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Nutritional mitigation of heatwave stress in European seabass, *Dicentrarchus labrax*: Metabolic, cellular, and molecular responses

Md Jakiul Islam[a,b,*], Oscar Puebla[a,b], Andreas Kunzmann[a]

[a] Leibniz Centre for Tropical Marine Research (ZMT), Fahrenheitstraße 6, Bremen 28359, Germany
[b] Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky Universität Oldenburg, Ammerländer Heerstraße 114-118, Oldenburg, Germany

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ABSTRACT

Climate change is fueling the perilous combination of heatwaves and prolonged drought and is now a reality affecting living organisms, including fish. The study aimed to investigate the effects of four dietary supplements on the European seabass, *Dicentrarchus labrax*, during an experimental heatwave. A total of 240 fish were allocated randomly to 15 tanks. Fish were fed for 56 days with diets supplemented with vitamins C (4.0 g kg^{-1}) & E (3.5 g kg^{-1}), propolis (4.5 g kg^{-1}), phycocyanin (0.03 g kg^{-1}), β-glucan (3.0 g kg^{-1}) and a control diet, and then exposed to a 18 days heatwave (32 ^{o}C). Multilayer of physiological parameters were measured before and after extreme warm exposure. Before the experimental heatwave, fish weight gain did not differ significantly between the four tested diets and the control group. However, during extreme warm exposure, serum triglycerides, total protein, cholesterol, and expression of Igf1 and TNF-1α were significantly higher in fish fed diets supplemented with vitamins C & E, propolis, and phycocyanin than in fish fed control diet ($p < 0.05$). In contrast, serum cortisol, AST, ALT, LDH, γGGT, BUN, and creatine levels were found to be significantly lower ($P<0.05$) in fish fed diets supplemented with vitamins C & E, propolis, and phycocyanin. After heat stress, HSP70, and GLUT2 expression increased at varying degrees in fish fed all tested diets in comparison to the control diet, whereas FADS2 expression decreased. Nevertheless, the gene expression response was different in the kidneys. Overall, most of the tested parameters indicate that diets supplemented with vitamins C & E, propolis, or phycocyanin are beneficial for European seabass during exposure to extremely warm temperatures (32°C). These supplements alleviated the adverse effects of the heatwave, improving weight gain and metabolic activities at the cellular and molecular levels.

1. Introduction

Climate change is a global threat to the environment, biodiversity, and ecosystems that is becoming more prevalent (Weiskopf et al., 2020). Anthropogenically-driven climate change is increasing the frequency and intensity of heatwaves and is predicted to become more severe in the future (IPCC, 2018; Korell et al., 2020; Weiskopf et al., 2020). Heatwaves in Europe have resulted in record-breaking temperatures (Lhotka and Kyselý, 2022; Molina et al., 2023; Sánchez-Cueto et al., 2023). Moreover, Europe is rapidly warming, twice the rate of the global average over the last four decades, with devastating consequences (Turnau et al., 2022; Vautard et al., 2023). The consequences were particularly severe in the last couple of years, with widespread droughts and heatwaves impacting agriculture and aquaculture. For example, France saw its driest January-to-September period ever recorded, and Spain's water reserves plummeted to just over 40% capacity (Paddison, 2023). Heatwave stress in Europe poses significant challenges to aquaculture and fisheries, affecting the health of aquatic ecosystems, and the productivity of aquaculture industries (Geffroy et al., 2023; Lattos et al., 2022; Sánchez-Cueto et al., 2023). The Mediterranean region, for example, is one of the most vulnerable to climate change (Intergovernmental Panel on Climate Change, 2023). In the southern part of the Mediterranean, an increased pattern of extreme heatwaves and droughts has been observed, creating hydrological stress (Pérez-Palazón et al., 2018; Pisano et al., 2020; Raymond et al., 2019; Seager et al., 2019). Consequently, in this region, both wild and aquaculture fish have been facing environmental and physiological challenges (Brierley and Kingsford, 2009; Islam et al., 2020a; Weiskopf et al., 2020). For instance,

* Correspondence to: Leibniz Centre for Tropical Marine Research (ZMT), Bremen 28359, Germany.
E-mail address: md.jakiul.islam@uni-oldenburg.de (M.J. Islam).

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during periods of exceptionally high summer temperatures, entire intertidal coastal communities have experienced a collapse across thousands of kilometers in the Northwest Mediterranean region (Garrabou et al., 2009; Sorby et al., 2018). As a major abiotic factor, temperature affects fish physiology from genes to cells and to metabolic pathways, organs, and individuals (Islam et al., 2021b; Madeira et al., 2016; Tejpal et al., 2014). However, the potential cascading effects of extreme heatwaves on fish and how to mitigate them are poorly understood (Dahlke et al., 2020; Islam et al., 2022; Weiskopf et al., 2020). Thus, effective adaptation measures are essential for building resilience and ensuring the long-term sustainability of aquaculture and fisheries in the face of climate change.

Several management approaches (e.g. pond shedding, shifting aquaculture depths or sites, selecting more heat-tolerant species) can be employed to mitigate the effect of extreme heatwaves on fish (Galappaththi et al., 2020; Maulu et al., 2021). However, these options are not always feasible. Dietary management strategies such as supplementation of functional feed ingredients have also been reported as a promising option to ameliorate the response to heatwave-exerted stress in teleosts (Hassaan et al., 2019; Kumar et al., 2018; Turchini and Nie, 2021; Župan et al., 2015). Dietary ingredients such as vitamin C, vitamin E, pyridoxine, propolis, lecithin, phycocyanin, tryptophan, and β-glucan are effective in mitigating stress effects in fishes (Alrashada et al., 2023; Bahi et al., 2023; Do-Huu et al., 2023; Rahman et al., 2023; Singh et al., 2023; Xu et al., 2023). For example, an improvement in growth and physiological responses was observed in gilthead seabream *Sparus aurata* (Ibarz et al., 2010), Indian carp *Cirrhinus mrigala* (Tejpal et al., 2014) and *Labeo rohita* (Kumar et al., 2018, 2014), and tilapia *Oreochromis niloticus* (Dawood et al., 2020; Hassaan et al., 2019) fed diets supplemented with the above-mentioned ingredients. Dietary supplements and immunostimulants, e.g. beehive extracts (reviewed in Farag et al., 2021) as propolis, (Alrashada et al., 2023) algae extracts as phycocyanin (AbdAllah et al., 2023; Mamun et al., 2023; Sayed et al., 2023; Youssef et al., 2023), and vitamins C (ascorbic acid) and E (tocopherol) (Chen et al., 2003; Harabawy and Mosleh, 2014; Ortuño et al., 2003) have been getting increased attention for the mitigation of thermal stress in fishes. For fishes, several studies reported general beneficial effects using vitamin C at 0.05–4.0 g kg^{-1}; vitamin E at 1.2–3 kg^{-1} (Gao et al., 2014); propolis at 2–10 g kg^{-1} (Farag et al., 2021; Islam et al., 2022); phycocyanin at 0.2–0.50 g kg^{-1} (Elabd et al., 2020; Hassaan et al., 2021; Jin et al., 2020; Zhang et al., 2020); and β-glucan at 0.5–5 g kg^{-1} (Hassaan et al., 2021; Neamat-Allah et al., 2021; Yang et al., 2021). However, there is limited knowledge regarding the impact of these supplements on fish under heatwave-induced stress.

Propolis (beehive extract) is rich in antiseptic, antimicrobial, antioxidant, and anti-inflammatory substances. Animals fed propolis-supplemented diets show improved growth, antioxidative, and immune functions (Alrashada et al., 2023; Kaplan and Erdoğan, 2021a; Kelestemur et al., 2012). Diets supplemented with phycocyanin (*Spirulina platensis* and other algae extract) have been found to improve the physiology, immunity, and growth of fish (Kizhakkekarammal Puthiyedathu et al., 2022; Rahman et al., 2023; Yap et al., 2021). In animals, phycocyanin can inhibit inflammation by blocking proinflammatory cytokines and also ameliorate oxidative stress (AbdAllah et al., 2023; Mamun et al., 2023; Sayed et al., 2023; Xu et al., 2024). However, the direct use of phycocyanin has not been widely used as a feed supplement to mitigate thermal stress in fish. Besides, vitamins C & E are considered natural antioxidants to safeguard against oxidative damage in animals. Due to synergistic effects, the combined use of these two vitamins is recommended in animal diets (Chen et al., 2004; Dawood and Koshio, 2018; Gao et al., 2014; Shiau and Hsu, 2002). Moreover, β-glucan extracted from yeast, specifically *Saccharomyces cerevisiae*, has been found effective in animals including fish to improve physiological performances, growth, and immunity (Do-Huu et al., 2023; Neamat-Allah et al., 2021; Porter et al., 2023).

European seabass, *Dicentrarchus labrax*, is a eurythermic and euryhaline fish, and it ranks among the most significant aquaculture species in both Europe and the Mediterranean area. This fish grows best between 22 and 24 ^{o}C (Person-Le Ruyet et al., 2004; Vinagre et al., 2012c). Feed intake, physiology, and growth have all been reported to be reduced in European seabass exposed to temperatures above 29 ^{o}C (Person-Le Ruyet et al., 2004). Fish exposed to temperatures above 32 ^{o}C have a higher mortality rate (Islam et. al., 2020a). However, during the summer, the water temperature of shallow waterbodies can sometimes exceed 32–33 ^{o}C, making resident fish including the European seabass vulnerable to these extreme heatwave events and compromising their physiological ability (Dülger et al., 2012; Islam et al., 2020b; Moyano et al., 2017). Thus, it is important to increase the ability of this fish to endure summer heatwaves, especially in farming conditions (Galappaththi et al., 2020; Heath et al., 2012; Holsman et al., 2020; Turchini and Nie, 2021). In a previous study with the same species, we observed that fish fed diets enriched with vitamins C, E, propolis, and phycocyanin exhibited improved resilience during heatwave events, as evidenced by growth performance, blood cell counts, erythrocytic abnormalities, and oxidative stress response (Islam et al., 2021a). This initial finding motivated us to go deeper down by analyzing additional tissues, organs, and parameters from the same experiment. Our focus extended to key physiological processes that were not previously explored, all of which are addressed in this study.

Due to the interactive relationship among growth, metabolism, physiology, and molecular networks, a multilayer of biomarkers [ions ($Na+$, $k+$, $Cl-$), metabolites (glucose, triglycerides, protein, cholesterol, urea, creatinine), and cellular enzymes (aspartate transaminase, alanine transaminase, lactate dehydrogenase, and ɣ-glutamyl transaminase) are considered reliable to assess stress status in fish (Esmaeili, 2021; Islam et al., 2022). Moreover, the expression of HSP70, TNF-α, and Igf1 genes are considered good indicators for stress, immunity, and fish growth (Islam et al., 2022). Besides, FADS2, SREBP1, and GLUT2 gene expression is considered a reliable indicator of metabolic responses in fish (Castro et al., 2015; Geay et al., 2010). Experimental exposure to extreme heatwave events indicates that the European seabass is not capable to endure this stress (Islam et al., 2020b; Vinagre et al., 2012a). Thus, for this fish, sustainable aquaculture management strategies are essential to face climate change-induced extreme heatwave events (Galappaththi et al., 2020; Sarà et al., 2018). Exploration of nutrient supplementation that may mitigate the effect of extreme heatwave events is essential in this respect (D'Abramo and Slater, 2019; Herrera et al., 2019; Kumar et al., 2018; Maulu et al., 2021). This study investigated the effects of diets supplemented with propolis, vitamins C (as ascorbic acid) and E (α-tocopherol), β-glucans, and phycocyanin on European seabass to test whether these help fish to fare better during extreme heatwave events. A range of parameters linked with growth, metabolism, and physiological fitness were measured to test this hypothesis.

2. Materials and methods

2.1. Source of experimental dietary supplements and formulation

The data presented in this study were generated from a previously published experiment (Islam et al., 2021a). Pure propolis (Bioland, Germany), vitamin C (ascorbic acid), and vitamin E (tocopherol) (Sigma Aldrich, Germany) were used as dietary supplements. *Spirulina platensis* extract (Spirulysat®, MiAL, Germany) was used as phycocyanin (99.99%) supplement. Highly pure β-D-glucan extracted from brewers' yeast (100% *Saccharomyces cerevisiae*) was used as β-glucan supplement. Five diets meeting the nutritional requirements of European seabass, but differing in supplementary contents [propolis, phycocyanin, β-glucan, vitamin C & E together, and without supplement (control diet), respectively] were formulated following Islam et al. (2021a) (Table 1). Ingredients were finely mixed and pelleted (2.0 mm) following the cold extrusion method and stored at -4 ^{o}C until use. The formulated diets are

Table 1
Formulation of experimental diets and nutritional profile (g kg^{-1}).

Dietary ingredients (g kg^{-1})	Dietary formulation				
	Control	Vitamins C&E	Propolis	Phycocyanin	β-glucan
Fishmeal LT	280.00	280.00	280.00	280.00	280.00
Fishmeal 60	200.00	200.00	200.00	200.00	200.00
Fish hydrolysate	25.0	25.0	25.0	25.0	25.0
Soya concentrate	50.0	50.0	50.0	50.0	50.0
Gluten (wheat)	55.0	55.0	55.0	55.0	55.0
Gluten (corn)	50.0	50.0	50.0	50.0	50.0
Soybean meal	90.0	90.0	90.0	90.0	90.0
Wheatmeal	55.0	55.0	55.0	55.0	55.0
Peas (Whole)	50.0	50.0	50.0	50.0	50.0
Fish oil	135.0	135.0	135.0	135.0	135.0
Vitamin and minerals	10.0	10.0	10.0	10.0	10.0
Vitamin-C	-	4.0	-	-	-
Vitamin-E	-	3.5	-	-	-
Propolis	-	-	4.5	-	-
Phycocyanin	-	-	-	0.03	-
β-glucan	-	-	-	-	3.0
Nutritional composition (g kg^{-1})					
Dry matters	951.10	964.10	932.20	974.90	967.70
Crude proteins	549.10	540.70	553.00	534.30	540.30
Crude lipids	189.20	183.40	170.10	160.50	177.10
Ash	108.60	105.60	109.30	107.50	108.60
Carbohydrate	153.10	170.30	144.00	197.70	174.60
Phosphorous	14.40	13.90	14.40	14.00	141.00
Gross energy (kJ g^{-1})	233.10	231.50	232.10	230.40	232.00

hereafter mentioned as "propolis," "vitamin C & E," "phycocyanin," "β-glucan," and "control". The nutritional compositions of formulated diets were tested by SPAROS, the company that prepared them, according to AOAC (1995).

2.2. Experimental fish and rearing conditions

The experiment took place at the Alfred Wegener Institute for Polar and Marine Research (AWI) in Bremerhaven, Germany. The facility used a temperature-controlled recirculatory aquaculture system with fifteen fiberglass rectangular tanks with a 60 L water holding capacity. The tanks were supplied with a continuous flow of filtered seawater at a rate of 4.0 L per minute. Water quality was maintained with biofilters, UV lights, lava stones, continuous aeration, and thermostatically controlled heaters and coolers. European seabass fingerlings (approximately 7 g in weight) were sourced from Les Poissons du Soleil Hatchery, located in France. After two weeks of acclimatization to the experimental setup (22 °C, 30 PSU, 14:10 h light: dark regime), 240 juvenile fish (initial body weight: 8.92 ± 1.34 g, max=13.0, min=6.4, n=240) were randomly assigned into 15 tanks (5 treatments x 3 replicates; 16 fish tank^{-1}). The tested diets were randomly applied to triplicate fish groups (Fig. 1). After 56 days of feeding trial, fish were exposed to a simulated extreme heatwave event (32 °C). To simulate extreme heatwave stress, the water temperature was increased (~3.5 °C day^{-1}) from 22 °C to 32 °C and was maintained for 18 days. The duration of the heatwave stress was counted since the temperature reached 32°C. Throughout the experiment, fish were hand-fed with tested diets twice a day (9.00 and 16.00 h) to apparent visual satiety. Uneaten feed and faces were cleaned daily. To ensure optimal water quality, the following parameters were maintained: DO (>6.0 ppm), NH_3 (<0.05 mg L^{-1}), NO_3^-, and NO_2^- (<0.2 mg L^{-1}). Additionally, about 50–60% of temperature-adjusted seawater was exchanged two times a week to limit the buildup of nitrogenous excretion. The experiment was conducted following the EU directive for animal experiments (EU Directive 2010/63/EU) approved by the Animal Ethics Committee (authorization code TVA 153).

