Cardiac function in fishes

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Importance of cardiac function to respiratory metabolism & performance

• Some basic principles
• Responses to reduced O₂ supply: hypoxia
• Air-breathing in fishes
• Responses to increased O₂ demand: SDA and aerobic exercise

Some basic principles
A limited knowledge base

- 28,000 fish species, more than half of all chordates/vertebrates
- Comparative morphological information on ~1000 species
- Detailed information on cardiac physiology for less than 100 species
- Knowledge base on 2 or 3, rainbow trout being the physiological model.

Ventilation and perfusion in fishes

Cardiac and ventilatory activity are regulated in response changes in oxygen supply and demand

Autonomic control of the heart in fishes

- Intrinsic heart rate is derived from pacemaker cells in the sino-atrial node
- Heart is innervated by the vagus, cranial nerve X
- Inhibitory cholinergic fibres (all gnathostomes): slow the heart
- Excitatory adrenergic fibres (teleosts "up"): accelerate the heart
- Circulating catecholamines
Fishes have a single circulation

The fish heart is an aerobic organ

Reduced oxygen supply:
Hypoxia
Aquatic hypoxia

Water contains 20–40 times less $O_2$ than air
- Increased temperature and salinity both cause marked reductions in $O_2$ availability
- Microbial respiration can deplete $O_2$ quite rapidly in water

$O_2$ diffuses ~ 10,000 times more slowly through water
- Density stratification can maintain hypoxic zones in lakes and seas

Hypoxia (oxygen availability) has been a potent evolutionary force for fishes

Fishes can sense oxygen levels in the water and in their blood

Chemosensory cells are believed to be neuro-epithelial cells in fish gills, with nerve connections to sensory medulla

Fishes try to regulate their aerobic metabolism in hypoxia
Hyperventilation contributes to the regulation of aerobic metabolism

- The increased water flow across gills decreases the inspired-expired PO₂ difference
- It raises mean water-arterial PO₂ difference
- Raises arterial PO₂ and therefore haemoglobin saturation
- Other more complex effects (respiratory alkalosis)

Stimulation of oxygen receptors causes reflex cardiac and ventilatory responses

Cardiac slowing is sympathetic cholinergic inhibition. The impulses are carried by the cardiac branch of the vagus nerve, which slows the pacemaker cells.
• In most species (but not all), the reduction in heart rate is accompanied by an increase in stroke volume, so that cardiac output remains more or less constant.
• This is because of increased filling time, and increased venous tone
• This is associated with an increase in systemic blood pressure, due to systemic vasoconstriction.

Does bradycardia also contribute to the regulation of aerobic metabolism?

Proposed advantages for gas exchange:
- Increased residence time of blood in the gills increases time for diffusion
- Increased pulse pressure increases effective area for gas exchange
  - opens poorly perfused vascular spaces in the gill lamellae
  - creates a more even blood flow within the lamellae
  - recruits unperfused lamellae

Does bradycardia contribute to the regulation of aerobic metabolism?

Mixed results:
- In dogfish, pharmacological blockade of the response (atropine) caused a decline in arterial oxygen content (Taylor et al. 1977) and gill diffusive conductance (Barratt and Taylor 1985)
- In trout, this blockade was without effect on blood gases (Desforges and Perry 2006)
**Is bradycardia to protect the heart itself?**
Farrell (2007)

Potential benefits:
- increases blood residence time in the heart: more time for diffusion
- improved cardiac contractility through the negative force-frequency effect.
- the increase in stroke volume will stretch the cardiac chambers, potentially reducing diffusion distance for oxygen.
- reduce cardiac oxygen demand by reducing cardiac dP/dt and cardiac power output

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Why hypoxic bradycardia in fishes?
If reflex bradycardia contributes to gill O2 transfer and uptake in hypoxia, then:
- abolition of the response should impair the ability to regulate aerobic metabolic rate
- this should be evident as a decline in critical PO2 (Pcrit)

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**Approach:**
- Measure instantaneous oxygen uptake and cardiac activity simultaneously
- Expose fish to progressive hypoxia
- Investigate effects on Pcrit of abolishing bradycardia
- Look for effects on cardiac function below Pcrit

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European eel
Pharmacological inhibition

Atlantic cod
Cardiac vagotomy
Overall results

Bradycardia did not initiate until below Pcrit in eels or cod
Abolition of bradycardia had no effect on Pcrit in eels or cod
Ventilatory responses were attenuated in denervated cod
Some atropinised eels showed cardiac arrhythmia in deep hypoxia
All denervated cod lost equilibrium in deep hypoxia

Why hypoxic bradycardia in fishes?

