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RESEARCH

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The effect of two rearing systems and water exchange rates on growth, welfare and robustness of juvenile lumpfish

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Abstract

The aim of the study was to investigate the effect of different tank designs and water exchange rates on growth, stress, skin and gill health in juvenile lumpfish in order to validate if different rearing regimes could increase welfare and robustness of farmed juvenile lumpfish. Experimental trials were conducted to investigate how lumpfish growth, condition factor, skin and gill mucus activity, cortisol and serotonin activity was affected by rearing in two systems (tank (T), raceway (R)) and two water exchange rates (high (T-H): 1350 L h^{-1}; (R-H): 780 L h^{-1} and low (T-L): 450 L h^{-1}; (R-L): 260 L h^{-1}) during a 66 days laboratory trial. Furthermore, potential carryover effects on performance at sea were investigated by studying growth, condition and welfare of the same fish in small scale sea pens A combination of tanks and low water exchange rates (T-L) produced significantly smaller lumpfish, with significantly lower specific growth rate, and lower condition factor. Fish reared in raceways had significantly higher baseline plasma cortisol levels and elevated values of brainstem serotonergic activity compared to fish reared in tanks. There was an indication of lower defence activity in skin mucus cells in raceways at high water exchange, suggesting that raceways at high water exchange may have more favourable environmental conditions. The rearing conditions in the different tank designs at the hatchery stage did not seem to affect fish welfare during the subsequent stage in sea cages. Based on the significantly smaller size, slower growth rate and a condition factor below the threshold which represents acceptable fish welfare in lumpfish, rearing of juvenile lumpfish in tanks at the low water exchange rate (150% exchange of tank water per hour) is considered sub-optimal compared to exchange rates of 450% in tanks and 200% or 600% in raceways.

Keywords Lumpfish, Growth, Shallow raceways, Tanks, Mucus, Cortisol

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Introduction

Recently, biological delousing using cleaner fish has developed as an alternative approach to treating sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar.* Cleaner fish are less stressful to the salmon and can even improve salmon growth and quality [29, 40, 41, 44]. The common lumpfish, *Cyclopterus lumpus*, has been identified as a suitable cleaner fish species for Atlantic salmon aquaculture [26–28, 45]. They remain active feeders in cold water and can be ready for deployment to sea cages within 4 months post hatch, compared to ~ 18 months for wrasse species [9, 26, 28]. Lumpfish have become an increasingly popular lice control alternative and are now commonplace in Atlantic salmon farms in Norway, Scotland, Iceland, Ireland, and the Faroes [3, 4, 13, 24]. The lumpfish aquaculture industry is relatively new and faces its own challenges. In particular, there are major ethical concerns surrounding lumpfish welfare in the Atlantic salmon industry [7, 8, 48]. There are reports of high cleaner fish mortalities in salmon aquaculture, mostly due to handling, emaciation from poor feeding and disease outbreaks [7, 41, 48].

Cleaner fish welfare has become a pressing concern due to reports of high mortalities and disease outbreaks in salmon farms [7, 48]. It has been reported that lumpfish can lose body condition within the first six weeks of transfer to sea cages, suggesting that conditions throughout production and deployment are not adapted enough to ensure the transfer of robust lumpfish to sea cages [31]. However, there is limited information in the literature on how commercial rearing conditions of juvenile lumpfish [33, 44] affect their robustness and performance in hatcheries, as well as potential carryover effects at salmon rearing units at sea.

Preliminary trials at our research facility have found that rearing lumpfish in raceways systems may produce more robust individuals with higher condition factor when transferred to Atlantic salmon sea cages and body condition has recently been suggested as an operational welfare indicator for lumpfish [7, 17]. However, there is no information in the literature yet to corroborate these observations, although previous studies [20, 30] have recommended to study the optimal water current speed or water exchange rates for lumpfish juvenile production.

Verified welfare indicators are essential for documenting the effects of different farming conditions on fish welfare and robustness. Mucosal epithelium is the first immune barrier facing environmental challenges such as pollutants, bacteria, and parasites, and the living cells in mucosal barriers continuously react to these challenges [10]. Skin health parameters can therefore be good indicators of functional disorders [53], reduced fish welfare [50], and robustness [34].

An emerging body of literature suggests that maintaining the stress response in teleost fish [49] over prolonged periods (i.e., chronic stress) may lead to allostatic overload [32]. This condition occurs when normal stress-coping mechanisms become maladaptive or even detrimental [37, 38]. Serotonin (5-hydroxytryptamine; 5-HT), a neurotransmitter in the brain, plays a pivotal role in stress coping and social behaviour among vertebrates [16, 42, 46, 55], and as such it serves as a central mediator of allostatic processes [5]. In line with this view, studies in salmonid fishes have shown that chronic stress induces changes in central 5-HTergic neurochemistry and the cortisol responsiveness to stress [22, 39, 54]. Accordingly, neuroendocrine responses to a standardized stressor have been suggested as indicators of allostatic overload and compromised welfare in fish [22, 39].

With the aim of increasing robustness of farmed juvenile lumpfish, we compared how different rearing systems (circular, raceway) and water exchange rates (low, high) in the hatchery affects growth, stress, skin and gill health in juvenile lumpfish. Furthermore, potential carryover effects on performance at sea were investigated by studying growth, condition and welfare of the same fish in small scale sea pens.

Materials and methods

The first part of the study was carried out between 28 February and 4 May 2023 as a laboratory study at the research facility of Akvaplan-niva (Forsknings- og Innovasjonsenter Kraknes, FISK), Tromsø, Norway, and the second part (follow-up study) from May to August 2023 at Gifas research station at Gildeskål, Nordland.

Experimental fish and maintenance

Ten families of lumpfish were reared in summer 2022 using 10 different males and 10 different females. Nine males and nine females originated from previous generations of lumpfish families produced at FISK. Two wild lumpfish, an additional male and female, were caught near Hekkingen, Kvaløya, in May 2022 and used for breeding. Eggs were stripped from the females and fertilised in May 2022. Fertilised eggs were incubated until hatching began at 280–310 degree-days (7–8 July). The fish were start fed in 90 l circular incubators for 98 days (40 cm width × 90 cm depth), after that moved to raceways (13–14 October) at average weight of 0.75 g. All rearing units were on a flow-through system and supplied with full-salinity, filtered seawater. Temperature and dissolved oxygen levels were monitored daily, and tanks were under 24-h light photoperiod. Juveniles were fed using 24-h automatic feeders. Between 23 and 25 January 2023, all fish were vaccinated with AMARINE micro 3–1 (Pharmaq AS, Oslo, Norway). A sub-population

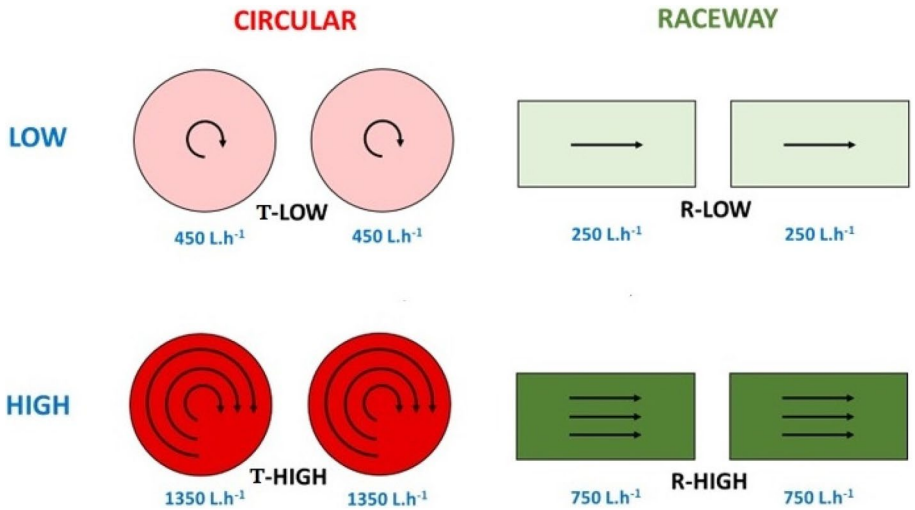

Fig. 1 Experimental set-up of the laboratory trial. Juvenile lumpfish reared in circular tanks (T) or raceways (R) and at two water exchange rates (high (TH): 1350 L h^{-1} (RH): 780 L h^{-1}; and low (TL): 450 L h^{-1}; (RL): 260 L h^{-1}) for 66 days

Table 1 Measured water current (cm s^{-1}) in raceways (R) with high (H) and low (L) water exchange

	Front	Middle	Back	Average
R-H (780 L h^{-1}, 600%)	1.5	1.3	1.2	1.3
R-L (260 L h^{-1}, 200%)	1.2	1.7	1.1	1.3

of lumpfish ($N=40$ in each experimental group) were tagged intraperitoneally using passive integrated transponder (PIT) tags (2×12 mm) between 13 and 16 February 2023.

