Effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status

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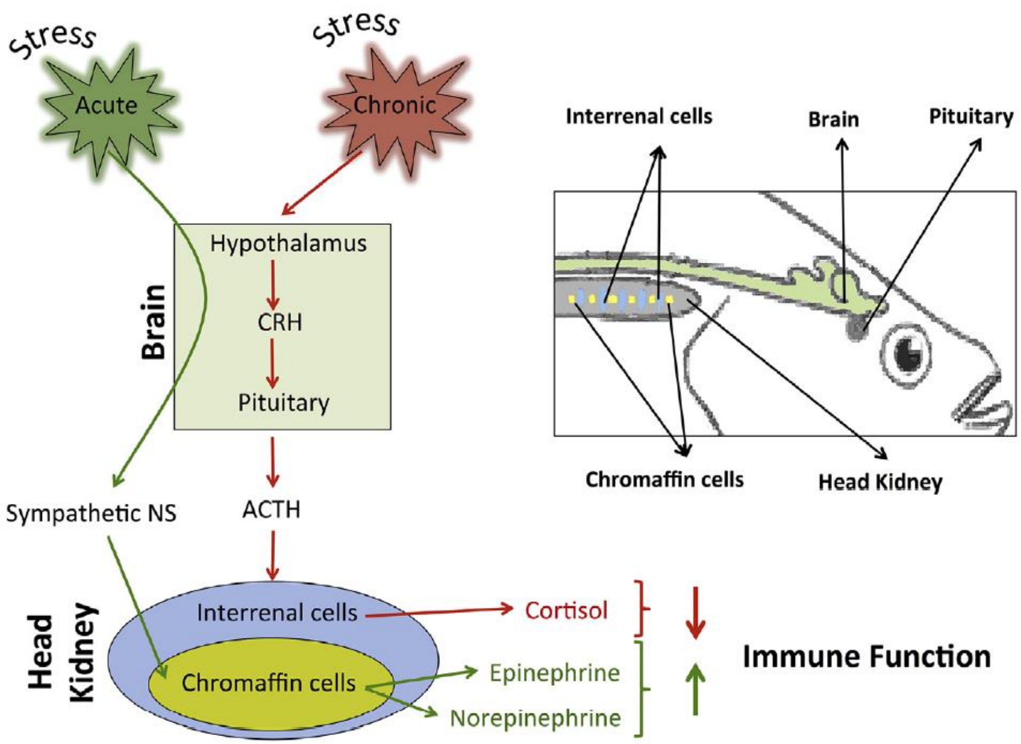
Context

- Pikerperch is a valuable candidate for diversification of inland aquaculture in European countries
- But its development is still limited by sudden and unpredictable mortalities

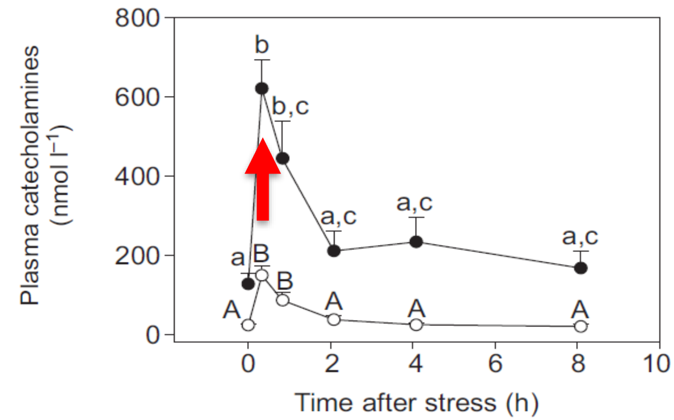
Among the main bottlenecks:

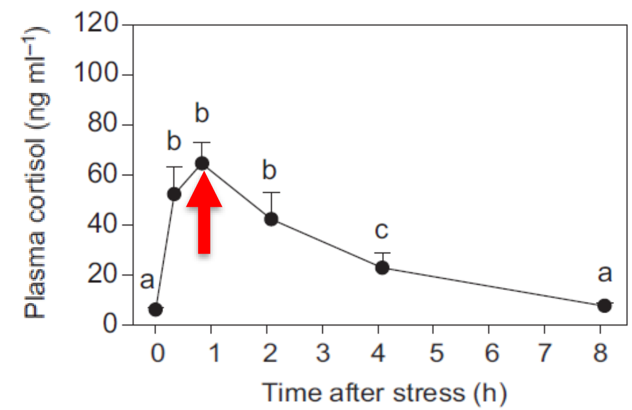
- High stress responsiveness
- Frequent grading manipulations to reduce growth depensation and cannibalism behaviour
- Absence of standardized rearing conditions
- Low level of domestication

- Stress is unavoidable in aquaculture production systems
- Stress induces physiological, endocrine and immunological responses to maintain homeostasis



Nardocci et al, 2014





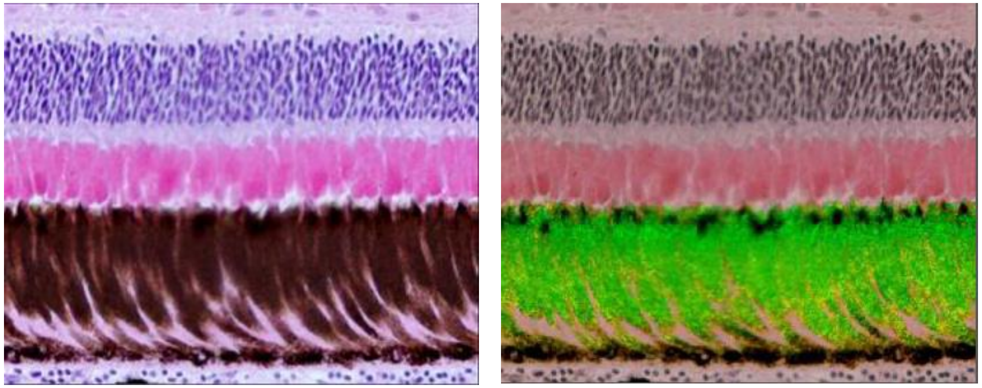
- Metabolic changes
- Osmoregulatory disturbance
- Immune modulation

Stress… in pikeperch

	Basal cortisol levels (ng/ml)	Cortisol levels 1-hour after an acute stress
Pikeperch & walleye	± 30 ng/ml	±230 ng/ml
Coho salmon	< 25 ng/ml	±175 ng/ml
Rainbow trout	< 25 ng/ml	± 40 ng/ml

- Highly responsive/sensitive to environmental changes: ***advantage or constraint in aquaculture?***
- Specialized retina with a *tapetum lucidum*, a light-reflecting layer increasing the eye sensitivity: **effects of unsuitable light characteristics?**

Retina



Guanine in the retinal tapetum

Dubielzig D. (UW-Madison)

Objectives of the WP 22
Growth & husbandry – pikeperch

- WP22.1 – Effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status
- WP22.2 – Characterization of pikeperch growth, immune and physiological status in farm conditions
- WP22.3 – Effects of pikeperch domestication level and geographical origin on growth and stress sensitivity

WP22.1

Effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status

Materials & Methods

Eight factors considered as relevant for the welfare of pikeperch

Factors	Modality	References
Photoperiod	10 L : 14 D	Pourhosein Sarameh et al., 2012
	24 L : 0 D	Teletchea et al., 2009
Light intensity	10 lux	Luchiari et al., 2006
	100 lux	
Light spectrum	White	Luchiari et al., 2009
	Red	
Rearing density	15kg/m^3	Steenfeldt et al., (2010) in Dalsgaard et al., 2013
	30kg/m^3	
Temperature	21 °C	Dalsgaard et al., 2013
	26 °C	Wang et al., 2009
Oxygen saturation	60 %	Dalsgaard et al., 2013
	90 %	
Alimentation	Semi-floating	Steenfeldt et al., (2010) in Dalsgaard et al., 2013
	Sinking	
Handling	Yes	Arlinghaus, 2007
	No	

Factorial fractional design

Experimental unit = tank

8 factors * 2 modalities/factors

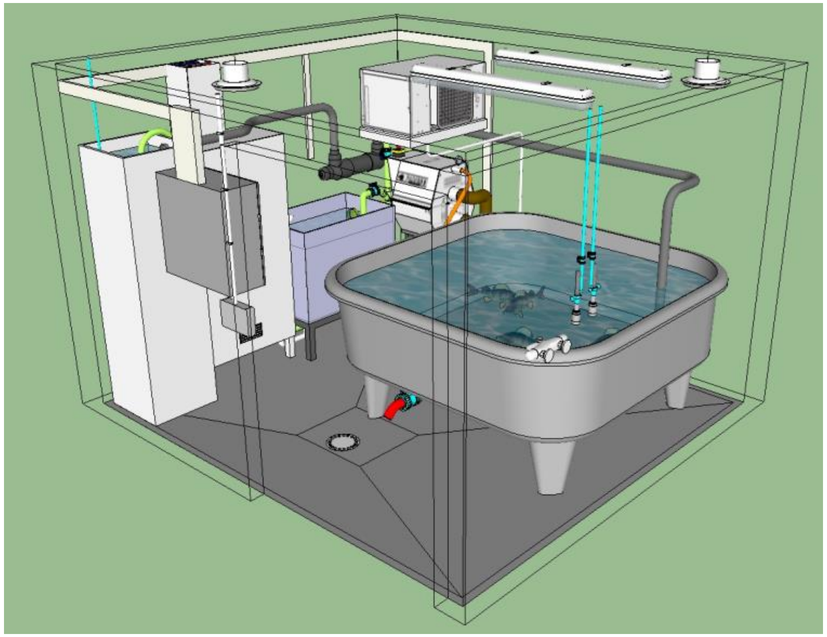
↓

2^8 = 256 combinations

Using factorial fractional design

↓

16 different combinations



Screening approach

Organisation of the fractional factorial design

Tank	Light intensity	Stocking density	Light spectrum	Photoperiod	Water temperature	Feed type	Grading	Oxygen saturation
1	10	15	white	24	21	sinking	Y	90
2	100	[illegible]	Red	10	26	floating	N	60
3	100	15	white	24	21	sinking	N	60
4	100		Red	10	21	sinking	N	90
5	10	15	Red	10	21	sinking	Y	60
6	10	15	white	10	21	floating	N	90
7	100	15	Red	24	21	floating	Y	90
8	10	15	white	24	26	floating	Y	60
9	100	15	white	10	26	sinking	Y	90
10	100	30	white	10	21	floating	Y	60
11	100	30	white	24	26	floating	N	90
12	10	30	Red	10	26	floating	Y	90
13		30	Red	24	26	sinking	Y	60
14	10	30	Red	24	21	floating	N	60
15	10	30	white	10	26	sinking	N	60
16	10	15	Red	24	26	sinking	N	90