2.3. Sample collections

Following 56 days of feeding, a total of three fish were collected per replicate tank. Fish were sampled at three designated sampling points: the day before the onset of heatwave stress, as well as on day 9 and day 18 of heatwave exposure at 32°C. These three-time points are subsequently denoted as "Day 0," "Day 9," and "Day 18," respectively. Each sampling point resulted in nine fish per treatment. Before sampling, fish underwent a 24-h fasting period to ensure they were dissected in the post-absorptive condition. Immediately before sampling, fish were anesthetized by immersion in an overdose of tricaine methane sulfonate (MS-222, 50 mg L^{-1}). Following anesthesia, measurements of fish length and weight were taken, and two blood samples were drawn through a caudal vein puncture. The first portion of the blood sample was used in the previously published study (Islam et al., 2021a), and the second potion was analyzed for this study. Blood samples obtained from individuals of each replicate tank were pooled together to get a sufficient

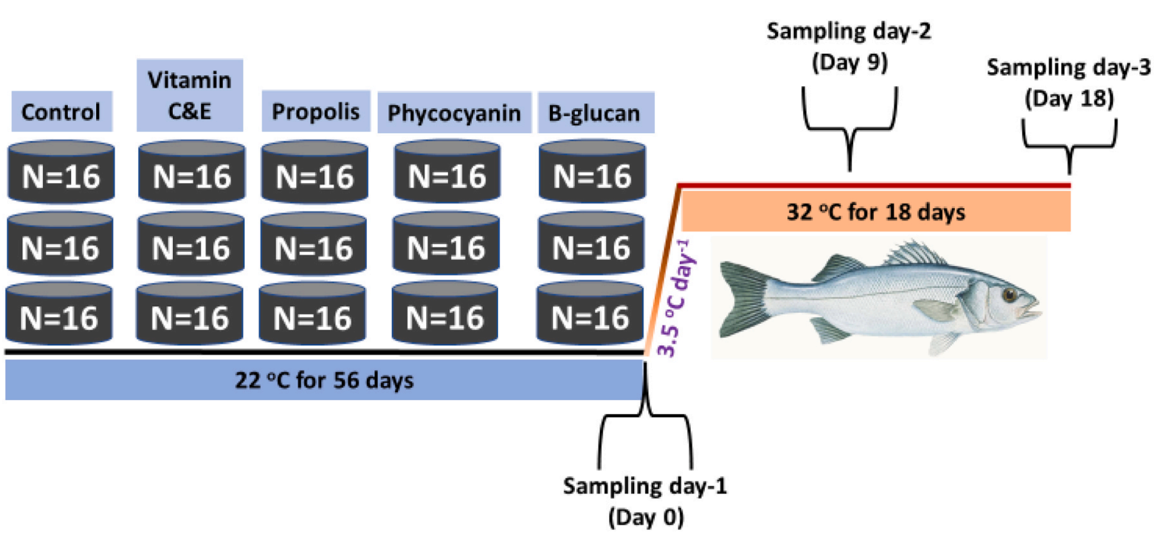

Fig. 1. Design of experiment and sampling: Fish were sampled on day 0, day 9, and day 18 of heatwave exposure (32 °C). The feeding trial took place from days 1–56 at 22°C. On day 56, the 1st sampling was conducted (the day before initiating heatwave stress, Basal, "Day-0"). Temperature was gradually increased to 32°C from days 56–59, followed by the heatwave stress from days 60–77 at 32°C. On day 68, the second sampling occurred (stress day 9, Day 9), and on day 77, the third sampling was performed (stress day 18, "Day 18").

amount for analysis of measured parameters (Dawood et al., 2020; Hossain et al., 2016). Serum was obtained from blood through centrifugation at 4000xg for 15 min. To lessen the effect of handling stress on hematological parameters, blood withdrawal was completed within 5 mins of anesthetizing the fish. Samples for Kidney and liver (mid-part) were collected from the same fish used to get the blood samples. Tissue samples were rapidly frozen in liquid N_2 and then preserved at -80 ^oC until further analyses.

2.4. Measurement of growth, metabolic, and cellular stress responses

Fish lengths and weights were recorded at the beginning of the feeding experiment and prior to the initiation of heatwave stress to test whether the diet supplements affected growth in the absence of heat stress. To calculate the average weight of fish in each replicate tank, the bulk weight of all fish in the tank was divided by the individual number. Weight gain (%) was calculated as [(final weight−initial weight) × 100/ initial weight] (Islam et al., 2021a). Our previously published study (Islam et al., 2021a) showed that diet supplements have an effect on growth during the heatwave event, so these analyses are not repeated here. Serum metabolic products (glucose, triglycerides, cholesterol, protein, lactate, urea, creatin, and urea), serum osmotic ions (Na+, K+, Cl-), and cellular enzymes [aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), ɣ-glutamyl transaminase (γGGT)] were measured using an automated blood analyzer (Fuji Dri-CHEM NX500i). Serum cortisol content was measured with a commercial kit (Cortisol Saliva ELISA, IBL International, Germany) previously used and validated for the same species used in this experiment following the manufacturer's instructions (Islam et al., 2020a).

2.5. Gene expression

We selected specific genes as representatives to assess specific molecular responses across various physiological networks. HSP70 was chosen to investigate heat shock protein response (Clerico et al., 2019), TNF-α for immune system activity (Azeredo et al., 2015), IGF1 for growth-related responses (Islam et al., 2022), and FADS2 and SREBP1 for lipid metabolism (Geay et al., 2010). GLUT2 was selected to assess energy metabolism (Castro et al., 2015), specifically carbohydrate metabolism. Using sterile scissors and forceps, around ~45 mg of tissue samples for the Kidney and liver (mid-part) were also collected from the same fish used to get the blood samples. Tissue samples were rapidly frozen in liquid N_2 and then preserved at -80 ^oC until gene expression analysis. Total RNA was extracted following the manufacturer's recommendations using RNA Miniprep (Monarch, USA). Around ~30 mg tissue sample was used to extract total RNA. Genomic DNA contamination was reduced with the kit provided DNAase I. RNA quality and quantity were assessed using gel electrophoresis alongside a 1.0 kb DNA ladder and spectrophotometry (Tecan, Switzerland). Complementary DNA (cDNA) synthesis was carried out using 1 µg of the obtained total RNA employing RevertAid cDNA synthesis kit (Thermo Fisher Scientific) and oligo (dT) 18 primer. Gene expression levels were quantified through real-time quantitative PCR (qPCR) using CFX Manager™ (Bio-Rad, USA). The analyses were conducted with a mixture consisting using 1 µl of the diluted cDNA (1:10), 0.5 µl of each primer (10 µM), SYBR Green (12.5 µl), 5.5 µl DEPC-treated water (Thermo Fisher Scientific) resulting in a total volume of 20 µl. Previously used and validated gene primers used for the same species were employed in the present study (Table 2). Thermal cycling began with an initial incubation at 95°C for 3 min, followed by 40 cycles consisting of denaturation at 95°C for 15 s, annealing at 59°C, for 30 s, and extension at 72°C for 20 s. Following the PCR cycle, melting curves were monitored to verify the amplification of a single fragment. Quantification of target mRNA expression was conducted using the elongation factor 1α (El 1α) gene as the reference. To reduce interindividual variability, mRNA expression analyses were conducted on pooled muscle and pooled liver samples (Assefa et al., 2020). To add more statistical robustness, technical replicates were not pooled during gene expression calculation. Finally, quantitative mRNA expression was determined by employing the $2^{(\text{-delta delta CT})}$ method following the principles of normalized relative quantification (Rao et al., 2013; Steibel et al., 2009).

2.6. Statistical analysis

Data were checked for normality and homoscedasticity with Kolmogorov-Smirnov and Levene tests, respectively. In cases of violation of the homoscedasticity assumption and normal distribution, data were log-transformed to better meet the assumption of normality. To understand the effects of diet, heatwave stress, and their interactions, all data were statistically compared with a two-way MANOVA, where a 2×2 factorial design was followed with diet and duration as fixed factors. For growth data, a one-way ANOVA was used to assess growth performances among dietary groups. Both types of ANOVA were followed by the Bonferroni correction for multiple comparisons. A statistical significance level of 0.05 was employed to reject the null hypothesis.

3. Results

3.1. Growth performance

Before starting the temperature increase, fish fed diet supplemented with propolis exhibited higher weight gain (%) compared to the control diet (Fig. 2), but this difference was not statistically significant (p=0.258) and growth performance was not affected by the diet overall before the start of the heatwave.

Table 2
Realtime RT-PCR primers details.

Gene	Primer sequence (5´-3´)		Fragment length (bp)	Annealing Temp	Primer efficiency	Accession No.	References
HSP70	Forward:	GTCTGGACAAAGGCAAGAGC	181	59 °C	104.0%	MG711592.1	(Enes et al., 2006)
	Reverse:	TTGTGAGAGGGCCAAGAGAA					
TNF-α	Forward:	GCCAAGCAAACAGCAGGAC	77	60 °C	105.0%	DQ200910	(Azeredo et al., 2015)
	Reverse:	ACAGCGGATATGGACGGTG					
Igf1	Forward:	ATGTACTGTGCACCTGCCAA	106	59 °C	90.0%	GQ924783.1	(Islam et al., 2020a)
	Reverse:	CTTTGTGCCCTGCGGTACTA					
SREBP1	Forward:	CTGGAGCCAAAACAGAGGAG	100	60 °C	101.0%	FN677951	(Geay et al., 2010)
	Reverse	GACAGGAAGGAGGGAGGAAG					
GLUT2	Forward:	GAGCCCACGGTACCTTTACA	165	60 °C	101.5%	EF014277	(Castro et al., 2015)
	Reverse	CGGATCAAAGAAAGGATGGA					
FADS2	Forward:	CCTTCACTGCTTTCATCCCAA	202	60 °C	104.0%	EU439924	(Geay et al., 2010)
	Reverse	CCCAGGTGGAGGCAGAAGAA					
El-1α	Forward:	AGATGACCACGAGTCTCTGC	127	60 °C	105.0%	FM019753	(Mitter et al., 2009)
	Reverse:	CTTGGGTGGGTCGTTCTTG					

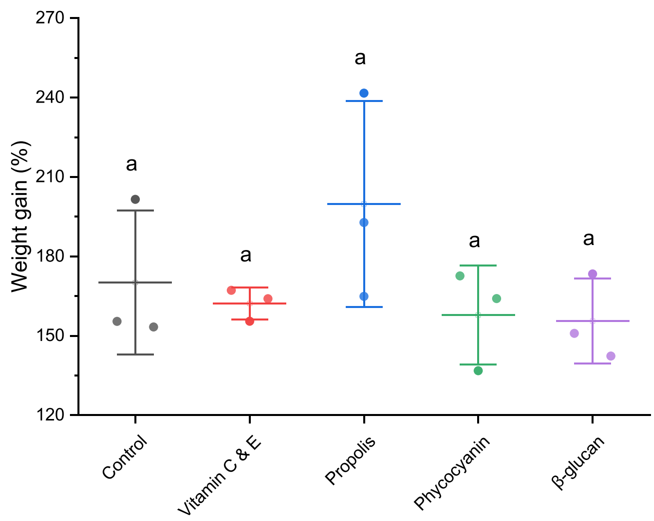

Fig. 2. Weight gain (%) during the 56-day acclimation period (prior to starting the heatwave stress) of European seabass juveniles fed the test diets. Values are means of triplicate group ± SD, n=3. Different letters refer to significantly different values.

3.2. Serum osmotic parameters

Before starting the thermal stress (Day 0), significantly decreased Na+, K+, and Cl- content was observed in fish fed vitamins C & E supplemented diets ($p<0.05$). On days 9 and 18 of heatwave stress, significantly higher Na+ and Cl- content was observed ($p<0.05$) in the fish fed control diet, followed by diets supplemented with vitamins C & E, propolis, phycocyanin, and β-glucan. During heatwave stress, K+ content was found to be significantly lower ($p<0.05$) in fish fed control and β-glucan supplemented diets. Osmotic ion concentrations significantly varied ($p < 0.05$) among sampling days (Table 3).

3.3. Serum energy storage parameters

On days 9 and 18 of heatwave exposure, cortisol was significantly lower ($p<0.05$) in fish fed vitamin C & E, propolis, and phycocyanin supplemented diets compared to fish fed control diet (Fig. 4 A). Serum triglycerides level was also significantly affected by dietary supplements. On day 0, triglyceride content was found to be significantly decreased ($p<0.05$) in fish fed propolis, vitamins C & E, and β-glucan supplemented diets compared to fish fed control diet. In contrast, on days 9 and 18, decreased triglyceride values were observed in fish fed with control and β-glucan supplemented diets (Fig. 4B). Serum protein content did not have an effect prior to the heatwave stress (Fig. 4 C). During heatwave stress, higher protein content was observed in fish fed with propolis, phycocyanin, and vitamin C & E supplemented diets, but this difference was not significant (Fig. 4C). On day 0, total cholesterol was found to be significantly higher in control diet fed fish. In contrast, on days 9 and 18, cholesterol content was significantly decreased in fish fed to control and β-glucan supplemented diets (Fig. 4D). Energy storage content differed significantly ($p < 0.05$) among sampling days (Table 3).

3.4. Serum metabolites

Before starting thermal stress (Day 0), fish fed with tested diets did not differ significantly in blood urea nitrogen (BUN) and creatine levels (Fig. 5). On days 9 and 18 of heatwave stress exposure, BUN and creatine content were significantly lower ($p<0.05$) in fish fed diets supplemented with vitamin C & E, propolis, and phycocyanin compared to fish fed control diet and β-glucan supplementation (Fig. 5 A, B). Moreover, significant differences were observed in the tested metabolites measured at different sampling days (Table 3).

3.5. Cellular stress responses

On day 0, AST content was higher in fish fed diets supplemented with vitamins C & E, followed by propolis, control, phycocyanin, and β-glucan supplemented diets. However, on days 9 and 18, the AST content was significantly lower in fish fed diets containing vitamins C & E, propolis, phycocyanin, and β-glucan supplemented diet compared to those fed the control diet (Fig. 6 A). For ALT content, before the heatwave stress, the values were significantly influenced by dietary supplements compared to the control. However, on days 9 and 18, the ALT level was significantly lower in fish feed diets supplemented with propolis, vitamins C & E, and phycocyanin compared to the control diet (Fig. 6 B). On day 0, LDH and γ-GGT values were not influenced by dietary supplements. However, on days 9 and 18, fish fed diets supplemented with vitamins C & E, propolis, and phycocyanin had significantly lower LDH and γ-GGT levels compared to fish fed control diet (Fig. 6 C, D). Moreover, significant differences were noted in the cellular stress responses measured at different sampling days (Table 3).

3.6. Relative gene expression

3.6.1. Stress, growth, and metabolic genes in muscle and liver

On days 0 and 9, HSP70 expression (a marker of stress) in the muscle and liver did not significantly vary within treatments and tended to stay stable until day 9. On day 18, HSP70 expression in fish muscle and liver of all tested dietary groups tended to increase. On day 18, HSP70 expression in fish fed with propolis was significantly higher than in other tested diets, including the control (Fig. 7 A). Expression of muscle and liver FADS2 [a protein responsible for LC-PUFA biosynthesis] and SREBP1 were affected by dietary supplementation. On day 18, relatively higher expression of FADS2 was observed in fish fed with vitamins C & E supplemented diet. Whereas, on days 9 and 18, relatively higher expression of SREBP1 was observed in fish fed with vitamins C & E supplemented diet (Fig. 7 B, C). During extreme warm heat exposure, fish fed control diets and the diet supplemented with vitamins C & E exhibited higher expression of SREBP-1 compared to fish fed propolis, phycocyanin, and β-glucan supplemented diets (Fig. 7 C). Expression of GLUT type 2 (GLUT2) responded to dietary supplementation. On day 0, GLUT2 was higher in fish fed β-glucan, followed by phycocyanin and propolis. On days 9 and 18, significant upregulation of GLUT2 was observed in fish fed propolis diet ($p<0.05$) compared to fish fed control diet. Besides, both β-glucan and phycocyanin contributed to GLUT2 upregulation compared to the control (Fig. 7 D). On day 0 (prior stress), IGF1 expression levels increased in fish fed the vitamins C & E supplemented diet compared to fish fed the control diet. On day 9, fish fed propolis and phycocyanin supplemented diets exhibited significantly higher expression of IGF1 than fish fed control diet ($p<0.05$). Whereas, on day 18, fish fed propolis exhibited significantly higher ($p<0.05$) levels of IGF1 expression (Fig. 7 E). On day 0, dietary treatments did not affect TNF-1α, expression. In contrast, on days 9 and 18, a significant increase in TNF-1α transcripts was observed in fish fed phycocyanin and propolis supplemented diets, compared to the fish fed control diet (Fig. 7 F). The differences in gene expression on different sampling days are reported in Table 3.

3.6.2. Stress, growth, and metabolic genes in the kidney

On days 0 and 9, HSP70 expression in the kidneys of fish fed with five tested diets did not differ significantly and tended to stay stable until day 9. On day 18, HSP70 mRNA expression levels in fish from all diet groups tended to increase. By day 18, significant upregulation of HSP70 mRNA was observed in fish fed propolis and β-glucan supplemented diets compared to those fed control diet (Fig. 8 A). Expression of the FADS2 gene was not affected in this tissue by any dietary treatment (Fig. 8 B), but mRNA transcripts of SREBP1 and GLUT2 were affected in fish of all five dietary groups. On day 0, fish fed vitamin C & E supplemented diet showed significantly higher upregulation of SREBP1 and GLUT2 than

Table 3
Two-way MANOVA test results of measured parameters in European seabass, *Dicentrarchus labrax* fed control, vitamins C & E, propolis, phycocyanin, and β-glucan supplemented diets after following an experimental heatwave exposure (32 °C). Probability values (p) <0.05 were considered significant.

