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Commercial-scale Validation of Temperature-step Rearing on Growth Physiology in Turbot, *Scophthalmus maximus*

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Abstract

The aim of this study was to investigate the possible benefit of "temperature-steps" (T-steps) rearing for juvenile turbot (initial weight 15.1 g) under realistic production scale and to determine whether initial growth advantage is maintained throughout the rearing period to market size. One group (called T-step 22-19-16) of juvenile turbot was reared at three different temperatures, that is, 22 C (from 17 to 60 g) followed by 19 C (from 60 to 100 g) and 16 C (>100 g); another group (called T-step 19-16) at two temperatures, that is, 19 C (from 17 to 100 g) and lowered to 16 C (>100 g); and the third group (called C16) at one constant temperature, that is, 16 C. Relative growth was significantly higher in the two T-step groups, with the T-step 19-16 showing the highest overall growth. Feed conversion efficiency was highest in the 19-16 group. Only minor effects of the experimental rearing on blood physiology were found, with one notable exception of inverse relationship between plasma glucose and growth. Overall, these findings indicate that a short interval of rearing fish at high temperature during the early juvenile phase may have a long-term effect on biomass increment in turbot. This is an important finding for the turbot industry.

In juvenile turbot, *Scophthalmus maximus* (Rafinesque), growth rate is significantly influenced by temperature, following a pattern typical of most fish species (cf. Cuenco et al. 1985; Fonds et al. 1992; Björnsson et al. 2001; Imsland and Jonassen 2002). Fish typically show a rapid increase in relative growth rate as the temperature rises, passing through a peak at optimum temperature for growth ($T_{opt}G$) and falling rapidly at temperatures beyond $T_{opt}G$ (cf. Imsland et al. 1996, 2000, 2006). A common finding in studies examining the effect of temperature and size on growth is that $T_{opt}G$ shifts to lower temperatures as fish increase in size. The findings of different temperature optima for different size classes together with the downward trend of the $T_{opt}G$ with size can be summarized in what can be called the "stepwise temperature hypothesis." Instead of using constant rearing temperature, one uses specific "temperature-steps" (T-steps) where the fish are reared at optimum temperatures defined for each size class, that is, temperature should be lowered following changes in fish size, mimicking a mechanism suggested for wild turbot (Aneer and Westin 1990; Déniel 1990). An ontogenetic shift in optimal temperature for growth

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has, thus, consequences for the natural distribution of different life stages of that species as well as for rearing under culture conditions. The $T_{opt}G$ for juvenile turbot is highly size dependent and drops rapidly in the first 6–8 mo of the juvenile period. For juveniles <50 g, $T_{opt}G$ is reported to be from 20 to 22 C (Imsland et al. 2000, 2001), for 50- to 100-g juveniles $T_{opt}G$ is reported around 19 C (Burel et al. 1996; Imsland et al. 1996), and for juveniles >100 g $T_{opt}G$ is reported around 16–17 C (Imsland et al. 2006). In the present study, it was tried to mimic this ontogenetic drop in $T_{opt}G$ by rearing fish at different T-steps and comparing growth, feed conversion efficiency (FCE), and blood physiology in these groups. Monitoring of blood physiological response was included to investigate if, and to what extent, the hydromineral and acid–base status (sodium, potassium, pH, CO_2, bicarbonate), metabolic status (glucose), and general blood physiology (haematocrit, haemoglobin levels) of turbot were affected by progressive changes in temperature.

There are two new aspects in this trial compared to the published literature: first, the long-term follow-up of individually tagged fish, and second, the implementation at a commercial farm level as the experiment was done under realistic production scale for a large part of a production cycle. The aim of this study was to investigate the possible benefit of "T-steps" rearing for juvenile turbot under realistic production scale and to determine whether initial growth advantage is maintained throughout the rearing period up to market size.

Materials and Methods

Preexperimental Protocol

Juvenile turbot of mixed parental background (eight families) were used in this experiment. The eggs were spawned and hatched at the Icelandic Marine Research Institute, Grindavik, Iceland, and the larvae and juveniles reared under intensive conditions. In February 2005, a batch of approximately 5200 turbot juveniles (mean size 2.5 g) were brought to the commercial turbot farm Sæbýli, SW-Iceland, and reared at 17–18 C and continuous light in one tank (bottom area 10 m^2) until tagging. The fish were hand-fed twice daily with formulated dry feed (Dan-Ex 1562). Pellet size of 3 mm was used at the start of the experiment, with gradual introduction of bigger pellets according to fish size and producers' recommendations. Tanks were supplied with seawater (30%) pumped from boreholes. To maintain the desired temperature, a heat exchanger was used to regulate the inlet water temperature to each of the tanks. To maintain a sufficient oxygen level in the tanks, the inlet water into each tank was oxygenated with liquid oxygen. Oxygen saturation was higher than 80% at all times.