Experimental facilities

Ecotron with white spectrum (UL)

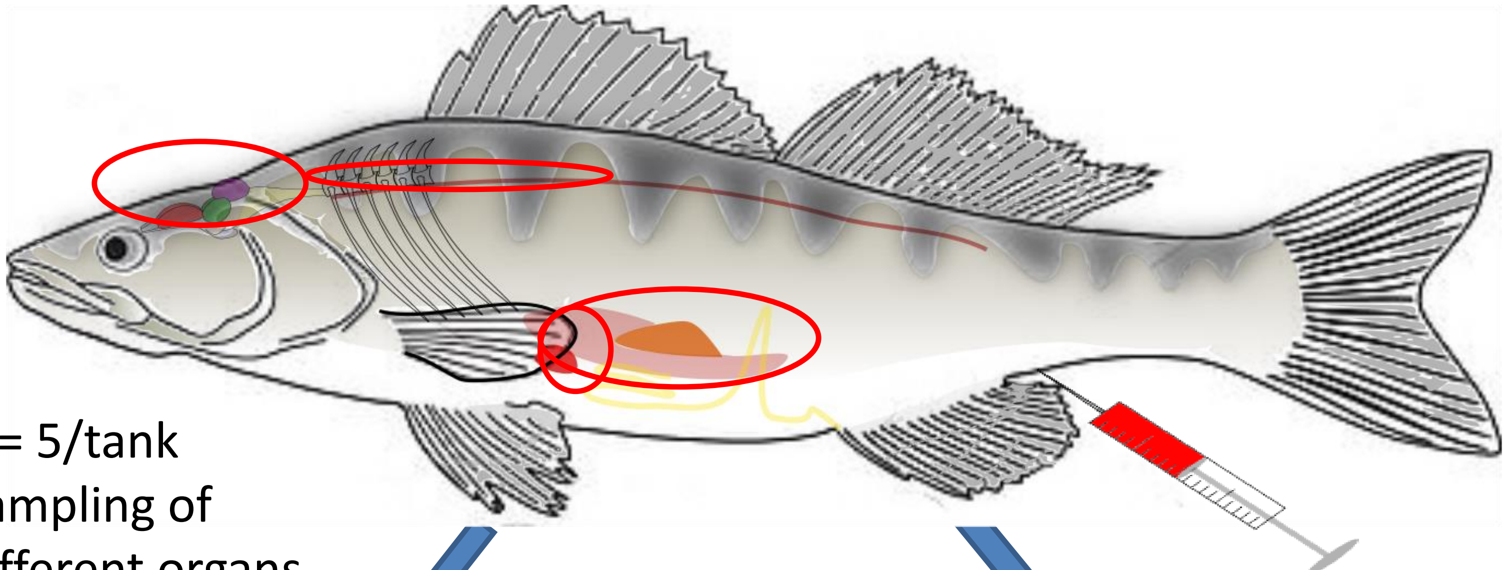
Ecotron with red spectrum (UL)

Experimental design and analyses

D-30 — D0 — D36 — D63

Acclimation | Short-term effects | Long-term effects

(1) *Husbandry variables: survival, growth, FCR,…*



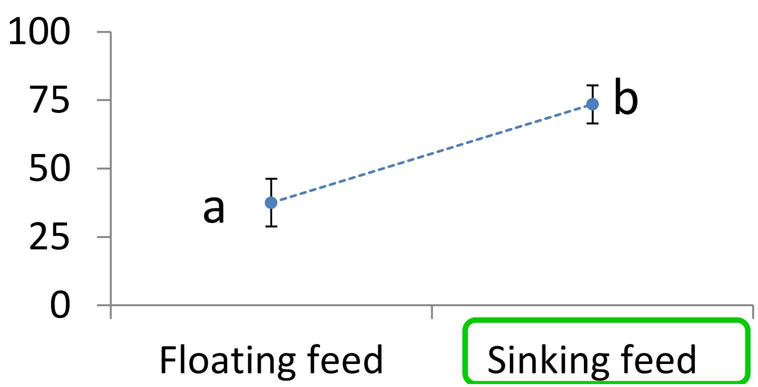
- n = 5/tank
- Sampling of different organs

(2) *Stress markers:*
- Plasma cortisol
- Plasma glucose
- Brain neurotransmitters

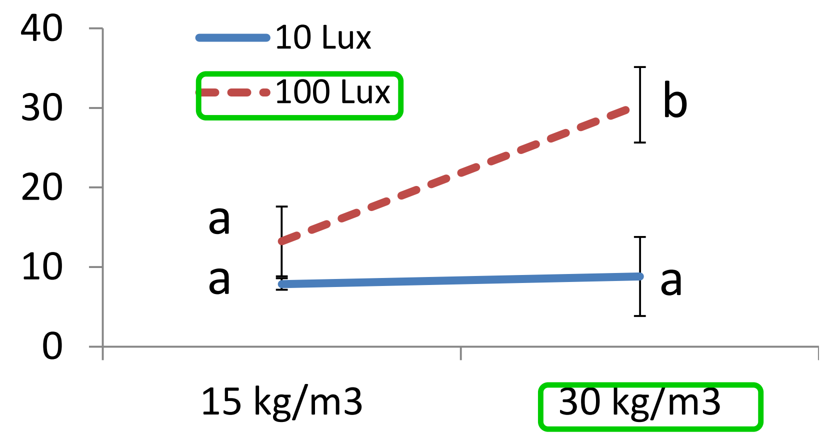
(3) *Immune parameters:*
- Plasma lysozyme activity
- Complement activity (ACH50)
- Gene expressions (lysozyme,…)