	Measured parameters	Days					Diets					Days x Diets				
		DF	DF (Error)	F-statistic	p-value	Π^2 (Partial)	DF	DF (Error)	F-statistic	p-value	Π^2 (Partial)	DF	DF (Error)	F-statistic	p-value	Π^2 (Partial)
Serum ions	Na^+	2	30	4.549	0.019	0.233	4	30	164.090	<0.001	0.956	8	30	66.716	<0.001	0.947
	Cl-	2	30	132.017	<0.001	0.898	4	30	102.782	<0.001	0.932	8	30	36.376	<0.001	0.907
	K+	2	30	78.194	<0.001	0.839	4	30	28.794	<0.001	0.793	8	30	28.025	<0.001	0.882
Serum metabolites	Triglycerides	2	30	88.101	<0.001	0.855	4	30	23.336	<0.001	0.757	8	30	15.509	<0.001	0.805
	Total Protein	2	30	22.348	<0.001	0.598	4	30	4.049	<0.001	0.351	8	30	3.085	0.012	0.451
	γGGT	2	30	121.132	<0.001	0.890	4	30	43.847	<0.001	0.854	8	30	14.731	<0.001	0.797
	BUN	2	30	75.286	<0.001	0.834	4	30	35.557	<0.001	0.826	8	30	8.192	<0.001	0.686
	ALT	2	30	23.123	<0.001	0.607	4	30	93.578	<0.001	0.926	8	30	13.392	<0.001	0.781
	LDH	2	30	0.149	0.862	0.010	4	30	37.606	<0.001	0.834	8	30	24.951	<0.001	0.869
	AST	2	30	0.893	0.420	0.056	4	30	10.547	<0.001	0.584	8	30	7.212	<0.001	0.658
	Cholesterol	2	30	200.093	<0.001	0.930	4	30	16.667	<0.001	0.690	8	30	15.848	<0.001	0.809
	Creatine	2	30	78.627	<0.001	0.840	4	30	30.077	<0.001	0.800	8	30	11.619	<0.001	0.756
	Cortisol	2	30	112.362	<0.001	0.882	4	30	22.802	<0.001	0.752	8	30	10.310	<0.001	0.733
Liver tissue	HSP70	2	30	50.452	<0.001	0.771	4	30	4.671	0.005	0.384	8	30	4.287	0.002	0.533
	TNF-1α	2	30	8.184	0.001	0.353	4	30	7.002	<0.001	0.483	8	30	8.479	<0.001	0.693
	Igf1	2	30	26.977	<0.001	0.643	4	30	24.413	<0.001	0.765	8	30	12.608	<0.001	0.771
	FADS2	2	30	14.555	<0.001	0.492	4	30	4.873	0.004	0.394	8	30	3.700	0.004	0.497
	GLUT2	2	30	6.537	0.004	0.304	4	30	4.139	0.009	0.356	8	30	1.650	0.152	0.306
	SREBP1	2	30	4.076	0.027	0.214	4	30	5.161	0.003	0.408	8	30	0.967	0.480	0.205
Kidney tissue	HSP70	2	30	123.003	<0.001	0.891	4	30	3.823	0.013	0.338	8	30	1.618	0.161	0.301
	TNF-1α	2	30	0.574	0.569	0.037	4	30	3.273	0.024	0.304	8	30	0.611	0.761	0.140
	Igf1	2	30	6.528	0.004	0.303	4	30	1.320	0.285	0.150	8	30	1.266	0.297	0.252
	FADS2	2	30	0.191	0.827	0.013	4	30	1.833	0.149	0.196	8	30	1.472	0.209	0.282
	GLUT2	2	30	5.692	0.008	0.275	4	30	0.769	0.554	0.093	8	30	1.091	0.396	0.225
	SREBP1	2	30	18.642	<0.001	0.554	4	30	0.509	0.730	0.064	8	30	1.239	0.312	0.248
	Igf1	2	30	4.549	0.019	0.233	4	30	164.090	<0.001	0.956	8	30	66.716	<0.001	0.947
MANOVA results (Wilks' lambda, λ)																
Effects		50	12	23.239	<0.001	0.990	100	26.325	11.264	<0.001	0.976	200	61.583	5.816	<0.001	0.943

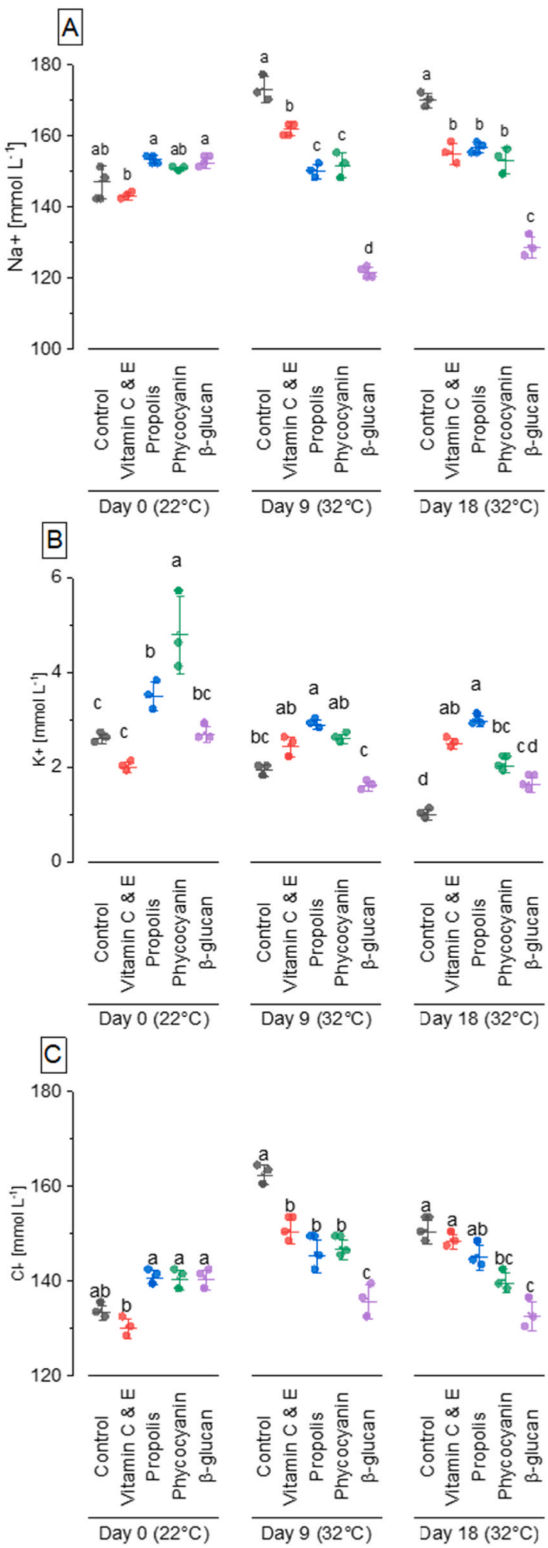

Fig. 3. Serum ionic concentrations in European seabass juvenile fed the test diets while acclimatized for 56 at 22 °C days following 18 days of heatwave stress (32 °C). A. Na+, B. K+, and C. Cl-. Values are presented as triplicates (mean ± SD, n=3). Different letters indicate statistically significant differences.

fish fed control diet ($p<0.05$, Fig. 8 C). For these genes, decreasing trends in expression were observed during extreme warm stress, with no significant differences among treatments (Fig. 8 C, D). On day 0, IGF1 transcripts were found to be up-regulated in fish fed vitamins C & E, phycocyanin, propolis, and β-glucan supplemented diets. Whereas, on day 18, IGF1 was found to be downregulated in all tested diets compared to days 0 and 9 (Fig. 8 E). TNF-1α was found to be higher in fish fed vitamins C & E, propolis, phycocyanin, and β-glucan supplemented diets during the non-stress period (day 0) (Fig. 8 F). On day 18 of heatwave exposure, fish fed on diets supplemented with vitamins C & E, and β-glucan diets exhibited higher expression of TNF-1α compared to fish fed control diet, although these differences were not significant (Fig. 8 F). The differences observed with studied genes in different sampling days are reported in Table 3.

4. Discussion

Current and projected global climate change trends are one of the foremost concerns for the aquaculture industry. Ongoing global warming is becoming one of the major challenges for sustainable aquaculture development worldwide as species are often farmed at temperatures close to their upper physiological thresholds (Le et al., 2020). Extreme heatwaves exerted by climate change already have negative effects on aquaculture in some regions and are predicted to become more severe and frequent (Geffroy et al., 2023; Lattos et al., 2022; Sánchez-Cueto et al., 2023). As the aquaculture industry is moving forward with the agenda to increase and diversify aquaculture production, rising temperatures will impact fish farming, affecting fish health and welfare. Temperature directly affects fish physiology and can result in disease outbreaks and increased mortality within aquaculture systems, or decreased production, with significant financial impacts (Alfonso et al., 2020; Cheung and Frölicher, 2020; Madeira et al., 2016; Piatt et al., 2020; Smith et al., 2023). Previous studies have highlighted the adverse effects of extreme heatwave events on European seabass and other cultured fishes (Ashaf-Ud-Doulah et al., 2020; Islam et al., 2022, 2020b, 2020a; Majharul et al., 2020; Shahjahan et al., 2022, 2017). The impact of climate change on aquaculture varies by species and ecoregion. These changes will have direct, indirect, and complex impacts on the physiology, productivity, and survival of aquaculture species and associated ecological processes. One of the keys to maintain future aquaculture productivity is to mitigate the effect of climate-change-induced extreme temperatures. To alleviate the detrimental effects of extreme temperature events, it is important to prioritize research in the development of new aquafeed formulations to aid aquaculture species to overcome the physiological stress exerted by heatwaves. Our study focused on exploring dietary supplementation strategies to mitigate the adverse impacts on fish during extreme heatwaves. Before implementation of effective dietary mitigation measures, on-farm experiments are essential to assess their effect. However, considering the inherent uncertainties and uncontrollable confounding factors in on-farm experiments, comprehending the impacts of dietary supplementation to mitigate heatwave stress in fish proves exceedingly challenging. Thus, understanding the effectiveness of dietary interventions necessitates further research before on-farm experimentation and dissemination of findings to the aquafarmers. The findings from the current study highlight that fish diets enriched with propolis (0.45%), vitamins C (0.35%) & E (0.40%), and phycocyanin (0.03%) have the potential to improve fish physiological performance during extreme heatwave events. In a prior study, European seabass fed diets enriched with vitamins C, E, propolis, and phycocyanin, exhibited enhanced resilience during heatwave events. This was substantiated by improved growth performance, blood cell counts, erythrocytic abnormalities, and oxidative stress response (Islam et al., 2021a). This observation prompted us to conduct a more comprehensive analysis by examining additional tissue and organs with different parameters from the same experiment to substantiate and validate the findings further.

In the present study, in agreement with the measured biochemical, metabolic, cellular, and molecular responses to stress and metabolism, fish weight gain (growth performance) was comparatively higher in fish fed a diet supplemented with propolis. However, for this study, before the onset of heatwave stress, fish growth performance among tested

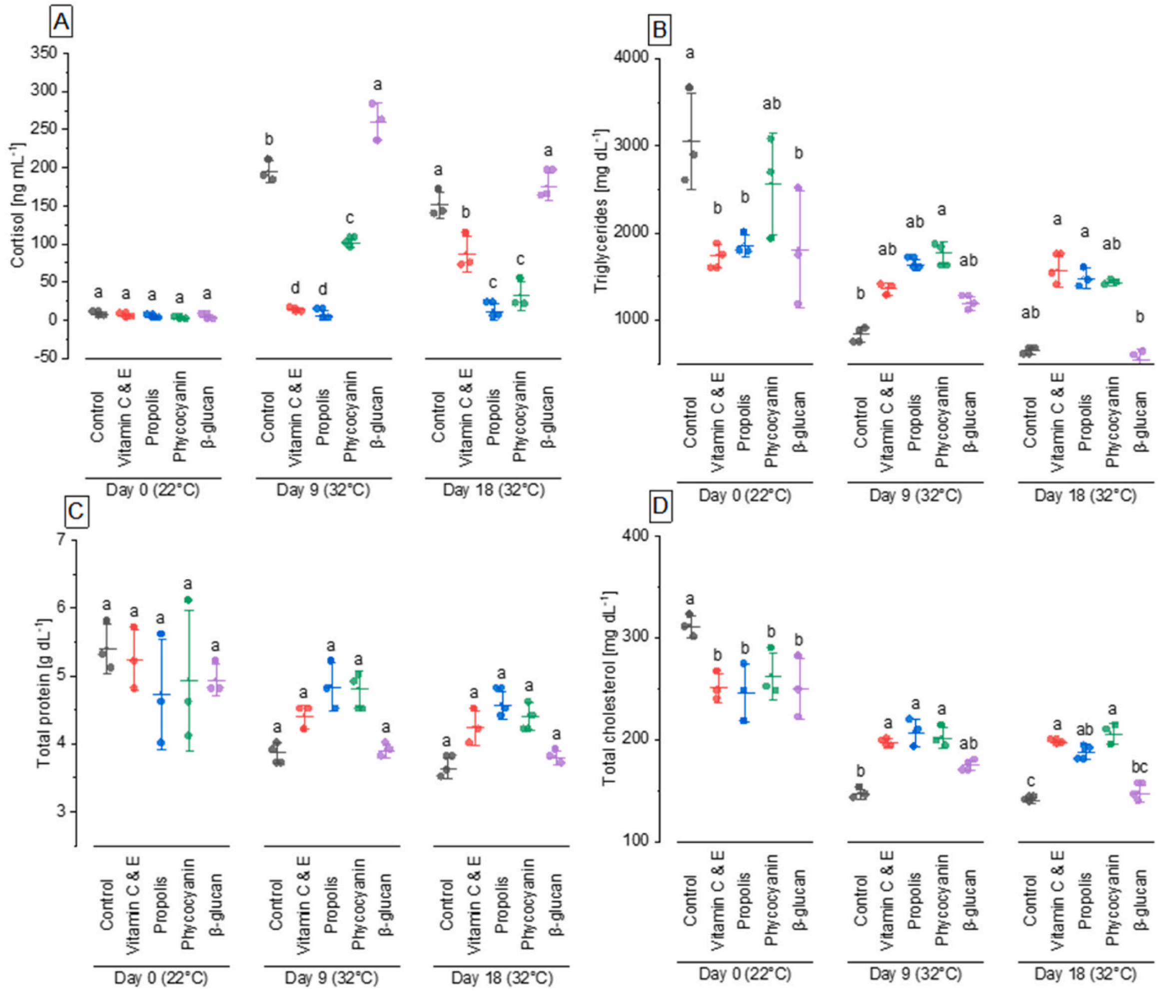

Fig. 4. Responses of serum energy storage in European seabass juvenile fed the test diets while acclimatized for 56 days at 22 °C following 18 days of heatwave stress (32 °C). A. cortisol, B. Triglycerides, C. Total protein, and D. Total cholesterol. Values are presented as triplicates (mean ± SD, n=3). Different letters indicate statistically significant differences.

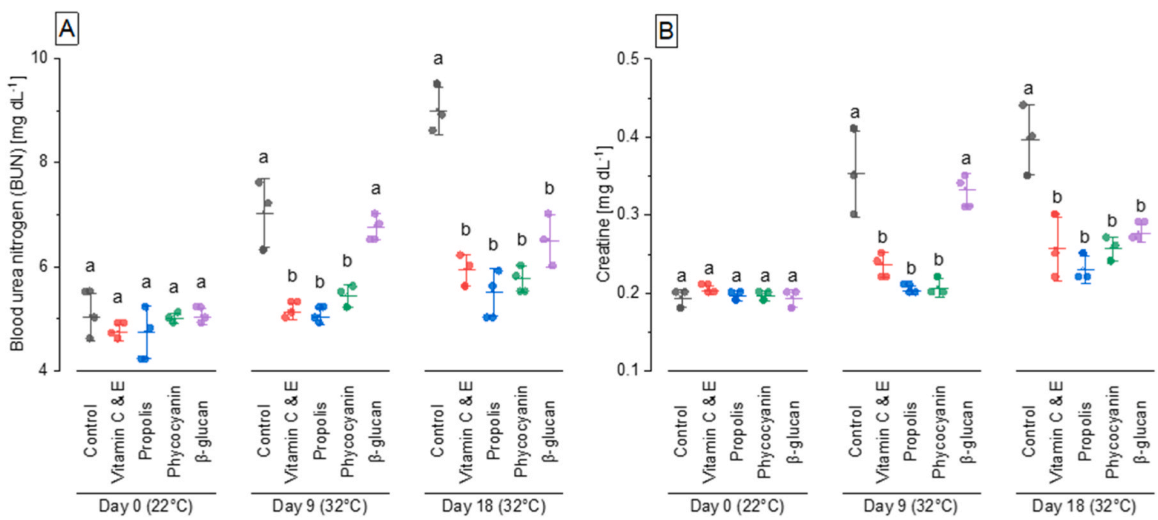

Fig. 5. Responses of serum metabolites in European seabass juveniles fed the test diets while acclimatized for 56 days at 22 °C following 18 days of heatwave stress (32 °C). A. BUN, B. Creatine, C. Cortisol, and D. Lactate. Values are presented as triplicates (mean ± SD, n=3). Different letters indicate statistically significant differences.