Experimental design

The first part of the present study consisted of a laboratory trial and was carried out for 66 days from February 28 to May 4, 2023, at FISK, Tromsø, Norway. At the start of the laboratory trial on February 28, 2023, the fish were randomly allocated to 8 experimental units, of which four units were raceways (R) and four units were deep square tanks with rounded corners (T). The following four experimental groups were established: R-H (raceways with high water exchange rate), R-L (raceways with low water exchange rates), T-H (tanks with high water exchange rate), T-L (tanks with low water exchange rate) (Fig. 1). There were two replicate units per group and 70 fish per unit. Water consumption and water velocities for the different units are given in Tables 1 and (raceways) and 2 (tanks).

The dimensions for the raceways (LSR) were: length 220 cm, width 40 cm, and depth 15 cm. This provided a water volume of 72 L and a bottom area of 0.48 m^2. The measurement points for water velocity were at a depth of 9 cm, 20 cm in front of the inlet, the middle of the raceway, and 20 cm in front of the outlet. The dimensions for the raceway inlet were: 33 cm long 32 mm blinded pipe with a row of 3 mm holes facing the inlet wall, perforated plate with 1.5 mm perforation fixed 10 cm from the inlet wall forming an inlet chamber. The dimensions for the tanks were: 60×60 cm, with a depth of 60 cm. This gave a water volume of 220 L and a bottom area of 0.36 m^2. Including the tank walls, which fish were observed sitting on, each tank provided a surface area of 1.8 m^2. The tanks were square with rounded corners. The measurement points for water velocity were located at the inlet, the middle of the tank, and behind the inlet at the bottom (61 cm depth) and top (31 cm depth). The dimensions for the inlet were: 55 cm long blinded 32 mm pipe with a row of 5 mm holes,

Table 2 Measured water current (cm s^{-1}) tanks (T) with high (H) and low (L) water exchange

	Behind inlet, bottom	Behind inlet, top	Centre, bottom	Centre, top	In front of inlet, bottom	In front of inlet, top	Average
T-H (1350 L h^{-1}, 450%)	8.3	5.0	6.3	5.0	8.7	6.8	6.7
T-L (450 L h^{-1}, 150%)	1.2	0.9	2.0	0.9	3.8	1.5	1.7

fixed vertically 10 cm from the corner wall. The water jet from the inlet was angled at 45 degrees towards the tank wall. The diameter of the outlet sieve was 25 cm.

One week prior to the start of the experiment, all lumpfish were individually weighed, and standard length measured, before being randomly distributed between the 8 experimental units. Weight was recorded to the nearest 0.2 g and standard length was measured to the nearest 1 mm. Standard length was measured from the mouth of the fish to the end of the caudal peduncle. Seven lumpfish from each family were used in each experimental unit ($N=70$ in each unit) to minimise genetic influence on the results. The total biomass of tagged fish was calculated for each tank. Untagged lumpfish were weighed in bulk and added to each tank to maintain a consistent stocking density of 25 kg m^{-3} throughout the trials.

The experimental trials at FISK began on 28 February 2023 and ran for 66 days. Weight and total length measurements for all tagged lumpfish were recorded biweekly. An additional round of weight and total length measurements were measured, prior to the tagged lumpfish being transferred to GIFAS in Nordland, Norway for 70 days follow up trial in small-scale sea pens.

During the trial period the lumpfish were fed a 50% mixture of 1.5 and 3 mm of Amber Neptune (52.5% protein, 15.5% lipid, 8.5% ash, Skretting AS, Stavanger, Norway) at 2% body weight using 24-h automatic feeders (FIAP clock feeder pro 3 kg/12 h; Sterner Fish Tec AS, Vestby, Norway). All raceways and tanks were flow-through and provided with full salinity seawater at ambient temperature throughout the trials. The water current speed of each tank was set to a predetermined value using a handheld flow meter (Hontzsch Flow Measuring Technology, Waiblingen, Germany) and the water intake valve was adjusted to maintain the desired water current speed. Water flow was monitored routinely throughout the trials and adjustments were made when required. Temperature and oxygen were measured daily in all raceways and tanks. Average oxygen saturation in raceways was 97.7% (max/min = 112%/72%). Four measurements showed oxygen saturation below 80%. The average oxygen saturation in tanks was 100.7% (max/min = 120%/79%). One measurement showed oxygen saturation below 80%. Average temperature in all rearing units during the trial period was 4.9 °C (max/min = 5.7 °C/4.5 °C).

After transfer to Gifas research station all lumpfish were reared in 1000 L tanks until transfer to salmon farming cages on 6 June (average weight 114 g) and kept in cages (5 × 5x5 m) together with salmon (weight of 480.3 ± 18.9 g, mean ± SD) until 14 August 2023. At termination of the trial operational welfare indicators, according to an index defined in [7, 17] were scored for all fish.

Growth and condition factor

All tagged fish were individually weighed every two weeks during the six-week laboratory trial. Specific growth rate (SGR, % d^{-1}) of individual lumpfish was calculated using the following formula from [19]:

$$SGR = (eg - 1) \times 100$$

where $g = (\ln (W_2) - \ln (W_1)/(t_2 - t_1)$ and W_2 and W_1 are weights on days t_2 and t_1 respectively.

Condition factor, K, was calculated using the following formula and growth coefficients (3.516 and 2.559) from [17]:

$$K = (103.516 \times W)/(10 \times L)2.559$$

where W is the weight of the fish in grams and L is the corresponding total length in cm. Fulton's condition factor is not suitable for lumpfish [6] and so K was interpreted following [6, 17].

Mucosal barrier analyses

Mucosal barrier analyses of skin and gills was done from; 5 fish randomly selected from the base population at the onset of the trial on 23 February; from 5 fish of each experimental group ($N_{total}=20$) on 21 April; and from 5 fish of each previously laboratory experimental group ($N_{total}=20$, all fish were individually tagged so they could be traced to the original rearing group).

For each euthanized fish, a dorsal skin biopsy of 1.5 × 2.0 cm was taken from the right side between two rows of ossicles and second gill arch from the right gill was excised. The samples were fixed in 4% phosphate-buffered formalin prior to processing and analysis for mucosal barrier health by the standardized Veribarr™ method [11, 12, 43] (Quantidoc, Bergen, Norway). The method provides a measure of mucous cell status, which is a good indicator of functional disorders, reduced fish welfare [50], and robustness in lumpfish [34]. The same fish sampled for mucosal barrier were also examined for a set of operational welfare indicators, according to an index defined in [7, 17].

Cortisol and serotonin activity

To assess baseline stress levels, five fish from each of the experimental groups (R-H, R-L, T-H, and T-L) were sampled directly from two tanks per experimental group (two fish from one tank and three fish from another tank). The fish were euthanized in a lethal bath of 500 mg/L MS 222 (Tricaine-S, Pharmaq Analytic, Oslo, Norway). Following this, operational welfare indicators were scored during weighing and length measurements. Blood was then sampled from the caudal vein with heparinized syringes,

and the brains were excised. The telencephalon and brain stem (excluding the cerebellum) were immediately dissected out and frozen on liquid nitrogen.

After baseline sampling, the fish were exposed to a stress test. This involved netting eight fish ($n=4$ per tank) from the T-L and T-H groups, and eight fish ($n=4$ per tank) from the R-L and R-H groups. They were then individually confined for 30 min within a 0.8 L container with a height and diameter of 15 cm. The water in the containers was aerated during the stress test. Immediately after confinement, the length and weight of the fish were measured, and blood and brains were sampled as before.

The blood samples were centrifuged for 5 min at 4 °C, 8000 g, and the plasma was frozen and stored at − 20 °C for later analysis of cortisol levels. Brain samples were wrapped in aluminium foil and snap frozen in liquid nitrogen for future examination.

