

# DIVERSIFY: WP 22.1

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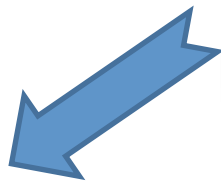
Effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status



# DIVERSIFY: WP 22 context



High potential for European aquaculture



**aquapri**  
handpicked luxury seafood



  
**Asialor**  
ÉLEVAGE DE PERCHES ET SANDRES

≠



High mortality in juveniles

# DIVERSIFY: Goals of the WP 22

Find out the optimal combinations between environmental and husbandry practices for improving the growth and survival rates as well as the welfare of pikeperch



DIVERSIFY: Multifactorial experiment

What are the best conditions  
for pikeperch rearing ?



## Husbandry and environmental stressors

Factor	Modality	References
Photoperiod	10 L : 14 D	Pourhosein Saramah 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 <sup>3</sup>	Steenfeldt et al., (2010) in Dalsgaard et al., 2013
	30kg/m <sup>3</sup>	
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	

# DIVERSIFY: Factorial fractional design

Experimental unit = tank

8 factors \* 2 modalities/factors

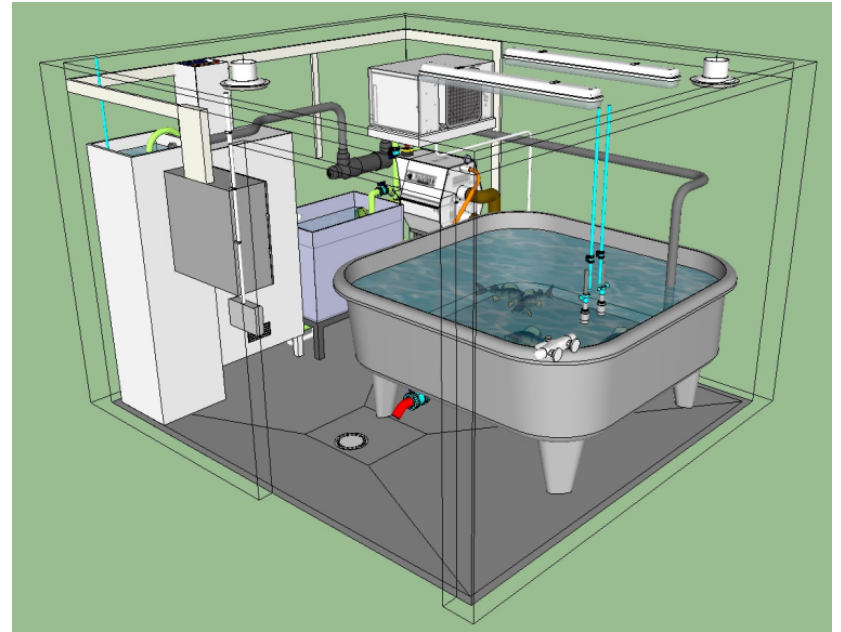


$2^8 = 256$  combinations

Using factorial  
fractional design



16 different combinations



## Screening approach

# DIVERSIFY: Factorial fractional design

- Complete design =  $2^4$   
=> 16 EU
- Factorial fractional design =  $2^{8-4}$

Main factors				
A	B	C	D	
	1	1	1	1
	1	1	1	-1
	1	1	-1	1
	1	1	-1	-1
	1	-1	1	1
	1	-1	1	-1
	1	-1	-1	1
	1	-1	-1	-1
	-1	1	1	1
	-1	1	1	-1
	-1	1	-1	1
	-1	1	-1	-1
	-1	-1	1	1
	-1	-1	1	-1
	-1	-1	-1	1
	-1	-1	-1	-1



E = ABC
F = -BCD
G = ABD
H = -ACD



Main factors defined by aliasing factors				
A	B	C	D	
E (ABC)	F (-BCD)	G (ABD)	H (-ACD)	
	1	-1	1	-1
	1	1	-1	1
	-1	1	1	1
	-1	-1	-1	-1
	-1	1	-1	-1
	-1	-1	1	1
	1	-1	-1	1
	1	1	1	-1
	-1	-1	-1	1
	-1	1	1	-1
	-1	1	-1	-1
	1	1	-1	-1
	1	-1	1	1
	1	1	1	1
	1	-1	-1	-1
	-1	-1	1	-1
	-1	1	-1	1

