



BENCHMARKING OF HEALTH PROMOTING ADDITIVES TO SUPPORT HEAT STRESS TOLERANCE OF EUROPEAN SEABASS *Dicentrarchus labrax*

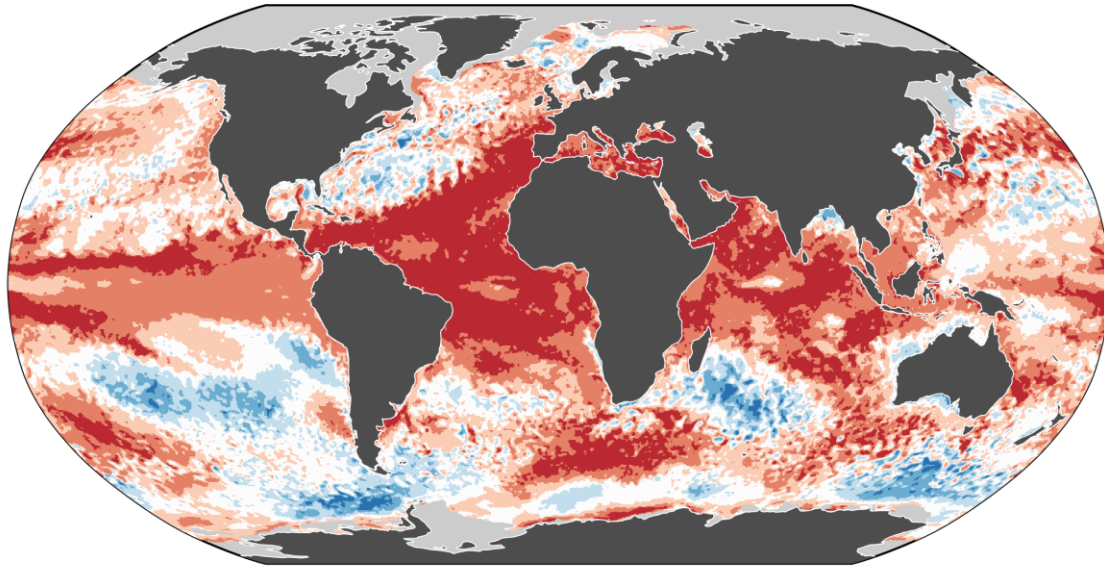
Robles-Arozarena, R., Chen, I-Tung, Bermúdez Villaescusa, L., Nuez-Ortin, W.



Heat waves in major aquaculture regions create unstable and stressful conditions for the fish

Sea surface temperature percentiles for February 2024

Data: ERA5 1979-2024 • Reference period: 1991-2020 • Credit: C3S/ECMWF



Sea ice & ice shelves

Coldest	Much colder than avg	Colder than avg	Near avg	Warmer than avg	Much warmer than avg	Warmest
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PROGRAMME OF
THE EUROPEAN UNION



June 2025 is the 3rd warmest June for the global Ocean following 2023 and 2024

July 8, 2025 • General News

Marine heatwaves continue to intensify and expand, with June 2025 breaking records for sea surface temperatures and heatwave extent across Europe and the global ocean. Intense heatwaves are impacting 62% of the Mediterranean. While some regions show signs of easing, others remain under severe thermal stress.

Direct effects of climate change on productivity of European aquaculture

Alhambra Martínez Cubillo, et al. *[full author details at the end of the article]*

<https://doi.org/10.1007/s10499-021-00694-6>

Summer Mortality Syndrome Affecting Cultured European Seabass at Kafrelsheikh Province, Egypt



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Mofeed Yousef Gouda³

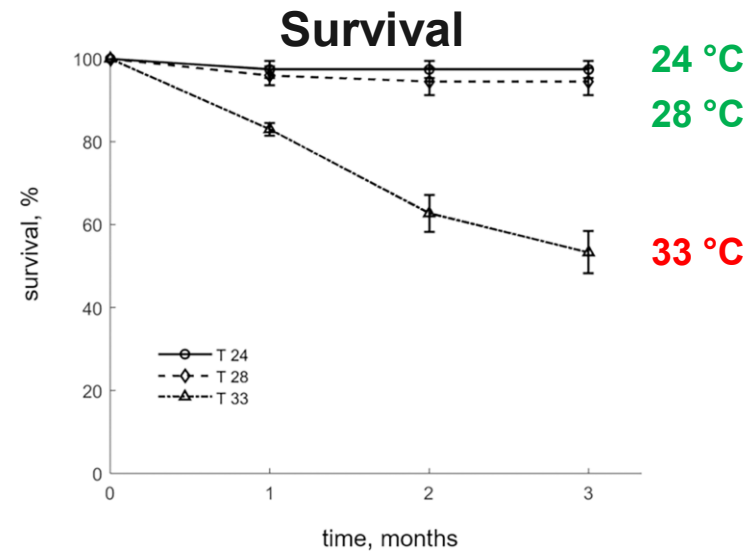
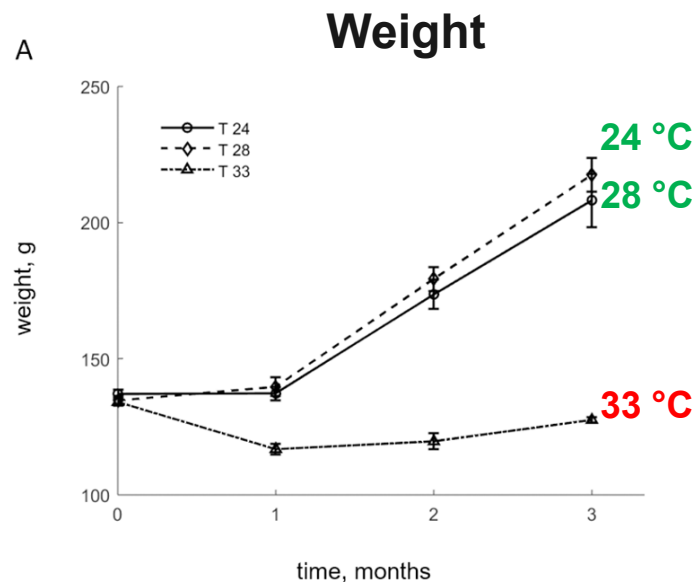