Analysis of brain serotonin neurochemistry and plasma cortisol

Brain stem samples were weighed and homogenized in 400 µL of perchloric acid (PCA) solution (0.4 M PCA and 0.1 mM EDTA) with a Sonopuls ultrasonic homogenizer (Bandelin, Germany). Homogenates were then centrifuged for 10 min at 4 °C at 14000 × g. Supernatants of centrifuged samples were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-EC). The HPLC mobile phase consisted of 73.9 mM NaH2PO4, 0.1 mM Na2EDTA, and 0.58 mM sodium 1-octanesulfonate in deionized water with 15.3% (v/ v) methanol, pH 3.0. A Supelcosil LC-18-DB column (15 cm × 4.6 mm, 5 µM) (Supelco, Bellefonte, PA, USA) was used for the chromatographic separation and electrochemical detection of the separated compounds was performed using an ESA Coulochem II detector. The detection system included a double analytical cell (5011A) with oxidation potentials set at + 200 mV (first electrode) and -350 mV (second electrode). A conditioning cell (5020) at + 300 mV was used before the analytical cell. The levels of serotonin (5-HT), its main oxidative metabolite 5-hydroxyindoleacetic acid (5-HIAA) were quantified. Quantification was carried out by comparing peak areas with those of the corresponding standards. The ratios between 5-HIAA and 5-HT were then calculated as indirect measures of the activity of the serotonergic neurons, respectively.

Cortisol in plasma was analyzed using a commercially available DetectX® cortisol enzyme immunoassay kit (Arbor Assays, Ann Arbor, MI, USA) following the manufacturers protocol. The absorbance of the prepared ELISA plate was read in a plate reader at 450 nm and the concentrations were calculated using the four-parameter logistics curve.

Statistical analysis

All statistical analyses were performed using STATISTICA™ 14.0 and R-software [47]. Shapiro–Wilk and Levene's tests were used to assess normality of distributions and homogeneity of variances [56]. Three-way nested analysis of variance (ANOVA) was used to test for differences in mean weight, SGR, K and skin and gill mucus parameters of lumpfish between rearing systems, and water exchange rates. Replicates were nested within rearing system and water exchange rates. Effects on the neuroendocrine stress response were investigated by three-way ANOVAs, with stress (baseline or stress tested), rearing unit form (T or R) and water exchange rate (H or L) as grouping variables. The serotonergic activity (5-HIAA/5-HT ratio) in telencephalon and brainstem, and plasma cortisol levels were continuous variables. Data on plasma cortisol levels were log transformed to obtain normal distribution. Significant ANOVA was followed by the Student Newman-Keuls post-hoc test (SNK) to identify differences between groups. A significance level of $P<0.05$ was chosen for all statistical tests.

Ethical statement

The laboratory rearing experiment was undertaken as part of an MSc research dissertation program through the University of the Highlands and Islands (UHI) and was approved by the Animal Welfare and Ethics Committee of UHI (Application ID: ETH2223-0263). The present experiment was conducted under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority (FOTS ID 20189). The experiment has been conducted in accordance with the laws and regulations controlling experiments on live animals in Norway, i.e. the Animal Protection Act of 20 December 1974, No. 73, chapter VI Sects. 20–22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of 15 January 1996.

Results

Growth – laboratory trial

The fish in the T-H group were significantly smaller at the start of the laboratory trial (SNK post hoc test, $P<0.05$, Fig. 2). The lumpfish in the T-L group had a mean weight ± SE of 62.5 ± 16.7 g at the termination of the laboratory trial and was significantly smaller compared to all the other experimental groups (SNK post hoc test, $P<0.05$, Fig. 2).

The lowest SGR was found in lumpfish in both low water exchange groups (T-L and R-L) at each time point of the trial period (Table 3). The T-L group exhibited the significantly lowest SGR of all groups from 14 March onwards (SNK post hoc test, $P<0.05$, Table 3). Apart from the period between 14 and 28 March no statistically

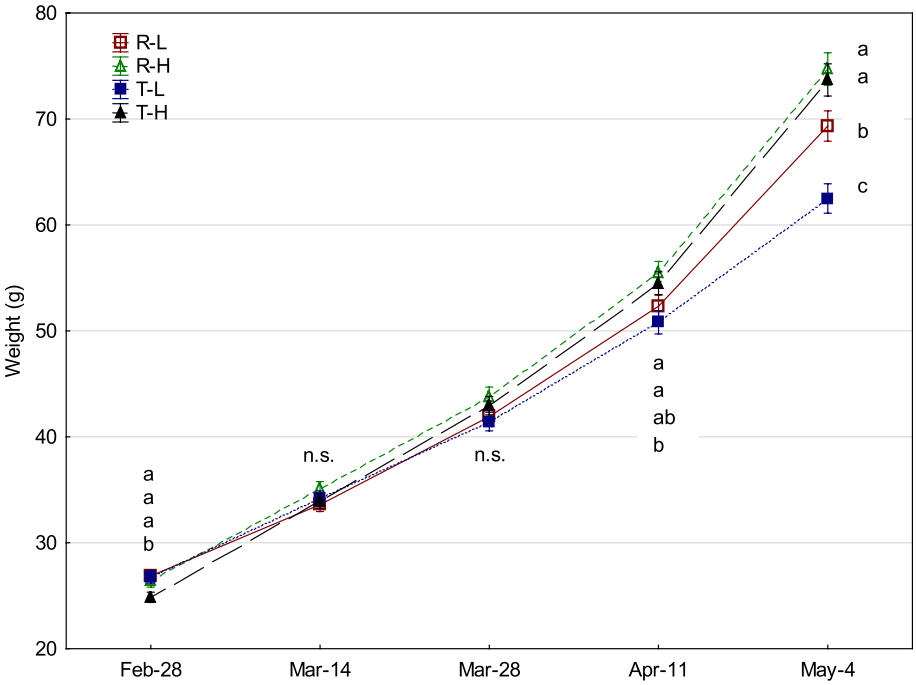

Fig. 2 Mean weight (g) of lumpfish throughout the laboratory phase of the project. Values represent mean average, and vertical whiskers indicate standard error of mean (SEM). Letters indicate significant differences (Student Newman-Keuls post hoc test, $P < 0.05$) between the experimental groups

Table 3 Mean (SE) SGR (% d^{-1}) of lumpfish reared under four different treatments throughout the laboratory phase of the project. Letters indicate significant differences (Student Newman-Keuls post hoc test, $P < 0.05$) between the experimental groups at the same rearing period (horizontal)

	Tank-Low	Tank-High	Raceway-Low	Raceway-High
28 Feb. – 14 Mar	1.72 (0.05)^{b}	2.17 (0.04)^{a}	1.74 (0.03)^{b}	2.19 (0.03)^{a}
15 Mar. – 28 Mar	1.38 (0.05)^{c}	1.74 (0.03)^{a}	1.61 (0.03)^{b}	1.62 (0.03)^{b}
29 Mar. – 11 Apr	1.46 (0.04)^{c}	1.81 (0.02)^{a}	1.59 (0.03)^{b}	1.74 (0.03)^{a}
12 Apr. – 11 May	0.98 (0.03)^{c}	1.38 (0.02)^{a}	1.24 (0.02)^{b}	1.42 (0.03)^{a}

significant differences in SGR was found between the two high water exchange groups. Overall SGR during the 66 days tank trial was 1.58%$^{-d}$ and 1.65%$^{-d}$ for R-H and T-R respectively.

There were no statistically significant differences in condition factor (K) at the start of the experiment (three-way nested ANOVA, $P > 0.25$, Fig. 3). The general picture, consistent throughout the experiment, was that a higher exchange rate, especially in raceway (R-H) resulted in a higher K. On the other hand, T-L group exhibited the lowest condition factor during most of the experiment. On 14 March, the condition factor was significantly higher (SNK post hoc test, $P < 0.05$) in the groups with high water exchange raceways (R-H, 1.80) and tanks (T-H, 1.79). On 11 April, the condition factor was significantly highest in the R-H group (1.93). At the end of the trial on 4 May, the R-H group had the highest (SNK post hoc test, $P < 0.05$) condition factor (1.98) and the T-L group the lowest (1.84). There was no statistically significant difference between R-L (1.90) and T-H (1.91) groups.

