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

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M: Atlas of fathead minnow normal histology; http://aquaticpath.umd.edu/fhm/resp.html

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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 mm^2 / g tissue
- Systolic blood pressure: about 44 mm Hg
- Gill irrigation: 5-20 L H_2O / kg BM / h
- Opercular beat counts: 40-60 / minute

Henry's law

$$VO_2 = \alpha \, PO_2$$

VO_2 = O_2 concentration in ml/L (or mg/L)

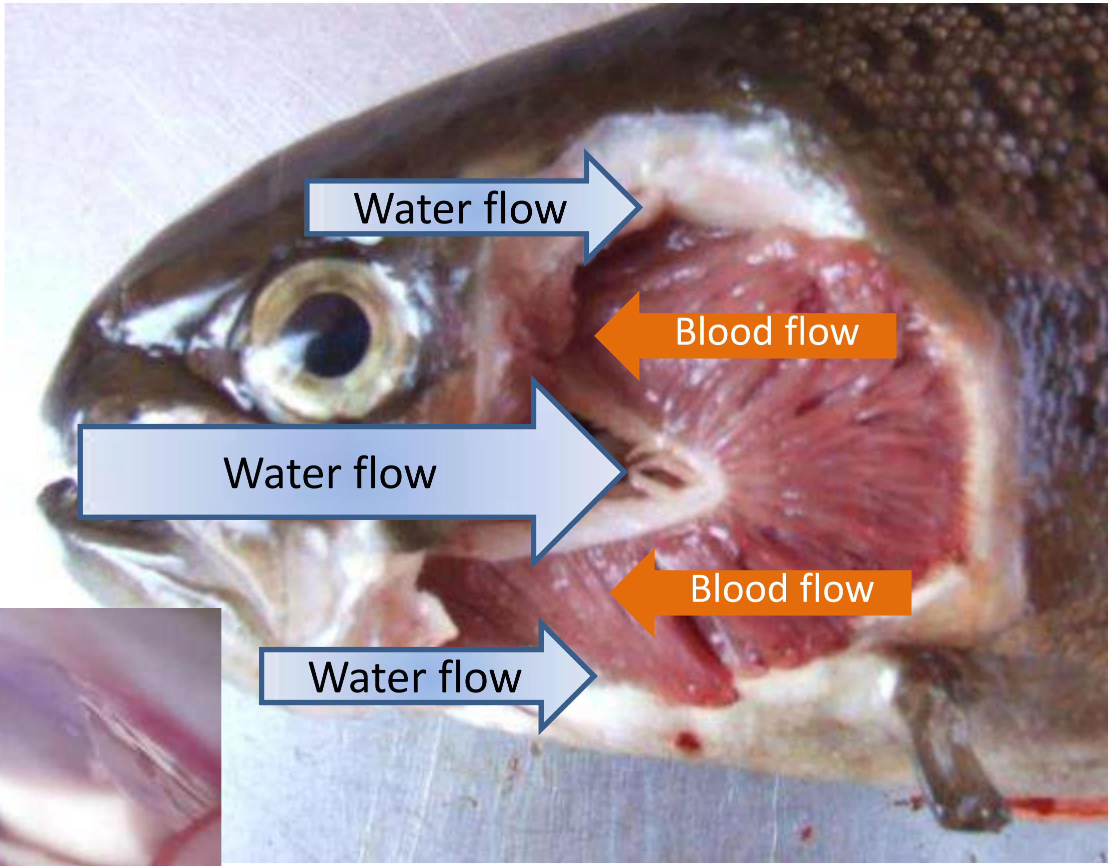
α = solubility coefficient: the volume of O_2 dissolved in water: ml O_2/L/atm

PO_2 = PO_2 partial pressure (atm)

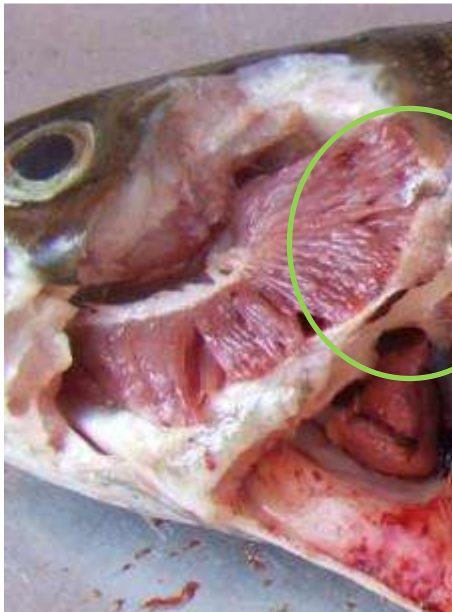
Metabolism of trout vs turtle

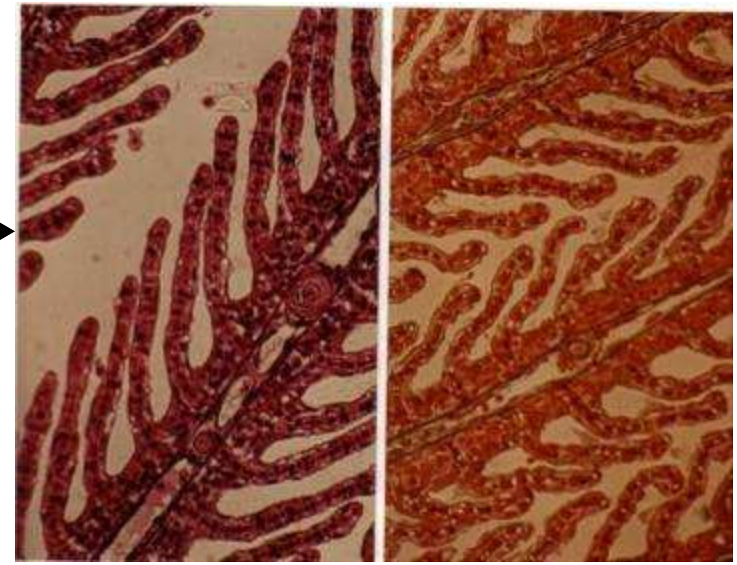
	Trout	Turtle
Oxygen requirement	~ 5 ml / min / kg	~ 5 ml / min / kg
Ventilation volume	600 ml H_2O / min / kg	50 ml air / min / kg
Routine costs for ventilation	10 %	2 %

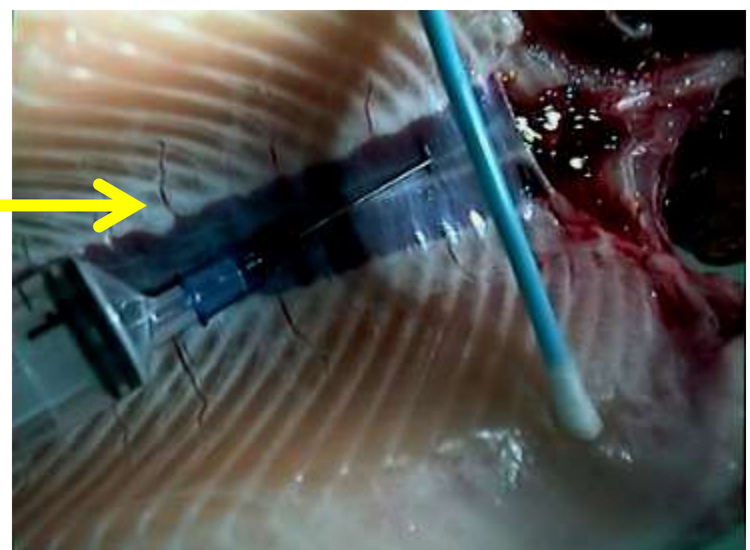
ref. D


Water flow
Blood flow
Water flow
Blood flow
Water flow

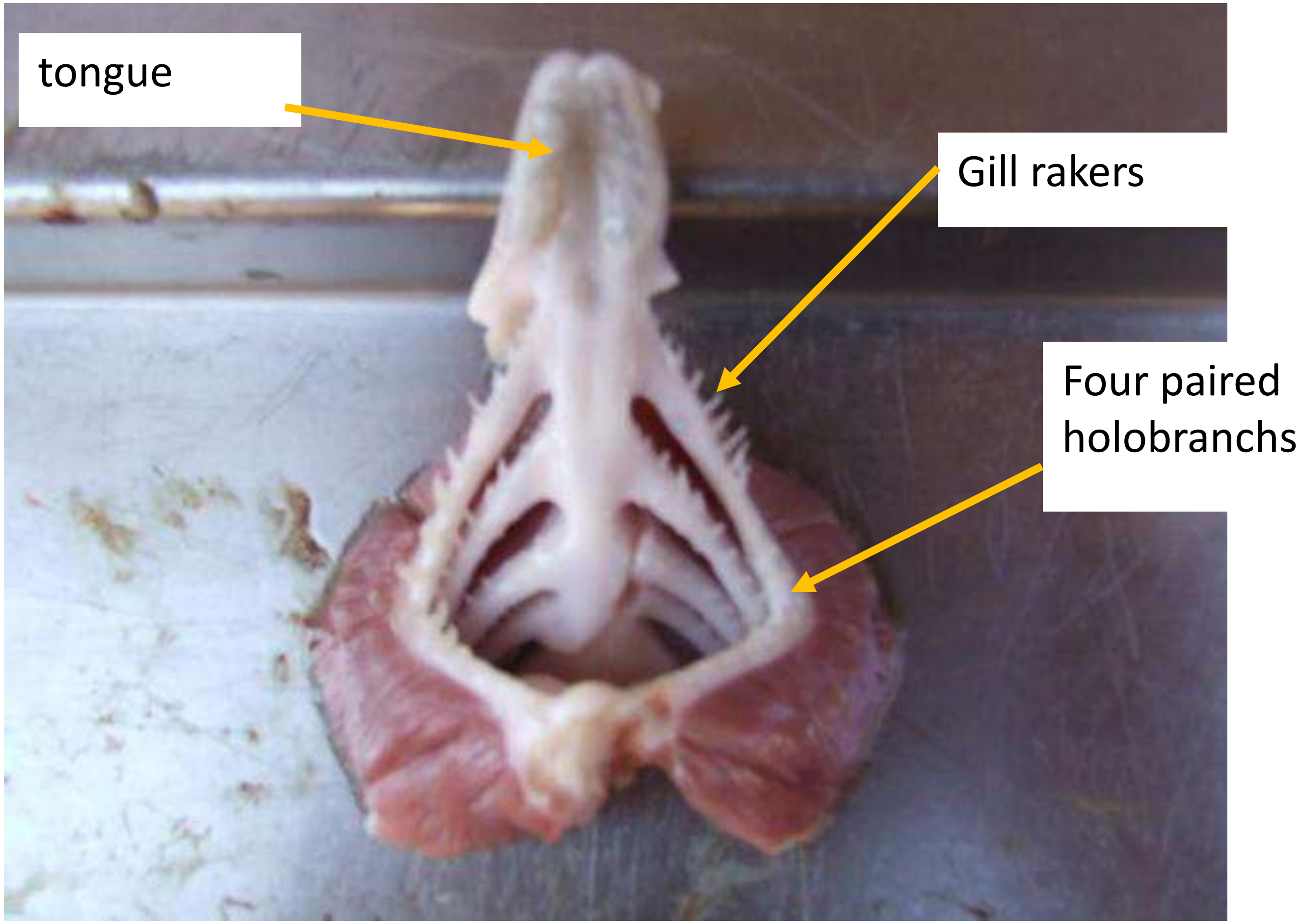
Gills and kidney: Osmoregulatory organs







PAIRED HOLOBRANCHS



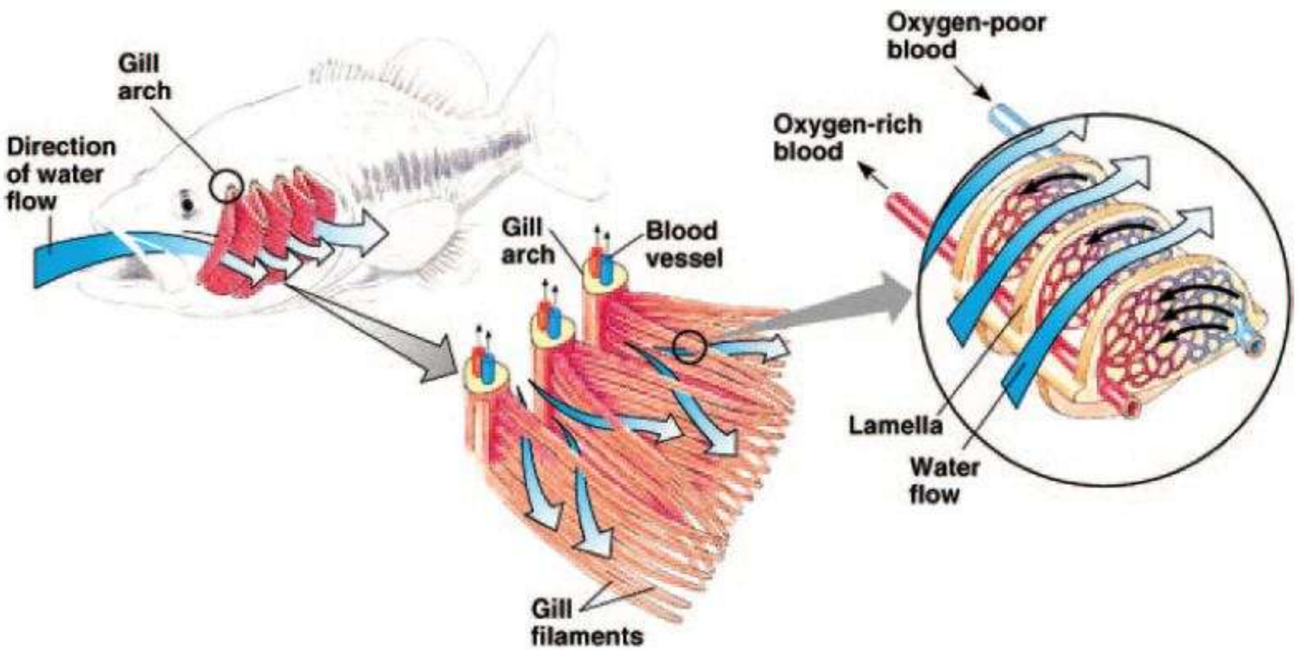
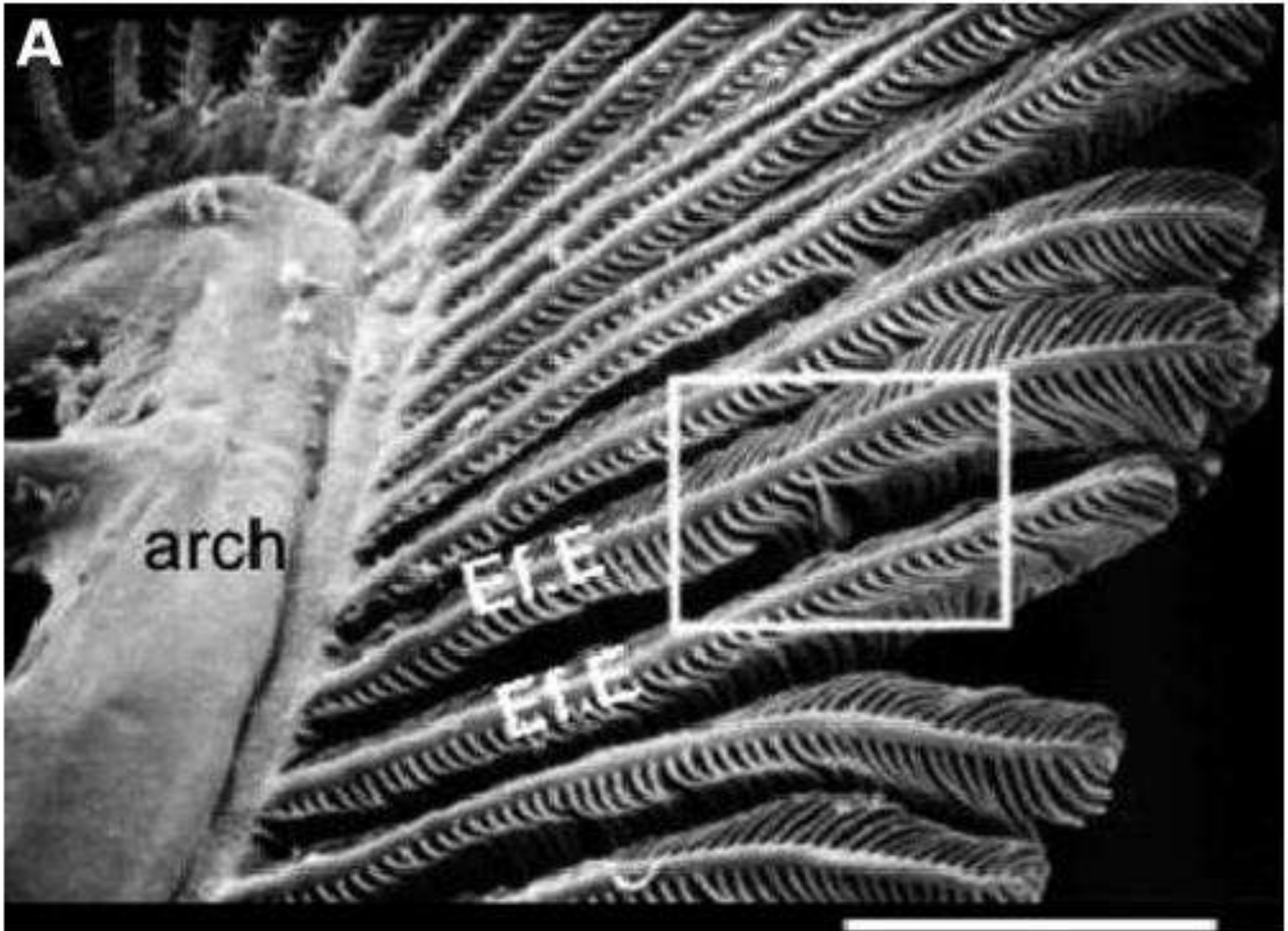
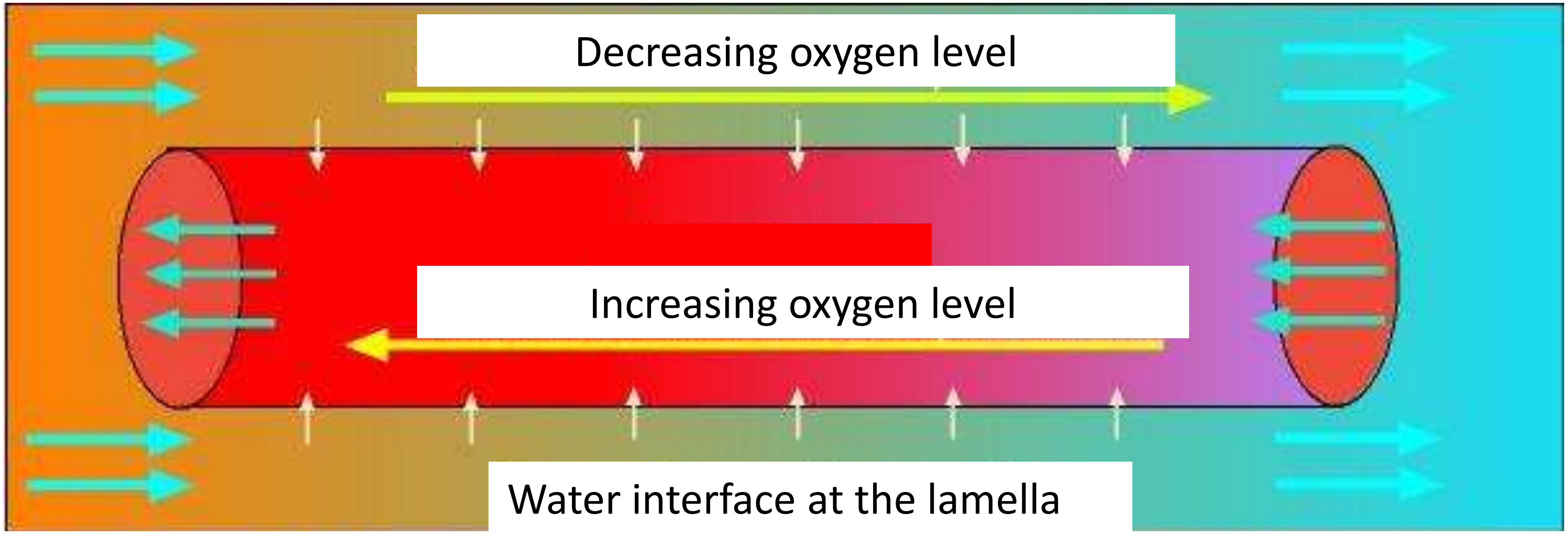


FIG. 1. Schematic of the teleost fish gill. See text for details. [From Campbell and Reece (81).]

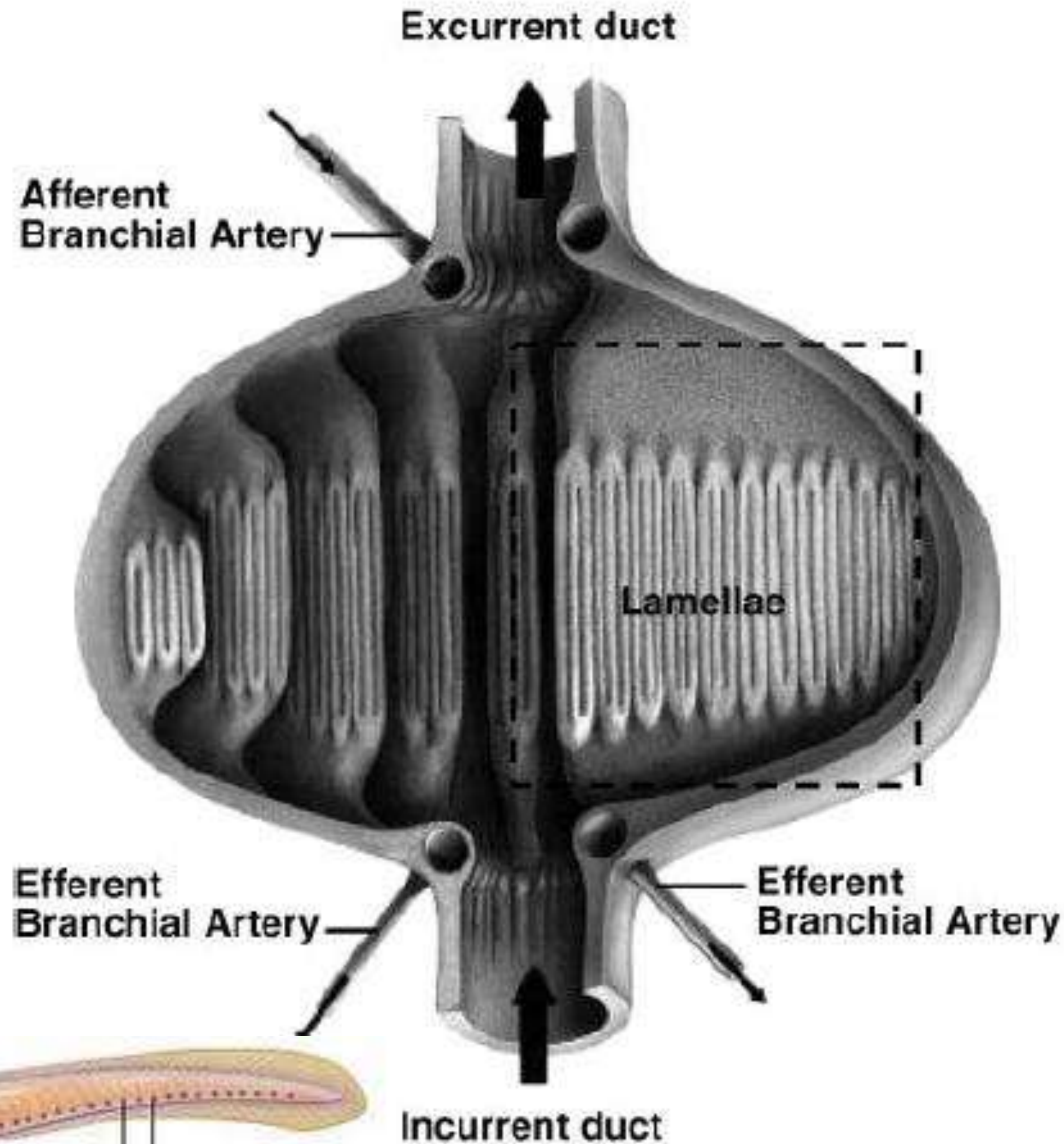

A
arch
Ef.E
Ef.E

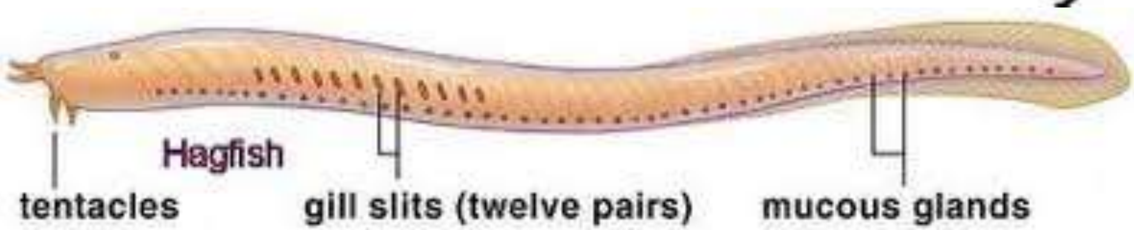
Countercurrent exchange

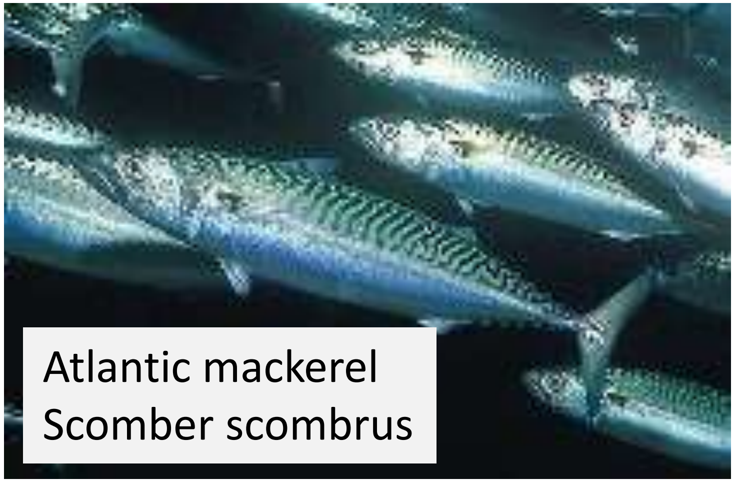
The **difference** in oxygen partial pressure PO_2 between water and plasma strongly influences the uptake of oxygen at the gill lamella / water interface!



Hagfish gill pouch







Atlantic mackerel
Scomber scombrus

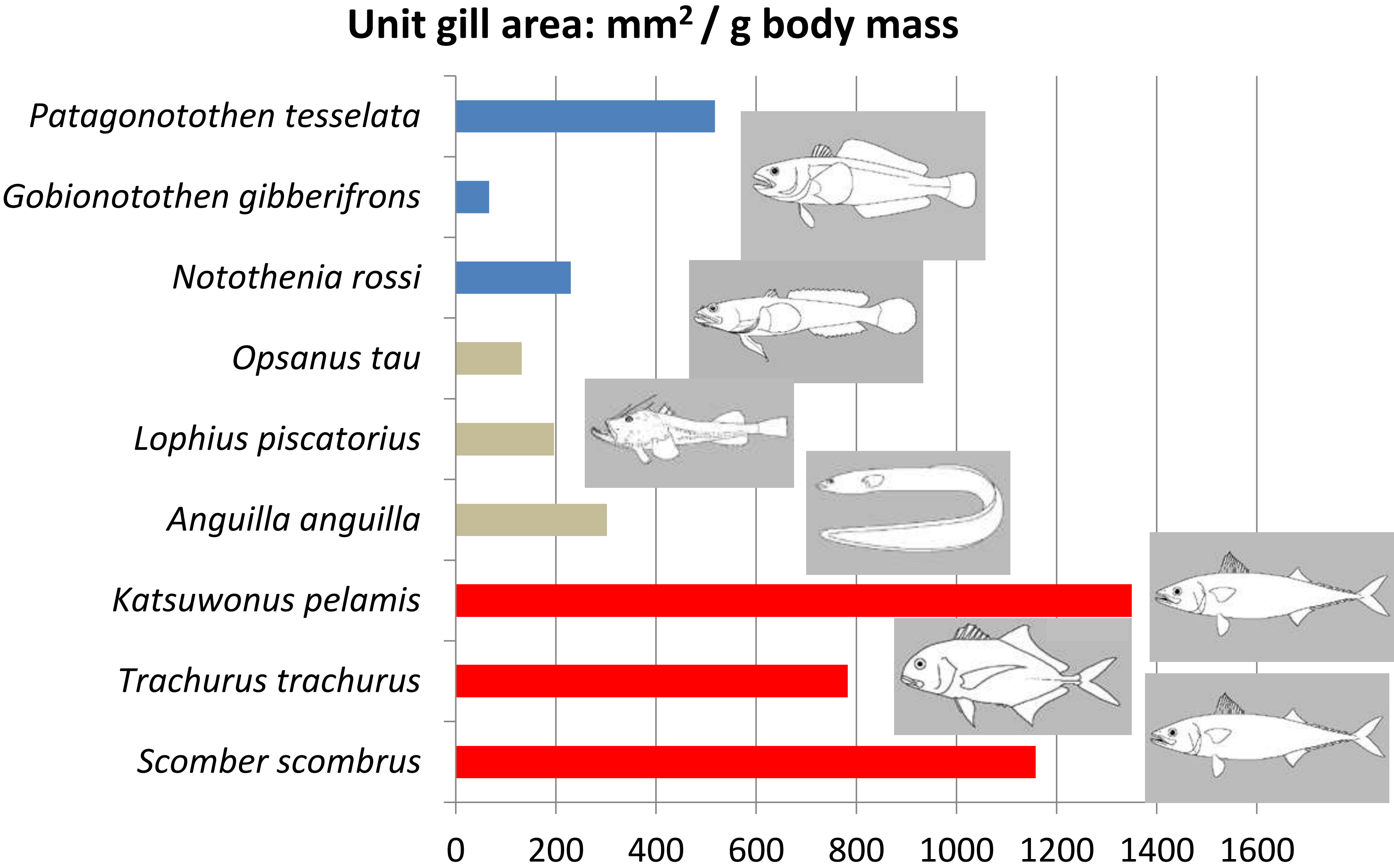
Horse mackerel
Trachurus trachurus

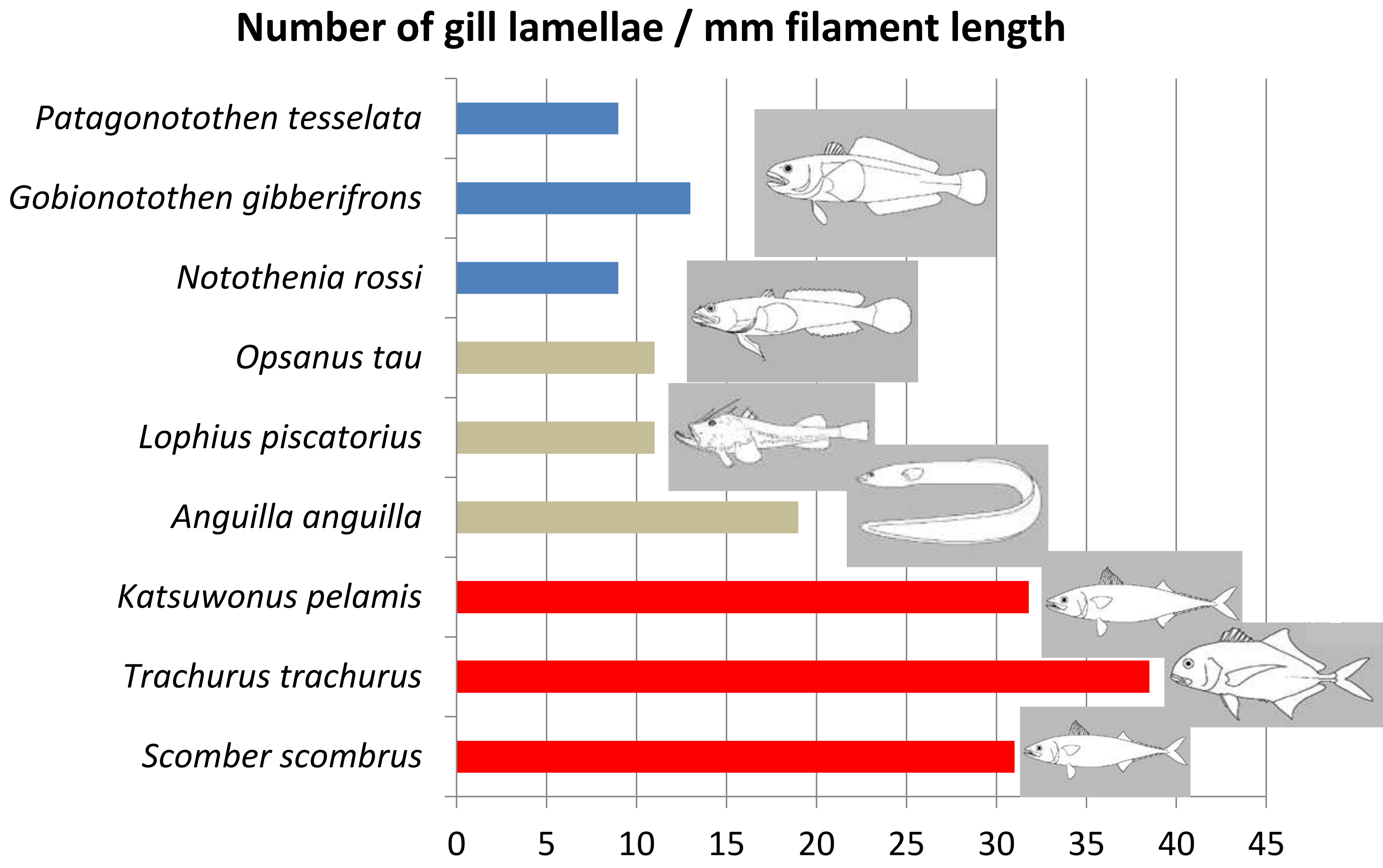
Skipkack tuna, *Katsuwonus pelamis*

Oyster toadfish
Opsanus tau

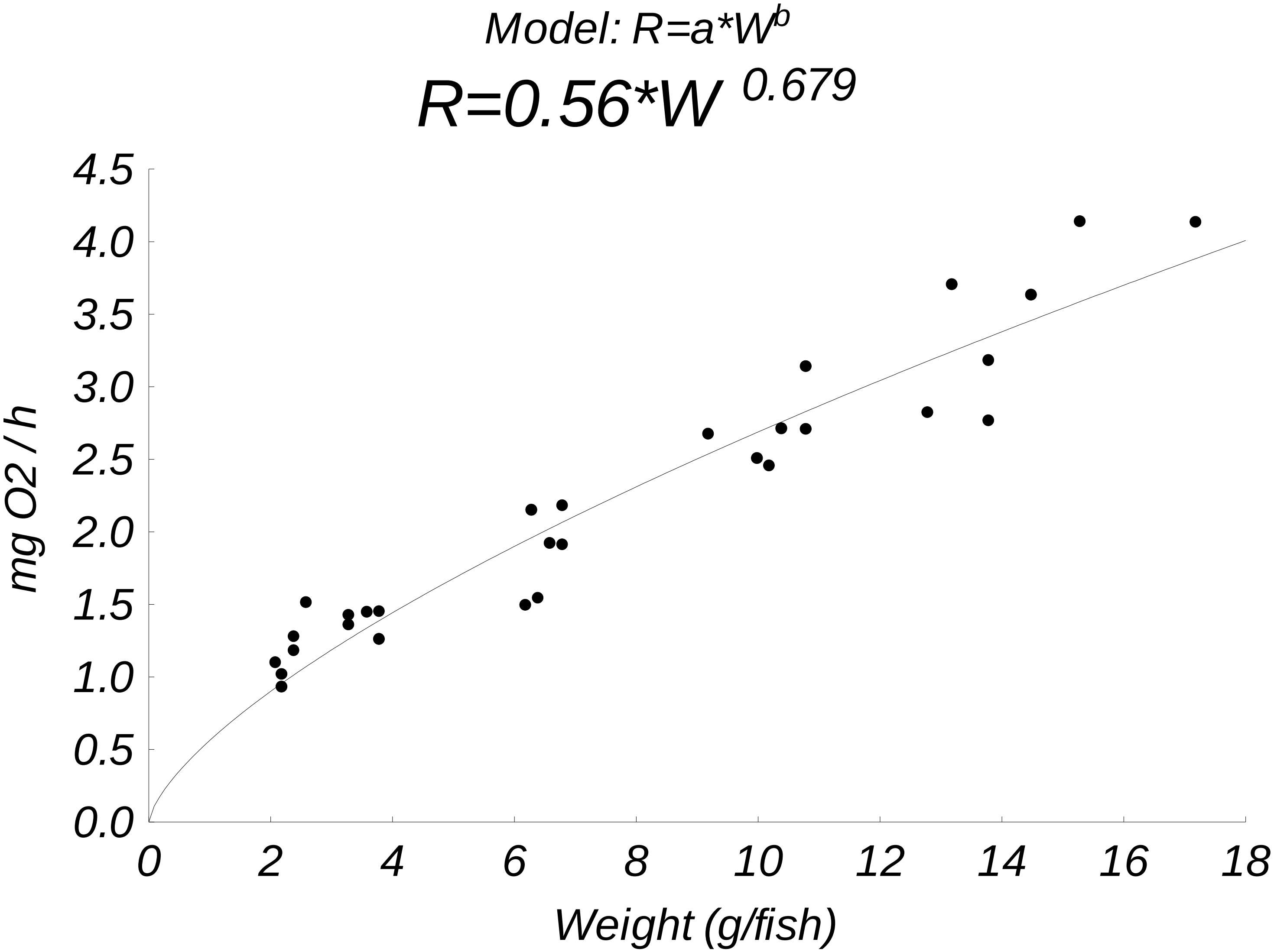
Eel, *Anguilla japonica*

Angler
Lophius piscatorius


Unit gill area: mm² / g body mass
Patagonotothen tesselata
Gobionotothen gibberifrons
Notothenia rossi
Opsanus tau
Lophius piscatorius
Anguilla anguilla
Katsuwonus pelamis
Trachurus trachurus
Scomber scombrus
0
200
400
600
800
1000
1200
1400
1600