Experimental Design

The experiment was carried out from April 12, 2005, until April 11, 2006. Initially, the fish were distributed into three rectangular tanks (bottom area 12 m^2) with similar initial density in all tanks (approximately 1.5 kg/m^2). On April 5, 2005, 88–110 fish in each tank (a total of 305 fish, mean size 17.2 g) were tagged intraperitoneally with Trovan tags and distributed randomly into the three tanks with one experimental temperature in each tank (see below). All growth measurements are based on these tagged fish. The three experimental groups were defined as follows:

One treatment group was reared at approximately 16 C throughout the whole experimental period (mean temperature 16.1 C, maximum 17.4 C, minimum 14.4 C, SD 0.8 C). This group is called C16.

The second group was reared at 19 C from April 14 to August 30, 2005 (mean temperature 18.8 C, maximum 19.7 C, minimum 17.8 C, SD 0.4 C), and from August 31, 2005, to April 11, 2006, this group was reared at 16 C (mean temperature 16.4 C, maximum 17.4 C, minimum 14.4 C, SD 0.8 C). This group is called T-step 19-16.

The third group was reared at 22 C from April 14 to June 18, 2005 (mean temperature 22.0 C, maximum 23.8 C, minimum 20.1 C, SD 0.8 C). From June 18 to August 30, 2005, this group was reared at 19 C (mean temperature 18.6 C, maximum 19.3 C, minimum 17.8 C, SD 0.3 C), and from August 31, 2005, to April 11, 2006, this

group was reared at 16 C (mean temperature 16.4 C, maximum 17.4 C, minimum 14.4 C, SD 0.8 C). This group is called T-step 22-19-16.

In September 2005, all fish were transferred to two commercial-size tanks at Silfurstjarnan NE-Iceland (bottom area 18 m^2) with identical rearing regimes. Here all groups were reared together in two tanks with identical temperature regime. As a result of mechanical failure, temperature in all three groups was lowered to 11 C between and September 5 and 29, 2005. Mean temperature in the rearing period from September 2005 to April 2006 was 16.3 C (maximum 19.8 C, minimum 11.2 C, SD 1.2 C).

The tagged fish were weighed individually at a monthly interval (April to August 2005) and later at 2–3 mo intervals (September 2005 to April 2006). During each measurement, the number and total weight of the untagged fish in each group was recorded so that the total biomass in each tank could be calculated. Feed was provided from automatic feeders for 8 h daily (0800–1600 h). In addition, the fish were occasionally hand-fed to ensure satiation. The amount of eaten feed was registered on a daily basis. Uneaten feed was collected by filtering the outlet water, but this amount was almost always negligible because of strict control of feeding.

Feed Data

Total feed consumption (C) was calculated as total feed supplied – total remaining feed in the effluent water. C was calculated on a daily basis and then summarized for each experimental period. FCE was calculated as biomass gain per weight unit of consumed feed.

$$\mathrm{FCE} = (B_2 - B_1)/C$$

where C is total feed consumption (g dry matter) in the period and B_1 and B_2 are fish biomass (g wet weight) on days t_1 (start) and t_2 (final), respectively. Feed data were not available for two periods during the trial period (e.g., September 2005 to October 2005, February 2006 to April 2006; see Table 1).

Blood Physiology

To monitor the possible effect of different temperature regimes on blood physiology, blood samples were collected from the caudal vessels of eight individually tagged fish from each experimental group in July 2005 and April 2006 and analyzed using an i-STAT Portable Clinical Analyzer. The analyzer was used in conjunction with EC8+ disposable cartridges, measuring blood sodium, potassium content, glucose, hematocrit, pH level, partial pressure of CO_2 (pCO_2), and displaying calculated values of blood bicarbonate content, total carbon dioxide concentration, and hemoglobin.

Data Analysis and Statistical Methods

Specific growth rate (SGR) of the tagged fish was calculated according to the formula $\mathrm{SGR} = (e^g - 1) \times 100$, where $g = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ and W_2 and W_1 are wet weights (g) at days t_2 and t_1, respectively. One-way ANOVA (Zar 1984) was applied to calculate the effect

TABLE 1. *Feed conversion efficiency for the three experimental groups when reared at different temperatures (April to August 2005) and when reared together (September 2005 to April 2006) at one temperature.*

Period	Experimental groups (separate temperatures)			All groups (one temperature)
	C16	T-step 19-16	T-step 22-19-16	
April 2005 to May 2005	1.14	1.50	1.33	
May 2005 to June 2005	1.27	1.68	1.17	
June 2005 to August 2005	1.35	1.49	1.54	
September 2005 to October 2005				NA
October 2005 to November 2005				1.42
November 2005 to February 2006				0.92
February 2006 to April 2006				NA

NA = data not available.

of different temperature regimes on mean weights, SGR, and FCE. Two-way analysis of covariance (ANCOVA), where the fish weight was used as a covariate and the temperature regimes as independent variable, was applied to calculate the effect of different experimental regimes on blood physiology. In case of significant ANOVA and ANCOVAs, Student–Newman–Keuls multiple comparison tests were used to locate differences among treatments (Zar 1984).

