

# TNF-A, IL-6, HSP-70, fish growth hormone, and growth performance of sea trout, *Salmo trutta* (Actinopterygii: Salmoniformes: Salmonidae) after long-term dietary administration of $\beta$ -glucan and BGN-2

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## Abstract

The presently reported study intended to examine the effect of the oral administration of an immunomodulator  $\beta$ -glucan and  $\beta$ -glucan-containing product (BGN-2) on the levels of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), fish heat shock protein 70 (HSP-70), and fish growth hormone (GH) as well as growth performance of cultured sea trout juveniles. The sea trout is a migratory sea-run brown trout, *Salmo trutta* Linnaeus, 1758. The fish (total of 15 000) were divided into four experimental and one control group including control (consisting of basal diet) (D1); basal diet + 1 g kg<sup>-1</sup>  $\beta$ -glucan (D2); basal diet + 3 g kg<sup>-1</sup>  $\beta$ -glucan (D3); basal diet + 6 g kg<sup>-1</sup> BGN-2 (D4); basal diet + 14 g kg<sup>-1</sup> BGN-2 (D5). The fish fed D4 and D5 diets showed significantly higher IL-6, HSP-70, and GH expression levels than other treatments ( $P < 0.05$ ). Sea trout fed D4 and D5 diets showed significant improvements in growth performance compared to the fish fed the control diet. In conclusion, our results suggest that dietary supplementation with the product BGN-2 provides great immunostimulation and could be used as an effective measure to improve growth performance in juvenile sea trout.

## Keywords

glucan, immunostimulants, sea trout

## Introduction

The decline of wild salmonid populations is a direct result of extensive human influence and the consequences of climate change on their natural environment (Fraser 2008). The sea trout is a historically significant fish species for Latvia. The sea trout is a migratory sea-run brown trout, *Salmo trutta* Linnaeus, 1758. Latvia has a long tradition of artificial rearing salmonid fishes, dating back more than 130 years. The principal species have been

“Baltic” Atlantic salmon, *Salmo salar* Linnaeus, 1758) and sea trout (brown trout), *Salmo trutta* (Purvina et al. 2019). Currently, Latvia is actively involved in the artificial reproduction and conservation of the natural sea trout population. During the juvenile stage, sea trout exhibit slow growth. According to the *Restocking plan of fish resources, 2017–2020 (Latvia)* (Kučinskis and Dūklavs 2016) state fish hatcheries can release sea trout when the mean weight of the group reaches 15 g, with the smallest fish in the group weighing at least 13 g. It is of utmost

importance for Latvian state fish hatcheries to rear high-weight and healthy sea trout on time.

Therefore, there is a pressing need for well-structured breeding and rearing initiatives that emphasize nutritional approaches. This includes the study of feeding behaviors and the development of initial diets that are crafted from ecologically sustainable components (Fraser 2008; Hoffmann et al. 2021). One of the most persuasive immunity boosters in aquaculture is  $\beta$ -glucan (Ringø et al. 2011; Vetvicka et al. 2013). In order to increase disease resistance (thereby reducing the use of antibiotics), improve growth performance and weight gain, and support fish health immunostimulants have been used as dietary additives in cultured fish (Vallejos-Vidal et al. 2016).

Immunological studies prove that the immune system of fish is comparable to those of mammals, characterized by different types of leukocytes, complement factors, immunoglobulins (Ig), toll-like receptors (TLRs), major histocompatibility complex (MHC), T-cell receptors (TCR), and cytokines (Sakamoto and McCormick 2006; Yada 2007; Firdaus-Nawi and Zamri-Saad 2016).  $\beta$ -glucan stands out as an effective immunostimulant due to its remarkable ability to bind to various receptors on immune cells, primarily leukocytes.  $\beta$ -glucan is detected by complement receptor C3 and TLRs. This binding triggers a cascade of immune responses, including heightened bactericidal activity, increased cytokine production, and enhanced cellular survival capacity. Upon encountering these immune cell surface receptors,  $\beta$ -glucan prompts the release of cytokines (e.g., tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ); interleukins, interferons, lymphokines, and chemokines) (Brown et al. 2003; Hadiuzzaman et al. 2022). This process has been observed to activate immunocompetent cells in fish, such as monocytes, macrophages, and neutrophils, which subsequently engage in pathogen elimination through mechanisms like phagocytosis, oxidative burst, and cytotoxic killing activities. Additionally,  $\beta$ -glucan contributes to the generation of immunological memory and specific antibodies by activating T and B lymphocytes (Hadiuzzaman et al. 2022).

Researchers have demonstrated that  $\beta$ -glucan can modulate essential biochemical markers, such as serum hemoglobin, serum protein, and total hemocyte count, as well as crucial immunological parameters like lysozyme activity, phagocytic activity, oxidative burst activity, and phenoloxidase activity. This leads to a more robust immune profile, making it highly valuable in the treatment of fish and aquatic organisms. Fish supplemented with  $\beta$ -glucan have shown reduced sensitivity in genes associated with acute inflammatory reactions (Falco et al. 2012; Miest et al. 2016; Hadiuzzaman et al. 2022).

TNF- $\alpha$  and interleukin-6 (IL-6) are among the most significant cytokines produced by macrophages and monocytes and are listed as a humoral immune component (Calder 2007). TNF- $\alpha$  is a multifunctional cytokine involved in systemic inflammation and has pro-inflammatory and anti-inflammatory properties (Costa et al. 2011; Zou and Secombes 2016; Eggestøl et al. 2020). It also

plays a major role in the regulation of immune responses, hematopoiesis, multiple homeostatic, and controlling infection (Atzeni and Sarzi-Puttini 2013; Hong et al. 2013).

Heat shock proteins (HSPs) are a family of highly conserved stress proteins that are produced by cells in response to stressful conditions and are indicators of cellular stress and animal health status (Cara et al. 2005). HSP expression is influenced by a variety of biological stressors, such as infectious pathogens, and abiotic factors, such as sudden changes in water temperature and environmental pollutants (Iwama et al. 1998). HSPs are classified by their molecular weight (kilodalton): HSP40, HSP70, etc. (Li and Srivastava 2003; Kumar et al. 2015).