# DIVERSIFY: Factorial fractional design

$$1 = ABCE = -BCDF = ABDG = -ACDH$$

$$A = BCE = -ABCDF = BDG = -CDH$$

$$B = ACE = -CDF = ADG = -ABCDH$$

$$C = ABE = -BDF = ABCDG = -ADH$$

$$D = ABCDE = -BCF = ABG = -ACH$$

$$AB = CE = -ACDF = DG = -BCDH$$

$$AC = BE = -ABDF = BCDG = -DH$$

$$AD = BCDE = -ABCF = BG = -AH$$

$$BC = AE = -DF = ACDG = -ABDH$$

$$BD = ACDE = -CF = AG = -ABCH$$

$$CD = ABDE = -BF = ABCG = -AH$$

$$ABC = E = -ADF = CDG = -BDH$$

$$ABD = DCE = -ACF = G = -BCH$$

$$ACD = BDE = -ABF = BCG = -H$$

$$BCD = ADE = -F = ACG = -ABH$$

$$ABCD = DE = -AF = CG = -BH$$

Main effects

 Alone

2 ways interactions

 Aliased

3 ways and + interactions

 H0

# DIVERSIFY: Multifactorial experiment

## Experimental design using Planor (Kobilinsky)

Ecotron (n°)	Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type of aliment	Handling	Oxygen saturation
<b>1</b>	10	30	white	24	21	sinking	Y	90
<b>2</b>	100	15	red	10	26	floating	N	60
<b>3</b>	100	15	white	24	21	sinking	N	60
<b>4</b>	100	30	red	10	21	sinking	N	90
<b>5</b>	10	15	red	10	21	sinking	Y	60
<b>6</b>	10	15	white	10	21	floating	N	90
<b>7</b>	100	15	red	24	21	floating	Y	90
<b>8</b>	10	15	white	24	26	floating	Y	60
<b>9</b>	100	15	white	10	26	sinking	Y	90
<b>10</b>	100	30	white	10	21	floating	Y	60
<b>11</b>	100	30	white	24	26	floating	N	90
<b>12</b>	10	30	red	10	26	floating	Y	90
<b>13</b>	100	30	red	24	26	sinking	Y	60
<b>14</b>	10	30	red	24	21	floating	N	60
<b>15</b>	10	30	white	10	26	sinking	N	60
<b>16</b>	10	15	red	24	26	sinking	N	90

# DIVERSIFY: Multifactorial experiment

## Deeper look into our experimental design

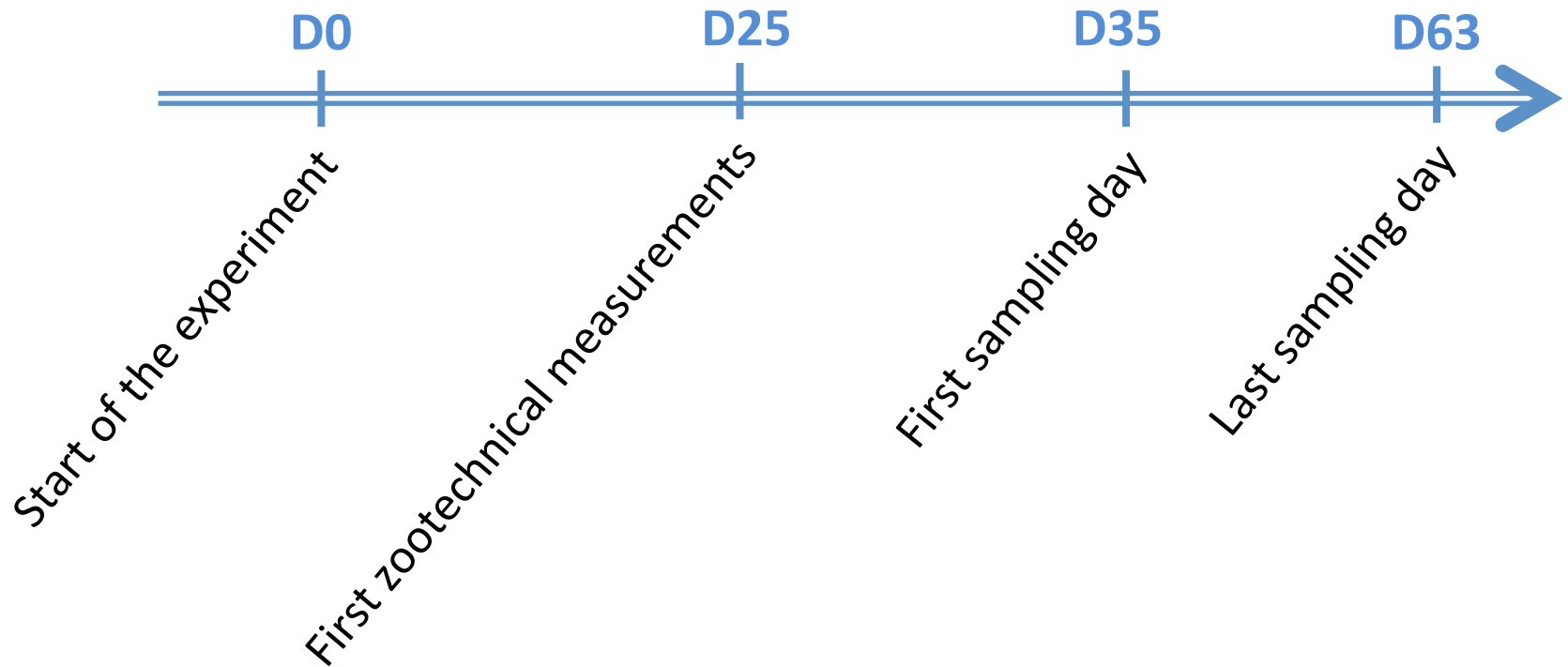
Ecotron	Light intensity	Rearing density	Light spectrum	Photoperiod	Temperature	Alimentation	handling	Oxygen
1	10 lux	30 kg/m <sup>3</sup>	White	24 L : 0 D	21 °C	Sinking	Yes	90%
5	10 lux	15 kg/m <sup>3</sup>	Red	10 L : 14 D	21 °C	Sinking	Yes	60%
7	100 lux	15 kg/m <sup>3</sup>	Red	24 L : 0 D	21 °C	Floating	Yes	90%