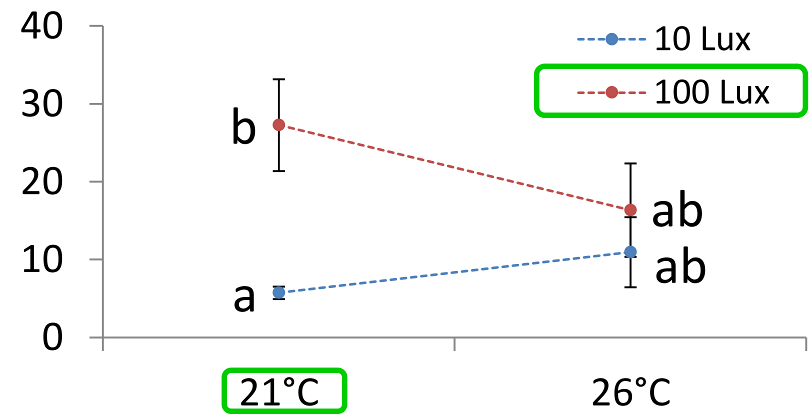
Results

- ***Relative Growth Rate (%)***



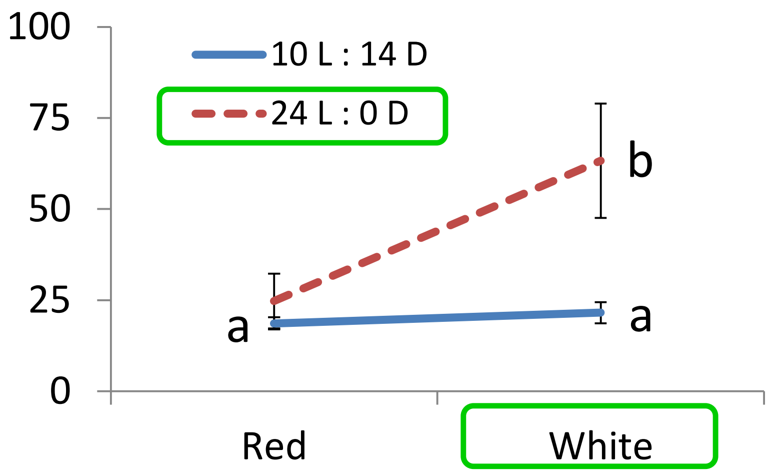
- ***Mortality rate (%)***

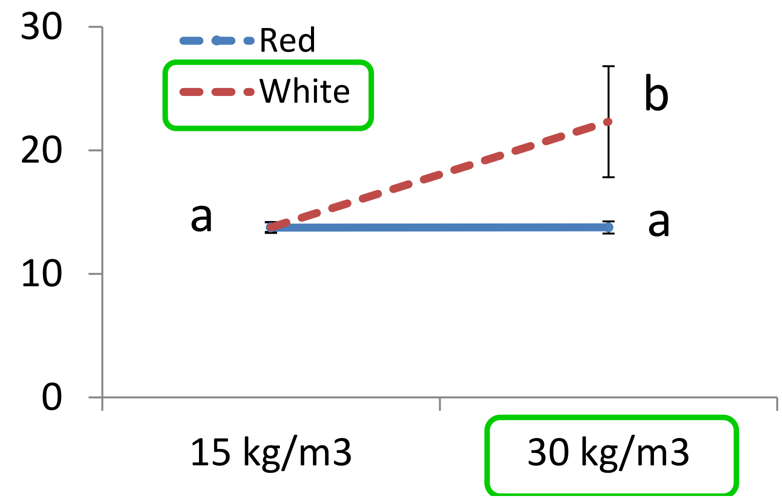


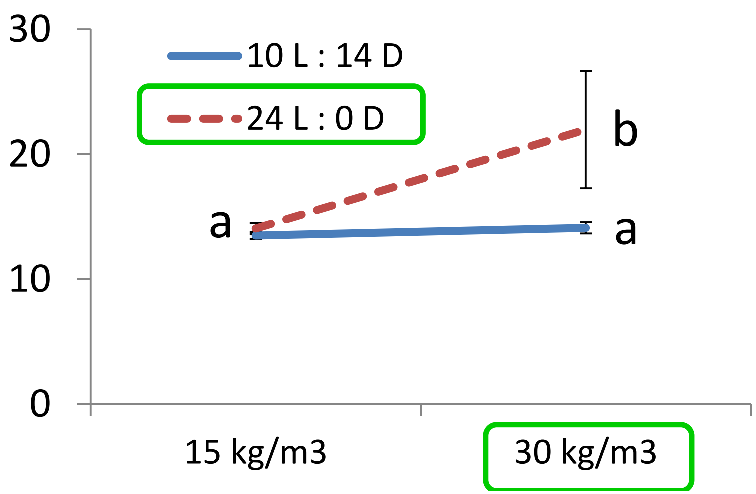


Results

- ***Plasma cortisol (ng/ml)***



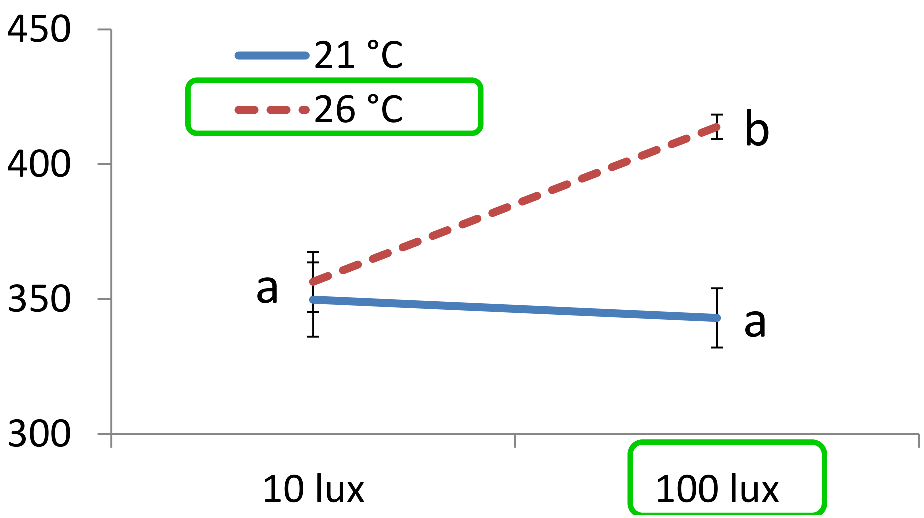




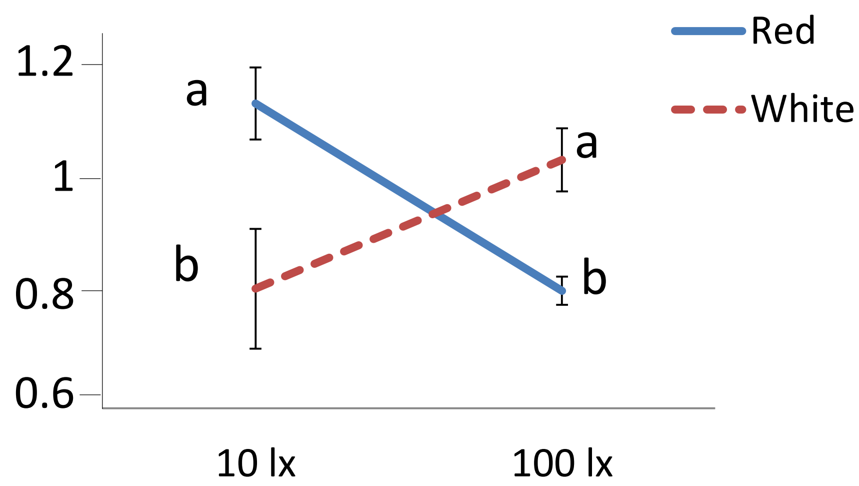
➢ High plasma cortisol levels due to white light, long photoperiod and high stocking density

Results

- ***Plasma glucose (µg/ml)***



- ***Brain serotonergic activity (5-HIAA/5-HT)***



➢ High glucose levels when high light intensity associated with high temperature

➢ Higher activity related to higher stress level

Ecotron	Biomass gain (g)	RGR (%)	GMR (%)
1	7238	81	4,32
2	2888	56,9	2,86
3	3059	84,9	12,86
4	17	53,7	30,94
5	1494	41	7,14
6	-609	0	7,14
7	-1210	21	24,29
8	1996	57,4	10
9	3216	102,8	12,86
10	-2056	57,4	41,01
11	1770	63,4	17,99
12	-1764	22,8	23,74
13	1534	62,4	31,65
14	3007	26,5	4,32
15	9042	80	2,88
16	3511	81,9	7,14

3 combinations look promising for pikeperch aquaculture

- **High biomass gain**
- **High growth**
- **Low mortality**

Tested in a confirmation experiment

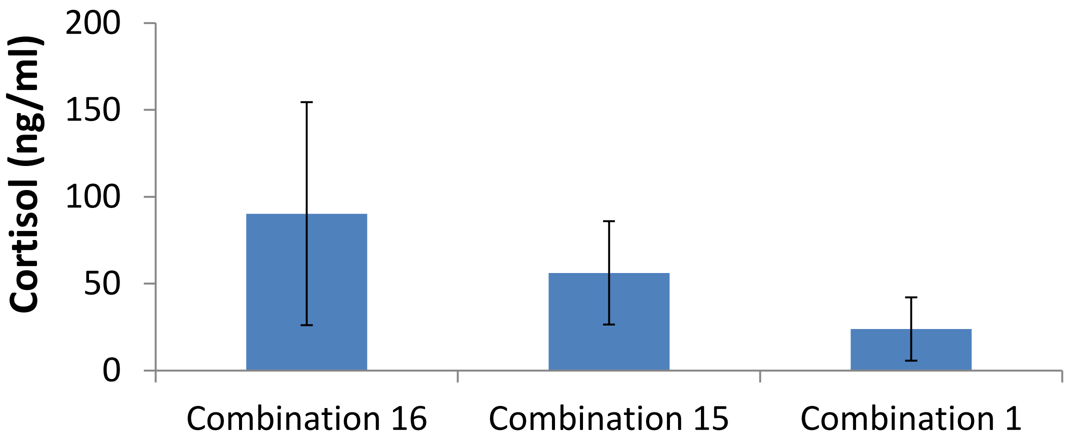
Confirmation experiment

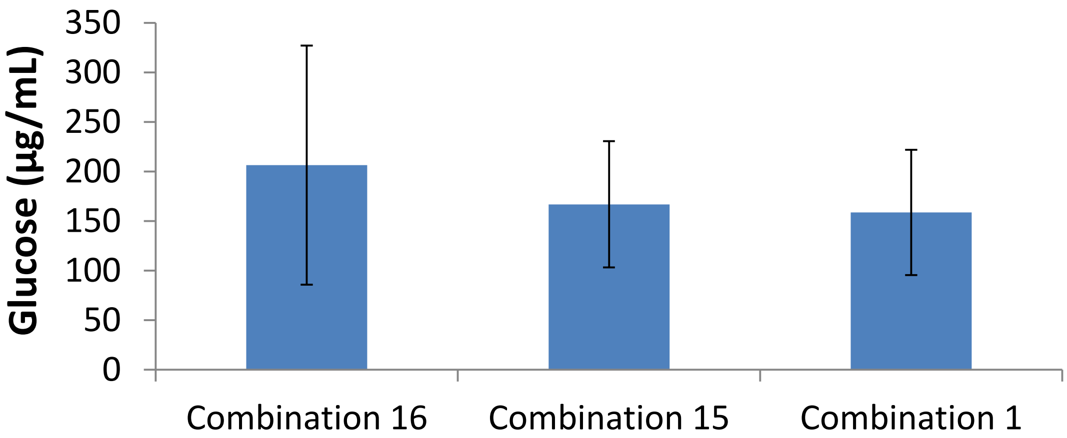
- Objective: determining the disease resistance after bacterial exposure following exposure to selected conditions

Tank	Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type feed	Grading	Oxygen saturation
16	**10**	15	Red	24	26	**sinking**	N	90
15	**10**	30	White	10	26	**sinking**	N	60
1	**10**	30	White	24	21	**sinking**	Y	90

- 3 replicates per condition
- 3 separated recirculating aquaculture systems
- Duration = 36 days
- Bacteria challenge, *Aeromonas salmonicida Achromogen*: 10^7 CFU/100g fish
- Mortality during 10 days after bacteria injection