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
0
5
10
15
20
25
30
35
40
45

Oxygen consumption in grunter

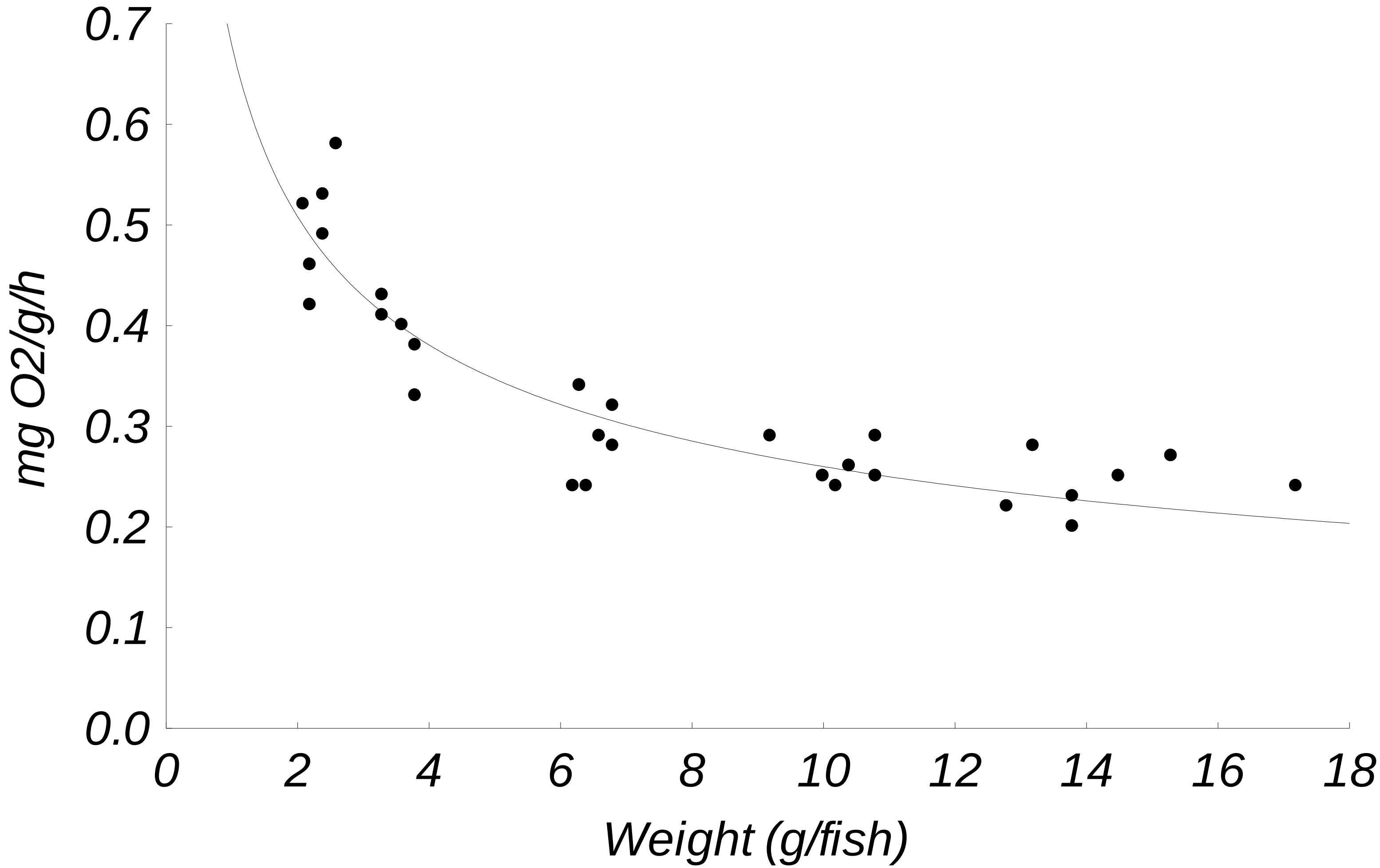


Data are from John Radull’s thesis

Oxygen consumption in grunter

Model: $R=a*W^{b}$

$$R=0.67*W^{-.042}$$



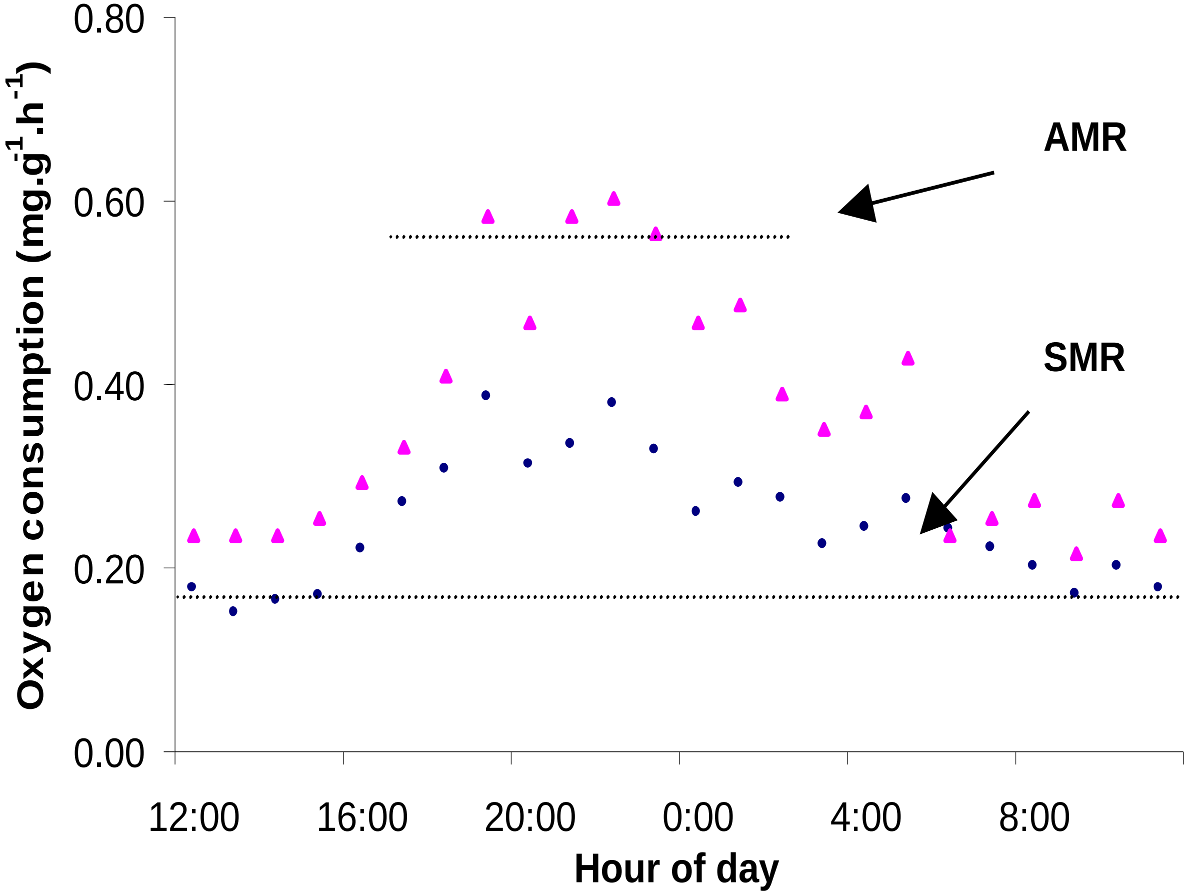
Data are from John Radull's thesis

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?

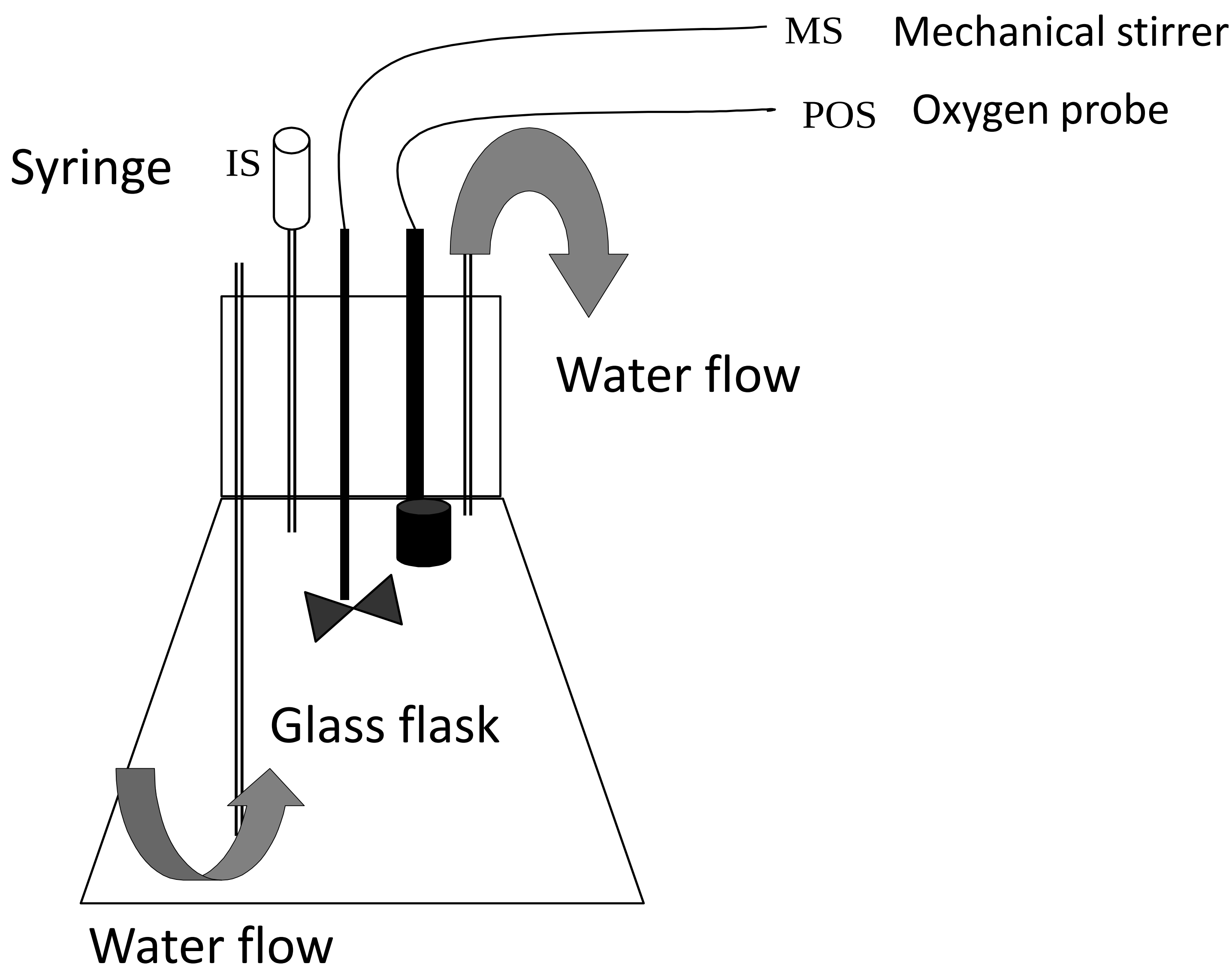
Metabolic rate in fish

- Standard metabolic rate (SMR)
- Routine metabolic rate (RMR)
- Active metabolic rate (AMR)
- Metabolic scope (MS): $MS = SMR – AMR$


0.80
0.60
0.40
0.20
0.00
Oxygen consumption (mg.g-1.h-1)
AMR
SMR
12:00
16:00
20:00
0:00
4:00
8:00
Hour of day

ref. F

Intermittent respirometry



ref. G

Physiology of the stress response

An interesting and important relationship between the stress response and respiration

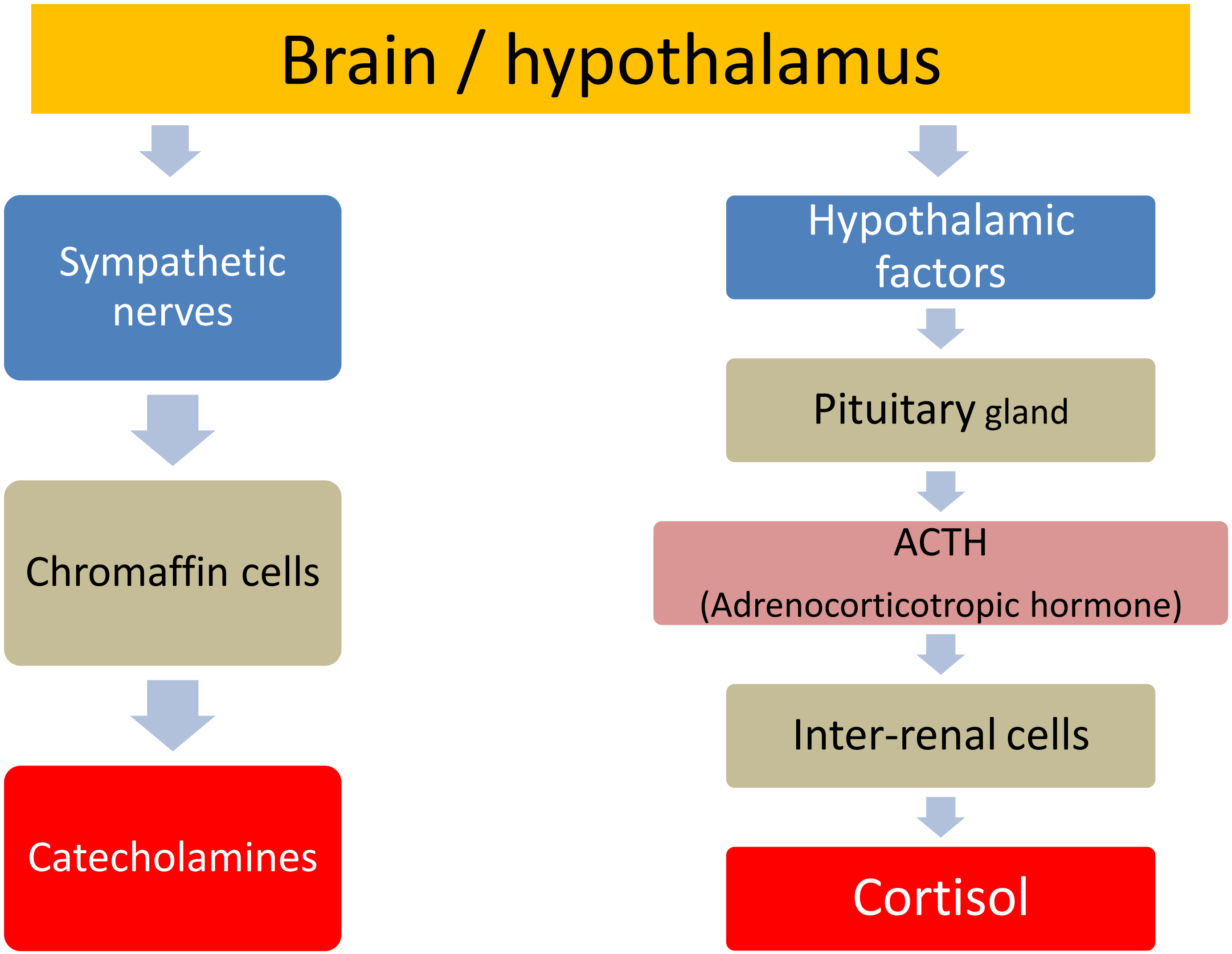
The stress response

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

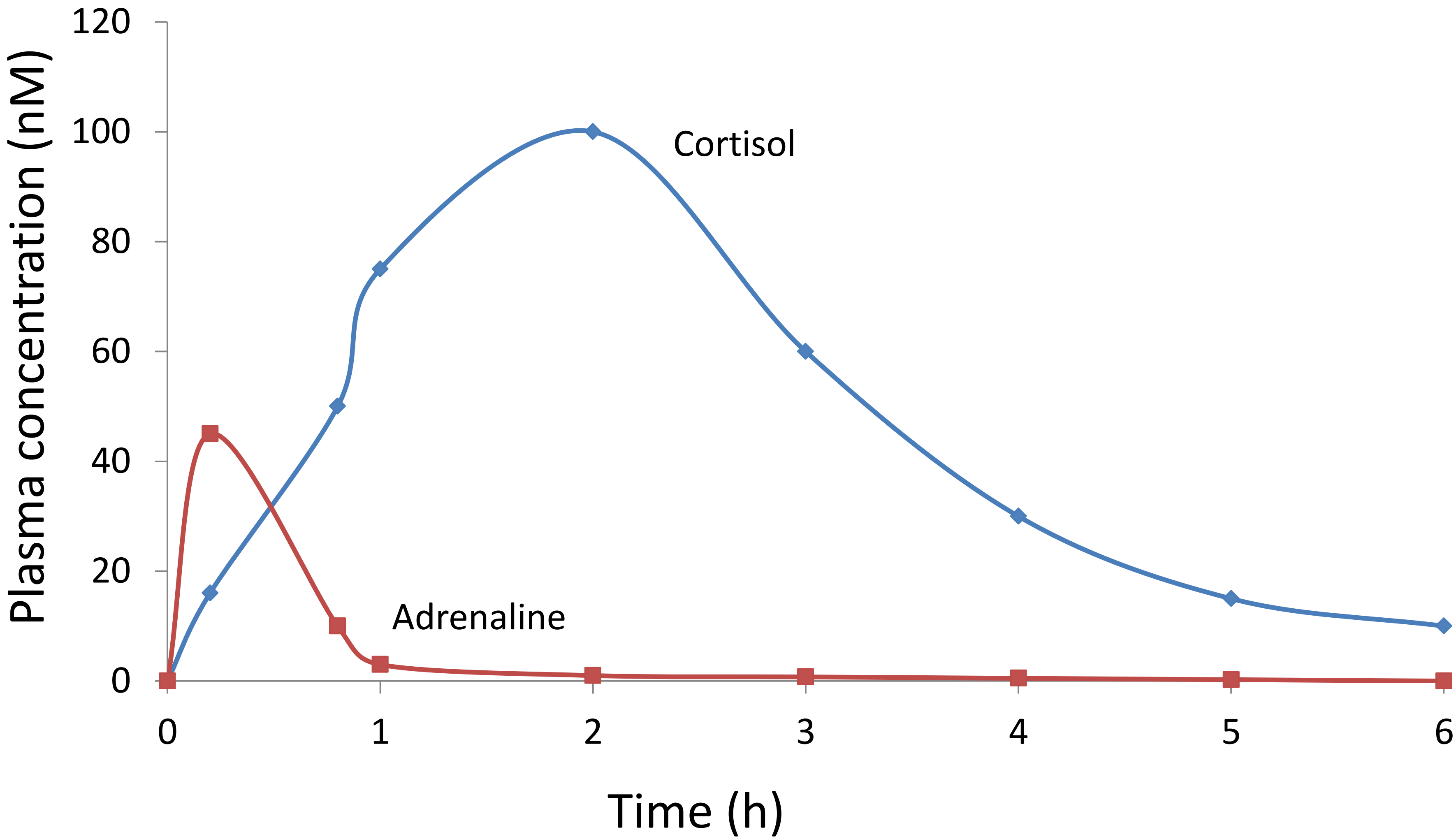
- Secondary stress response: Biochemical and physiological changes (i.e.,: release of stress hormones).

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

Endocrinology of the stress response



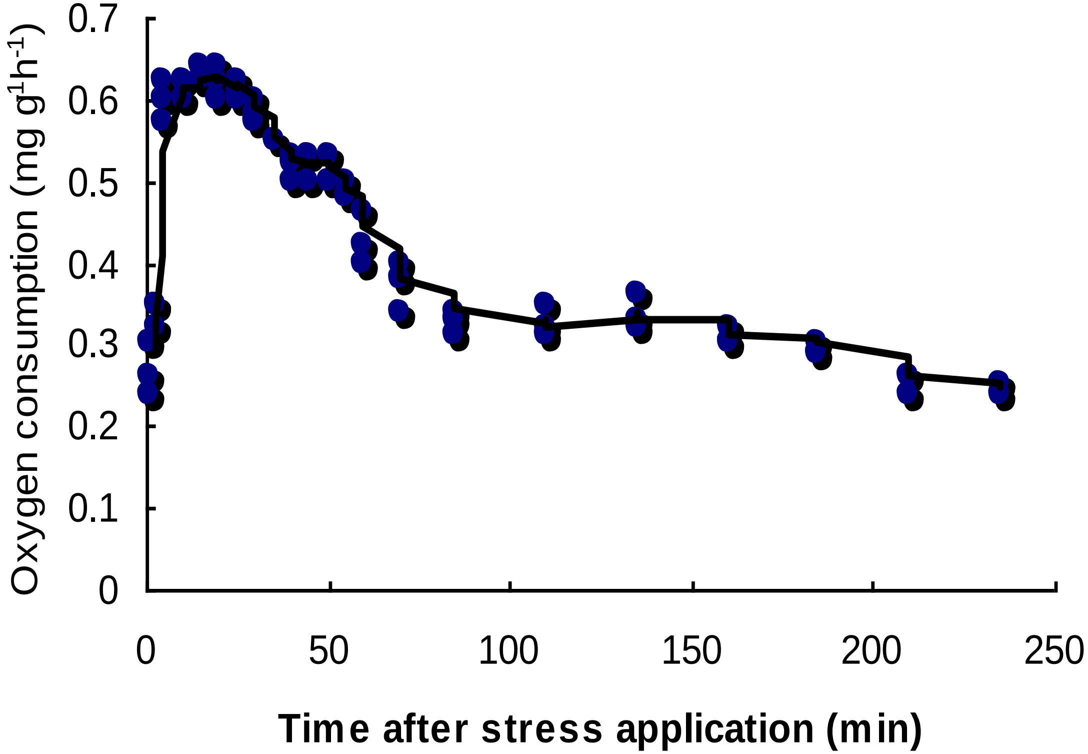
Generalised change in adrenaline and cortisol concentration in fish plasma following a stressor



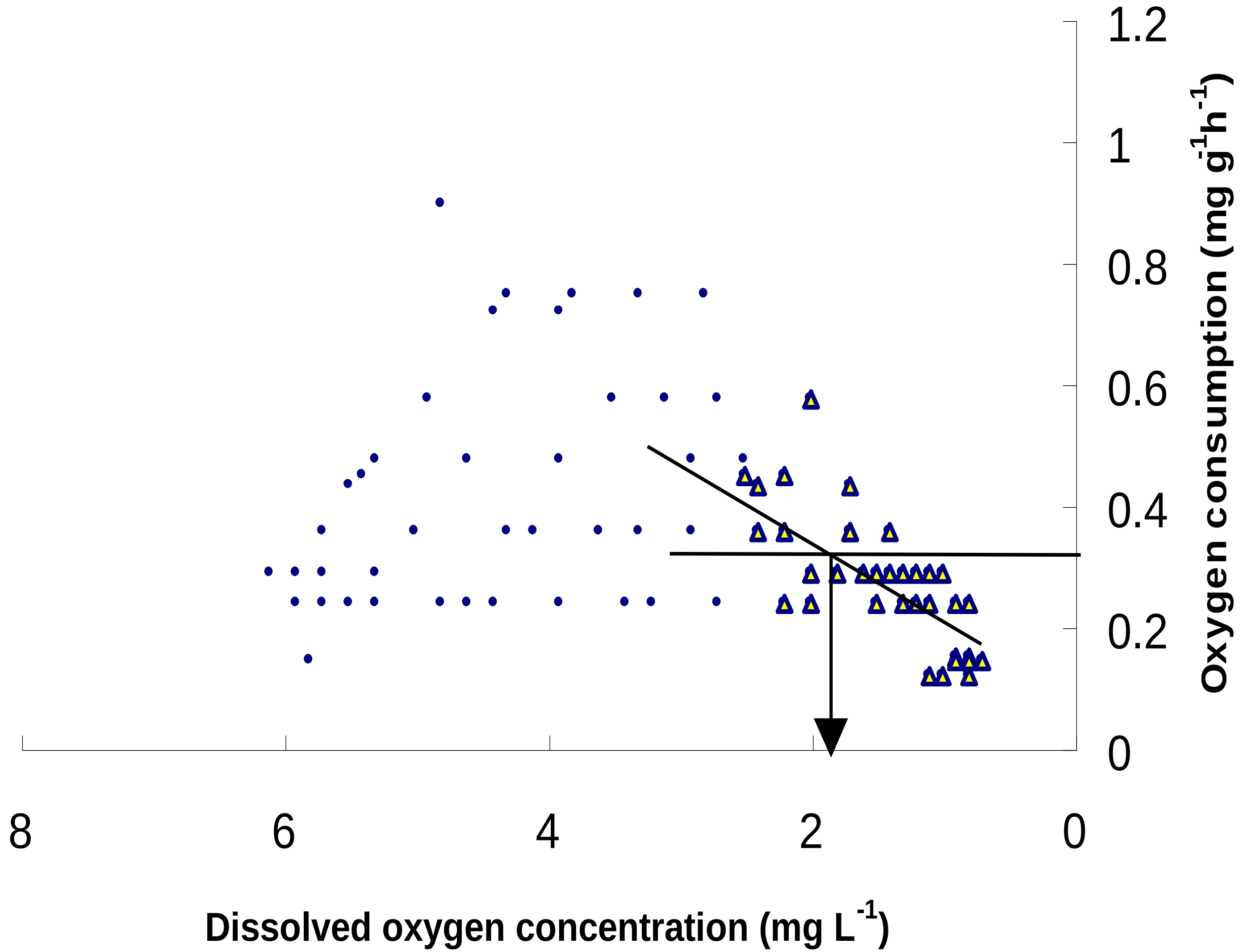
The main effects of elevated cortisol levels

- 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

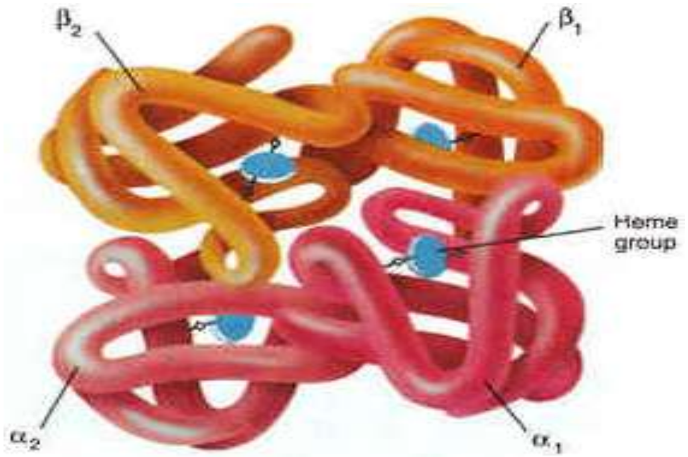

1.2
1
0.8
0.6
0.4
0.2
0
Oxygen consumption (mg g-1h-1)
8
6
4
2
0
Dissolved oxygen concentration (mg L-1)

ref. F

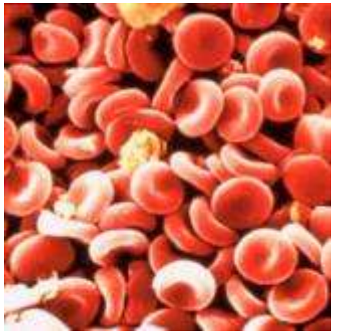
Respiration

Gas exchange

Respiration

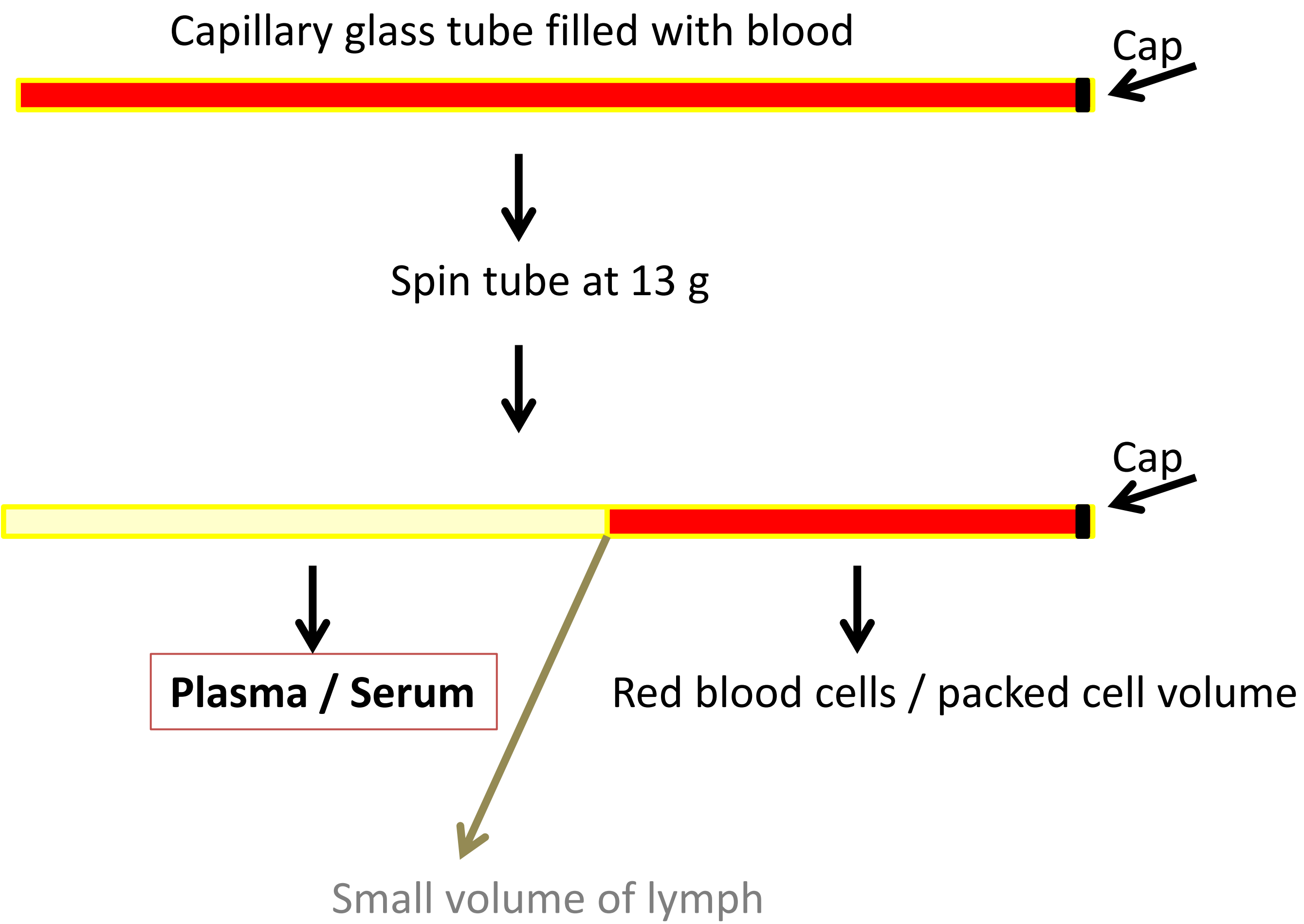


Hemoglobin (Hb)



- 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?**

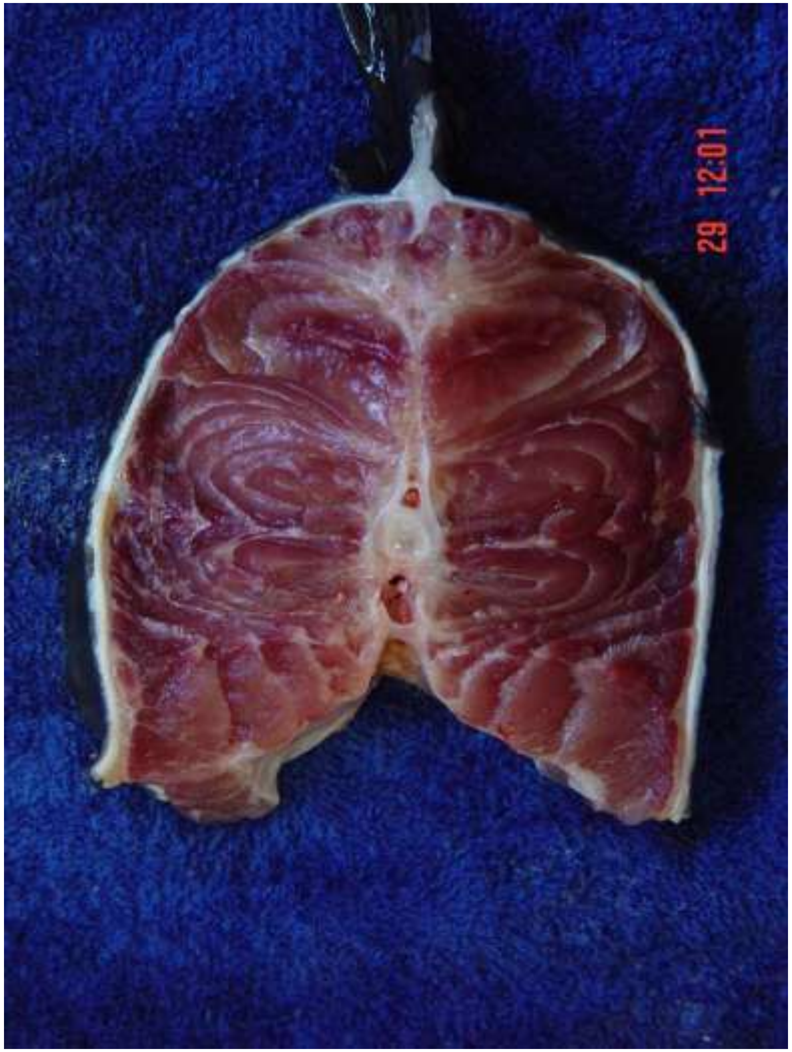
Blood and serum

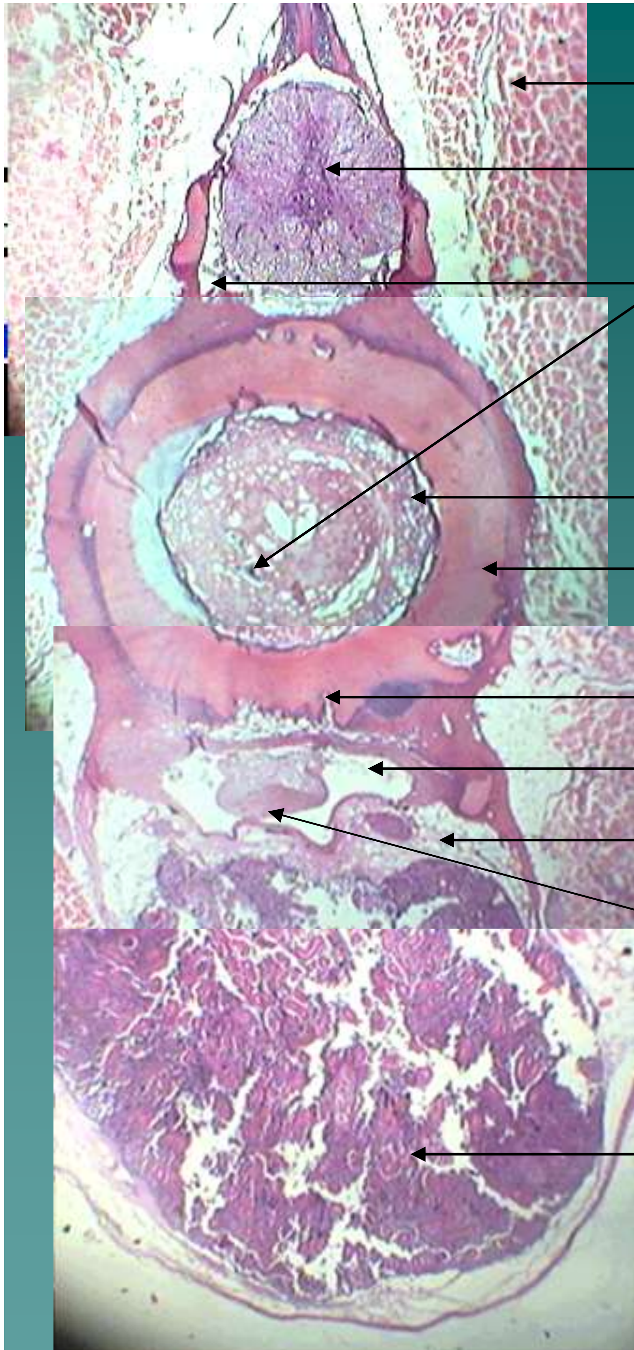


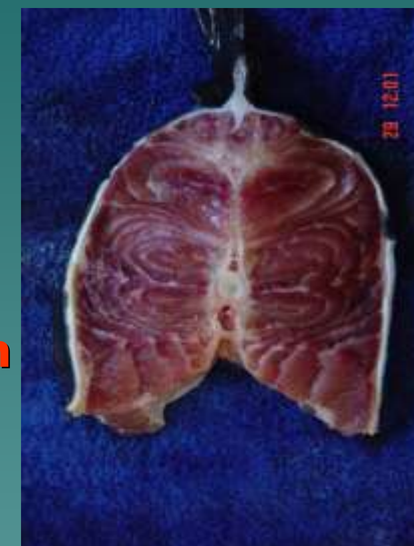
Oxygen carrying capacity depends on …

- Number of red blood cells
- Concentration of haemoglobin within the red blood cells
- Oxygen binding properties of haemoglobin
- Partial pressure of oxygen

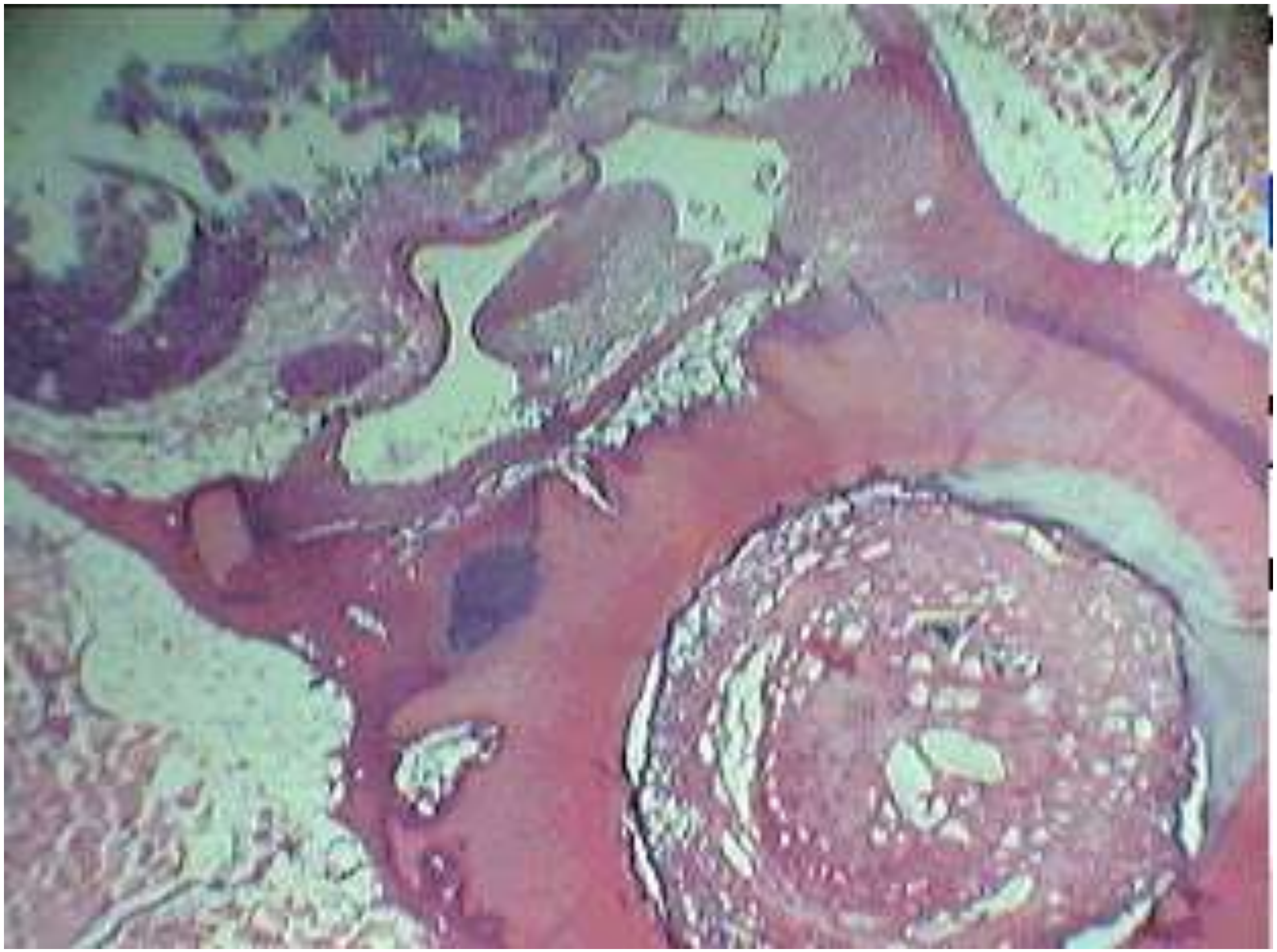
Where do red blood cells come from?

