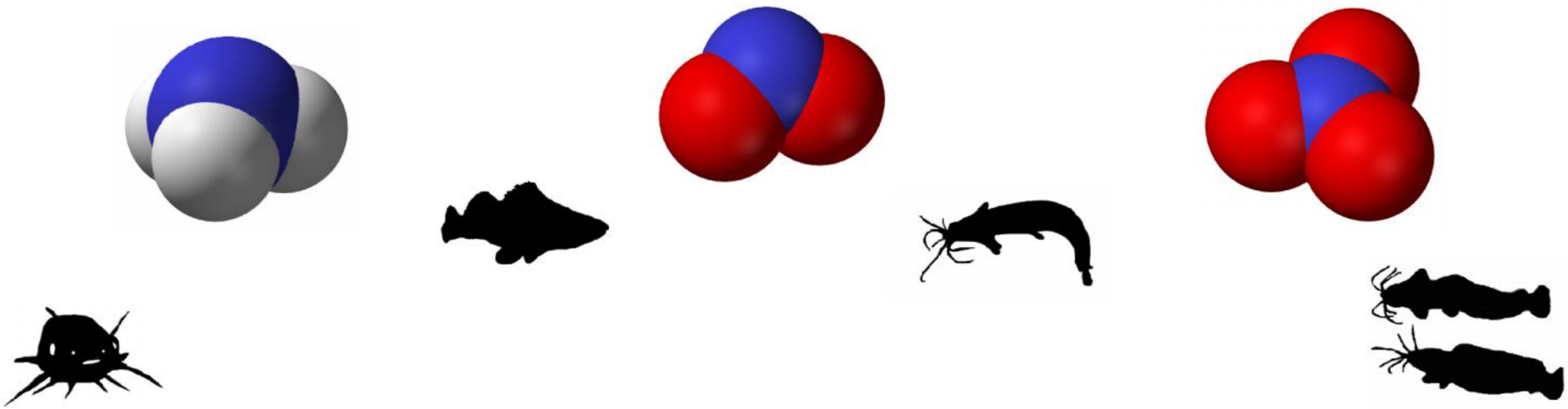
Radbound University Nijmegen

The impact of elevated water nitrogenous wastes concentration on physiology, growth and feed intake of African catfish (*Clarias gariepinus*) and pikeperch (*Sander Lucioperca*) in recirculating systems

JAC Roques , E Schram, W Abbink , G Flik , H van de Vis



Recirculating systems (RAS)

RAS offers the possibility to achieve a high production with minimal ecological impact (Martins *et al*, 2010)

- -Low dependence on water resources (reuse of up to 95% of the water)
- -Reduced environmental impact
- -Culture at high densities
- -'Optimal' control over all steps of the culture (including stunning and killing)

Recirculating systems (RAS)

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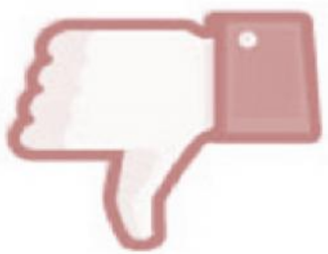
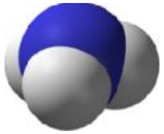


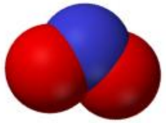
- High investment and maintenance costs (De Ionno *et al*, 2006)
- Labor intensive
- Potential accumulation of nitrogenous waste products

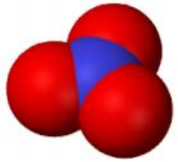
Recirculating systems (RAS)

RAS offers the possibility to achieve a high production with minimal ecological impact (Martins *et al*, 2010)

With the expansion of the aquaculture industry and especially the RAS, the attention for the well-being of the fish has strongly increased

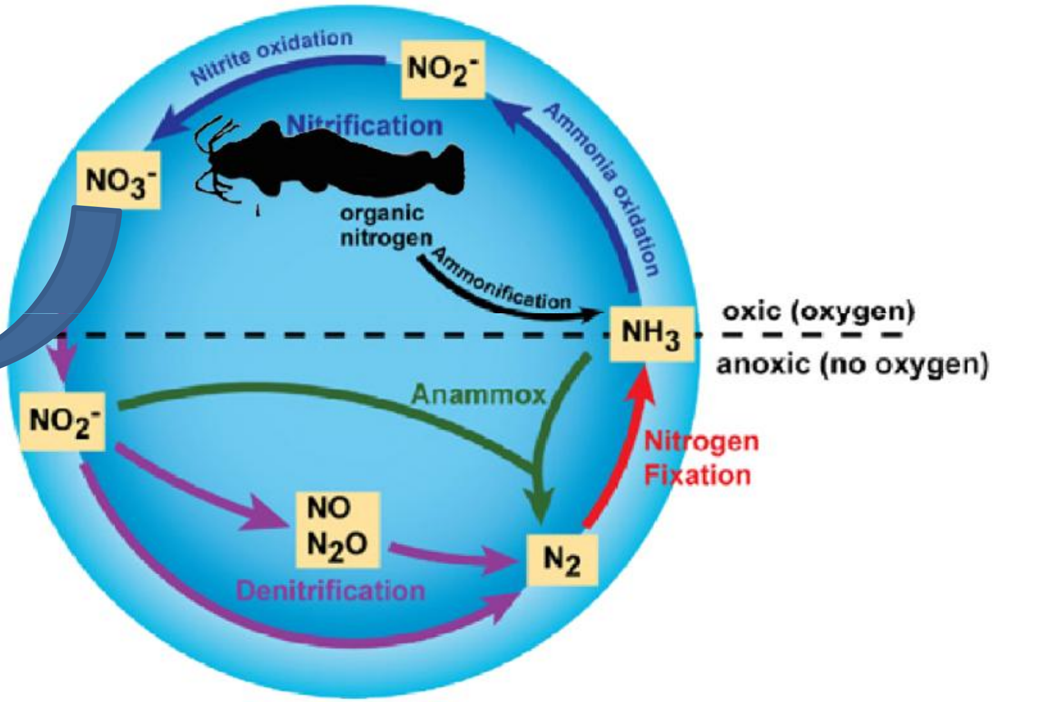






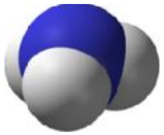
Nitrogen cycle in RAS

Potential accumulation

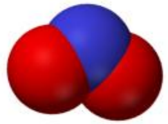


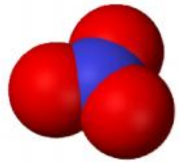
Fish produce ammonia through catabolism of amino acids