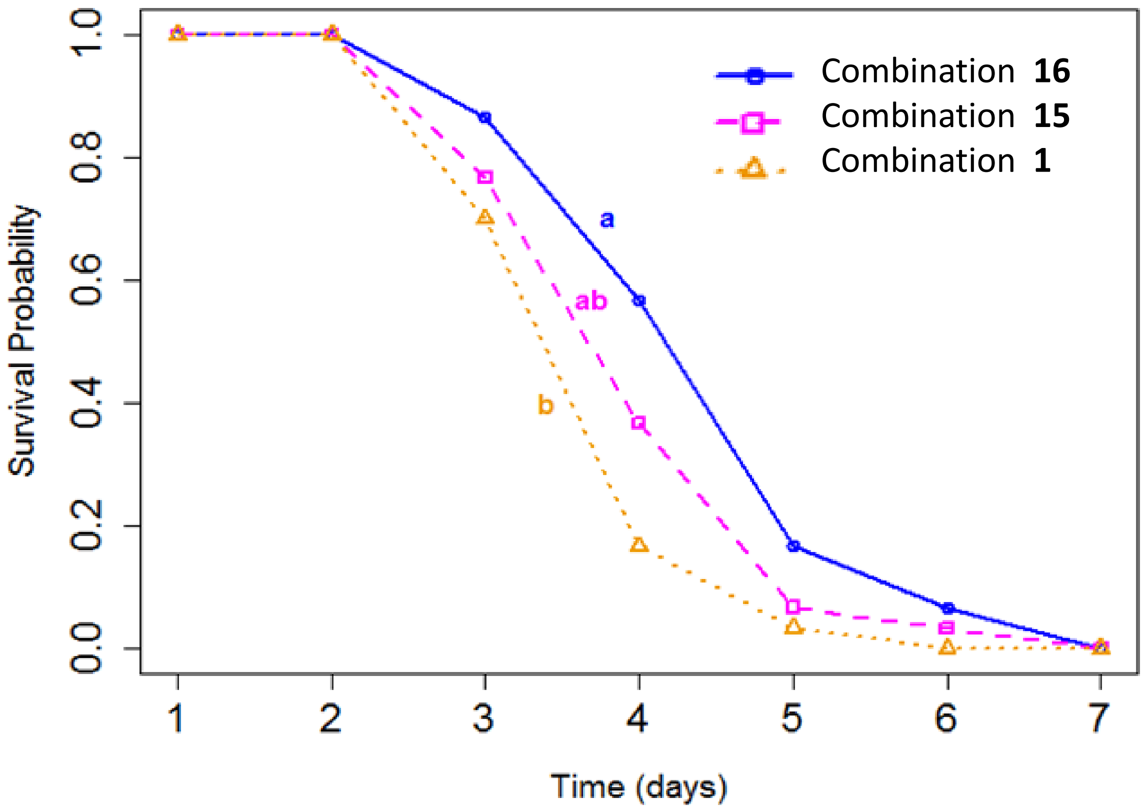
- Physiological status





No significant effects of combinations

- Mortality after bacterial challenge



LT50 (Lethal Time) (days)	
16	**4.2**
15	3.6
1	3.4

	16	15	1
Intensity	**10**	10	10
Density	**15**	30	30
Spectrum	**R**	W	W
Photop.	**24**	10	24
Temp.	**26**	26	21
Grading	**N**	N	Y
O2	**90**	60	90

➢ Modalities of combination 16 with **red spectrum** were the most protective indicating a higher disease resistance

Conclusions of WP22.1

- Light characteristics and, to a lesser extent, temperature and density are directive environmental factors for pikeperch welfare
- Low light intensity and red light spectrum seem less stressful since stress markers (cortisol, glucose, brain neurotransmitters) tended to decrease
- The optimal environmental and husbandry factor-modalities defined need more validation under experimental and commercial aquaculture conditions

WP22.2

Validation of optimal rearing variables under commercial farm conditions

Objectives of WP22.2

- To validate in commercial farm conditions identified as optimal for pikeperch rearing (WP 22.1) by assessing growth related parameters and physio-immunological status of pikeperch at different developmental stages (from 10 g to 500 g)

- To determine the stress sensitivity of fish reared under red or white light spectrum to grading manipulations

Material & Methods

Facilities and Fish

- Production of 8100 juveniles up to 11g in Fish2Be facilities
- Distribution of fish into 6 tanks (vol. 2000 L) in a RAS

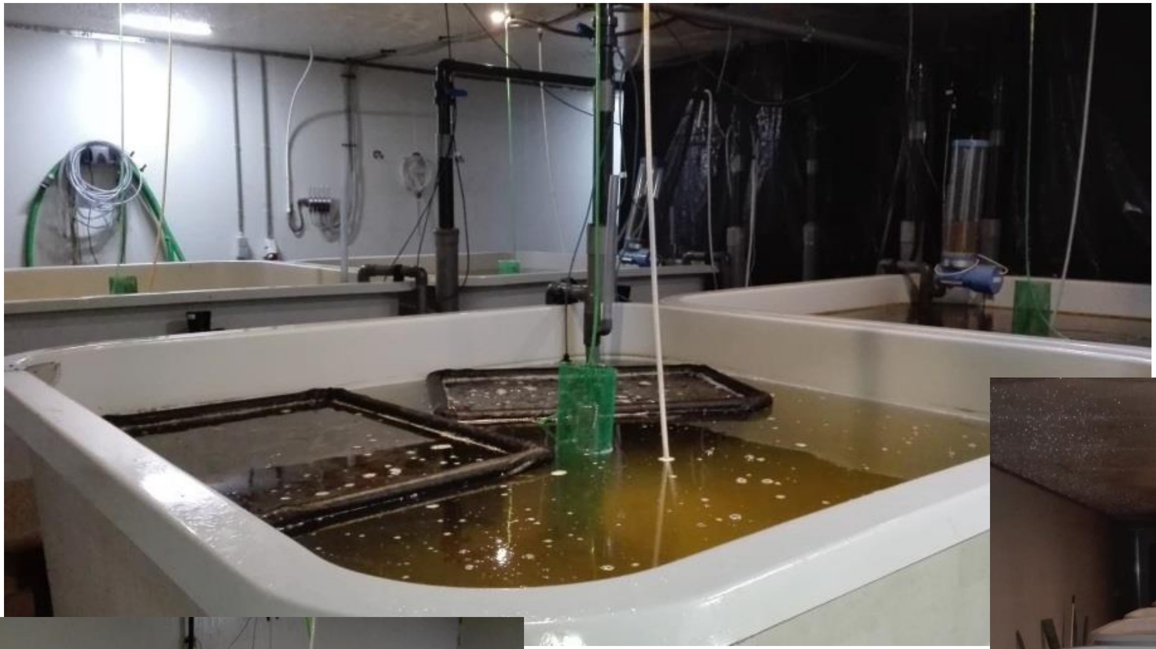
Rearing conditions

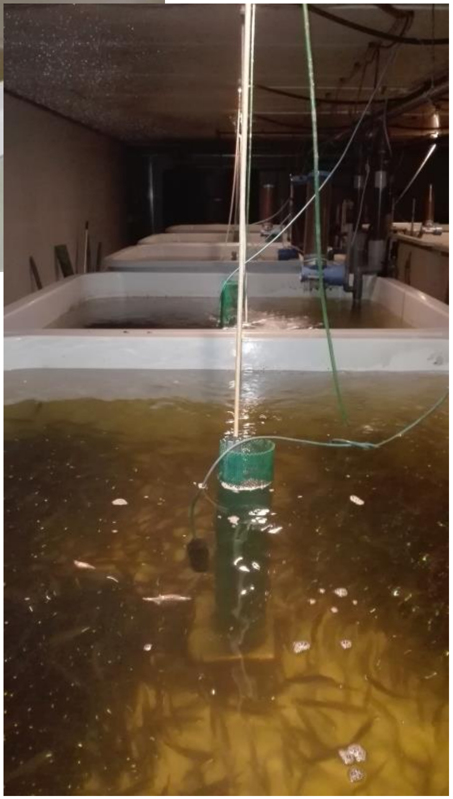
- Based on WP 22.1 results:
    - Temperature: 21° C
    - Light intensity: 10 Lux – Light spectrum: white vs Red – Photoperiod: 12L-12D
- Three 800L-tanks/group
- Size grading on days 49 and 83 to reduce size heterogeneity

Analytical endpoints

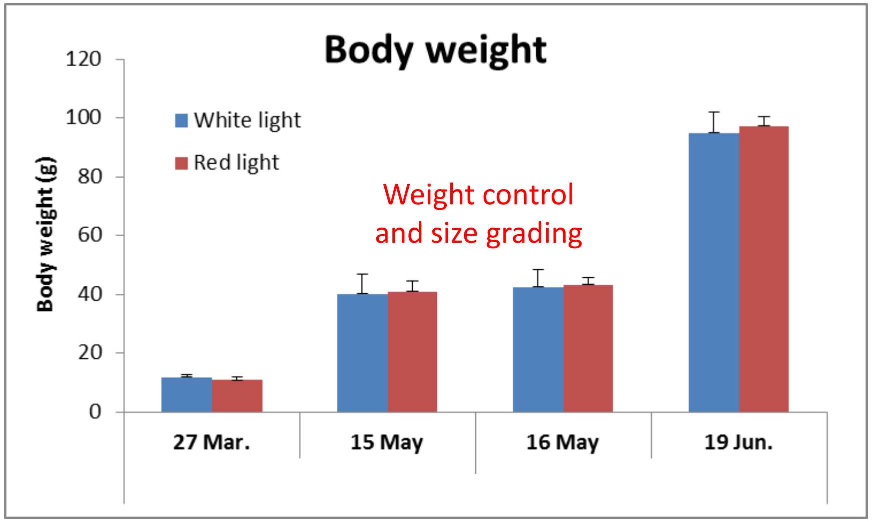
- Growth (SGR) and weight heterogeneity
- Stress indicators (cortisol, glucose)
- Immune indicators (lysozyme and peroxidase activities)