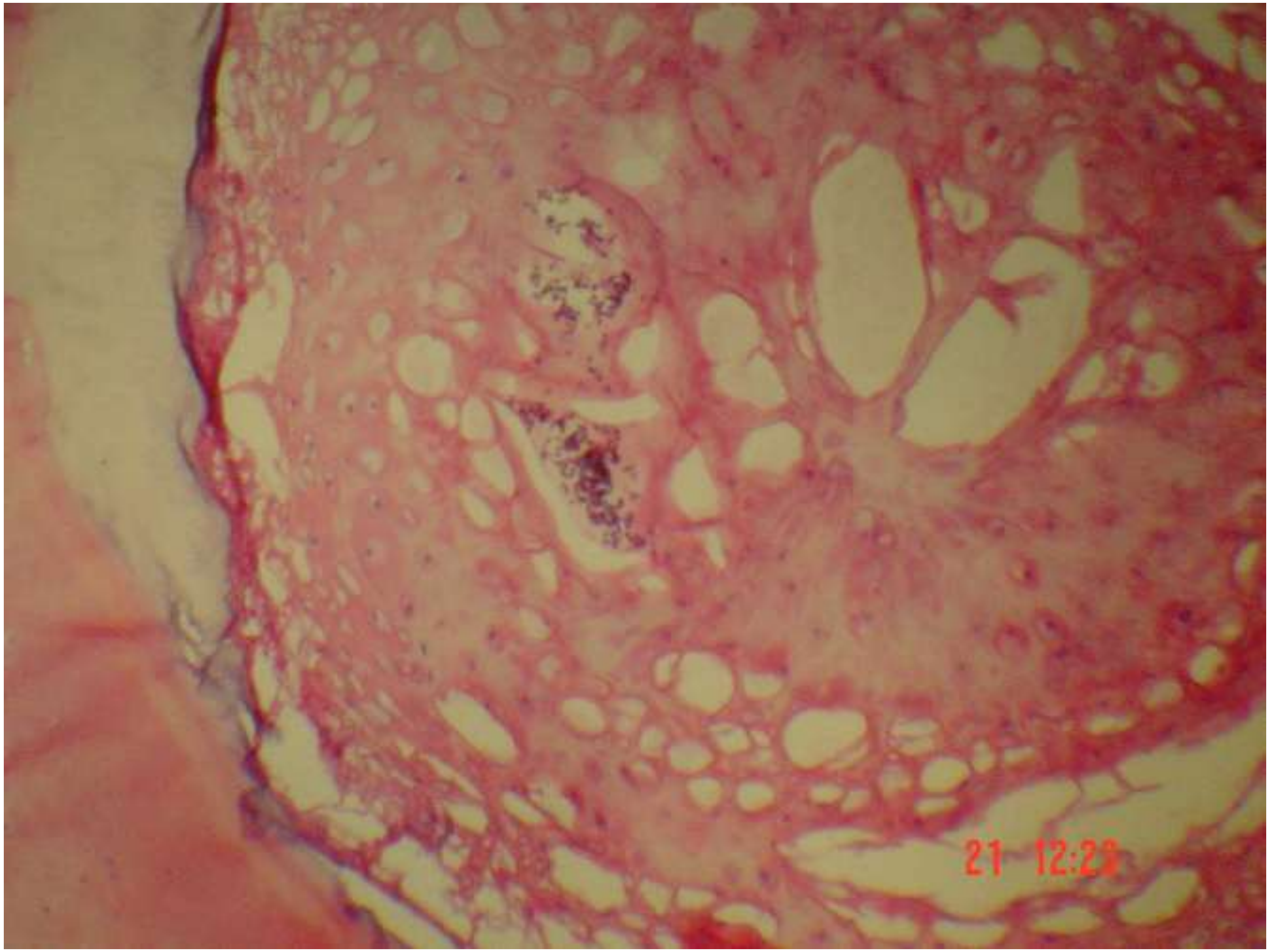

29 12:01





HAEMOPOIETIC ORGAN IN RAINBOW TROUT





Carbon dioxide transport – the chemistry of CO_2 in water

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Carbon dioxide

Carbonic acid

Bicarbonate ion

Carbonic anhydrase speeds up carbon dioxide dissociation in the cell

Extracellular

Slow reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Carbonic anhydrase

Fast reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Red blood cell

At the tissues

Carbonic anhydrase

Fast reaction

HCO_3^- transport to plasma in exchange for Cl^-

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Drop in pH !

Hemoglobin changes state and releases $\mathbf{O_2}$

O_2 diffuses to plasma and tissues

At the gills

Carbonic anhydrase

Fast reaction

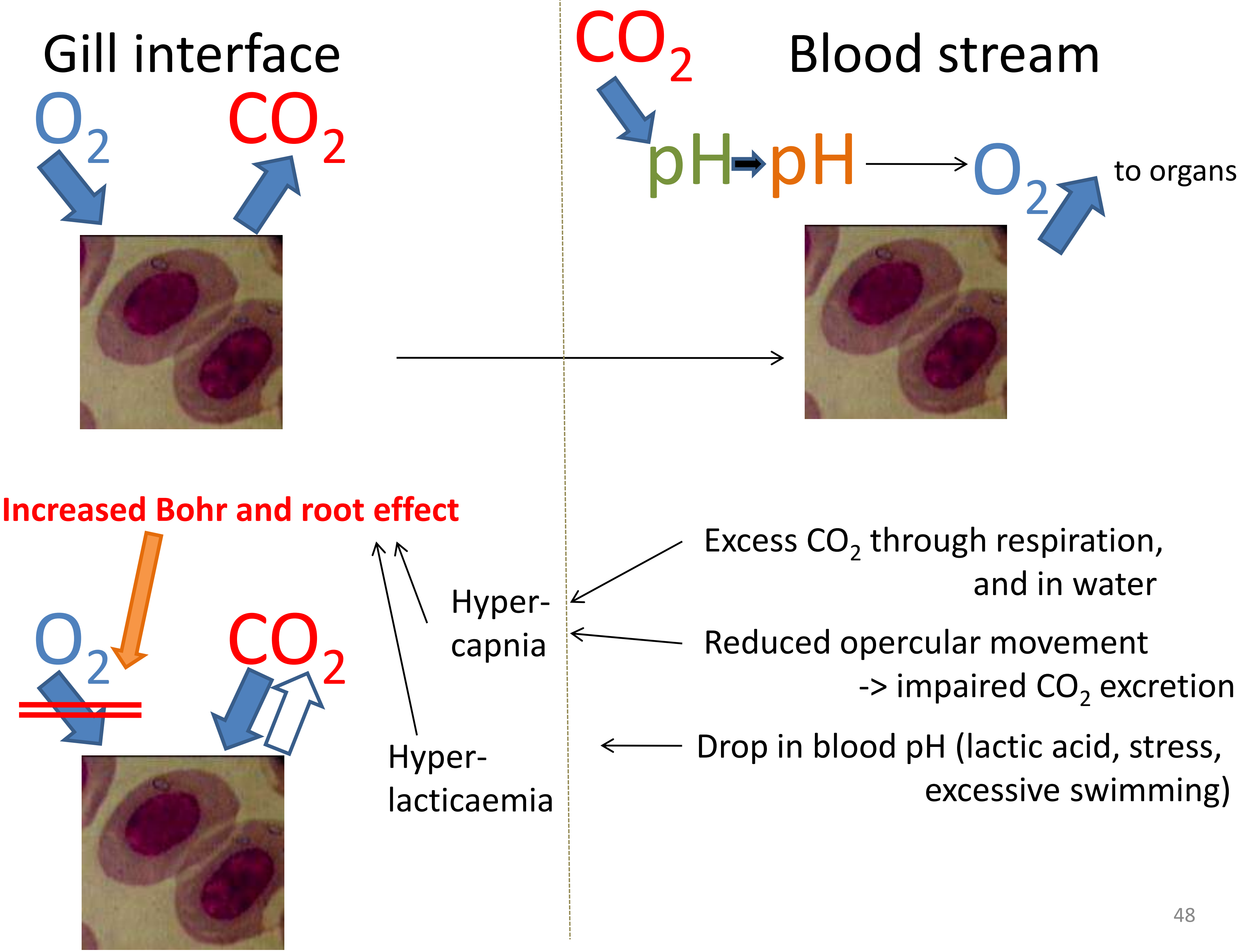
CO_2 diffusion to plasma and then to the water

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

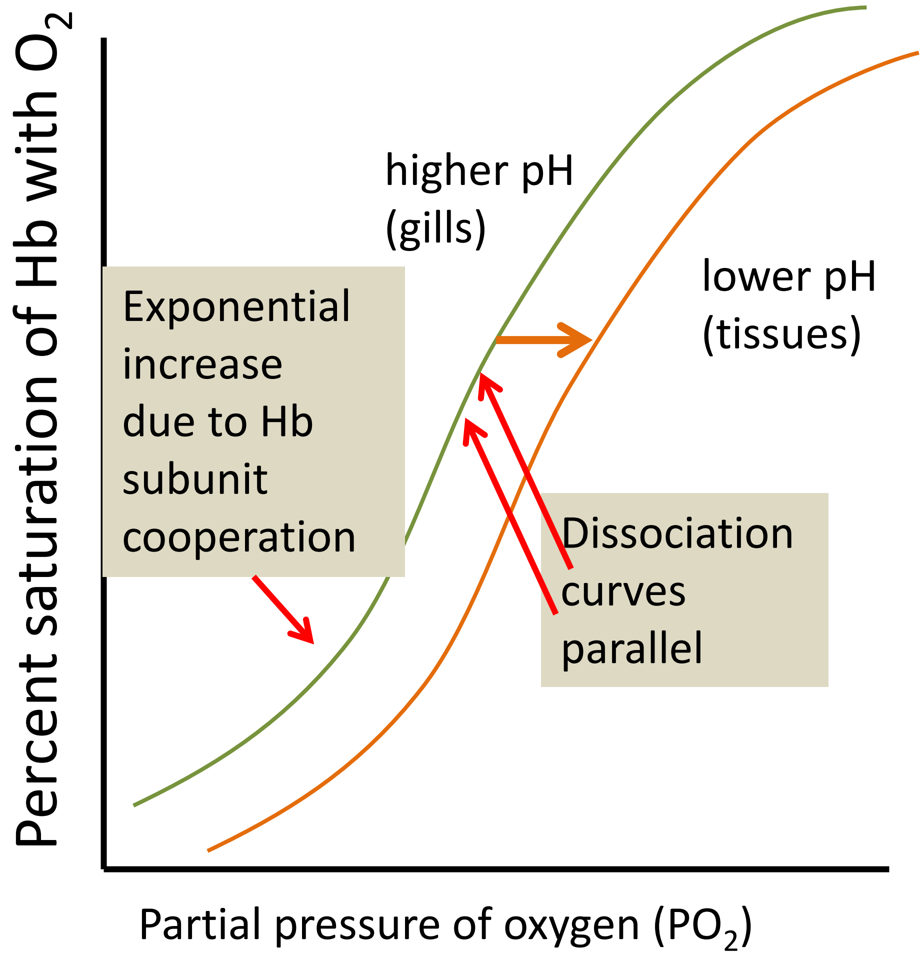
HCO_3^- transport to blood cell in exchange for Cl^-

Release of CO_2 increases pH

Hemoglobin changes state and is ready to pick up **O_2** at the gills.


Gill interface
Blood stream
O2
CO2
CO2
pH
pH
O2
to organs
Increased Bohr and root effect
O2
CO2
Hyper-
capnia
Hyper-
lacticaemia
Excess CO2 through respiration,
and in water
Reduced opercular movement
-> impaired CO2 excretion
Drop in blood pH (lactic acid, stress,
excessive swimming)

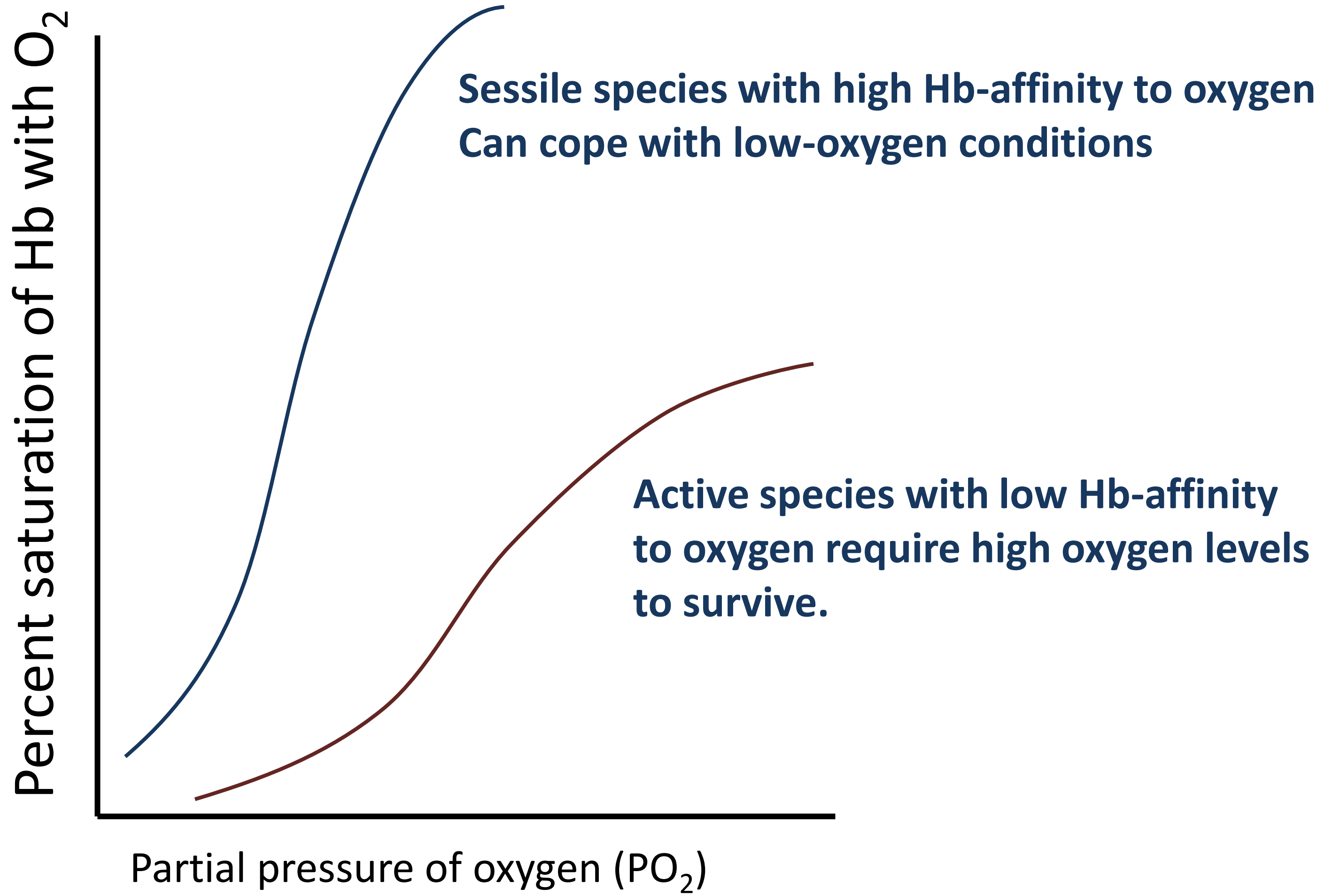
Bohr effect



Reduced affinity under acidic conditions

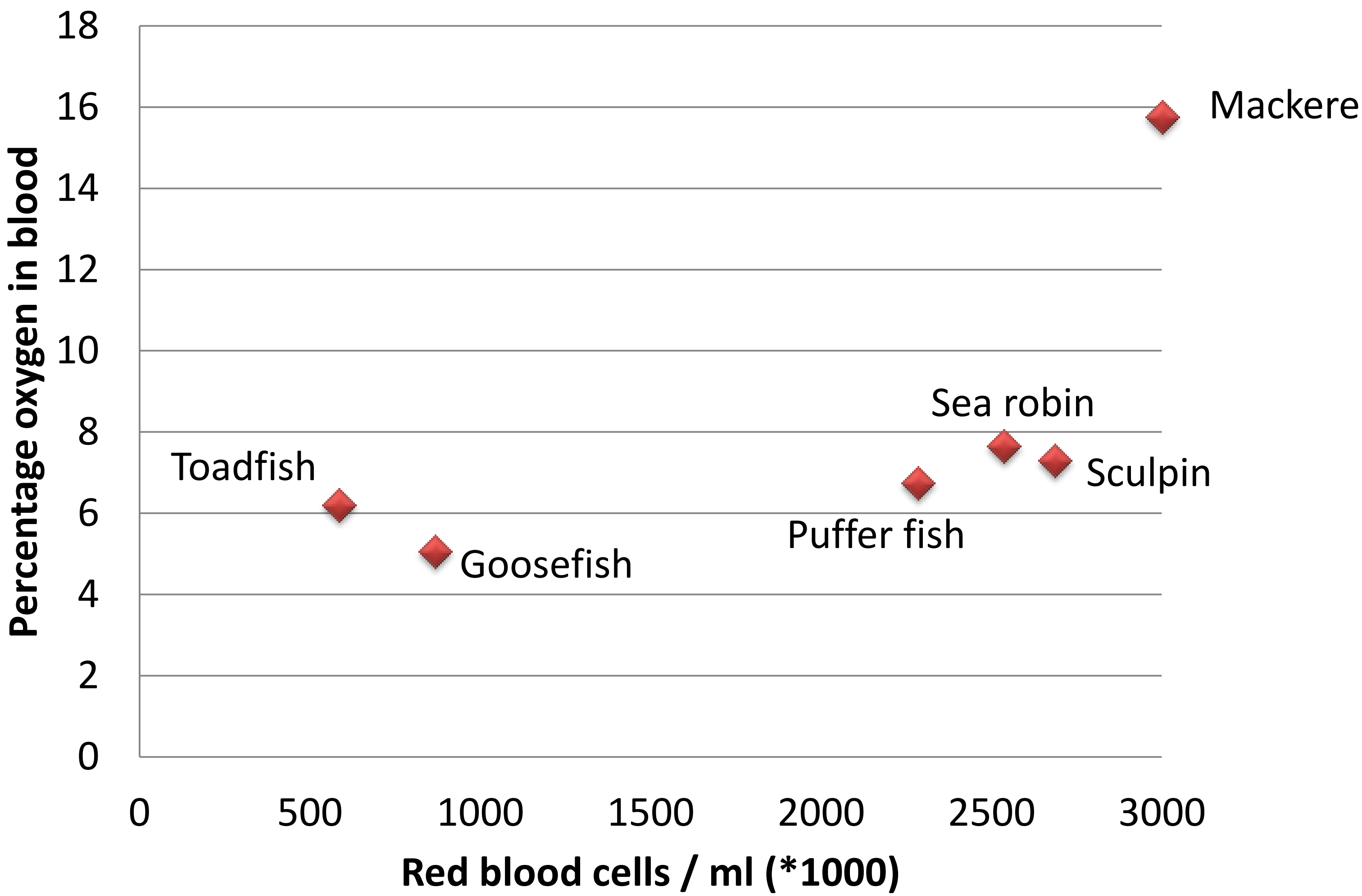
ref. B

Bohr effect – species differences



Reduced affinity under acidic conditions

ref. B



Mackerel
Sea robin
Sculpin
Toadfish
Puffer fish
Goosefish
Percentage oxygen in blood
Red blood cells / ml (*1000)
0
2
4
6
8
10
12
14
16
18
0
500
1000
1500
2000
2500
3000

ref. E

Sea robin (*Prionotus* spp)

Toadfish (*Opsanus* spp)

Root effect

Mackerel (*Scomber scombrus*)

Why is the Root effect unique to fish?

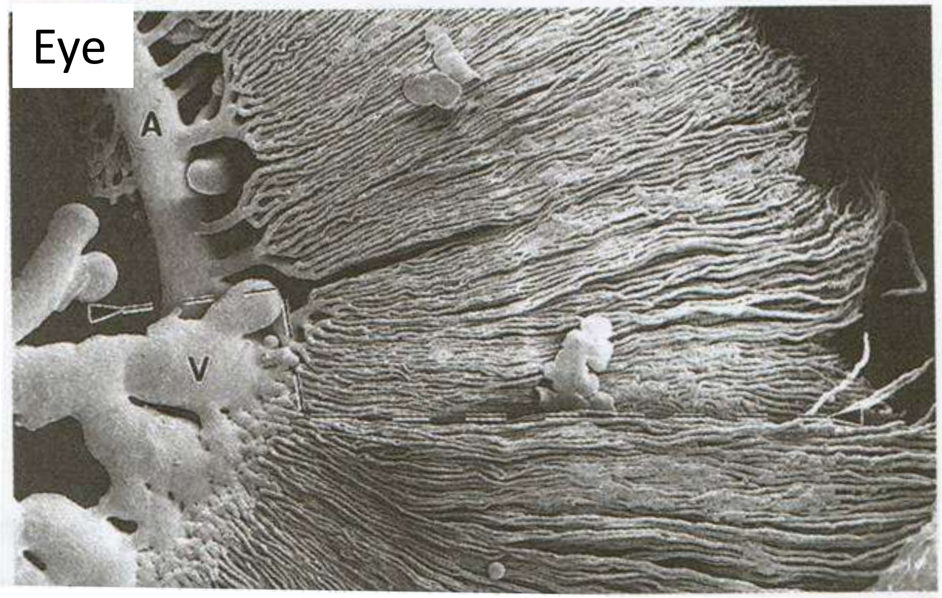
- Oxygen supply to the retina
- Gas bladder function

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

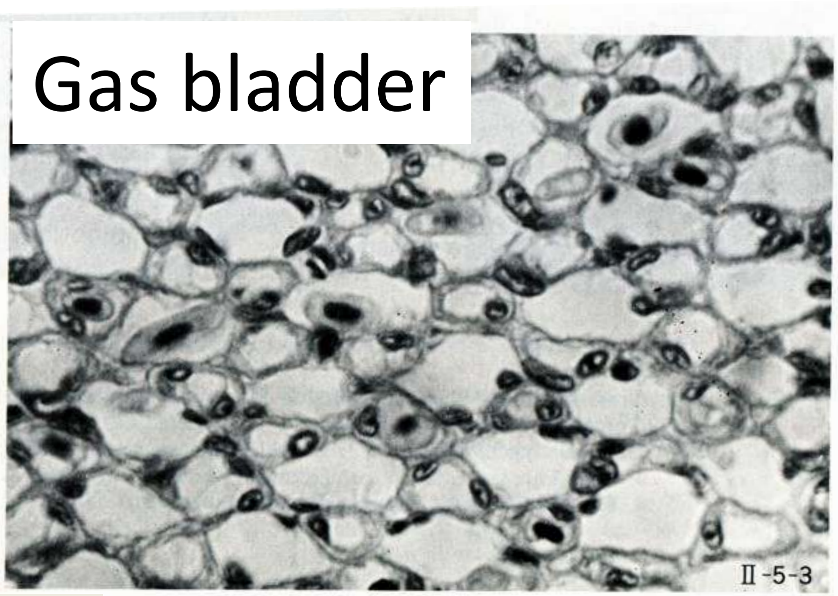
Eye

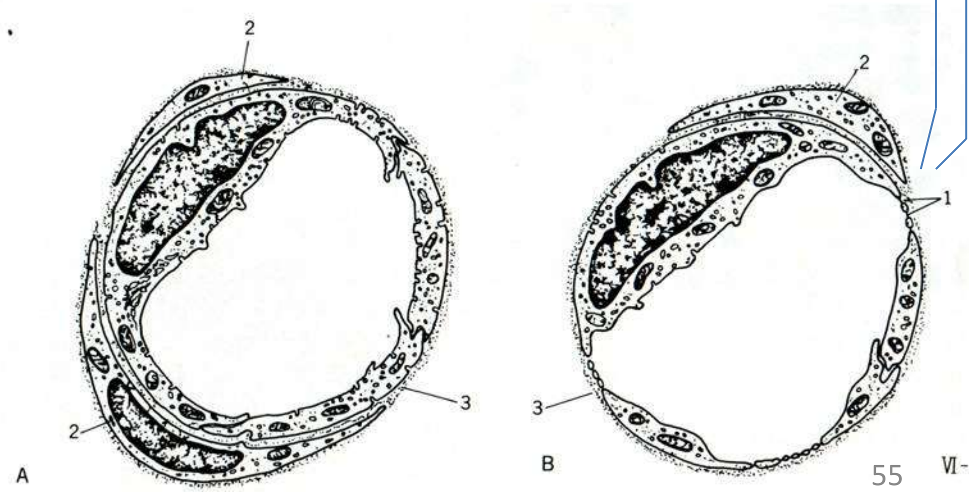


Example: gas bladder rete in eel

- 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

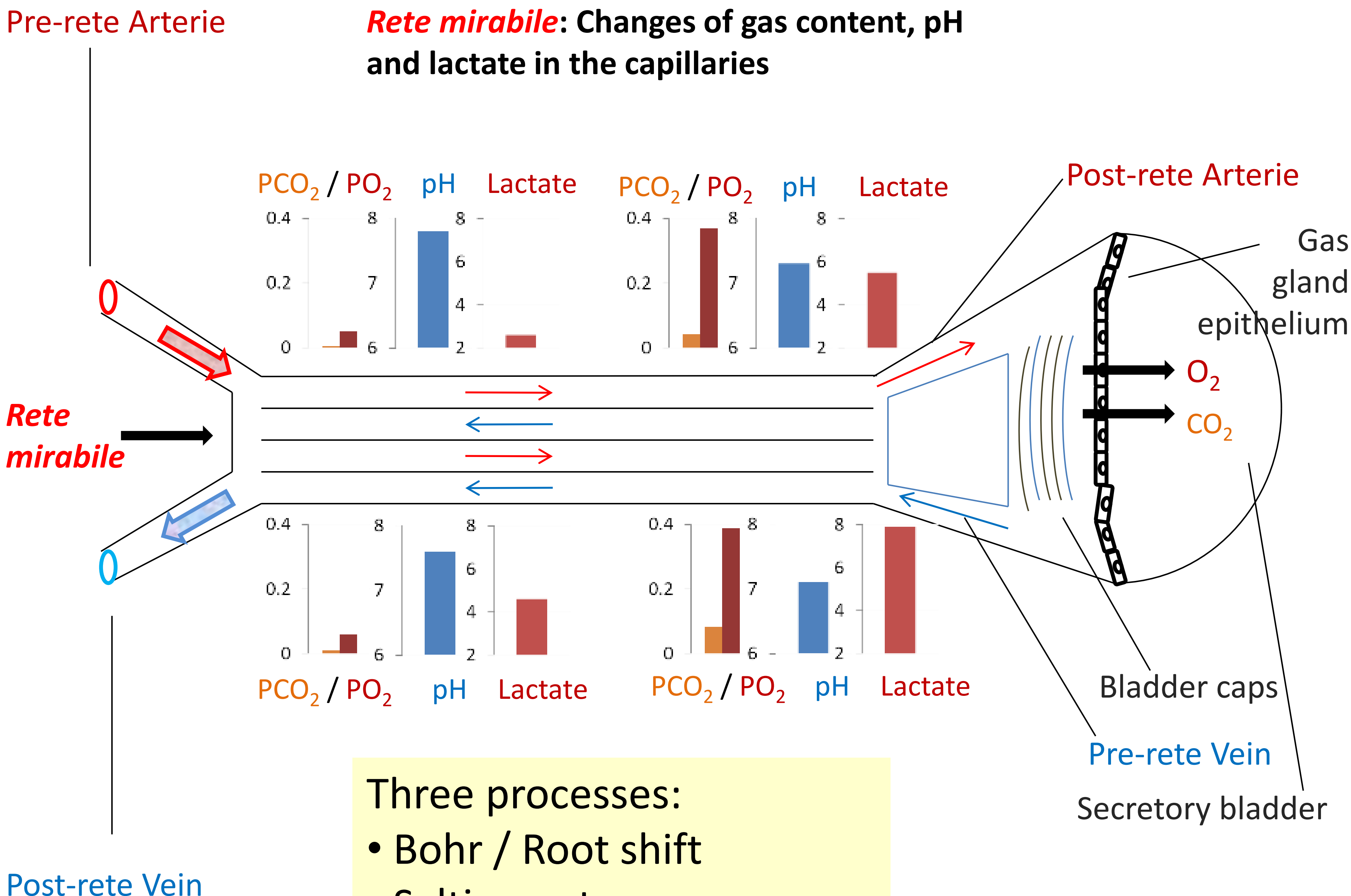
Gas bladder





ref. B

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

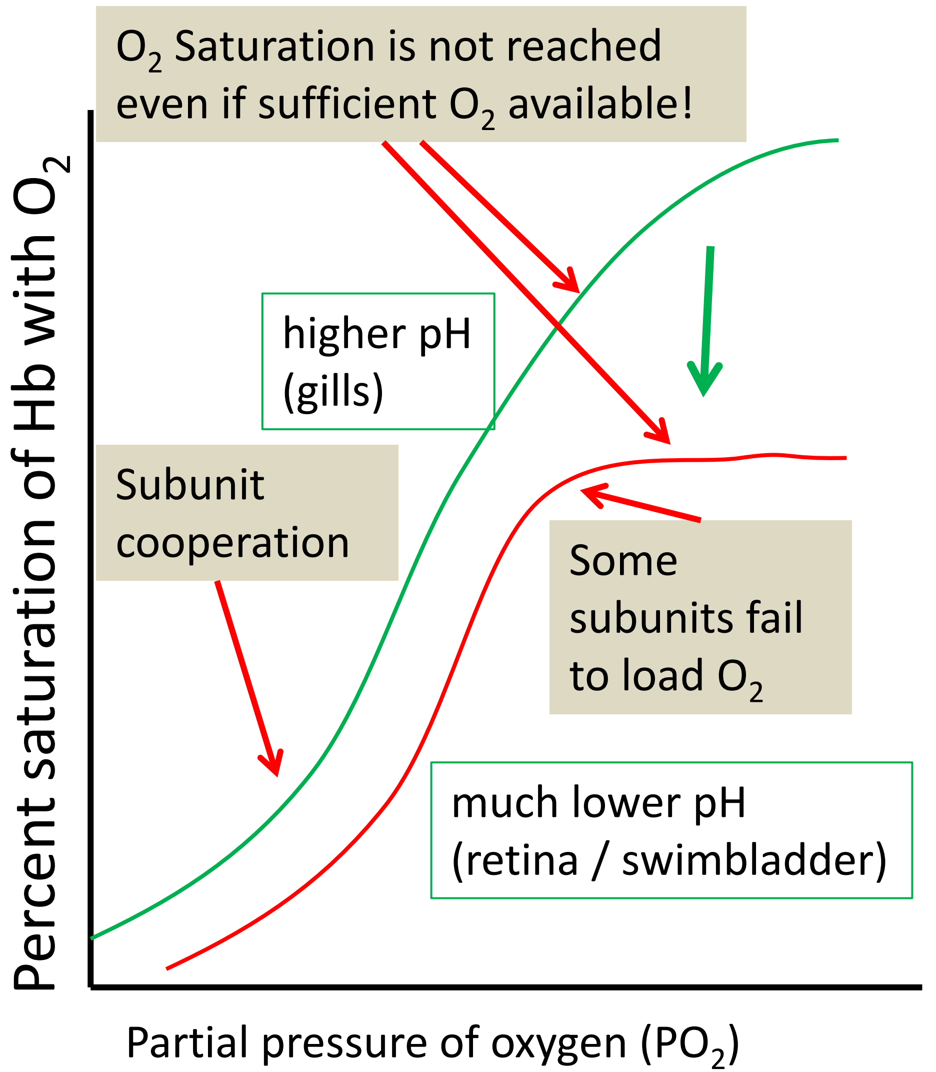


Three processes:

- Bohr / Root shift
- Salting out
- Counter-current diffusion

ref. D

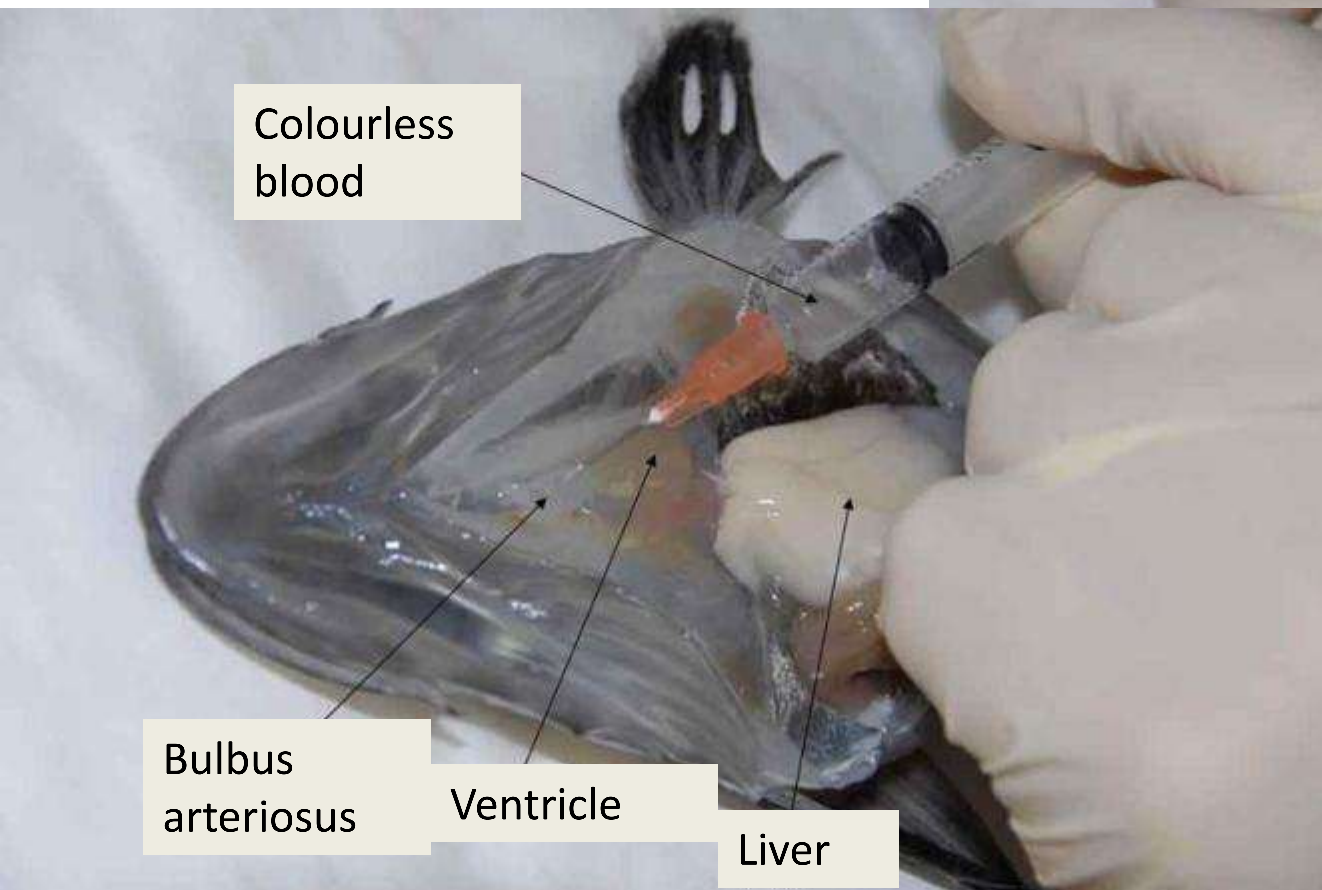
Root effect



Decreased capacity under acidic conditions

Ice fish

- White gills
- Almost transparent blood
- No haemoglobin



Channichthyids

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 are 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

- O_2-consumption = V_g x $[O_2]$ x % extraction
  - O_2-consumption = mg O_2 /kg / h
  - V_g = Gill water flow in ml O_2 / kg / h
  - $[O_2]$ = O_2 (mg/L) in water pumped over gills

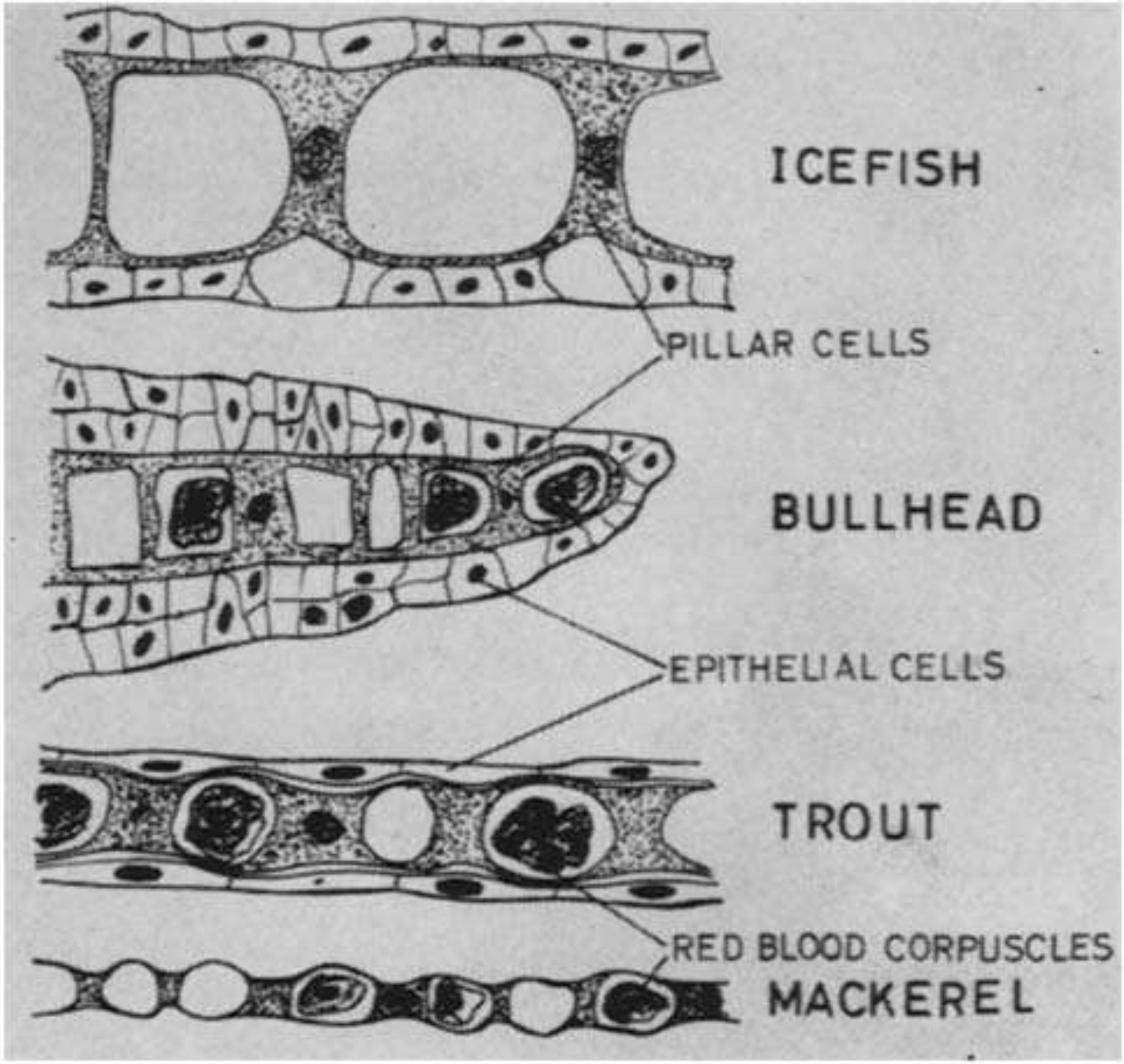
If % extraction is low (= no haemoglobin carrier!), V_g should increase. V_g in ice fishes *is* relatively high. However, this is only part of the explanation.

The Fick principle

- O_2-consumption = Q x [(A-V) x O_2]
  - O_2-consumption = mg O_2 /kg / h
  - Q = Cardiac output (ml / kg / h)
  - A and V = O_2 (mg/L) in ***A***rterial and ***V***enal 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.



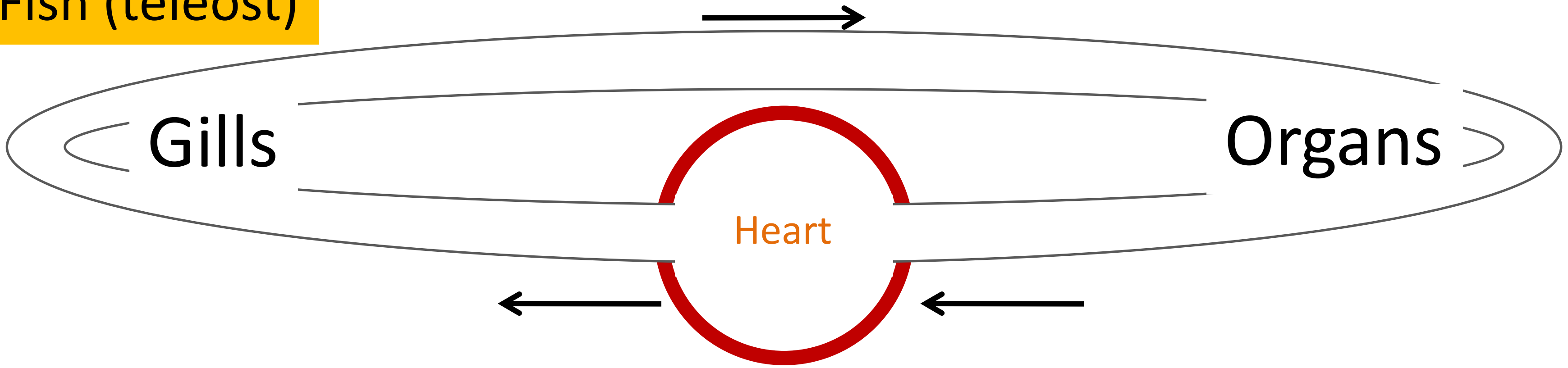
STEEN and BERG, *Comp. Biochem. Physiol.,* 1966, *Vol.* 18, pp. 517 *to* 526

Adaptations in ice fishes

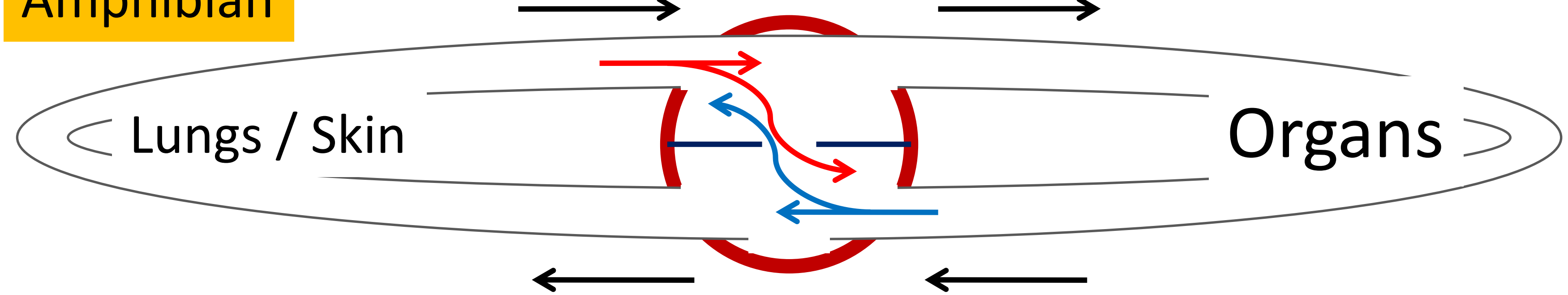
- Relatively high cardiac output / large hearts
- Relatively high blood flow to the gills
- Modification of secondary gill lamellae to increase diameter
- Lower blood pressure
- Ice fishes make use of *perfusion in addition* to *diffusion* to pick up $O_{2.}$

The circulatory system

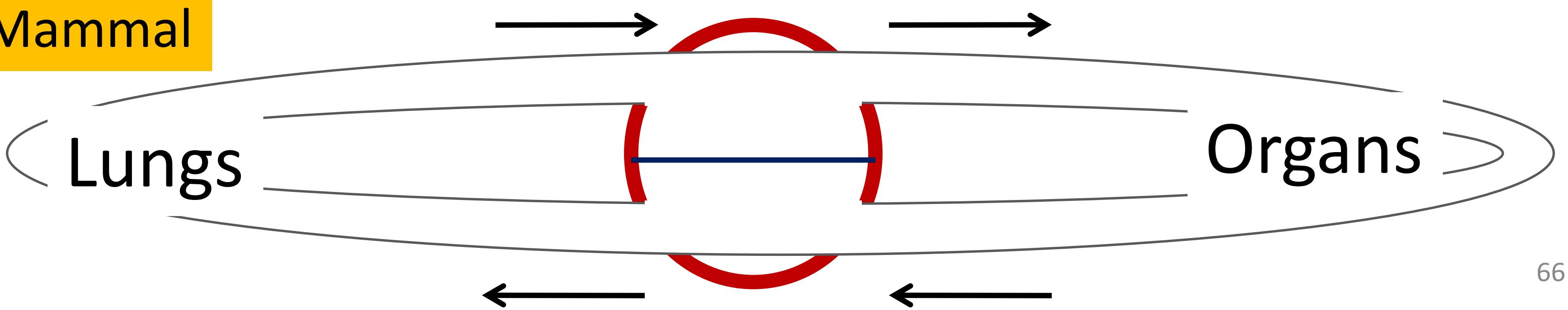
Fish (teleost)



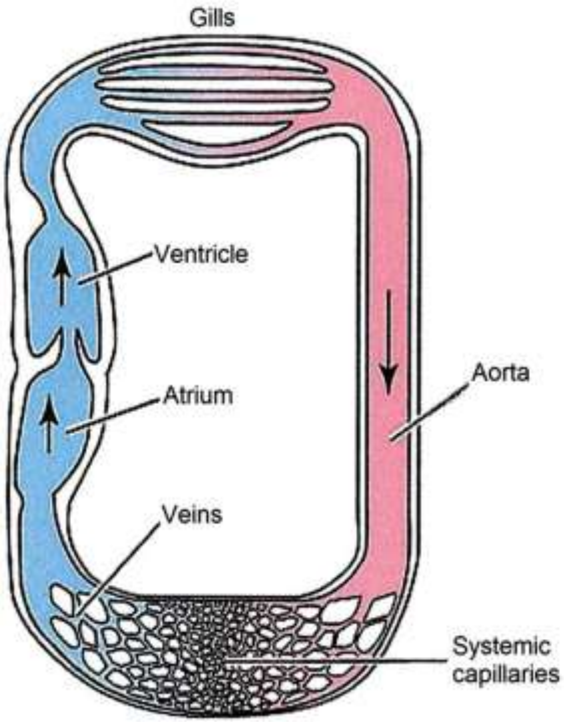
Amphibian



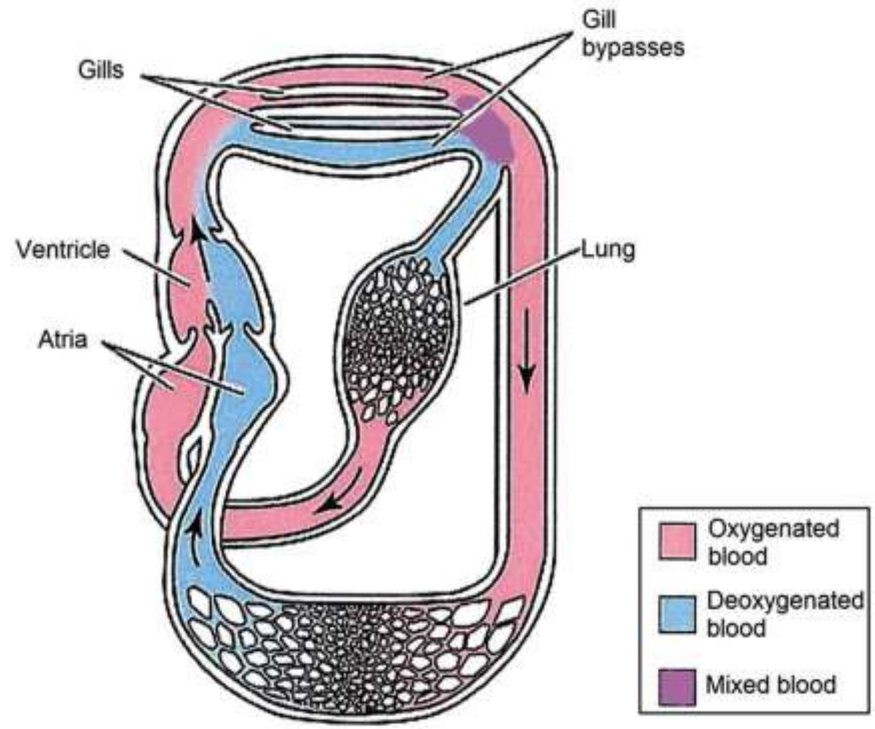
Mammal

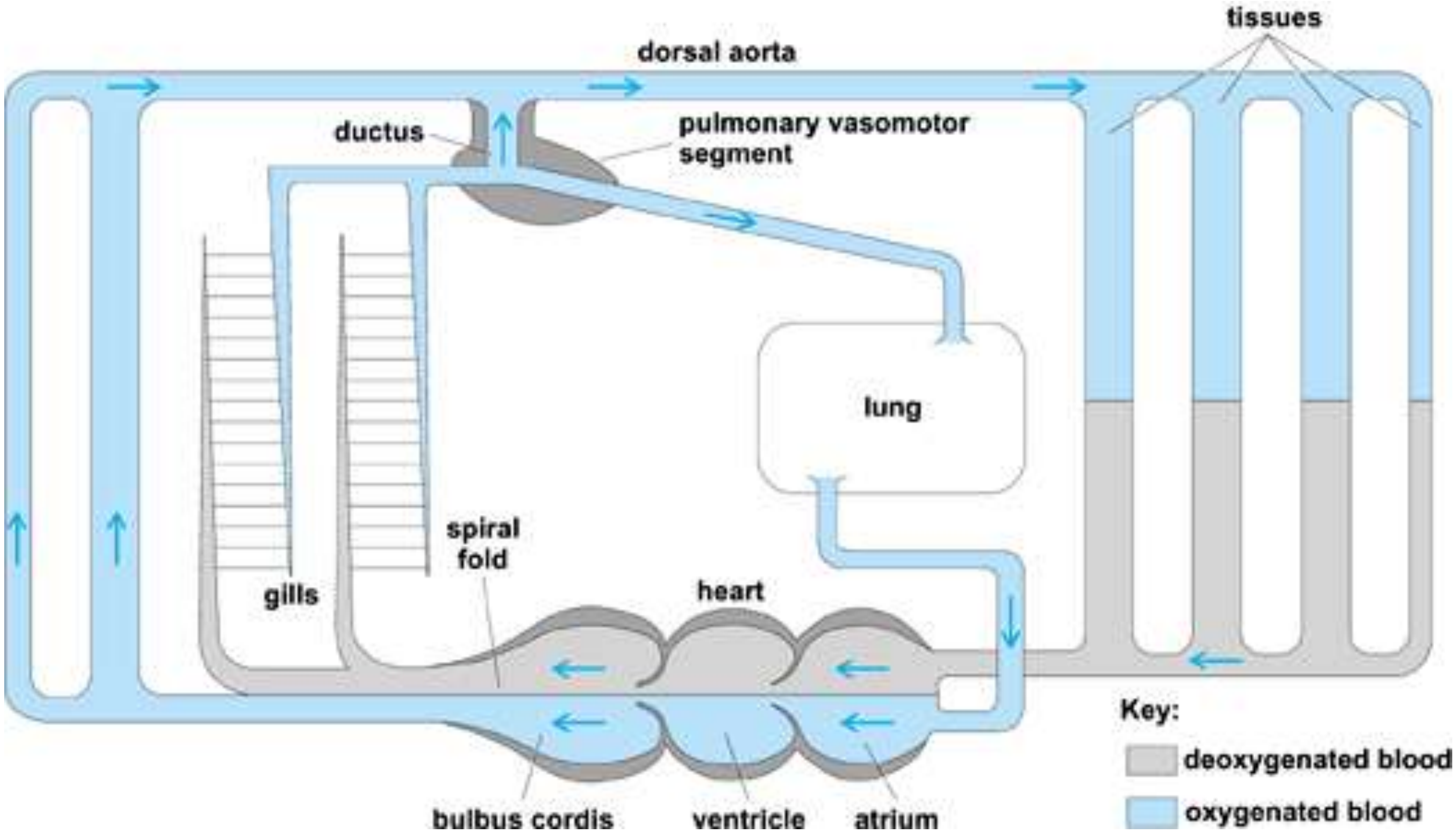


Fish



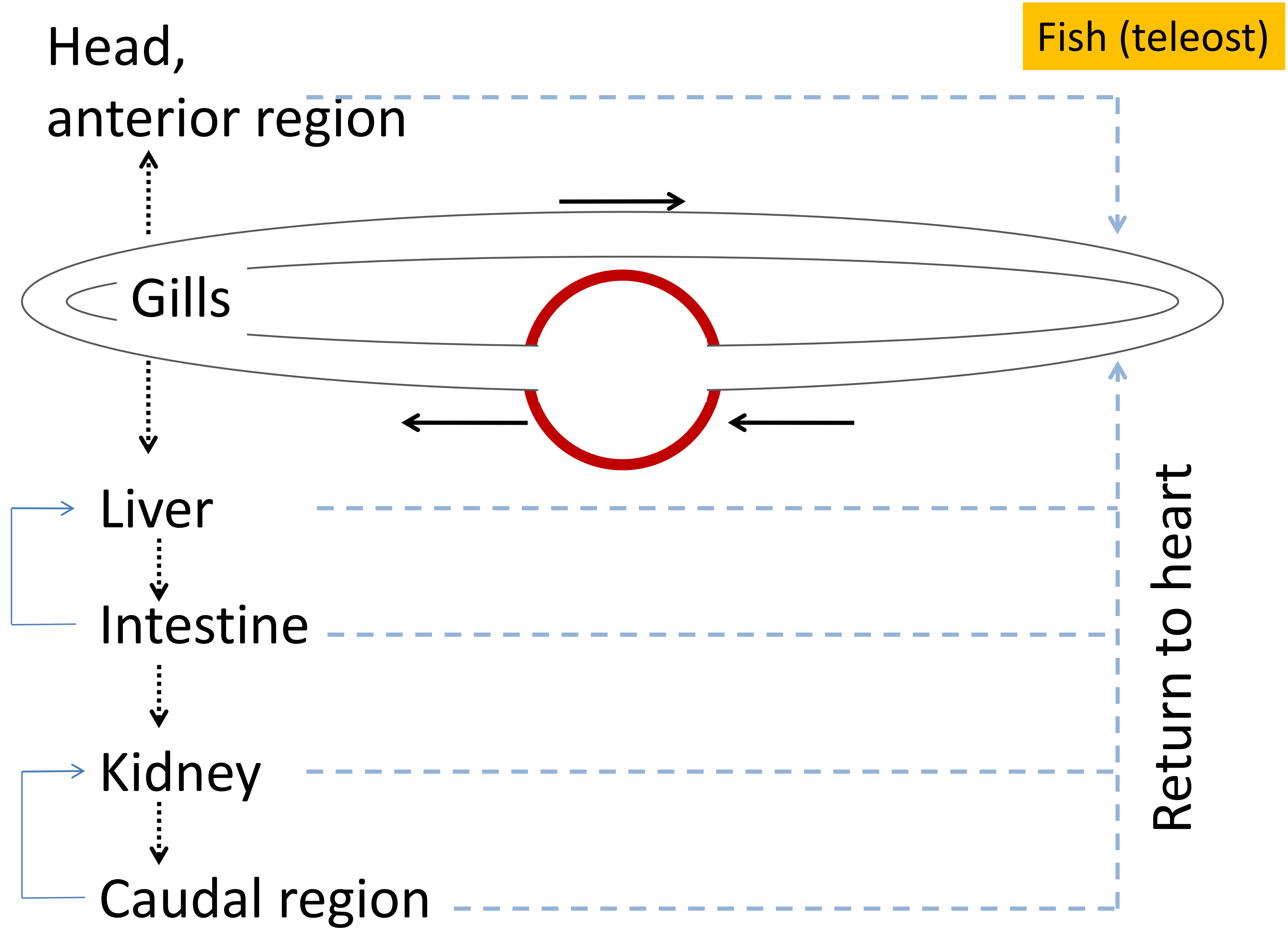
Lungfish





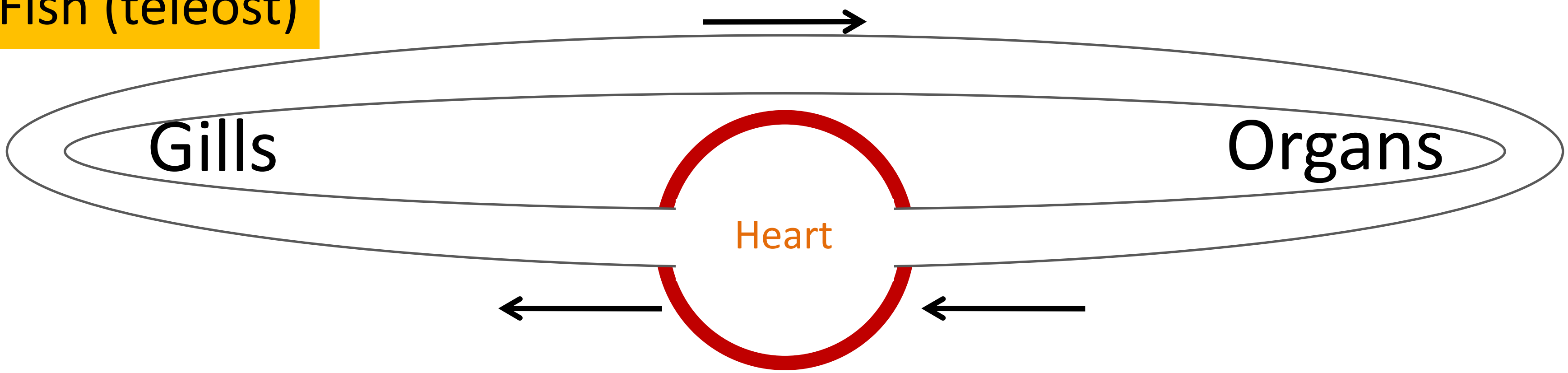
African lungfish, *Protopterus*. The blood is directed to the dorsal aorta or lungs depending on whether the fish is breathing in air or water. (*After D. Randall et al., eds., Eckert Animal Physiology, 4th ed., W. H. Freeman, New York, 1997*)

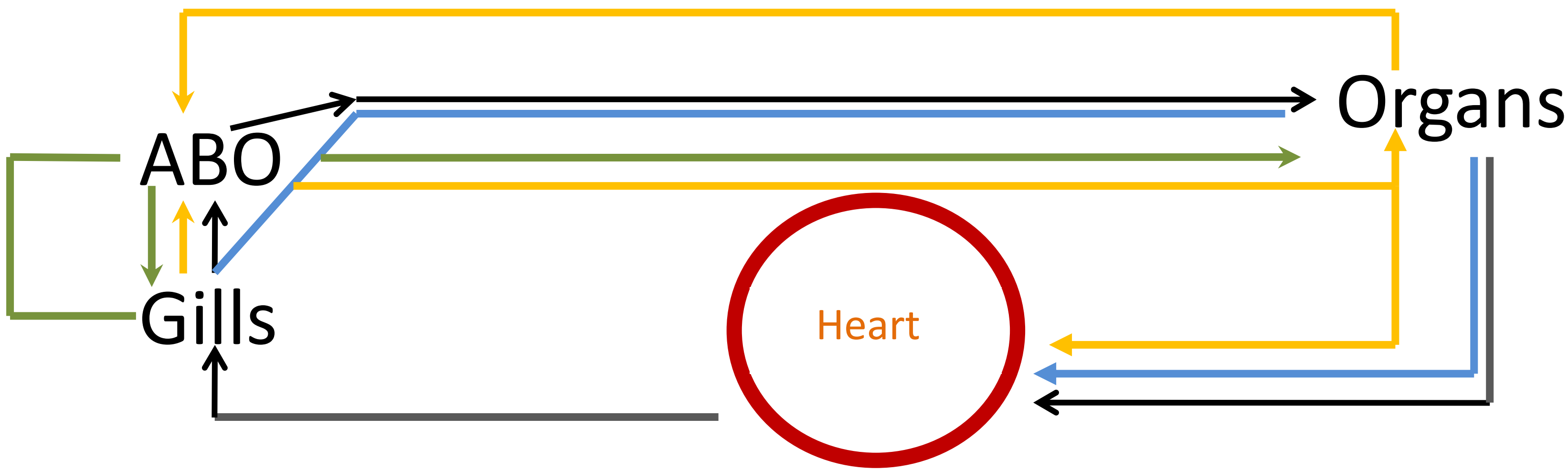
[From: http://accessscience.com](http://accessscience.com)



Fish (teleost)
Head,
anterior region
Gills
Liver
Intestine
Kidney
Caudal region
Return to heart

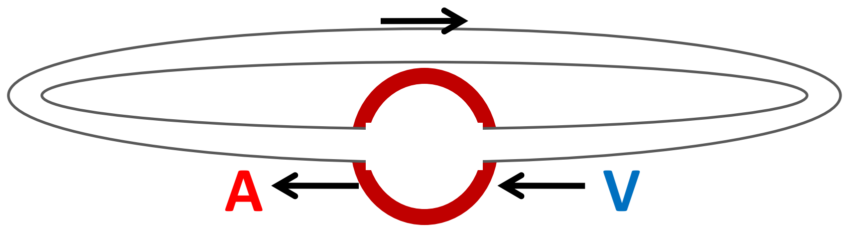
Fish (teleost)

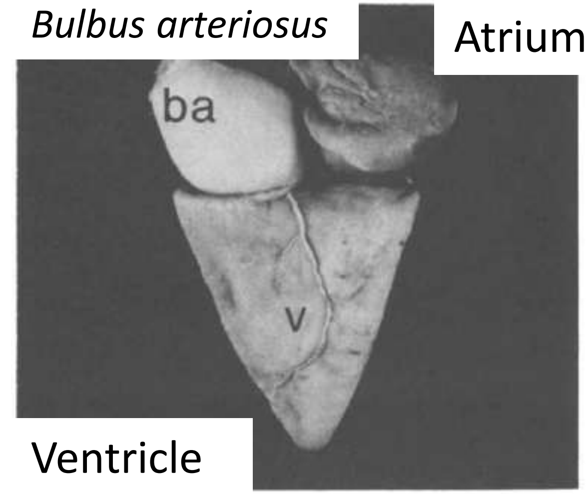




Fish (teleost with ABO – generalised) – Colours resemble adaptations

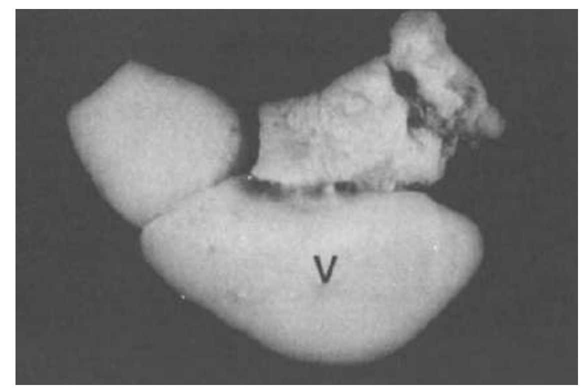
ABO = air breathing organ



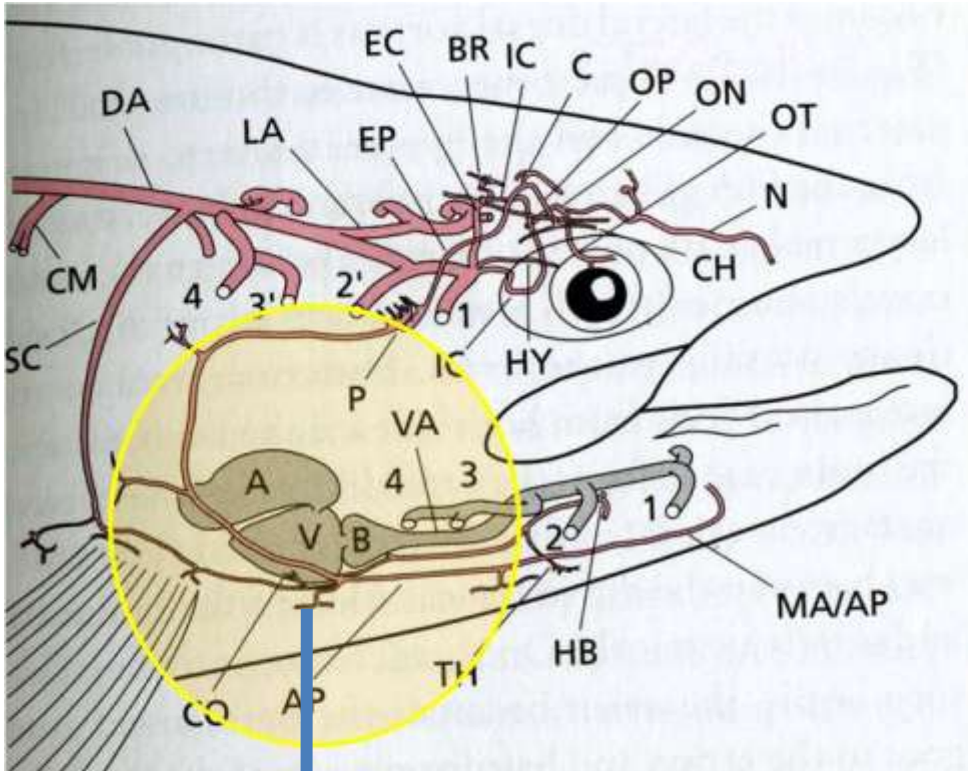


Unique features of the heart in fish

- 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

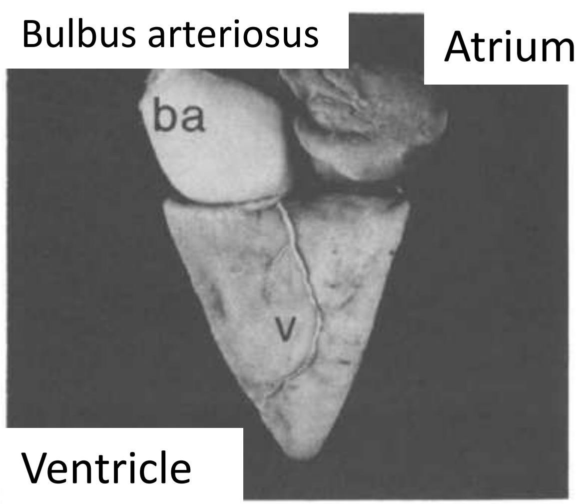


ref. A



Blood circulation in the head of trout

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



Bulbus arteriosus

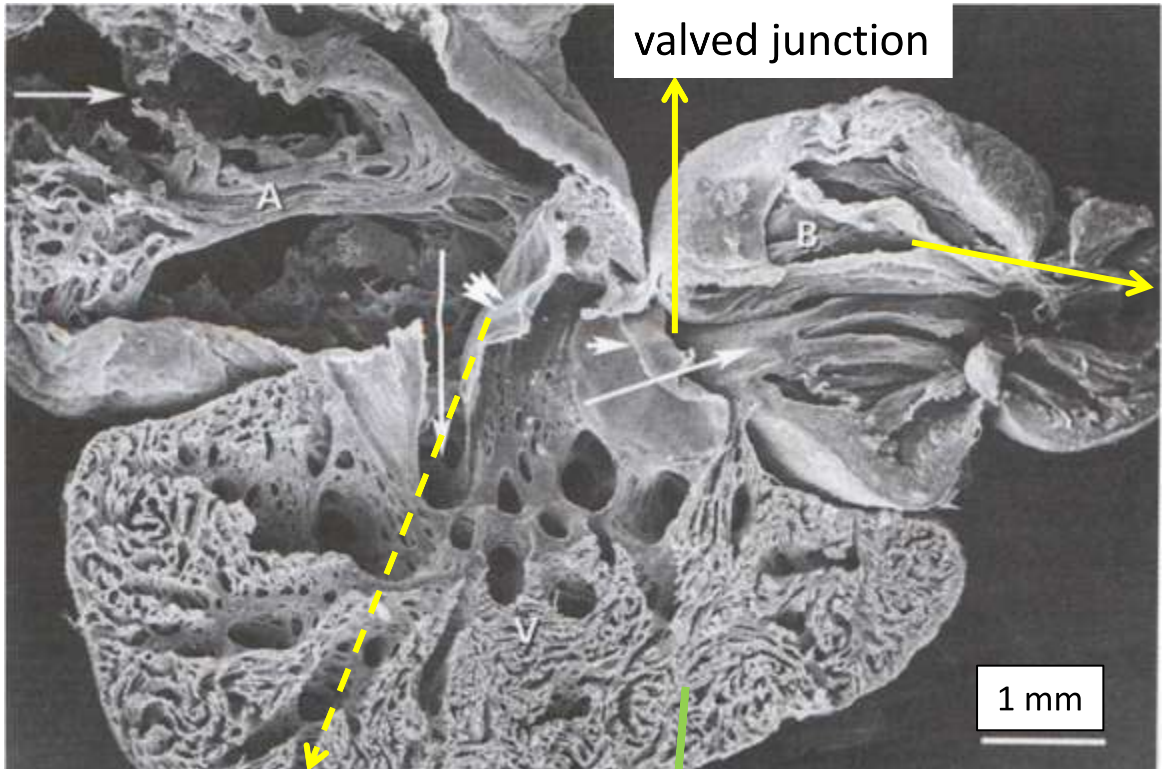
- Thick elastic walls, smooth muscle
- Stores up to 100% of stroke volume to control pulse pressure