Fish growth hormone (GH) has a role in feeding, growth-promoting, immune function, and osmoregulation in teleost fish (Lal and Singh 2005; Sakamoto and McCormick 2006). Smith (1956) was the first to describe the positive effects of GH treatment on salinity tolerance, and increased capacity of brown trout (*Salmo trutta*). Later it became known that this consequence was due to the ability of GH to raise the size and number of gill chloride cells, Na(+)/K(+)/2Cl(-) cotransporter (NKCC) and Na(+)/K(+)-ATPase (NKA), branchial ion transporters necessitated in salt secretion (Tipsmark et al. 2002; McCormick et al. 2009). For anadromous salmonids, GH plays an essential role in parr-smolt transformation and adaptation for seawater entry (Sakamoto and McCormick 2006). Smoltification includes many metabolic and behavioral changes, as well as the development of hypoosmoregulatory ability (Stefansson et al. 1991).

The presently reported study intended to investigate the immunomodulatory effects of two *Saccharomyces cerevisiae*-derived (yeast)  $\beta$ -glucan products on juvenile sea trout. These products included a commercially available purified  $\beta$ -glucan product (Angel Yeast Co., Ltd., China), as well as a biological product known as BGN-2 (JP Biotechnology, Ltd., Latvia), using a patented technological method. BGN-2 comprises derivatives of the yeast *S. cerevisiae* obtained during the ethanol production process from grain (Revina et al. 2019, 2020).

In the presently reported study, the hypothesis suggests that the prolonged administration of various oral doses of  $\beta$ -glucan products exerts an influence on the expression of TNF- $\alpha$ , IL-6, HSP-70, and GH, potentially enhancing both the health and growth performance of sea trout.

## Materials and methods

**Experimental fish and culture condition.** The research was developed at the state fish farm Pelci of the Institute of Food Safety, Animal Health and Environment BIOR, Latvia (56°55'16.3"N, 021°58'28.6"E) (Pelci) using riverine water.

At the farming trial, 15 000 sea trout juveniles ( $2.3 \pm 0.30$  g, mean weight  $\pm$  SE) were randomly placed into five 1.8 m<sup>3</sup> tanks ( $n = 3000$  in each tank), with a volume of 1.2 m<sup>3</sup>, in a flow-through rearing system. The

quality of water was regularly monitored. The fish were acclimated for 2 weeks.

All of the sea trout used in the trial were planned to be released in natural watercourses in April of the same year, according to the *Restocking plan of fish resources 2017–2020, Latvia* (Kučinskis and Dūklavs 2016). This study was carried out in strict accordance with the recommendations of the national regulations on ethics and research in Latvia (Rozenkopfs and Rudze 2003). All efforts were made to minimize animal suffering according to the guidelines of Latvian law and EU directive No. 2010/63/EU (Anonymous 2010).

**Fish diet and preparation of feed.** A basal diet was formulated according to the nutrition requirement of sea trout (National Research Council 1999; Kamalam et al. 2020). The basal diet was the commercial dry diet for fish Aller Futura EX (Aller Aqua, Ltd., Poland) (appropriated size according to Aller Aqua manufacturer recommendations). The specific composition of the formulation is considered proprietary information held by Aller Aqua. However, based on the information provided on the label, the feed appears to contain a mixture of ingredients, including fish meal, fish oil, functional ingredients, grain products, krill meal, single-cell proteins, vegetable proteins, vitamins, and minerals. To prepare experimental diets,  $\beta$ -glucan powder (Angel Yeast Co, Ltd., China; extracted from *S. cerevisiae*) and BGN-2 powder (JP Biotechnology, Ltd., Latvia) were added to the basal diet. The experimental diets were top-coated with rapeseed (*Brassica napus*) oil, mixed with supplements and air-dried (Revina et al. 2019, 2020).

Five experimental diets were designed as follows: control (consisting of basal diet) (D1), basal diet + 1 g kg<sup>-1</sup>  $\beta$ -glucan (D2), basal diet + 3 g kg<sup>-1</sup>  $\beta$ -glucan (D3), basal diet + 6 g kg<sup>-1</sup> BGN-2 (D4), and basal diet + 14 g kg<sup>-1</sup> BGN-2 (D5). The dosage of Angel yeast product was 1 g kg<sup>-1</sup> and 3 g kg<sup>-1</sup>; the dosage of JP Biotechnology product (BGN-2) was 6 g kg<sup>-1</sup> and 14 g kg<sup>-1</sup>, equivalent to the final concentration of pure  $\beta$ -glucan in the final feed in a ratio of 1 g kg<sup>-1</sup> and 3 g kg<sup>-1</sup> (according to JP Biotechnology data).

The feed was delivered by an automatic fish feeder following a feeding regime of 4 times/hour, with 4 s feeding/time, at approximately 2% of body weight per day.

**Enzyme-linked immunosorbent assay.** Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (CSB-E13254Fh), fish growth hormone (GH) (CSB-E12121Fh), fish heat shock protein-70 (HSP-70) (CSB-E16327Fh), and interleukin-6 (IL-6) (CSB-E13258Fh) levels were determined in samples of the muscle tissue homogenates via enzyme-linked immunosorbent assay (ELISA), using commercially available kits for fish from CUSABIO (Wuhan, China, <http://www.cusabio.com>). Samples were taken five times, from September to January. Each month, three pooled samples ( $n = 5$  fish in one pooled sample) from each experimental group were collected.

Collection, storage, and analysis of samples were carried out according to CUSABIO manufacturer's instructions (see above). Supernatants were stored at  $-80^{\circ}\text{C}$  for further analyses. Concentrations were measured at 450 nm on a Thermo Labsystems Multiskan Ascent 354 Microplate Reader (Thermo Labsystems Inc., USA).

**Evaluation of growth performance.** The weight and length of 50 randomly selected fish from each group were measured individually every 30 days, throughout the treatment period (Hopkins 1992; Aviva and Watson 2013).

