref. B
**Rete mirabile**: Changes of gas content, pH and lactate in the capillaries

Three processes:
- Bohr / Root shift
- Salting out
- Counter-current diffusion
Partial pressure of oxygen (PO$_2$)

Percent saturation of Hb with O$_2$

Root effect

O$_2$ Saturation is not reached even if sufficient O$_2$ available!

higher pH (gills)

Subunit cooperation

Some subunits fail to load O$_2$

much lower pH (retina / swimbladder)

Decreased capacity under acidic conditions.
**Ice fish**

- White gills
- Almost transparent blood
- No haemoglobin

![Image of ice fish with labels for Bulbus arteriosus, Ventricle, Liver, Colourless blood]
An interesting case: respiration in icefishes

• Some species, i.e., Nototheniidae, do not have haemoglobin
• Many fish take up oxygen across the skin (up to 35%) – do icefish employ this strategy?
• Icefish gill morphology does not differ much from that of most other fishes
• Surface area is similar to that of other species
• In this case, which factors determine $O_2$ uptake?
We observe

• Gill histology in ice fishes does not contribute to oxygen uptake.

So how do they do it?
The Fick principle

• \( \text{O}_2\)-consumption = \( V_g \times [\text{O}_2] \times \% \text{ extraction} \)
  – \( \text{O}_2\)-consumption = mg \( \text{O}_2 \)/kg / h
  – \( V_g \) = Gill water flow in ml \( \text{O}_2 \)/ kg / h
  – \([\text{O}_2]\) = \( \text{O}_2 \)(mg/L) in water pumped over gills

If \% extraction is low (= no haemoglobin carrier!), \( V_g \) should increase. \( V_g \) in ice fishes *is* relatively high. However, this is only part of the explanation.
The Fick principle

- $O_2$-consumption = $Q \times [(A-V) \times O_2]$
  - $O_2$-consumption = mg $O_2$/kg/h
  - $Q$ = Cardiac output (ml/kg/h)
  - $A$ and $V$ = $O_2$ (mg/L) in Arterial and Venal blood

Ice fishes without haemoglobin dissolve $O_2$ in plasma, thus, they have a low oxygen-carrying capacity.

Prediction: $Q$ should be relatively high. Data show a 7-fold increase in $Q$ relative to many other species.
Adaptations in ice fishes

- Relatively high cardiac output / large hearts
- Relatively high blood flow to the gills
- Modification of secondary gill lamellae to increase diameter
- Lower blood pressure
- Ice fishes make use of *perfusion* *in addition to diffusion* to pick up O$_2$. 
The circulatory system
African lungfish, *Protopterus*. The blood is directed to the dorsal aorta or lungs depending on whether the fish is breathing in air or water. (*After D. Randall et al., eds., Eckert Animal Physiology, 4th ed., W. H. Freeman, New York, 1997*)

*From: http://accessscience.com*
Fish (teleost)

Gills

Heart

ABO

Organs

ABO = air breathing organ

Fish (teleost with ABO – generalised) – Colours resemble adaptations
Unique features of the heart in fish

- Cells of the heart muscle receive poorly oxygenated venous blood (V).
- Single ventricle produces low pressure (<40 mm Hg)
- Lack of coronary circulation
- Relatively high ventricular stroke volume
- No valves at the venous inflow
- Pacemaker
Blood circulation in the head of trout

Gray: pre-branchial arteries (afferent)
Red: post-branchial arteries (efferent)

*Bulbus arteriosus*
- Thick elastic walls, smooth muscle
- Stores up to 100% of stroke volume to control pulse pressure

**Ventricle**
- Pumps blood to *bulbus arteriosus*
- Pumped volume = stroke volume (in fish)
Type trabecular heart of an air breathing catfish

- A = Atrium
- V = Ventricle
- B = Bulbus arteriosus

Entrance from the *sinous venosous*

Valved junction

Longitudinal trabeculae (mechanical strength)

White arrow = direction of blood flow

Trabeculae

Spongy myocardium of the ventricle

1 mm

ref. B
• Myocardial fibres in the **spongy** tissue layer
• Organised in fascicles
• Fascicles form branching lacunae
• Large surface area in fish due to lack of blood supply