Ventricle

- Pumps blood to *bulbus arteriosus*
- Pumped volume = stroke volume (in fish)

ref. A, B

Type trabecular heart of an air breathing catfish



Entrance from the *sinous venosous*

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

Longitudinal trabeculae (mechanical strength)

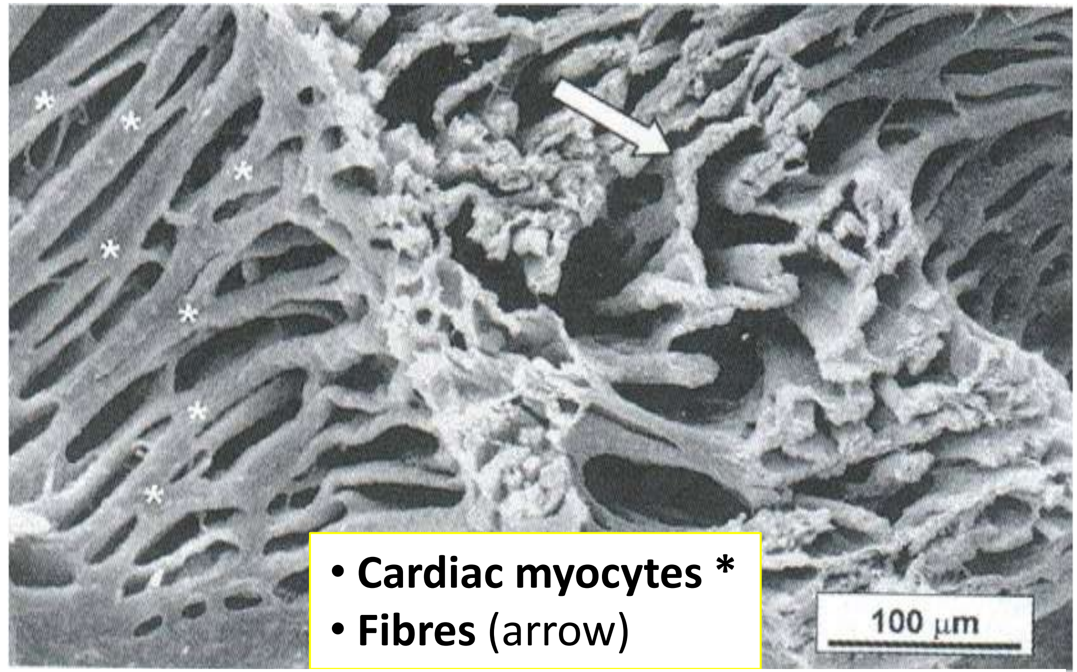
White arrow = direction of blood flow

Trabeculae

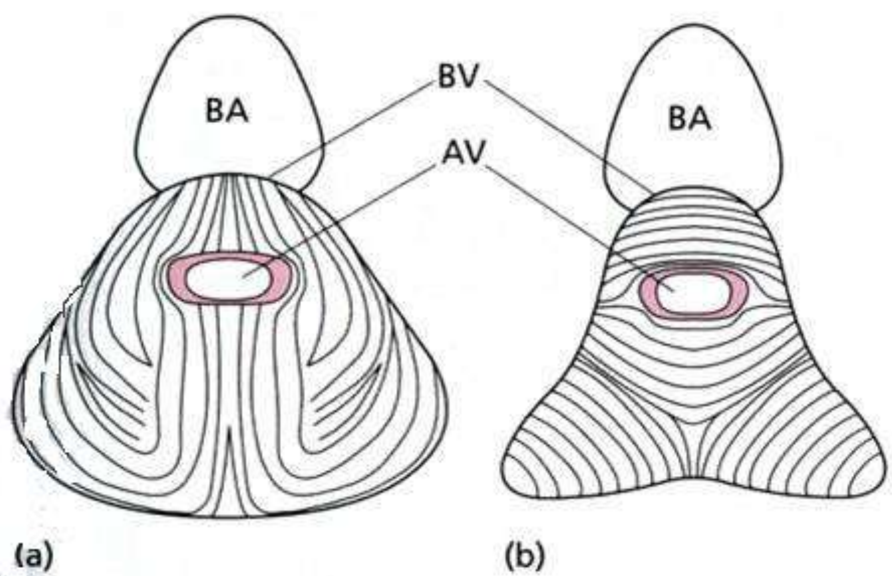
Spongy myocardium of the ventricle

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

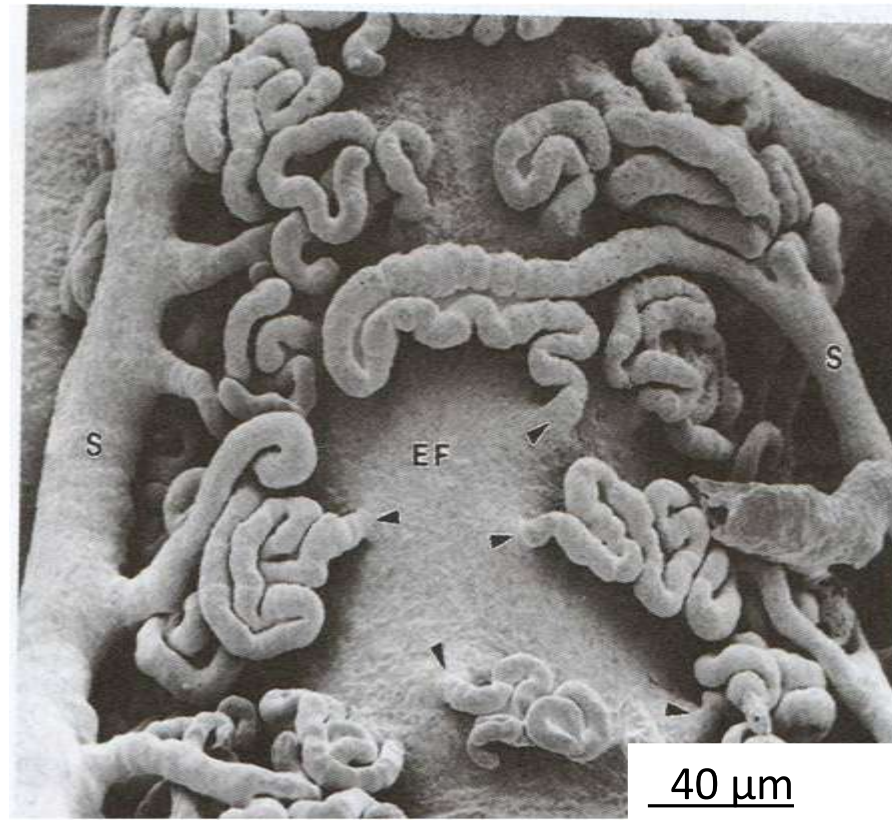


BA = *Bulbus arteriosus*
AV = Atrioventricular valve
BV = Bulboventricular ring
(a)= outer layer
(b)= inner layer

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?



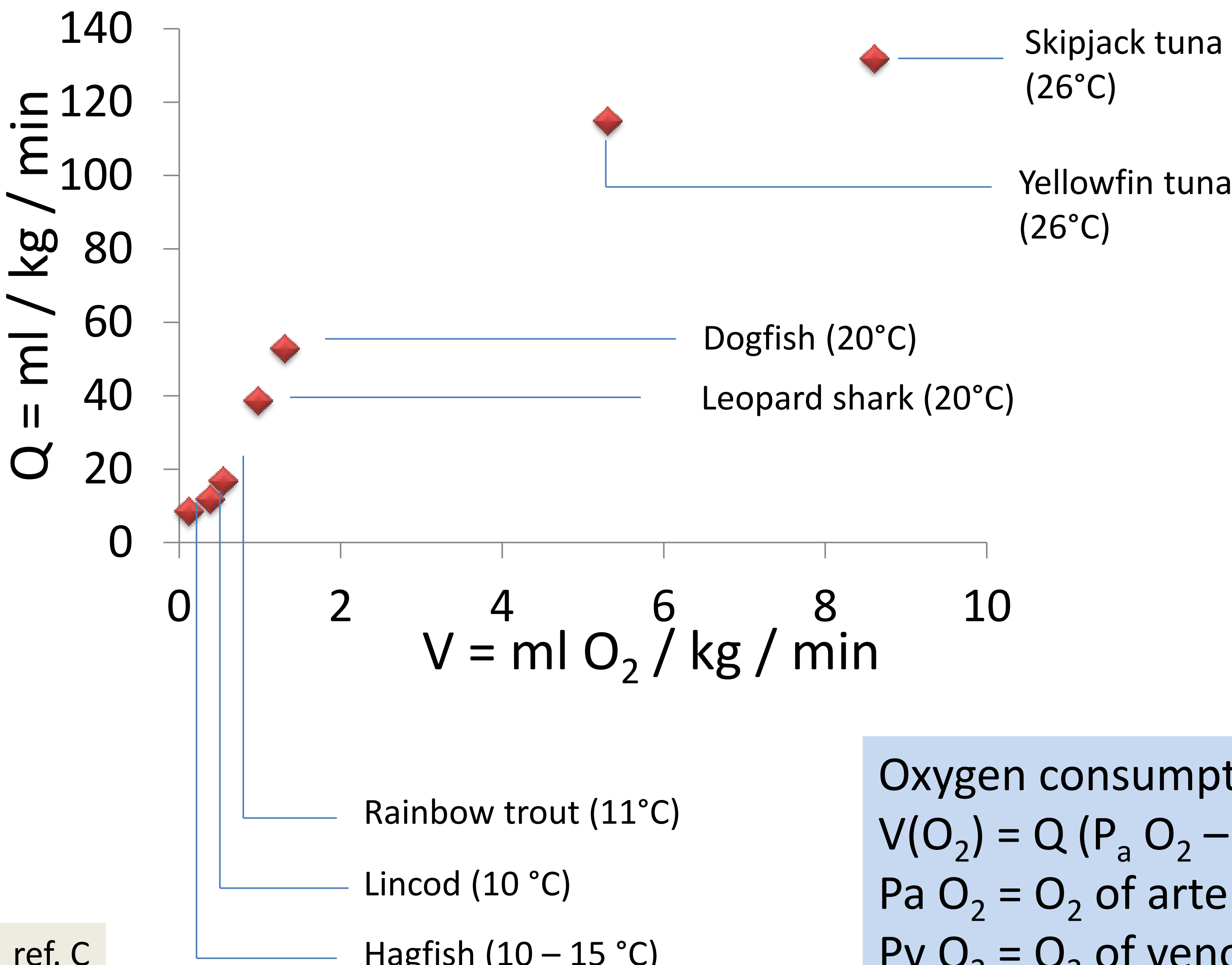
ref. C

Blood flow – the basic definitions

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

$$F = \frac{\pi r^4 \delta P}{8 L \eta}$$

Oxygen consumption (V) and cardiac output (Q)



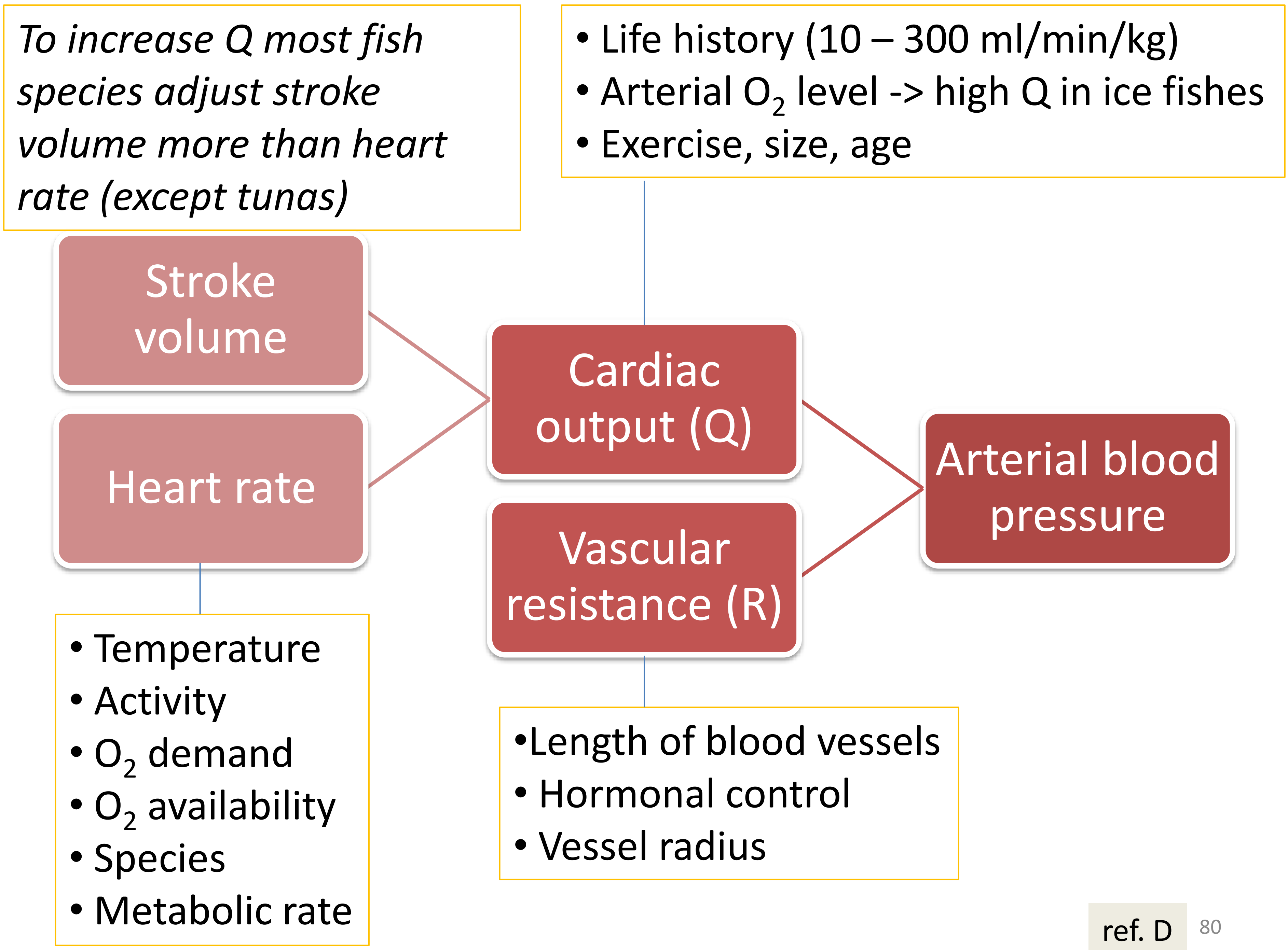
ref. C

Oxygen consumption

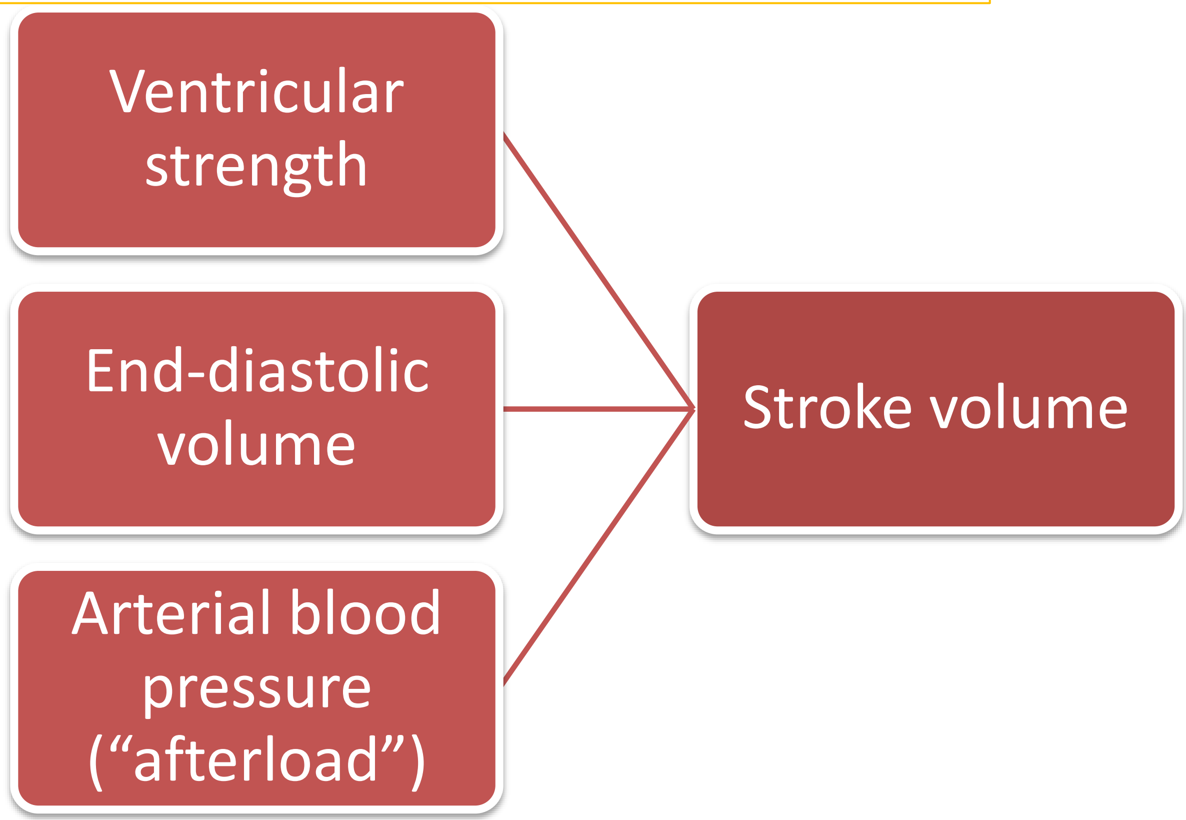
$V(O_2) = Q\,(P_a\,O_2 - P_v\,O_2)$

$Pa\ O_2 = O_2$ of arterial blood

$Pv\ O_2 = O_2$ of venous blood


To increase Q most fish species adjust stroke volume more than heart rate (except tunas)
• Life history (10 – 300 ml/min/kg)
• Arterial O_2 level -> high Q in ice fishes
• Exercise, size, age
Stroke volume
Heart rate
Cardiac output (Q)
Vascular resistance (R)
Arterial blood pressure
• Temperature
• Activity
• O_2 demand
• O_2 availability
• Species
• Metabolic rate
•Length of blood vessels
• Hormonal control
• Vessel radius
ref. D

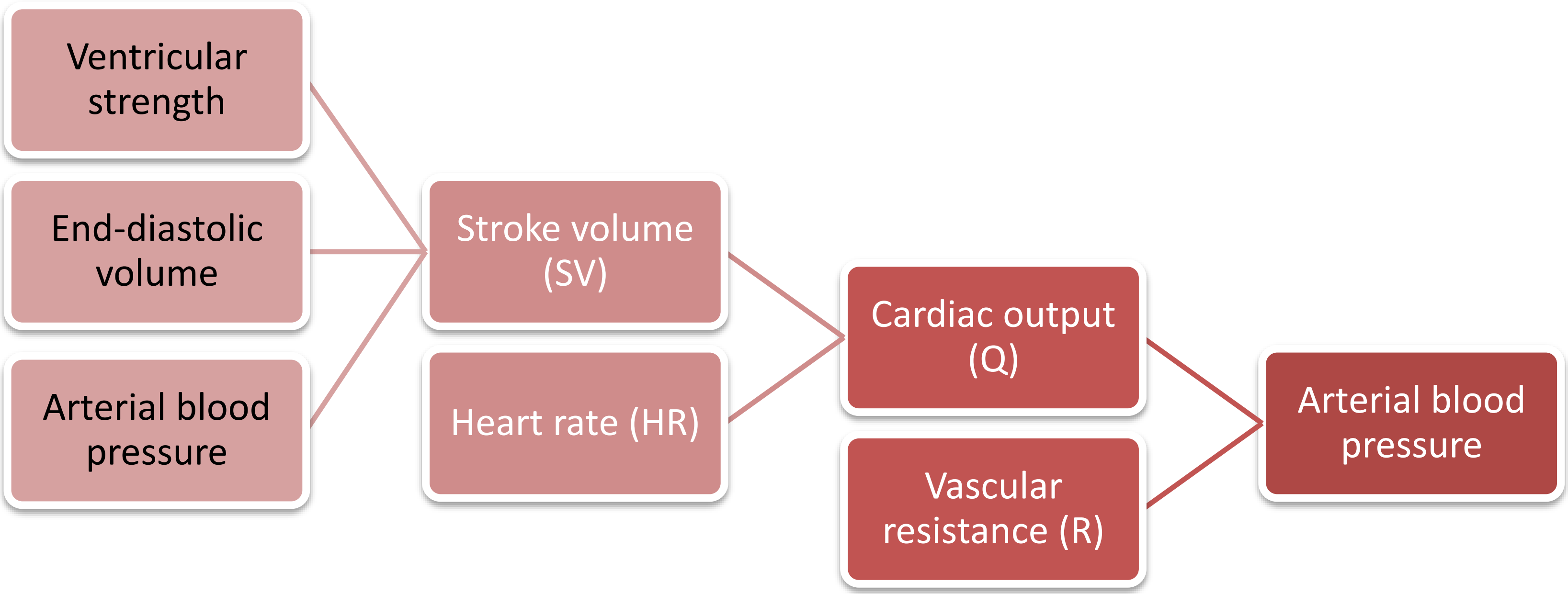
- Energy of contraction is a function of muscle fibre length.
- A fish heart can pump almost its own volume
- Greater ventricular filling = greater stroke volume



- Filling time
- Filling pressure

- Operational pressure (mm Hg) is species specific

ref. D



Examples

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

ref. D

Endocrinology

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

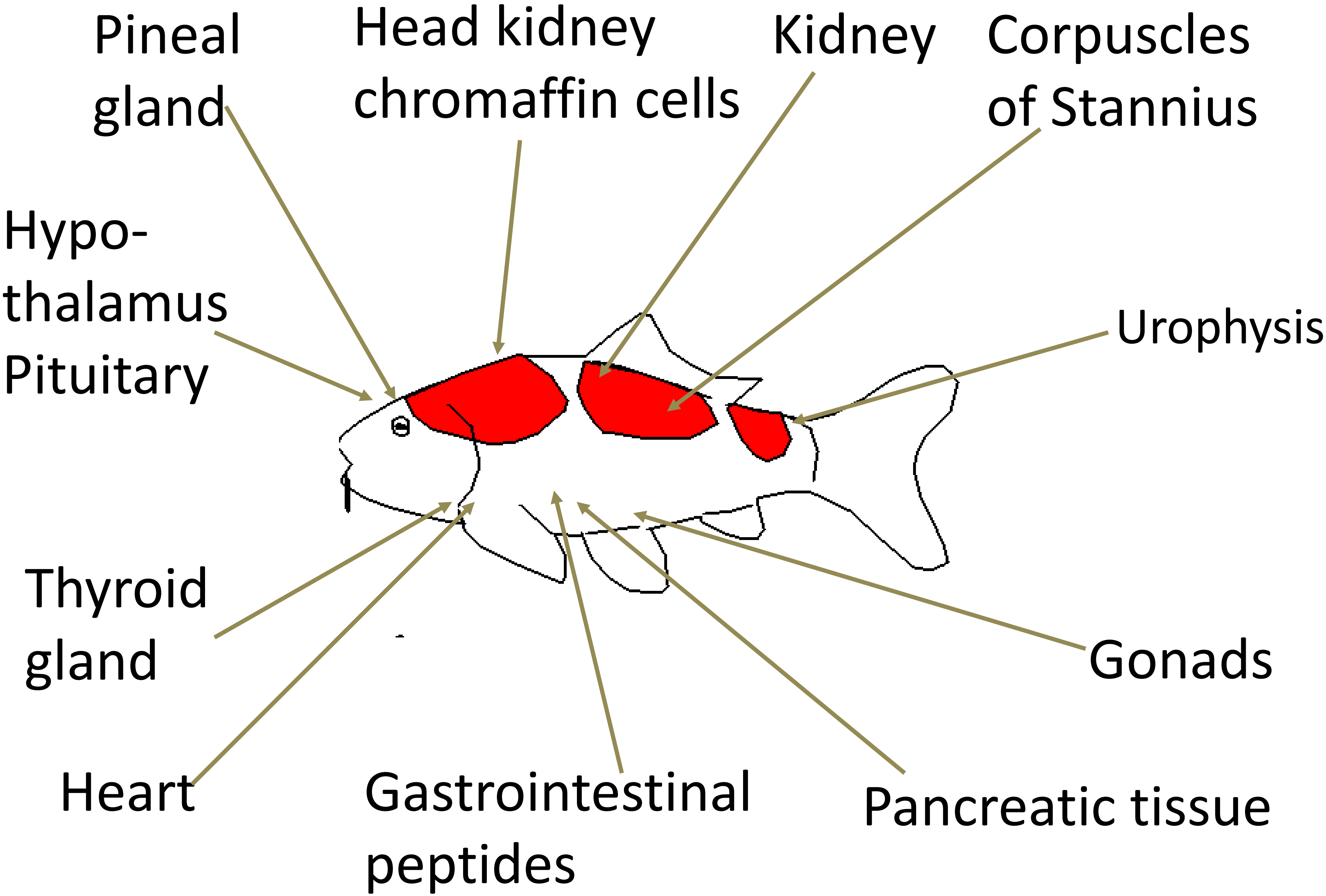
Richly vascularised

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
Head kidney chromaffin cells
Kidney
Corpuscles of Stannius
Hypo-thalamus Pituitary
Urophysis
Thyroid gland
Gonads
Heart
Gastrointestinal peptides
Pancreatic tissue

Hormones as messengers

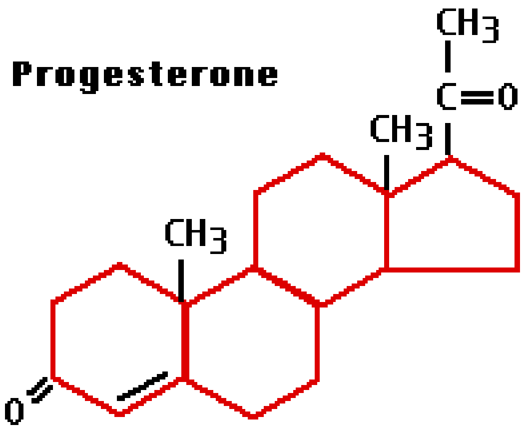
- Endocrine
  - Affect distant parts in the body
  - Transported via the circulating blood
- Paracrine
  - Local effects / diffusion
- Pheromonal
  - Secretion into the environment
- Autocrine
  - Work in the cell in which they are produced

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.

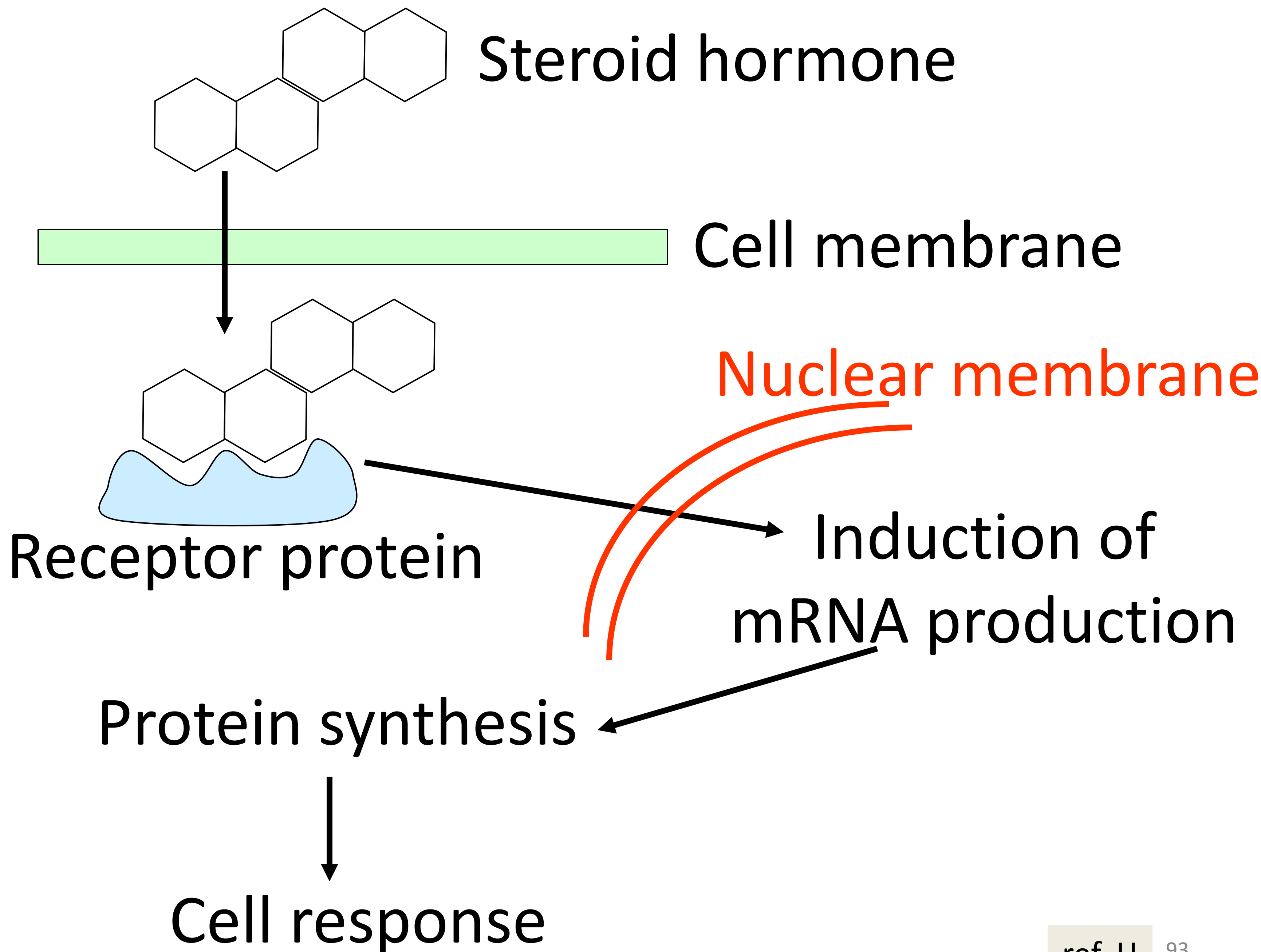
Classification of hormones

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



Steroids / steroid-related hormones

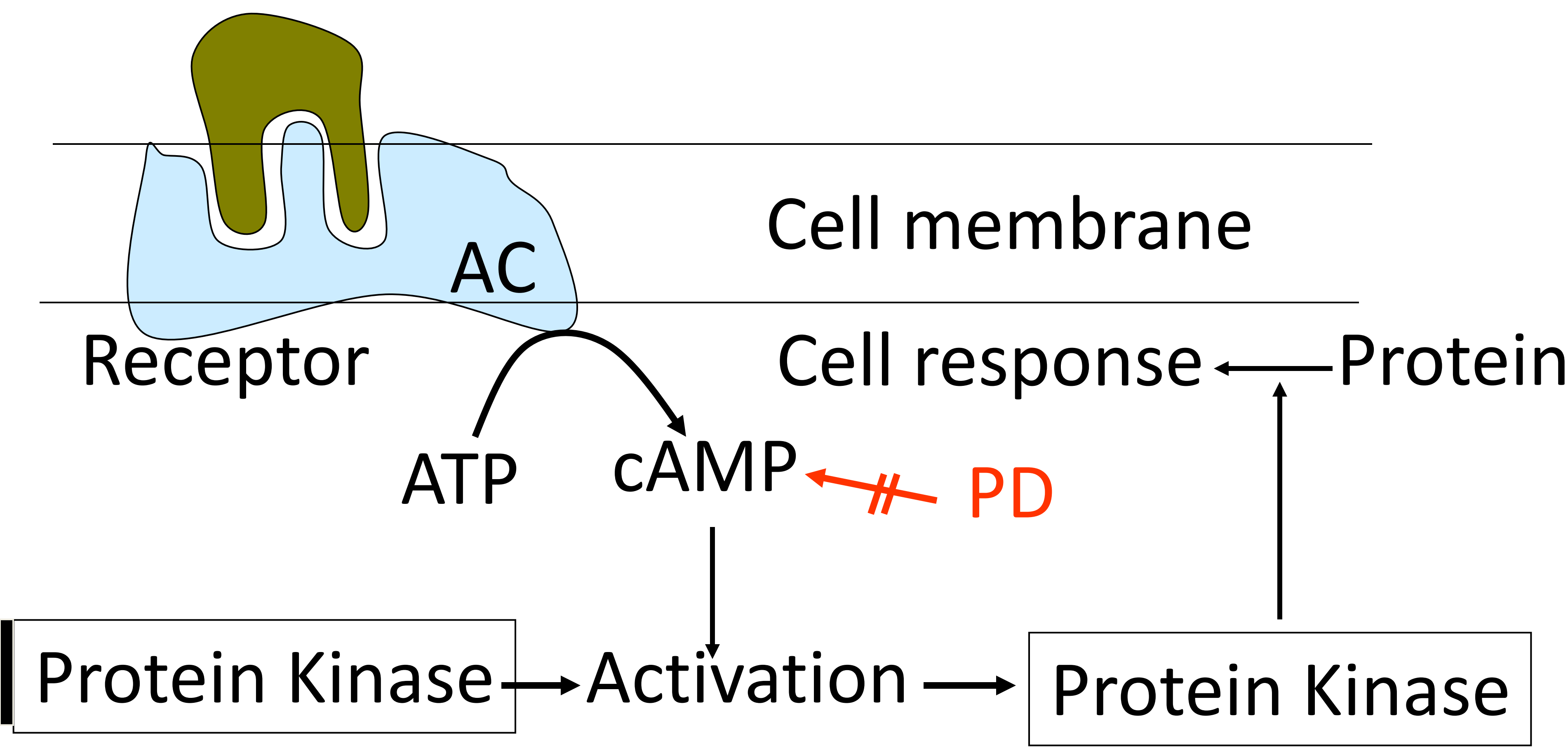
- Lipid soluble.
- All steroids are cholesterol derivates. 4-ring structure.
- Producing organs: Mainly gonads, but in fish also adrenal cortex.