Conclusions so far:
- Mixed evidence for contribution to gas exchange
- Abolition of bradycardia does not influence regulation of aerobic metabolism
- Bradycardia starts below Pcrit
- Abolition of bradycardia has some negative effects on cardiac and respiratory function below Pcrit
- There may be a role for protection of the heart?

Still needed:
An experiment simultaneously measuring O₂ uptake, cardiac activity, and arterial and venous blood gases
Fish typically use gills for taking up O\textsubscript{2} from the water.

Gas exchanges take place by diffusion - a passive process where molecules move from higher to lower partial pressures.

Air can contain much more oxygen than water.
Air-breathing may have evolved independently multiple times in the bony fishes

About 450 species among 25,000 bony fishes
The comparative physiology of air-breathing fishes: Insights into vertebrate evolution?

Some believe that air-breathing organs evolved in fish in fresh waters susceptible to seasonal hypoxia
Under these conditions, O2 levels would get lower with depth in the water column.

What was the selective pressure for the evolution of air-breathing in fishes?

Evolution of air-breathing in freshwater

Fish may have first come to the surface layer where the O2 would be the richest, to skim the surface water and this may have led to them also gulping air.
This would supplement gill respiration and may have been the selective force for all of the evolutionary experiments we see in air breathing.
Diversity in accessory air-breathing organs

- **gas bladders:**
  - primitive bony fishes
  - primitive teleosts
- **true lungs:**
  - polypterids
  - dipnoi (lungfishes)

- **climbing perch**
- **electric eel**
- **indian catfish**

Diversity in accessory air-breathing organs

- **gut and digestive tract:**
  - modern teleosts
All air-breathing fish use a buccal force pump

Amphibious air-breathing fishes: the mudskippers

Aquatic hypoxia stimulates air-breathing in every species studied to date
Air-breathing fish can regulate oxygen uptake independently of water oxygen availability

Air-breathing is a reflex driven by receptors in the gills

Spotted gar, Lepisosteus oculatus (Smetske 1989)

Bowfin, Amia calva (McKenzie et al. 1991)

Jeju, Hopleythrinus unzaniatus (Lopez et al. 2010)

Cardiac reflexes in air-breathing fishes are complex. Hypoxia can cause bradycardia, tachycardia, or no change. Not enough is known about these at present.

There are complex changes in cardiac activity associated with air-breathing itself.

Variation in cardiac activity in association with air-breathing
An extreme example:
The marbled swamp eel
*Synbranchus marmoratus*

Skals et al. (2006)
Huge increases in heart rate and cardiac output during air-breathing
Require a large increase in venous tone to ensure venous return

The Asian swamp eel, *Monopterus albus*

Iversen et al. 2011

Control and functional significance of the changes in heart rate

- Graham (1997) suggested that heart rate changes are qualitatively similar to mammalian respiratory sinus arrhythmia (RSA).
- RSA comprises inspiration tachycardia followed by expiration bradycardia, and is due to modulation of vagal cholinergic inhibition.
- Tachycardia associated with increased cardiac output and perfusion of air-breathing organ (*Synbranchus* and *Lepidosiren*), indicating that facilitate oxygen uptake from the ABO?
Autonomic control of the heart in vertebrates

- Intrinsic heart rate is derived from pacemaker cells in the sino-atrial node.
- Heart is innervated by the vagus, cranial nerve X.
- Inhibitory cholinergic fibres (all gnathostomes): slow the heart.
- Excitatory adrenergic fibres (teleosts “up”): accelerate the heart.
- Circulating catecholamines.
- "Respiratory sinus arrhythmia": heart rate variations linked to lung ventilation in mammals.
- RSA is cholinergically mediated; integrates cardiac and ventilatory activity.

"RSA" in the jeju: autonomic regulation and functional significance

- Exposed to extreme hypoxia – fish rely on air-breathing.
- Pharmacological blockade with propranolol (β-adrenergic) and atropine (muscarinic cholinergic).
- Investigated effects on heart rate variability and consequent regulation of routine aerobic metabolism.