Growth performance indices including weight gain ( $W_G$ ) [g], coefficient of variation (CV) [%], size heterogeneity of the weight (SH), specific growth rate (SGR) [%day<sup>-1</sup>], and Fulton's condition factor ( $K$ ) were calculated using the following formulae inspired by Hopkins (1992), Afrooz et al. (2014) and Gora et al. (2019):

$$W_G = W_F - W_I$$

$$\text{CV} = (\text{SD}_{\text{IW}} \times W_{\text{IW}}^{-1}) \times 100$$

$$\text{SH} = \text{CV}_F \times \text{CV}_I^{-1}$$

$$\text{SGR} = (\text{Ln } W_F - \text{Ln } W_I) \times t^{-1} \times 100$$

$$K = (W \times 10^5) \times L^{-3}$$

where  $W_F$  is the mean final weight of fish [g],  $W_I$  is the mean initial weight [g],  $\text{SD}_{\text{IW}}$  is the standard deviation of individual weight,  $W_{\text{IW}}$  is the mean individual weight [g],  $\text{CV}_F$  is the final coefficient of variation,  $\text{CV}_I$  is the initial coefficient of variation,  $t$  is the time of rearing [day],  $W$  is the weight, and  $L$  is the mean total length.

**Statistical analysis.** The results were analyzed statistically by the R (version 3.6.2) environment with RStudio software (Wickham et al. 2023). The results were presented as mean  $\pm$  SEM. The normality and homogeneity of the data were checked with the tests of Shapiro–Wilk's and Bartlett, respectively when the data passed the tests, they were compared by one-way analysis of variance (ANOVA), followed by Duncan's test ( $P < 0.05$ ). Otherwise, was done data logarithmic transformation (Aviva and Watson 2013; Wickham et al. 2023).

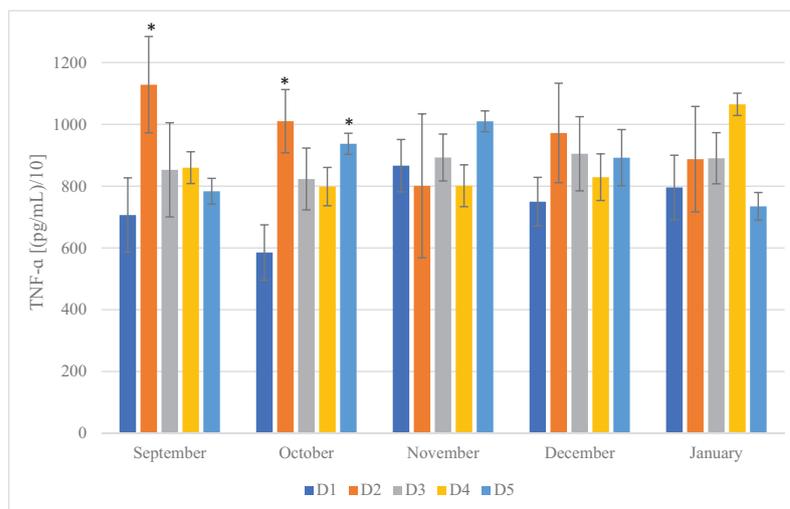
## Results

Mean TNF- $\alpha$ , IL-6, GH, and HSP-70 concentrations are presented in Table 1. All the examined parameters were lower in the control group (D1). However, the mean TNF- $\alpha$  did not exhibit statistically significant differences ( $P < 0.05$ ) among the diet groups throughout the study period. In turn, the TNF- $\alpha$  concentration in the D2 group was significantly higher in September and October in comparison to the control group (Fig. 1). In September, it reached its maximum level within the D2 group.

**Table 1.** Expression of TNF- $\alpha$ , IL-6, GH, and HSP-70 in juveniles of sea trout, *Salmo trutta*, subjected to long-term dietary administration of  $\beta$ -glucan and BGN-2 in experimental groups (treatments).

Group	Parameter			
	TNF- $\alpha$ [(pg/mL)/10]	IL-6 [pg/mL]	GH [pg/mL]	HSP-70 [pg/mL]
D1	740.67 $\pm$ 44.37 <sup>a</sup>	172.83 $\pm$ 0.91 <sup>b</sup>	3371.42 $\pm$ 16.88 <sup>c</sup>	239.01 $\pm$ 1.17 <sup>c</sup>
D2	959.83 $\pm$ 70.68 <sup>a</sup>	176.36 $\pm$ 1.16 <sup>ab</sup>	3436.01 $\pm$ 36.50 <sup>bc</sup>	267.84 $\pm$ 5.82 <sup>ab</sup>
D3	872.75 $\pm$ 42.28 <sup>a</sup>	178.28 $\pm$ 0.89 <sup>ab</sup>	3479.20 $\pm$ 14.72 <sup>abc</sup>	263.96 $\pm$ 4.63 <sup>abc</sup>
D4	870.69 $\pm$ 35.00 <sup>a</sup>	179.07 $\pm$ 0.77 <sup>a</sup>	3674.71 $\pm$ 60.07 <sup>ab</sup>	291.62 $\pm$ 6.06 <sup>a</sup>
D5	871.43 $\pm$ 33.59 <sup>a</sup>	180.81 $\pm$ 0.71 <sup>a</sup>	3722.67 $\pm$ 62.40 <sup>a</sup>	257.82 $\pm$ 2.53 <sup>bc</sup>

Values are mean  $\pm$  SEM. Values in a row with different superscripts are significantly different ( $P < 0.05$ ). Abbreviations: TNF- $\alpha$  = tumor necrosis factor-alpha, IL-6 = interleukin-6, GH = fish growth hormone, HSP-70, fish heat shock protein, BGN-2 =  $\beta$ -glucan containing product; Diets: D1 = control (consisting of basal diet), D2 = basal diet + 1 g kg<sup>-1</sup>  $\beta$ -glucan, D3 = basal diet + 3 g kg<sup>-1</sup>  $\beta$ -glucan, D4 = basal diet + 6 g kg<sup>-1</sup> BGN-2, D5 = basal diet + 14 g kg<sup>-1</sup> BGN-2.



\*, statistically significant values ( $P < 0.05$ ) compared to control

**Figure 1.** Concentrations of TNF- $\alpha$  (tumor necrosis factor-alpha) in juveniles of sea trout, *Salmo trutta*, subjected to dietary administration of  $\beta$ -glucan and BGN-2 ( $\beta$ -glucan containing product) in consecutive months of the experiment.

Our findings indicate that the mean IL-6 concentration was significantly higher in the D4 and D5 groups compared to D1, with no significant difference observed when compared to D2 and D3. The lowest mean IL-6 levels were observed in D1 (Table 1). Notably, IL-6 reached its maximum level in D5 (Fig. 2). Additionally, the IL-6 levels in D5 significantly increased in November.