Steroid hormone
Cell membrane
Nuclear membrane
Receptor protein
Induction of mRNA production
Protein synthesis
Cell response

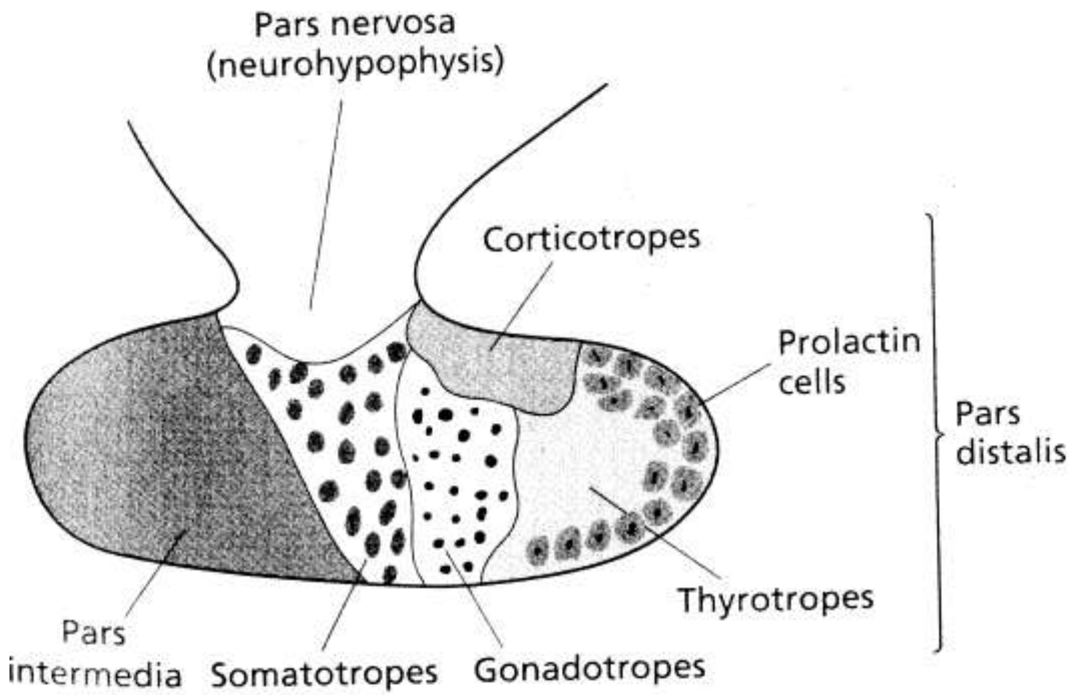
ref. H

AC = Adenylate cyclase
cAMP = cyclic AMP
PD = Phosphodiesterase

Peptide hormone



ref. H

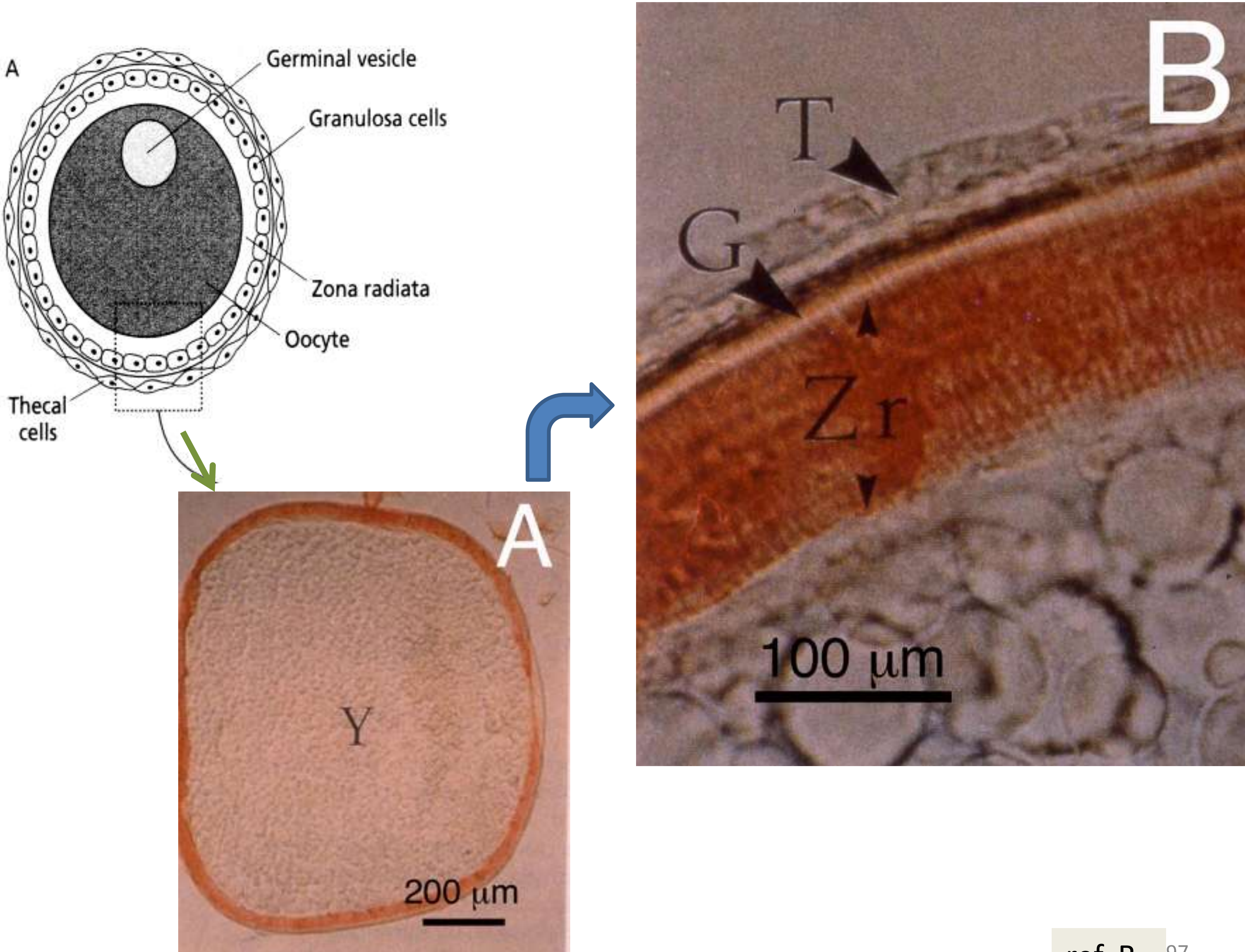


Generalized diagram of different regions and cellular zonation of trophic hormone producing cells in the adenohypophysis of a teleost fish.

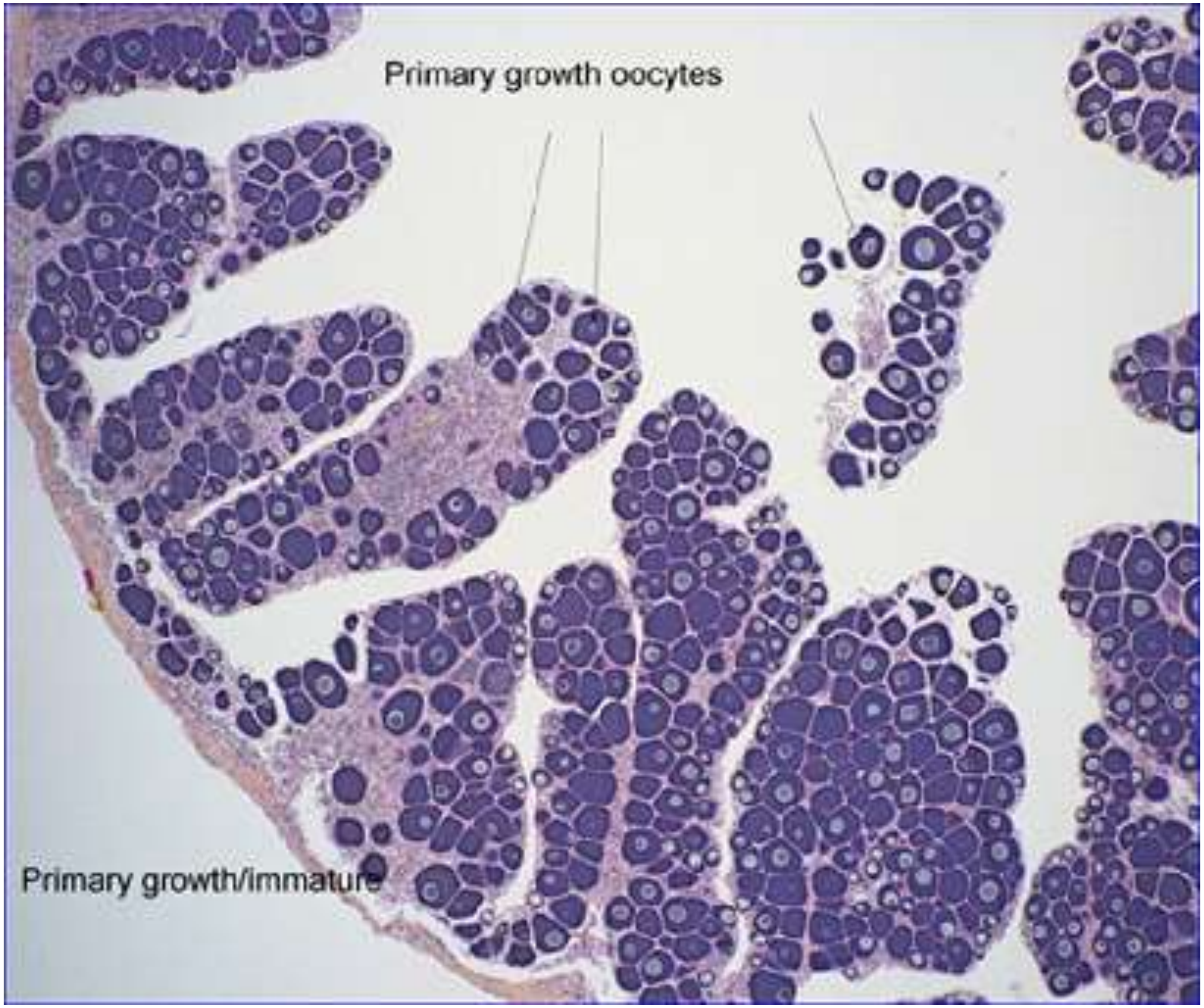
ref. B

Endocrinology of reproduction

Maturation and ovulation of oocytes


A
Germinal vesicle
Granulosa cells
Zona radiata
Oocyte
Thecal cells
A
Y
200 µm
B
T
G
Zr
100 µm

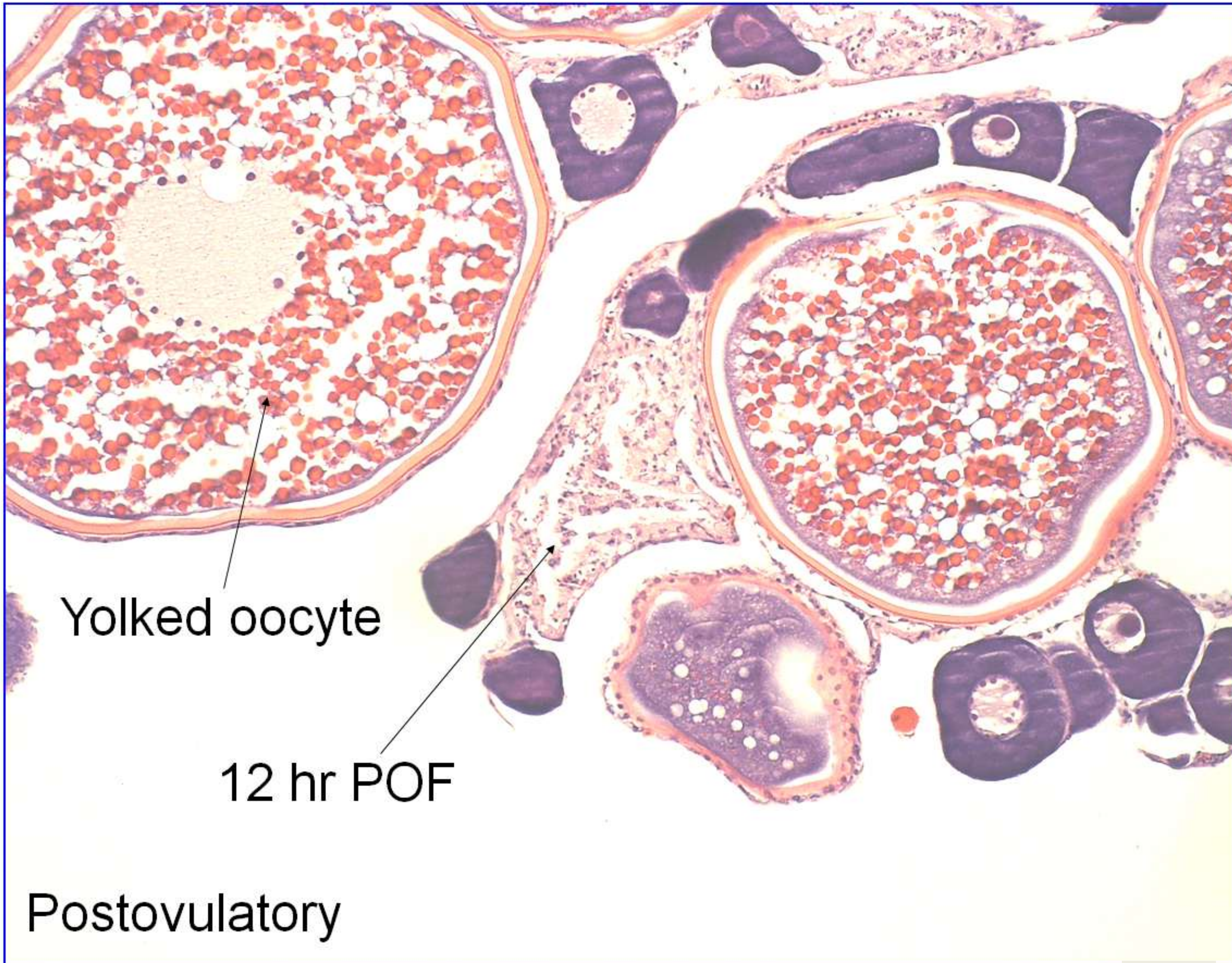
ref. B,



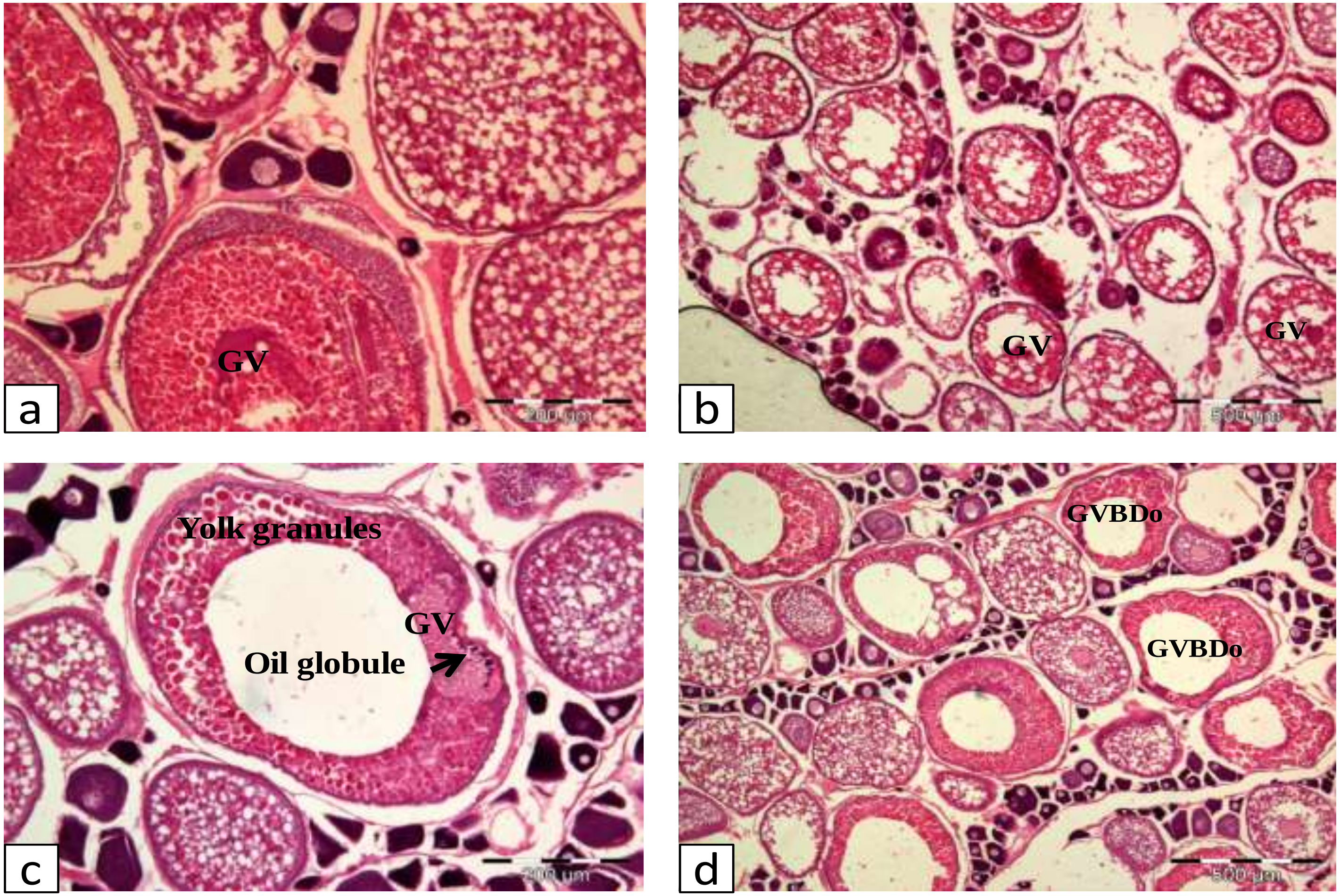
An Overview of the Histological Study of Marine Finfish; Fish and Wildlife Research Institute, Florida, USA;

ref. I

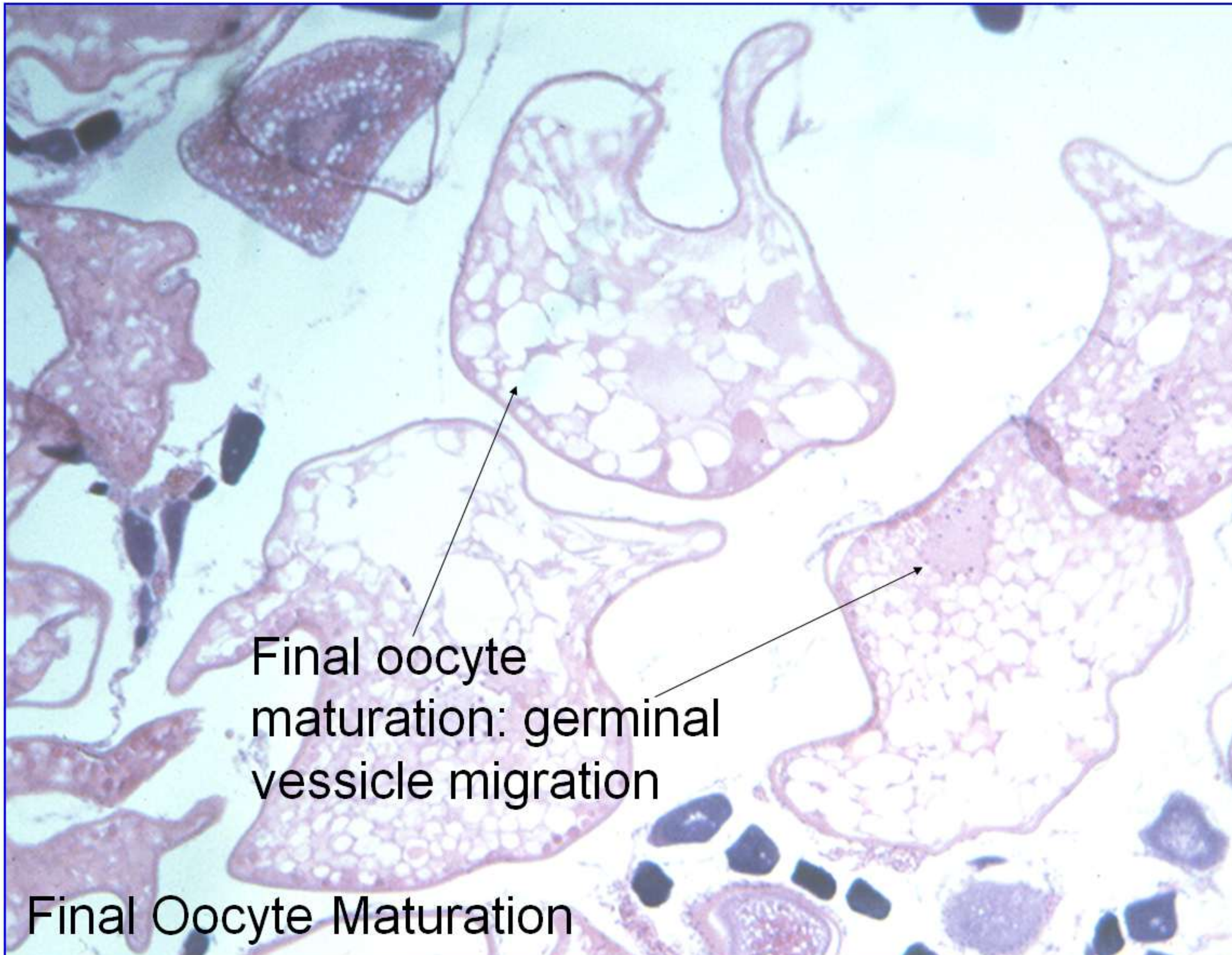
ref. I



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, GVBDo = germinal yolk vesicle break-down oocyte


Final oocyte
maturation: germinal
vessicle migration
Final Oocyte Maturation

Examples for predictive factors

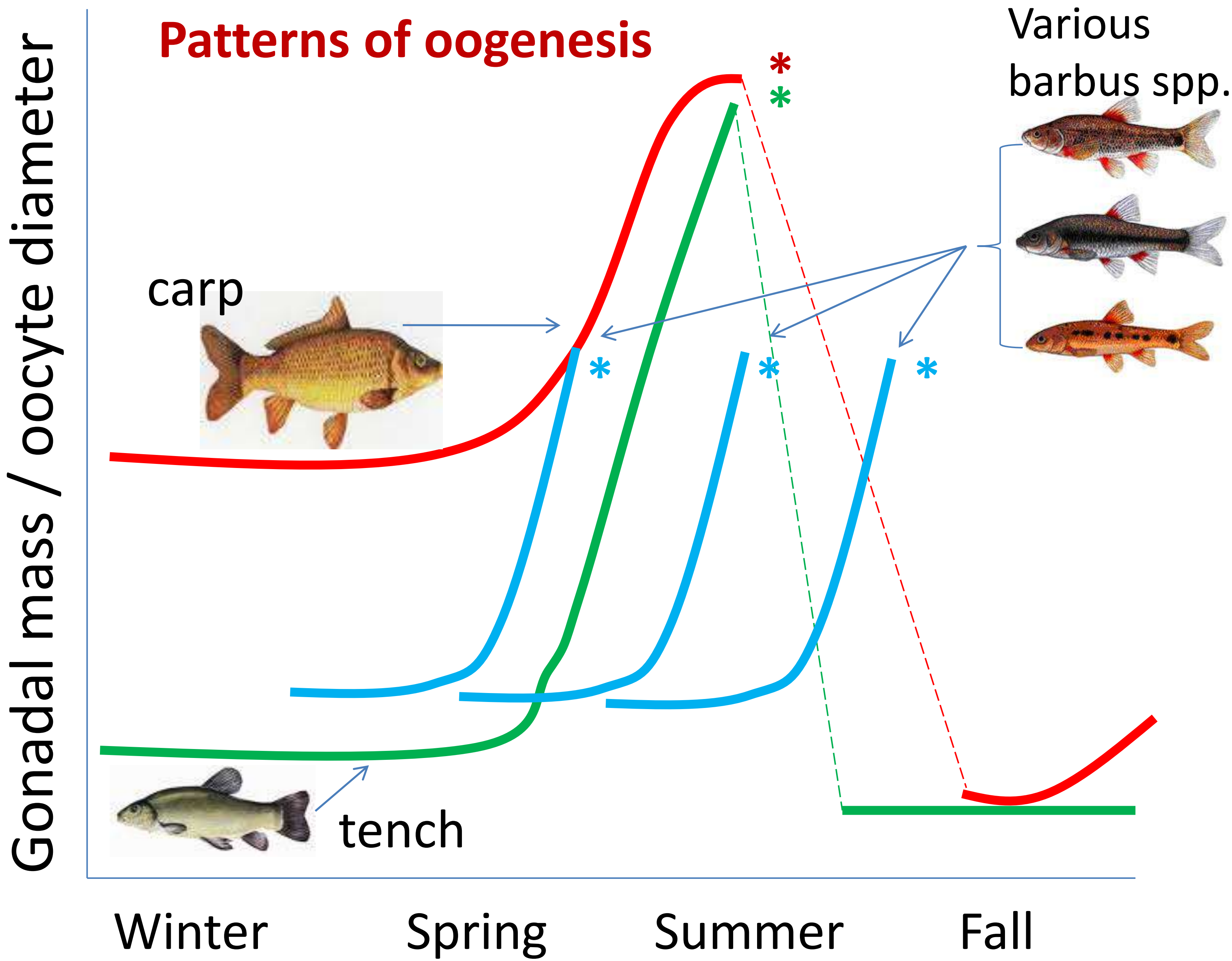
- Changes in day length
- Slow changes in temperature
- Other factors in the environment depending on the stages of gonadal development

Modifying factors

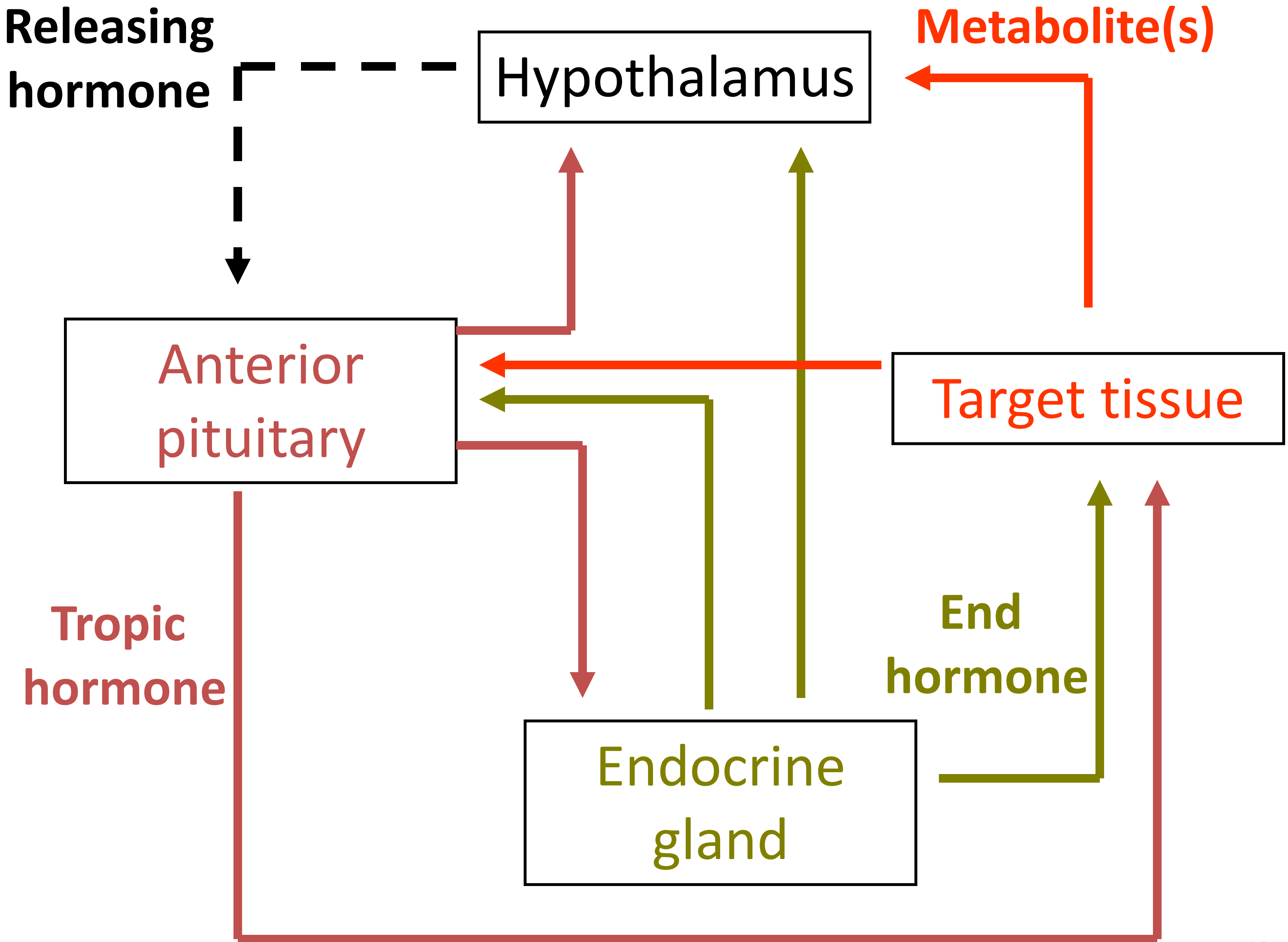
- Water quality
- Lunar cycle
- Broodstock nutrition
- Social interactions

Synchronising cues

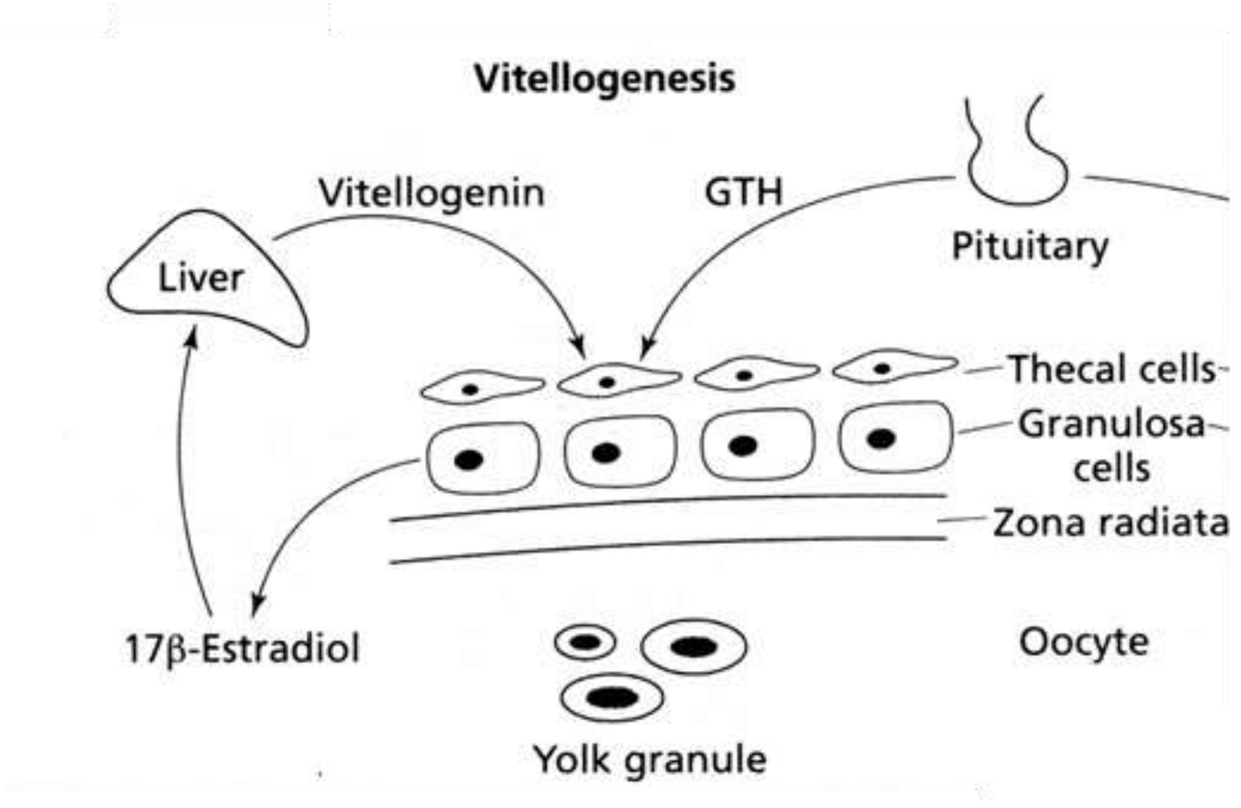
- Water quality changes
- Floods
- Rapid temperature changes
- Atmospheric changes

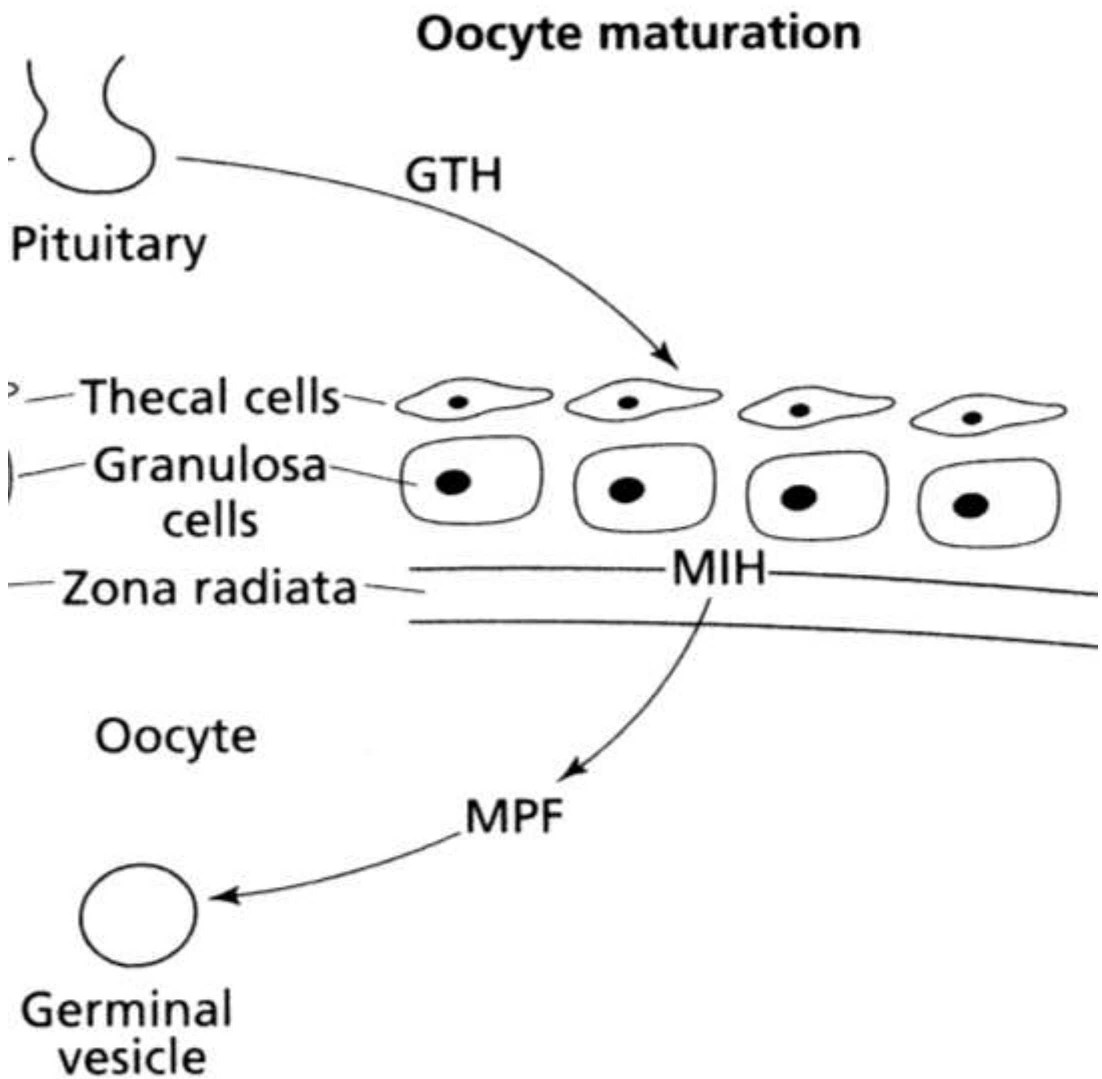


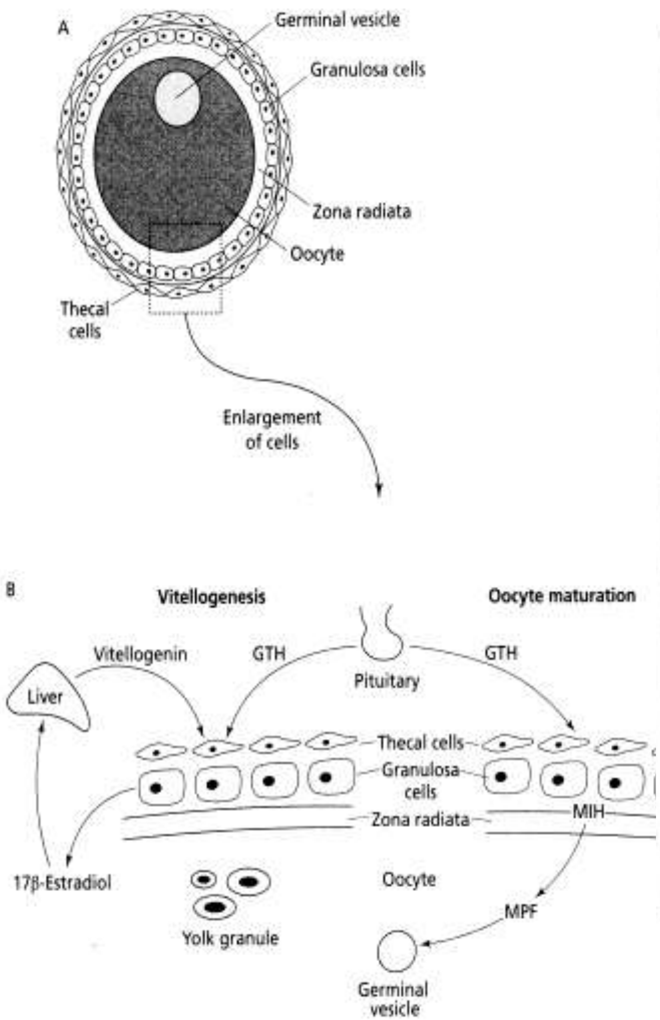
Patterns of oogenesis
Gonadal mass / oocyte diameter
carp
tench
Various
barbus spp.
Winter
Spring
Summer
Fall

