Nitrogen Excretion in Fish

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Lecture Outline:
Nitrogen Excretion In Fish

Excretion Strategies
• Ammoniotelism
• Ureotelism
• Ammoniotelism to Ureotelism

Detoxification Strategies
• Partial Amino Acid Catabolism
• Glutamine Synthesis

Gulf Toadfish (Opsanus beta)
Nitrogen Excretion

• Most nitrogenous wastes in fish are produced and excreted as ammonia or urea.

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{H—N—H} & \quad \text{H}_2\text{N—C—NH}_2 \\
\text{Ammonia} & \quad \text{Urea}
\end{align*}
\]

Ammoniotelism: Ammonia Excretion

• Animals that excrete their nitrogenous wastes primarily as ammonia (NH₃) are ammoniotelic.
  - Most fish (including agnathans and most teleosts)
• About 80 to 90% of their nitrogenous wastes are excreted as ammonia and the remainder as urea.

Goldfish (Carassius auratus)
What is Ammonia ($NH_3$)?

- Weak base
- Highly soluble
- Can diffuse passively across epithelia (e.g. gill)
  - In solution, it exists as 2 species:
    \[
    NH_3 + H_3O^+ \leftrightarrow NH_4^+ + H_2O
    \]
    However, in fish tissue about 95% of total ammonia exists as $NH_4^+$. $NH_4^+$ cannot diffuse across epithelia.
    (side note: pK of $NH_3$ = 9-10; fish blood = pH 7.4)

- Highly toxic at high concentrations

Why is it toxic?

- increases internal pH
- can inhibit key enzymes required for energy generation (destabilizes proteins)
- $NH_4^+$ substitutes for $K^+$ in ion transporters, (e.g. $Na^+-K^+-ATPases$) disrupting electrochemical gradients.
In general, fish are much more resistant to build-up of internal ammonia than terrestrial vertebrates.

For example,

Fish $\rightarrow$ 100 - 200 µM (up to 1000 µM)
Humans $\rightarrow$ 40 µM (up to 80 µM)

Ammonia Production

• Ammonia is generally produced by two catabolic processes:

1) Amino Acid Catabolism (mainly)

2) Purine Catabolism (trace)
Amino Acid Catabolism

• Majority of ammonia in fish is produced by the catabolism of amino acids

• Requires little to no energy

• α-Ketoacids (e.g. pyruvate) generated can be used for:
  - Production of energy (e.g. in Krebs Cycle)
  - Gluconeogenesis
  - Lipogenesis

• The primary mechanism for amino acid catabolism in fish is transdeamination.

Amino Acid Catabolism: Transdeamination

Step 1: Transamination:

Any Amino Acid + α-Ketoglutarate → Aminotransferase (AT) → α-Ketoacid + Glutamate

• Amino group (NH₂) of any amino acid is transferred to α-ketoglutarate to form glutamate.
Amino Acid Catabolism: Transdeamination

Step 2: Deamination

![Diagram of transdeamination involving Glutamate Dehydrogenase, resulting in ammonia release.]

- Amino group of glutamate is released as ammonia.

Amino Acid Catabolism: Transdeamination

- Typically, 50 to 70% (up to 99% in goldfish) of ammonia produced by transdeamination occurs in the liver.
- The rest originates in the kidney, muscle, gill and intestine.

*How is Ammonia Excreted???*
How is Ammonia Excreted?

- Unlike most vertebrates, >80% of nitrogenous wastes are excreted by the gills, with only trace amounts excreted by the kidney as urine.

Ammonia Excretion in FW Fish Gill

- Passive diffusion of NH₃ into water (transcellularly or paracellularly)

\[ \text{NH}_4^+ \xrightarrow{\text{Gill Epithelium}} \text{NH}_3 \]

\[ \text{WATER} \]

\[ \text{BLOOD} \]

\[ \text{High NH}_3 \rightarrow \text{Low NH}_3 \]
Ammonia Excretion in FW Fish Gill

- **NH₃ Trapping**: Protons pumped out of the gill combine with NH₃ to produce impermeable NH₄⁺.

Ammonia Excretion in FW Fish Gill

- **CO₂ is converted to HCO₃⁻ and H⁺ by the enzyme carbonic anhydrase.**
Ammonia Excretion in **FW** Fish Gill

**Review**

1. **Passive diffusion** of NH₃ into water (trancellularly or paracellularly)

2. **NH₃ Trapping**: Gill water is acidified by protons pumped out of the gill by an H⁺-ATPase. Protons combine with NH₃ to produce impermeable NH₄⁺ and maintain NH₃ gradient. (High NH₃ in blood, low in water)

3. **Protons** are produced by carbonic anhydrase from CO₂.