A significantly higher GH concentration was observed in D5 (Fig. 3), followed by D4, D3, D2, and D1 groups, indicating a positive correlation with the diet group. Also, in the D4 group, GH is higher, compared to the control group (D1).

TNF- $\alpha$ , IL-6, and GH levels did not vary significantly in the D2 and D3 groups during the whole trial of this study compared to the control.

In September, there was no significant difference in HSP-70 between the trial groups and the control. From October to the end of the study, the concentration of HSP-70 in the D4 group was the highest, it was significantly higher than the control group. Also, a high concentration was observed in the D2 group (Fig. 4).

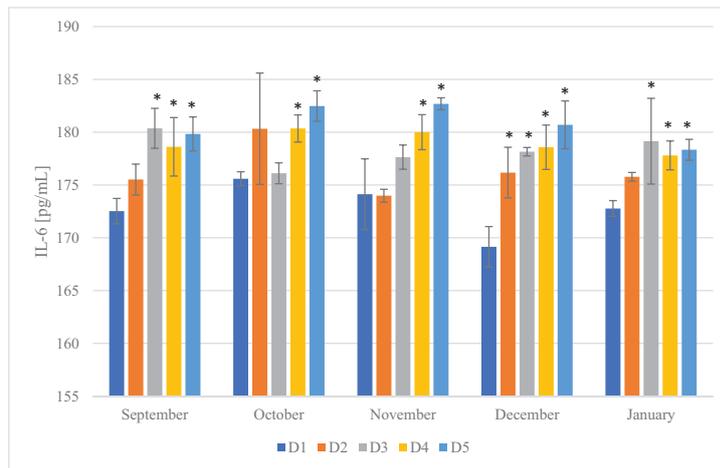
Growth performance, mean final weight, weight gain, coefficient of variation, size heterogeneity, specific growth rate, and Fulton's condition factor are presented in Table 2.

Initially, there were no statistically significant differences in the initial mean weight among the diet groups. However, at the end of the study, it was observed that the final mean weight in groups D4 and D5 was significantly higher compared to the control group (D1). Consequently, the weight gain of fish from groups D4 and D5 was higher.

The same was observed for the specific growth rates (SGR) where fish from D4 and D5 displayed an increased SGR compared to other diet groups. The final mean length in D4 and D5 was significantly higher compared to D1 and D2.

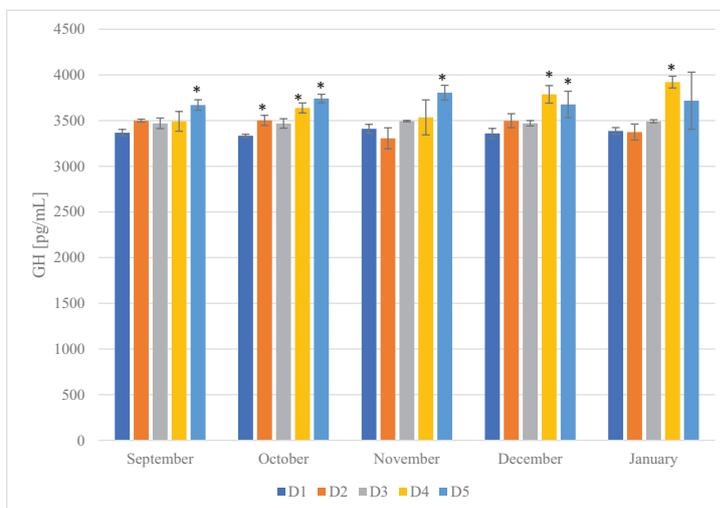
Fulton's condition factor ( $K$ ) was significantly affected by dietary treatment. The lowest  $K$  was recorded in D4 and D5, while the highest value was observed in D1 and D2. The results obtained at the end of the study showed that fish fed the D2 and D3 did not show a statistically significant difference ( $P < 0.05$ ) from fish fed the control diet. The results showed that the growth performance of sea trout in the control group was at the lowest level and showed a significant difference ( $P < 0.05$ ) with the D4 and D5 diet groups.

Results of other aspects of these experiments have been reported in previous papers (Revina et al. 2019, 2020).



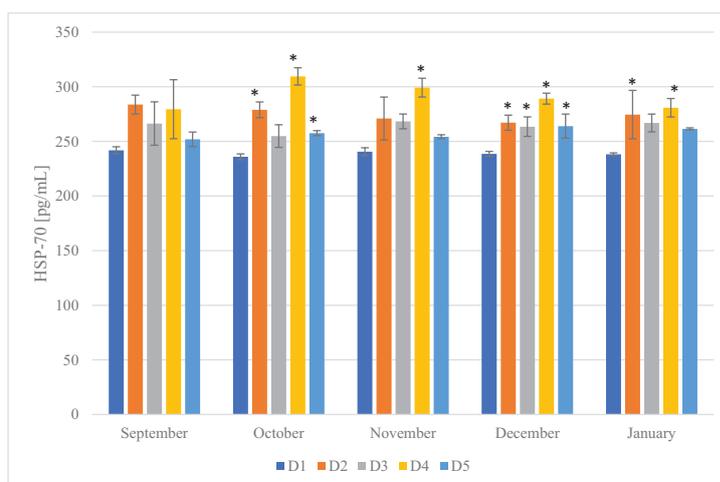
\*, statistically significant values ( $P < 0.05$ ) compared to control

**Figure 2.** Concentrations of IL-6 (interleukin-6) in juveniles of sea trout, *Salmo trutta*, subjected to dietary administration of  $\beta$ -glucan and BGN-2 ( $\beta$ -glucan containing product) in consecutive months of the experiment.



\*, statistically significant values ( $P < 0.05$ ) compared to control

**Figure 3.** Concentrations of GH (fish growth hormone) in juveniles of sea trout, *Salmo trutta*, subjected to dietary administration of  $\beta$ -glucan and BGN-2 ( $\beta$ -glucan containing product) in consecutive months of the experiment.