Final biomass, water use and stocking density in laboratory trial

Calculations of final biomass, specific water consumption (water use, L/kg fish/min) and stocking density for the experimental groups are given Table 4. Both for raceways (R) and tanks (T) the specific water consumption at high water exchange (H) was threefold the rate at low (L) water consumption (Table 4). Final stocking density in raceways was approximately threefold higher than the density in tanks, both in terms of volume specific density (kg/m^3) and area specific density (kg/m^2, Table 4). The area of tank walls is relevant to include since lumpfish prefers resting at tank walls (and other smooth vertical surfaces). There was little difference in final stocking

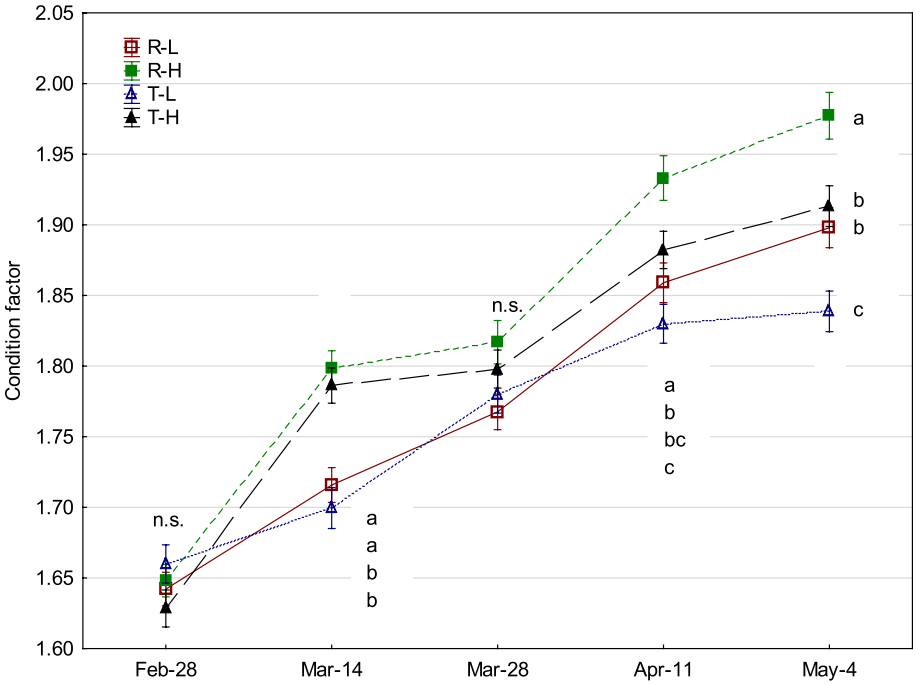

Fig. 3 Condition factor (K) of lumpfish reared under four different treatments (T-L, T-H, R-L, R-H) throughout the laboratory phase of the project. Values represent mean average, and vertical whiskers indicate standard error of mean (SEM). Letters indicate significant differences (Student Newman-Keuls post hoc test, $P < 0.05$) between the experimental groups

Table 4 Biomass, specific water consumption, biomass density in the experimental groups at the end of the laboratory experiment

Experimental groups	Final biomass (kg)	Final water use (L/kg/min)	Final stocking density (m^3)	Final stocking density (m^2)
R-H	5.6	2.32	77.8	11.7
R-L	5.2	0.83	72.2	10.8
T-H	5.5	4.07	25.1	3.1
T-L	4.7	1.60	21.3	2.6

density between high- and low water exchange groups (H and L) within the respective tank designs (R and T).

Mucosal barrier analyses

Skin

There was a statistically significant increase in mucus parameter values in the skin (mucous cell area, volumetric density and defence activity) from the first to the second sampling in April in all groups (Fig. 4). However, there was a decrease in mucus parameter values in the skin in the Tanks groups (T-L and T-H) from April to August. The skin of lumpfish in the R-H group in August had mucus cells with a high volumetric density in large numbers (defence activity or barrier status) (Fig. 4B-C).

Gills

There was a statistically significant increase in mucus parameter values in gills (Fig. 5) from the first sampling in February to the sampling 21 April, in all groups. Subsequently, there was a statistically significant decrease in mucus parameter values in gills from 21 April to August in all groups. Gills of lumpfish in the R-H group had significantly more mucus cells with high volumetric density (defence activity or barrier status) in August (Fig. 5B-C).

Cortisol and serotonin activity

Independently of rearing system, there were significant interaction effects between water exchange rate and stress on plasma cortisol ($P < 0.005$). This was reflected in significantly higher baseline cortisol levels in fish reared in tanks with high water exchange rate (R-H and T-H) compared to fish reared in low water exchange rate (T-L and R-L) ($P < 0.005$, Fig. 6). Furthermore, the stress test resulted in statistically significant elevated cortisol values in fish reared in low water exchange rate (L) ($P < 0.001$), as well as in fish reared in tanks with high water exchange rate (H) ($P < 0.001$, Fig. 6). There were no statistically significant differences in cortisol response to the stress test between the L and H reared fish ($P > 0.80$). There were also statistically significant interaction effects between rearing unit form and stress, which were independent

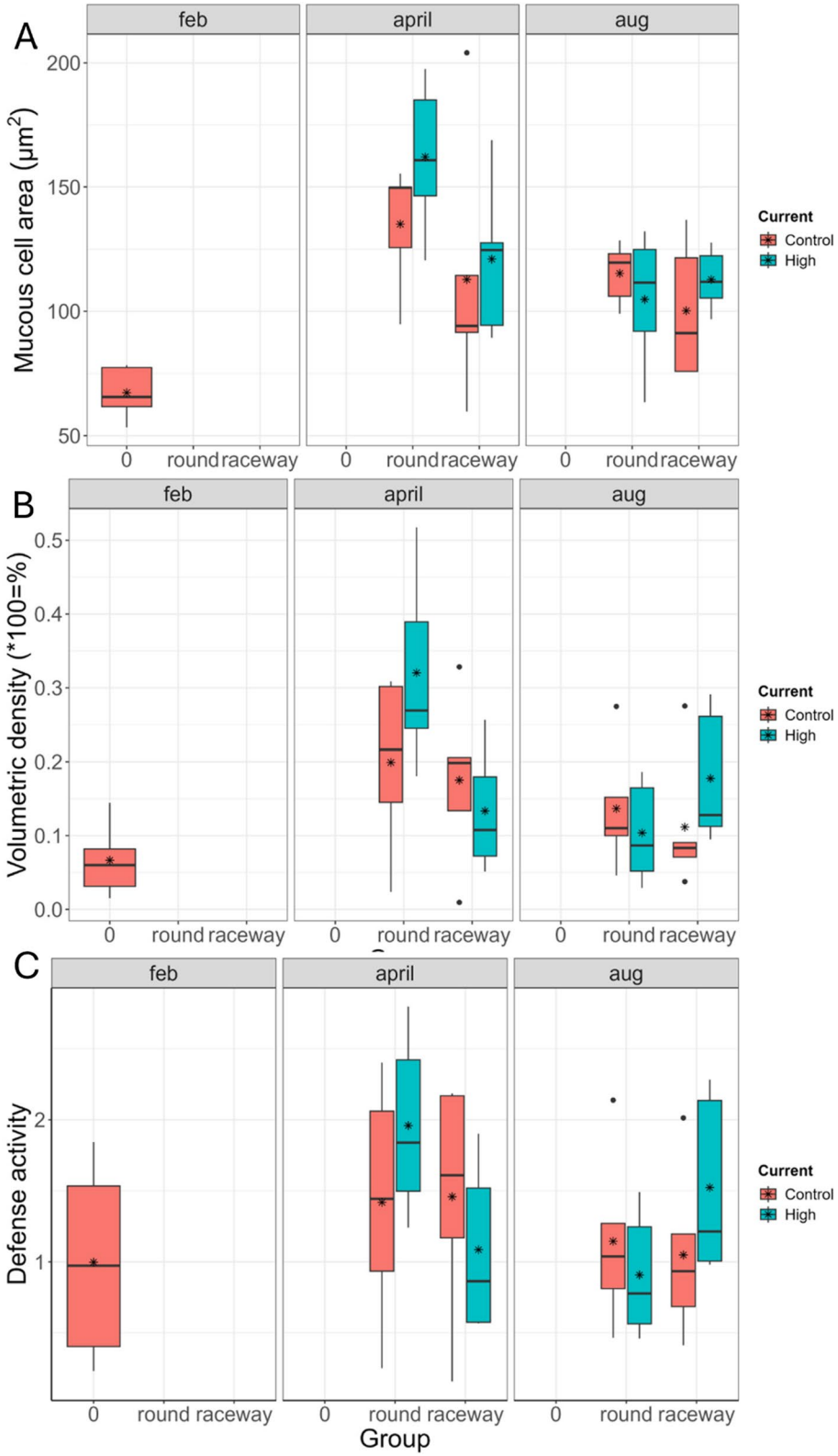

Fig. 4 Overview of lumpfish mucosal protection of the lateral skin in the four experimental groups. The asterisk and the line in the middle of the box plot are the average and median of the group, respectively. **A** Average mucus cell size in square micrometers. **B** Mean epithelium volumetric cell density (*100 = %). **C** Average estimated defence activity of mucus cells