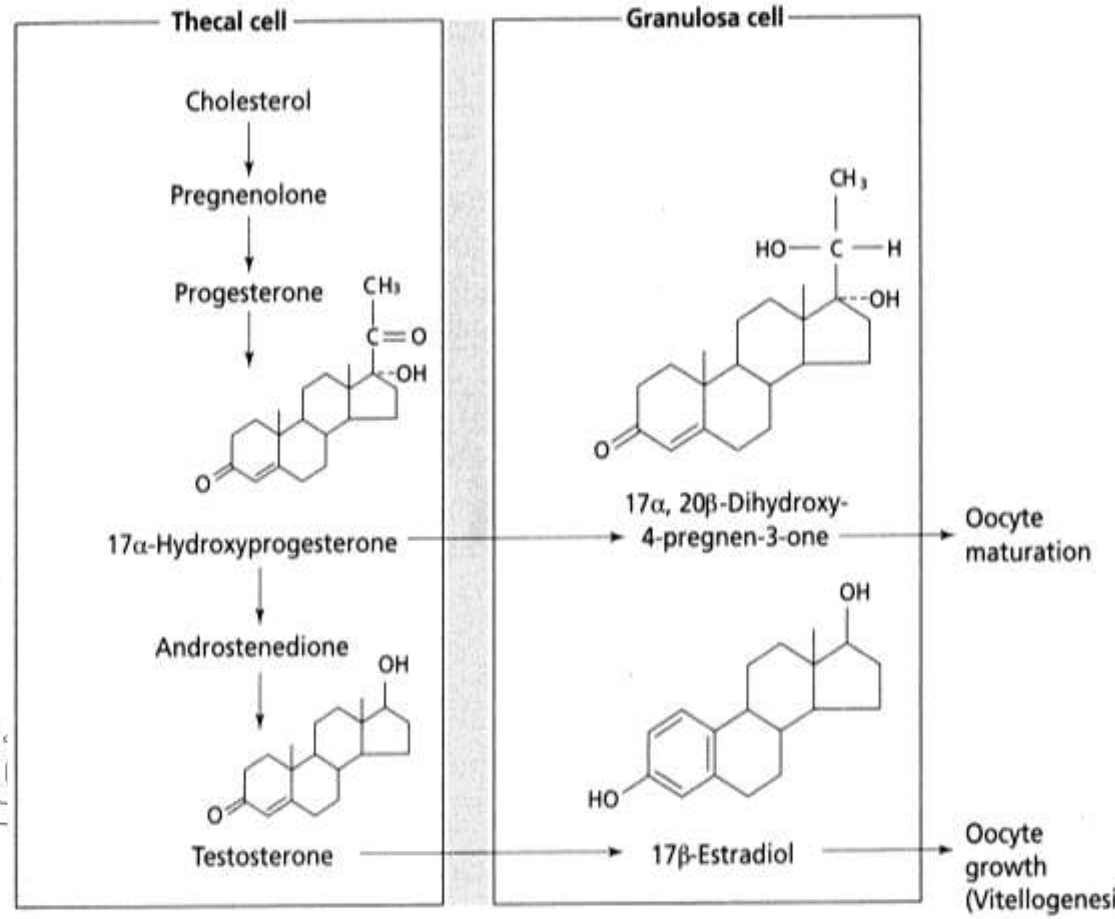


Adopted from Jobling 1998

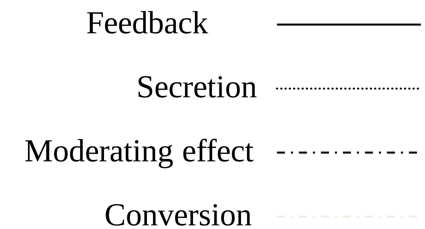

Vitellogenesis
Vitellogenin
GTH
Pituitary
Liver
Thecal cells
Granulosa cells
Zona radiata
17β-Estradiol
Oocyte
Yolk granule

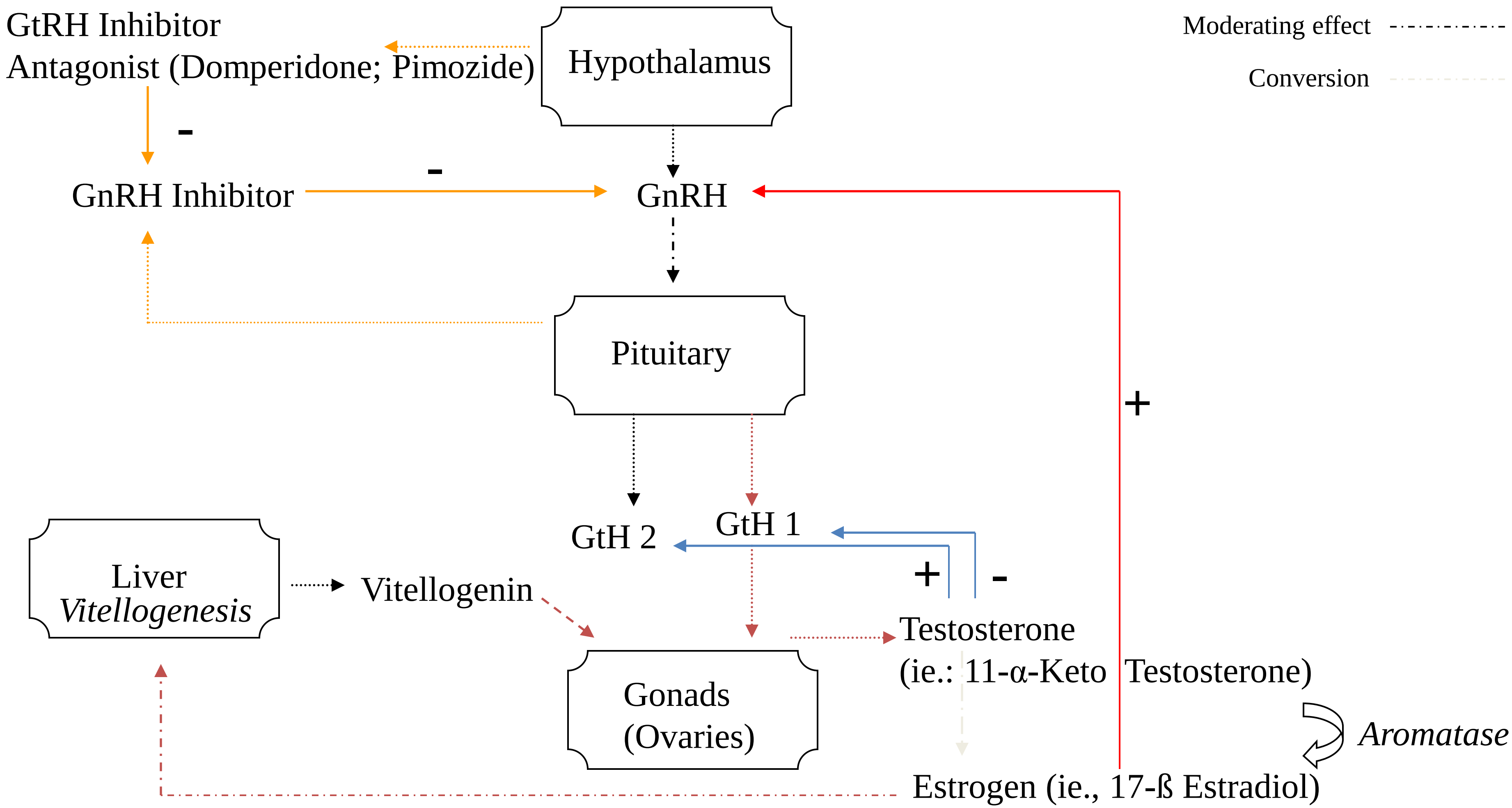

Oocyte maturation
GTH
Pituitary
Thecal cells
Granulosa
cells
MIH
Zona radiata
Oocyte
MPF
Germinal
vesicle


A
Germinal vesicle
Granulosa cells
Zona radiata
Oocyte
Thecal cells
Enlargement of cells
B
Vitellogenesis
Oocyte maturation
Vitellogenin
GTH
GTH
Pituitary
Liver
Thecal cells
Granulosa cells
Zona radiata
MIH
17β-Estradiol
Oocyte
MPF
Yolk granule
Germinal vesicle


Thecal cell
Granulosa cell
Cholesterol
Pregnenolone
Progesterone
17α-Hydroxyprogesterone
Androstenedione
Testosterone
17α, 20β-Dihydroxy-4-pregnen-3-one
17β-Estradiol
Oocyte maturation
Oocyte growth (Vitellogenesis

Summary of oocyte maturation / ovulation





GtH = Gonadotropin Hormone

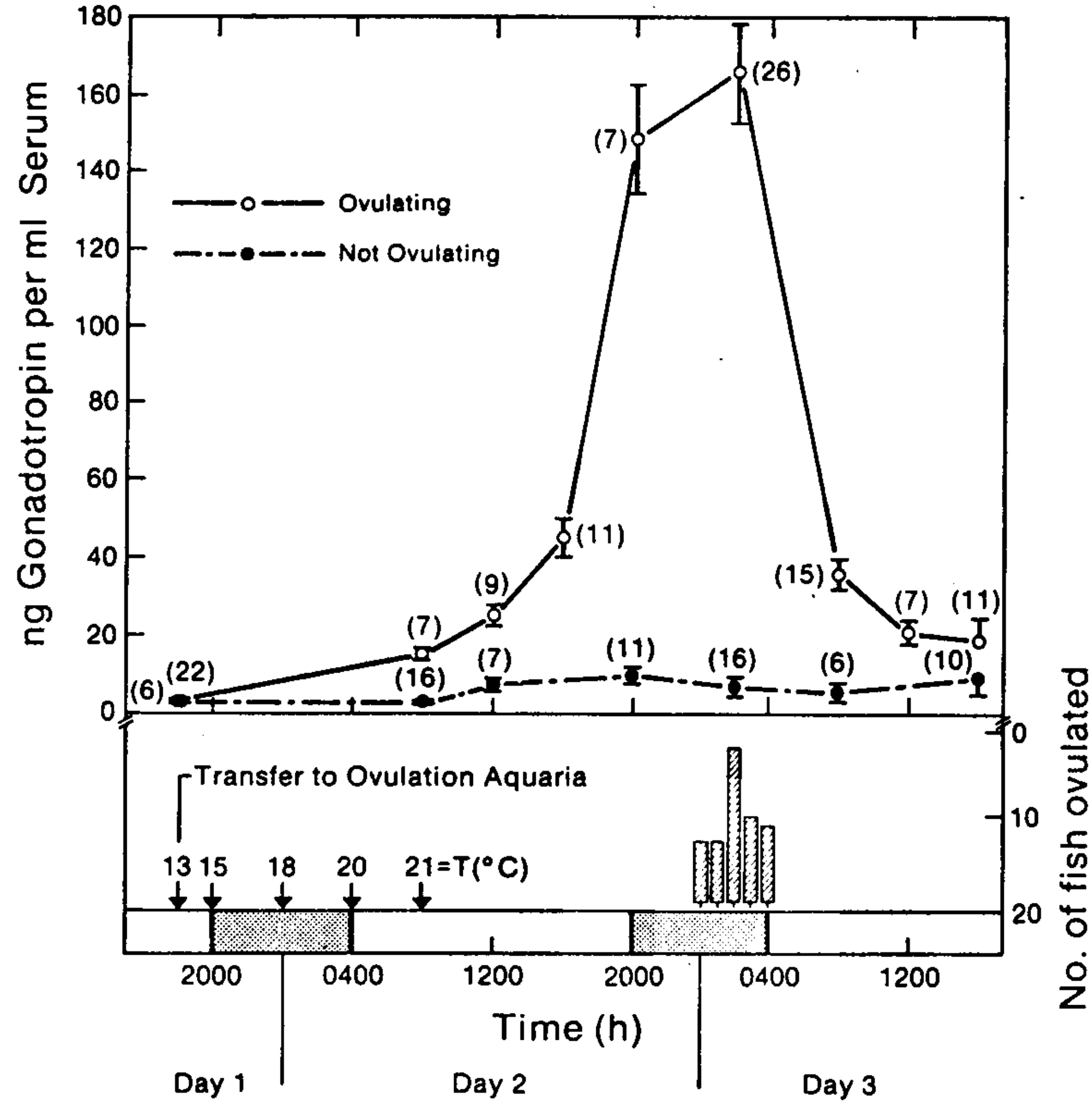
GnRH = Gonadotropin-Releasing Hormone

Case example 1: Goldfish (*Carassius auratus*)

- Environmental cues
- Behavioural cues
- Endocrinological control
- Pheromonal control

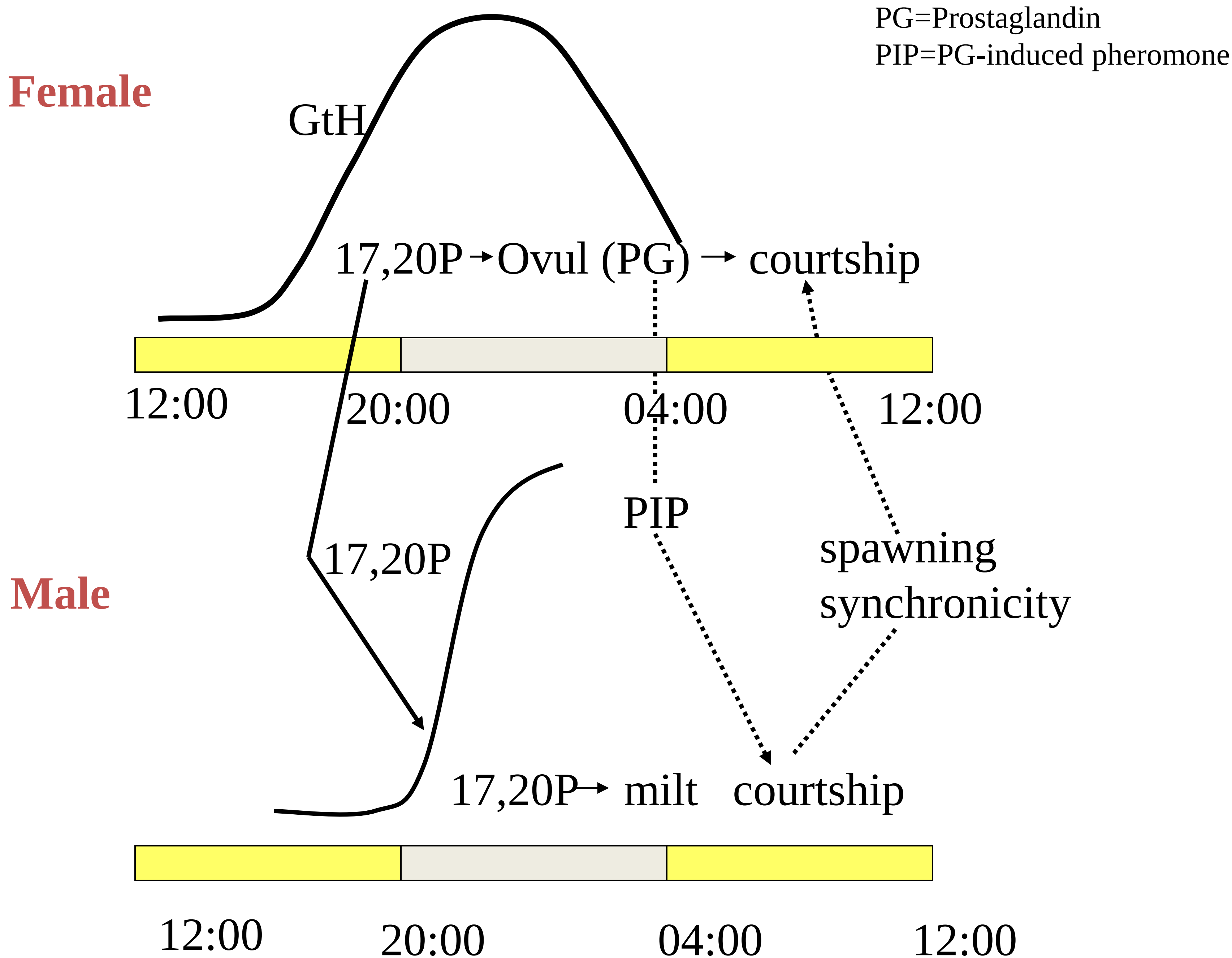
Aspects of goldfish reproduction

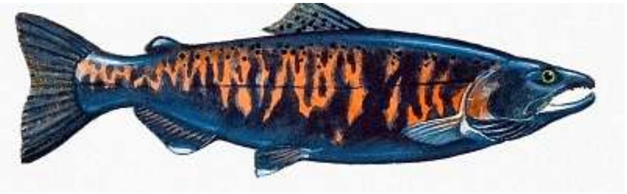
- Pre-ovulatory waiting phase
- Influence of a diurnal rhythm
- Spawning follows ovulation very tightly
- Three cues for initiation of ovulation:
  - Vegetation
  - Temperature
  - Presence of males
- But scotophase is an overriding factor



ng Gonadotropin per ml Serum
Ovulating
Not Ovulating
Transfer to Ovulation Aquaria
13 15 18 20 21=T(°C)
Time (h)
Day 1
Day 2
Day 3
No. of fish ovulated

ref. J


PG=Prostaglandin
PIP=PG-induced pheromone
Female
GtH
17,20P → Ovul (PG) → courtship
12:00
20:00
04:00
12:00
PIP
17,20P
spawning
synchronicity
Male
17,20P → milt courtship
12:00
20:00
04:00
12:00
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

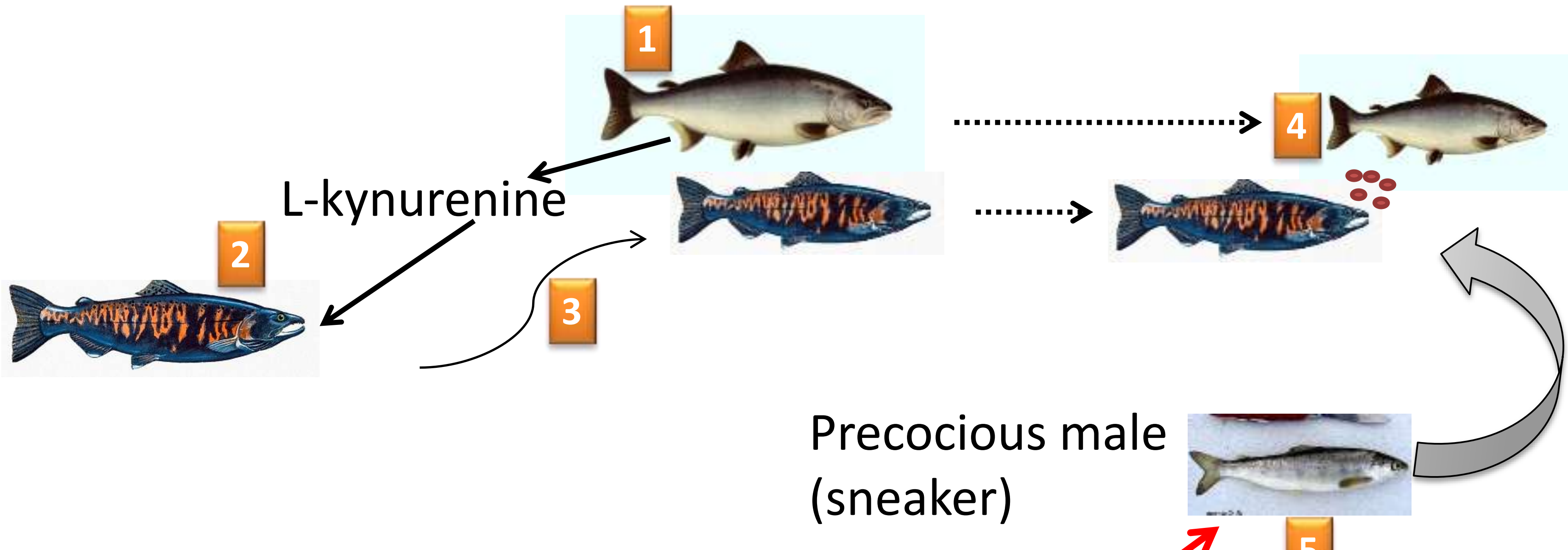
Fresh water	Fresh water	Sea water	Fresh water
			 

Salmon parr: precocious sneakers

Fresh water	Fresh water	Fresh water	Fresh water
			 

Masu salmon: reproductive behaviour

Testosterone -> upstream migration in females 1
Testosterone -> nest digging behaviour in females 4



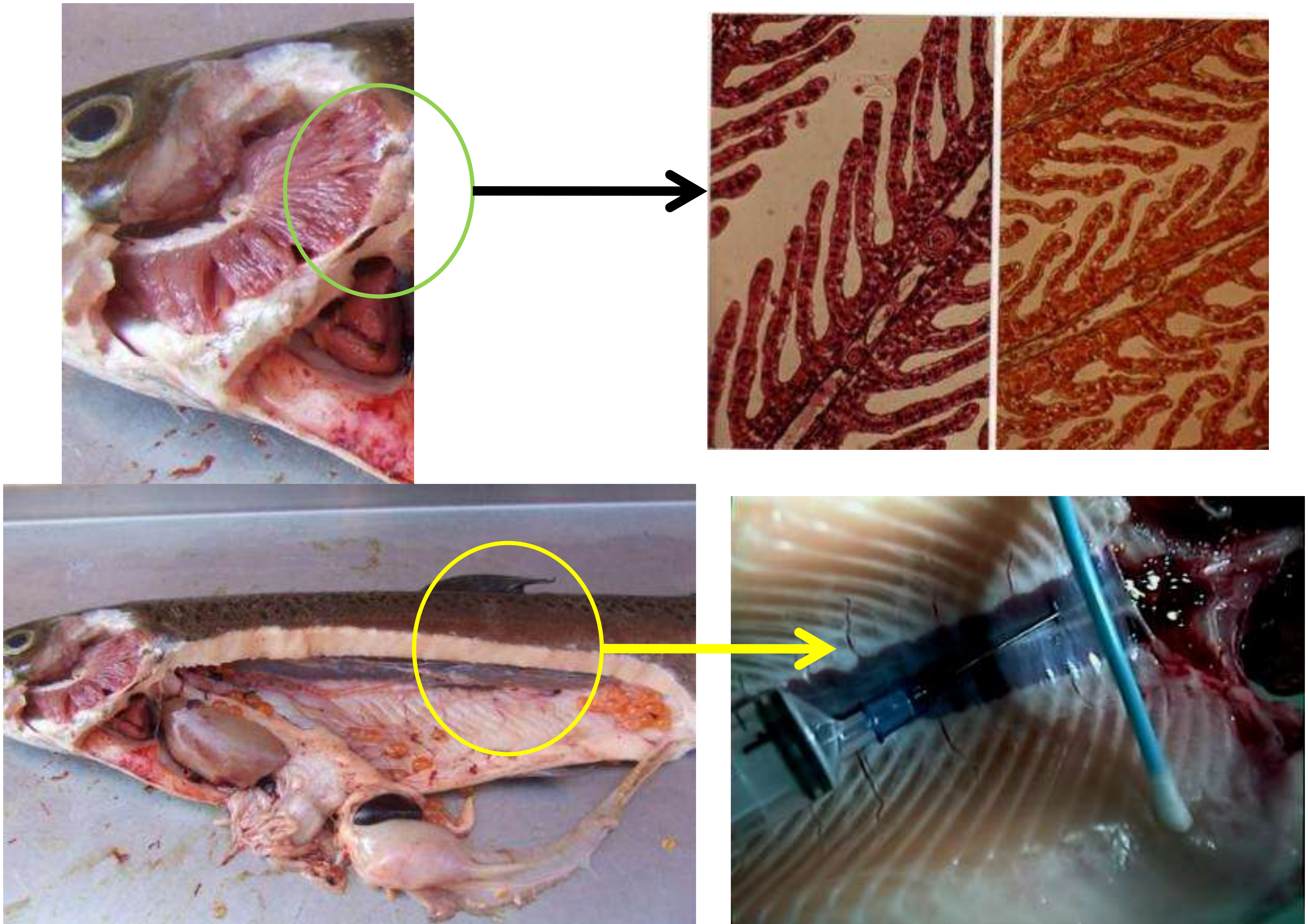
Testosterone -> induces upstream migration in males, including precocious sneakers

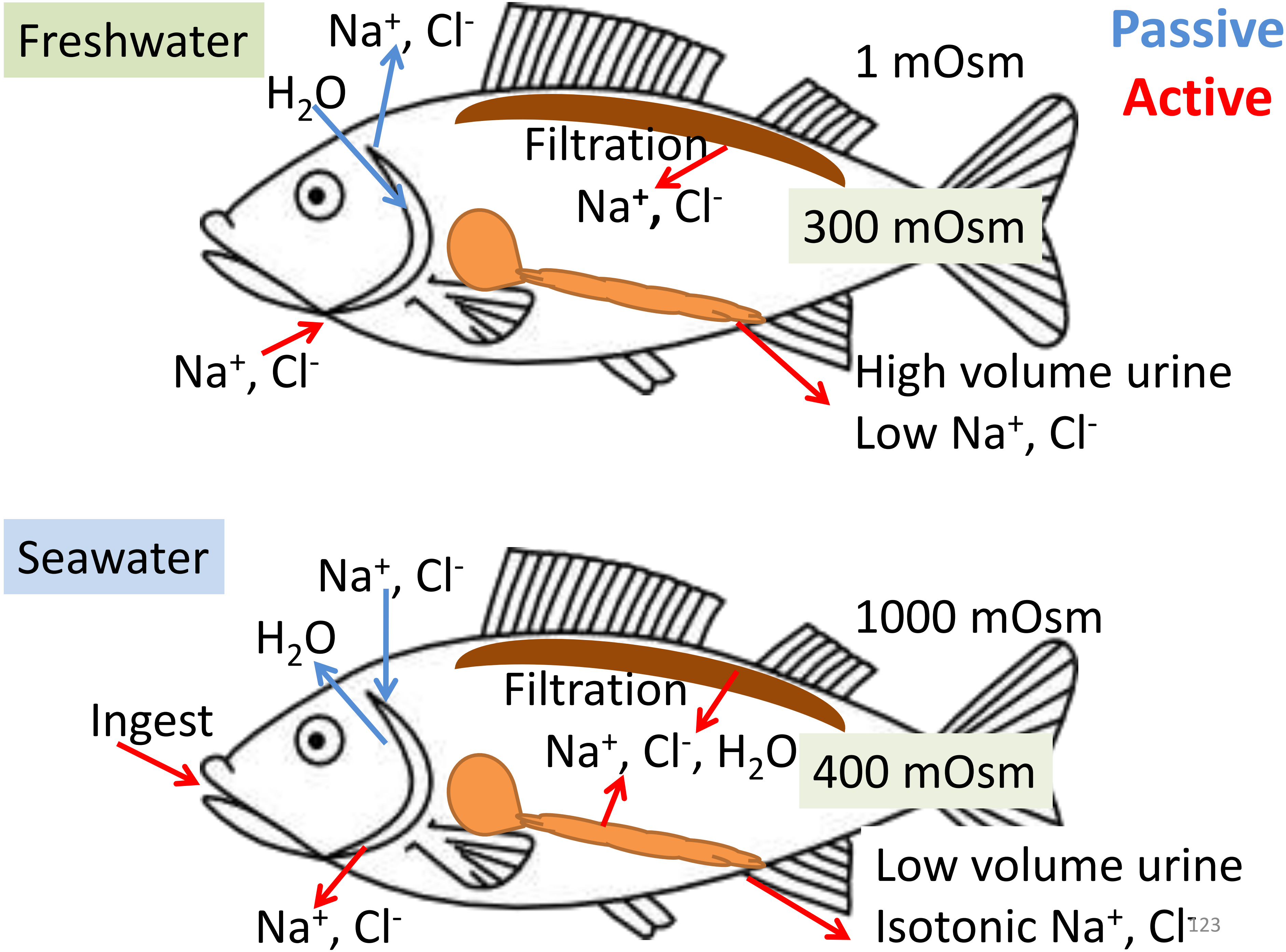
Osmoregulation

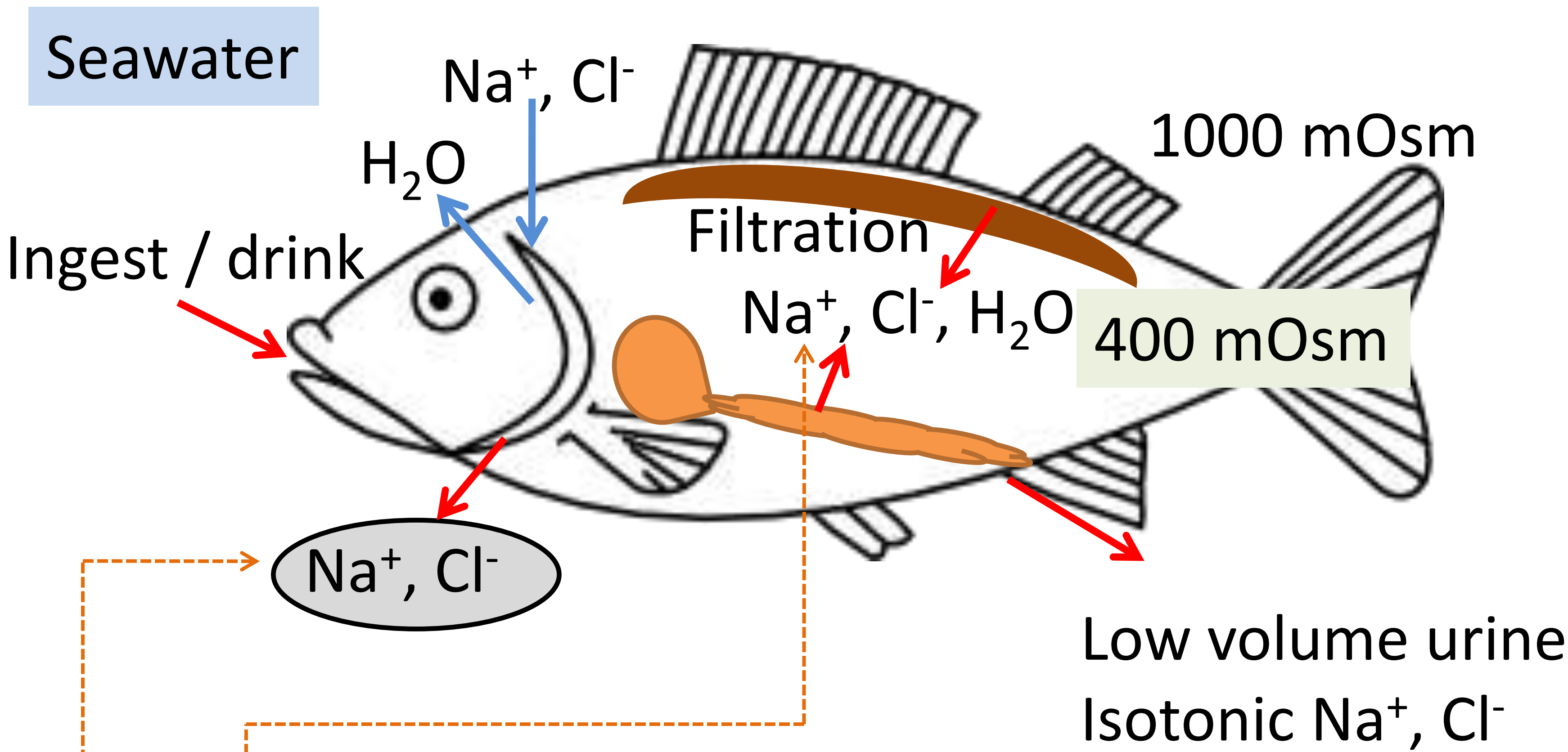
$$Osmol/L = \sum \lambda_i n_i C_i$$

- λ = Osmotic coefficient (ranges from 0 -1)
- n = Number of particles (ions) into which a molecule dissociates (example: NaCl = 2; glucose = 1)
- C = the molar concentration of the solute
- i = The index *i* represents the identity of a particular solute

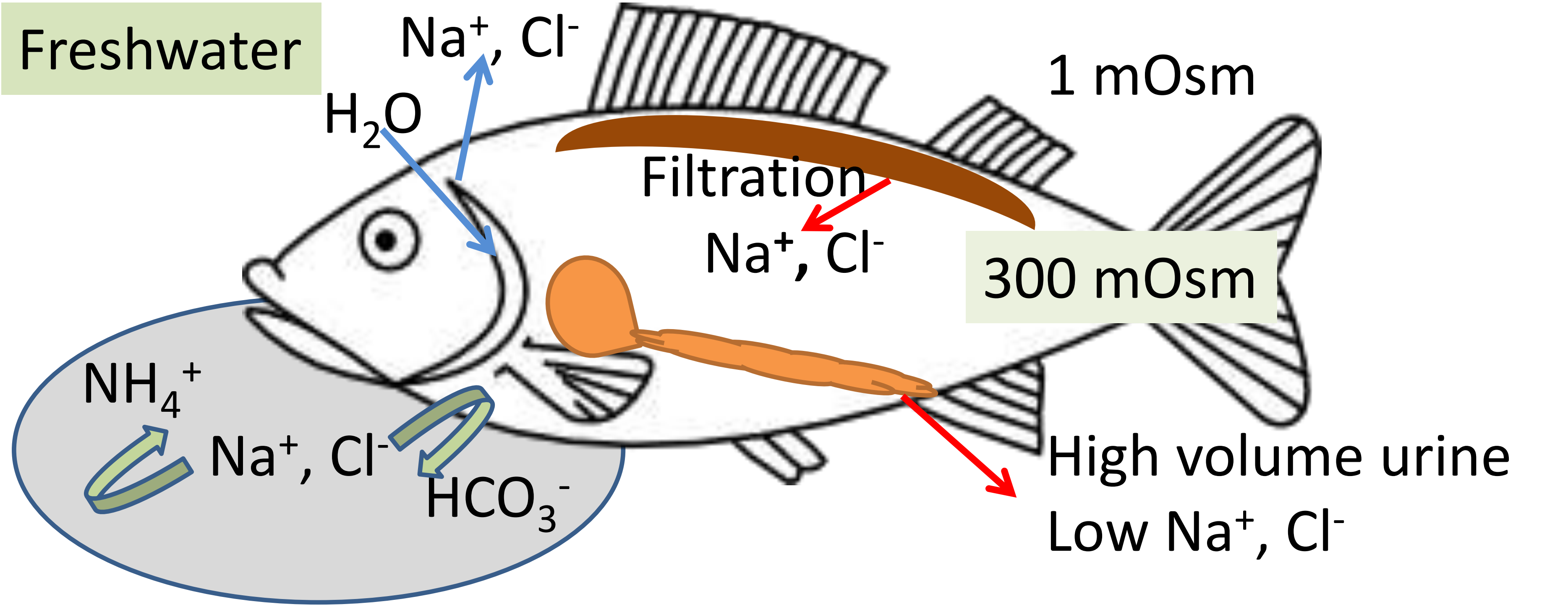
Gills and kidney: Osmoregulatory organs



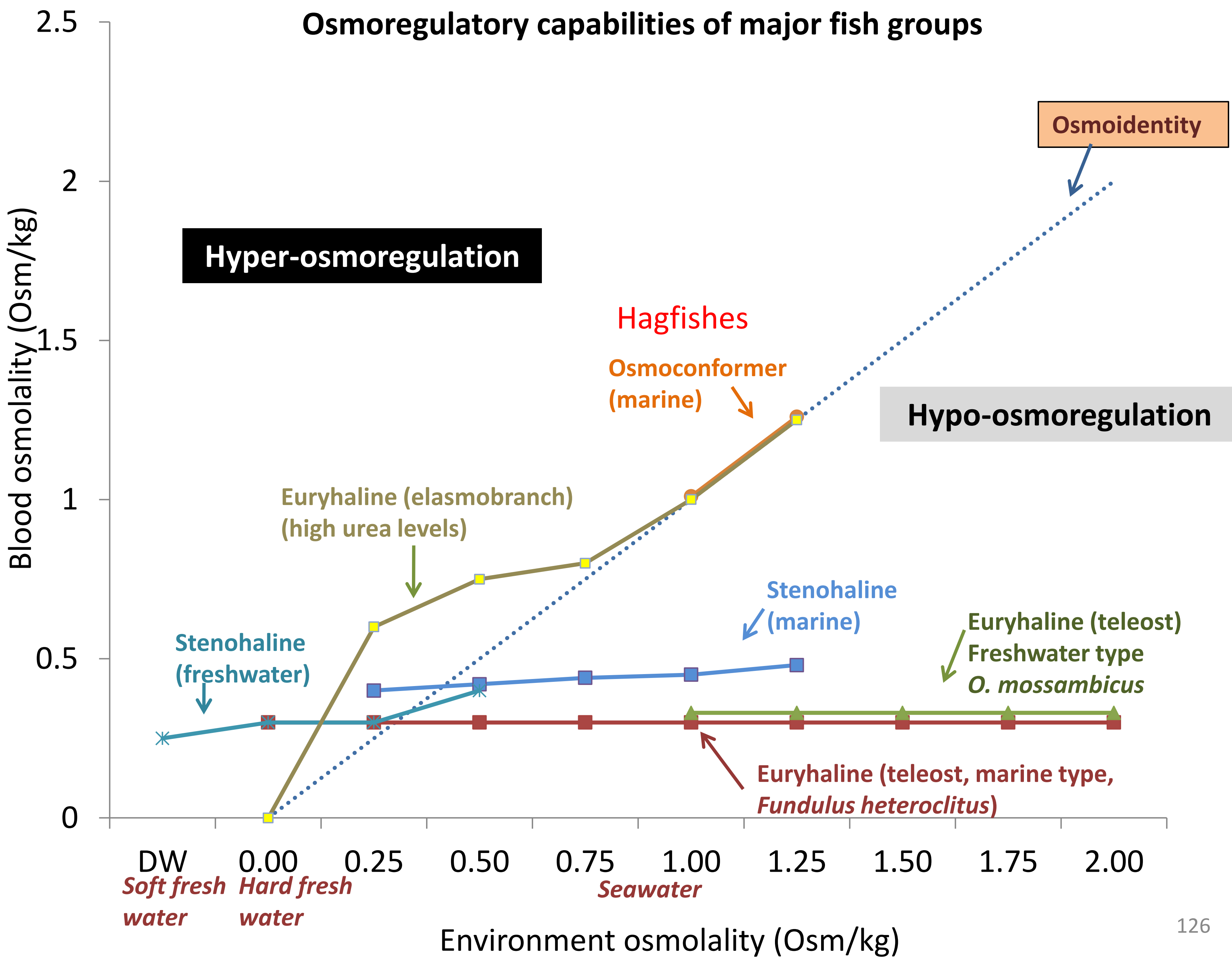

Freshwater
Na^+, Cl^-
H_2O
Filtration
Na^+, Cl^-
1 mOsm
300 mOsm
Na^+, Cl^-
High volume urine
Low Na^+, Cl^-
Passive
Active
Seawater
Na^+, Cl^-
H_2O
Ingest
Filtration
Na^+, Cl^-, H_2O
1000 mOsm
400 mOsm
Na^+, Cl^-
Low volume urine
Isotonic Na^+, Cl^-



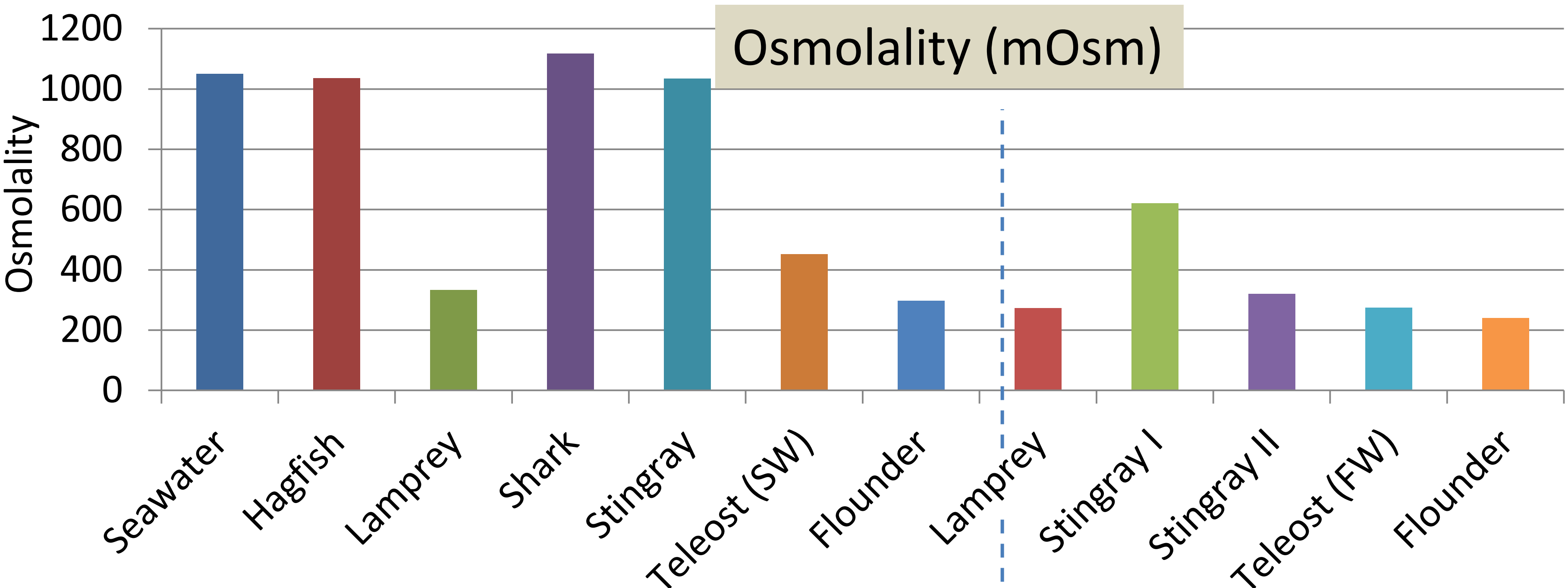
1. Plasma hyposmotic to seawater; approximately 60% lower
2. Constantly dehydrated and loaded with salt
3. Up to 90% of Na^+ and Cl^- is removed in the intestine
4. Dehydration forces organism to excrete small volume of urine
5. Urine is isosmotic to plasma **and** seawater; **not** hyperosmotic
6. There is **active** extra-renal excretion of salts via the gills!