\*, statistically significant values ( $P < 0.05$ ) compared to control

**Figure 4.** Concentrations of HSP-70 (fish heat shock protein) in juveniles of sea trout, *Salmo trutta*, subjected to dietary administration of  $\beta$ -glucan and BGN-2 ( $\beta$ -glucan containing product) in consecutive months of the experiment.

**Table 2.** Growth performance of juveniles of sea trout, *Salmo trutta*, subjected to long-term dietary administration of  $\beta$ -glucan and BGN-2 in experimental groups (treatments) by the end of trial.

Group	Parameter							
	$W_I$	$W_F$	$L_F$	$W_G$	CV	SH	SGR	$K$
D1	2.50 $\pm$ 0.21 <sup>a</sup>	11.84 $\pm$ 0.78 <sup>c</sup>	9.33 $\pm$ 0.24 <sup>a</sup>	9.34	28.76	0.90	0.73	1.60 $\pm$ 0.11 <sup>a</sup>
D2	2.59 $\pm$ 0.17 <sup>a</sup>	13.31 $\pm$ 0.84 <sup>bc</sup>	9.50 $\pm$ 0.24 <sup>a</sup>	10.72	27.50	1.10	0.77	1.58 $\pm$ 0.10 <sup>a</sup>
D3	2.67 $\pm$ 0.21 <sup>a</sup>	16.18 $\pm$ 0.77 <sup>abc</sup>	11.12 $\pm$ 0.32 <sup>ab</sup>	13.51	20.79	0.69	0.85	1.18 $\pm$ 0.12 <sup>ab</sup>
D4	2.67 $\pm$ 0.17 <sup>a</sup>	16.76 $\pm$ 0.64 <sup>ab</sup>	11.91 $\pm$ 0.28 <sup>b</sup>	14.09	16.55	0.66	0.86	0.99 $\pm$ 0.04 <sup>b</sup>
D5	2.76 $\pm$ 0.16 <sup>a</sup>	19.88 $\pm$ 0.74 <sup>a</sup>	12.81 $\pm$ 0.26 <sup>b</sup>	17.12	16.15	0.73	0.93	0.94 $\pm$ 0.04 <sup>b</sup>

Values (of  $W_I$ ,  $W_F$ ,  $L_F$ , and  $K$ ) are mean  $\pm$  SE; mean values with different superscript letters in a row are significantly different ( $P < 0.05$ ), according to Duncan's multiple range tests. Abbreviations:  $W_I$  = initial mean weight [g],  $W_F$  = final mean weight [g],  $L_F$  = final mean length [cm],  $W_G$  = weight gain [g], CV = coefficient of variation [%], SH = size heterogeneity (weight), SGR = specific growth rate [% day<sup>-1</sup>],  $K$  = condition factor, BGN-2 =  $\beta$ -glucan containing product; Diets: D1 = control (consisting of basal diet), D2 = basal diet + 1 g kg<sup>-1</sup>  $\beta$ -glucan, D3 = basal diet + 3 g kg<sup>-1</sup>  $\beta$ -glucan, D4 = basal diet + 6 g kg<sup>-1</sup> BGN-2, D5 = basal diet + 14 g kg<sup>-1</sup> BGN-2.

## Discussion

The use of natural immunostimulants offers important advantages for aquaculture. These substances enhance the immune response and strengthen disease resistance, contributing to improved health and overall well-being in aquatic organisms. Furthermore, these products are environmentally friendly, biodegradable, and safe for humans (Vetvicka et al. 2013; Rodrigues et al. 2020). Also,  $\beta$ -glucan is widely acknowledged as a beneficial growth promoter for fish (Ji et al. 2017).

In teleost fishes, such as Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), the administration of  $\beta$ -glucans enhances the regenerative capacity of immunosuppressed cells, thereby bolstering their ability to combat infectious diseases (Petit and Wiegertjes 2016). Research investigations have revealed that the immunomodulating effects of  $\beta$ -glucan can be characterized as follows: (1) Prebiotic effects of  $\beta$ -glucan involve an indirect form of immunomodulation, encompassing the fermentation of  $\beta$ -glucan by native bacteria, resulting in alterations in microbial composition and a shift in the production of short-chain fatty acid (SCFA) metabolites within the gastrointestinal tract (GIT) of fish. (2) In addition,  $\beta$ -glucan contributes to the direct immunomodulation of the host through receptor-mediated recognition mechanisms occurring within the GIT of fish (Petit et al. 2022). This, in turn, leads to improved digestion and increased nutrient absorption efficiency from their diets (Dawood et al. 2020).

While the exact mechanism by which  $\beta$ -glucan influences the fish's immune system remains uncertain, there is substantial evidence indicating its ability to boost phagocytic activity and increase the expression of cytokines in different types of immune cells, including macrophages, dendritic cells, and neutrophils (Di Domenico et al. 2017).

The results of our studies are consistent with other authors (Kim et al. 2009, Vetvicka et al. 2013), who have demonstrated that the impact of  $\beta$ -glucan on cytokine expression is rapid and does not necessitate long-term administration. The effect becomes evident within the initial months of application. The level of IL-6 remained significantly higher in the D4 and D5 groups compared to the

control group throughout the study. This can be attributed to  $\beta$ -glucan's engagement with  $\beta$ -glucan receptors, leading to the enhancement of various immune functions, including the release of cytokines such as IL-6 (Rodrigues et al. 2020).

The inclusion of  $\beta$ -glucan at a dose of 1 g kg<sup>-1</sup> (D2) in the diet leads to a significant rise in TNF- $\alpha$  levels during the months of September and October. In subsequent months, the expression of TNF- $\alpha$  in all groups remains without significant statistical difference. Similar results were obtained by other researchers (Sealey et al. 2008; Rodriguez et al. 2009), who proved that  $\beta$ -glucan had no impact on the expression of TNF- $\alpha$ . TNF- $\alpha$  is mainly produced by activated macrophages, natural killer (NK) cells, and lymphocytes (Atzeni and Sarzi-Puttini 2013), it is expressed in most examined teleost tissue (Eggestøl et al. 2020). Our hypothesis suggests that  $\beta$ -glucan supplements improve the functional capabilities of macrophages, leading to a slight increase in TNF- $\alpha$  levels, but not reaching statistical significance. It is possible, that if fish were infected with pathogens, the release of TNF- $\alpha$  might be higher. In addition, some studies have confirmed a significant increase in TNF- $\alpha$  during prolonged stress (Dawood et al. 2020). Since our fish were maintained under optimal conditions, this factor is excluded. Regrettably, there is currently a dearth of information regarding these potential effects of  $\beta$ -glucan in fish.