Kroupova *et al*, 2005; van Kessel *et al*, 2010; van Rijn *et al*, 2006

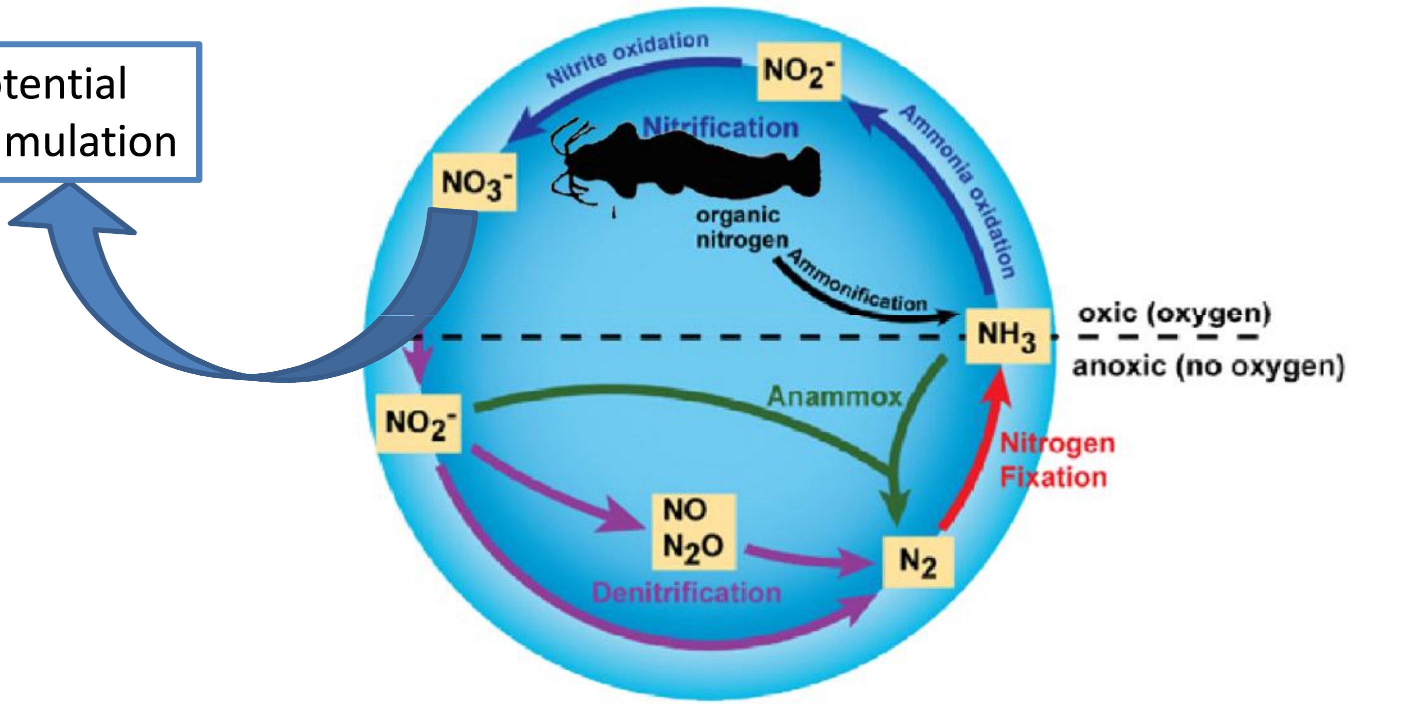


Nitrogen cycle in RAS





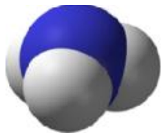
Potential accumulation



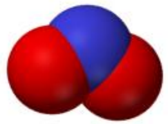
In RAS NH_3, is ultimately converted to NO_3^- (via NO_2^-) in aerobic biofilters

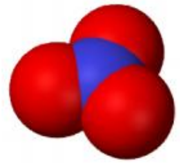
→ accumulation of NO_3^-(up to 70 mM)