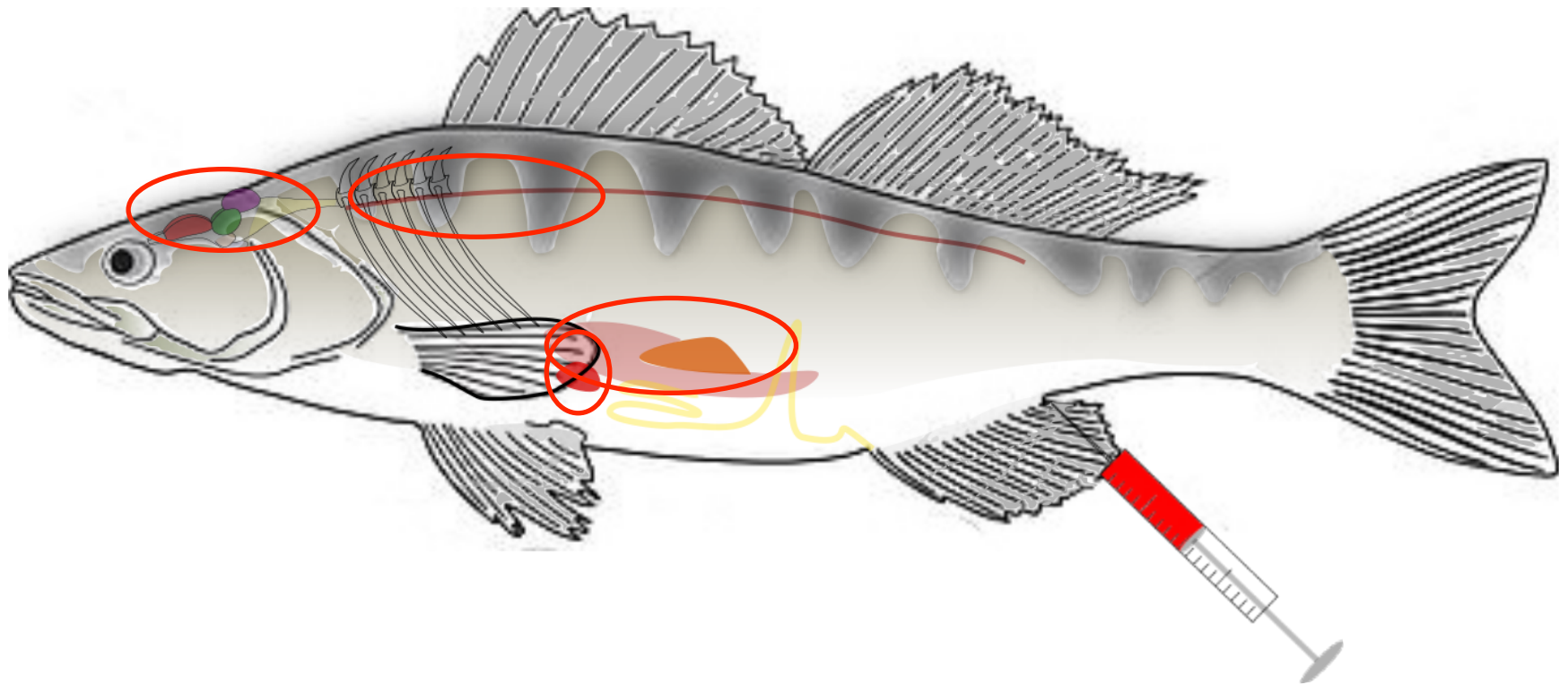
# DIVERSIFY: Multifactorial experiment

## Timeline



# DIVERSIFY: Multifactorial experiment

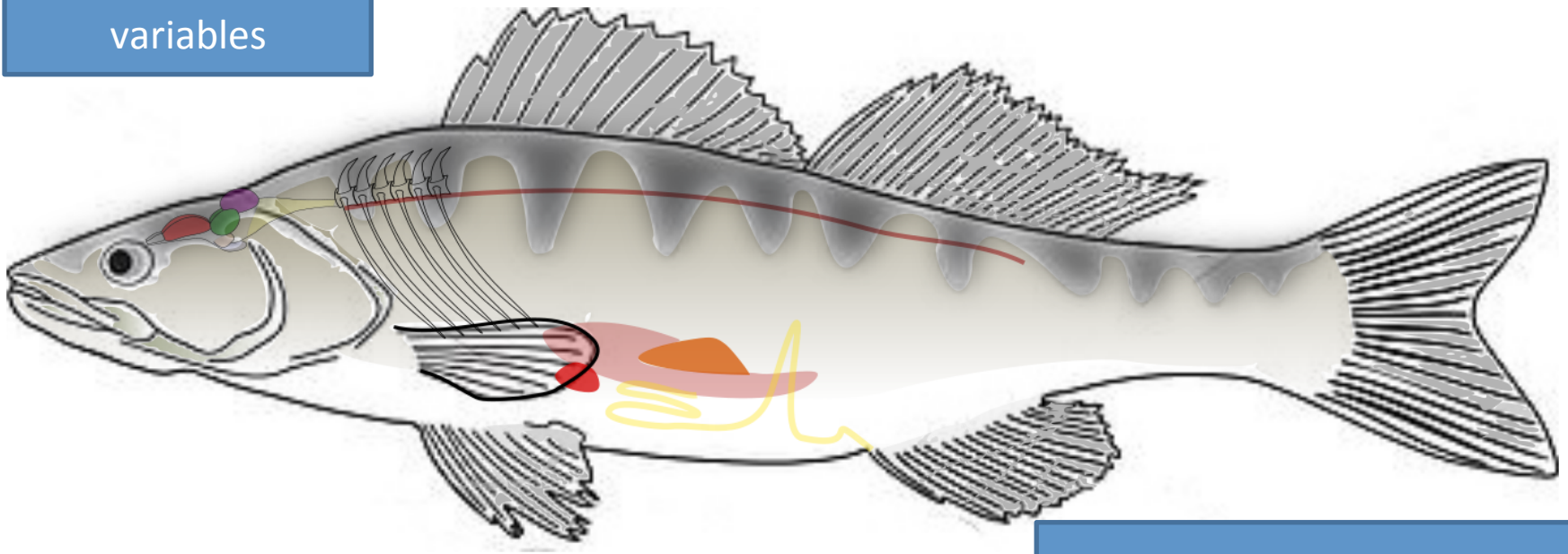
## Fish sampling



# DIVERSIFY: Multifactorial experiment

## Analyses

Zootechnical  
variables



Assays :

- Plasma cortisol
- Plasma glucose
- Lysosomal activity
- Complement activity

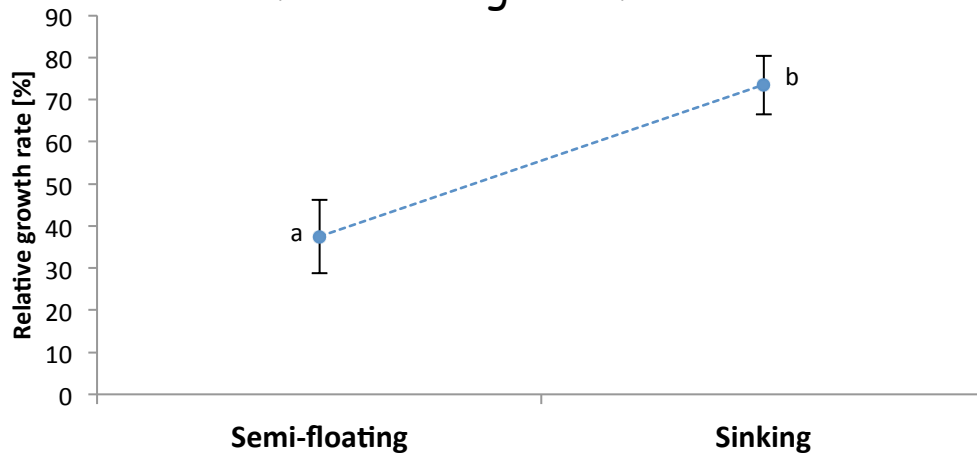
# Summary of the results

Ecotron	Biomass gain	RGR (%)	GMR (%)	Cortisol		Glucose	
	(g)			D35 (ng/ml)	D63 (ng/ml)	D35 (µg/ml)	D63 (µg/ml)
1	7238	81	4,32	84,05	31,38	456,9	317,7
2	2888	56,9	2,86	17,25	12,82	377,5	426,9
3	3059	84,9	12,86	20,29	14,78	355,1	371,7