GH plays a crucial role in regulating various physiological functions of fish such as development, growth, osmoregulation, immune systems, reproduction function, etc. (Lal and Singh 2005; Abdollahnejad et al. 2015). In the presently reported study, it was found that the inclusion of the BGN-2 product in the diet had a significantly positive impact on GH expression in groups D4 and D5. This positive GH expression can help explain the increase in growth parameters of sea trout observed in the presently reported and previous studies, especially during the winter months (Revina et al. 2020). However, in contrast, our study did not show any significant beneficial effect of pure  $\beta$ -glucan on GH expression in groups D2 and D3. Our results are consistent with other authors, who suggested that pure  $\beta$ -glucan does not have a significant impact on GH.

More authors demonstrated that dietary  $\beta$ -glucan has played a major role in regulating stress- and immune-re-

lated factors through expressing HSP-70 (Ji et al. 2017; Salah et al. 2017). HSP-70 is typically expressed in the cells of fish that have experienced environmental stressors (Dawood et al. 2020). In the initial month of the study, no significant effect was observed when adding  $\beta$ -glucan additives to the sea trout diet (Fig. 4). However, a notable effect was observed with the BGN-2 product at a dose of  $6 \text{ g kg}^{-1}$  (D4). Additionally, a substantial impact was seen in D2.

This study highlights the beneficial outcomes resulting from the oral administration of the BGN-2 product at doses of  $6 \text{ g kg}^{-1}$  and  $14 \text{ g kg}^{-1}$  on the growth performance of sea trout. These findings align with previous research (Hoang et al. 2018), which also demonstrated the growth-enhancing effects of  $\beta$ -glucan in various aquaculture species. Fish from D1, D2, D3, and D4 groups exhibited excellent condition. According to several studies, Fulton's condition factor for salmonids is considered excellent when it falls within the range of 0.95 to 1.44 (Mahmoudi et al. 2014; Wali et al. 2019).

This study adds to the growing body of evidence supporting the efficacy of  $\beta$ -glucan, specifically the BGN-2 product, in promoting the growth of sea trout. The results suggest that the administration of  $\beta$ -glucan products at the specified doses can positively influence the growth

parameters of sea trout, contributing to the development of more efficient and sustainable aquaculture practices.

In summary, all of our research (Revina et al. 2019, 2020) strongly recommends incorporating the BGN-2 product at a dosage of  $6 \text{ g kg}^{-1}$  into the sea trout diet. This approach enhances the immunomodulatory response, and overall health, and represents an effective strategy for increasing sea trout production. Our study affirms that the utilization of BGN-2 products has a positive impact on the welfare, innate immune system, and growth performance of sea trout.

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## References

- Abdolahnejad Z, Pourkazemi M, Khoshkholgh MR, Yarmohammadi M (2015) Expression of growth hormone gene during early development of Siberian sturgeon (*Acipenser baerii*). *Molecular Biology Research Communications* 4: 181–188. <https://doi.org/10.22099/mbr.2015.3166>
- Afroz E, Gholamreza E, Seyed ARH (2014) Length–weight relationship and Fultons condition factor of *Carasobarbus luteus* (Heckel, 1843) in Hoor Al-azim wetland. *Journal of Ecology and the Natural Environment* 6(7): 238–243. <https://doi.org/10.5897/JENE2014.0449>
- Anonymous (2010) Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The European Parliament and the Council of the European Union. *Official Journal of the European Union* L 276/33.
- Atzeni F, Sarzi-Puttini P (2013) Tumor Necrosis Factor. Pp. 229–231. In: Brenner's Encyclopedia of Genetics. 2nd edn. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-374984-0.01594-1>
- Aviva P, Watson P (2013) *Statistics for veterinary and animal science*. 3rd edn. British Medical Journal Publishing Group, Wiley-Blackwell, 408 pp.
- Brown GD, Herre J, Williams DL, Willment JA, Marshall ASJ, Gordon S (2003) Dectin-1 mediates the biological effects of  $\beta$ -glucans. *Journal of Experimental Medicine* 197(9): 1119–1124. <https://doi.org/10.1084/jem.20021890>
- Calder PC (2007) Immunological parameters: What do they mean? *Journal of Nutrition* 137(3): 773S–780S. <https://doi.org/10.1093/jn/137.3.773S>
- Cara JB, Aluru N, Moyano FJ, Vijayan MM (2005) Food-deprivation induces HSP70 and HSP90 protein expression in larval gilthead sea bream and rainbow trout. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 142(4): 426–431. <https://doi.org/10.1016/j.cbpb.2005.09.005>
- Costa MM, Maehr T, Diaz-Rosales P, Secombes CJ, Wang T (2011) Bioactivity studies of rainbow trout (*Oncorhynchus mykiss*) interleukin-6: Effects on macrophage growth and antimicrobial peptide gene expression. *Molecular Immunology* 48(15–16): 1903–1916. <https://doi.org/10.1016/j.molimm.2011.05.027>
- Dawood MAO, Metwally AES, El-Sharawy ME, Atta AM, Elbially ZI, Abdel-Latif HMR, Paray BA (2020) The role of  $\beta$ -glucan in the growth, intestinal morphometry, and immune-related gene and heat shock protein expressions of Nile tilapia (*Oreochromis niloticus*) under different stocking densities. *Aquaculture* 523: e735205. <https://doi.org/10.1016/j.aquaculture.2020.735205>
- Domenico DJ, Canova R, Soveral FL, Nied CO, Costa MM, Frandoloso R, Kreutz LC (2017) Immunomodulatory effects of dietary  $\beta$ -glucan in silver catfish (*Rhamdia quelen*). *Pesquisa Veterinaria Brasileira* 37(1): 73–78. <https://doi.org/10.1590/s0100-736x2017000100012>
- Eggsetøl HØ, Lunde HS, Haugland GT (2020) The proinflammatory cytokines TNF- $\alpha$  and IL-6 in lumpfish (*Cyclopterus lumpus* L.)—Identification, molecular characterization, phylogeny and gene expression analyses. *Developmental and Comparative Immunology* 105: 103608. <https://doi.org/10.1016/j.dci.2020.103608>
- Rozenkopfs O, Rudze L (2003) National regulations on ethics and research in Latvia. European Commission, Directorate-General for Research and Society. Office for Official Publications of the European Communities, Luxembourg.
- Falco A, Frost P, Miest J, Pionnier N, Irnazarow I, Hoole D (2012) Reduced inflammatory response to *Aeromonas salmonicida*