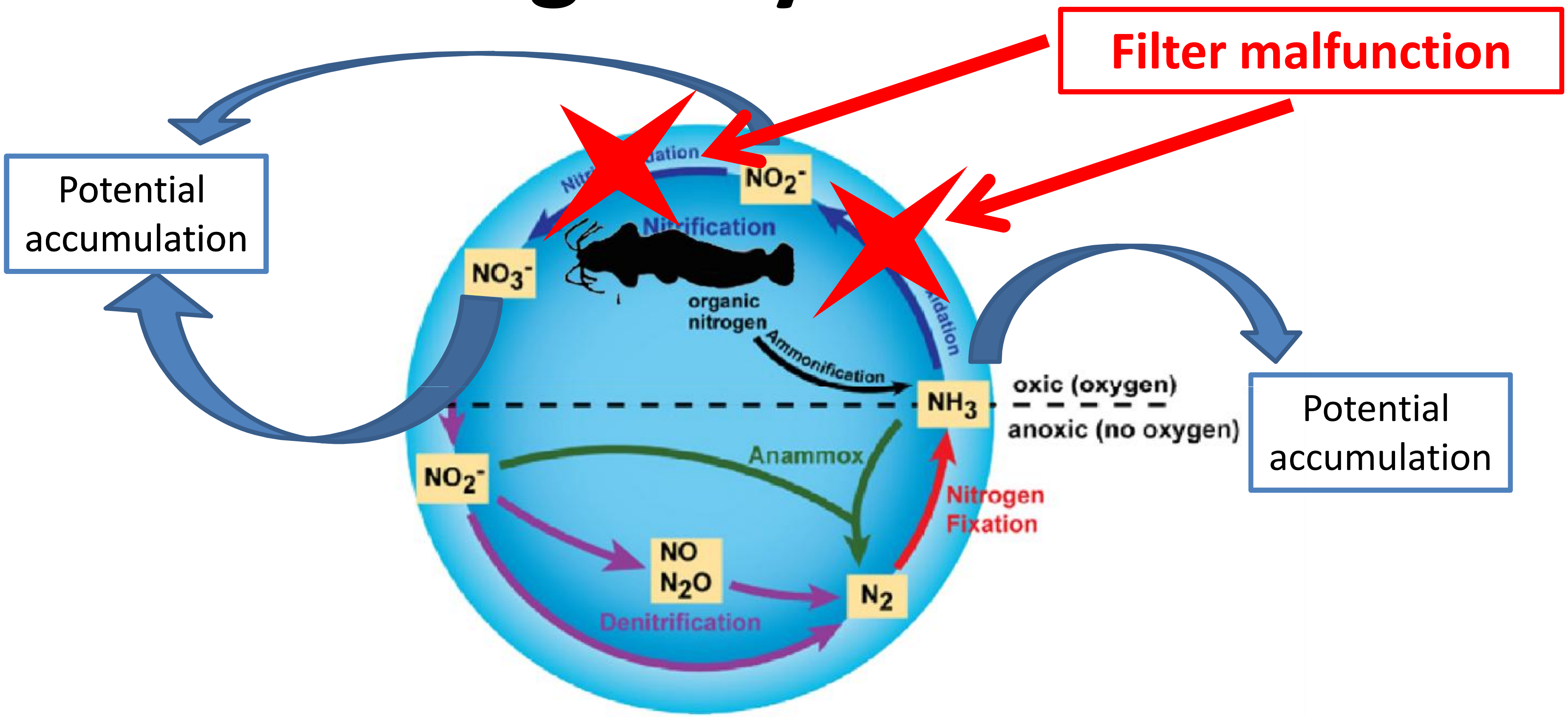
Kroupova *et al*, 2005; van Kessel *et al*, 2010; van Rijn *et al*, 2006



Nitrogen cycle in RAS



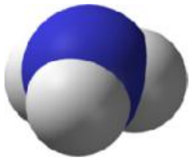


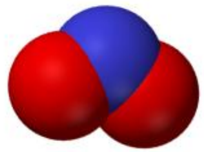


In RAS NH_3, if problem occurs in aerobic biofilters

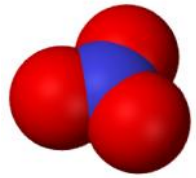
→ potential accumulation of NH_3 or NO_2^-, in addition to NO_3^-

Kroupova *et al*, 2005; van Kessel *et al*, 2010; van Rijn *et al*, 2006





Ammonia, nitrite, nitrate



All nitrogenous waste products are more or less toxic to fish

Elevated ambient ammonia has various (neuro)toxic effects:

physiological disturbances → **reduced growth** → **mortality**

Nitrate and nitrite toxicity are considered similar

→ conversion of haemoglobin to methaemoglobin

Nitrate is considered less toxic than nitrite (lower branchial permeability)

Bœuf *et al*, 1999; Camargo *et al*, 2005; Kroupova *et al*, 2005; Stormer *et al*, 1996; Wilkie *et al*, 2002

Clarias gariepinus

Aim of these studies

Sander lucioperca

- Determine the impact of chronic exposure to nitrogenous waste products in RAS on the welfare of two important species for the Dutch aquaculture industry :

  - African catfish (~2000-4000 t/year, FAO 2015)
  - Juvenile pike perch, introduced to diversify aquaculture

- Parameters investigated:
  - (stress) physiology
  - growth impairment

(Le-Francois *et al*, 2002; (van Duijn *et al*, 2010))