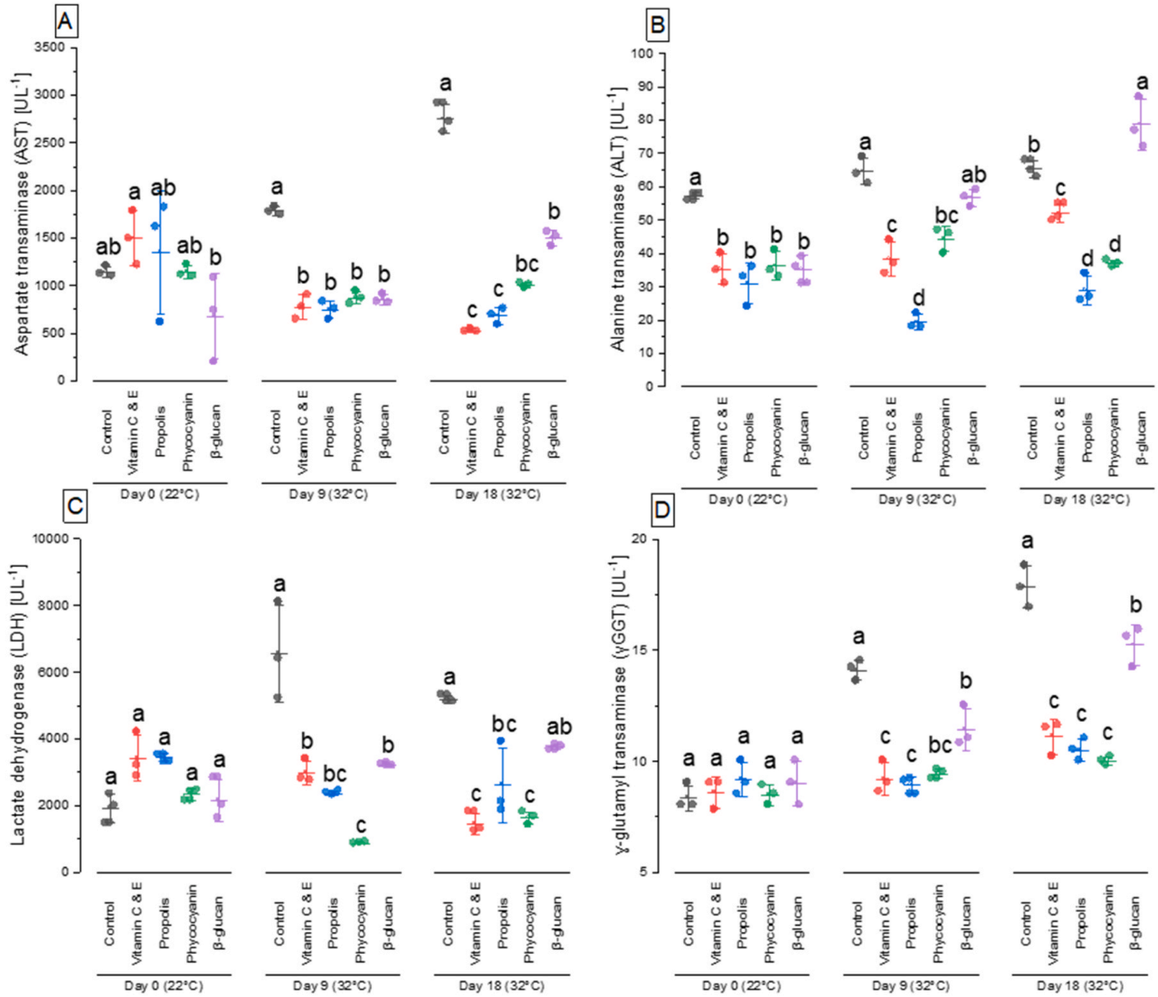

Fig. 6. Responses of cellular enzymatic activities in European seabass juvenile fed the test diets while acclimatized for 56 days at 22 °C following 18 days of heatwave stress (32 °C). A. AST, B. ALT, C. LDH D. γ-GGT. Values are presented as triplicates (mean ± SD, n=3). Different letters indicate statistically significant differences.

dietary groups did not differ significantly compared to the control diet. Thus, the tested supplements do not appear to have a confounding effect outside of the heatwave stress. A comparatively higher, albeit not significant so, weight gain was observed in fish fed with propolis supplemented diet. This may be attributed to the improved intestinal health, immunity, digestion, and absorption resulting from the supplementation of this ingredient (Abdelnour et al., 2020; Do-Huu et al., 2023; Panettieri et al., 2020). Several studies have been conducted to explore the impacts of supplementation with vitamin C, 0.05–4.0 g kg^{-1}; vitamin E, 1.2–3 kg^{-1} (Gao et al., 2014); propolis, 2–10 g kg^{-1} (Farag et al., 2021; Islam et al., 2022); phycocyanin, 0.2–0.50 g kg^{-1} (Elabd et al., 2020; Hassaan et al., 2021; Jin et al., 2020; Zhang et al., 2020); β-glucan, 0.5–5 g kg^{-1} (Hassaan et al., 2021; Neamat-Allah et al., 2021; Yang et al., 2021). These dosages were applied to a range of fishes with varying experimental designs and objectives (Alfons et al., 2023; Bahi et al., 2023; Mamun et al., 2023; Satiro et al., 2024; Singh et al., 2023; Xu et al., 2023). However, there is limited knowledge regarding the impact of these supplements on fish under heatwave-induced stress. However, the influences of tested dietary supplements on European seabass coping with heatwave stress have not yet been widely reported. As a result, direct comparisons of our research results with those of others were not always possible. However, these ingredients were observed to enhance fish fitness to a certain extent. This was evident through improvements in growth performance, and survival rate during the heatwave, along with enhanced liver function and metabolic activities at both cellular and molecular levels (Islam et al., 2021a).

In this study, during extreme warm stress exposure, European seabass fed with control diet exhibited significantly higher sodium and chloride ions, which was in contrast to potassium concentration. In fish, higher concentrations of sodium and chloride ions during thermal stress indicate ionic dysfunction (Hwang et al., 2011; Islam et al., 2020b; Vargas-Chacoff et al., 2018). Potassium is essential for maintaining the osmotic balance between cells and their surroundings, regulating acid-base balance, transmitting neural impulses, and facilitating muscular contraction. Changes in potassium homeostasis have the potential to disrupt the neurological system and cause cardiac disruption (Evans, 1993; Gonzalez and Mcdonald, 2000; Palmer, 2014; Vargas-Chacoff et al., 2018). Temperature has a significant impact on electrolyte and osmotic balance in aquatic ectotherms. This is due to the fact that metabolically-driven ion transport is substantially more responsive to temperature fluctuations compared to passive ionic diffusion (Gonzalez and Mcdonald, 2000). For the present study, fish fed with control and β-glucan supplemented diets might be unable to maintain osmotic balance, which requires activating ionic pumps to maintain osmotic homeostasis. This stressor or disturbance in fish has the potential to activate the hypothalamic–pituitary–adrenal axis within the neuroendocrine system, leading to the release of stress hormones such as corticosteroids. Thus, in turn, initiates alteration in electrolyte concentrations and osmoregulation (Hassaan et al., 2019). Increased cortisol levels are associated with ionic imbalances, necessitating

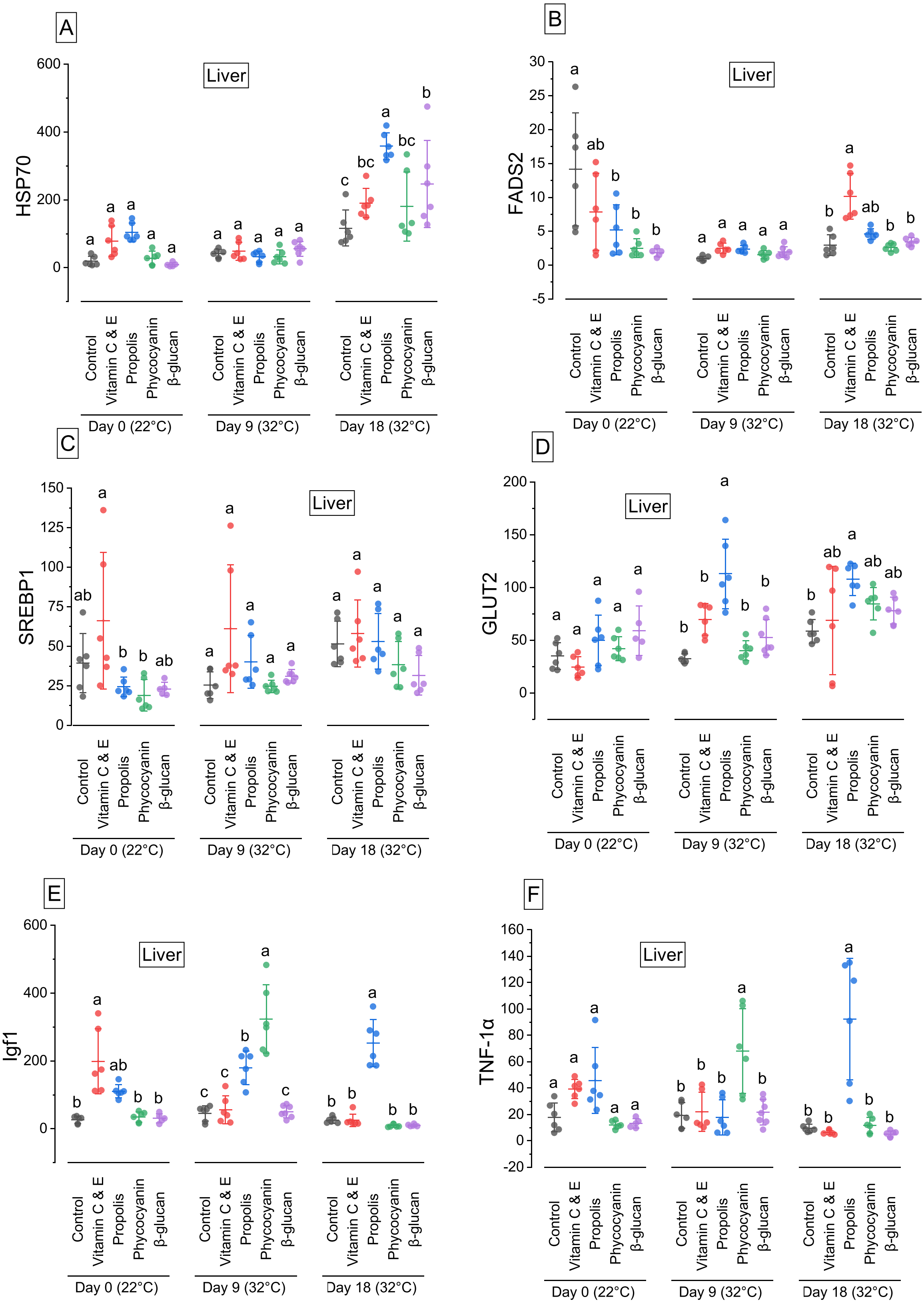

Fig. 7. Genes expression in liver of European seabass fed tested diets prior (Day 0) and after heatwave exposure (Days 9, and 18) of heatwave exposure (32 °C). A. HSP70, B. FADS2, C. SREBP-1, D. GLUT2, E. Igf1, and F. TNF-1α. On each sampling day, bars labeled with different letters refer significant differences among the dietary groups ($p < 0.05$). Values are presented as mean ± SD, n=6.

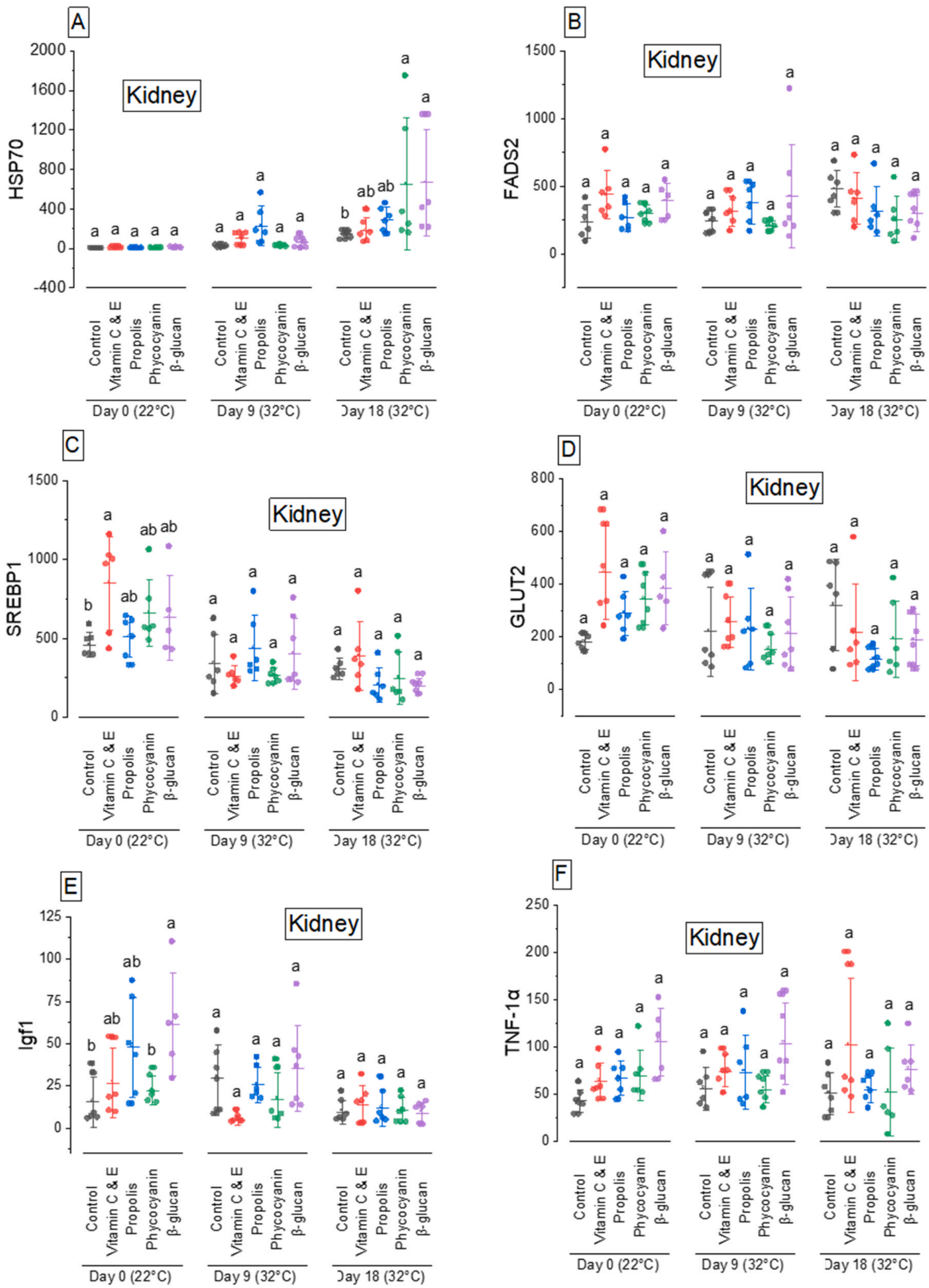

Fig. 8. Genes expression in kidney of European seabass fed tested diets prior (Day 0) and after heatwave exposure (Days 9, and 18) of heatwave exposure (32 °C). A. HSP70, B. FADS2, C. SREBP-1, D. GLUT2, E. Igf1, and F. TNF-1α. On each sampling day, bars labeled with different letters refer to significant differences among the dietary groups ($p < 0.05$). Values are presented as mean ± SD, n=6.

increased energy to maintain osmotic balances leading to compromised growth (Árnason et al., 2013; Bœuf and Payan, 2001; Islam et al., 2020b). Furthermore, reduced serum protein and elevated cortisol, BUN, and creatine support ionic dysfunction. Consistent with previous research findings, fish are required to expend additional energy to maintain physiological homeostasis in adverse environments. This increased energy expenditure can lead to reduced growth, physiological imbalances, and disruptions in ionic regulation (Alfons et al., 2023; Herrera et al., 2009; Islam et al., 2020b). Previous studies based on the impact of these ingredients on fish have focused on feed acceptability, growth performance, and physiological responses (Alrashada et al., 2023; Do-Huu et al., 2023; Medagoda et al., 2023; Porter et al., 2023; Singh et al., 2023; Xu et al., 2023; Youssef et al., 2023). However, the combined effect of thermal stress and remediation through dietary manipulation is largely unexplored. We know less about the joint effects of dietary supplementation and fish's physiological responses to extreme temperatures in terms of ionic regulation. Further studies are required to unveil the mechanisms.

Measurement of blood biochemistry provides information on physiological responses and nutritional state (Esmaeili, 2021; Shahjahan et al., 2022). To the best of our knowledge, no prior studies have investigated the impacts of diets enriched with vitamins C & E, propolis, phycocyanin, and β-glucan on serum metabolites, energy storage, cellular stress responses, and gene expression in fish exposed to extreme heatwaves. In this study, before starting extreme temperature stress (day 0), serum cortisol content was significantly lower than on days 9 and 18. This indicates that none of the diets trigger cortisol responses. During extreme warm exposure, the highest cortisol level was found in fish fed the control diet and the lowest in fish fed propolis, phycocyanin, and vitamins C & E supplemented diets. In the present study, the increased cortisol levels in fish fed the control diet while exposed to heatwave stress could be attributed to thermal adaptation leading to the higher enzymatic and metabolic reaction rate (Alfons et al., 2023; Alfonso et al., 2020; Tang et al., 2022). Energy storage (glucose) and anaerobic metabolites (lactate) levels were significantly influenced by the diets tested, the duration of extreme temperatures of exposure, and the interactions between these factors (Islam et al., 2021a). This could potentially indicate an increased energy demand required to counteract the stress effects (Alfons et al., 2023; Barton, 2002; Schreck and Tort, 2016). A comparable trend was reported for European seabass subjected to temperature fluctuation (Islam et al., 2020a; Varsamos et al., 2006) and in other species reviewed in Islam et al. (2022). The effect of propolis (Alrashada et al., 2023; Eslami et al., 2022; Hassaan et al., 2019; Hassanien et al., 2023) and phycocyanin (Abdel-Latif et al., 2022; Hassaan et al., 2021; Sayed et al., 2023) on cortisol levels in fish subjected to stress has been reported. The authors suggested that aquafeed supplemented with propolis and phycocyanin might inhibit oxidative stress and peroxidation of unsaturated fatty acids, thus preventing the reduction of cholesterol in fish exposed to thermal stress. In our study, on days 9 and 18 of temperature stress, the reduced concentration of cholesterol in fish fed diets supplemented with vitamins C&E, propolis, and phycocyanin substantiates the potent antioxidant activity of these supplements. On days 9 and 18, fish fed diets supplemented with vitamins C & E and propolis demonstrated notably decreased serum antioxidant responses in comparison to those fed the control diet (Islam et al., 2021a) further supporting decreased oxidative stress.