- infection in common carp (*Cyprinus carpio* L.) fed with  $\beta$ -glucan supplements. *Fish and Shellfish Immunology* 32(6): 1051–1057. <https://doi.org/10.1016/j.fsi.2012.02.028>
- Firdaus-Nawi M, Zamri-Saad M (2016) Major components of fish immunity: A review. *Pertanika Journal of Tropical Agricultural Science* 39: 393–420.
- Fraser DJ (2008) How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1(4): 535–586. <https://doi.org/10.1111/j.1752-4571.2008.00036.x>
- Gora AH, Ambasankar K, Sandeep KP, Rehman S, Agarwal D, Ahmad I, Ramachandran K (2019) Effect of dietary supplementation of crude microalgal extracts on growth performance, survival and disease resistance of *Lates calcarifer* (Bloch, 1790) larvae. *Indian Journal of Fisheries* 66(1): 64–72. <https://doi.org/10.21077/ijf.2019.66.1.79076-09>
- Hadiuzzaman M, Moniruzzaman M, Shahjahan M, Bai SC, Min T, Hossein Z (2022)  $\beta$ -glucan: Mode of action and its uses in fish immunomodulation. *Frontiers in Marine Science* 9: e905986. <https://doi.org/10.3389/fmars.2022.905986>
- Hoang DH, Lam HS, Nguyen CV (2018) Efficiency of dietary  $\beta$ -glucan supplementation on growth, body composition, feed, and nutrient utilization in juveniles of pompano fish (*Trachinotus ovatus*, Linnaeus, 1758). *Israeli Journal of Aquaculture—Bamidgeh* 70. <https://doi.org/10.46989/001c.20924>
- Hoffmann L, Rawski M, Pruszyńska-Oszmałek E, Kołodziejcki P, Mazurkiewicz J (2021) Environmentally sustainable feeding system for sea trout (*Salmo trutta* m. *trutta*): Live food and insect meal-based diets in larval rearing. *Aquaculture Reports* 21: 100795. <https://doi.org/10.1016/j.aqrep.2021.100795>
- Hong S, Li R, Xu Q, Secombes CJ, Wang T (2013) Two types of TNF- $\alpha$  exist in teleost fish: Phylogeny, expression, and bioactivity analysis of Type-II TNF- $\alpha$ 3 in rainbow trout *Oncorhynchus mykiss*. *Journal of Immunology* 191(12): 5959–5972. <https://doi.org/10.4049/jimmunol.1301584>
- Hopkins K (1992) Reporting fish growth: A review of the basics. *Journal of the World Aquaculture Society* 23(3): 173–179. <https://doi.org/10.1111/j.1749-7345.1992.tb00766.x>
- Iwama GK, Thomas PT, Forsyth RB, Vijayan MM (1998) Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8(1): 35–56. <https://doi.org/10.1023/A:1008812500650>
- Ji L, Sun G, Li J, Wang Y, Du Y, Li X, Liu Y (2017) Effect of dietary  $\beta$ -glucan on growth, survival and regulation of immune processes in rainbow trout (*Oncorhynchus mykiss*) infected by *Aeromonas salmonicida*. *Fish and Shellfish Immunology* 64: 56–67. <https://doi.org/10.1016/j.fsi.2017.03.015>
- Kamalam BS, Rajesh M, Kaushik S (2020) Nutrition and feeding of rainbow trout (*Oncorhynchus mykiss*). Pp. 299–332. In: Ninawe AS, Dhanze JR, Dhanze R, Indulkar ST (Eds.) *Fish nutrition and its relevance to human health*. CRC Press, London, UK. <https://doi.org/10.1201/9781003107583>
- Kim YS, Ke F, Zhang QY (2009) Effect of  $\beta$ -glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. *Fish and Shellfish Immunology* 27: 336–340. <https://doi.org/10.1016/j.fsi.2009.06.006>
- Kučinskis M, Dūklavs J (2016) Par Zivju resursu mākslīgās atražošanas plānu 2017.–2020. gadam. Ministru kabineta rīkojums Nr. 684. [Restocking plan of fish resources 2017–2020. Order of the Cabinet of Ministers No. 684.] *Latvijas Vēstnesis* Nr. 227. <https://www.vestnesis.lv/op/2016/227.12>
- Kumar A, Ashraf S, Goud TS, Grewal A, Singh SV, Yadav BR, Upadhyay RC (2015) Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. *Journal of Thermal Biology* 51: 55–64. <https://doi.org/10.1016/j.jtherbio.2015.03.006>
- Lal B, Singh AK (2005) Immunological and physiological validation of enzyme-linked immunosorbent assay for growth hormone of the Asian catfish, *Clarias batrachus*. *Fish Physiology and Biochemistry* 31(2–3): 289–293. <https://doi.org/10.1007/s10695-006-0038-x>
- Li Z, Srivastava P (2003) Heat shock proteins. *Current Protocols in Immunology* 58: Appendix 1, Appendix 1T. <https://doi.org/10.1002/0471142735.ima01ts58>
- Mahmoudi R, Soltani M, Abbas M, Sohrab RG, Abolghasem K (2014) Morphometric relationship between length–weight, length–length and condition factor in farmed rainbow trout (*Oncorhynchus mykiss*). *Bulletin of Environment. Pharmacology and Life Sciences* 3(4): 215–220.
- McCormick SD, Regish AM, Christensen AK (2009) Distinct freshwater and seawater isoforms of Na<sup>+</sup>/K<sup>+</sup> +ATPase in gill chloride cells of Atlantic salmon. *Journal of Experimental Biology* 212(24): 3994–4001. <https://doi.org/10.1242/jeb.037275>
- Miest JJ, Arndt C, Adamek M, Steinhagen D, Reusch TBH (2016) Dietary  $\beta$ -glucan (MacroGard®) enhances survival of first feeding turbot (*Scophthalmus maximus*) larvae by altering immunity, metabolism and microbiota. *Fish and Shellfish Immunology* 48: 94–104. <https://doi.org/10.1016/j.fsi.2015.11.013>
- National Research Council (1999) *Nutrient requirements of fish*. National Academies Press, Washington, DC, USA, 128 pp. <https://doi.org/10.17226/2115>
- Petit J, Wiegertjes GF (2016) Long-lived effects of administering  $\beta$ -glucans: Indications for trained immunity in fish. *Developmental and Comparative Immunology* 64: 93–102. <https://doi.org/10.1016/j.dci.2016.03.003>
- Petit J, de Bruijn I, Goldman MRG, van den Brink E, Pellikaan WF, Forlenza M, Wiegertjes GF (2022)  $\beta$ -glucan-induced immunomodulation: A role for the intestinal microbiota and short-chain fatty acids in common carp. *Frontiers in Immunology* 12: 761820. <https://doi.org/10.3389/fimmu.2021.761820>
- Purvina S, Medne R, Bajinskis J, Kondratyeva N (2019) Success of long term salmonid restocking in Latvia. *Environmental and Experimental Biology* 17: 31–32.
- Revina O, Revins V, Cirule D, Valdovska A (2019) The effect of dietary  $\beta$ -glucans supplements on the haematological parameters of the sea trout. *Research For Rural Development* 247–251. <https://doi.org/10.22616/rrd.25.2019.037>
- Revina O, Avsejenko J, Revins V, Sargautis D, Cīrle D, Valdovska A (2020) Effect of dietary supplementation with  $\beta$ -glucan on growth performance and skin-mucus microbiota of sea trout (*Salmo trutta*). *Fisheries and Aquatic Life* 28(3): 155–165. <https://doi.org/10.2478/aopf-2020-0019>
- Ringø E, Olsen RE, Vecino JLG, Wadsworth S (2011) Use of immunostimulants and nucleotides in aquaculture: A review. *Journal of Marine Science: Research and Development* 02(1): 1–22. <https://doi.org/10.4172/2155-9910.1000104>
- Rodrigues MV, Zanuzzo FS, Koch JFA, Oliveira CAF, Sima P, Vetvicaka V (2020) Development of fish immunity and the role of  $\beta$ -glu-