Mean heart rate over 3 air-breath cycles in hypoxia and following autonomic blockade.

n = 3 breaths on 7 fish.
Effects of hypoxia and autonomic blockade on mean heart rate and R-R interval, over 3 air-breath cycles.

**Tones based on R-R interval:**
- Adrenergic + 41%
- Cholinergic - 267%

Effects of hypoxia and autonomic blockade on mean heart rate and R-R interval, over 3 air-breath cycles.

Pharmacological blockade had no effect on efficacy of air-breathing.

No significant changes in O₂ uptake per breath.

"Respiratory sinus arrhythmia" in air-breathing fishes

- "RSA" is mostly modulation of inhibitory cholinergic tone (jeju, african lungfish, asian swamp eel, marbled swamp eel)
- Pharmacological blockade had no effect on air-breathing efficiency in deep hypoxia (jeju, marbled swamp eel)
- Problem with the protocol - cholinergic blockade tends to cause a large increase in cardiac output (marbled swamp eel). Cardiac vagotomy?
Facultative vs obligate air breathers

Facultative: air-breathing is a supplement to gill ventilation in times when there is insufficient oxygen uptake from water.

Obligate: The fish must air-breathe and will asphyxiate if denied access to air. This is because the gills have become so reduced in surface area.

Obligate air-breather: Adult Pirarucu (Arapaima gigas)

Water-breather: Aruana (Osteoglossum bicirrhosum)

Why would the gills have become so reduced?

Vascular anatomy: blood leaving the ABO returns to the heart and must pass through the gills before reaching the systemic circulation. Oxygen may be lost across the gills. Under these conditions, it is an advantage to reduce the gills or modify their structure.

Or to modify the circulation...

Teleost

Syngnathidae

Hypogeophyidae

Dipnoi

Bird-mammal
Snakehead (*Channa argus*)

Ishimatsu and Itazawa, *JCP*, 1983

**Posterior aorta perfuses the gills**

**Anterior aorta perfuses the "lungs"**

**No sinoatrial valve**  
*Channa argus*  
Ishimatsu and Itazawa, *JCP*, 1983

**Evidence that blood flows are partially separated**

"Because oxygen-rich pulmonary blood mixes with oxygen-poor systemic blood before entering the heart of air-breathing fishes, lung ventilation may supply the myocardium with oxygen and expand aerobic exercise capabilities." (Farmer, 1999)

**Did air-breathing evolve to oxygenate the heart??**

“Because oxygen-rich pulmonary blood mixes with oxygen-poor systemic blood before entering the heart of air-breathing fishes, lung ventilation may supply the myocardium with oxygen and expand aerobic exercise capabilities.” (Farmer, 1999)
Increased oxygen demand: Specific dynamic action and aerobic exercise

Exercise can cause a 10-fold increase in metabolic rate (larval coral reef fishes are most extreme example)

Digestion (specific dynamic action) can cause a 5-fold increase (cod are most extreme example measured to date)

Post-prandial hyperemia: feeding raises cardiac output and gut blood flow

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>QG (PD)</th>
<th>QG (R)</th>
<th>Finding Regime</th>
<th>Time Period</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chironomus riparius</td>
<td>13-16</td>
<td>5-44</td>
<td>15-25</td>
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<td>Holocentrus antilleanus</td>
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<tr>
<td>Parachironomus</td>
<td>12-18</td>
<td>5-14</td>
<td>15-20</td>
<td>24</td>
<td></td>
<td></td>
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<tr>
<td>Enallagma maculatum</td>
<td>12-18</td>
<td>5-14</td>
<td>15-20</td>
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<tr>
<td>Alnocoris viridis</td>
<td>12-18</td>
<td>5-14</td>
<td>15-20</td>
<td>24</td>
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</tbody>
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QG measured in coeliacomesenteric artery, which is the first caudal branch of the dorsal aorta

Increased gut blood flow seems to be controlled in large part by the enteric nervous system (Seth and Axelsson 2010)
In European sea bass, hyperemia is due to relaxation of cholinergic tone, no evidence of NANC factors

Aerobic exercise in fishes is powered by "red" oxidative slow-twitch muscles

Measuring exercise performance in fishes
Oxygen uptake during Ucrit exercise

- A linear increase in tailbeat frequency causes increased swimming speed, which is associated with an exponential increase in O$_2$ uptake
- There is an asymptote in O$_2$ uptake as fish fatigue
- Aerobic scope usually 4 - 10 time higher than standard metabolic rate