4	17	53,7	30,94	18,76	14,39	263,5	340,2
5	1494	41	7,14	15,34	13,91	424,2	378,9
6	-609	0	7,14	16,24	14,01	342,0	365,2
7	-1210	21	24,29	17,06	13,42	272,8	342,7
8	1996	57,4	10	59,64	13,14	321,9	359,2
9	3216	102,8	12,86	25,73	13,18	371,2	411,3
10	-2056	57,4	41,01	17,00	14,26	339,5	317,5
11	1770	63,4	17,99	89,12	28,71	327,9	405,3
12	-1764	22,8	23,74	23,20	12,83	304,1	324,6
13	1534	62,4	31,65	47,18	14,81	466,5	411,8
14	3007	26,5	4,32	16,73	13,01	271,5	337,5
15	9042	80	2,88	27,22	14,95	419,9	373,1
16	3511	81,9	7,14	18,01	14,88	374,7	368,6

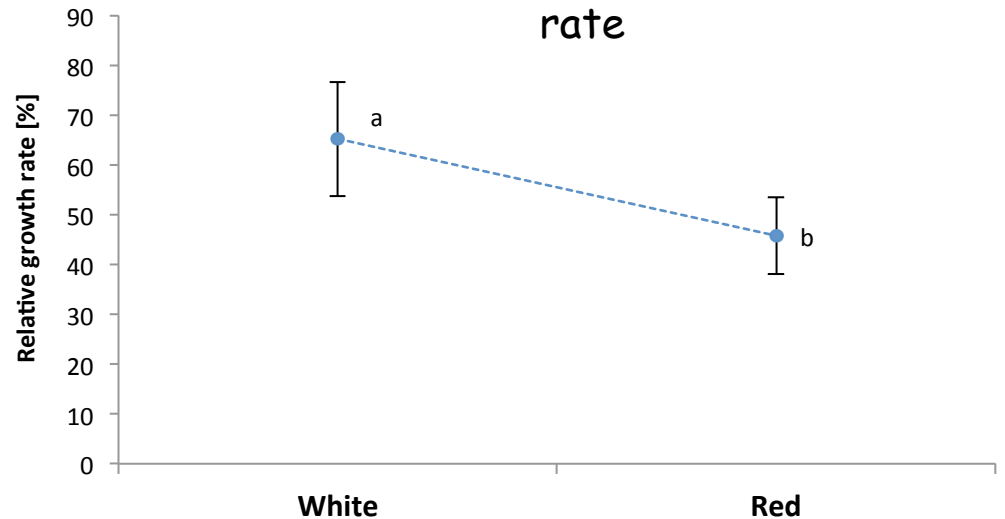
**3 combinations look promising for pikeperch aquaculture !!!**