Ibrahim Mohamed Aboyadak¹

<https://doi.org/10.3389/fmars.2021.717360>

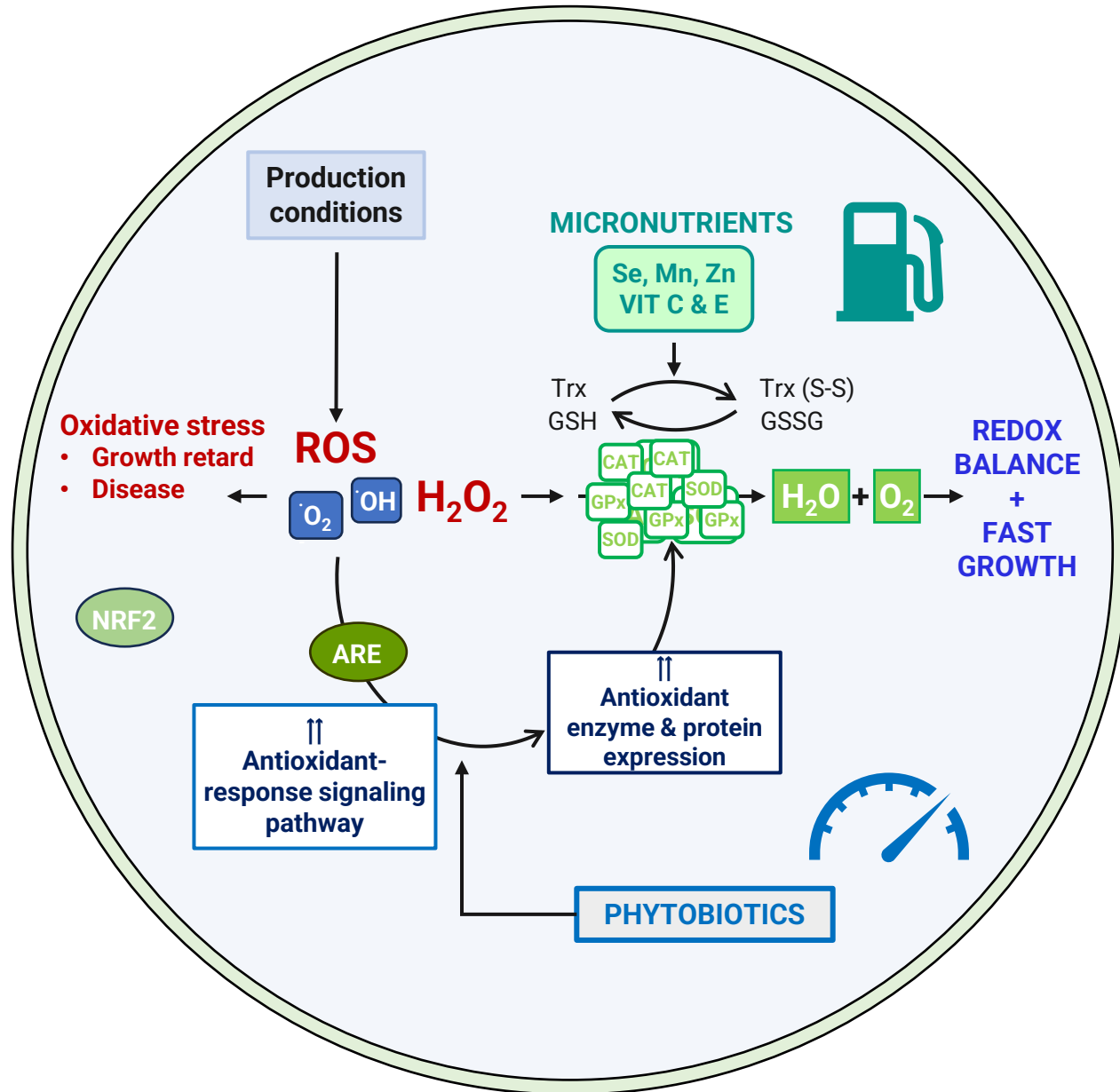
European sea bass (*Dicentrarchus labrax*) performance and survival at high temperatures

- European seabass can tolerate a wide range of temperatures, from 5 to 28 °C, and they can live in various salt levels, from freshwater to full-strength seawater. (FAO, 2024)
- European seabass can sustain high performance up to 28 °C. Critical survival thresholds are close to 33 °C, where aerobic capacity begins to decline, leading to a decrease in overall performance (Stavrakidis-Zachou et al., 2022)



<https://doi.org/10.1371/journal.pone.0272510>.

REDOX BALANCE



➤ ANTIOXIDANT MICRONUTRIENTS

- ✓ Cofactors for antioxidant enzymes
- ✓ Enhance endogenous activity
- ✓ Regenerates other antioxidants

➤ PHYTOBIOTICS

- ✓ Upregulate expression of antioxidant enzymes
- ✓ Enhance endogenous activity

Quick Tip!

ROS: Reactive Oxygen Species are chemical derivatives of molecular oxygen that include free radicals like superoxide (O_2^-), hydroxyl radical ($OH^\cdot$), singlet oxygen (1O_2), and non-radical species like hydrogen peroxide (H_2O_2)

Objective:

Benchmarking the efficacy of feed additives to counteract heat stress effects in farmed seabass (*Dicentrarchus labrax*).



M & M



Ingredients g/100 g	Control	HeatPro	H1	R2	K8
Fish meal superprime	20,00	20,00	20,00	20,00	20,00
Soybean meal 60%	13,70	13,70	13,70	13,70	13,70
soybean meal 50%	12,70	12,70	12,70	12,70	12,70
Wheat flour	16,60	16,40	16,50	16,58	16,30
Corn gluten flour	13,00	13,00	13,00	13,00	13,00
Rapeseed meal	9,00	9,00	9,00	9,00	9,00
Fish oil	13,30	13,30	13,30	13,30	13,30
MCP	0,55	0,55	0,55	0,55	0,55
Taurine	0,05	0,05	0,05	0,05	0,05
Lysine	0,15	0,15	0,15	0,15	0,15
DL-Methionine	0,06	0,06	0,06	0,06	0,06
Magnesium oxide	0,05	0,05	0,05	0,05	0,05
Vitamin C35	0,04	0,04	0,04	0,04	0,04
Vit & Min Premix	0,80	0,80	0,80	0,80	0,80
Heat Pro	--	0,20	--	--	--
H1	--	--	0,10	--	--
R2	--	--	--	0,02	--
K8	--	--	--	--	0,30
Crude Protein (%)	45,66	45,47	45,77	45,68	45,84
Crude Fat (%)	16,45	16,49	16,64	16,7	16,87
Ash (%)	7,49	7,77	7,88	7,59	7,36
Moisture (%)	5,81	5,96	6,46	6,29	6,6