Experimental setups

-30 L tanks partially covered

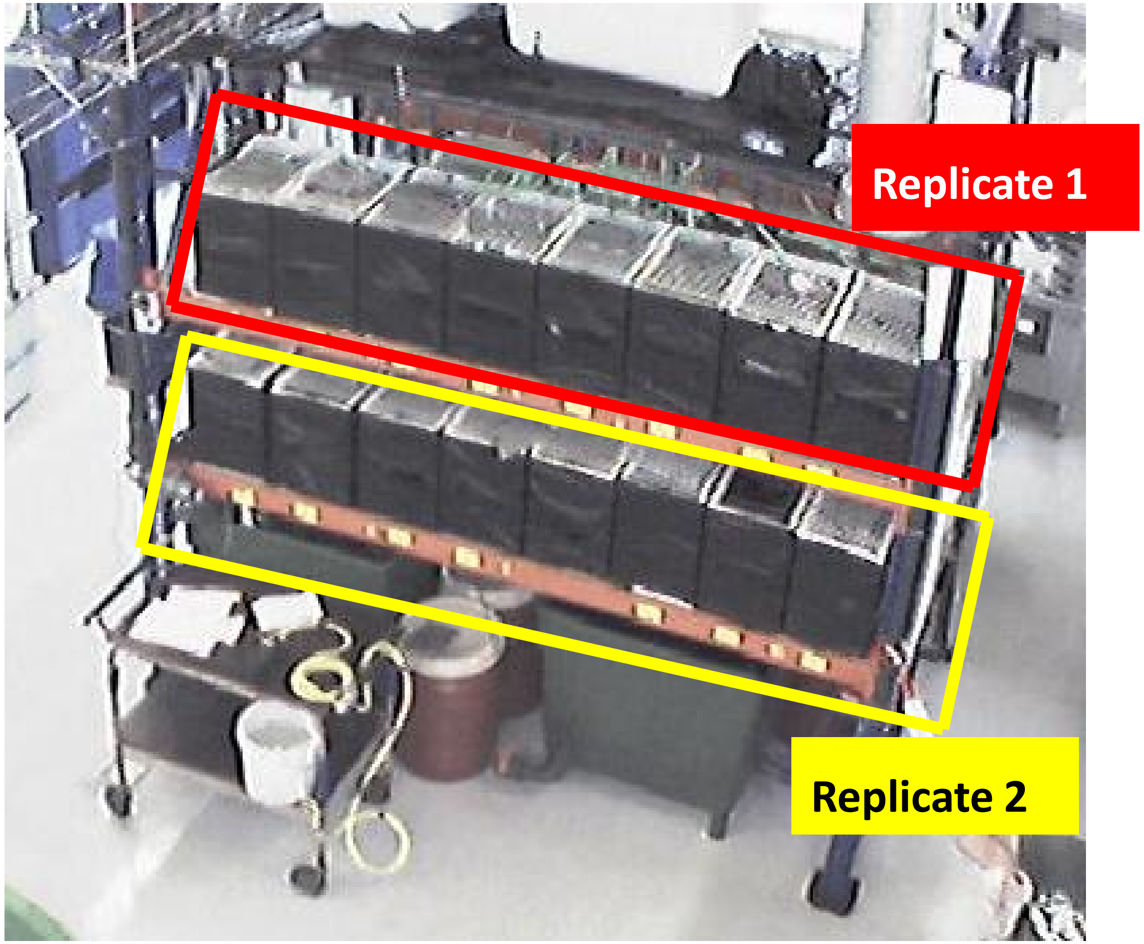
- 12 catfish / 24 pike perch per tank

- water quality checked daily

- fed to satiety twice / day

- 5-8 experimental concentrations,
  in duplicate

(T=0 and pair fed treatments)

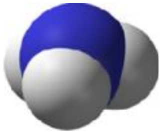


Roques, Schram *et al*, 2010; 2014a;2014b; 2015

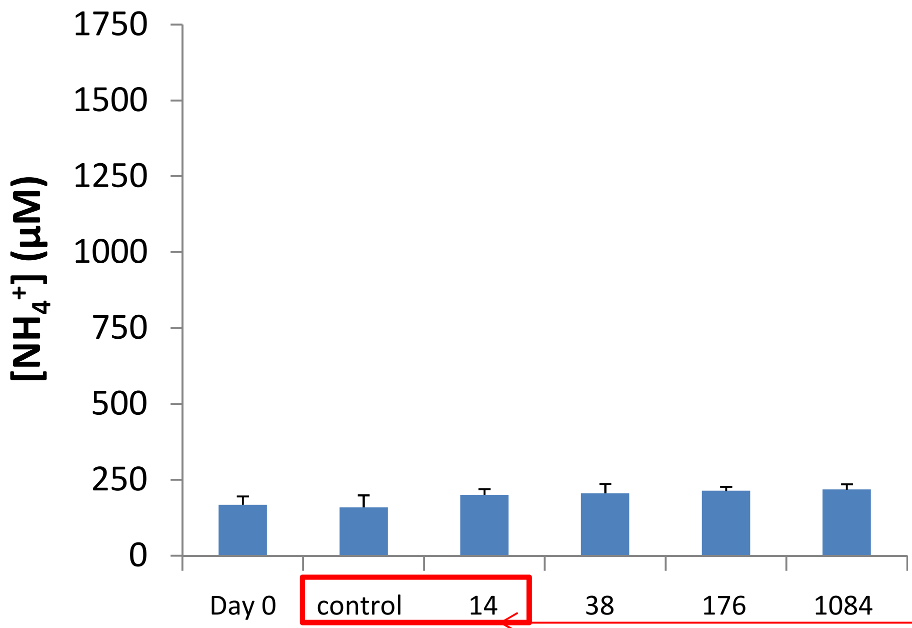
Experimental setups

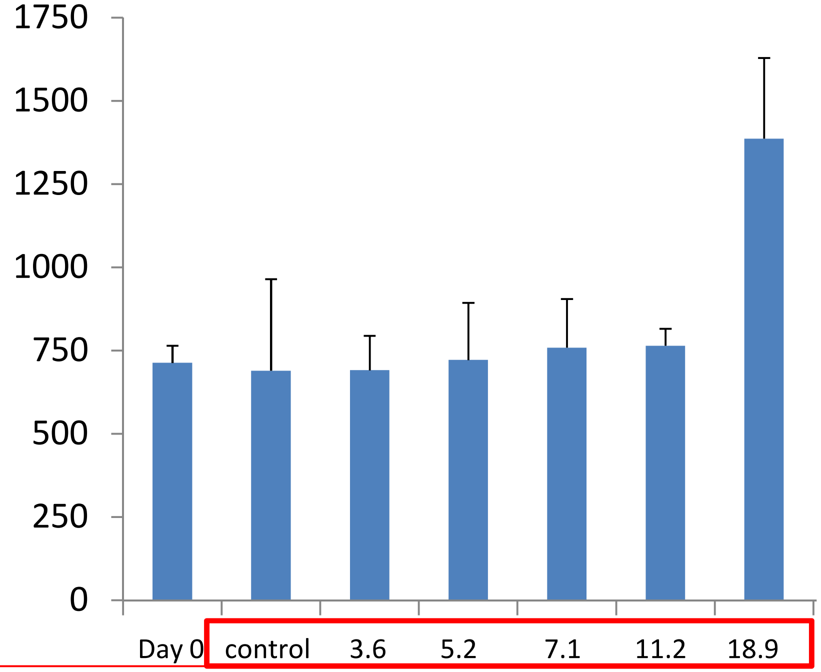
			
NH_3	[concentration range] Exposure time (d) Initial density (kg/m^3)	4 - 1084 **µM** 34 65.8	0.9 - 18.9 **µM** 42 14.2
NO_3^-	[concentration range] Exposure time (d) Initial density (kg/m^3)	0.4 - 27.0 **mM** 42 66.9	0.1 - 25.6 **mM** 42 21.6
NO_2^-	[concentration range] Exposure time (d) Initial density (kg/m^3)	6 - 928 **µM*** 28 84.7	Not studied