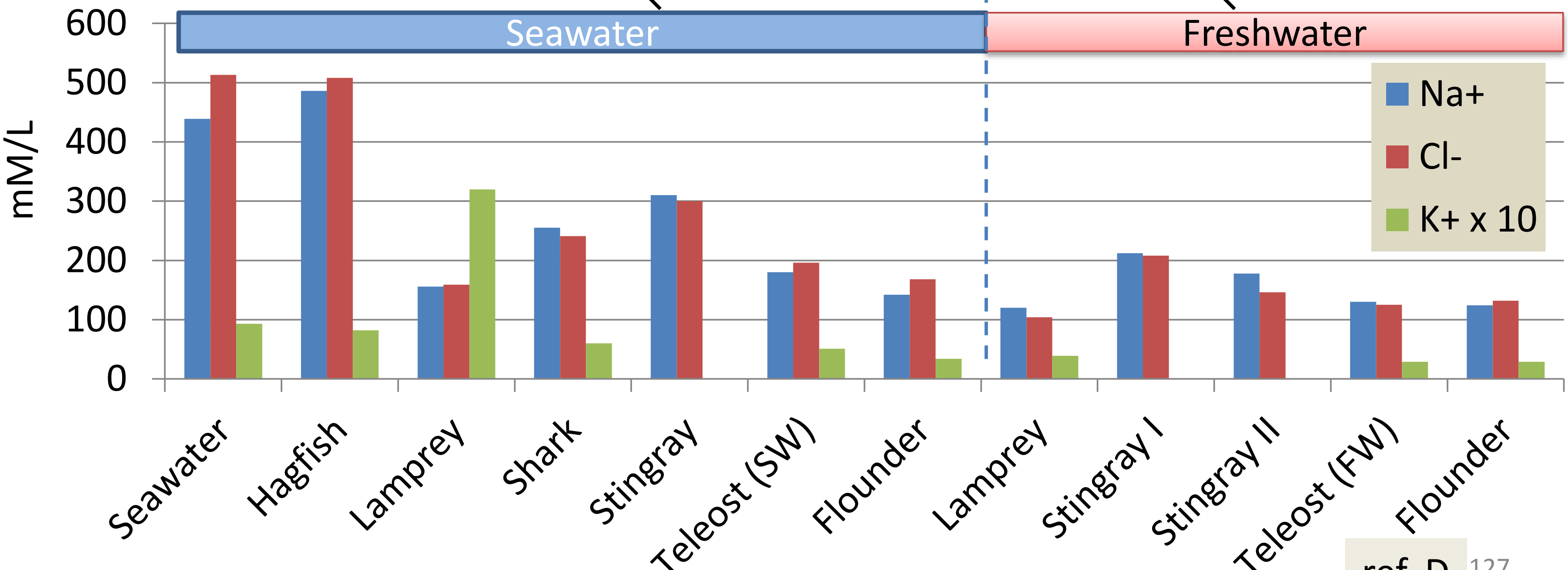


1. Plasma hyperosmotic to freshwater
2. Over-hydrated and salt-depleted
3. Large volumes of dilute urine
4. Take up Cl^- from the environment in exchange for HCO_3^-
5. Take up Na^+ in exchange for ammonia (NH_4^+)



Osmoregulatory capabilities of major fish groups
Blood osmolality (Osm/kg)
2.5
2
1.5
1
0.5
0
Osmoidentity
Hyper-osmoregulation
Hagfishes
Osmoconformer (marine)
Hypo-osmoregulation
Euryhaline (elasmobranch) (high urea levels)
Stenohaline (marine)
Euryhaline (teleost) Freshwater type *O. mossambicus*
Stenohaline (freshwater)
Euryhaline (teleost, marine type, *Fundulus heteroclitus*)
DW
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
Soft fresh water
Hard fresh water
Seawater
Environment osmolality (Osm/kg)


Osmolality (mOsm)
Osmolality
1200
1000
800
600
400
200
0
Seawater
Hagfish
Lamprey
Shark
Stingray
Teleost (SW)
Flounder
Lamprey
Stingray I
Stingray II
Teleost (FW)
Flounder

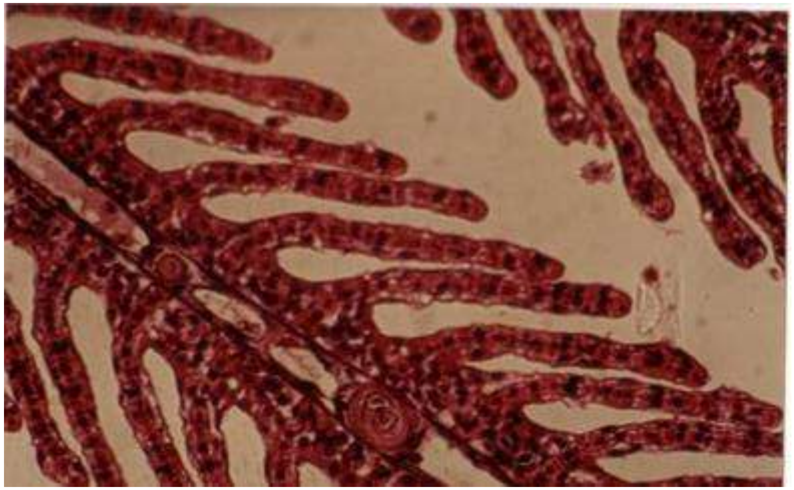

Seawater
Freshwater
mM/L
600
500
400
300
200
100
0
Na+
Cl-
K+ x 10
Seawater
Hagfish
Lamprey
Shark
Stingray
Teleost (SW)
Flounder
Lamprey
Stingray I
Stingray II
Teleost (FW)
Flounder

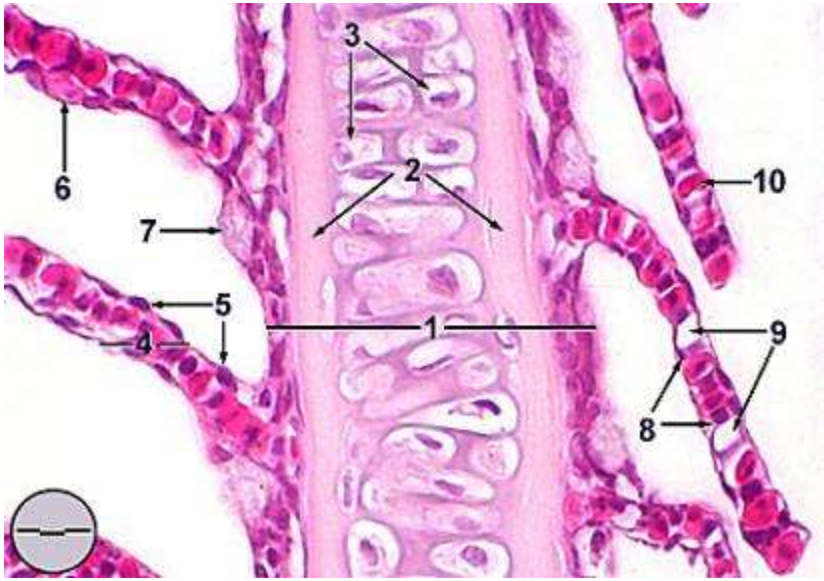
ref. D

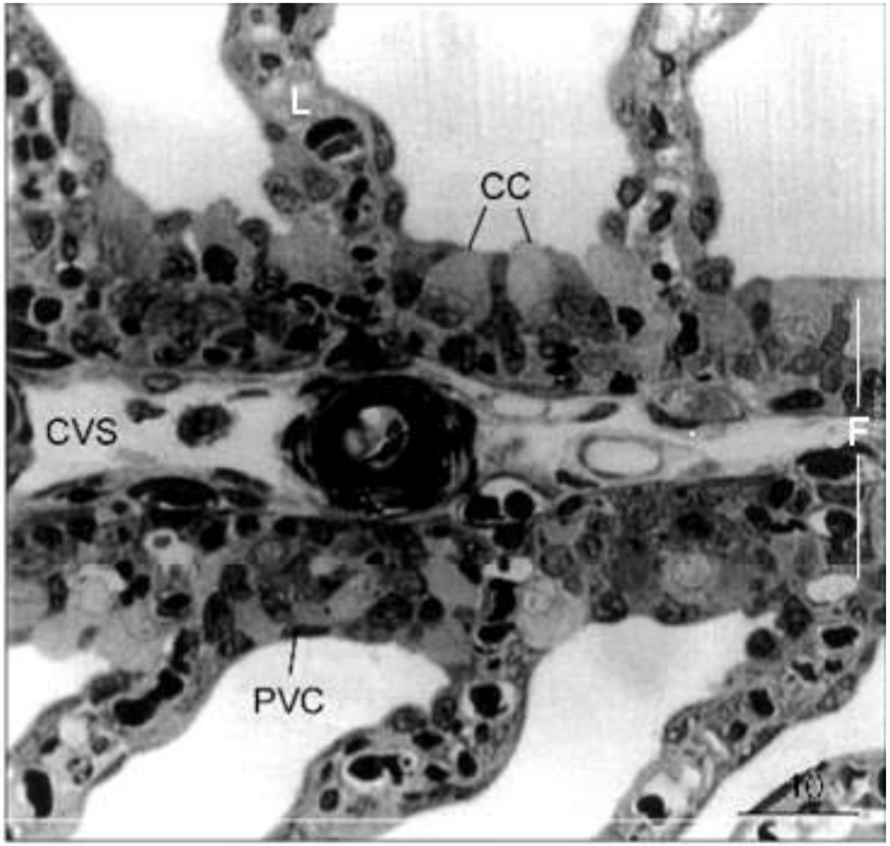
Active transport?

Where and how?

Form and function of the chloride cell

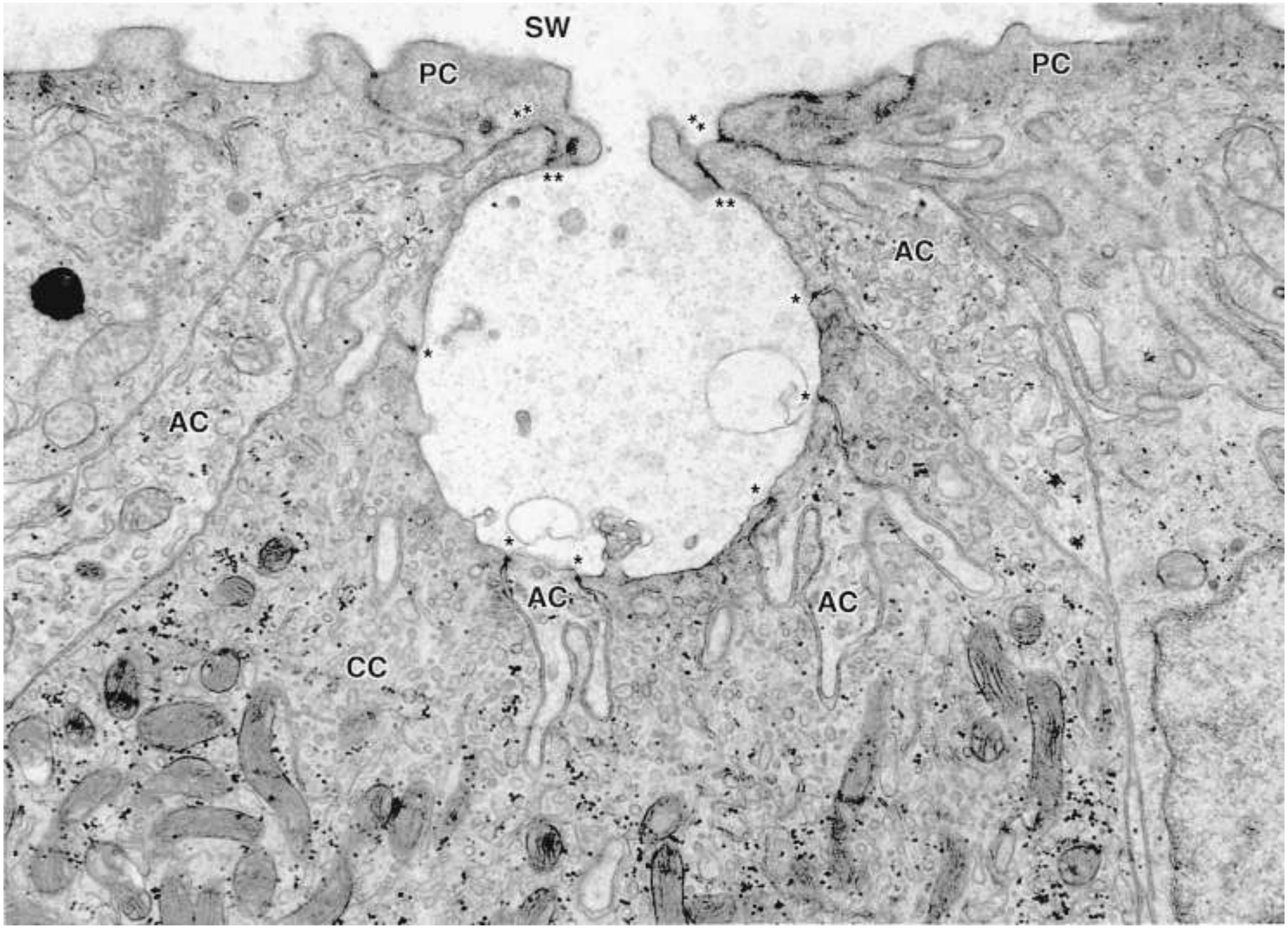






1. primary lamella; 2. extracellular cartilaginous matrix; 3. chondrocytes; 4. secondary lamella; 5. epithelial cell; 6. mucous cell; **7. chloride cell**; 8. pillar cell; 9. lacuna (capillary lumen); 10. red blood cells within lacuna.

ref. M



ref. B

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

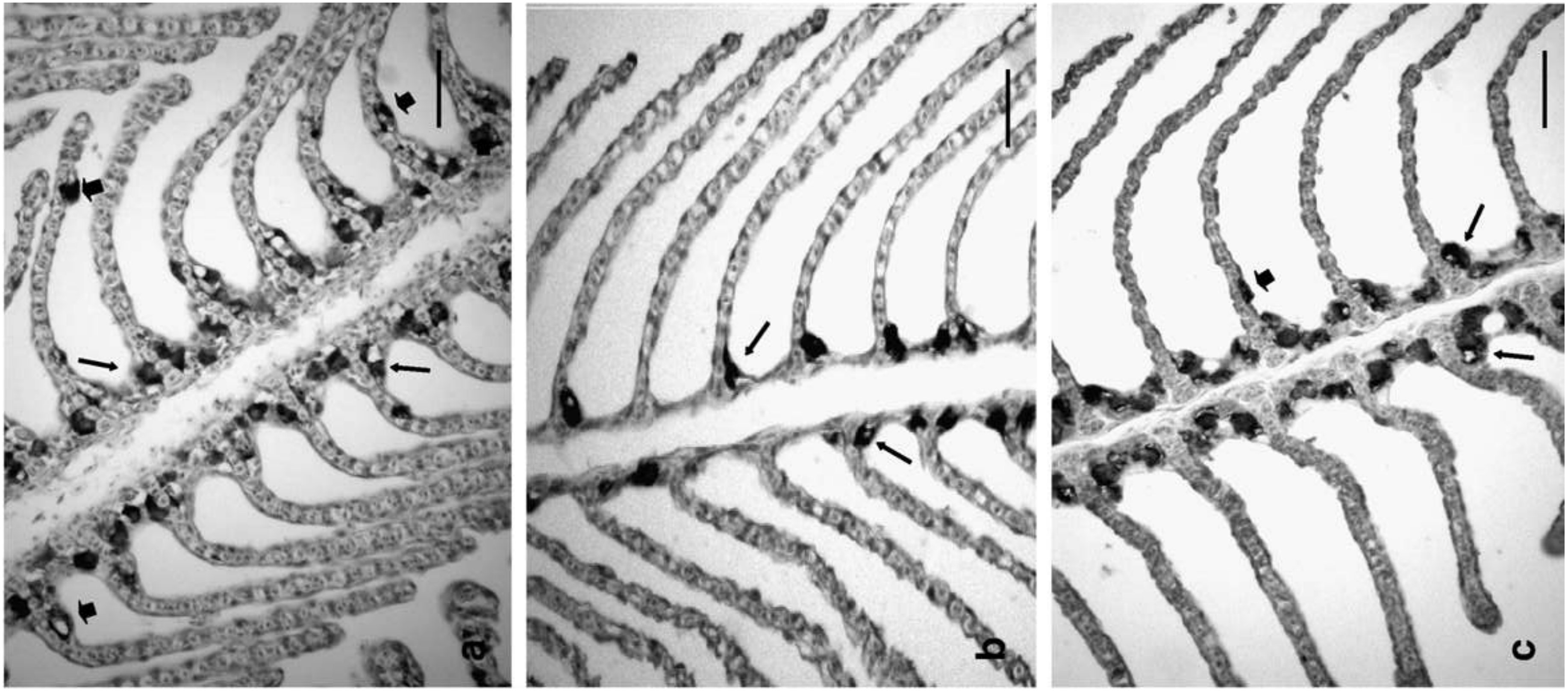
Salinity tolerance of Australian snapper - a case study

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

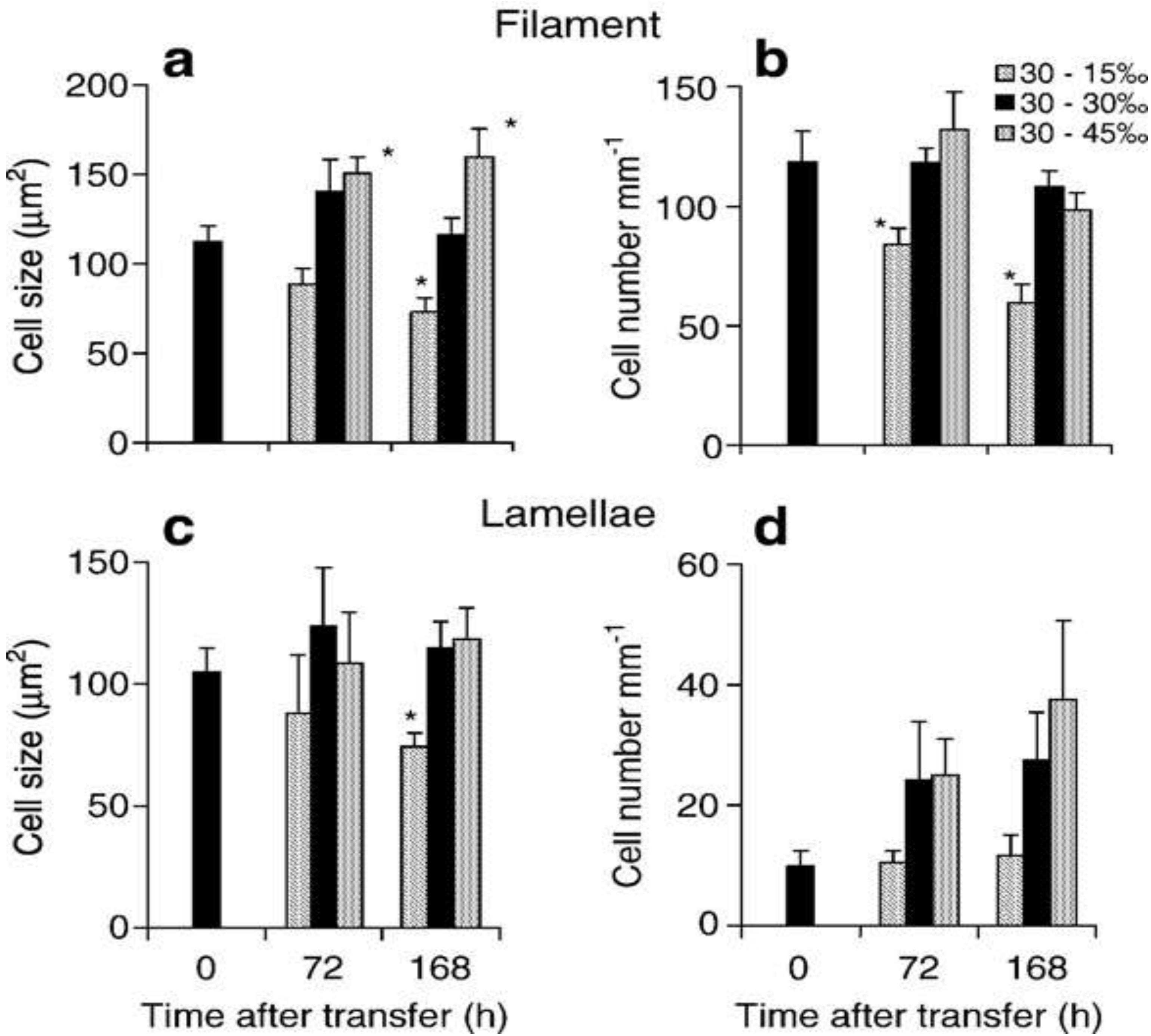
ref. N

Gill sections 168 h after transfer



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

ref. N


Filament
a
b
Cell size (μm²)
Cell number mm⁻¹
30 - 15‰
30 - 30‰
30 - 45‰
Lamellae
c
d
0
72
168
Time after transfer (h)

ref. N

Salinity tolerance of Australian snapper - a case study

Conclusions

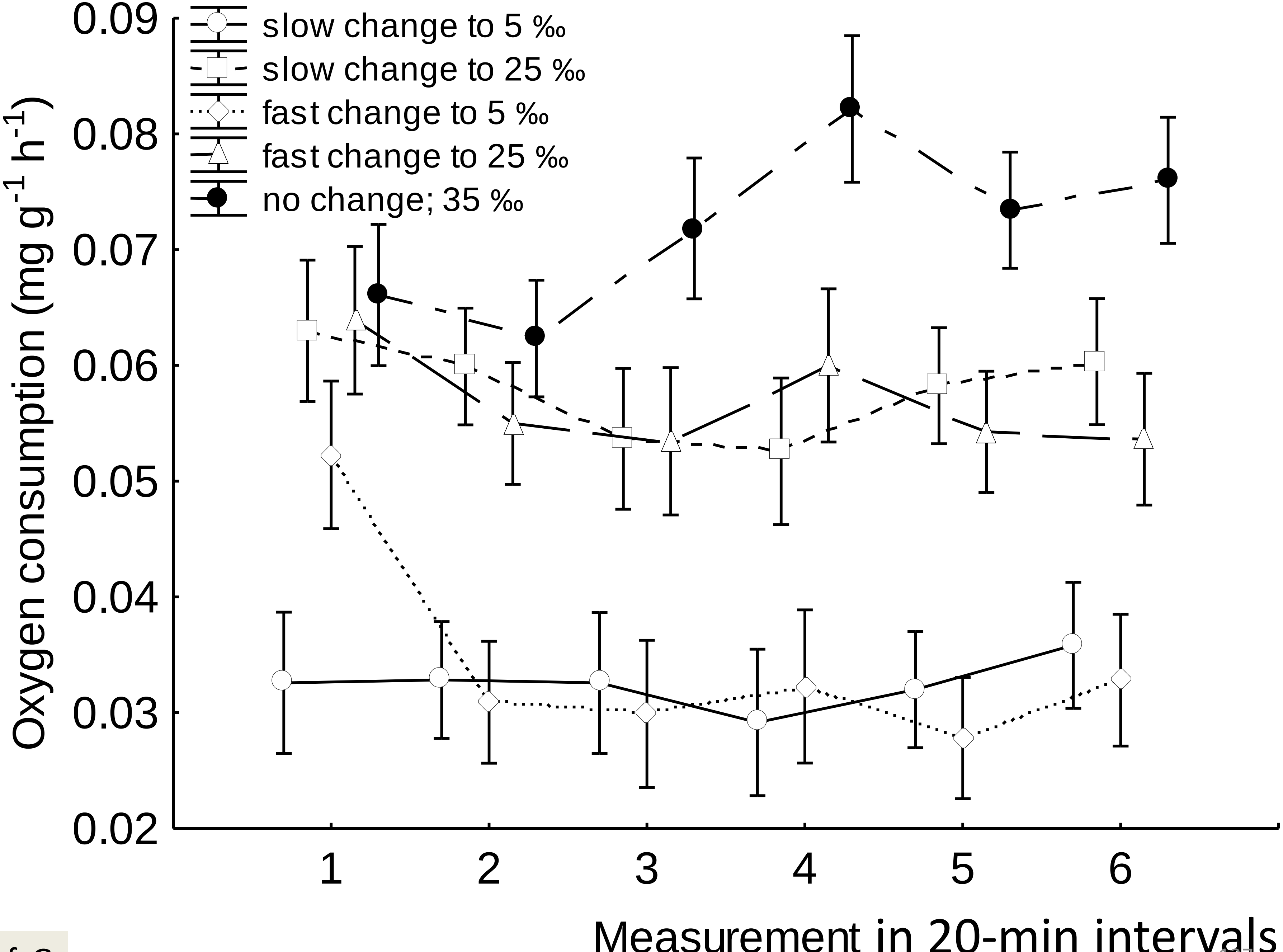
- The species shows good tolerance to fast changes in salinity
- Serum osmolality for Na^+ and Cl^- increased (15 -> 45 ppt) and decreased within 24 h and was restored
- Lamellar chloride cells also play a role in homeostasis

ref. N

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

ref. G



slow change to 5 ‰
slow change to 25 ‰
fast change to 5 ‰
fast change to 25 ‰
no change; 35 ‰
Oxygen consumption (mg g-1 h-1)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
1
2
3
4
5
6
Measurement in 20-min intervals

ref. G

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

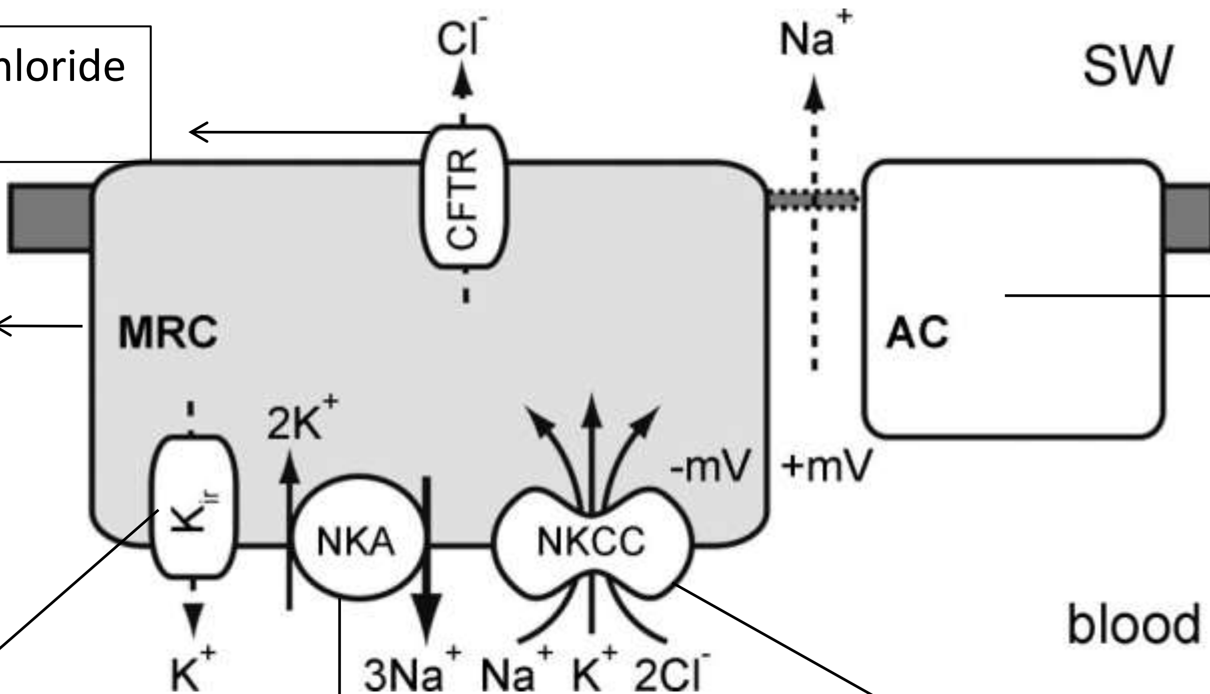
Saltwater

Extrusion of chloride via channel

Chloride cell

500 mM NaCl
0 mV

Accessory cell



150 mM NaCl
+ 35-40 mV

K^+ - correcting channel

1. Channels extra K^+ into the blood

Na^+, K^+, $2Cl^-$ co-transporter

1. Accumulates Cl^- into the cell
2. Excess Cl^- can exit via anion channel

Na^+, K^+-ATPase

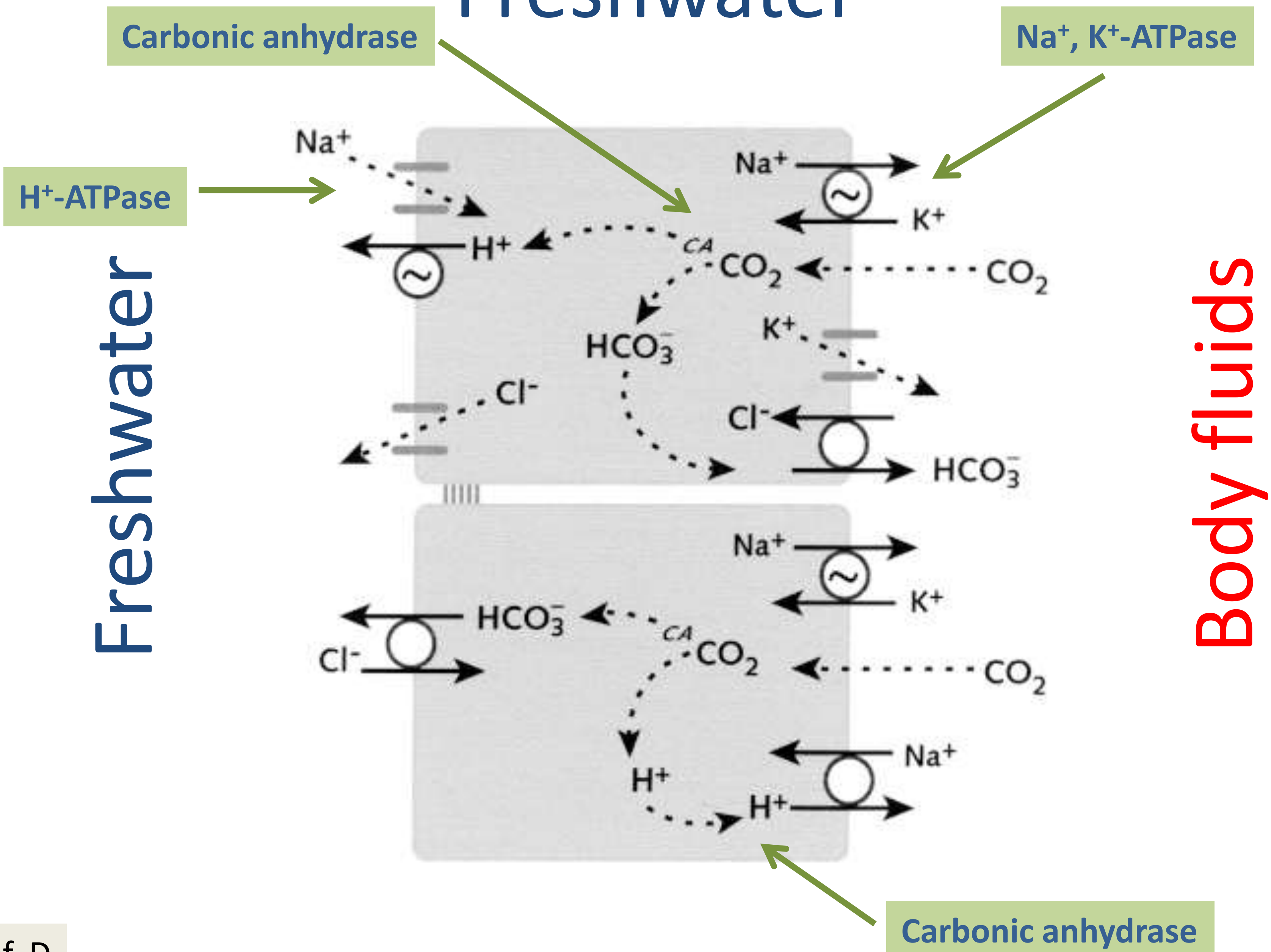
1. Keeps intracellular Na^+ low and K^+ high

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^+-ATPase**: Assists in exchange of H^+ for Na^+ (uptake)
  - **Na^+, K^+-ATPase**: Assists in moving Na^+ into blood
  - **Carbonic anhydrase**: Generates HCO_3^- to be exchanged for Cl^-

Freshwater



ref. D