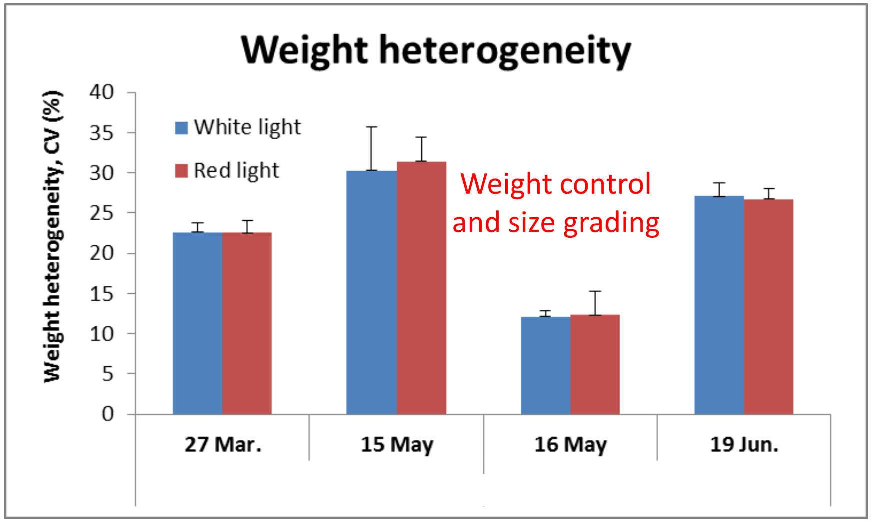
Facilities at Fish2Be – size grading

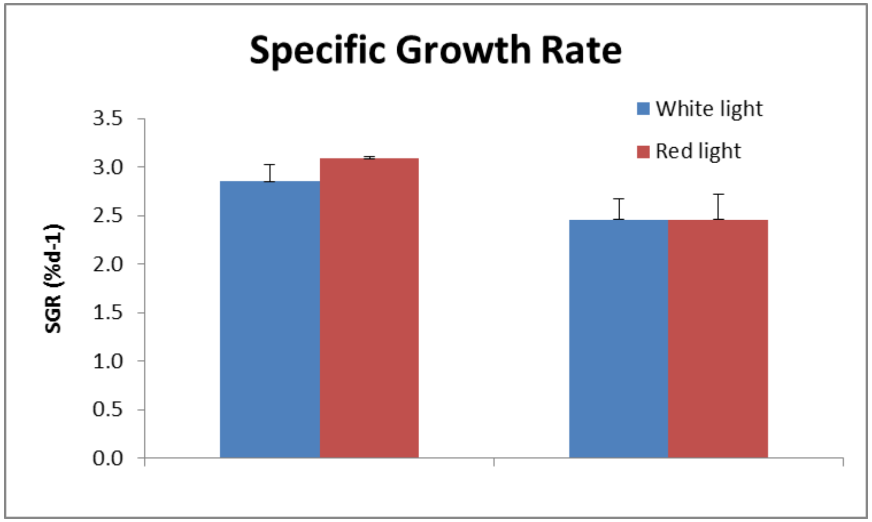


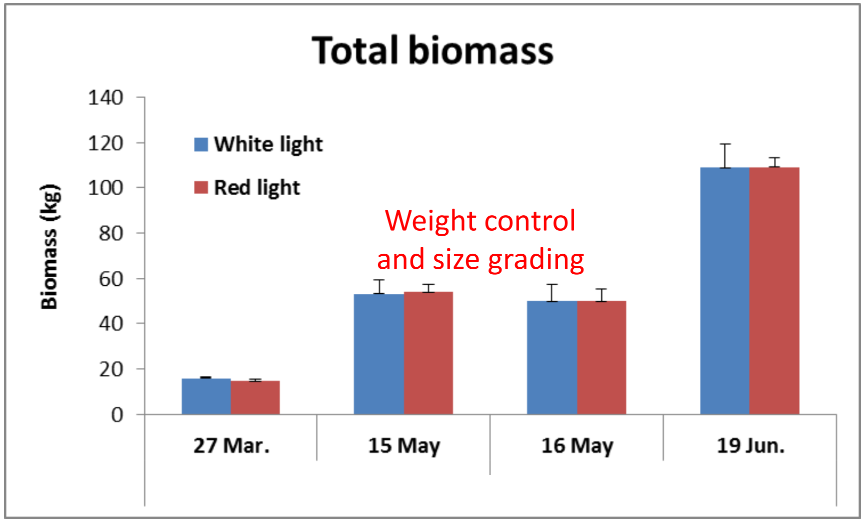


Results

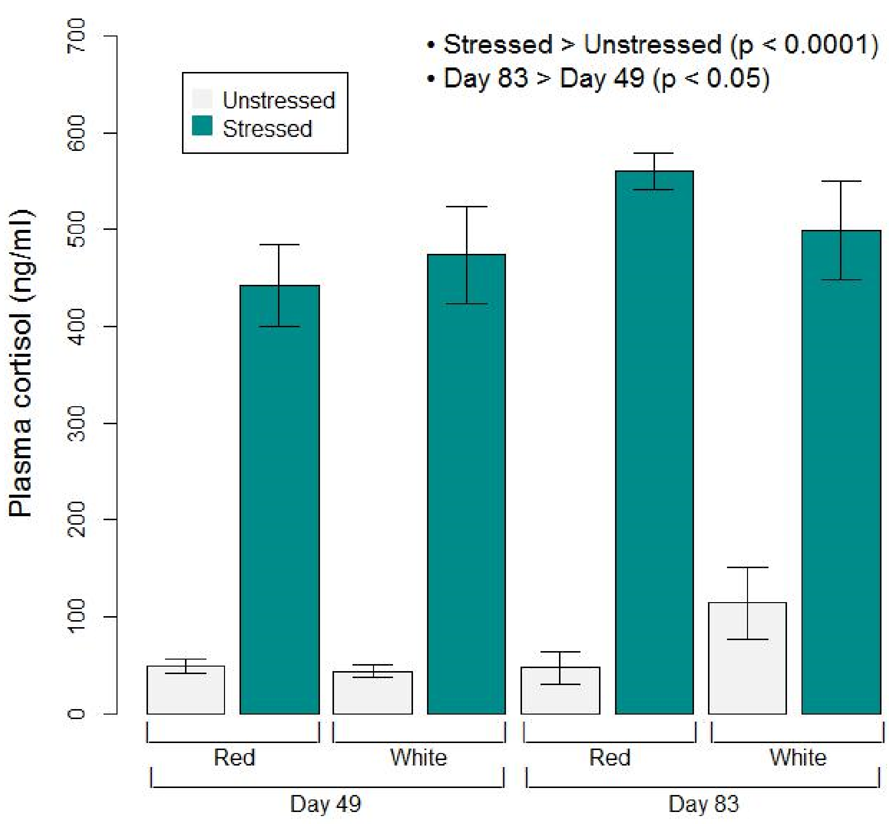

Body weight
Body weight (g)
120
100
80
60
40
20
0
White light
Red light
Weight control
and size grading
27 Mar.
15 May
16 May
19 Jun.


Weight heterogeneity
Weight heterogeneity, CV (%)
40
35
30
25
20
15
10
5
0
White light
Red light
Weight control
and size grading
27 Mar.
15 May
16 May
19 Jun.

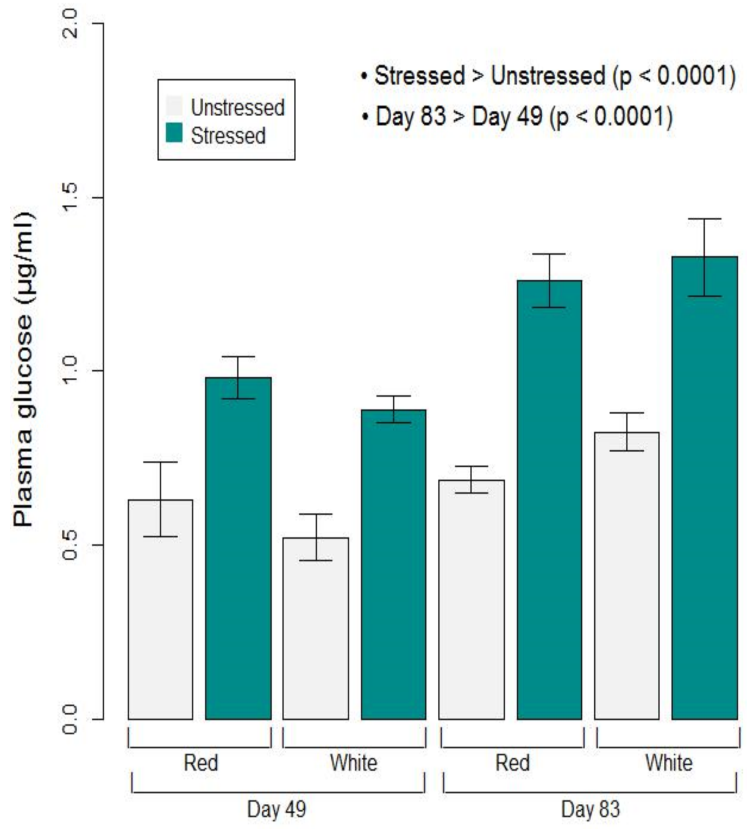

Specific Growth Rate
SGR (%d-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
White light
Red light


Total biomass
Biomass (kg)
140
120
100
80
60
40
20
0
White light
Red light
Weight control
and size grading
27 Mar.
15 May
16 May
19 Jun.