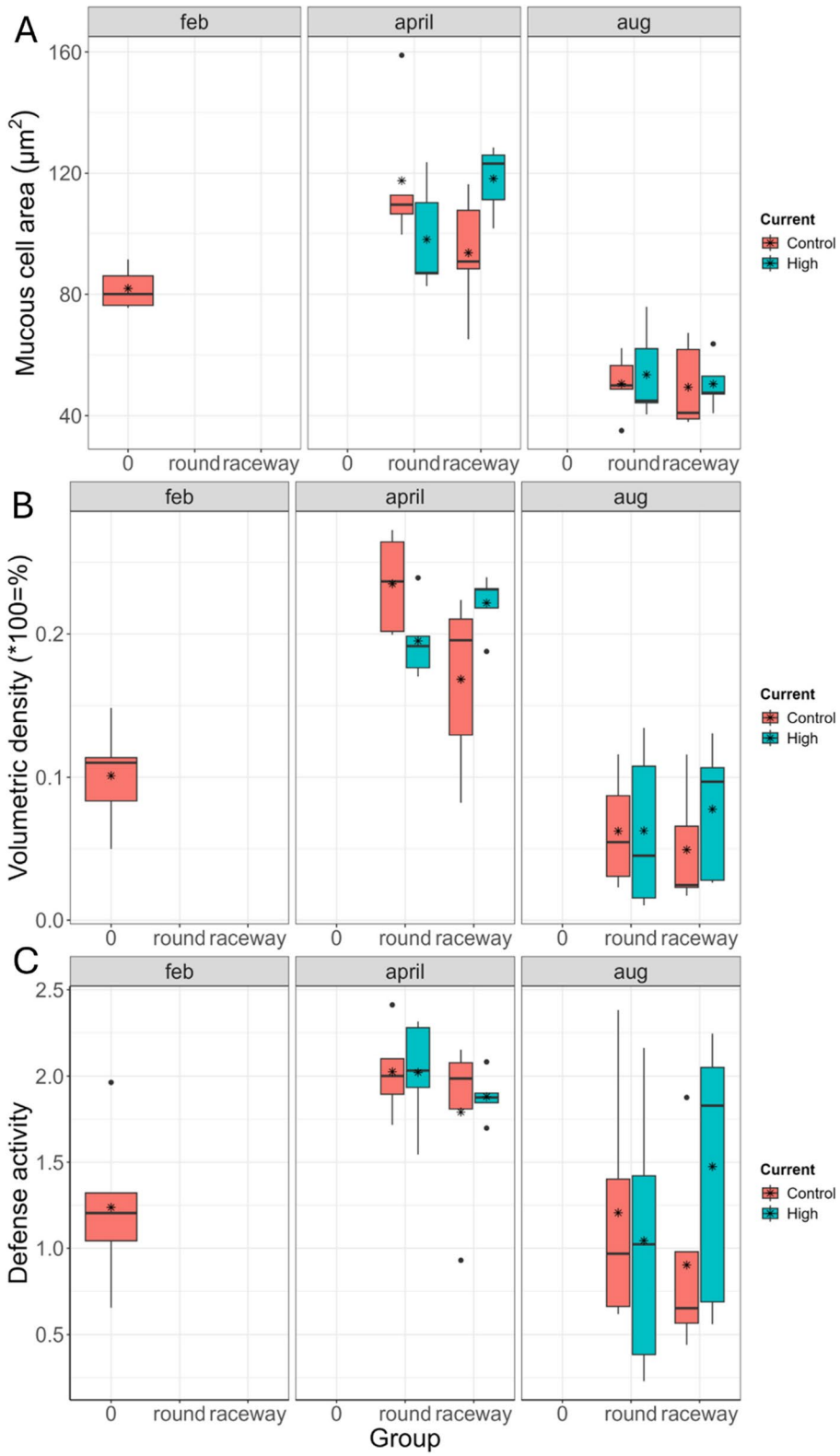

Fig. 5 Overview of lumpfish mucosal protection of gill lamellae in the four experimental groups. The asterisk and the line in the middle of the box plot are the average and median of the group, respectively. **A** Average mucus cell size in square micrometers. **B** Mean epithelium volumetric cell density (*100 = %). **C** Average estimated defence activity of mucus cells

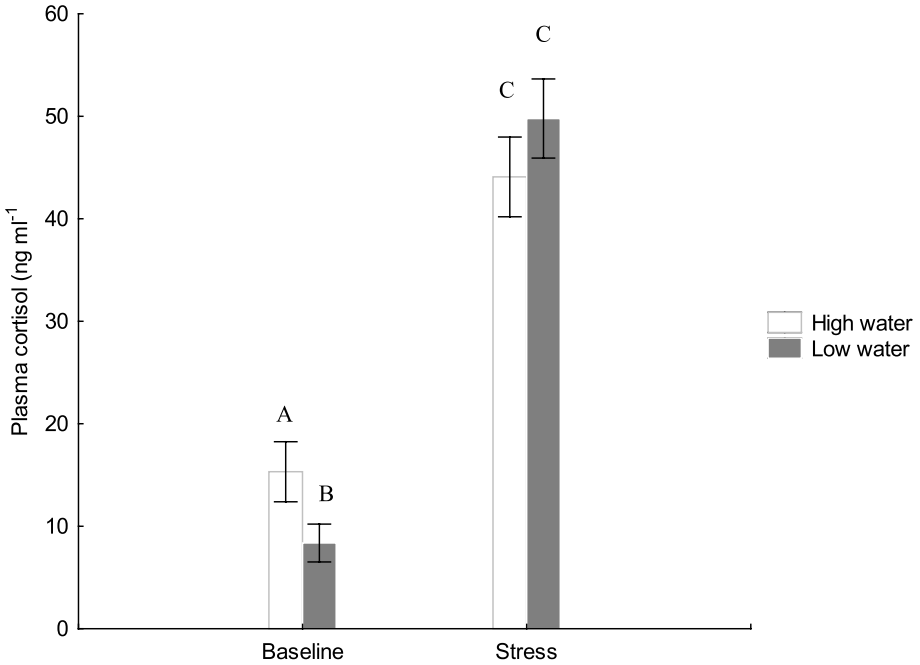

Fig. 6 Plasma cortisol (ng mL^{-1}) in juvenile lumpfish prior to and after stress test (confinement) at two water exchange rates (high (H)—1350 L h^{-1} and low (L)- 450 L $h.^{-1}$). Mean values which do not share a letter were found to be significantly different (Student Newman-Keuls post hoc test, $P < 0.05$)

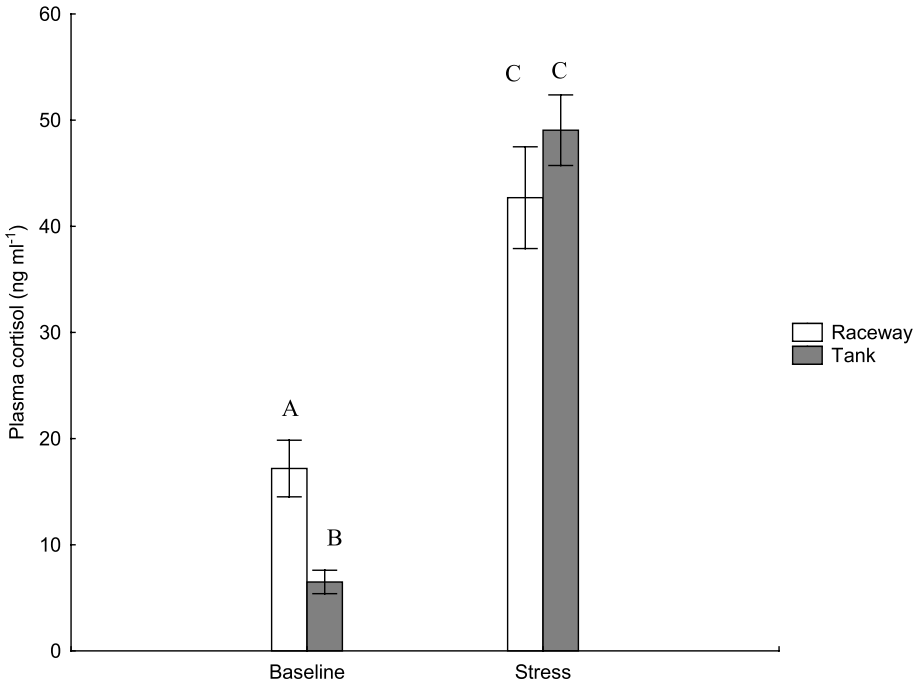

Fig. 7 Plasma cortisol (ng $mL.^{-1}$) in juvenile lumpfish reared in circular tanks (T) or raceways (R) prior to and after stress test (confinement). Mean values which do not share a letter were found to be significantly different (Student Newman-Keuls post hoc test, $P < 0.05$)