**extra group: high concentration + NaCl: possible attenuation effect of NaCl on nitrite toxicity*



Plasma [NH_4^+]





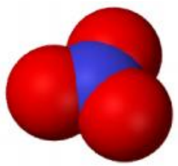
Capability to maintain low plasma [NH_4^+]

On-set of ammonia defense mechanisms

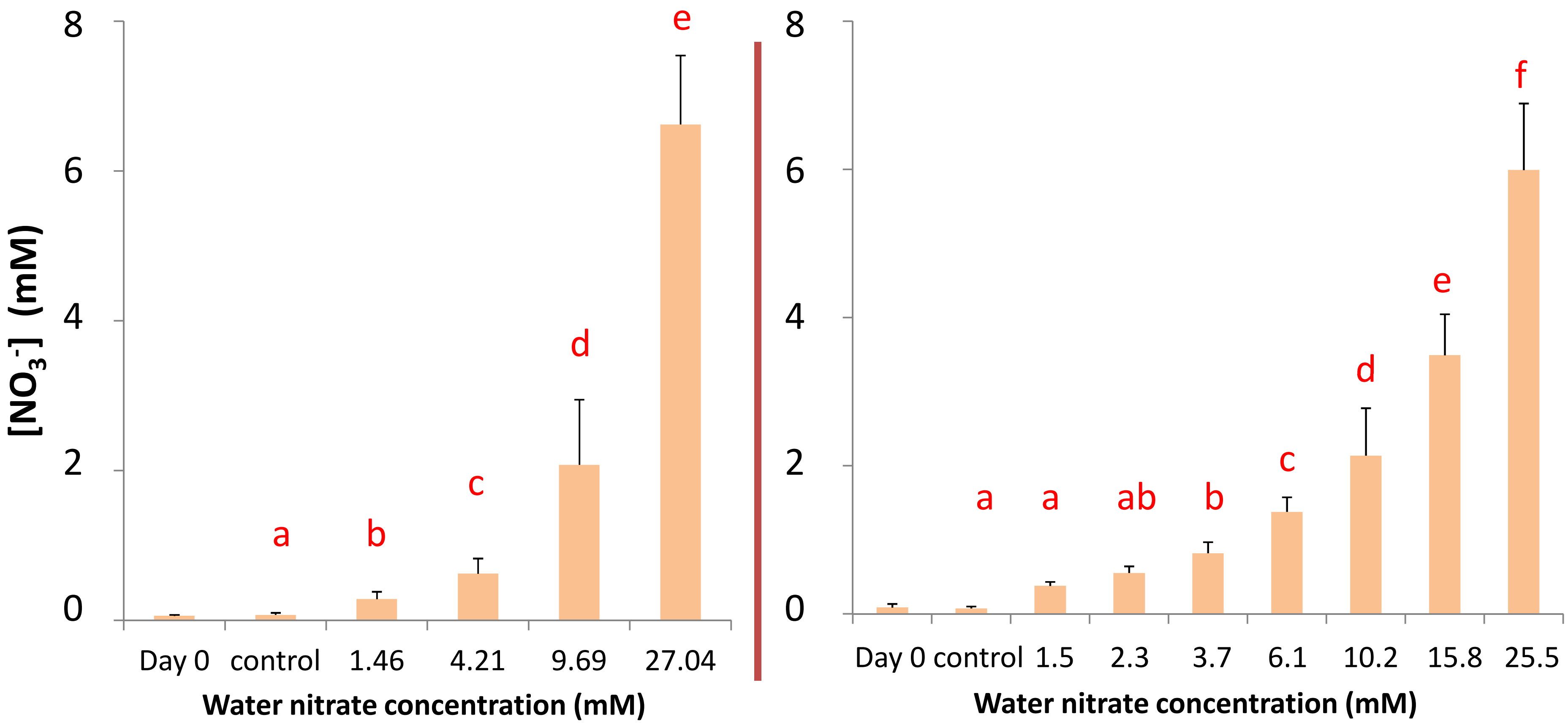
Maintain low plasma [NH_4^+] up to 11.2 µM

No defense mechanisms

Roques, Schram *et al*, 2010; 2014b



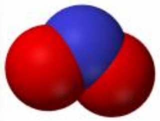
Plasma [NO_3^-]



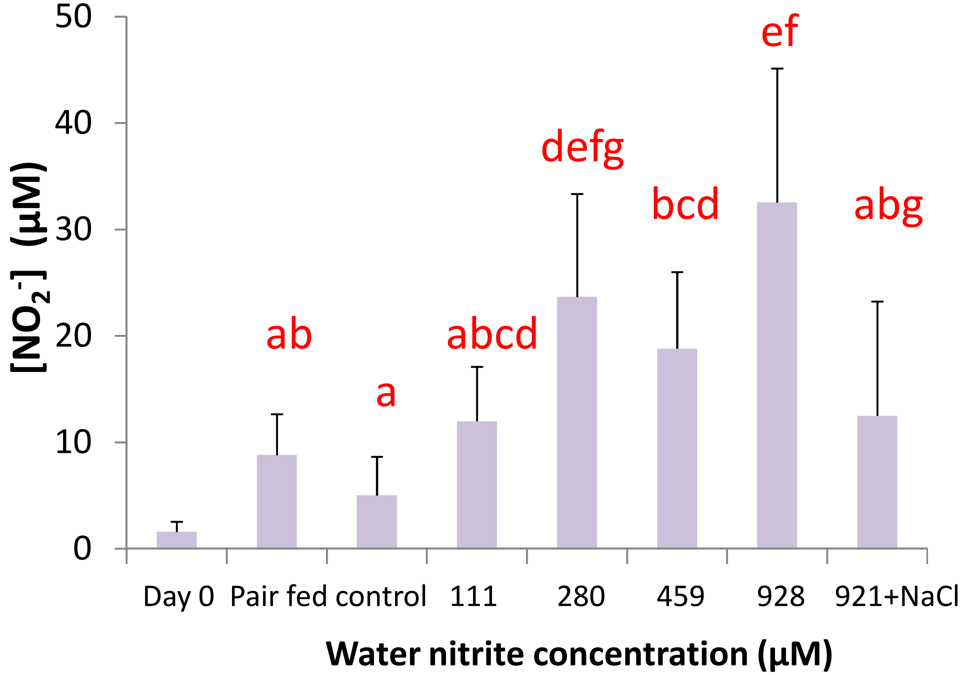
Gradual increase of plasma [NO_3^-] during exposure to high external nitrate