# Statistical analysis: zootechnical parameters (D63)

Sinking food significantly increases the relative growth rate

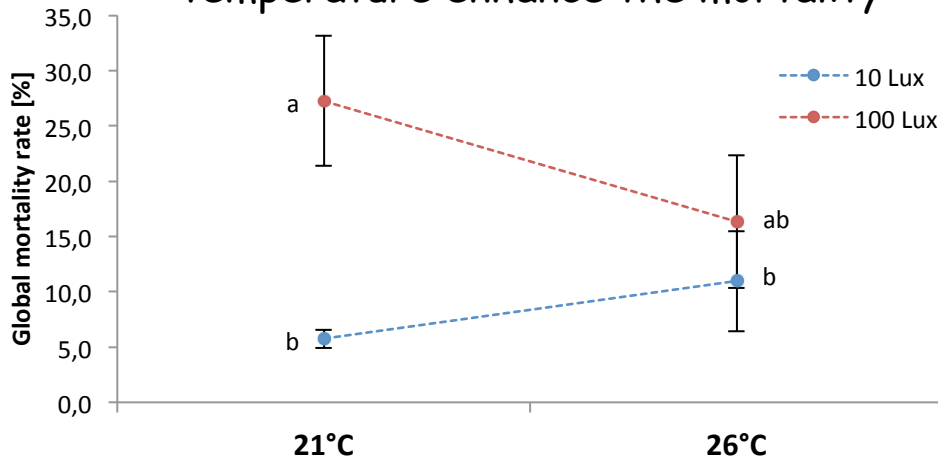


White light promotes the relative growth rate

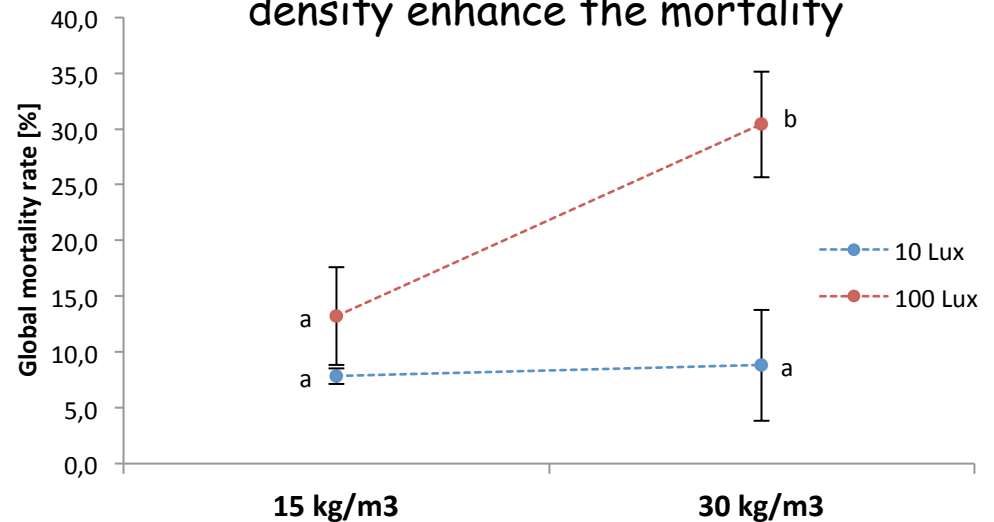


# Statistical analysis: zootechnical parameters (D63)

Higher light intensity and low temperature enhance the mortality



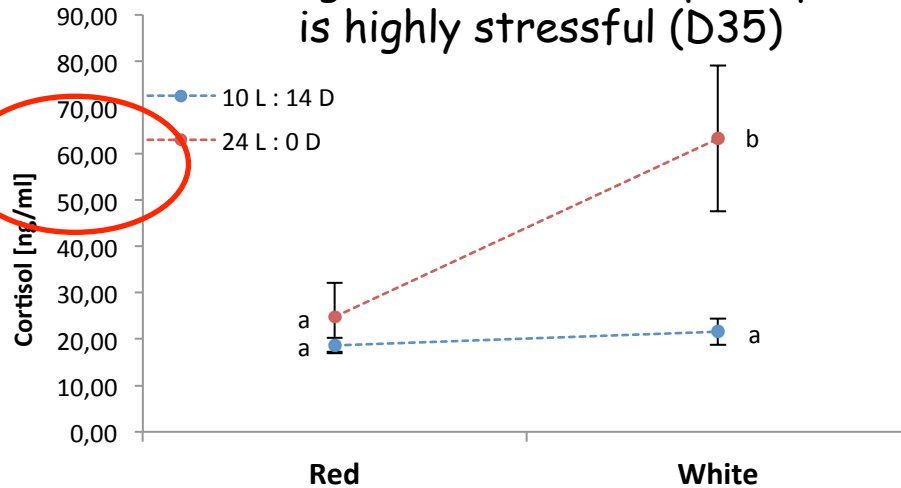
Higher light intensity and high density enhance the mortality