of water exchange rate ($P<0.001$, Fig. 7). Furthermore, the stress test resulted in statistically significant elevated cortisol values compared to baseline in the R groups and the T groups ($P<0.01$, Fig. 7). There were no statistically significant differences between the R and T reared fish in cortisol response to the acute stress test ($P<0.85$, Table 5). There were no significant statistically significant interacting effects between stress, rearing system, and water exchange rate (Table 5).

Independently of water exchange rate, rearing unit form affected brainstem serotonergic activity significantly with elevated values in fish reared in R compared to fish reared in T ($P<0.005$, Fig. 8). The three-way ANOVA with stress, rearing unit form, water exchange rate as independent grouping variables did not indicate any other statistically significant effects on brainstem serotonergic activity (Table 5).

Follow up trial in sea pens: growth and welfare

The fish in the T-H group were significantly larger at the start of the sea pen study and lumpfish in the T-L group were the smallest (SNK post hoc test, $P<0.01$, Fig. 9). This trend continued throughout the study period with the lumpfish in the T-H group having a significantly higher mean weight ± SE of 216.8 ± 7.93 g and lumpfish in the T-L group having the lowest mean weight ± SE of

Table 5 Results from three-way ANOVAs with water exchange rates (high or low), rearing unit form (tank or raceway) and stress (baseline or exposed to an acute stress test) as grouping factors. Plasma cortisol or brainstem serotonergic activity (the ratio between serotonin [5-HT] and its immediate catabolite 5-Hydroxyindoleacetic acid [5-HIAA]) were continues variables

	Plasma cortisol	Brain stem [5-HIAA]/[5-HT]
Water exchange rate	**$F_{(1, 36)}=5.5$, $P<0.05$**	$F_{(1, 34)}=0.4$, $P<0.6$
Raring unit form	**$F_{(1, 36)}=14$, $P<0.001$**	**$F_{(1, 34)}=7.6$, $P<0.01$**
Stress	**$F_{(1, 36)}=190$, $P<0.001$**	$F_{(1, 34)}=2.4$, $P<0.13$
Water exchange rate x rearing unit form	$F_{(1, 36)}=0.2$, $P<0.6$	$F_{(1, 34)}=0.15$, $P<0.7$
Water exchange rate x stress	**$F_{(1, 36)}=11$, $P<0.005$**	$F_{(1, 34)}=0.02$, $P<0.9$
Raring unit form x stress	**$F_{(1, 36)}=25$, $P<0.001$**	$F_{(1, 34)}=0.98$, $P<0.33$
Water exchange rate x Rearing unit form x Stress	$F_{(1, 36)}=0.02$, $P<0.85$	$F_{(1, 34)}=0.34$, $P<0.6$

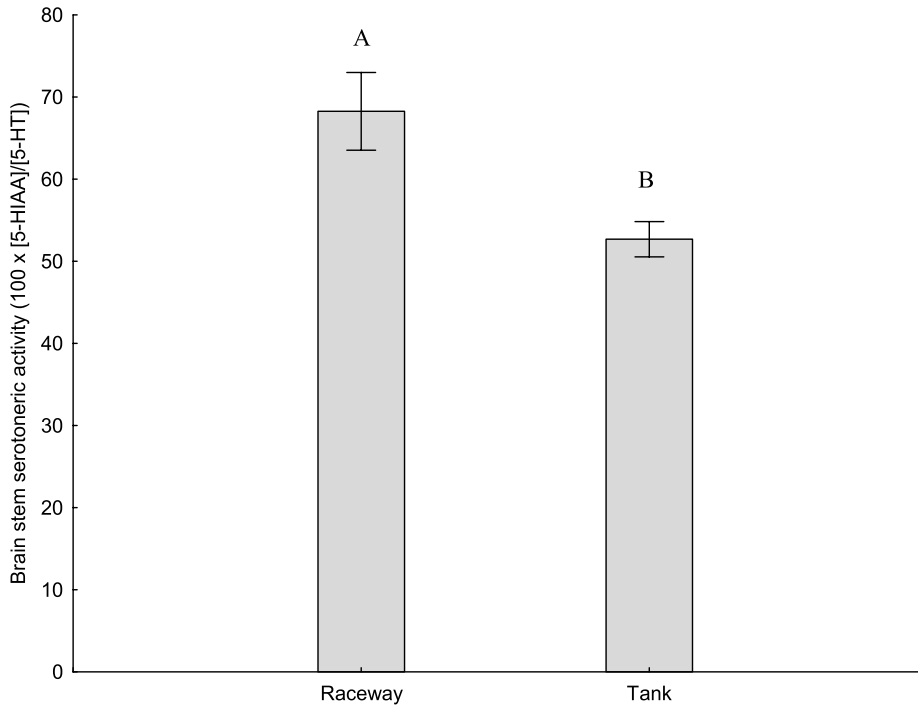

Fig. 8 Brain stem serotoneric activity (100 × [5-HIAA / 5-HT]) in juvenile lumpfish reared in shallow raceways (R) or circular tanks (T). Mean values which do not share a letter were found to be significantly different (Student–Newman–Keuls post hoc test, $P<0.05$)

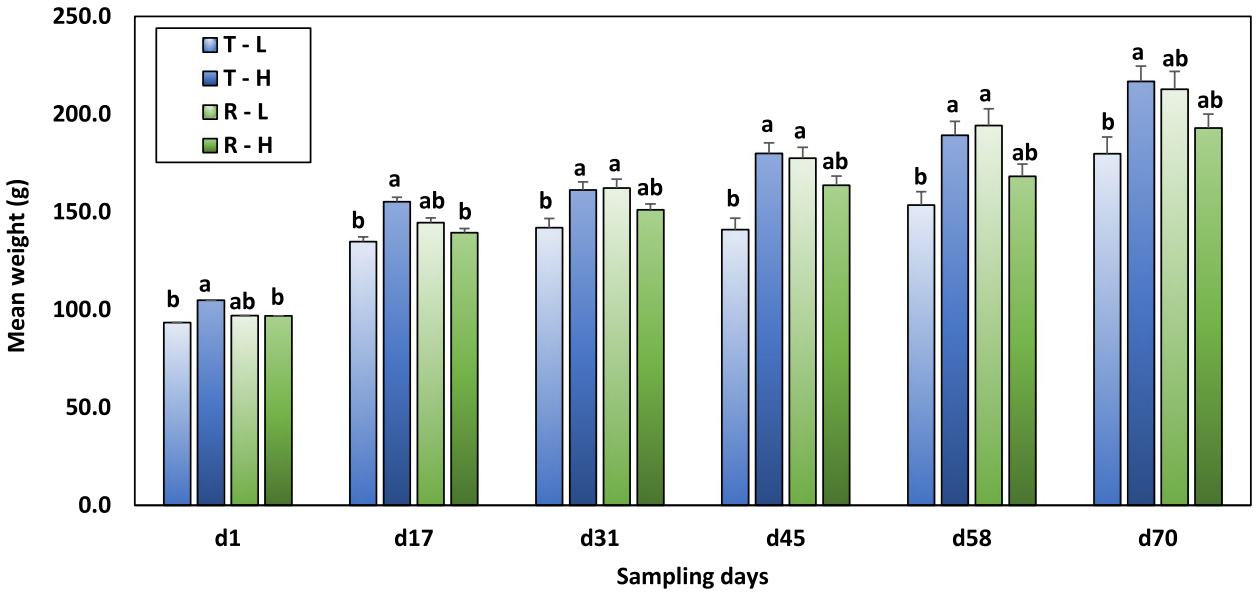

Fig. 9 Mean weight of the four experimental groups during a 70 day follow-up trial in small sea pens. Values represent means and vertical whiskers indicate standard error of mean (SEM). Mean values which do not share a letter were found to be significantly different Student–Newman–Keuls post hoc test, $P < 0.05$)

179.8 ± 8.59 g at the end of the study at day 70 (SNK post hoc test, $P < 0.05$, Fig. 9).