Results

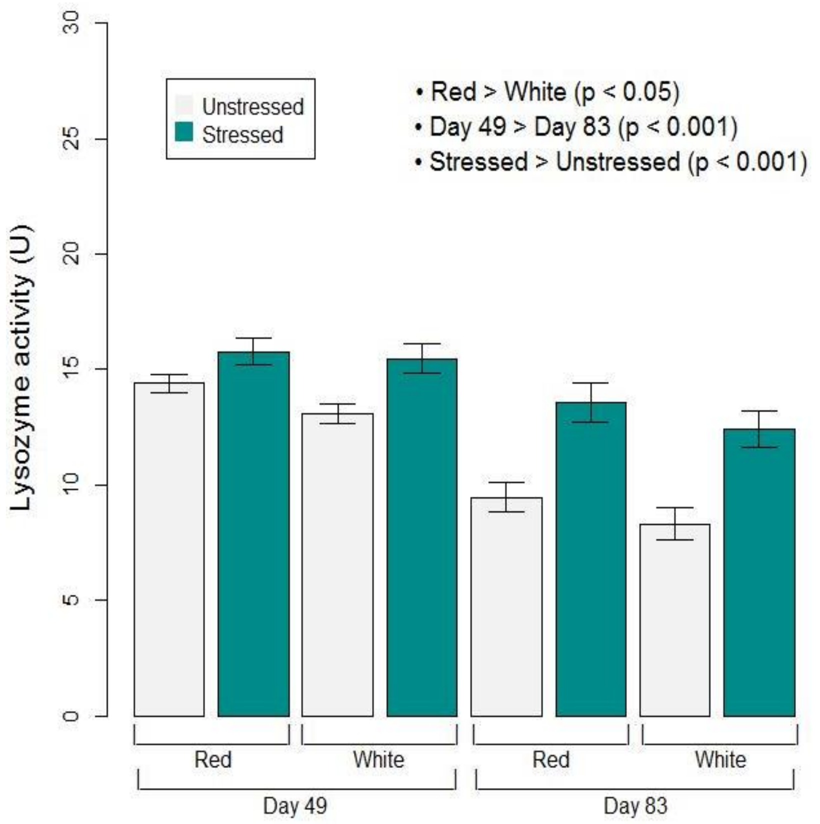


• Stressed > Unstressed (p < 0.0001)
• Day 83 > Day 49 (p < 0.05)
Unstressed
Stressed
Plasma cortisol (ng/ml)
0
100
200
300
400
500
600
700
Red
White
Red
White
Day 49
Day 83

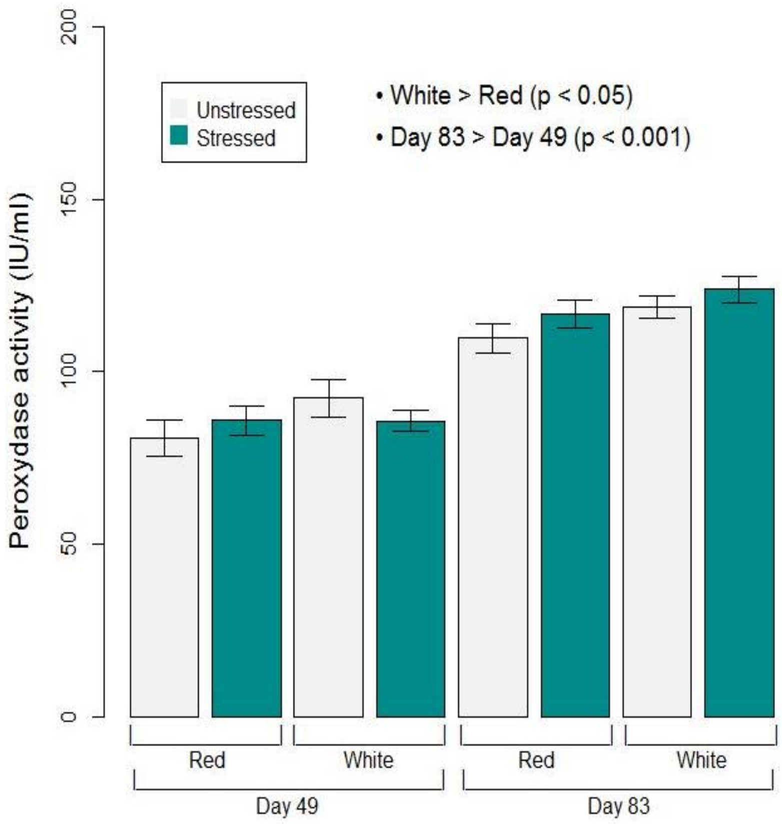


• Stressed > Unstressed (p < 0.0001)
• Day 83 > Day 49 (p < 0.0001)
Unstressed
Stressed
Plasma glucose (µg/ml)
0.0
0.5
1.0
1.5
2.0
Red
White
Red
White
Day 49
Day 83

Results



Lysozyme activity (U)
30
25
20
15
10
5
0
Unstressed
Stressed
• Red > White (p < 0.05)
• Day 49 > Day 83 (p < 0.001)
• Stressed > Unstressed (p < 0.001)
Red
White
Red
White
Day 49
Day 83



Peroxydase activity (IU/ml)
200
150
100
50
0
Unstressed
Stressed
• White > Red (p < 0.05)
• Day 83 > Day 49 (p < 0.001)
Red
White
Red
White
Day 49
Day 83

Conclusions of WP22.2

- Significant effects of size grading on stress markers
- No significant effects of light spectrum under low light intensity
  - Similar growth performance
  - Similar levels of cortisol and glucose before and after size grading
- Differential effects of light spectrum on immune markers
  - Red light enhanced lysozyme activity
  - White light enhanced peroxidase activity
- What are the consequences of light spectrum on immune competence of pikeperch?

WP22.3

Effects of domestication level and geographical origin on stress, immune response and growth performances

Materials & Methods

Fish

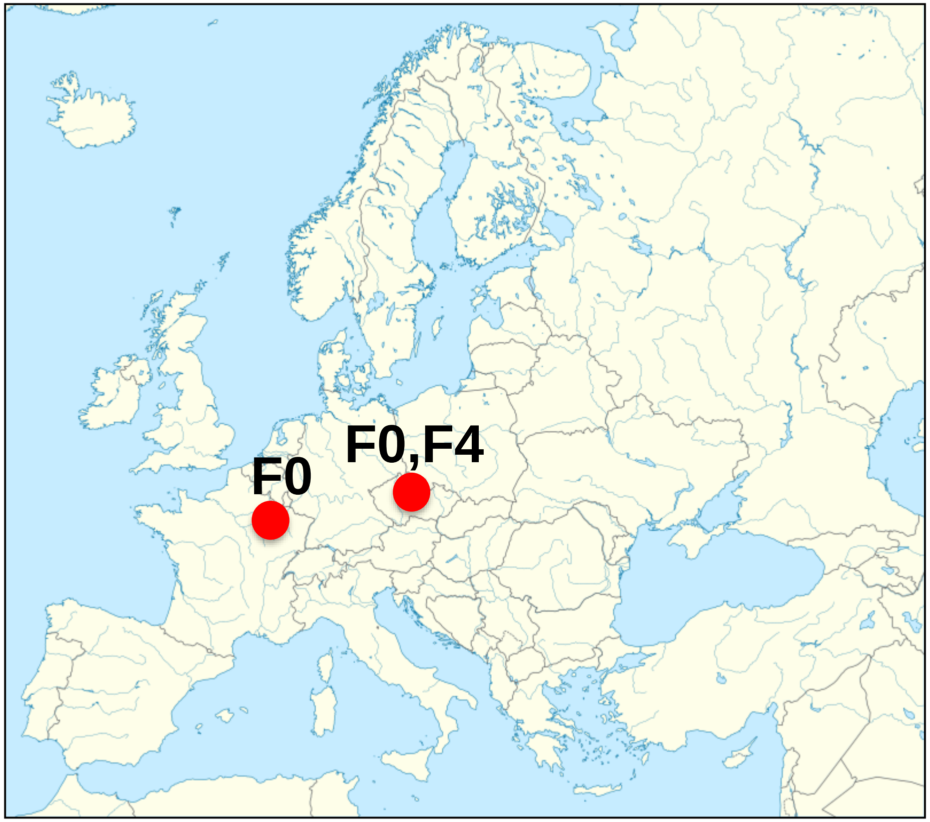
- Two European wild populations: Western Europe (Lindre, France) *vs* Central Europe (Czech Republic)
- Two domestication levels : F0 vs F4

Facilities

- Transfert to UL facilities at the larval stage
- Larval rearing under same conditions
- Three 800L-tanks/group
- 5 kg/m^3 initial biomass