- can in immune responses. *Molecules* 25(22): e5378. <https://doi.org/10.3390/molecules25225378>
- Rodríguez I, Chamorro R, Novoa B, Figueras A (2009)  $\beta$ -glucan administration enhances disease resistance and some innate immune responses in zebrafish (*Danio rerio*). *Fish and Shellfish Immunology* 27(2): 369–373. <https://doi.org/10.1016/j.fsi.2009.02.007>
- Rozenkopfs O, Rudze L (2003) National regulations on ethics and research in Latvia. European Commission, Directorate-General for Research and Society. Office for Official Publications of the European Communities, Luxembourg.
- Sakamoto T, McCormick SD (2006) Prolactin and growth hormone in fish osmoregulation. *General and Comparative Endocrinology* 147(1): 24–30. <https://doi.org/10.1016/j.ygcen.2005.10.008>
- Salah AS, Nahas AFE, Mahmoud S (2017) Modulatory effect of different doses of  $\beta$ -1,3/1,6-glucan on the expression of antioxidant, inflammatory, stress and immune-related genes of *Oreochromis niloticus* challenged with *Streptococcus iniae*. *Fish and Shellfish Immunology* 70: 204–213. <https://doi.org/10.1016/j.fsi.2017.09.008>
- Sealey WM, Barrows FT, Hang A, Johansen KA, Overturf K, LaPatra SE, Hardy RW (2008) Evaluation of the ability of barley genotypes containing different amounts of  $\beta$ -glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. *Animal Feed Science and Technology* 141(1–2): 115–128. <https://doi.org/10.1016/j.anifeedsci.2007.05.022>
- Smith WDC (1956) The role of the endocrine organs in the salinity tolerance of trout. *Memoirs of the Society for Endocrinology* 5: 83–101.
- Stefansson SO, Björnsson BT, Hansen T, Haux C, Taranger GL, Saunders RL (1991) Growth, parr–smolt transformation, and changes in growth hormone of Atlantic salmon (*Salmo salar*) reared under different photoperiods. *Canadian Journal of Fisheries and Aquatic Sciences* 48(11): 2100–2108. <https://doi.org/10.1139/f91-249>
- Tipsmark CK, Madsen SS, Seidelin M, Christensen AS, Cutler CP, Cramb G (2002) Dynamics of Na<sup>+</sup>,K<sup>+</sup>,2Cl<sup>-</sup> cotransporter and Na<sup>+</sup>,K<sup>+</sup>-ATPase expression in the branchial epithelium of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). *Journal of Experimental Zoology* 293(2): 106–118. <https://doi.org/10.1002/jez.10118>
- Vallejos-Vidal E, Reyes-López F, Teles M, MacKenzie S (2016) The response of fish to immunostimulant diets. *Fish and Shellfish Immunology* 56: 34–69. <https://doi.org/10.1016/j.fsi.2016.06.028>
- Vetvicka V, Vannucci L, Sima P (2013) The effects of  $\beta$ -glucan on fish immunity. *North American Journal of Medical Sciences* 5(10): e580. <https://doi.org/10.4103/1947-2714.120792>
- Wali A, Tasaduq SH, Balkhi MH, Bhat BA, Bhat FA, Qadri S, Mohamad I (2019) Morphometry and length–weight relationship of rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (*Salmoniformes: Salmonidae*) from Kashmir. *Journal of Entomology and Zoology Studies* 7: 1653–1656.
- Wickham H, Çetinkaya-Rundel M, Golemund G (2023) R for data science: Import, tidy, transform, visualize, and model data. 2nd edn. O'Reilly Media, Sebastopol, CA, USA, 576 pp.
- Yada T (2007) Growth hormone and fish immune system. *General and Comparative Endocrinology* 152(2–3): 353–358. <https://doi.org/10.1016/j.ygcen.2007.01.045>
- Zou J, Secombes C (2016) The function of fish cytokines. *Biology* 5(2): e23. <https://doi.org/10.3390/biology5020023>