# Statistical analysis: physiological status

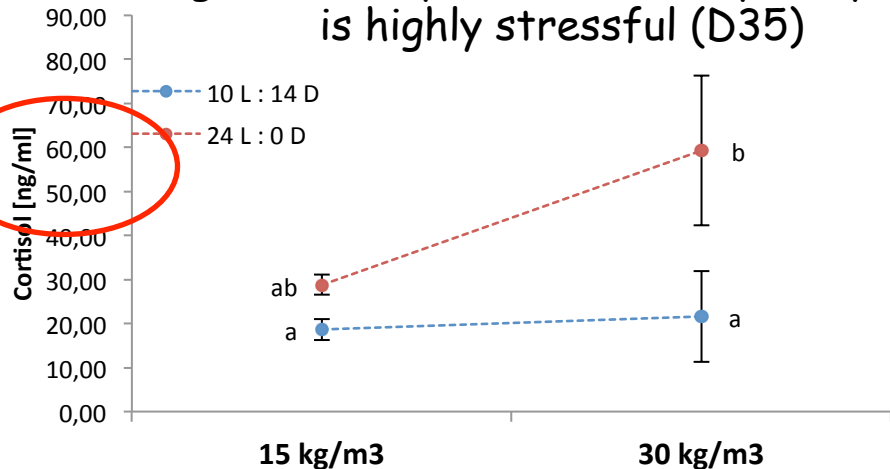
White light with constant photoperiod is highly stressful (D35)



Cortisol levels significantly decreased after 63 days until basal levels (15-20 ng/ml)



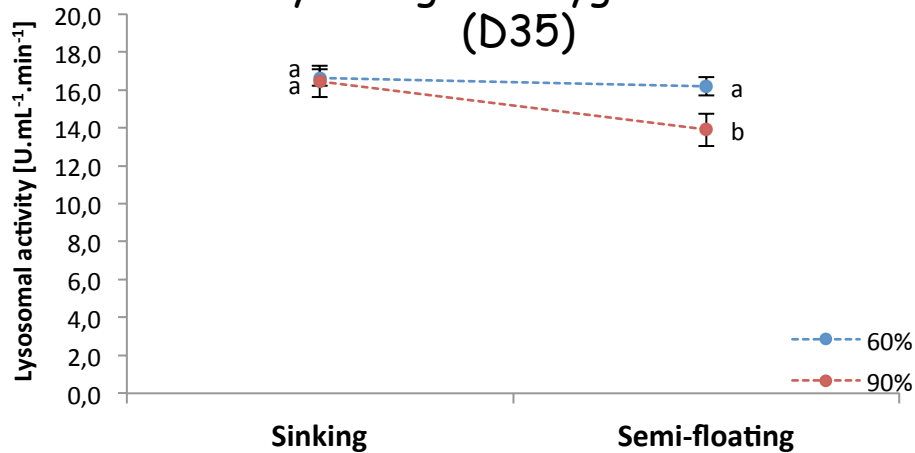
Higher density with constant photoperiod is highly stressful (D35)



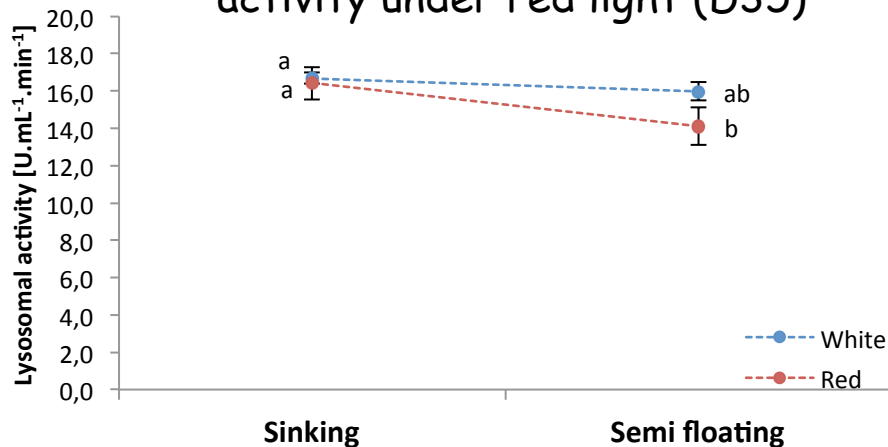
Acclimation of pikeperch juveniles!!

# Statistical analysis: physiological status

Floating food decreases lysosomal activity at higher oxygen saturation (D35)



Floating food decreases lysosomal activity under red light (D35)



In general, immune status increased after 63 days



Acclimation of pikeperch juveniles!!

# DIVERSIFY: Confirmation experiment

Selection of 3 “promising” combinations based on zootechnical and physiological variables

Ecotron (n°)	Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type of aliment	Handling	Oxygen saturation
<b>1</b>	10	30	white	24	21	sinking	Y	90
<b>15</b>	10	30	white	10	26	sinking	N	60
<b>16</b>	10	15	red	24	26	sinking	N	90