Ammonia Excretion in **SW** Fish Gill

- **Passive diffusion** of NH₃ into water (trancellularly and paracellularly) and **passive diffusion** of NH₄⁺ (paracellularly via “leaky” junctions).

SW is well buffered → NH₃ trapping not possible.
Ammonia Excretion in SW Fish Gill

Gill Epithelium

\[ \text{NH}_4^+ \xrightarrow{(K^+)} \text{NH}_4^+ \]

\[ \text{NH}_4^+ \text{ATPase} \]

\[ \text{Na}^+ \]

\[ \text{BLOOD} \]

\[ \text{WATER} \]

• \( \text{NH}_4^+ \) is pumped into gill by substituting for \( K^+ \) at a basolateral \( \text{Na}^+-\text{K}^+-\text{ATPase} \).

Ammonia Excretion in SW Fish Gill

Gill Epithelium

\[ \text{NH}_3 \]

\[ \text{NH}_4^+ \xrightarrow{(K^+)} \text{NH}_4^+ \]

\[ \text{ATPase} \]

\[ \text{HNE} \]

\[ \text{H}^+ \]

\[ \text{Na}^+ \]

\[ \text{BLOOD} \]

\[ \text{WATER} \]

• \( \text{NH}_4^+ \) is pumped out of the gill by substituting for \( H^+ \) at an apical \( H^+-\text{Na}^+ \) exchanger (HNE).
Ammonia Excretion in SW Fish Gill

Review

1. Passive diffusion of NH$_3$ into water (trancellularly or paracellularly) and passive diffusion of NH$_4^+$ (paracellularly via “leaky” junctions).

Remember: SW is well buffered → NH$_3$ trapping not possible

2. Active transport of NH$_4^+$ into the gill by replacing K$^+$ in Na$^+$-K$^+$-ATPases

3. Active transport of NH$_4^+$ into the water by replacing H$^+$ in HNEs.

Ureotelism: Urea Excretion

- Animals that excrete their nitrogenous wastes primarily as urea are ureotelic.

  - Elasmobranchs, coelacanths and a few teleosts.

Coelacanth
(Latimeria chalumnae)

Dogfish
(Squalus acanthias)
What is Urea?

- Highly soluble
- Ability to diffuse across epithelia (e.g. gill) depends on the species
  - e.g. elasmobranchs
    - high cholesterol: lipid membrane
      $\rightarrow$ impedes diffusion

- At high concentrations, much less toxic than ammonia

\[
\begin{align*}
\text{O} \\
\parallel \\
\text{H}_2\text{N}--\text{C}--\text{NH}_2 \\
\text{Urea}
\end{align*}
\]

Urea Production

- **Urea** is generally produced by two processes:

  1) **Ornithine-Urea Cycle (OUC)**
  2) **Uricolysis**
     - Most fish (including teleosts)
     - Breakdown of uric acid
Ornithine-Urea Cycle (OUC)

- Only elasmobranchs, coelacanths and a few teleostean fish that survive periods of air exposure or alkaline environments.
- Assumed that OUC genes encoding enzymes necessary for the cycle have been lost from the genome of most teleosts.
- However, high OUC enzyme activity detected in many teleosts during embryonic stages ➢ OUC genes are silenced in adult stages.

Ornithine-Urea Cycle (OUC)

- Carbamoyl phosphate synthase (CPSase) converts glutamine to carbamoyl phosphate, which is the first substrate fed into the OUC to produce urea.
  - Requires energy.
  - It occurs primarily in the liver.
Urea Excretion and Retention at the Gill

- Like ammonia, **urea excretion** occurs at the **gill**.

- However, **urea retention** also occurs at the **gill** for marine fish that retain urea as an **osmolyte** to increase body osmolarity e.g. elasmobranchs.
Passive diffusion of urea into water transcellularly and paracellularly in marine fish via “leaky” junctions.

Active transport of urea out of the gill by an Na\(^+\) dependent, secondary active urea transporter (UT).
Urea Excretion in FW and SW Fish Gill

Review

① Passive diffusion of urea into water trancellularly and paracellularly in marine fish via “leaky” junctions only.