**Compact** myocardium of active fishes

BA = *Bulbus arteriosus*
AV = Atrioventricular valve
BV = Bulboventricular ring
(a) = outer layer
(b) = inner layer

ref. B
The mysterious secondary circulatory system in fishes

- A system of very small capillaries branching (►) off mostly from efferent filamental gill arteries (EF)
- Secondary arterioles (S) are too small to transport red blood cells
- Volume of blood can make up to 20% of total blood volume.
- Blood flow under hormonal control
- Function unclear
  - Lymph precursor?
  - Osmoregulation?
  - Pool of reserve blood?
  - Some nutrient supply?

ref. C
Blood flow – the basic definitions

- $F = \text{Flow} [\text{ml / min}]$
- $P_a = \text{Arterial pressure} [\text{mm Hg}]$
- $P_v = \text{Venous pressure} [\text{mm Hg}]; \text{nb: } P_v \approx 0$
- $\delta P = P_a - P_v$
- $R = \text{Vascular resistance} [\text{mm Hg / ml}]$
- $\eta = \text{Viscosity} [\text{Pascal sec}]$
- $L = \text{Length of the blood vessel} [\text{mm}]$

$$F = \frac{\pi r^4 \delta P}{8 L \eta}$$
Oxygen consumption (V) and cardiac output (Q)

\[ V = \text{ml O}_2 / \text{kg} / \text{min} \]
\[ Q = \text{ml} / \text{kg} / \text{min} \]

**Oxygen consumption**

\[ V(O_2) = Q \left( P_{a O_2} - P_{v O_2} \right) \]

- **Pa O**₂ = O₂ of arterial blood
- **Pv O**₂ = O₂ of venous blood

- **Rainbow trout** (11°C)
- **Lincod** (10 °C)
- **Hagfish** (10 – 15 °C)
- **Dogfish** (20°C)
- **Leopard shark** (20°C)
- **Skipjack tuna** (26°C)
- **Yellowfin tuna** (26°C)
To increase Q most fish species adjust stroke volume more than heart rate (except tunas)

- Life history (10 – 300 ml/min/kg)
- Arterial O₂ level -> high Q in ice fishes
- Exercise, size, age

- Temperature
- Activity
- O₂ demand
- O₂ availability
- Species
- Metabolic rate

Cardiac output (Q)
- Length of blood vessels
- Hormonal control
- Vessel radius

Arterial blood pressure
• Energy of contraction is a function of muscle fibre length.
• A fish heart can pump almost its own volume
• Greater ventricular filling = greater stroke volume

• Filling time
• Filling pressure

stroke volume

Ventricular strength

End-diastolic volume

Arterial blood pressure ("afterload")

• Operational pressure (mm Hg) is species specific

ref. D
### Ventricular strength

### End-diastolic volume

### Arterial blood pressure

### Stroke volume (SV)

### Heart rate (HR)

### Cardiac output (Q)

### Vascular resistance (R)

### Arterial blood pressure

---

**Examples**

<table>
<thead>
<tr>
<th></th>
<th>Q (ml/min/kg)</th>
<th>HR (beats/min)</th>
<th>SV (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carp, resting, 6°C</td>
<td>9</td>
<td>7</td>
<td>1.22</td>
</tr>
<tr>
<td>Carp, resting, 15°C</td>
<td>15</td>
<td>17</td>
<td>0.77</td>
</tr>
<tr>
<td>Trout, resting, 11°C</td>
<td>17.6</td>
<td>38</td>
<td>0.46</td>
</tr>
<tr>
<td>Dogshark, resting, 19°C (2.8 kg)</td>
<td>52.5</td>
<td>43</td>
<td>1.21</td>
</tr>
<tr>
<td>Flounder, resting, 10°C</td>
<td>16</td>
<td>34</td>
<td>0.50</td>
</tr>
<tr>
<td>Sturgeon, resting, 19°C</td>
<td>36</td>
<td>48</td>
<td>0.83</td>
</tr>
<tr>
<td>Tuna, 26°C; 1.4 kg</td>
<td>115</td>
<td>97</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*ref. D*
Endocrinology
Objectives of the following lectures

- A comparative overview of hormones in fishes
- Reproductive endocrinology
  - The effect of pollutants on fish endocrinology
- Endocrinology of osmoregulation
Why do we study endocrinology?