Support of metabolism during exercise

SUSTAINED AEROBIC = increased O$_2$/nutrient demand by red muscle

- **Cardiovascular system**: 3-fold increase in cardiac output coupled with decreased peripheral resistance:
  - 8-fold increase in red muscle perfusion (O$_2$, nutrients)
  - 3-fold increase in gill perfusion (lamellar recruitment)^
  - 3-fold increase in arterio-venous pO$_2$ difference^

Ventilatory system: up to 10-fold increase in water flow over the gills^
- Up to 10-fold increase in O$_2$ uptake at the gills

CARDIOVASCULAR RESPONSES
Control of cardiovascular responses to exercise

- Tachycardia is mostly due to release of vagal cholinergic inhibition, some evidence of adrenergic contribution
- Decreased peripheral resistance is under partial $\alpha$-adrenergic control (probably also local factors)

What limits aerobic exercise performance in fishes?

- Not gill gas transfer: arterial blood is fully saturated at Ucrit
- Not red muscle oxygen delivery: red muscle oxygen levels are high at Ucrit
- Not red muscle fatigue: little evidence of metabolite accumulation
- Is it the heart?

Venous oxygen tensions decline dramatically during exercise

[Graph showing venous oxygen tensions ($PvO_2$) vs. swimming speed, with a fatigue threshold marked.]
Rainbow trout selected for good or poor swimming performance have differences in their underlying cardiac performance

<table>
<thead>
<tr>
<th></th>
<th>Good condition</th>
<th>Poor condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial (mmHg)</td>
<td>124±12</td>
<td>86±10</td>
</tr>
<tr>
<td>Venous (mmHg)</td>
<td>23±4</td>
<td>20±4</td>
</tr>
<tr>
<td>Arterial/venous</td>
<td>1.28</td>
<td>1.11</td>
</tr>
</tbody>
</table>

**in-vivo**
- 24% ↓ in aerobic scope
- 30% ↓ in max cardiac output
- 38% ↓ in max O2 transport

**in-vitro**
- 24% ↓ in max cardiac output
- 32% ↓ in maximum cardiac power

**Cardiac responses to SDA and aerobic exercise: conclusions**
- Feeding hyperemia appears to be regulated by release of vagal cholinergic inhibition
- No evidence for NANC factors at peak SDA (sea bass)
- Exercise tachycardia is also largely due to release of vagal cholinergic inhibition
- Venous oxygen supply to the heart may be the factor which limits aerobic exercise performance (trout)
- This hypothesis is very difficult to test

**Air-breathing during exercise**

Gymnus n. 7, swimming at 10 cm s⁻¹ (mass 65g, bodylength 230 mm)
Modified swimming respirometer

Water $O_2$ uptake: intermittent respirometry
Air $O_2$ uptake: monitored by micro-optode
Standard $U_{crit}$ protocol (10 cm s$^{-1}$ steps each 30 min)
Filmed to measure a-b frequency
N = 7 fish; mass 77 ± 4 g; bodylength 231 ± 4 mm

Air-breathing during exercise

$Gymnotus$ n. 7, swimming at 40 cm s$^{-1}$
(mass 65g, bodylength 230 mm)

Exercise with versus without access to air

<table>
<thead>
<tr>
<th></th>
<th>without access</th>
<th>with access</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{crit}$ (cm s$^{-1}$)</td>
<td>47 ± 4</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>routine $O_2$ uptake</td>
<td>134 ± 20</td>
<td>170 ± 25</td>
</tr>
<tr>
<td>maximum $O_2$ uptake</td>
<td>368 ± 24</td>
<td>350 ± 22</td>
</tr>
<tr>
<td>net scope</td>
<td>234 ± 12</td>
<td>181 ± 21</td>
</tr>
</tbody>
</table>

*metabolic rates in mg $O_2$ kg$^{-1}$ h$^{-1}$*
Air-breathing during exercise in hypoxia

Gymnotus n. 7, swimming at 50 cm s\(^{-1}\) in hypoxia (mass 65g, bodylength 230 mm)

Aerobic exercise in hypoxia, with or without access to air

Conclusions

- Gymnotus air-breathes during aerobic exercise
- In aquatic normoxia, it can achieve the same aerobic performance when denied access to air
- Does this indicate that air-breathing does not improve oxygen supply to the heart?
- In aquatic hypoxia, air-breathing allows it to achieve the same aerobic performance as in normoxia