Significantly lower SGR was found in lumpfish in the T-L at day 45 and day 58 (SNK post hoc test, $P > 0.05$, Fig. 10). Significantly highest SGR was seen in the R-L (between 0.89 and 1.06) group at day 45 and day 58 (Fig. 10, $P > 0.05$).

Overall group welfare scores were similar for all four groups at the end of the study period (Fig. 11A). Between 3 and 10% of the lumpfish sampled were classified as

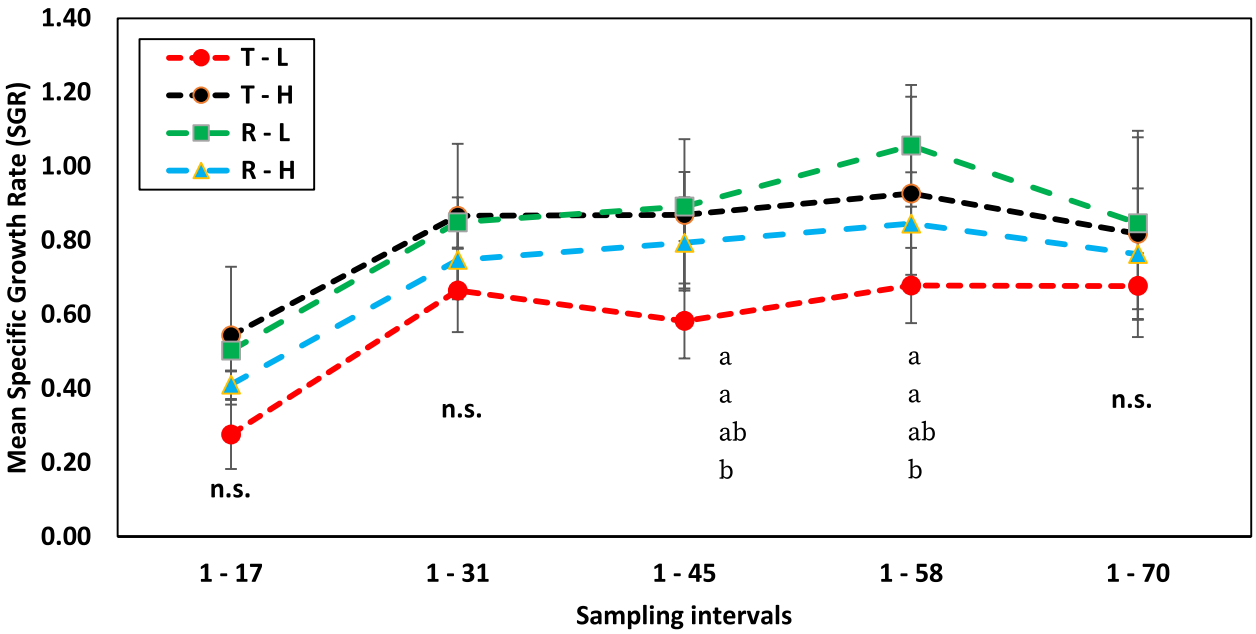

Fig. 10 Mean cumulative Specific Growth Rate (SGR) calculated for each of the four experimental groups during a 70 day follow-up trial in small sea pens. Values represent means and vertical whiskers indicate standard error of mean (SEM). Mean values which do not share a letter were found to be significantly different Student–Newman–Keuls post hoc test, $P < 0.05$). n.s. (not significant)

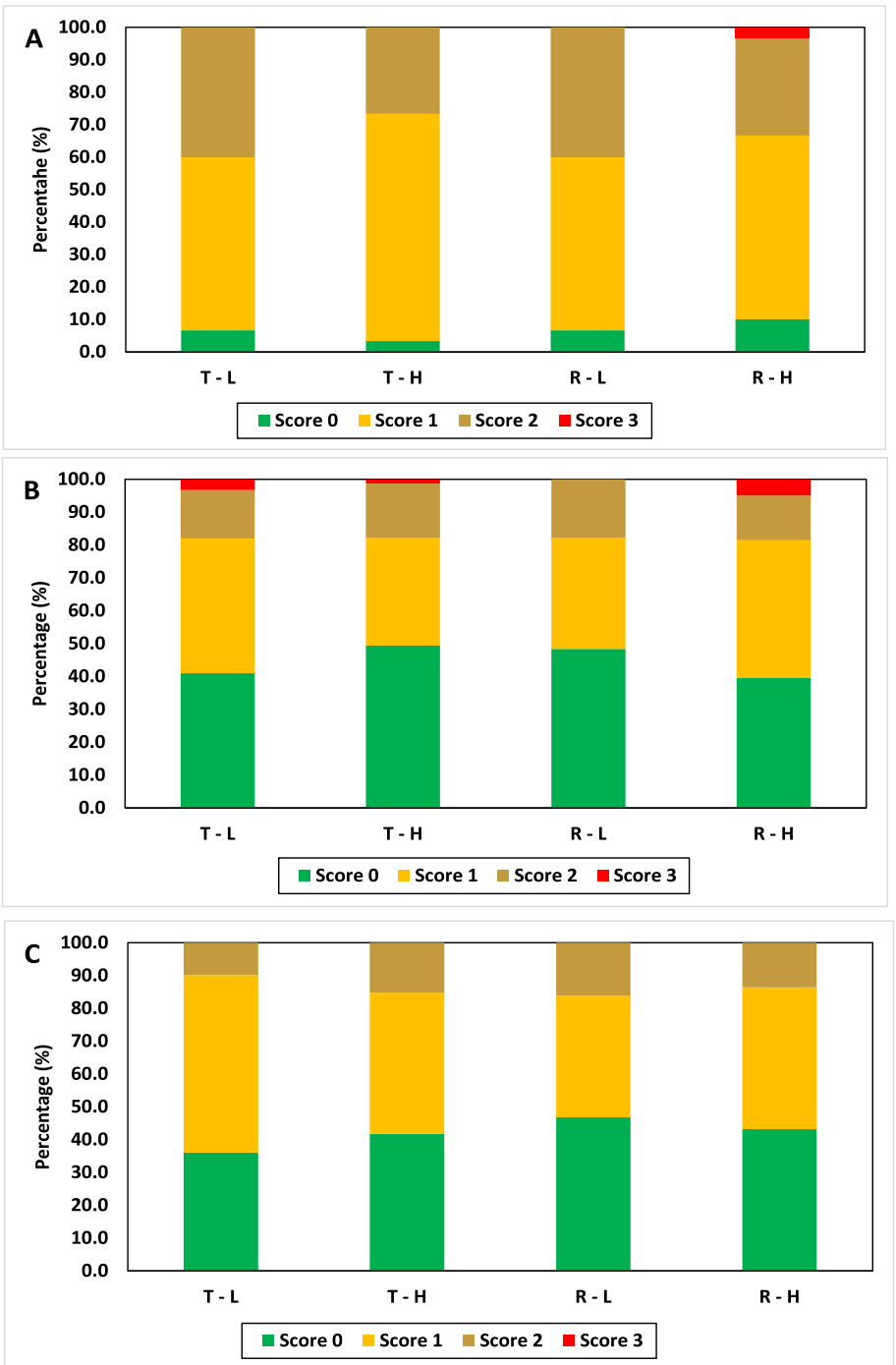

Fig. 11 Percentage of lumpfish from each treatment assessed as having **A**) either good, slight reduction, clear reduction, or severe reduction for overall group welfare score, **B**) Caudal fin damage and **C**) other fin damage at day 70 in the follow-up sea pen trial

having good welfare (score 0). whereas between 53 and 70% of the lumpfish were scored as having a slight reduction in welfare (score 1). A clear reduction in overall welfare score (score 2) was observed in all four groups with between 27% for group T-H and 40% for both groups T-L and R-L (Fig. 11A). There was 3% of lumpfish from group R-H with severe reduction in welfare (score 3).