No on-set nitrate defence mechanisms; lower toxicity than ammonia

Roques, Schram *et al*, 2014a;2014b

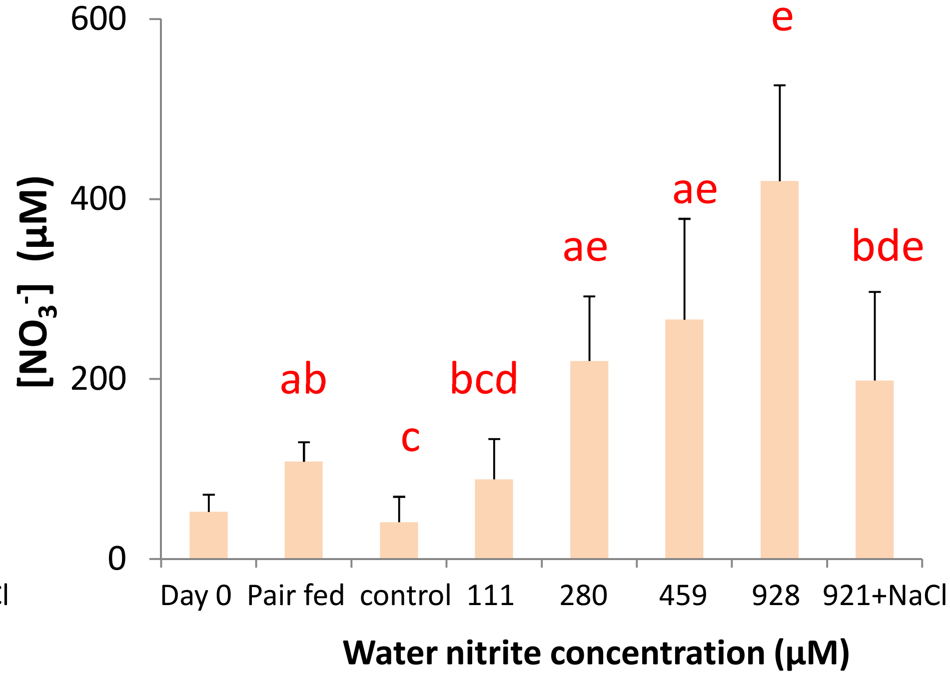


Plasma $[NO_2^-]$



Gradual increase of plasma $[NO_2^-]$, yet limited

Plasma $[NO_3^-]$
(NO2- exposure)



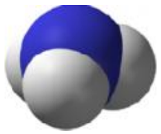
Gradual increase of plasma $[NO_3^-]$

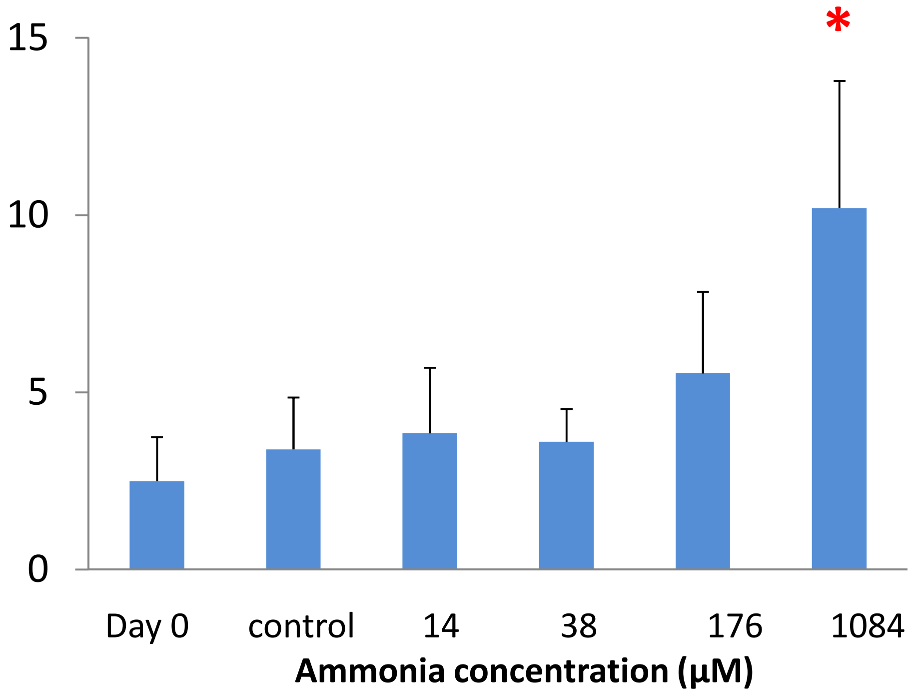
➢Internal detoxification of nitrite into less toxic nitrate

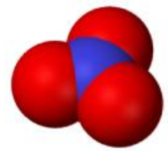
Slight attenuation effect of NaCl on plasma $[NO_2^-]$ and $[NO_3^-]$