The current study exhibited that triglycerides and cholesterol content tend to decrease in fish fed all four diets, but during heatwave exposure, fish fed vitamins C & E, propolis, and phycocyanin supplemented diets were able to maintain relatively higher triglycerides and cholesterol levels. From this output, we anticipated that fish fed with diets supplemented with vitamins C & E, propolis, and phycocyanin could maintain higher energy storage during thermal stress to respond to extreme warm stress. The transfer of triglycerides and cholesterol from the liver to peripheral cells lessens lipid peroxidation damage caused by heat shock (Fredenrich and Bayer, 2003; Gomez Isaza et al., 2019). Thus, the rise in serum triglyceride and cholesterol levels in this study revealed that vitamins C & E, propolis, and phycocyanin play an essential role in the protection of liver tissue damage. Triglycerides and cholesterol are primarily generated in the liver and are monitored to assess nutritional, and (lipid) metabolic states (Greene and Selivonchick, 1987; Madeira et al., 2016; Rossi et al., 2017). Under long-term warm and heatwave stress, deviation in normal triglycerides levels is considered an indicator of liver dysfunction, disrupted lipid, and fatty acid mobilization as well as circulation among the organs and tissue (Castro et al., 2015; Greene and Selivonchick, 1987; Liu et al., 2019). These changes enhance hepatic aminotransferase activity, subsequently amplifying the cortisol stress response, and promoting catabolism of protein at the expense of normal physiological activity and development, implying stressful situations in animals (Eslami et al., 2022; Jentoft et al., 2005). Interestingly, in the current study, a reduction in total plasma protein was seen in fish fed with control and β-glucan diets, a good indicator to assume the activation of protein catabolism. When protein catabolism occurs, the fish suffers from immunological dysfunction and protein breakdown, which predisposes the fish to diseases and death (Chadwick and McCormick, 2017; Nemova et al., 2016; Pérez-Sánchez et al., 2017). For this study, significantly higher expression of upregulation of the immune gene (TNF-1α) and growth gene (IGF1) in fish fed with propolis and phycocyanin supplemented diets indicated that fish were in better condition during extreme warm stress.

Changes in hepatic enzyme activity, such as ALT, AST, LDH, and ɣ-GGT, serve as indicators of changes in liver function (Mahmoud et al., 2023; Shahjahan et al., 2022). During heatwave simulation, fish fed vitamins C & E, propolis, and phycocyanin supplemented diets resulted in decreased activities of ALT, AST, LDH, and ɣ-GGT. Increased erythrocytic abnormalities, lysozyme activities, and white blood cell count were observed in fish fed the control diet and β-glucan supplemented diet (Islam et al., 2021a) corroborating with the present findings of a higher cellular stress response. The increased liver enzymatic activity of in fish fed control diet indicates the production of hepatic transferase and enzyme leakage due to thermal stress (Farag et al., 2021; Mahmoud et al., 2023). Our findings are consistent with previous research on European seabass (Šegvić-Bubić et al., 2013), and Nile tilapia, *O. niloticus* (Alrashada et al., 2023; Hassaan et al., 2019), which found that fish fed with propolis supplemented diet had decreased levels of liver enzymes. Reductions in ALT, AST, ALP, and LDH in fish fed with vitamins C & E, propolis, and phycocyanin supplemented diets demonstrated that these ingredients have hepatoprotective roles. Unchanged BUN and creatine levels in fish fed these diets further support the assumption. A decreased amount of BUN and creatine also refers to better liver and kidney function and metabolic function as well (Islam et al., 2021b; Peres et al., 2014; Roche and Bogé, 2000; Wagner and Congleton, 2004). We propose that the absence of changes in liver functioning pertains to the safe use of vitamins C & E, propolis, and phycocyanin in European seabass feeding. Besides, we recommend exploring other ingredients like betaine, dietary seaweed (*Dictyota dichotoma*), dietary dragonhead fish, and *Myristica fragrans* (nutmeg) alongside probiotics in future studies. These readily available ingredients have already shown promise in improving fish growth, stress tolerance, and immune response, making them worthy candidates for further investigation against temperature stress (Heshmatfar et al., 2023; Mahmoudi et al., 2022; Musavi et al., 2022; Safari et al., 2022b, 2022a; Vakili et al., 2023).

In general, farmed fish receive abundant and high-quality nutrition, resulting in a substantial concentration of polyunsaturated fatty acids (PUFA) (Castro et al., 2015; Martins et al., 2021; Zak and Manzon, 2019). In our study, prior to starting warm stress, comparatively higher upregulation of liver FADS2 and SREBP1 genes was observed in fish fed with control diet than in fish fed propolis, phycocyanin, and β-glucan supplemented diets. In the normal metabolic state, this high amount of PUFA requires more antioxidants to protect lipids from oxidative damage (Araújo-Luna et al., 2018; Zak and Manzon, 2019). However, the present research has shown that during the initial stages of experimental heatwave, fish feed intake drops (Md J Islam, personal observation), resulting in decreased antioxidant intake from dietary sources, rendering fish more susceptible to the adverse effects of heat stress events. As a consequence, aquaculture fish become more susceptible to oxidative stress during heatwave events (Islam et al., 2022; Torno et al., 2018; Truzzi et al., 2018). Thermal exposure activates antioxidant machinery in different tissues of European seabass (Islam et al., 2022; Pereira et al., 2017; Vinagre et al., 2012b), largemouth bass, *Micropterus salmoides* (Sun et al., 2020), and rock goby, *Gobius paganellus* (Choe et al., 2017; Paul et al., 2021). In this study, fish fed diets supplemented with propolis, vitamins C & E, and phycocyanin exhibited reduced transaminase activity and serum metabolites. This indicates a decreased level of (oxidative) stress and physiological dysfunction in European seabass. Conversely, during heatwave exposure, fish fed the control and β-glucan diets demonstrated significantly higher levels of cortisol, AST, ALT, LDH, ɣGGT, BUN, and creatine, justifying the increased physiological stress and metabolic dysfunction. Antioxidants protect PUFA and lipids against oxidation (Firsov et al., 2019; Pokorný and Parkányiová, 2019). Vitamins C and E have been shown to protect oxidation and promote stress tolerance (Li et al., 2023; Medagoda et al., 2023; Rathore et al., 2023; Singh et al., 2023; Xu et al., 2023). This might be due to the protective effects of vitamins C and E on fish during extreme warm stress. L-ascorbate (vitamin C) inhibits/reduces tocopheroxy free radicals and regenerates α-tocopherol (vitamin E) (Hajirezaee et al., 2020; Pokorný and Parkányiová, 2019; Xiao et al., 2023; Xu et al., 2023). The recycling process is thermodynamic and exergonic (Amorati et al., 2002; Vissers and Das, 2018). Temperature dependence impedes vitamin E regeneration during extreme warm exposure, and fishes necessitate a higher dosage of vitamins C and E to prevent/reduce oxidative stress. Further research into measuring vitamin C and E levels in the various tissues of fish subsequently feeding with different dosages of vitamins C and E supplemented diets might help to better understand this. Propolis contains a range of antioxidants, vitamins, and minerals that have been shown to increase digestive enzyme cofactors (reviewed in de la Cruz-Cervantes et al., 2018; Farag et al., 2021). These combinations promote improved digestion and absorption, resulting in improved growth. Other studies have found that the inclusion of propolis in fish diets boosted fish growth, immunity, and physiological performance in European seabass (Hassaan et al., 2019; Islam et al., 2021a), Nile tilapia, *T. niloticus* (Alrashada et al., 2023; Mafra et al., 2022), sturgeon, *Huso huso* (Eslami et al., 2022), African Catfish, *Clarias gariepinus* (Alfons et al., 2023; Nowosad et al., 2023), sea bream, *Sparus aurata* (Kaplan and Erdoğan, 2021b), and rainbow trout, *Oncorhynchus mykiss* (Liu et al., 2020). The present study also found considerably decreased blood transaminase and metabolites in fish fed with propolis and phycocyanin supplemented diets, which is consistent with earlier research (de Mattos et al., 2019; Hassaan et al., 2021). Phycocyanin, propolis, and β-glucan are likewise antioxidants and immunomodulators (Bacha et al., 2017; Bonfim-Mendonça et al., 2017; De Morais et al., 2018; Elabd et al., 2020). However, in this study, β-glucan was unable to manage or avoid extreme heatwave stress. This might be attributed to reduced antioxidant capacity compared to vitamin C & E, propolis, and phycocyanin, or the dosages employed in this study were inadequate. Further research is necessary to determine the β-glucan quantity required to generate the desired effects.

HSP70, a molecular chaperone and a highly conserved protein with a broad protective function in animals is essential for cellular and protein homeostasis. This gene prevents protein denaturation, and aggregation and aids in the refolding of misfolded proteins (Clerico et al., 2019; Fernández-Fernández and Valpuesta, 2018). On days 0 and 9, HSP70 was stable, which indicates either, fish were able to maintain physiological homeostasis prior to the onset of extreme heatwave stress or fish were incapable of activating this. During excessive stress periods, animals sometimes fail to activate the molecular mechanism to check the unfolded protein (Cho et al., 2021; Place et al., 2004; Yamashita et al., 2010). In contrast, on day 18 of heatwave exposure, higher HSP70 expression was observed in liver and kidney tissues of fish fed vitamins C & E, propolis, phycocyanin, and β-glucan supplemented diets, indicating a relatively higher protein repairing activity than fish fed the control diet. On days 9 and 18, both IGF1 and TNF-1α genes were upregulated in fish fed propolis and phycocyanin supplemented diets, corroborated with HSP7 expression. Significantly decreased expression of HSP70 and upregulation Igf1 gene in fish fed vitamins C & E, propolis, and phycocyanin supplemented diets were also observed in the muscle tissue of European seabass (Islam et al., 2021a) further justifying the findings in this study. Fish respond to stressors at the cellular level by stimulating proteins, energy metabolism, and phospholipid de novo synthesis, as well as stimulating nuclear and mitochondrial transcription (Eymann et al., 2020; Hassaan et al., 2021; O'Brien, 2011). Furthermore, among others, fish stimulate the FADS2, SREBP1, and GLUT2 genes to maintain balances in energy metabolism, antioxidant levels, tissue repair, and immunity (Bouaziz et al., 2017; Donaldson et al., 2008; Geay et al., 2010; Shi et al., 2018; Vanderplancke et al., 2014). Increased mitochondrial activity leads to increased ATPase activity, which enhances energy demand and HSP70 operation to safeguard cell machinery. A crucial mechanism for protecting cells from heat stress and disposing of damaged proteins via the ubiquitination and proteolysis pathways in the cell is the activation of HSP70 (Esmaeili et al., 2021; Lüders et al., 2000). In the present study, higher levels of serum cortisol, liver transaminase activities, FADS2, SREBP1, and GLUT2 gene upregulation in the liver were observed in fish fed vitamins C & E, propolis, phycocyanin, and β-glucan supplemented diets. These findings suggest that the elevated energy demands resulting from increased metabolic activities during warm stress were adequately addressed. Consequently, the heightened cortisol levels impact the release of growth hormone (GH) into the bloodstream, which is the primary mediator for Igf1 (Islam et al., 2020b). During extreme warm exposure (from days 9–18), increased Igf1 regulation was measured in fish fed propolis, justifying better growth and physiological performances. However, in the kidney, these gene expressions did not show the same shifting pattern as described above, nor did they demonstrate any synergism. Overall, on days 9 and 18 of heatwave exposure, decreased blood cortisol, transaminase, dehydrogenase, metabolites, and a comparatively higher upregulation of growth and immune genes were measured in fish fed with vitamins C & E, propolis, and phycocyanin. These contrasting tendencies suggest that European seabass fed with diets enriched with these ingredients fare substantially better during extreme warm exposure, a possible way to formulate climate-smart aquafeeds to lessen extreme weather events in aquaculture.

5. Conclusions

In conclusion, dietary incorporation of vitamins C & E, propolis, and phycocyanin through supplemented diets proved to be beneficial for European seabass subjected to extreme heat waves. During extreme warm exposure, diets supplemented with high levels of vitamin E (0.40%) and vitamin C (0.35%) together, or propolis (0.45%), or phycocyanin (0.030%) demonstrated the ability to lessen and mitigate the negative impact of heatwave stress responses. These ingredients were observed to enhance fish fitness to a certain extent, as indicated by improvements in weight gain during the heatwave (Islam et al., 2021a), liver function, and metabolic activities at both cellular and molecular levels. However, given the experimental design, the specific ingredients used, and the parameters measured, it is important to mention that the present study represents the initial exploration of these factors. Therefore, further research is necessary to gain a deeper understanding of the immunostimulatory and antioxidant properties of tested ingredients, as well as their optimal supplementation in summer diets for European seabass and other fish. Additional research on proteomic and metabolomic responses across various tissues could offer a more comprehensive understanding of fish physiology and nutritional status during exposure to extreme warm stress. Moreover, to get practical insights tailored to real-world conditions, validate research outcomes, and promote stakeholder involvement, on-farm experiments are essential. This will allow the assessment of possible constraints, enable long-term monitoring, and inform evidence-based decision-making for sustainable aqua farming practices.

CRediT authorship contribution statement

Md Jakiul Islam: Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Andreas Kunzmann:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Oscar Puebla:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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References

AbdAllah, H.M.L., Hamed, A.-A.S., Bakry, M.A., Eldin, I.M.G., AbdElal, M.N.A., Mohamed, D.T., Mohamady, S.N., 2023. Impact of Spirulina Supplementation On Some Immune And Biochemical Parameters Of Nile Tilapia Under Cold Stress. Alex. J. Vet. Sci. 79, 107. https://doi.org/10.5455/AJVS.164820.

Abdel-Latif, H.M.R., El-Ashram, S., Sayed, A.E.D.H., Alagawany, M., Shukry, M., Dawood, M.A.O., Kucharczyk, D., 2022. Elucidating the ameliorative effects of the cyanobacterium Spirulina *(Arthrospira platensis*) and several microalgal species against the negative impacts of contaminants in freshwater fish: A review. Aquaculture 554, 738155. https://doi.org/10.1016/j.aquaculture.2022.738155.

Abdelnour, S.A., Swelum, A.A., Salama, A., Al-Ghadi, M.Q., Qattan, S.Y.A., Abd El-Hack, M.E., Khafaga, A.F., Alhimaidi, A.R., Almutairi, B.O., Ammari, A.A., El-Saadony, M.T., 2020. The beneficial impacts of dietary phycocyanin supplementation on growing rabbits under high ambient temperature. Ital. J. Anim. Sci. 19, 1046–1056. https://doi.org/10.1080/1828051X.2020.1815598.

Alfons, M.S., Ibrahim, A.T.A., Harabawy, A.S.A., Al-salahy, M.B., Badr, G., 2023. Cytoprotective effect of propolis on heat stress induces alteration to histological, ultrastructural, and oxidative stress in catfish (*Clarias gariepinus*). Environ. Sci. Pollut. Res. 30, 114152–114165. https://doi.org/10.1007/s11356-023-30386-y.

Alfonso, S., Gesto, M., Sadoul, B., 2020. Temperature increase and its effects on fish stress physiology in the context of global warming. J. Fish. Biol. https://doi.org/10.1111/jfb.14599.

Alrashada, Y.N., Hassanien, H.A., Abbas, A.O., Alkhamis, S.A., Alkobaby, A.I., 2023. Dietary propolis improves the growth performance, redox status, and immune response of Nile tilapia upon a cold-stress challenge. PLoS One 18, e0293727. https://doi.org/10.1371/journal.pone.0293727.

Amorati, R., Ferroni, F., Lucarini, M., Pedulli, G.F., Valgimigli, L., 2002. A quantitative approach to the recycling of α-tocopherol by coantioxidants. J. Org. Chem. 67, 9295–9303. https://doi.org/10.1021/jo026501f.

AOAC, 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists., 16th ed. AOAC International, Arlington, VA, USA.

Araújo-Luna, R., Ribeiro, L., Bergheim, A., Pousão-Ferreira, P., 2018. The impact of different rearing condition on gilthead seabream welfare: Dissolved oxygen levels and stocking densities. Aquac. Res. 49, 3845–3855. https://doi.org/10.1111/ARE.13851.

Árnason, T., Magnadóttir, B., Björnsson, B., Steinarsson, A., Björnsson, B.T., 2013. Effects of salinity and temperature on growth, plasma ions, cortisol and immune parameters of juvenile Atlantic cod (*Gadus morhua*). Aquaculture 380–383, 70–79. https://doi.org/10.1016/j.aquaculture.2012.11.036.

Ashaf-Ud-Doulah, M., Mamun, A.Al, Rahman, M.L., Islam, S.M.M., Jannat, R., Hossain, M.A.R., Shahjahan, M., 2020. High temperature acclimation alters upper thermal limits and growth performance of Indian major carp, rohu, *Labeo rohita* (Hamilton, 1822). J. Therm. Biol. 93, 102738 https://doi.org/10.1016/j.jtherbio.2020.102738.

Assefa, A.T., Vandesompele, J., Thas, O., 2020. On the utility of RNA sample pooling to optimize cost and statistical power in RNA sequencing experiments. BMC Genom. 21, 1–14. https://doi.org/10.1186/s12864-020-6721-y.

Azeredo, R., Pérez-Sánchez, J., Sitjà-Bobadilla, A., Fouz, B., Tort, L., Aragão, C., Oliva-Teles, A., Costas, B., 2015. European Sea Bass (*Dicentrarchus labrax*) immune status and disease resistance are impaired by arginine dietary supplementation. PLoS One 10, e0139967. https://doi.org/10.1371/journal.pone.0139967.

Bacha, U., Nasir, M., Iqbal, S., Anjum, A.A., 2017. Nutraceutical, anti-inflammatory, and immune modulatory effects of β-glucan isolated from yeast, 8972678–8972678 Biomed. Res. Int. 2017. https://doi.org/10.1155/2017/8972678.