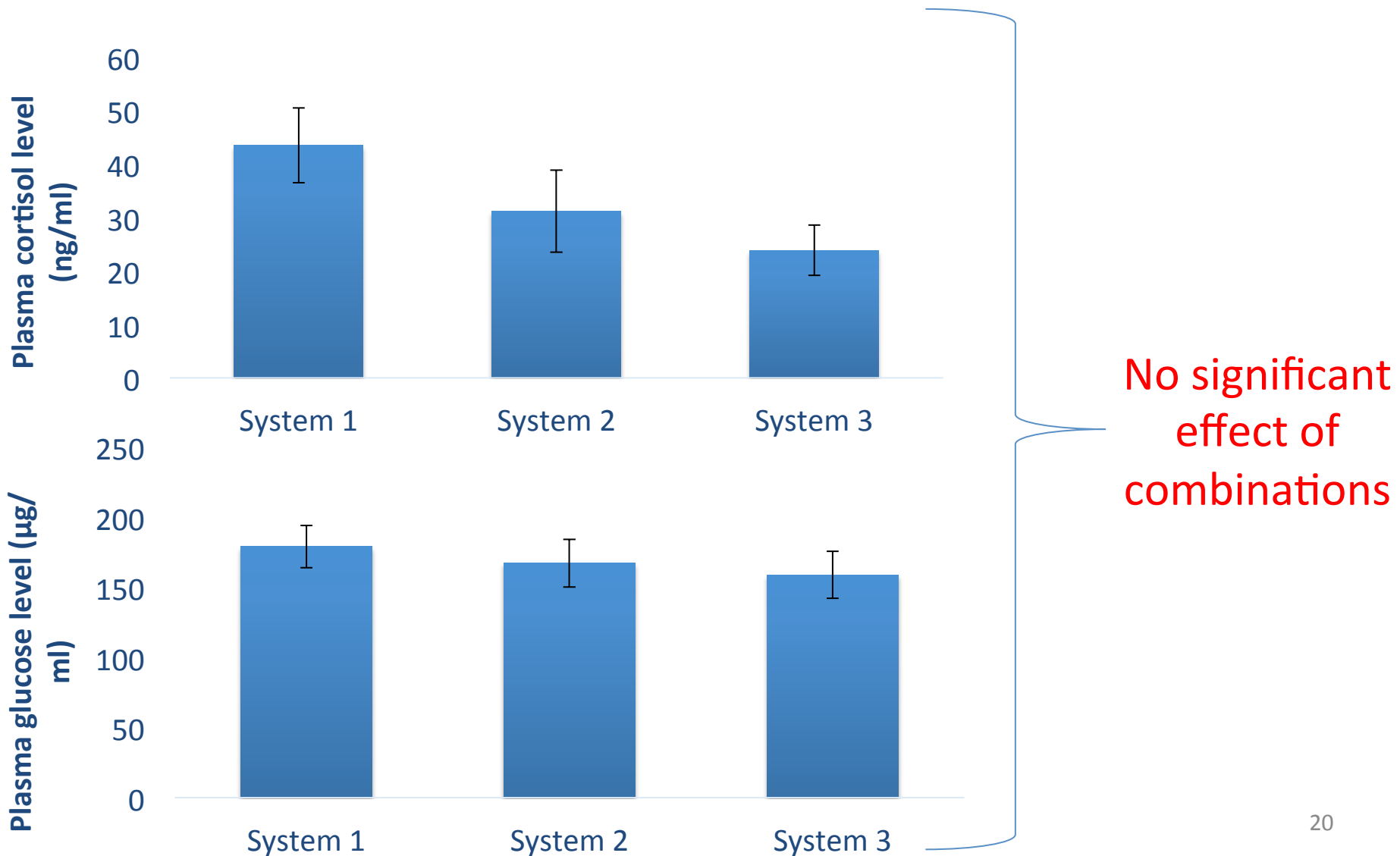
Confirmation experiment  
& bacterial challenge



System	Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type of aliment	Handling	Oxygen saturation
<b>1</b>	10	15	red	24	26	sinking	N	90
<b>2</b>	10	30	white	24	21	sinking	Y	90
<b>3</b>	10	30	white	10	26	sinking	N	60