• To understand fish physiology
• To manage fish health
• To understand and manipulate reproduction
• To evaluate the effect of pollutants on fish reproduction
• And more …
Glands

**Exocrine**
- Discharge of excretory products through ducts
- Examples: Intestinal glands, Sweat glands

**Endocrine**
- No ducts
- Richly vascularised
- Many cytoplasmatic organelles
- Interact with receptor cells
Endocrine system

- Chemical communication and regulation of ...
  - Development
    - Growth and maintenance
    - Larval development
    - Energy availability
  - Osmoregulation
  - Reproduction
  - Stress response
  - Digestion
  - Behaviour
Pineal gland
Hypothalamus
Pituitary
Head kidney chromaffin cells
Kidney
Corpuscles of Stannius
Urophysis
Gonads
Thyroid gland
Heart
Gastrointestinal peptides
Pancreatic tissue
Hormones as messengers

• **Endocrine**
  – Affect distant parts in the body
  – Transported via the circulating blood

• **Paracrine**
  – Local effects / diffusion

• **Pheromonal**
  – Secretion into the environment

• **Autocrine**
  – Work in the cell in which they are produced
Receptors

• These are large protein or glycoprotein molecules.
• Located at the target cells.
• They are constantly broken down or synthesised.
• Quantity affected by hormone levels.
• Down or up-regulation by hormones.
Classification of hormones

• Steroids / steroid-related hormones
• Tryrosine derivates
• Hormones with a peptide and glycopeptide structure
Steroids / steroid-related hormones

- Lipid soluble.
- All steroids are cholesterol derivates. 4-ring structure.
- Producing organs: Mainly gonads, but in fish also adrenal cortex.
Steroid hormone

Cell membrane

Receptor protein

Nuclear membrane

Induction of mRNA production

Protein synthesis

Cell response

ref. H
Peptide hormone → Receptor → Cell membrane

AC (Adenylate cyclase) → ATP → cAMP → Protein Kinase → Activation → Protein Kinase

Cell response

PD (Phosphodiesterase)
Generalized diagram of different regions and cellular zonation of trophic hormone producing cells in the adenohypophysis of a teleost fish.
Endocrinology of reproduction

Maturation and ovulation of oocytes
An Overview of the Histological Study of Marine Finfish; Fish and Wildlife Research Institute, Florida, USA;

ref. 1
Yolked oocyte

12 hr POF

Postovulatory

ref. I
Histological sections of GnRHa-induced wild-caught *L. niloticus* broodstock under-going final oocyte maturation; a-b) early oocyte maturation (centre GV); c) oocyte with fully formed oil globule with periphery GV; d) oocytes undergoing germinal vesicle breakdown, GV = germinal vesicle, black arrow head = multiple nucleoli, GVBDdo = germinal yolk vesicle break-down oocyte
Final oocyte maturation: germinal vessicle migration

Final Oocyte Maturation
Examples for predictive factors

• Changes in day length
• Slow changes in temperature
• Other factors in the environment depending on the stages of gonadal development
Modifying factors

- Water quality
- Lunar cycle
- Broodstock nutrition
- Social interactions
Synchronising cues

- Water quality changes
- Floods
- Rapid temperature changes
- Atmospheric changes
Patterns of oogenesis

Winter  Spring  Summer  Fall

Gonadal mass / oocyte diameter

Carp

Tench

Various barbus spp.