M & M

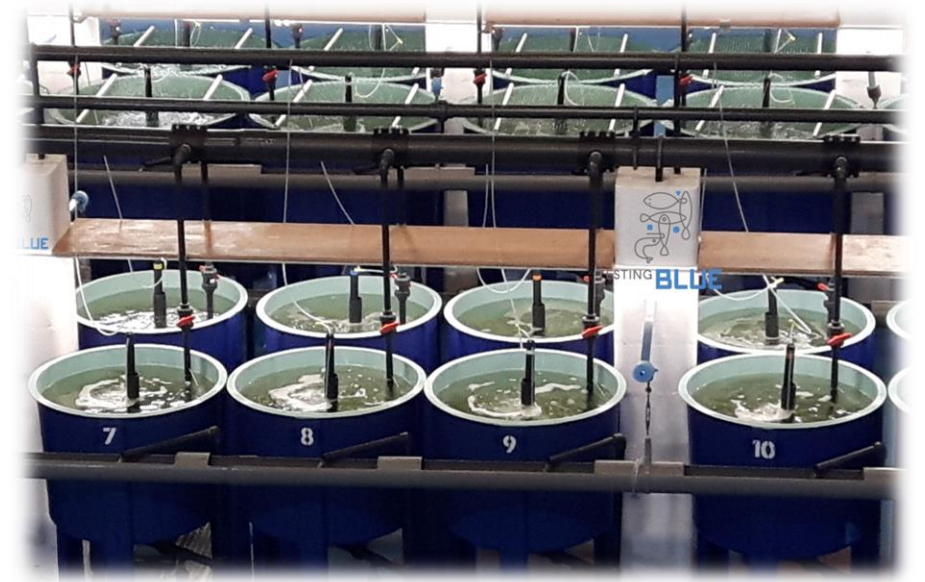


European seabass

Dicentrarchus labrax

Size: 144g

- RAS-14 fish/ 400L tank
 - 3 replicate
- Fed to satiation
 - 2 times/ day



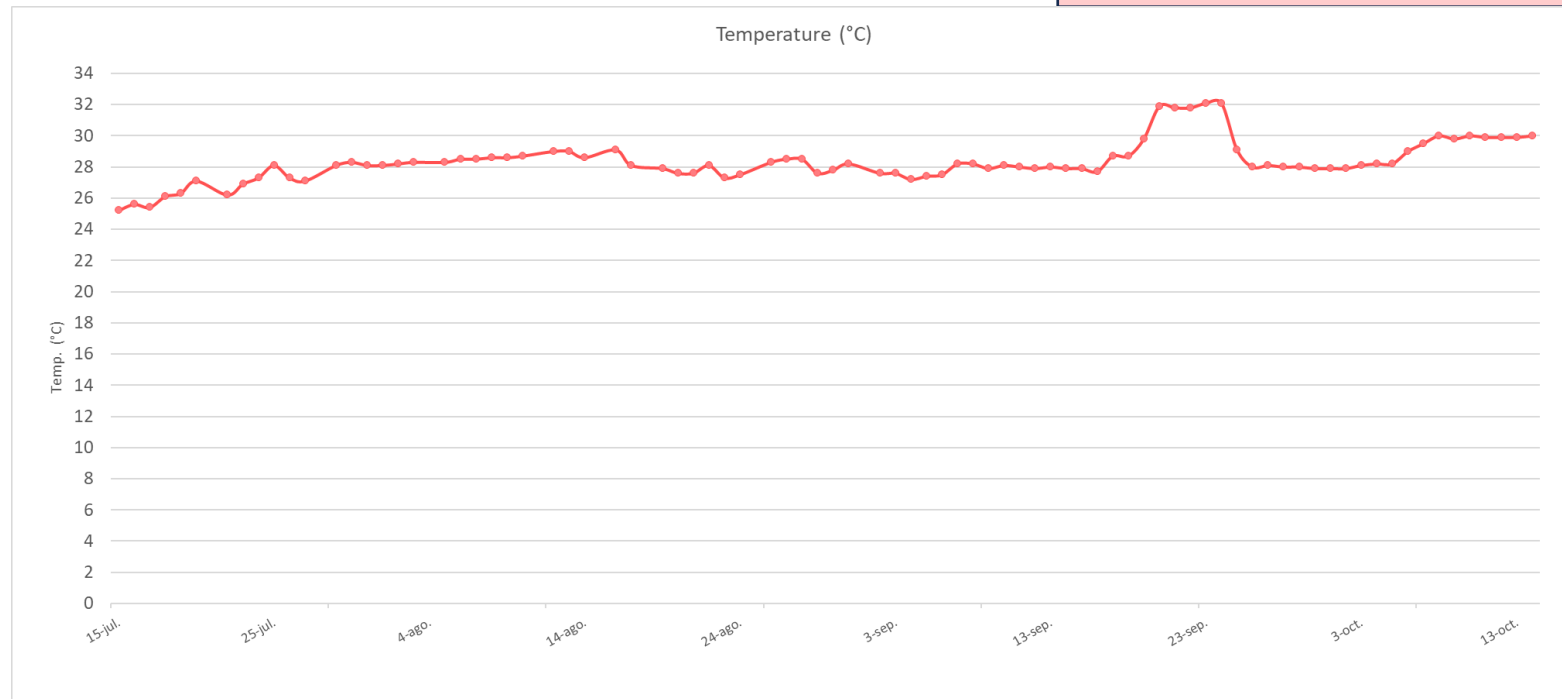
M & M



European seabass
Dicentrarchus labrax
Size: 144g

- 14 fish/ 400L tank
- 3 replicate
- Fed to satiation
- 2 times/ day

DOC	8 weeks	1 week	1 week	1 week
PHASE	REFEEDING AT OPTIMAL TEMP	HEAT AND FLUCTUATION STRESS		
TEMP	28 °C	32 °C	28 °C	30 °C



M & M



European seabass
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PHASE	REFEEDING AT OPTIMAL TEMP	HEAT AND FLUCTUATION STRESS		
TEMP	28 °C	32 °C	28 °C	30 °C
DIET	Experimental diets			

Quick Tip!

Cortisol: Stress signal hormone that shows the fish has sensed stress

Glucose: released in the blood for fast energy release

Lactate : By-product from muscles

MDA: biomarker for oxidative stress and lipid peroxidation

SOD: Neutralize the harmful free radical to H₂O₂

Catalase: Turn H₂O₂ to water and oxygen

GPX : Extra protection to eliminate H₂O₂

pNPB: Esterase linked to digestive and metabolic enzyme function

HSP70: Protects cells by repairing proteins under stress

IGF1: Key hormone promoting growth

Physiology stress markers:

➤ Cortisol, Glucose, Lactate, MDA

Anti-oxidant markers (enzyme):

➤ SOD, CAT, GPX, pNBP

Gene marker:

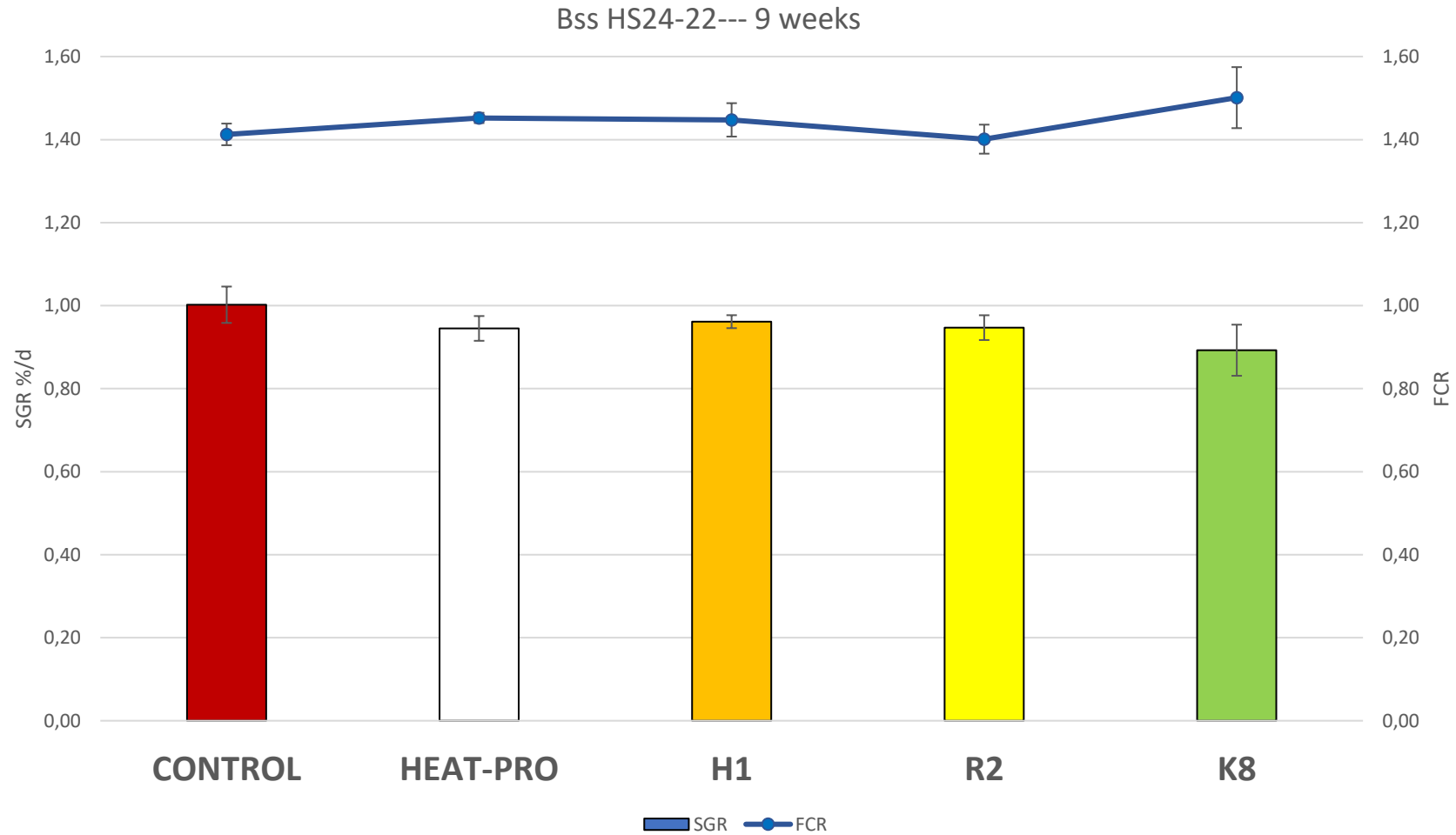
➤ SOD, HSP70, IGF1

End Sampling



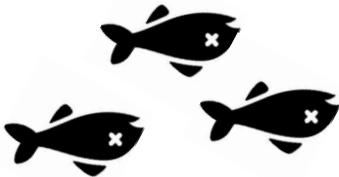
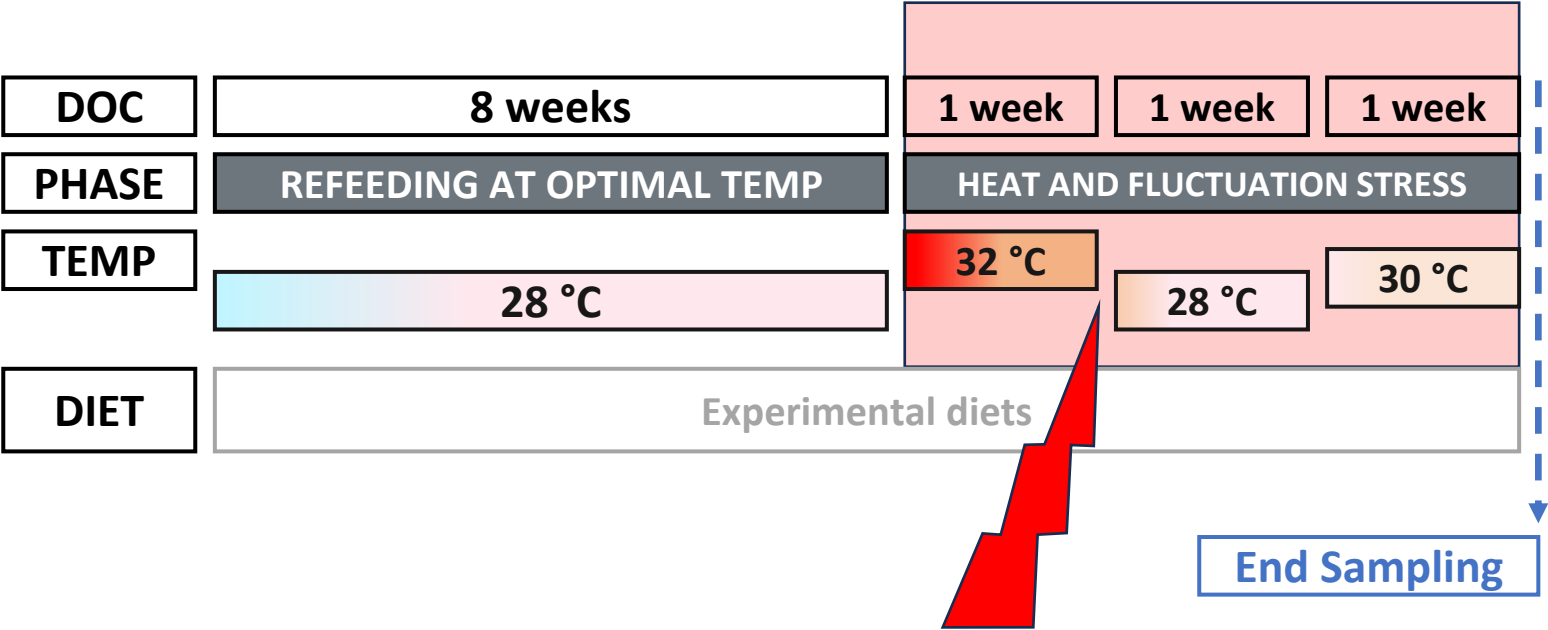
Results: 8 weeks 28°C