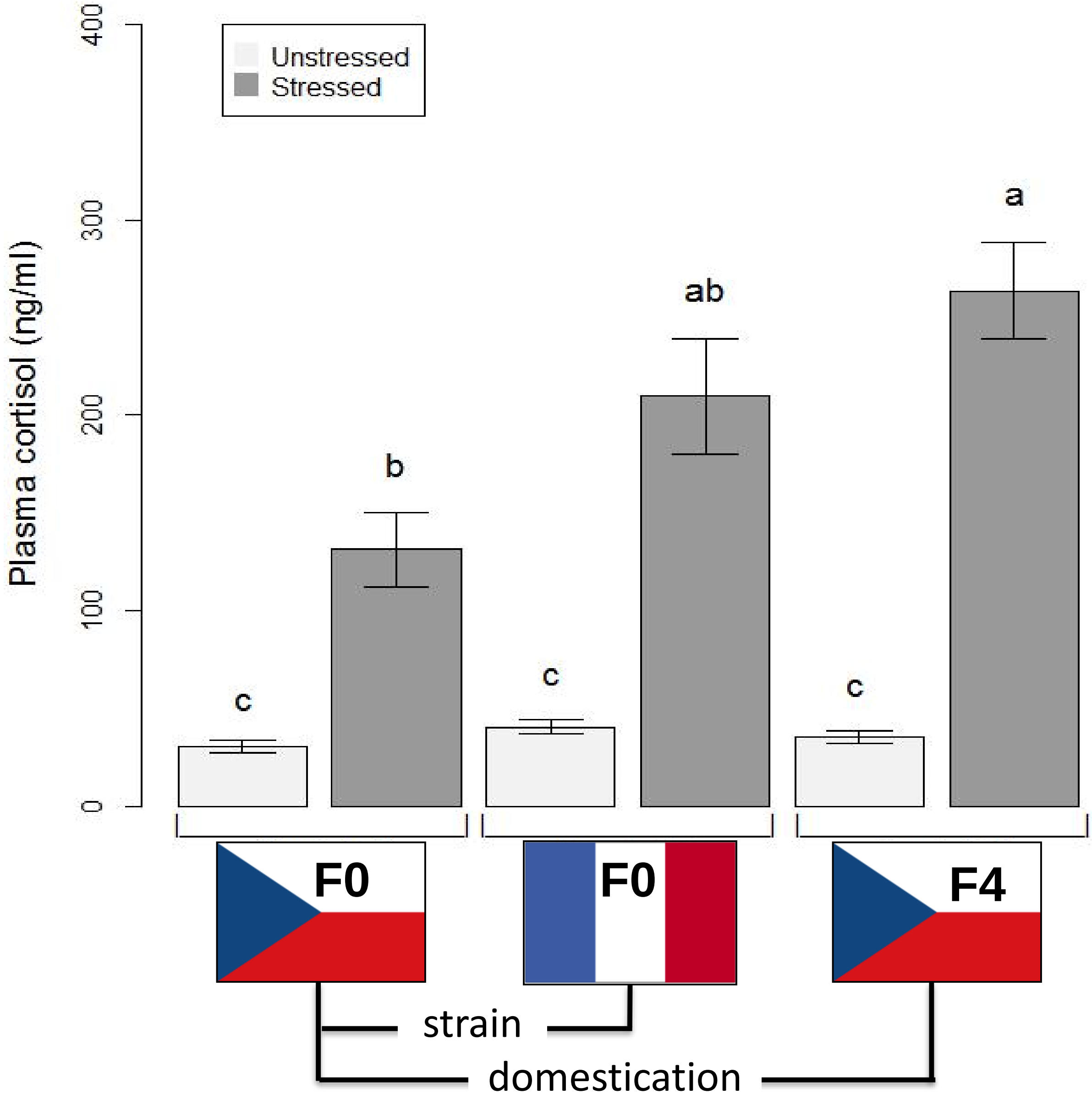
Stressor and biomarkers

- Net chasing stress during 30 s
- Stress indicators (cortisol, glucose)
- Immune indicators (lysozyme and peroxidase activities)



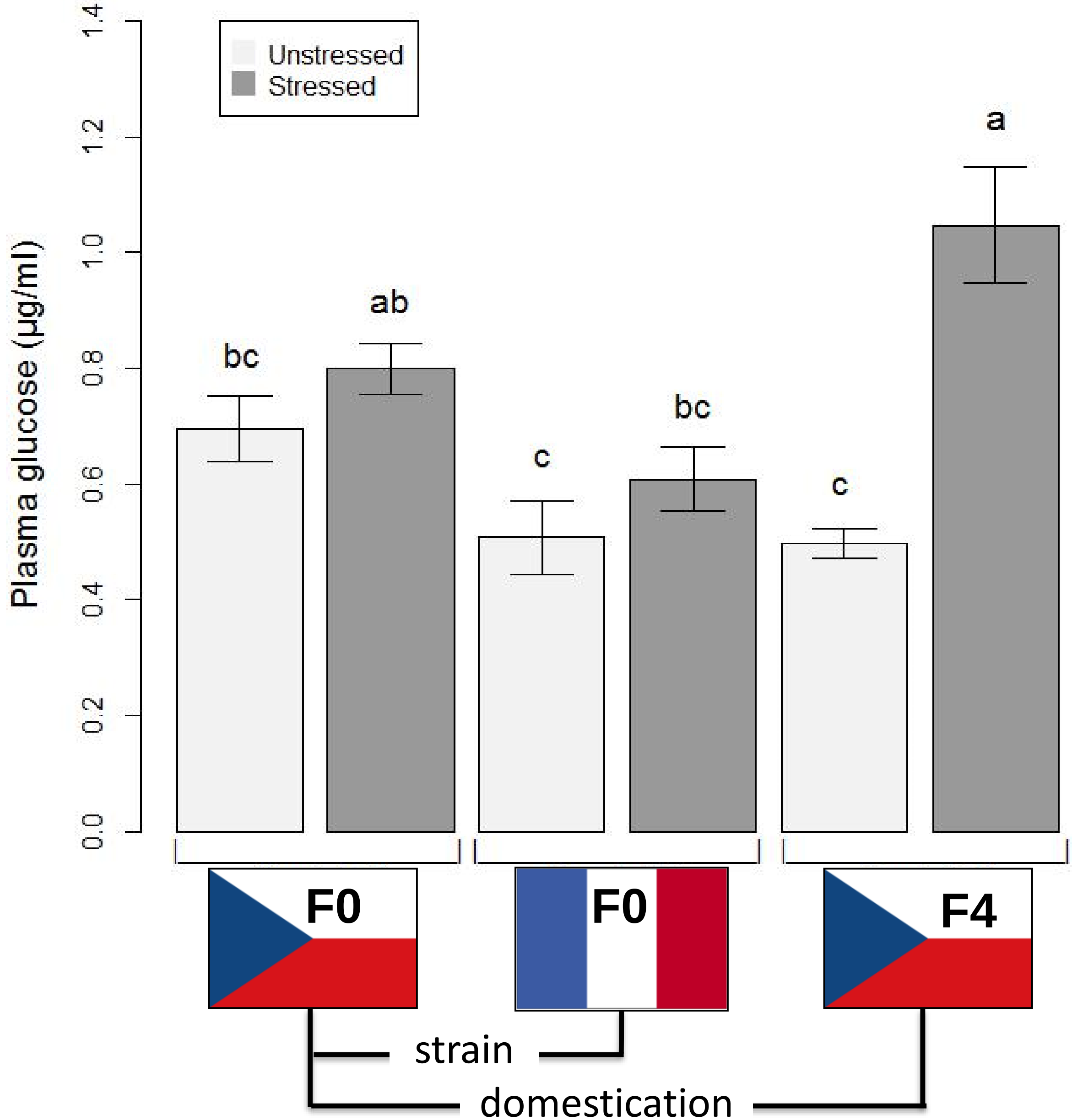
Facilities at University of Lorraine

Results



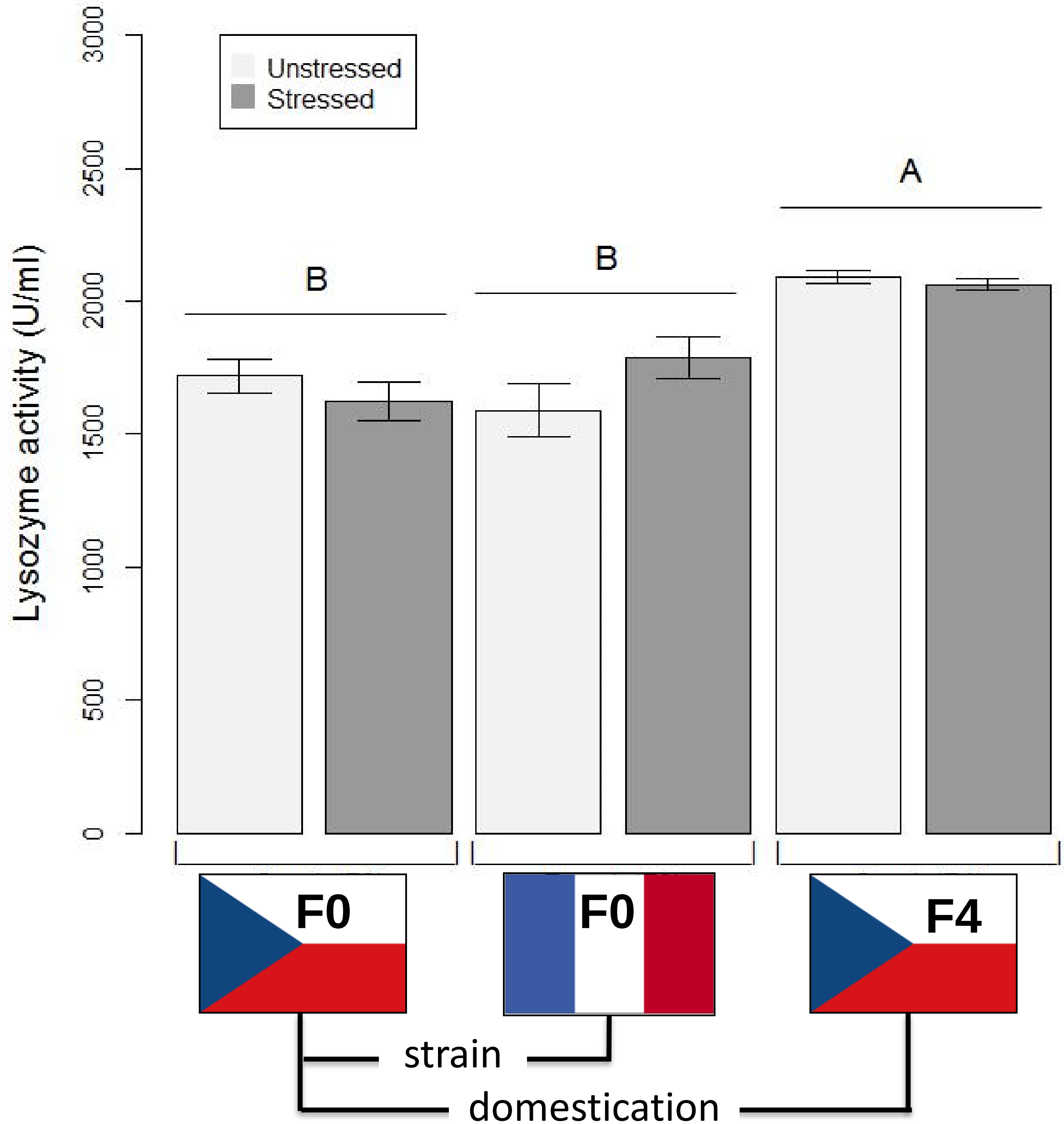
Plasma cortisol (ng/ml)
0
100
200
300
400
Unstressed
Stressed
c
b
c
ab
c
a
F0
F0
F4
strain
domestication