*
Hypothalamus

Endocrine gland

Anterior pituitary

Target tissue

Tropic hormone

Releasing hormone

End hormone

Metabolite(s)

Adopted from Jobling 1998
Summary of oocyte maturation / ovulation

GtRH Inhibitor
Antagonist (Domperidone; Pimozide)

Hypothalamus

GnRH

Pituitary

GtH 2

GtH 1

Liver

Vitellogenesis

Vitellogenin

Gonads
(Ovaries)

Testosterone

Testosterone (ie.: 11-α-Keto Testosterone)

Estrogen (ie., 17-ß Estradiol)

Aromatase

GtH = Gonadotropin Hormone

GnRH = Gonadotropin-Releasing Hormone
Case example 1: Goldfish (Carassius auratus)

- Environmental cues
- Behavioural cues
- Endocrinological control
- Pheromonal control
Aspects of goldfish reproduction

• Pre-ovulatory waiting phase
• Influence of a diurnal rhythm
• Spawning follows ovulation very tightly
• Three cues for initiation of ovulation:
  – Vegetation
  – Temperature
  – Presence of males
• But scotophase is an overriding factor
Female

GtH

17,20P → Ovul (PG) → courtship

12:00 20:00 04:00 12:00

Male

17,20P → PIP

17,20P → milt → courtship

12:00 20:00 04:00 12:00

PG = Prostaglandin
PIP = PG-induced pheromone

spawning synchronicity

ref. J
Case example: Masu salmon

- Migrational cues
- Behavioural cues
- Endocrinological control
- Sneaker behaviour
Masu salmon: two reproductive strategies in males

**Salmon parr: migratory form**
- Fresh water
- Fresh water
- Sea water
- Fresh water

**Salmon parr: precocious sneakers**
- Fresh water
- Fresh water
- Fresh water
- Fresh water
Masu salmon: reproductive behaviour

Testosterone $\rightarrow$ upstream migration in females
Testosterone $\rightarrow$ nest digging behaviour in females

L-kynurenine $\rightarrow$ upstream migration in females

Precocious male (sneaker)

Testosterone $\rightarrow$ induces upstream migration in males, including precocious sneakers
Osmoregulation
Osmol/L = \sum \lambda_i n_i C_i

- \lambda = \text{Osmotic coefficient (ranges from 0 -1)}
- n = \text{Number of particles (ions) into which a molecule dissociates (example: NaCl = 2; glucose = 1)}
- C = \text{the molar concentration of the solute}
- i = \text{The index } i \text{ represents the identity of a particular solute}
Gills and kidney: Osmoregulatory organs
Ingest Freshwater

Filtration

H$_2$O

Na$^+$, Cl$^-$

1 mOsm

High volume urine

Low Na$^+$, Cl$^-$

Seawater

Filtration

H$_2$O

Na$^+$, Cl$^-$

1000 mOsm

Low volume urine

Isotonic Na$^+$, Cl$^-$

123
1. Plasma hyposmotic to seawater; approximately 60% lower
2. Constantly dehydrated and loaded with salt
3. Up to 90% of Na\(^+\) and Cl\(^-\) is removed in the intestine
4. Dehydration forces organism to excrete small volume of urine
5. Urine is isosmotic to plasma and seawater; not hyperosmotic
6. There is active extra-renal excretion of salts via the gills!
1. Plasma hyperosmotic to freshwater
2. Over-hydrated and salt-depleted
3. Large volumes of dilute urine
4. Take up Cl\(^-\) from the environment in exchange for HCO\(_3^-\)
5. Take up Na\(^+\) in exchange for ammonia (NH\(_4^+\))
Osmoregulatory capabilities of major fish groups

- **Hyper-osmoregulation**
  - Hagfishes
  - Euryhaline (elasmobranch) (high urea levels)
  - Stenohaline (freshwater)

- **Hypo-osmoregulation**
  - Stenohaline (marine)
  - Euryhaline (teleost)
  - Freshwater type *O. mossambicus*
  - Euryhaline (teleost, marine type, *Fundulus heteroclitus*)

**Blood osmolality (Osm/kg)** vs. **Environment osmolality (Osm/kg)**
The graph shows the osmolality (mOsm) and concentrations of Na+, Cl-, and K+ for various species in seawater and freshwater environments.

**Seawater**
- Hagfish: High Na+ and Cl- concentrations, moderate K+.
- Lamprey: Moderate Na+ and Cl- concentrations, low K+.
- Shark: High Na+ and Cl- concentrations, low K+.
- Stingray: High Na+ and Cl- concentrations, moderate K+.
- Teleost (SW): High Na+ and Cl- concentrations, moderate K+.
- Flounder: Low Na+ and Cl- concentrations, low K+.