No negative effects

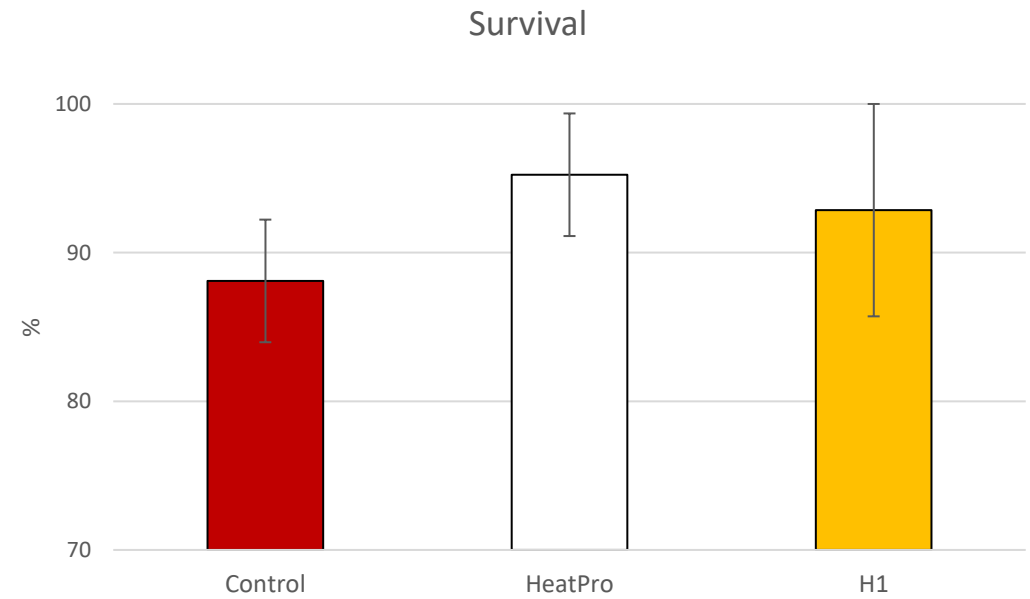
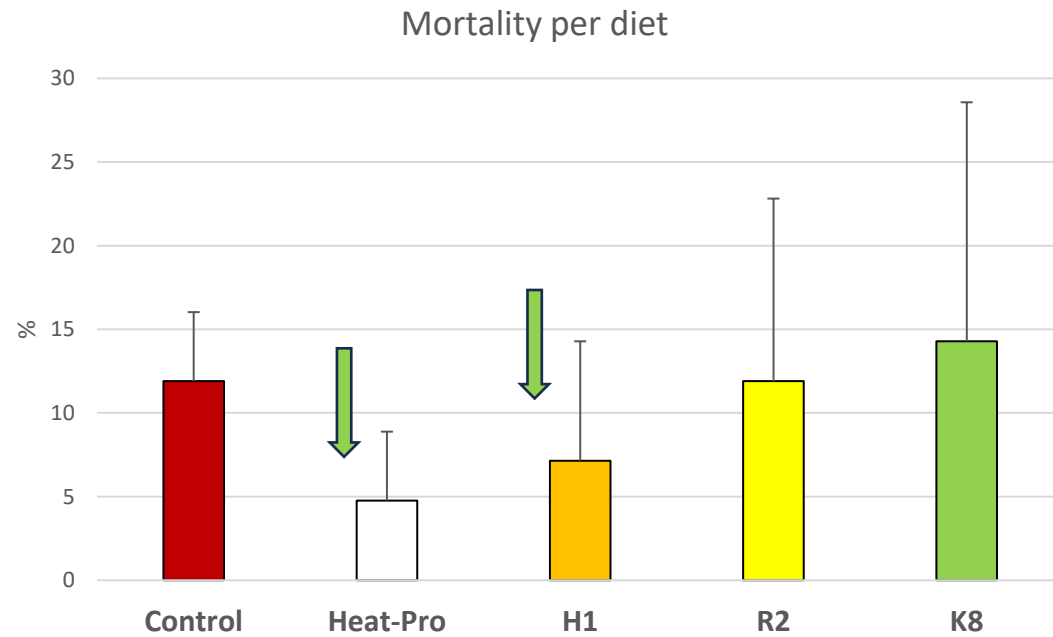




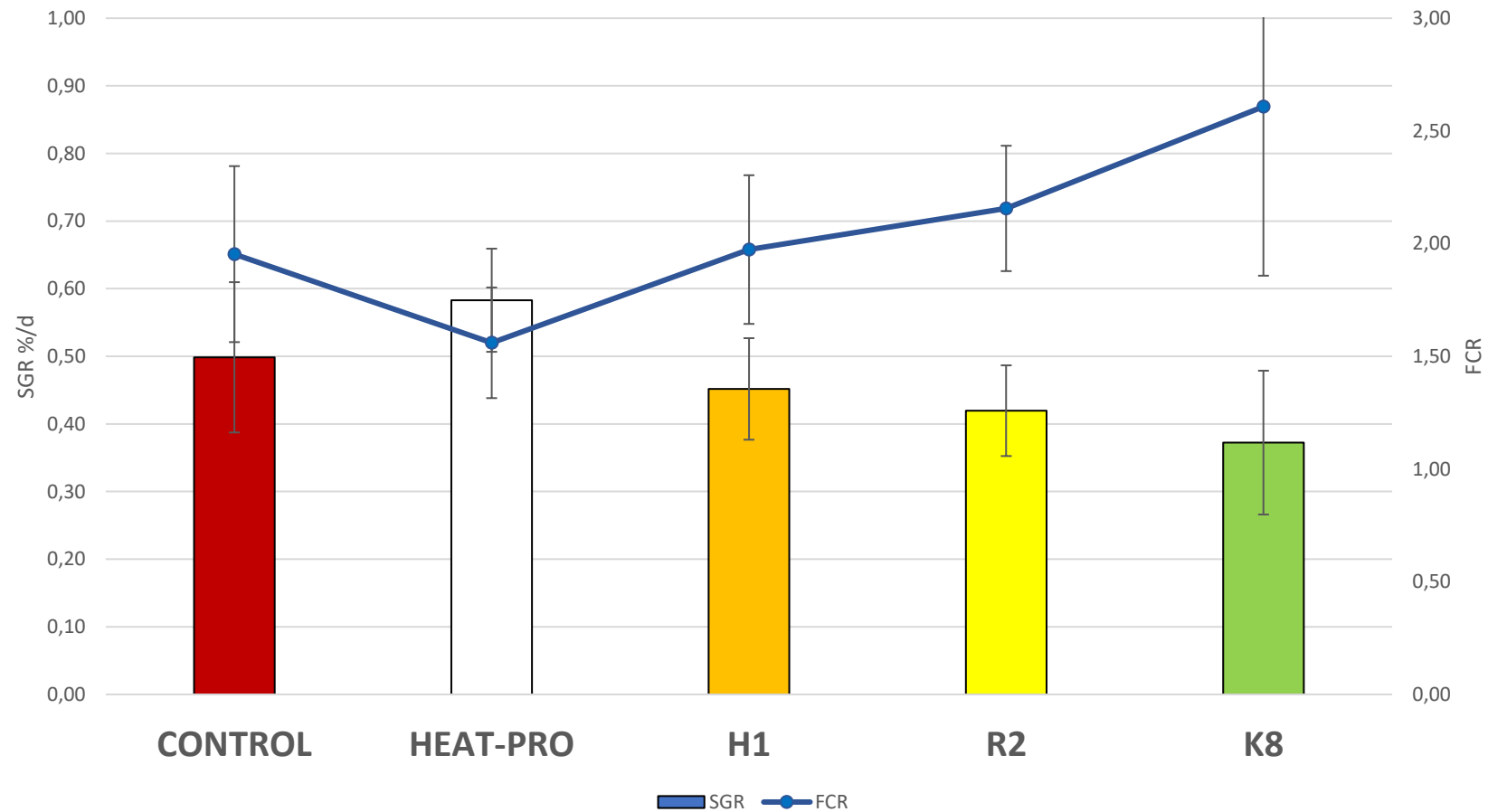
European seabass
Dicentrarchus labrax
Size: 144g