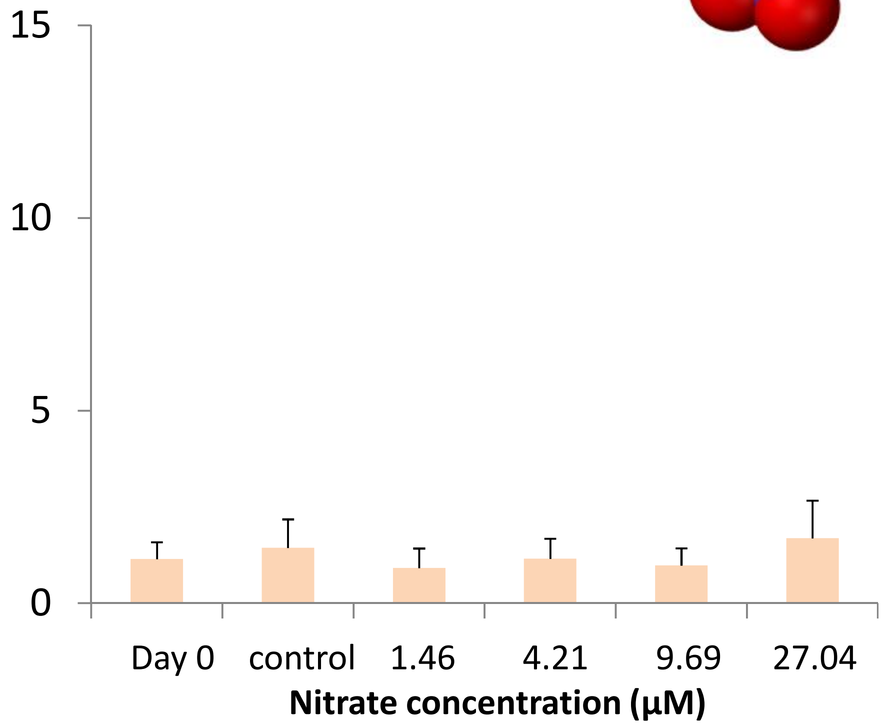
Roques, Schram *et al*, 2015

Physiological effect: Na^+/K^+ ATPase activity





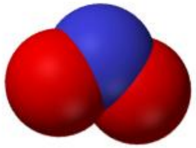




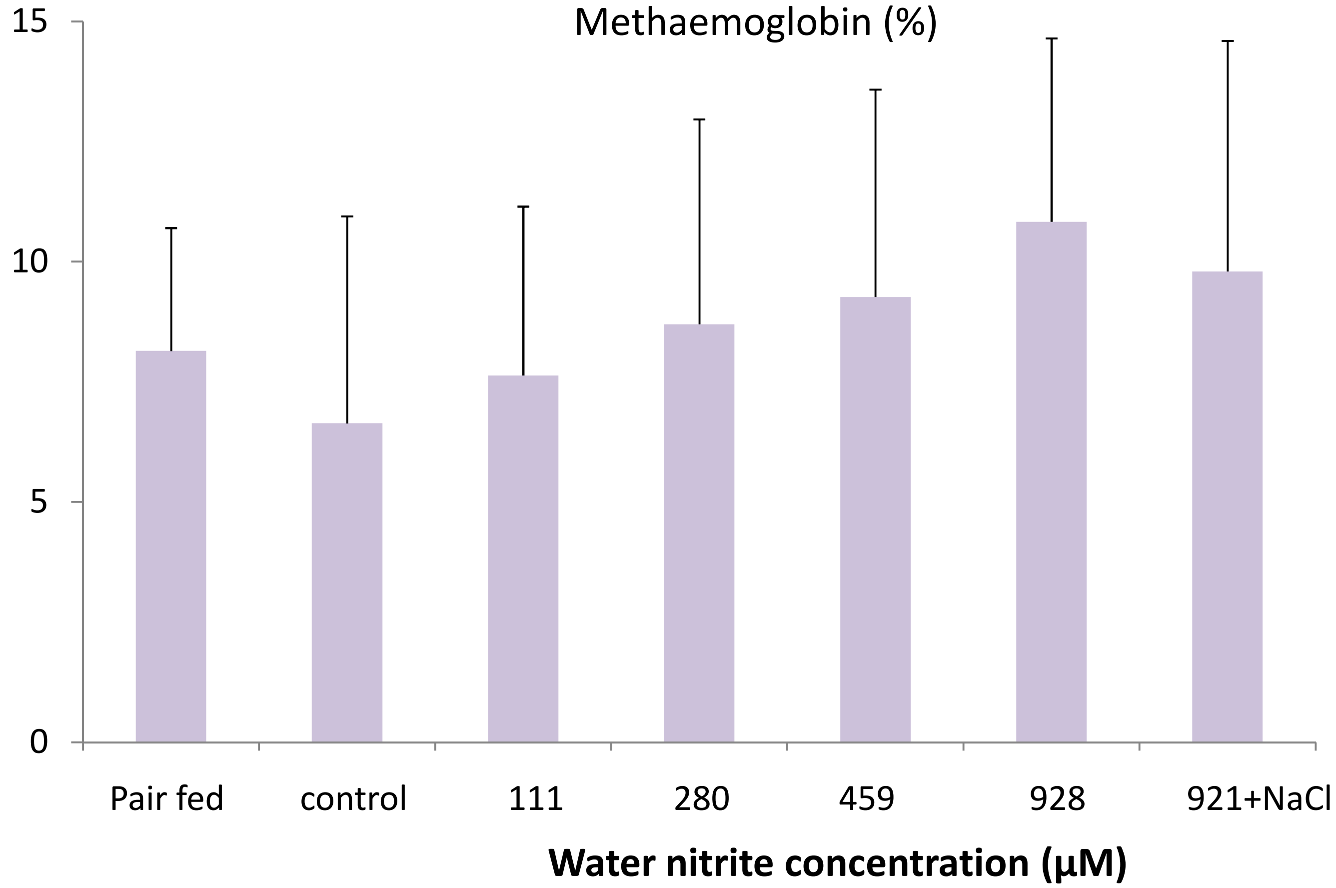
Increased activity in the highest group: defence mechanism: active branchial excretion

No role played in excretion (similar for NO_2^- and pike perch experiments)