There were similar trends in caudal fin damage observed for all four groups (Fig. 11B). Between 40 and 49% of the lumpfish sampled were found with intact

caudal fins at day 70 for all groups whereas, 33% and 42% had minor fin damage (score 1). Between 14 and 18% had moderate damage (score 2) and between 1 and 5% were observed with severe fin damage (score 3) from all groups except for R-L.

Damage to other fins was similar for all groups. Between 36 and 47% of lumpfish had no damage to other fins (Fig. 11C) and between 37 and 54% were recorded with slight damage (score 1). Between 10 and 16% of lumpfish from all groups had moderate damage to other fins (score 2) and no lumpfish were found with severe damage to other fins.

Discussion

Growth and condition factor

Raceways constitute a rearing system that may have advantages compared to tanks systems as they can be stacked, maximising hatchery space. Raceways have been used for other commercial fish species, particularly flatfish such as turbot (*Scophthalmus maximus* Rafinesque), Atlantic halibut (*Hippoglossus hippoglossus* L.) and spotted wolffish (*Anarhichas minor*), due to their benthic lifestyle [25, 57]. As such, raceways may be more suitable to the benthopelagic lumpfish with juveniles tending to spend the majority of time in shallow water, attached to seaweed and other surfaces, and thus may be better suited to shallow tanks with larger surface area like raceways [44]. However, as opposed to halibut and turbot, lumpfish also utilizes the tanks walls, which all together gives a larger surface area than raceways. In a previous study, it was shown that spotted wolffish reared in raceway systems experienced a 14% higher growth rate than individuals reared in round tanks, lending support to raceway as suitable rearing systems for benthopelagic species [25]. In the present study, the fish in the R-L group had 10% higher growth compared to T-L group. However, at the higher water velocities (R-H and T-H), growth was highest in the tanks (T-H, Table 3). Final weight was similar for R-H and T-H due to larger start weight for R-H.

Rearing system (tank vs. raceway) had a highly significant effect on lumpfish condition factor. Lumpfish from raceway systems had significantly higher condition factor in March, April and May, compared to lumpfish from the tank systems. However, according to a recent study, the condition factor values expressed in tank groups are still acceptable values for good welfare [7], which is also supported by the higher growth rate T-H despite lower condition factor. In the latter study, lumpfish with a condition factor > 1 were scored as 0 (good), lumpfish with a condition factor of 0.90–1.00 were scored as 1 (slightly emaciated), lumpfish with a condition factor of 0.75–0.90 were scored as 2 (clearly emaciated), and lumpfish with condition factor < 0.75 were scored as 3 (severely emaciated). However, in the study performed by Boissonnot et al. [7] the authors did not differentiate the welfare impact of condition factors above 1. However, if the relationship between welfare status and condition factor still are present above values of 1, this indicates a higher resistance to disease and robustness in lumpfish [7, 48] reared in raceway systems.

The neuroendocrine stress response

In this study, fish reared in tanks (T-L and T-H) generally exhibited lower baseline levels of plasma cortisol compared to those reared in raceways (R-L and R-H). Furthermore, independent of rearing system, fish kept in high water exchange rate showed elevated cortisol values in comparison with fish kept in low water exchange rate. Higher baseline cortisol levels in fish can indicate suboptimal rearing conditions [14]. The levels observed in both raceways and those with high water exchange were comparable to previously observed cortisol levels in lumpfish exposed to mild acute stressors [51]. Previous studies in salmonid fishes have shown that severe chronic stress can lead to a dampened cortisol response to an acute stressor [21, 39]. This has been interpreted as a weakened stress coping capability, allostatic overload, and compromised welfare [23, 36, 39]. The fact that the increased baseline cortisol in fish reared in raceways or in tanks at high water exchange were not mirrored in a significant dampened response to the acute stress test in this study could suggest that the stress experienced by fish reared in high water exchange rate or raceways did not reach a level that would induce allostatic type 2 overload, similar to what was reported in Staven et al. [52]. However, mean baseline cortisol levels on lumpfish reared in tank environments tend to vary and have been reported to range from 5 ng ml^{-1} [20] to 9 and 16 ng ml^{-1} [51, 52] and up to 20 ng ml^{-1} [35]. This indicates that lumpfish show variability in baseline cortisol levels depending on the rearing conditions, suggesting that even mild variations in tank environments can influence their stress physiology, in addition to other factors such as fish size and time of sampling during the day.

The brainstem serotonergic activity generally showed elevated values in fish reared in raceways compared to those reared in tanks. Numerous studies have demonstrated that chronic stress is linked with increased central 5-HT-ergic activity (reviewed by [2, 55]), which supports chronic stress in fish reared in raceways. Interestingly, in this study, the acute stress test did not result in increased serotonergic activity, an effect that was independent of rearing unit form and/or water exchange rate. In salmonid fishes, the inability of the central serotonergic system to respond to an additional stressor has been interpreted

as allostatic overload and compromised stress coping capability [22, 54]. Similarly, earlier reported serotonergic activity in lumpfish telencephalons after acute stress revealed miniscule effects [51], which suggests that serotonergic activity range varies between species, similar to the variation in range of cortisol levels observed among different fish species.

Mucus parameters in skin and gills of lumpfish

Generally, there was an elevation in most mucus parameters after 54 days in the tank experiment, and thereafter a reduction after 70 days in sea cages. The difference in the mucosal status in the tank experiment and subsequent stage in sea cages is most likely related to the response and plasticity in the mucosal surfaces to the different environmental condition [10]. Hence, the mucosal response in the cage environment is not expected to reflect the previous situation in tanks. Generally, there was a large individual variation in mucus parameter values, and it was not possible to conclude the effect of different tank designs and water exchange rates. However, the trend towards a lower defence activity in raceways at high water exchange (R-H) compared to tanks at high water exchange (T-H) give some indications of more favourable environmental conditions in the raceways.

Carryover effects after sea deployment

A lower SGR in the T-L group persisted for 70 days after sea transfer. This slower growth rate was also reflected in a lower weight at the end of the sea deployment period, suggesting a carry-over effect of hatchery rearing conditions on growth post-deployment. This result contrasts slightly with other studies that demonstrate compensatory growth in fish when unfavourable conditions cease to exist [1, 15, 18]. However, it is important to note that sea deployment in rearing cages may not be an optimal environment for lumpfish. Still, all groups were exposed to the same environmental factors after sea deployment. Thus, they should theoretically exhibit similar growth rates. The mechanisms underlying the carryover effects of rearing conditions on growth need further investigation.

Conclusion

Based on the statistically significant smaller size, statistically significant growth rate and a condition factor below the threshold which represents acceptable fish welfare in lumpfish, rearing of juvenile lumpfish in tanks at the low water exchange rate (150% exchange of tank water per hour) is considered sub-optimal compared to exchange rates of 450% in tanks and 200% or 600% in raceways. The stress indicators, plasma cortisol and brain serotoneric activity, indicates higher stress level in raceways and at higher water exchange rates (450% and 600%) independent of tank design. However, these effects are not reflected in negative growth response as fish at high water exchange in both tank designs showed the fastest growth. Despite large individual variation in the mucus cell parameters, there was an indication of lower defence activity in skin mucus cells in raceways at high water exchange compared to tanks at high water exchange, suggesting that raceways at high water exchange may have more favourable environmental conditions. The rearing conditions in the different tank designs at the hatchery stage did not seem to affect fish welfare during the subsequent stage in sea cages. Comparing the two different tank designs and water capacity set up, it is worth noting the difference in the tank surface area between the two designs, which gives differences in available space for resting and stocking densities (both per m^3 and m^2). However, the main differences between the two tank designs seemed to be related to water exchange rate.

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Authors' contributions

Albert Kjartan Dagbjartarson Imsland: Conceptualization, Funding, Supervision, Investigation, Methodology, Writing – original draft, review & editing; Christina Maple: Investigation, Methodology, Writing—original draft; Helena C. Reinardy: Supervision, Review & editing; Lauri Kapari: Investigation, Methodology; Erik Höglund: Conceptualization, Analyses, Review & editing; Trond Ivarjord: Investigation, Methodology; Patrick Reynolds: Investigation, Methodology, Analyses; Karin Pittman: Methodology, Analyses, Review & editing; Fredrik Staven: Methodology, Review & editing; Lauris Boissonnot: Methodology, Review & editing; Thor Magne Jonassen: Conceptualization, Investigation, Analyses, Writing – original draft, review & editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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