Results: mortality after 1 week at 32°C



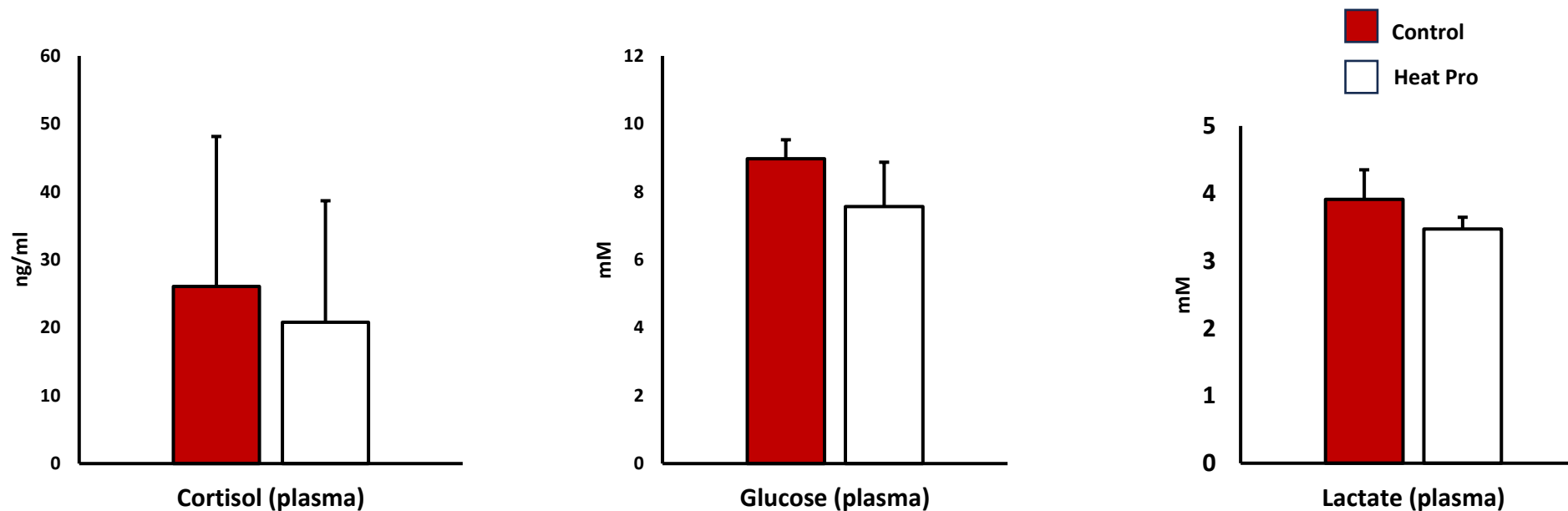
Results: SGR and FCR after mortality (heat fluctuation 28°C, 30°C)



Results: heat fluctuations

1 week	1 week	1 week
HEAT AND FLUCTUATION STRESS		
32 °C	28 °C	30 °C

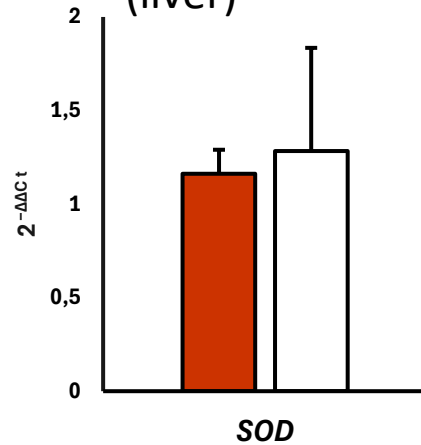
High-temperature stress (32°C) triggers mortality and **physiological stress response**



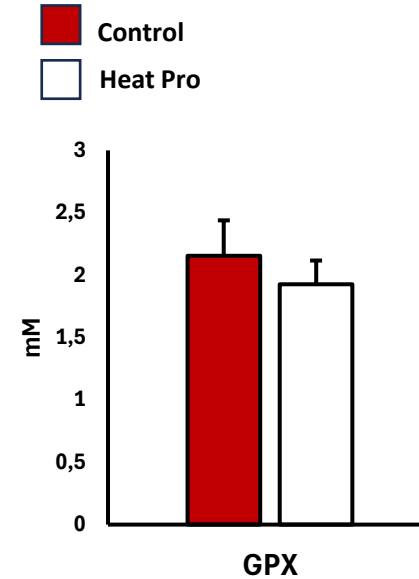
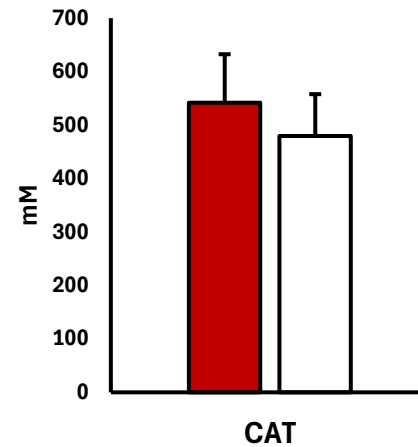
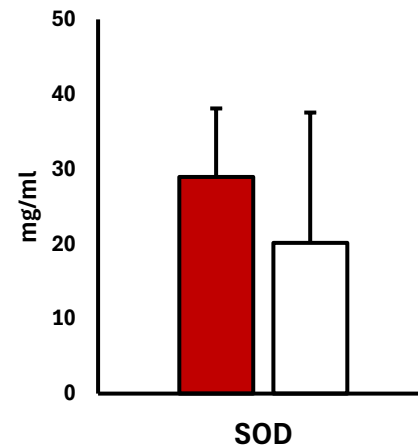
➤ *Supplementation mitigated stress effects, with lower stress responses under the challenge conditions*