**Freshwater**
- Lamprey: Low Na+ and Cl- concentrations, moderate K+.
- Stingray I: High Na+ and Cl- concentrations, moderate K+.
- Stingray II: High Na+ and Cl- concentrations, moderate K+.
- Teleost (FW): Low Na+ and Cl- concentrations, low K+.
- Flounder: Low Na+ and Cl- concentrations, low K+.

**Concentrations**
- mM/L (millimoles per liter) for Na+, Cl-, and K+.
Active transport?

Where and how?

Form and function of the chloride cell
1. primary lamella; 2. extracellular cartilaginous matrix; 3. chondrocytes; 4. secondary lamella; 5. epithelial cell; 6. mucous cell; 7. chloride cell; 8. pillar cell; 9. lacuna (capillary lumen); 10. red blood cells within lacuna.
Salinity tolerance of Australian snapper - a case study

Background and rationale

• Transport of euryhaline fish from FW -> SW causes proliferation of chloride cells

• Aquaculture of marine species
  – Often coastal sites / environmental fluctuations
  – Lack of information on rapid salinity change on chloride cell morphology

• Australian snapper life history includes estuaries
Salinity tolerance of Australian snapper - a case study

*Experimental design*

• Assess the effect of fast salinity changes
  – SW -> 15 ppt
  – SW -> 45 ppt

• Measure
  – Blood hematocrit
  – Blood serum chemistry
  – Morphology of chloride cells
Gill sections 168 h after transfer

A: 30 -> 30 ppt  
B: 30 -> 15 ppt  
C: 30 -> 45 ppt

ref. N
Salinity tolerance of Australian snapper - a case study

Conclusions

• The species shows good tolerance to fast changes in salinity

• Serum osmolality for Na\(^+\) and Cl\(^-\) increased (15 -> 45 ppt) and decreased within 24 h and was restored

• Lamellar chloride cells also play a role in homeostasis
White steenbras: Response to fast and slow changes in salinity

• The design
  – Fast and slow change from 35 ppt to 5 ppt and 25ppt
    • Gradual decrease: 3 – 9 h (depending on final value) and measurements after 2 days
    • Fast decrease: Change was effected within 10 minutes.
  – Oxygen consumption using intermittent respirometry
Measurement

Oxygen consumption (mg g\(^{-1}\) h\(^{-1}\))

- slow change to 5 \(^\circ\)C
- slow change to 25 \(^\circ\)C
- fast change to 5 \(^\circ\)C
- fast change to 25 \(^\circ\)C
- no change; 35 \(^\circ\)C

in 20-min intervals
Na\(^+\), K\(^+\)-ATPase

- Integral membrane protein
- Assists in translocating Na\(^+\) and K\(^+\)-ions across the cell membrane
- Transport produces a chemical and electrical gradient across the membrane
- Found in chloride cells, intestinal and renal cells
Na\(^+\), K\(^+\)-ATPase
1. Keeps intracellular Na\(^+\) low and K\(^+\) high

Na\(^+\), K\(^+\), 2Cl\(^-\) co-transporter
1. Accumulates Cl\(^-\) into the cell
2. Excess Cl\(^-\) can exit via anion channel

K\(^+\) - correcting channel
1. Channels extra K\(^+\) into the blood

Extrusion of chloride via channel

Chloride cell

Accessory cell

500 mM NaCl
0 mV

150 mM NaCl + 35-40 mV

ref. D
How do freshwater fish take up ions?

- FW: large gradient for both $Na^+$ and $Cl^-$
- $Na^+$ and $Cl^-$ are taken up independently of each other
- The enzymes involved are:
  - $H^+\text{-ATPase}$: Assists in exchange of $H^+$ for $Na^+$ (uptake)
  - $Na^+, K^+\text{-ATPase}$: Assists in moving $Na^+$ into blood
  - **Carbonic anhydrase**: Generates $HCO_3^-$ to be exchanged for $Cl^-$
Freshwater

Body fluids

Carbonic anhydrase

Na\(^+\), K\(^+\)-ATPase

H\(^+\)-ATPase

ref. D