Bahi, A., Ramos-Vega, A., Angulo, C., Monreal-Escalante, E., Guardiola, F.A., 2023. Microalgae with immunomodulatory effects on fish. Rev. Aquac. https://doi.org/10.1111/raq.12792.

Barton, B.A., 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525. https://doi.org/10.1093/ICB/42.3.517.

Bœuf, G., Payan, P., 2001. How should salinity influence fish growth? Comp. Biochem. Physiol. - C. Toxicol. Pharmacol. 130, 411–423. https://doi.org/10.1016/S1532-0456(01)00268-X.

Bonfim-Mendonça, P., de, S., Capoci, I.R.G., Tobaldini-Valerio, F.K., Negri, M., Svidzinski, T.I.E., 2017. Overview of β-Glucans from Laminaria spp.: Immunomodulation Properties and Applications on Biologic Models. Int. J. Mol. Sci. 18, 1629. https://doi.org/10.3390/IJMS18091629.

Bouaziz, M., Bejaoui, S., Rabeh, I., Besbes, R., El Cafsi, M.H., Falcón, J., 2017. Impact of temperature on sea bass, *Dicentrarchus labrax*, retina: Fatty acid composition, expression of rhodopsin and enzymes of lipid and melatonin metabolism. Exp. Eye Res. 159, 87–97. https://doi.org/10.1016/j.exer.2017.03.010.

Brierley, A.S., Kingsford, M.J., 2009. Impacts of Climate Change on Marine Organisms and Ecosystems. Curr. Biol. https://doi.org/10.1016/j.cub.2009.05.046.

Castro, C., Corraze, G., Pérez-Jiménez, A., Larroquet, L., Cluzeaud, M., Panserat, S., Oliva-Teles, A., 2015. Dietary carbohydrate and lipid source affect cholesterol metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. Br. J. Nutr. 114, 1143–1156. https://doi.org/10.1017/S0007114515002731.

Chadwick, J.G., McCormick, S.D., 2017. Upper thermal limits of growth in brook trout and their relationship to stress physiology. J. Exp. Biol. 220, 3976–3987. https://doi.org/10.1242/jeb.161224.

Chen, R., Lochmann, R., Goodwin, A., Praveen, K., Dabrowski, K., Lee, K.J., 2004. Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture 242, 553–569. https://doi.org/10.1016/j.aquaculture.2004.09.012.

Chen, R., Lochmann, R., Goodwin, A., Praveen, K., Dabrowski, K., Lee, K.J., 2003. Alternative complement activity and resistance to heat stress in golden shiners (*Notemigonus crysoleucas*) are increased by dietary vitamin C levels in excess of requirements for prevention of deficiency signs. J. Nutr. 133, 2281–2286. https://doi.org/10.1093/jn/133.7.2281.

Cheung, W.W.L., Frölicher, T.L., 2020. Marine heatwaves exacerbate climate change impacts for fisheries in the northeast Pacific. Sci. Rep. 10, 1–10. https://doi.org/10.1038/s41598-020-63650-z.

Cho, Y.S., Jeong, T.H., Choi, M.J., Kim, J.M., Lim, H.K., 2021. Heat shock protein 70 gene expression and stress response of red-spotted *(Epinephelus akaara*) and hybrid (E. akaara female × E. lanceolatus male) groupers to heat and cold shock exposure. Fish. Physiol. Biochem. 47, 2067–2080. https://doi.org/10.1007/s10695-021-00966-1.

Choe, J.R., Shin, Y.S., Choi, J.Y., Kim, T.H., Jung, M.M., Choi, C.Y., 2017. Effect of different wavelengths of light on the antioxidant and immunity status of juvenile rock bream, *Oplegnathus fasciatus*, exposed to thermal stress. Ocean Sci. J. 52, 501–509. https://doi.org/10.1007/s12601-017-0051-2.

Clerico, E.M., Meng, W., Pozhidaeva, A., Bhasne, K., Petridis, C., Gierasch, L.M., 2019. Hsp70 molecular chaperones: multifunctional allosteric holding and unfolding machines. Biochem. J. 476, 1653–1677. https://doi.org/10.1042/BCJ20170380.

D`Abramo, L.R., Slater, M.J., 2019. Climate change: Response and role of global aquaculture. J. World Aquac. Soc. https://doi.org/10.1111/jwas.12643.

Dahlke, F.T., Wohlrab, S., Butzin, M., Pörtner, H.-O., 2020. Thermal bottlenecks in the life cycle define climate vulnerability of fish. Sci. (80-.) 369, 65–70. https://doi.org/10.1126/science.aaz3658.

Dawood, M.A.O., Eweedah, N.M., El-Sharawy, M.E., Awad, S.S., Van Doan, H., Paray, B. A., 2020. Dietary white button mushroom improved the growth, immunity, antioxidative status and resistance against heat stress in Nile tilapia (*Oreochromis niloticus*). Aquaculture 523, 735229. https://doi.org/10.1016/j.aquaculture.2020.735229.

Dawood, M.A.O., Koshio, S., 2018. Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. Rev. Aquac. https://doi.org/10.1111/raq.12163.

de la Cruz-Cervantes, J.A., Benavides-González, F., Sánchez-Martínez, J.G., Vázquez-Sauceda, M., de la, L., Ruiz-Uribe, A.J., 2018. Propolis in Aquaculture: A Review of Its Potential. Rev. Fish. Sci. Aquac. https://doi.org/10.1080/23308249.2018.1424798.

de Mattos, B.O., López-Olmeda, J.F., Guerra-Santos, B., Ruiz, C.E., García-Beltrán, J.M., Ángeles-Esteban, M., Sánchez-Vázquez, F.J., Fortes-Silva, R., 2019. Coping with exposure to hypoxia: modifications in stress parameters in gilthead seabream (*Sparus aurata*) fed spirulina (*Arthrospira platensis*) and brewer's yeast (Saccharomyces cerevisiae). Fish. Physiol. Biochem. 45, 1801–1812. https://doi.org/10.1007/s10695-019-00677-8.

De Morais, M.G., Da Fontoura Prates, D., Moreira, J.B., Duarte, J.H., Costa, J.A.V., 2018. Phycocyanin from microalgae: Properties, extraction and purification, with some recent applications. Ind. Biotechnol. https://doi.org/10.1089/ind.2017.0009.

Do-Huu, H., Huynh, S.M., Nguyen, H.T.N., Pham, K.X., 2023. Effect of dietary β-glucan supplementation on growth performance, body composition, intestinal microbes, and capacity against pathogen of golden trevally (*Gnathanodon speciosus*, family Carangidae). J. World Aquac. Soc. 55, 77–88. https://doi.org/10.1111/jwas.13037.

Donaldson, M.R., Cooke, S.J., Patterson, D.A., Macdonald, J.S., 2008. Cold shock and fish. J. Fish. Biol. 73, 1491–1530. https://doi.org/10.1111/j.1095-8649.2008.02061.x.

Dülger, N., Kumlu, M., Türkmen, S., Ölçülü, A., Tufan Eroldoĝan, O., Asuman Yilmaz, H., Öçal, N., 2012. Thermal tolerance of European Sea Bass (*Dicentrarchus labrax*) juveniles acclimated to three temperature levels. J. Therm. Biol. 37, 79–82. https://doi.org/10.1016/j.jtherbio.2011.11.003.

Elabd, H., Wang, H.-P., Shaheen, A., Matter, A., 2020. Nano spirulina dietary supplementation augments growth, antioxidative and immunological reactions, digestion, and protection of Nile tilapia, *Oreochromis niloticus*, against *Aeromonas veronii* and some physical stressors. Fish. Physiol. Biochem. 46, 2143–2155. https://doi.org/10.1007/s10695-020-00864-y.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2006. Rapid metabolic adaptation in European sea bass (*Dicentrarchus labrax*) juveniles fed different carbohydrate sources after heat shock stress. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 145, 73–81. https://doi.org/10.1016/j.cbpa.2006.05.002.

Eslami, M., Zaretabar, A., Dawood, M.A.O., Mohammadzadeh, S., Ahmadifar, E., Sheikhzadeh, N., Shahriari Moghadam, M., Hoseinifar, S.H., Van Doan, H., 2022. Can dietary ethanolic extract of propolis alter growth performance, digestive enzyme activity, antioxidant, and immune indices in juvenile beluga sturgeon (*Huso huso*)? Aquaculture 552. https://doi.org/10.1016/j.aquaculture.2022.737939.

Esmaeili, M., 2021. Blood performance: a new formula for fish growth and health. Biol. (Basel) 10, 1236. https://doi.org/10.3390/BIOLOGY10121236/S1.

Esmaeili, M., Carter, C.G., Wilson, R., Walker, S.P., Miller, M.R., Bridle, A., Symonds, J. E., 2021. Proteomic investigation of liver and white muscle in efficient and inefficient Chinook salmon (*Oncorhynchus tshawytscha*): Fatty acid metabolism and protein turnover drive feed efficiency. Aquaculture 542, 736855. https://doi.org/10.1016/j.aquaculture.2021.736855.

Evans, D.H., 1993. Osmotic and ionic regulation in fish, CRC Marine science series. CRC-press, Boca Raton, Florida. US.

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A.H., Lannig, G., Sokolova, I.M., Aberhan, M., Pörtner, H.O., 2020. Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758)—explaining ecological findings under climate change. Mar. Biol. 167, 17. https://doi.org/10.1007/s00227-019-3620-3.

Farag, M.R., Abdelnour, S.A., Patra, A.K., Dhama, K., Dawood, M.A.O., Elnesr, S.S., Alagawany, M., 2021. Propolis: Properties and composition, health benefits and applications in fish nutrition. Fish. Shellfish Immunol. https://doi.org/10.1016/j.fsi.2021.06.010.

Fernández-Fernández, M.R., Valpuesta, J.M., 2018. Hsp70 chaperone: a master player in protein homeostasis. F1000Research 7, 1497. https://doi.org/10.12688/F1000RESEARCH.15528.1.

Firsov, A.M., Fomich, M.A., Bekish, A.V., Sharko, O.L., Kotova, E.A., Saal, H.J., Vidovic, D., Shmanai, V.V., Pratt, D.A., Antonenko, Y.N., Shchepinov, M.S., 2019. Threshold protective effect of deuterated polyunsaturated fatty acids on peroxidation of lipid bilayers. FEBS J. 286, 2099–2117. https://doi.org/10.1111/febs.14807.

Fredenrich, A., Bayer, P., 2003. Reverse cholesterol transport, high density lipoproteins and HDL cholesterol: recent data. Diabetes Metab. 29, 201–205. https://doi.org/10.1016/S1262-3636(07)70029-0.

Galappaththi, E.K., Ichien, S.T., Hyman, A.A., Aubrac, C.J., Ford, J.D., 2020. Climate change adaptation in aquaculture. Rev. Aquac. 12, 2160–2176. https://doi.org/10.1111/raq.12427.

Gao, J., Koshio, S., Ishikawa, M., Yokoyama, S., Mamauag, R.E.P., 2014. Interactive effects of vitamin C and E supplementation on growth performance, fatty acid composition and reduction of oxidative stress in juvenile Japanese flounder *Paralichthys olivaceus* fed dietary oxidized fish oil. Aquaculture 422–423 84–90. https://doi.org/10.1016/j.aquaculture.2013.11.031.

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J.G., Gambi, M.C., Kersting, D.K., Ledoux, J.B., Lejeusne, C., Linares, C., Marschal, C., Pérez, T., Ribes, M., Romano, J.C., Serrano, E., Teixido, N., Torrents, O., Zabala, M., Zuberer, F., Cerrano, C., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob. Chang. Biol. 15, 1090–1103. https://doi.org/10.1111/J.1365-2486.2008.01823.X.

Geay, F., Santigosa I Culi, E., Corporeau, C., Boudry, P., Dreano, Y., Corcos, L., Bodin, N., Vandeputte, M., Zambonino-Infante, J.L., Mazurais, D., Cahu, C.L., 2010. Regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 156, 237–243. https://doi.org/10.1016/j.cbpb.2010.03.008.

Geffroy, B., Sandoval-Vargas, L., Boyer-Clavel, M., Pérez-Atehortúa, M., Lallement, S., Isler, I.V., 2023. A simulated marine heatwave impacts European sea bass sperm quantity, but not quality. J. Fish. Biol. 103, 784–789. https://doi.org/10.1111/jfb.15327.

Gomez Isaza, D.F., Cramp, R.L., Smullen, R., Glencross, B.D., Franklin, C.E., 2019. Coping with climatic extremes: Dietary fat content decreased the thermal resilience of barramundi (*Lates calcarifer*). Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 230, 64–70. https://doi.org/10.1016/j.cbpa.2019.01.004.

Gonzalez, R.J., Mcdonald, D.G., 2000. Ionoregulatory responses to temperature change in two species of freshwater fish. Fish. Physiol. Biochem. 22, 311–317. https://doi.org/10.1023/A:1007837214327.

Greene, D.H.S., Selivonchick, D.P., 1987. Lipid metabolism in fish. Prog. Lipid Res. https://doi.org/10.1016/0163-7827(87)90008-7.

Hajirezaee, S., Mohammadi, G., Naserabad, S.S., 2020. The protective effects of vitamin C on common carp (*Cyprinus carpio*) exposed to titanium oxide nanoparticles (TiO2-NPs). Aquaculture 518, 734734. https://doi.org/10.1016/j.aquaculture.2019.734734.

Harabawy, A.S.A., Mosleh, Y.Y.I., 2014. The role of vitamins A, C, E and selenium as antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia, *Oreochromis niloticus*. Ecotoxicol. Environ. Saf. 104, 28–35. https://doi.org/10.1016/j.ecoenv.2014.02.015.

Hassaan, M.S., EL Nagar, A.G., Salim, H.S., Fitzsimmons, K., El-Haroun, E.R., 2019. Nutritional mitigation of winter thermal stress in Nile tilapia by propolis-extract: Associated indicators of nutritional status, physiological responses and transcriptional response of delta-9-desaturase gene. Aquaculture 511, 734256. https://doi.org/10.1016/j.aquaculture.2019.734256.

Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Sabae, S.A., Mahmoud, A.M.A., El-Haroun, E.R., 2021. Comparative study on the effect of dietary β-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in Nile tilapia, *Oreochromis niloticus*. Fish. Shellfish Immunol. 108, 63–72. https://doi.org/10.1016/j.fsi.2020.11.012.

Hassanien, H.A., Alrashada, Y.N., Abbas, A.O., Abdelwahab, A.M., 2023. Dietary propolis complementation relieves the physiological and growth deterioration induced by Flavobacterium columnare infection in juveniles of common carp (*Cyprinus carpio*). PLoS One 18, e0292976. https://doi.org/10.1371/journal.pone.0292976.

Heath, M.R., Neat, F.C., Pinnegar, J.K., Reid, D.G., Sims, D.W., Wright, P.J., 2012. Review of climate change impacts on marine fish and shellfish around the UK and Ireland. Aquat. Conserv. Mar. Freshw. Ecosyst. 22, 337–367. https://doi.org/10.1002/aqc.2244.

Herrera, M., Mancera, J.M., Costas, B., 2019. The use of dietary additives in fish stress mitigation: Comparative endocrine and physiological responses. Front. Endocrinol. (Lausanne). https://doi.org/10.3389/fendo.2019.00447.

Herrera, M., Vargas-Chacoff, L., Hachero, I., Ruíz-Jarabo, I., Rodiles, A., Navas, J.I., Mancera, J.M., 2009. Osmoregulatory changes in wedge sole (*Dicologoglossa cuneata* Moreau, 1881) after acclimation to different environmental salinities. Aquac. Res. 40, 762–771. https://doi.org/10.1111/j.1365-2109.2008.02147.x.

Heshmatfar, F., Safari, R., Shabani, A., Hoseinifar, S.H., Ghaffari, H., Shokohian, B., Ullah, M.R., Siddik, M.A.B., 2023. The effects of combined or singular administration of formic acid and *Pediococcus acidilactici* on stress resistance, growth performance, immune responses and related genes expression in common carp, *Cyprinus carpio*. Aquac. Rep. 29, 101474 https://doi.org/10.1016/j.aqrep.2023.101474.

Holsman, K.K., Haynie, A.C., Hollowed, A.B., Reum, J.C.P., Aydin, K., Hermann, A.J., Cheng, W., Faig, A., Ianelli, J.N., Kearney, K.A., Punt, A.E., 2020. Ecosystem-based fisheries management forestalls climate-driven collapse. Nat. Commun. 11, 1–10. https://doi.org/10.1038/s41467-020-18300-3.

Hossain, M.S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M., Ono, S., Fujieda, T., 2016. Comparison of the effects of inosine and inosine monophosphate on growth, immune response, stress resistance and gut morphology of juvenile red sea bream, *Pagrus major*. Aquaculture 458, 64–74.

Hwang, P.P., Lee, T.H., Lin, L.Y., 2011. Ion regulation in fish gills: Recent progress in the cellular and molecular mechanisms. Am. J. Physiol. - Regul. Integr. Comp. Physiol. https://doi.org/10.1152/ajpregu.00047.2011.

Ibarz, A., Blasco, J., Gallardo, M.A., Fernández-Borràs, J., 2010. Energy reserves and metabolic status affect the acclimation of gilthead sea bream (*Sparus aurata*) to cold. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 155, 319–326. https://doi.org/10.1016/j.cbpa.2009.11.012.

Intergovernmental Panel on Climate Change, 2023. Climate Change 2021 – The Physical Science Basis, Climate Change 2021 – The Physical Science Basis. Cambridge University Press. https://doi.org/10.1017/9781009157896.

IPCC, 2018. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development. [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterf. (eds.)]. Inpress.