Results: heat fluctuations

Antioxidant **GENE** expression
(liver)



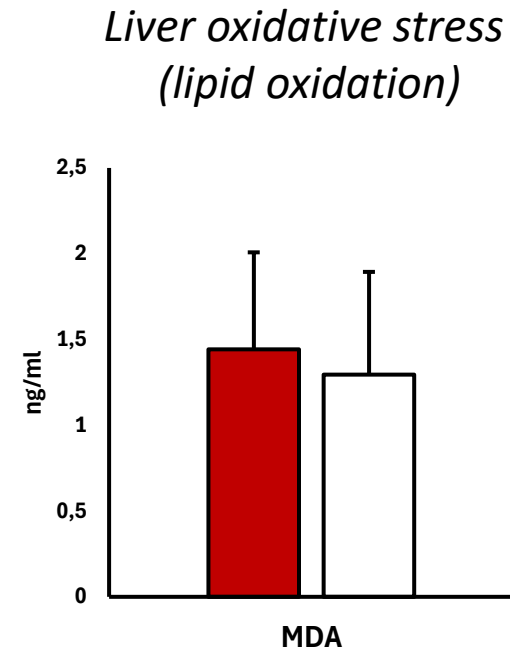
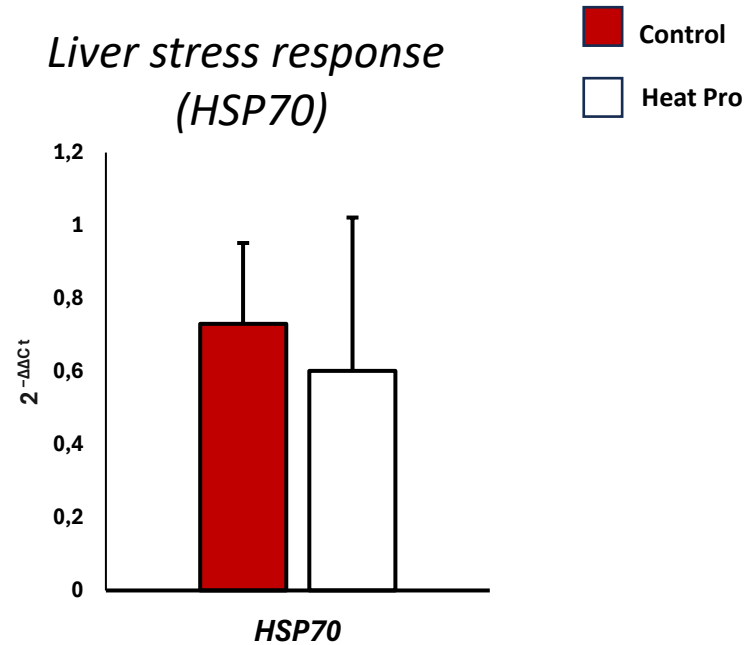
Antioxidant **ENZYME** activity (liver)



Different letters show that the results are statistically significant when analyzed by ANOVA test ($p < 0.05$) ($n=9$)

- At final sampling, Heat Pro treatment elevated **SOD gene expression**, while SOD enzyme activity was preserved due to a more balanced antioxidant status
- **Less ROS accumulation** due to the Heat Pro supplementation, less need for enzymatic activity (SOD, CAT, GPX), although the antioxidant defense system is prepared as shown by the increased in the SOD expression

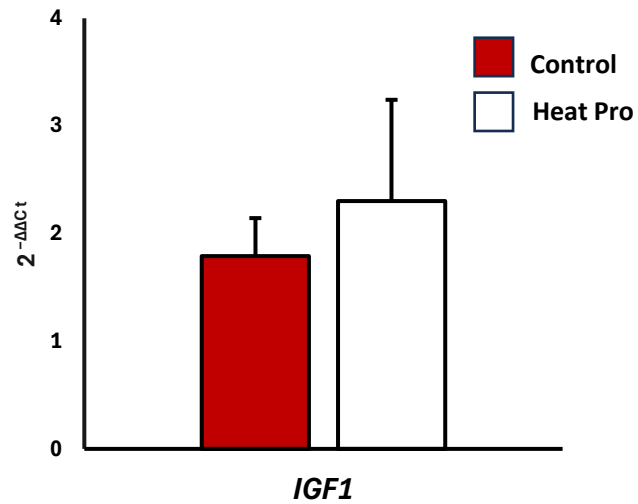
Results: heat fluctuations



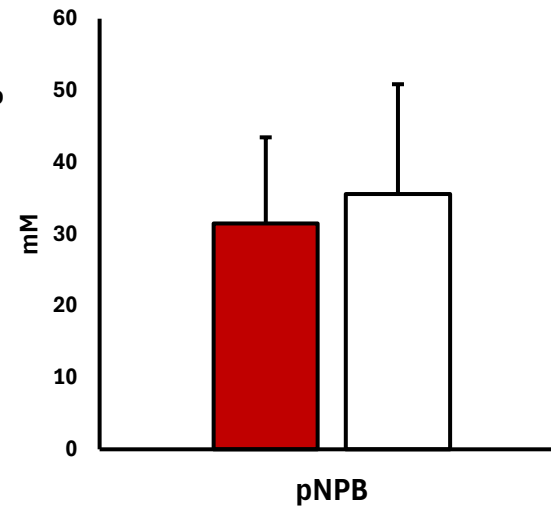
- Less ROS accumulation due to a more effective antioxidant defense system reduced oxidative damage (lower MDA) and cellular stress response (hsp70 gene expression)

Results: heat fluctuations

*Liver growth signal
(IGF-1 expression)*



*Liver metabolic activity
(pNPB enzyme)*



- Higher IGF1 expression and pNPB activity reflect improved anabolic signaling and metabolic efficiency
- Reduced physiological stress translated into better growth performance and feed efficiency

Key message:

- ✓ The inclusion of specific feed additive (Heat Pro) **increased the survival** of seabass during a heat stress period
- ✓ With the conditions tested in the trial, Heat Pro helps European seabass to cope with heat stress by **increasing transcriptional AOX defenses and reducing ROS accumulation**
- ✓ The inclusion of the additive **mitigates oxidative damage** and cellular stress, while simultaneously preserving the **energy for growth performance** (rather than for stress reduction)