② Basolateral Na⁺-K⁺-ATPases create a gradient of low Na⁺ in the gill epithelium and high Na⁺ in the blood.

③ Na⁺ diffuses down its concentration gradient via a urea transporter (UT) taking urea with it. Urea then diffuses out of the gill.

Urea Retention in Elasmobranch Gill

• Passive diffusion of urea into water transcellularly and paracellularly via “leaky” junctions.
**Urea Retention in Elasmobranch Gill**

- Active transport of urea back into the blood by an Na⁺ dependent, secondary active urea transporter (UT).

**Review**

1. Very little passive diffusion of urea into water tranccellularly due to high cholesterol:phospholipid basolateral membrane.
   - Some excretion paracellularly via “leaky” junctions only.

2. Basolateral Na⁺-K⁺-ATPases create a gradient of low Na⁺ in the gill epithelium and high Na⁺ in the blood.

3. Na⁺ diffuses down its concentration gradient via a urea transporter (UT) transporting urea back into the blood.
**Comparison at the Gill**

Urea Excretion

- FW and SW Fish Gill
- UT = Symporter

Urea Retention

- Elasmobranch Gill
- UT = Antiporter

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**Ureotelism vs. Ammoniotelism**

1) **Ammonia** takes little energy to produce/excrete; **Urea** production/excretion is energy expensive.

2) **Ammonia** requires a large volume of water for excretion since it occurs by diffusion; **Urea** requires less water for excretion - about 10x less water

Therefore, ureotelism is better suited for air-breathing fish, e.g. African Lungfish, which can live on land for extended periods and have limited access to water.
African Lungfish: Ammoniotelism to Ureotelism

- Can live for extended periods out of water in dried mucous cocoons, relying entirely on aerial respiration.
- In the water, it is ammoniotelic.
- On land, it shifts to ureotelism.
- Lack of water makes it impossible for the diffusion of ammonia from the gills.
- Uses OUC to convert toxic ammonia to urea for safer storage of nitrogenous wastes.
  \[ \text{Increases levels of active OUC enzymes} \]

Gulf Toadfish

- Under normal conditions \( \rightarrow \) Ammoniotelic
- Under stressful conditions (e.g. crowding, confinement to a small volume of water)
  \[ \rightarrow \text{Intermittently ureotelic} \] – excretes pulses of urea

- Transition is accompanied by an upregulation of active OUC enzymes in the liver.
- Stress hormone, cortisol, believed to be involved in regulating the transcription of enzymes required for the initiation of the OU cycle.
• **Intermittent pulses** of urea excretion (arrows) following confinement of a Gulf Toadfish (at time = 0).
  - Note the **negligible** ammonia excretion.

(Wood et. al., 2003)

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**Other Strategies to Defend Against Ammonia Toxicity on Land**

1) **Partial Amino Acid Catabolism**
   - Giant Mudskipper

2) **Glutamine Synthesis**
   - Four-Eyed Sleeper
Giant Mudskipper:
Partial Amino Acid Catabolism

- Air-breathing ammoniotelic teleost fish that can live in mud burrows.
- When exposed to air it does not switch to ureotelism
- Uses partial amino acid catabolism to generate energy, yet reduce production of ammonia.

- Prerequisites: 1) Alanine Aminotransferase (ALT)
  2) Malic Enzyme

Giant Mudskipper
*(Periophthalmodon schlosseri)*

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**Krebs Cycle**

- Pyruvate → Acetyl CoA → Citrate → Oxaloacetate → Malate → ATP
- NADH → Electron Transport Chain

[Diagram of the Krebs Cycle]
Partial Amino Acid Catabolism

Main point:

- Allows amino acids to be used as an energy source while on land without producing toxic ammonia, which would be difficult to excrete due to a lack of external water.
Four-Eyed Sleeper: Glutamine Synthesis

- Air-breathing ammoniotelic teleost fish that can live in crevices above river mouths
- When exposed to air it does not switch to ureotelism
- Uses the enzyme glutamine synthetase to combined NH$_3$ with glutamate forming glutamine.
- Glutamine $\rightarrow$ safer ammonia storage than urea
- Cost-effective:
  - only 2 mol ATP per NH$_3$ incorporated in glutamine
  - Vs. 2.5 mol ATP per NH$_3$ incorporated in urea

![Glutamine molecule](image)

Glutamine

Four-Eyed Sleeper

(*Bostrichyths sinensis*)

The End