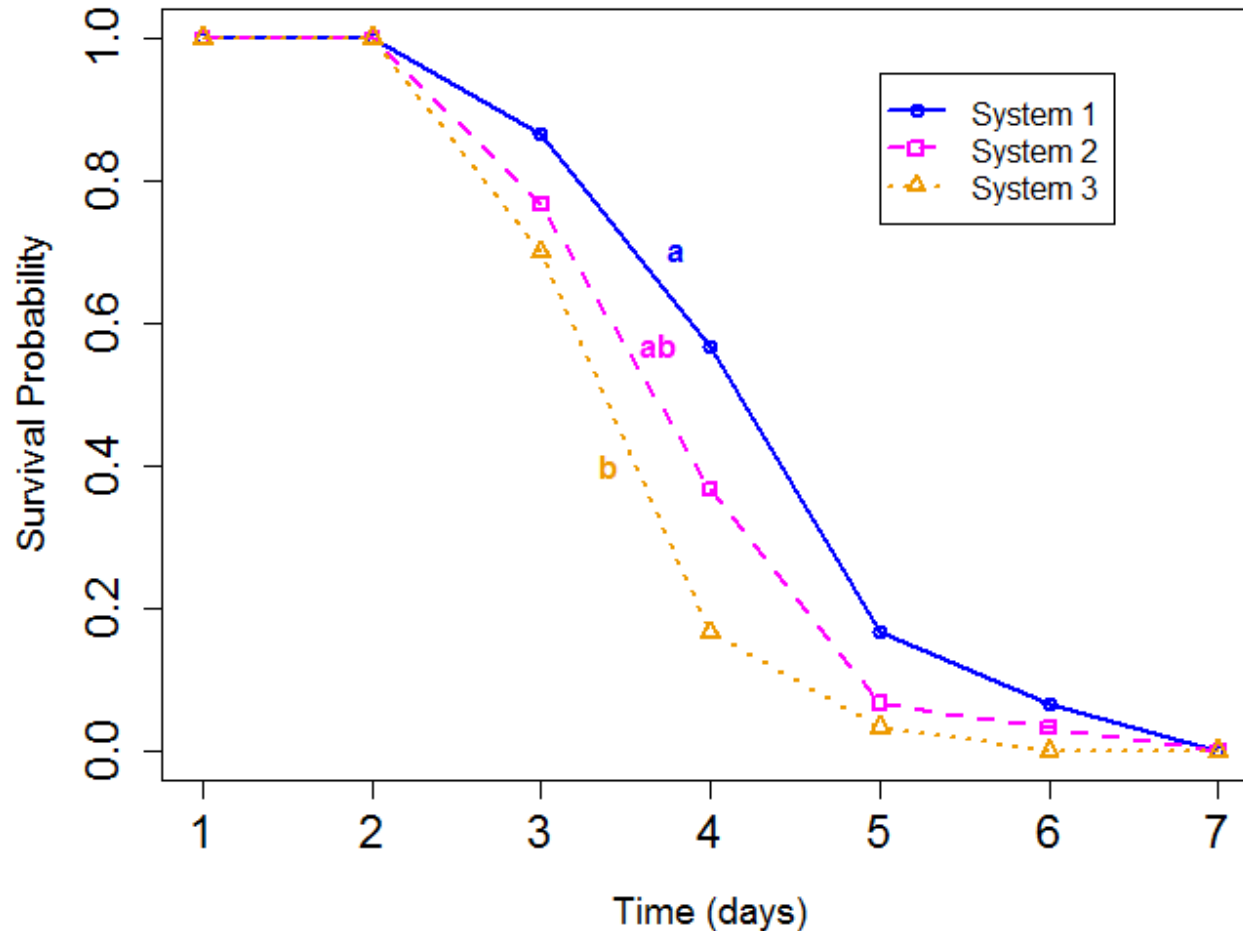
# DIVERSIFY: Confirmation experiment

## Results : physiological status



# DIVERSIFY: Confirmation experiment

Results : mortality after bacterial challenge



# DIVERSIFY: Conclusion

## Importance of light factors



**Bias of the type of food → How the fish has been fed in earlier stages? (Dalsgaard et al., 2013 and personal observations)**

## Our choice for the optimal rearing conditions:

Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type of aliment	Handling	Oxygen saturation
10	30	Red	10:14	26	???	N (but Y)	90

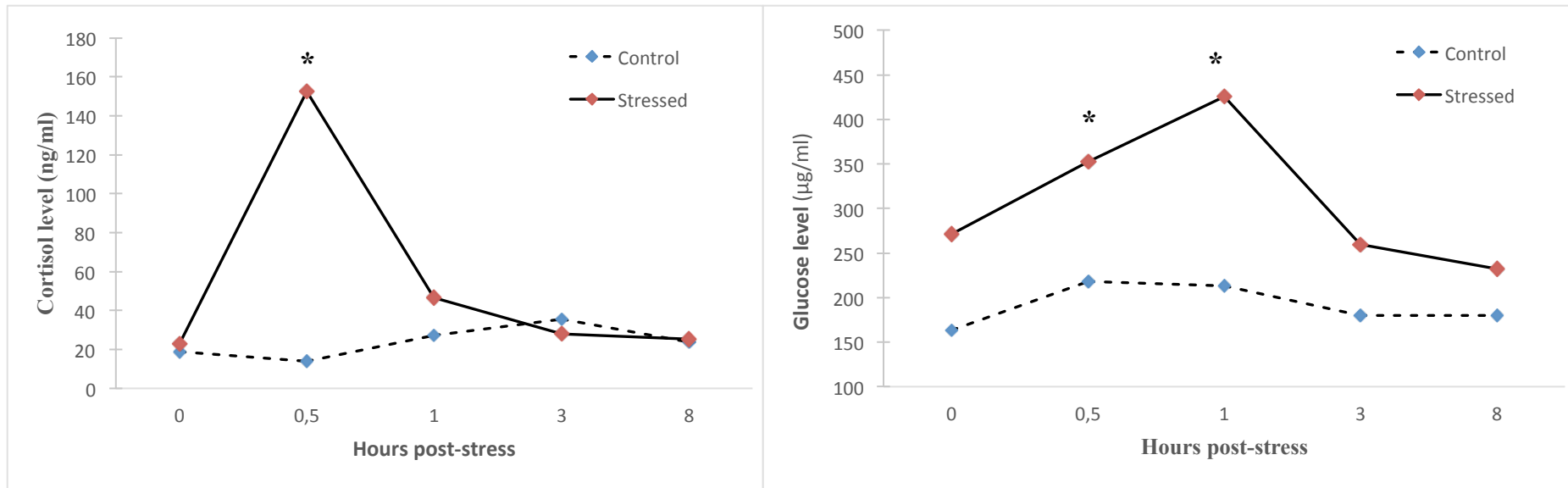




Thanks for your attention

# Preliminary studies

Physiological and immune responses in pikeperch exposed to repeatedly handling stress (1x/2weeks)



**Plasma cortisol and glucose peaked 30-60 min after handling-emersion but no effects on several selected immune markers (lysosomal and complement activities)**