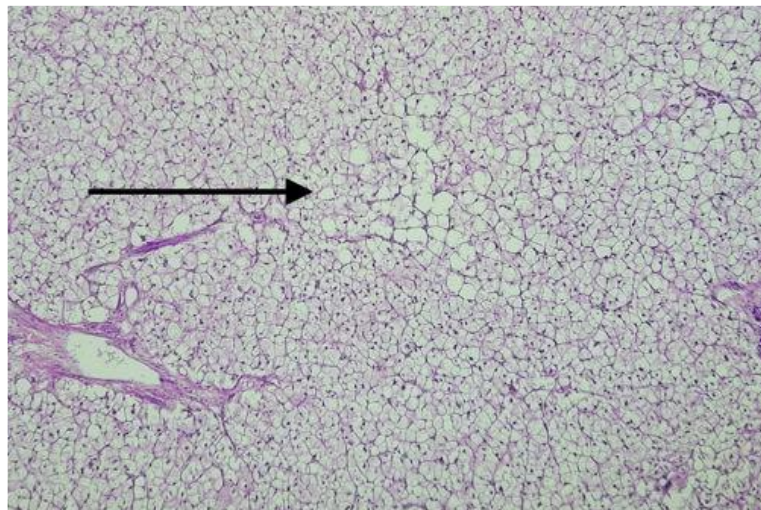
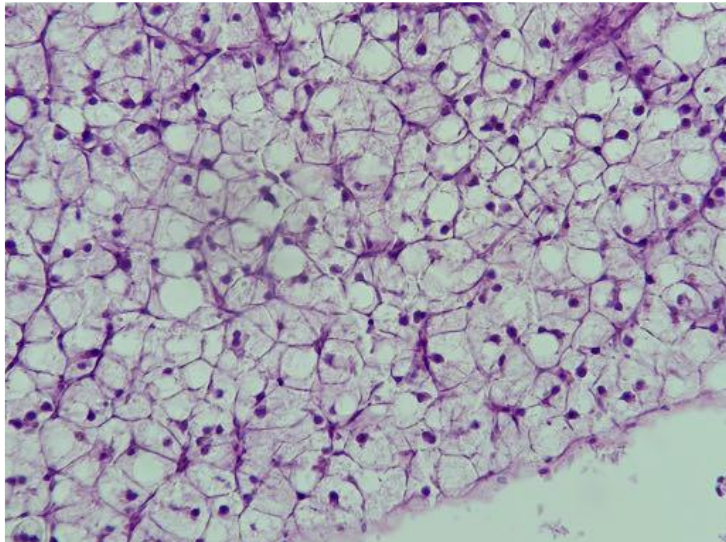
Thank you for your attention!

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Histopathology

All examined liver sections show vacuolation of hepatocytes and a predominance of intracytoplasmic macrovacuoles that on occasions caused and increase in size of the hepatocytes - hypertrophy-

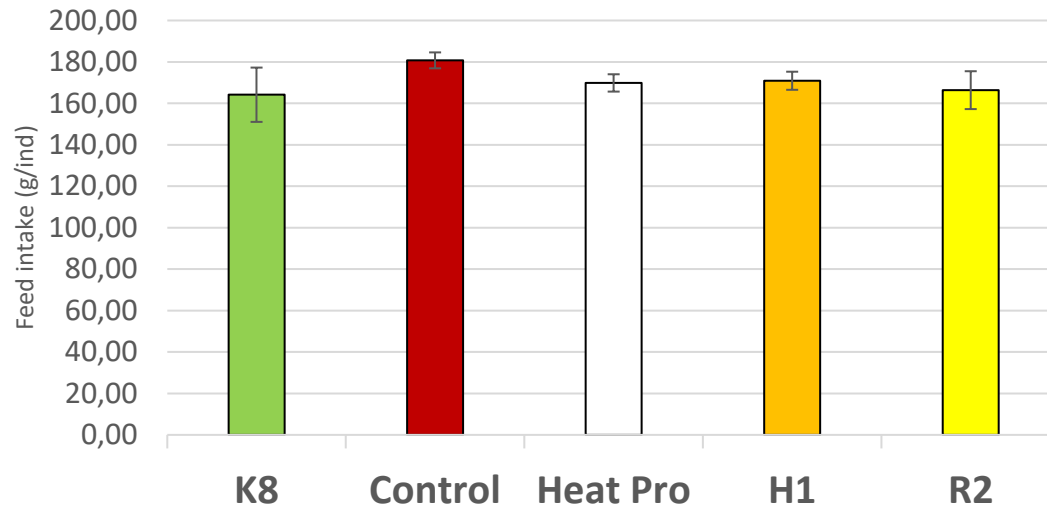


Liver

1. *Hepatocyte vacuolation (0-3)*
2. *Hepatitis (0-3)*
3. *Cholangitis*
4. *Degeneration /loss of cellular limits*
5. *Necrosis*

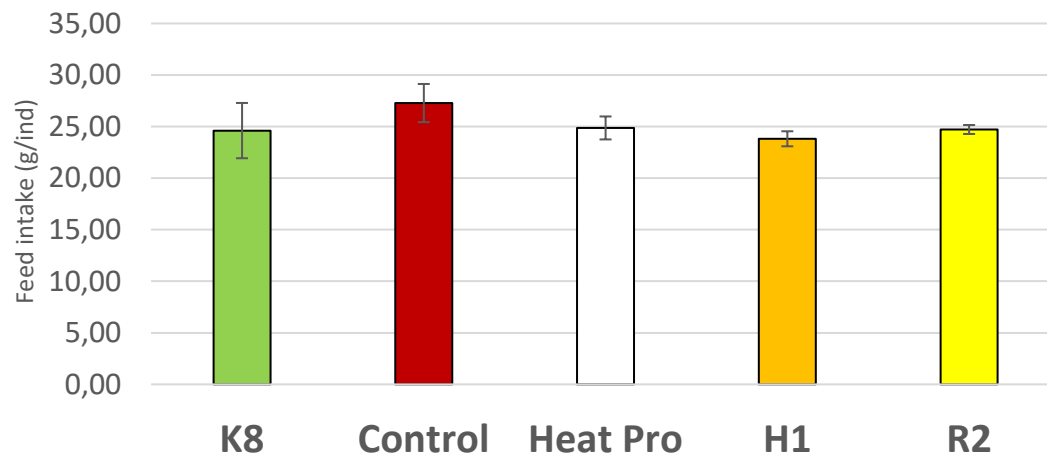
0 no incidence; 3 marked incidence

Feed intake -8 w-28°C period



Feeding in g/day/ fish:
3.21 to 2.93 g/d/f

Feed intake (post- mortality period, 2 w)



Feeding in g/day/ fish:
2.25- 2 g/d/f (30% less)