Results



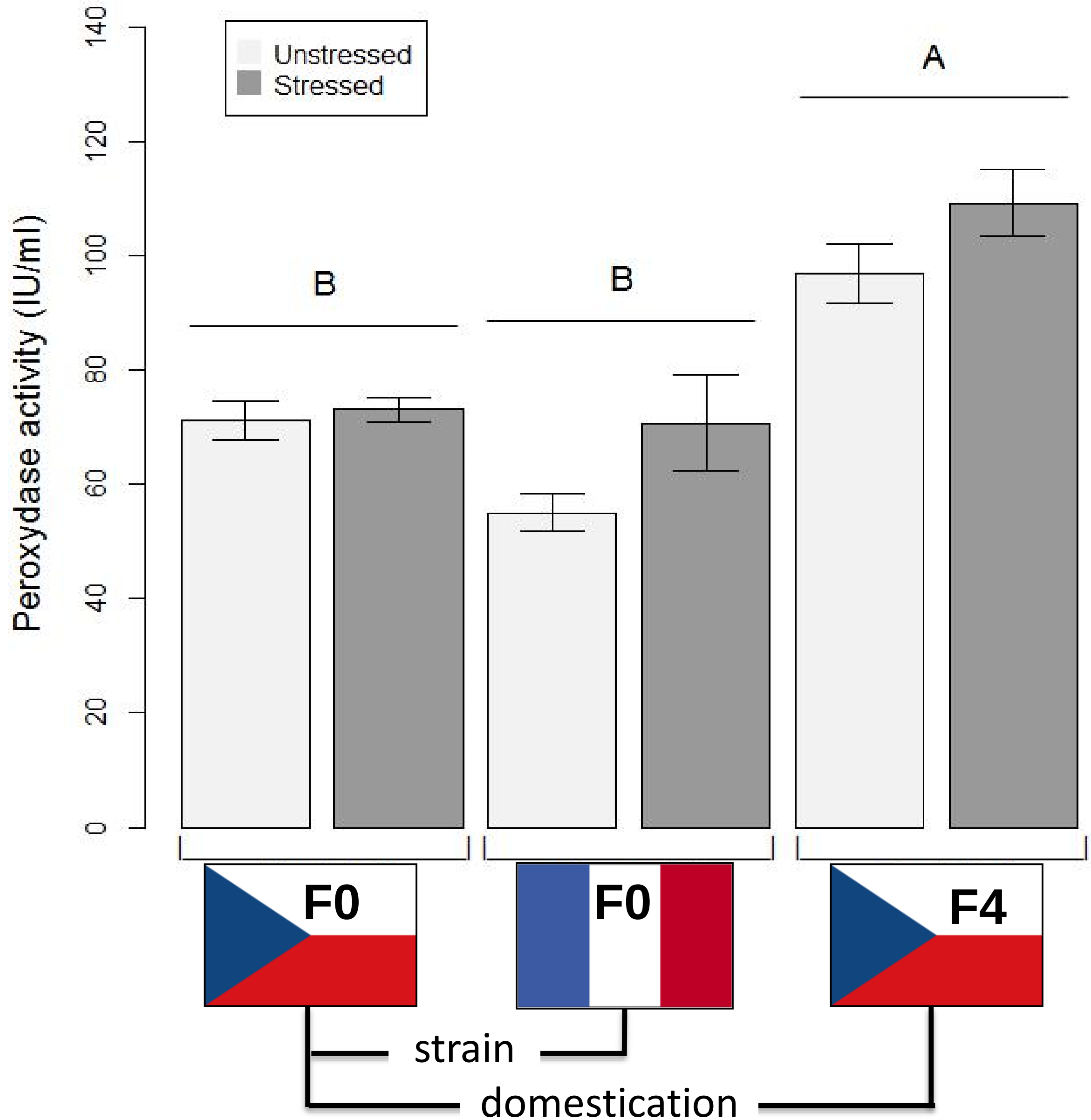
Plasma glucose (µg/ml)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Unstressed
Stressed
bc
ab
c
bc
c
a
F0
F0
F4
strain
domestication

Results



Lysozyme activity (U/ml)
0
500
1000
1500
2000
2500
3000
Unstressed
Stressed
B
B
A
F0
F0
F4
strain
domestication

Results



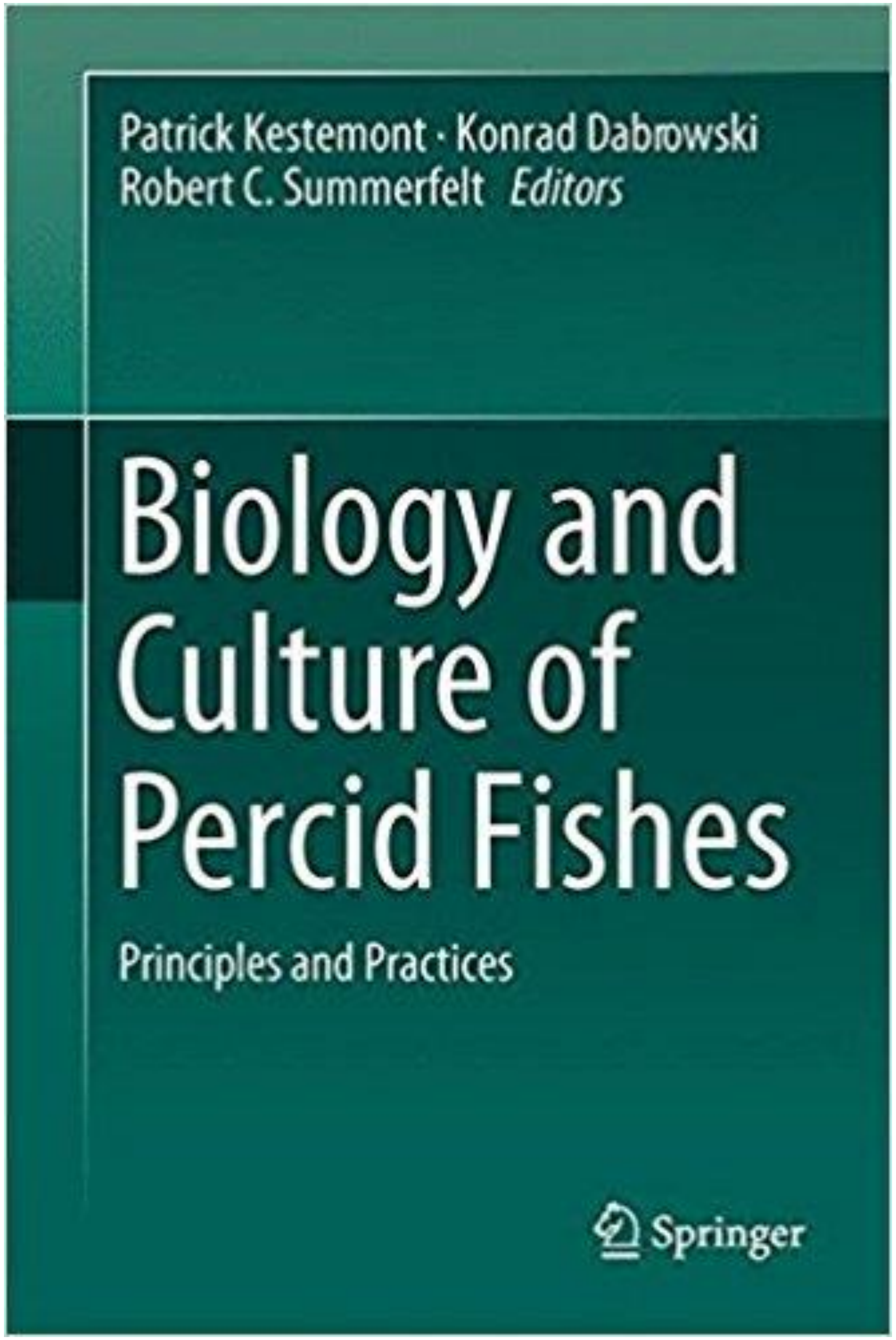
Peroxydase activity (IU/ml)
0
20
40
60
80
100
120
140
Unstressed
Stressed
B
B
A
F0
F0
F4
strain
domestication

Conclusions of WP22.3

- Influence of strain on stress responsiveness and immune response
  - No differences between wild French and Czech strains
  - Similar basal levels, similar acute responses after net chasing
- Influence of domestication level on stress responsiveness and immune response
  - No significant differences of basal levels between F0 and F4 Czech strains
  - Increased stress response in F4 strain after net chasing
  - Increased level of bactericidal immune activities in F4 strain
- These preliminary results suggest that domestication is likely to stimulate the stress axis, inducing a higher immune competence

Thanks for your attention

More info…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