Roques, Schram *et al*, 2010; 2014a



Physiology: blood methaemoglobin



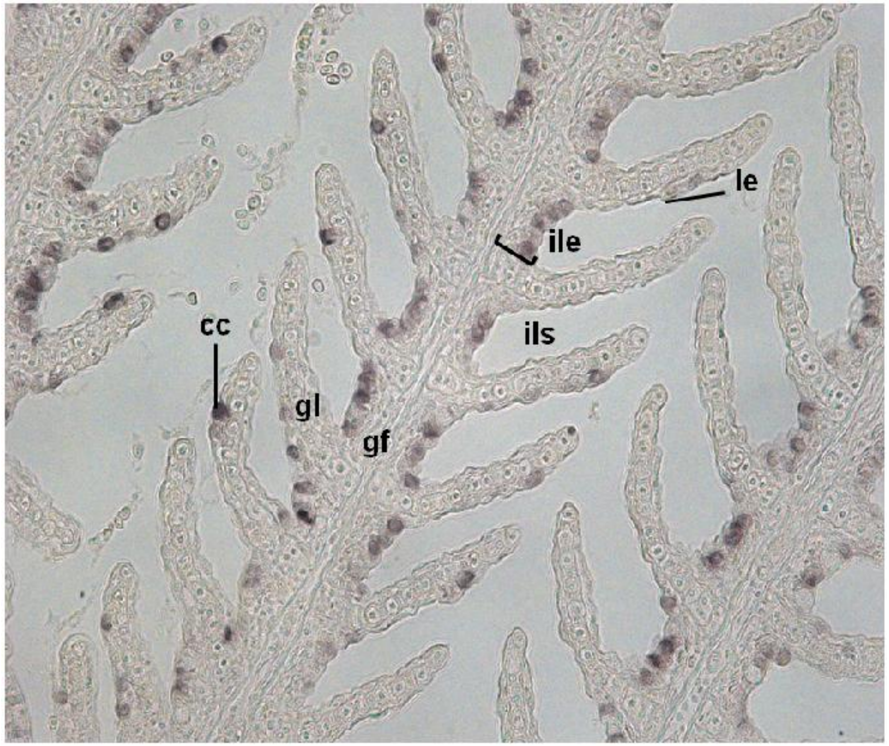
Note: sampled 2 fish at the beginning of the experiment

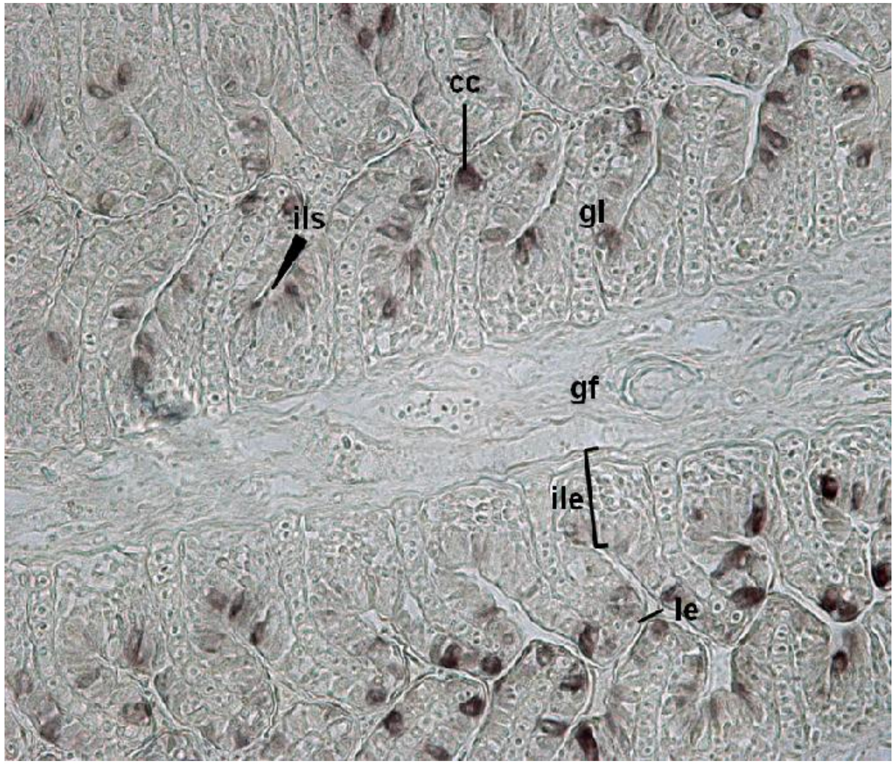
→ methaemoglobin value of 70% (459 µM group) and 95% (928 µM group)

→ adaptation (possibly linked to the internal NO_2^- → NO_3^- detoxification)

Roques, Schram *et al*, 2015

Branchial physiological and morphology





Control → **High ammonia**

- Defense mechanism against ammonia entry:

morphology drastically affected, but the fish can tolerate an survive

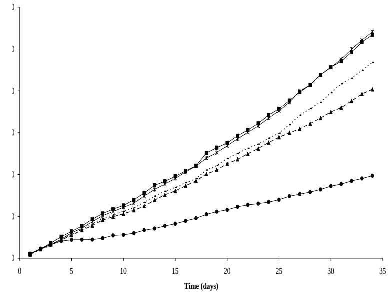
- Particularity of the African catfish: (facultative) air breather:

- gas exchange not impaired: other fish would not survive

suprabranchial organ

Roques, Schram *et al*, 2010

Cumulative food intake



		
NH_3	176 µM * 1084 µM **	11.2 µM * 18.9 µM **
NO_3^-	27 mM **	Not affected in the range studied (0.1 – 25.6 mM)
NO_2^-	Not affected in the range studied (6 – 928 µM)	Not studied

Roques, Schram *et al*, 2010; 2014a;2014b; 2015

Conclusions

Tolerance to toxic nitrogenous waste products is species and compound-specific

- Ammonia is a serious threat for African catfish and pike perch, the latter being the more sensitive

- With growth as readout, pike perch is rather tolerant to nitrate

- The nitrate and nitrite levels in RAS can affect growth performance of African catfish

Recommendations

		
NH_3	< 24 µM	< 3.4 µM
NO_3^-	< 10 mM	< 25 mM
NO_2^-	< 43 µM	*To be determined*

Roques, Schram *et al*, 2010; 2014a;2014b; 2015

Thank you for your attention

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