Islam, M.J., Kunzmann, A., Bögner, M., Meyer, A., Thiele, R., James Slater, M., 2020a. Metabolic and molecular stress responses of European seabass, *Dicentrarchus labrax* at low and high temperature extremes. Ecol. Indic. 112, 106118 https://doi.org/10.1016/j.ecolind.2020.106118.

Islam, M.J., Kunzmann, A., Henjes, J., Slater, M.J., 2021a. Can dietary manipulation mitigate extreme warm stress in fish? The case of European seabass, *Dicentrarchus labrax*. Aquaculture 545, 737153. https://doi.org/10.1016/j.aquaculture.2021.737153.

Islam, M.J., Kunzmann, A., Slater, M.J., 2022. Responses of aquaculture fish to climate change-induced extreme temperatures: A review. J. World Aquac. Soc. https://doi.org/10.1111/jwas.12853.

Islam, M.J., Kunzmann, A., Slater, M.J., 2021b. Extreme winter cold-induced osmoregulatory, metabolic, and physiological responses in European seabass

(*Dicentrarchus labrax*) acclimatized at different salinities. Sci. Total Environ. 771, 145202 https://doi.org/10.1016/j.scitotenv.2021.145202.
Islam, M.J., Slater, M.J., Kunzmann, A., 2020b. What metabolic, osmotic and molecular stress responses tell us about extreme ambient heatwave impacts in fish at low salinities: The case of European seabass, *Dicentrarchus labrax*. Sci. Total Environ. 749, 141458 https://doi.org/10.1016/j.scitotenv.2020.141458.
Jentoft, S., Aastveit, A.H., Torjesen, P.A., Andersen, Ø., 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 141, 353–358. https://doi.org/10.1016/j.cbpb.2005.06.006.
Jin, S.E., Lee, S.J., Kim, Y., Park, C.Y., 2020. Spirulina powder as a feed supplement to enhance abalone growth. Aquac. Rep. 17, 100318 https://doi.org/10.1016/j.aqrep.2020.100318.
Kaplan, Ç., Erdoğan, F., 2021a. Effect of dietary propolis on growth, body composition, and serum biochemistry of juvenile sea bream (*Sparus aurata*). Aquac. Int. 29, 553–563. https://doi.org/10.1007/S10499-020-00642-W/METRICS.
Kaplan, Ç., Erdoğan, F., 2021b. Effect of dietary propolis on growth, body composition, and serum biochemistry of juvenile sea bream (*Sparus aurata*). Aquac. Int. 1–11. https://doi.org/10.1007/s10499-020-00642-w.
Kelestemur, G.T., Seven, P.T., Yilmaz, S., 2012. Effects of dietary propolis and vitamin E on growth performance and antioxidant status in blood of juvenile Rainbow trout, *Oncorhynchus mykiss* (Teleostei: Salmoniformes) under different flow rates. Zoologia 29, 99–108. https://doi.org/10.1590/S1984-46702012000200001.
Kizhakkekarammal Puthiyedathu, S., Angel, J.R.J., Thirugnanamurthy, S., Suresh, S., Nathamuni, S., Raja, R.A., Kumar, S., Tomy, S., Dayal, J.S., Changaramkumarth Paran, B., Sawant, P.B., Mudagandur Shashi, S., Narinder Kumar, C., Kondusamy, A., 2022. Effect of dietary C-Phycocyanin on growth, survival, haematology, immune response, gut microbiome and disease resistance of Pacific white shrimp, *Penaeus vannamei*. Aquac. Res. 53, 6292–6309. https://doi.org/10.1111/ARE.16102.
Korell, L., Auge, H., Chase, J.M., Harpole, S., Knight, T.M., 2020. We need more realistic climate change experiments for understanding ecosystems of the future. Glob. Chang. Biol. https://doi.org/10.1111/gcb.14797.
Kumar, P., Pal, A.K., Sahu, N.P., Jha, A.K., Kumar, N., Christina, L., Priya, P., 2018. Dietary L-Tryptophan potentiates non-specific immunity in *Labeo rohita* fingerlings reared under elevated temperature. J. Therm. Biol. 74, 55–62. https://doi.org/10.1016/j.jtherbio.2018.03.010.
Kumar, P., Saurabh, S., Pal, A.K., Sahu, N.P., Arasu, A.R.T., 2014. Stress mitigating and growth enhancing effect of dietary tryptophan in rohu (*Labeo rohita*, Hamilton, 1822) fingerlings. Fish. Physiol. Biochem. 40, 1325–1338. https://doi.org/10.1007/s10695-014-9927-6.
Lattos, A., Papadopoulos, D.K., Feidantsis, K., Karagiannis, D., Giantsis, I.A., Michaelidis, B., 2022. Are Marine Heatwaves Responsible for Mortalities of Farmed *Mytilus galloprovincialis*? A Pathophysiological Analysis of Marteilia Infected Mussels from Thermaikos Gulf. Greece Anim. 12, 2805. https://doi.org/10.3390/ANI12202805/S1.
Le, M.H., Dinh, K.V., Nguyen, M.V., Rønnestad, I., 2020. Combined effects of a simulated marine heatwave and an algal toxin on a tropical marine aquaculture fish cobia (*Rachycentron canadum*). Aquac. Res. 51, 2535–2544. https://doi.org/10.1111/are.14596.
Lhotka, O., Kyselý, J., 2022. The 2021 European Heat Wave in the Context of Past Major Heat Waves. Earth Sp. Sci. 9 https://doi.org/10.1029/2022EA002567.
Li, X., Sun, J., Wang, L., Song, K., Lu, K., Zhang, L., Ma, X., Zhang, C., 2023. Effects of dietary vitamin E levels on growth, antioxidant capacity and immune response of spotted seabass (*Lateolabrax maculatus*) reared at different water temperatures. Aquaculture 565, 739141. https://doi.org/10.1016/J.AQUACULTURE.2022.739141.
Liu, S., Wang, D., Cao, Y., Lu, T., Liu, H., Li, S., 2020. Effects of propolis on the immune enhancement of the formalin-inactivated Aeromonas salmonicida vaccine. Aquac. Res. 51, 4759–4770. https://doi.org/10.1111/are.14822.
Liu, Y., Liu, J., Ye, S., Bureau, D.P., Liu, H., Yin, J., Mou, Z., Lin, H., Hao, F., 2019. Global metabolic responses of the lenok *(Brachymystax lenok*) to thermal stress. Comp. Biochem. Physiol. Part D. Genom. Proteom. 29, 308–319. https://doi.org/10.1016/j.cbd.2019.01.006.
Lüders, J., Demand, J., Höhfeld, J., 2000. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J. Biol. Chem. 275, 4613–4617. https://doi.org/10.1074/jbc.275.7.4613.
Madeira, D., Vinagre, C., Diniz, M.S., 2016. Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. Ecol. Indic. 63, 324–331. https://doi.org/10.1016/j.ecolind.2015.12.008.
Mafra, J.F., de Santana, T.S., Cruz, A.I.C., Ferreira, M.A., Miranda, F.M., Araújo, F.M., Ribeiro, P.R., Evangelista-Barreto, N.S., 2022. Influence of red propolis on the physicochemical, microbiological and sensory characteristics of tilapia (*Oreochromis niloticus*) salami. Food Chem. 394 https://doi.org/10.1016/j.foodchem.2022.133502.
Mahmoud, M.A., Kassab, M.S., Zaineldin, A.I., Amer, A.A., Gewaily, M.S., Darwish, S., Dawood, M.A.O., 2023. Mitigation of Heat Stress in Striped Catfish (*Pangasianodon hypophthalmus*) by Dietary Allicin: Exploring the Growth Performance, Stress Biomarkers, Antioxidative, and Immune Responses. Aquac. Res. 2023, 1–16. https://doi.org/10.1155/2023/8292007.
Mahmoudi, N., Safari, R., Shabani, A., Hoseinifar, S.H., Yazici, M., El-Haroun, E., 2022. Can dietary Dictyota dichotoma powder affect performance, serum, and mucus immune parameters, and antioxidant defense in Zebrafish (*Danio rerio*)? Aquac. Rep. 26, 101279 https://doi.org/10.1016/J.AQREP.2022.101279.
Majharul, I.S., Sultana, R., Imran, M., Jannat, M.F.T., Ashaf-Ud-Doulah, M., Rohani, M.F., Brown, C., Shahjahan, M., 2020. Elevated temperature affects growth and hemato-biochemical parameters, inducing morphological abnormalities of erythrocytes in Nile tilapia *Oreochromis niloticus*. Aquac. Res. 51, 4361–4371. https://doi.org/10.1111/are.14780.
Mamun, M.Al, Hossain, M.A., Saha, J., Khan, S., Akter, T., Banu, M.R., 2023. Effects of spirulina Spirulina platensis meal as a feed additive on growth performance and immunological response of Gangetic mystus *Mystus cavasius*. Aquac. Rep. 30, 101553 https://doi.org/10.1016/J.AQREP.2023.101553.
Martins, N., Diógenes, A.F., Magalhães, R., Matas, I., Oliva-Teles, A., Peres, H., 2021. Dietary taurine supplementation affects lipid metabolism and improves the oxidative status of European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture 531, 735820. https://doi.org/10.1016/j.aquaculture.2020.735820.
Maulu, S., Hasimuna, O.J., Haambiya, L.H., Monde, C., Musuka, C.G., Makorwa, T.H., Munganga, B.P., Phiri, K.J., Nsekanabo, J.D.M., 2021. Climate Change Effects on Aquaculture Production: Sustainability Implications, Mitigation, and Adaptations. Front. Sustain. Food Syst. https://doi.org/10.3389/fsufs.2021.609097.
Medagoda, N., Chotikachinda, R., Hasanthi, M., Lee, K.J., 2023. Dietary Supplementation of a Mixture of Nucleotides, β-Glucan and Vitamins C and E Improved the Growth and Health Performance of Olive Flounder, *Paralichthys olivaceus*, 302 8, 302 Fishes 2023 Vol. 8. https://doi.org/10.3390/FISHES8060302.
Mitter, K., Kotoulas, G., Magoulas, A., Mulero, V., Sepulcre, P., Figueras, A., Novoa, B., Sarropoulou, E., 2009. Evaluation of candidate reference genes for QPCR during ontogenesis and of immune-relevant tissues of European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 153, 340–347. https://doi.org/10.1016/j.cbpb.2009.04.009.
Molina, M.O., Gutiérrez, C., Ortega, M., Sánchez, E., 2023. Summer heatwaves, wind production and electricity demand in Southern Europe: Climatic conditions and impacts. Environ. Res. Commun. 5, 085005 https://doi.org/10.1088/2515-7620/acec37.
Moyano, M., Candebat, C., Ruhbaum, Y., Álvarez-Fernández, S., Claireaux, G., Zambonino-Infante, J.L.-L., Peck, M.A., 2017. Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. PLoS One 12, e0179928. https://doi.org/10.1371/journal.pone.0179928.
Musavi, M., Hasanpour, S., Safari, R., Imanpour, M.R., Gutiérrez, J., 2022. Combined effects of Pediococcus acidi lactici and natuzyme on growth performance, hematology and immunity indices in juvenile beluga (*Huso huso*). Aquac. Rep. 24, 101180 https://doi.org/10.1016/J.AQREP.2022.101180.
Neamat-Allah, A.N.F., Mahsoub, Y.H., Mahmoud, E.A., 2021. The potential benefits of dietary β-glucan against growth retardation, immunosuppression, oxidative stress and expression of related genes and susceptibility to *Aeromonas hydrophila* challenge in *Oreochromis niloticus* induced by herbicide pendimethalin. Aquac. Res. 52, 518–528. https://doi.org/10.1111/are.14910.
Nemova, N.N., Lysenko, L.A., Kantserova, N.P., 2016. Degradation of skeletal muscle protein during growth and development of salmonid fish. Russ. J. Dev. Biol. 47, 161–172. https://doi.org/10.1134/S1062360416040068.
Nowosad, J., Jasiński, S., Arciuch-Rutkowska, M., Abdel-Latif, H.M.R., Wróbel, M., Mikiewicz, M., Zielonka, Ł., Kotsyumbas, I.Y., Muzyka, V.P., Brezvyn, O.M., Dietrich, G., Kucharczyk, D., 2023. Effects of Bee Pollen on Growth Performance, Intestinal Microbiota and Histomorphometry in African Catfish. Animals 13. https://doi.org/10.3390/ANI13010132.
O'Brien, K.M., 2011. Mitochondrial biogenesis in cold-bodied fishes. J. Exp. Biol. 214, 275–285. https://doi.org/10.1242/jeb.046854.
Ortuño, J., Esteban, M.A., Meseguer, J., 2003. The effect of dietary intake of vitamins C and E on the stress response of gilthead seabream (*Sparus aurata* L.). Fish. Shellfish Immunol. 14, 145–156. https://doi.org/10.1006/fsim.2002.0428.
Paddison, L., 2023. Heat, drought and fire: Europe's year of extremes in 2022 will not be a 'one-off,' new report finds. CNN. URL ⟨https://edition.cnn.com/2023/06/19/europe/europe-state-of-climate-heat-drought-intl/index.html⟩ (accessed 2.27.24).
Palmer, B.F., 2014. Regulation Of Potassium Homeostasis. Clin. J. Am. Soc. Nephrol. 10, 1050–1060. https://doi.org/10.2215/CJN.08580813.
Panettieri, V., Chatzifotis, S., Messina, C.M., Olivotto, I., Manuguerra, S., Randazzo, B., Ariano, A., Bovera, F., Santulli, A., Severino, L., Piccolo, G., 2020. Honey Bee Pollen in Meagre (*Argyrosomus regius*) Juvenile Diets: Effects on Growth, Diet Digestibility, Intestinal Traits, and Biochemical Markers Related to Health and Stress. Animals 10, 231. https://doi.org/10.3390/ani10020231.
Paul, N., Novais, S.C., Silva, C.S.E., Mendes, S., Kunzmann, A., Lemos, M.F.L., 2021. Global warming overrides physiological anti-predatory mechanisms in intertidal rock pool fish Gobius paganellus. Sci. Total Environ. 776, 145736 https://doi.org/10.1016/j.scitotenv.2021.145736.
Pereira, L.F., Gonçalves, J.F.M., Peixoto, M.J., Domingues, A., Fonseca, S.B., Magalhães, F.A., Santos, G.A., Ozorio, R.O.A., 2017. Cross-effects of dietary probiotic supplementation and rearing temperature on antioxidant responses in European seabass [*Dicentrarchus labrax* (Linnaeus, 1758)] juveniles. J. Appl. Ichthyol. 33, 377–385. https://doi.org/10.1111/jai.13256.
Peres, H., Santos, S., Oliva-Teles, A., 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). Fish. Physiol. Biochem. 40, 1339–1347. https://doi.org/10.1007/s10695-014-9928-5.
Pérez-Palazón, M., Pimentel, R., Polo, M., 2018. Climate Trends Impact on the Snowfall Regime in Mediterranean Mountain Areas: Future Scenario Assessment in Sierra Nevada (Spain). Water 10, 720. https://doi.org/10.3390/w10060720.
Pérez-Sánchez, J., Terova, G., Simó-Mirabet, P., Rimoldi, S., Folkedal, O., Calduch-Giner, J.A., Olsen, R.E., Sitjà-Bobadilla, A., 2017. Skin mucus of gilthead sea bream (*Sparus aurata* l.). protein mapping and regulation in chronically stressed fish. Front. Physiol. 8, 34. https://doi.org/10.3389/fphys.2017.00034.
Person-Le Ruyet, J., Mahe, K., Le Bayon, N., Le Delliou, H., Mahé, K., Le Bayon, N., Le Delliou, H., 2004. Effects of temperature on growth and metabolism in a

Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquaculture 237, 269–280. https://doi.org/10.1016/J.AQUACULTURE.2004.04.021.

Piatt, J.F., Parrish, J.K., Renner, H.M., Schoen, S.K., Jones, T.T., Arimitsu, M.L., Kuletz, K.J., Bodenstein, B., García-Reyes, M., Duerr, R.S., Corcoran, R.M., Kaler, R.S.A., McChesney, G.J., Golightly, R.T., Coletti, H.A., Suryan, R.M., Burgess, H.K., Lindsey, J., Lindquist, K., Warzybok, P.M., Jahncke, J., Roletto, J., Sydeman, W.J., 2020. Extreme mortality and reproductive failure of common murres resulting from the northeast Pacific marine heatwave of 2014-2016. PLoS One. https://doi.org/10.1371/journal.pone.0226087.

Pisano, A., Marullo, S., Artale, V., Falcini, F., Yang, C., Leonelli, F.E., Santoleri, R., Nardelli, B.B., 2020. New evidence of Mediterranean climate change and variability from Sea Surface Temperature observations. Remote Sens 12, 132. https://doi.org/10.3390/RS12010132.

Place, S.P., Zippay, M.L., Hofmann, G.E., 2004. Constitutive roles for inducible genes: Evidence for the alteration in expression of the inducible hsp70 gene in Antarctic notothenioid fishes. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 287, 429–436. https://doi.org/10.1152/ajpregu.00223.2004.

Pokorný, J., Parkányiová, J., 2019. Lipids with Antioxidant Properties. In: Healthful Lipids. AOCS Publishing, pp. 273–300. https://doi.org/10.1201/9780429104497-13.

Porter, D., Peggs, D., McGurk, C., Martin, S.A.M., 2023. In-vivo analysis of Protec™ and β-glucan supplementation on innate immune performance and intestinal health of rainbow trout. Fish. Shellfish Immunol. 134, 108573 https://doi.org/10.1016/j.fsi.2023.108573.

Rahman, M., Mamun, M.A.Al, Rathore, S.S., Nandi, S.K., Abdul Kari, Z., Wei, L.S., Tahiluddin, A.B., Rahman, M.M., Manjappa, N.K., Hossain, A., Nasren, S., Alam, M. M.M., Bottje, W.G., Téllez-Isaías, G., Kabir, M.A., 2023. Effects of dietary supplementation of natural Spirulina on growth performance, hemato-biochemical indices, gut health, and disease resistance to Aeromonas hydrophila of Stinging catfish (*Heteropneustes fossilis*) fingerling. Aquac. Rep. 32, 101727 https://doi.org/10.1016/j.aqrep.2023.101727.

Rao, X., Huang, X., Zhou, Z., Lin, X., 2013. An improvement of the 2(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat. Bioinforma. Biomath. 3, 71–85.

Rathore, S.S., Hanumappa, S.M., Yusufzai, S.I., Suyani, N.K., Abdullah-Al-Mamun, M., Nasren, S., Sidiq, M.J., Hanumanthappa, S.K., Kalyani, R., 2023. Dietary Administration of Engineered Nano-selenium and Vitamin C Ameliorates Immune Response, Nutritional Physiology, Oxidative Stress, and Resistance Against *Aeromonas hydrophila* in Nile Tilapia (*Oreochromis niloticus*). Biol. Trace Elem. Res. 201, 4079–4092. https://doi.org/10.1007/S12011-022-03473-3/FIGURES/3.

Raymond, F., Ullmann, A., Tramblay, Y., Drobinski, P., Camberlin, P., 2019. Evolution of Mediterranean extreme dry spells during the wet season under climate change. Reg. Environ. Chang. 19, 2339–2351. https://doi.org/10.1007/s10113-019-01526-3.

Roche, H., Bogé, G., 2000. In vivo effects of phenolic compounds on blood parameters of a marine fish (*Dicentrarchus labrax*). Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 125, 345–353. https://doi.org/10.1016/s0742-8413(99)00119-x.

Rossi, A., Bacchetta, C., Cazenave, J., 2017. Effect of thermal stress on metabolic and oxidative stress biomarkers of *Hoplosternum littorale* (Teleostei, Callichthyidae). Ecol. Indic. 79, 361–370. https://doi.org/10.1016/j.ecolind.2017.04.042.

Safari, R., Hoseinifar, S.H., Imanpour, M.R., Hajibegloo, A., Sanchouli, H., Homayouni, M., Siddik, M.A.B., 2022a. The effects of multi-enzyme and betaine on growth performance, body composition haemato-immunological parameters and expression of growth-related genes in beluga (*Huso huso*). Aquaculture 549, 737784. https://doi.org/10.1016/J.AQUACULTURE.2021.737784.

Safari, R., Roosta, Z., Vakili, F., Rahmani, E., Sakhawat Hossain, M., Raeisi, M., Doan, H., Van, Paolucci, M., Hoseinifar, S.H., 2022b. Dietary dragonhead effects on growth, immunity and antioxidant and related genes expression in zebrafish (*Danio rerio*). Aquac. Rep. 27, 101384 https://doi.org/10.1016/J.AQREP.2022.101384.

Sánchez-Cueto, P., Stavrakidis-Zachou, O., Clos-Garcia, M., Bosch, M., Papandroulakis, N., Lladó, S., 2023. Mediterranean Sea heatwaves jeopardize greater amberjack's (*Seriola dumerili*) aquaculture productivity through impacts on the fish microbiota. ISME Commun. 3 https://doi.org/10.1038/S43705-023-00243-7.

Sarà, G., Gouhier, T.C., Brigolin, D., Porporato, E.M.D., Mangano, M.C., Mirto, S., Mazzola, A., Pastres, R., 2018. Predicting shifting sustainability trade-offs in marine finfish aquaculture under climate change. Glob. Chang. Biol. 24, 3654–3665. https://doi.org/10.1111/gcb.14296.

Satiro, T.M., Carli, G.C., Amorim, J.P. de A., Koch, J.F.A., Zanuzzo, F.S., Takahashi, L.S., 2024. Dietary β-glucan ameliorates metabolic stress caused by a high dietary carbohydrate level in Nile tilapia. Aquaculture 579, 740186. https://doi.org/10.1016/J.AQUACULTURE.2023.740186.

Sayed, A.E.D.H., Hamed, M., El-Sayed, A.A.A., Nunes, B., Soliman, H.A.M., 2023. The mitigating effect of Spirulina (*Arthrospira platensis*) on the hemotoxicity of gibberellic acid on juvenile tilapia (*Oreochromis niloticus*). Environ. Sci. Pollut. Res. 30, 25701–25711. https://doi.org/10.1007/s11356-022-23844-6.

Schreck, C.B., Tort, L., 2016. The Concept of Stress in Fish. In: Fish Physiology. Academic Press, pp. 1–34. https://doi.org/10.1016/B978-0-12-802728-8.00001-1.

Seager, R., Osborn, T.J., Kushnir, Y., Simpson, I.R., Nakamura, J., Liu, H., 2019. Climate variability and change of mediterranean-type climates. J. Clim. 32, 2887–2915. https://doi.org/10.1175/JCLI-D-18-0472.1.

Šegvić-Bubić, T., Boban, J., Grubišić, L., Trumbić, Ž., Radman, M., Perčić, M., Čož-Rakovac, R., 2013. Effects of propolis enriched diet on growth performance and plasma biochemical parameters of juvenile European sea bass (*Dicentrarchus labrax* L.) under acute low-temperature stress. Aquac. Nutr. 19, 877–885. https://doi.org/10.1111/anu.12032.

Shahjahan, M., Islam, M.J., Hossain, M.T., Mishu, M.A., Hasan, J., Brown, C., 2022. Blood biomarkers as diagnostic tools: An overview of climate-driven stress responses in fish. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2022.156910.

Shahjahan, M., Kitahashi, T., Ando, H., 2017. Temperature affects sexual maturation through the control of kisspeptin, kisspeptin receptor, GnRH and GTH subunit gene expression in the grass puffer during the spawning season. Gen. Comp. Endocrinol. 243, 138–145. https://doi.org/10.1016/J.YGCEN.2016.11.012.

Shi, H.J., Xu, C., Liu, M.Y., Wang, B.K., Liu, W.Bin, Chen, D.H., Zhang, L., Xu, C.Y., Li, X. F., 2018. Resveratrol improves the energy sensing and glycolipid metabolism of blunt snout bream Megalobrama amblycephalafed high-carbohydrate diets by activating the AMPK-SIRT1-PGC-1a network. Front. Physiol. 9, 1258. https://doi.org/10.3389/fphys.2018.01258.

Shiau, S.Y., Hsu, C.Y., 2002. Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus* x O. aureus. Aquaculture 210, 335–342. https://doi.org/10.1016/S0044-8486(01)00853-5.

Singh, P.K., Munilkumar, S., Sundaray, J.K., Santhanam, P., Sharma, A., Haque, R., Satheesh, M., 2023. Effect of selenium, vitamin C and highly unsaturated fatty acids-enriched Brachionus calyciflorus on growth, survival, physio-metabolic and anti-oxidative responses in *Anabas testudineus* (Bloch, 1792) larvae. Aquaculture 568, 739293. https://doi.org/10.1016/j.aquaculture.2023.739293.

Smith, K.E., Burrows, M.T., Hobday, A.J., King, N.G., Moore, P.J., Sen Gupta, A., Thomsen, M.S., Wernberg, T., Smale, D.A., 2023. Biological Impacts of Marine Heatwaves. Ann. Rev. Mar. Sci. 15, 119–145. https://doi.org/10.1146/annurev-marine-032122-121437.

Sorby, K.L., Green, M.P., Dempster, T.D., Jessop, T.S., 2018. Can physiological engineering/programming increase multi-generational thermal tolerance to extreme temperature events? J. Exp. Biol. 221, jeb174672. https://doi.org/10.1242/jeb.174672.

Steibel, J.P., Poletto, R., Coussens, P.M., Rosa, G.J.M., 2009. A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics 94, 146–152. https://doi.org/10.1016/j.ygeno.2009.04.008.

Sun, J.L., Zhao, L.L., Liao, L., Tang, X.H., Cui, C., Liu, Q., He, K., Ma, J.D., Jin, L., Yan, T., Zhou, J., Yang, S., 2020. Interactive effect of thermal and hypoxia on largemouth bass (*Micropterus salmoides*) gill and liver: Aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis. Fish. Shellfish Immunol. 98, 923–936. https://doi.org/10.1016/j.fsi.2019.11.056.

Tang, P.A., Stefansson, S.O., Nilsen, T.O., Gharbi, N., Lai, F., Tronci, V., Balseiro, P., Marnix, M., Ebbesson, L.O.E., 2022. Exposure to cold temperatures differentially modulates neural plasticity and stress responses in post-smolt Atlantic salmon (*Salmo salar*). Aquaculture 560, 738458. https://doi.org/10.1016/J.AQUACULTURE.2022.738458.

Tejpal, C.S., Sumitha, E.B., Pal, A.K., Shivananda Murthy, H., Sahu, N.P., Siddaiah, G.M., 2014. Effect of dietary supplementation of l-tryptophan on thermal tolerance and oxygen consumption rate in *Cirrhinus mrigala* fingerlings under varied stocking density. J. Therm. Biol. 41, 59–64. https://doi.org/10.1016/j.jtherbio.2014.02.008.

Torno, C., Staats, S., Michl, S.C., De Pascual-Teresa, S., Izquierdo, M., Rimbach, G., Schulz, C., 2018. Fatty acid composition and fatty acid associated gene-expression in gilthead sea bream (*Sparus aurata*) are affected by low-fish oil diets, dietary resveratrol, and holding temperature. Mar. Drugs 16, 1–22. https://doi.org/10.3390/md16100379.

Truzzi, C., Illuminati, S., Antonucci, M., Scarponi, G., Annibaldi, A., 2018. Heat shock influences the fatty acid composition of the muscle of the Antarctic fish *Trematomus bernacchii*. Mar. Environ. Res. 139, 122–128. https://doi.org/10.1016/j.marenvres.2018.03.017.

Turchini, G.M., Nie, P., 2021. The climate is still changing. Rev. Aquac. https://doi.org/10.1111/raq.12517.

Turnau, R., Robinson, W.A., Lackmann, G.M., Michaelis, A.C., 2022. Model Projections of Increased Severity of Heat Waves in Eastern Europe. Geophys. Res. Lett. 49, e2022GL100183 https://doi.org/10.1029/2022GL100183.

Vakili, F., Roosta, Z., Safari, R., Raeisi, M., Hossain, M.S., Guerreiro, I., Akbazadeh, A., Hoseinifar, S.H., 2023. Effects of dietary nutmeg (*Myristica fragrans*) seed meals on growth, non-specific immune indices, antioxidant status, gene expression analysis, and cold stress tolerance in zebrafish (*Danio rerio*). Front. Nutr. 9, 1038748. https://doi.org/10.3389/FNUT.2022.1038748/BIBTEX.

Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.L., Mazurais, D., 2014. Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). Mar. Biol. 162, 367–376. https://doi.org/10.1007/s00227-014-2601-9.

Vargas-Chacoff, L., Regish, A.M., Weinstock, A., McCormick, S.D., Oyarzún, R., Muñoz, J. L.P., Pontigo, J.P., Morera, F.J., Vargas-Chacoff, L., Rubio, V.C., Sánchez-Vázquez, F. J., Madrid, J.A., Bodinier, C., Lorin-Nebel, C., Charmantier, G., Boulo, V., Masroor, W., Farcy, E., Gros, R., Lorin-Nebel, C., 2018. Effect of combined stress (salinity and temperature) in European sea bass *Dicentrarchus labrax* osmoregulatory processes. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 215, 550–559. https://doi.org/10.1111/jfb.13683.

Varsamos, S., Flik, G., Pepin, J.F., Bonga, S.E.W., Breuil, G., 2006. Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax*. Fish. Shellfish Immunol. 20, 83–96. https://doi.org/10.1016/j.fsi.2005.04.005.

Vautard, R., Cattiaux, J., Happé, T., Singh, J., Bonnet, R., Cassou, C., Coumou, D., D'Andrea, F., Faranda, D., Fischer, E., Ribes, A., Sippel, S., Yiou, P., 2023. Heat extremes in Western Europe increasing faster than simulated due to atmospheric circulation trends, 2023 141 Nat. Commun. 14, 1–9. https://doi.org/10.1038/s41467-023-42143-3.

Vinagre, C., Madeira, D., Narciso, L., Cabral, H., Diniz, M., 2012a. Impact of climate change on coastal versus estuarine nursery areas: cellular and whole-animal indicators in juvenile seabass *Dicentrarchus labrax*. Mar. Ecol. Prog. Ser. 464, 237–243. https://doi.org/10.3354/meps09885.

Vinagre, C., Madeira, D., Narciso, L., Cabral, H.N., Diniz, M., 2012b. Effect of temperature on oxidative stress in fish: Lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. Ecol. Indic. 23, 274–279. https://doi.org/10.1016/j.ecolind.2012.04.009.

Vinagre, C., Narciso, L., Cabral, H.N., Costa, M.J., Rosa, R., 2012c. Coastal versus estuarine nursery grounds: Effect of differential temperature and heat waves on juvenile seabass, *Dicentrarchus labrax*. Estuar. Coast. Shelf Sci. 109, 133–137. https://doi.org/10.1016/j.ecss.2012.05.029.

Vissers, M.C.M., Das, A.B., 2018. Potential mechanisms of action for vitamin C in cancer: Reviewing the evidence, 809–809 Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.00809.

Wagner, T., Congleton, J.L., 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci. 61, 1066–1074. https://doi.org/10.1139/f04-050.

Weiskopf, S.R., Rubenstein, M.A., Crozier, L.G., Gaichas, S., Griffis, R., Halofsky, J.E., Hyde, K.J.W., Morelli, T.L., Morisette, J.T., Muñoz, R.C., Pershing, A.J., Peterson, D. L., Poudel, R., Staudinger, M.D., Sutton-Grier, A.E., Thompson, L., Vose, J., Weltzin, J.F., Whyte, K.P., 2020. Climate change effects on biodiversity, ecosystems, ecosystem services, and natural resource management in the United States. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137782.

Xiao, K., Wang, X., Wang, M. mang, Guo, H.X., Liu, W.Bin, Jiang, G.Z., 2023. Metabolism, antioxidant and immunity in acute and chronic hypoxic stress and the improving effect of vitamin C in the channel catfish (*Ictalurus punctatus*). Fish. Physiol. Biochem 1–14. https://doi.org/10.1007/s10695-023-01205-5.

Xu, A., Shang-Guan, J., Li, Z., Huang, Z., Shi, S., Ye, Y., 2023. Effects of dietary vitamin E on the growth performance, immunity and digestion of *Epinephelus fuscoguttatus* ♁ × *Epinephelus lanceolatus* ♂ by physiology, pathology and RNA-seq. Aquaculture 575, 739752. https://doi.org/10.1016/j.aquaculture.2023.739752.

Xu, F., Yang, F., Qiu, Y., Wang, C., Zou, Q., Wang, L., Li, X., Jin, M., Liu, K., Zhang, S., Zhang, Y., Li, B., 2024. The alleviative effect of C-phycocyanin peptides against TNBS-induced inflammatory bowel disease in zebrafish via the MAPK/Nrf2 signaling pathways. Fish. Shellfish Immunol. 145, 109351 https://doi.org/10.1016/J.FSI.2023.109351.

Yamashita, M., Yabu, T., Ojima, N., 2010. Stress Protein HSP70 in Fish. Aqua-Biosci. Monogr. 3, 111–141. https://doi.org/10.5047/absm.2010.00304.0111.

Yang, G., Qiu, H., Yu, R., Xiong, L., Yan, Q., Wen, C., Peng, M., 2021. Dietary supplementation of β-glucan, inulin and emodin modulates antioxidant response and suppresses intestinal inflammation of grass carp (*Ctenopharyngodon idellus*). Anim. Feed Sci. Technol. 272, 114789 https://doi.org/10.1016/j.anifeedsci.2020.114789.

Yap, J.K., Sankaran, R., Chew, K.W., Halimatul Munawaroh, H.S., Ho, S.H., Rajesh Banu, J., Show, P.L., 2021. Advancement of green technologies: A comprehensive review on the potential application of microalgae biomass. Chemosphere 281, 130886. https://doi.org/10.1016/J.CHEMOSPHERE.2021.130886.

Youssef, I.M.I., Saleh, E.S.E., Tawfeek, S.S., Abdel-Fadeel, A.A.A., Abdel-Razik, A.R.H., Abdel-Daim, A.S.A., 2023. Effect of Spirulina platensis on growth, hematological, biochemical, and immunological parameters of Nile tilapia (*Oreochromis niloticus*). Trop. Anim. Health Prod. 55, 1–14. https://doi.org/10.1007/s11250-023-03690-5.

Zak, M.A., Manzon, R.G., 2019. Expression and activity of lipid and oxidative metabolism enzymes following elevated temperature exposure and thyroid hormone manipulation in juvenile lake whitefish (*Coregonus clupeaformis*). Gen. Comp. Endocrinol. 275, 51–64. https://doi.org/10.1016/j.ygcen.2019.02.001.

Zhang, F., Man, Y.B., Mo, W.Y., Wong, M.H., 2020. Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. Rev. Aquac. 12, 582–599. https://doi.org/10.1111/raq.12341.

Župan, I., Tkalčić, S., Šarić, T., Čož-Rakovac, R., Strunjak-Perović, I., Topić-Popović, N., Kardum, M., Kanski, D., Ljubić, B.B., Matijatko, V., Poljičak-Milas, N., 2015. Supplementation with imuno-2865® in gilthead sea bream (*Sparus aurata* Linnaeus, 1758): Effects on hematological and antioxidant parameters. Fish. Shellfish Immunol. 47, 590–594. https://doi.org/10.1016/j.fsi.2015.09.049.