Individual growth trajectories were analyzed using a growth curve analysis (GCM) multivariate analysis of variance (MANOVA) model (Timm 1980; Chambers and Miller 1995). The model equation of the GCM had the form:

$$Y(n \times p) = X(n \times q)B(q \times p) + E(n \times p) \quad (1)$$

where $\boldsymbol{Y}(n \times p)$ are the growth at age vectors.

$$y = (y_1, y_2, \ldots, y_p) \quad (2)$$

for each p (age) measurement on n individual fish; $\boldsymbol{X}(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, that is, $q = \text{age}_p + \text{temperature regime}_i$ (i = C16, T-step 19-16, and T-step 22-19-16); $\boldsymbol{B}(q \times p)$ is the matrix of parameters estimated by the model; $\boldsymbol{E}(n \times p)$ is the matrix of deviations for each individual from the expected value of $\boldsymbol{Y} = \boldsymbol{XB}$.

A significance level (α) of 0.05 was used if not stated otherwise.

Results

Growth

The initial mean weight (±SD) was 17.2 g (±3.1) and did not differ between the three experimental groups (one-way ANOVA, $P > 0.6$). From Day 36 and throughout the experimental period, the two T-step groups (i.e., T-step 19-16 and T-step 22-19-16) showed overall higher mean weights compared to the C16 group (Student–Newman–Keuls test, $P < 0.05$; Fig. 1). The two T-step groups maintained their weight advantage throughout the experimental period (Fig. 1). Final mean weight was 16–18% larger in the two T-step groups. Mean

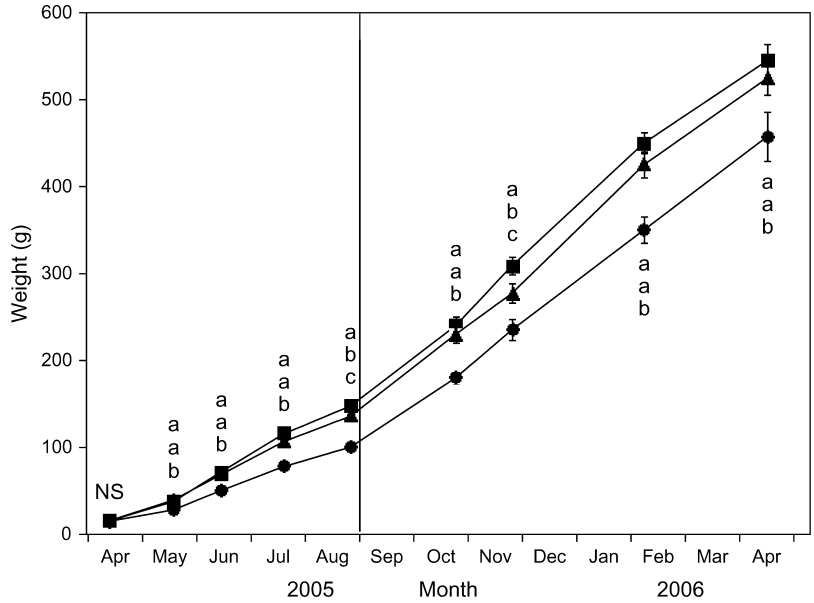

FIGURE 1. *Mean weight of juvenile turbot reared at three different temperature regimes. Vertical line indicating standard error may be obscured by symbol. Different letters indicate statistical differences (Student–Newman–Keuls test,* $P < 0.05$*), with "a" as the highest value. The black line indicates transfer from three different temperatures to one common rearing temperature. Symbols – C16, circles; T step 19-16, squares; T step 22-19-16, triangles.* N = *88–110 for each mean value; NS, not significant.*

individual growth trajectories were different (GCM, $MANOVA_{TEMPERATURE}$, Wilk's lambda $(\Lambda)_{12,\ 350} = 0.28$, $P < 0.001$) between the three temperature groups throughout the study period. Significant differences were also found in growth-at-period trajectories of the experimental groups ($MANOVA_{TEMPERATURE \times PERIOD}$, Wilk's $\Lambda_{10,\ 350} = 0.15$, $P < 0.01$) as growth declined between periods in all groups. Overall, the growth rate was significantly different between treatments in four of eight experimental periods (one-way ANOVA, $P < 0.05$; Fig. 2). During the period of different temperatures (i.e., April to August 2005), growth was 17–18% higher in the two T-step groups compared to the C16 group (Fig. 2).

Feed Conversion Efficiency

In the first part of the trial when the groups were reared at different temperatures, the FCE was higher in the T-step 19-16 group, compared to the two other groups (Student–Newman–Keuls test, $P < 0.05$, Table 1). Overall, the FCE was 14–20% higher in the T-step 19-16 group in this period (i.e., April to September 2005).

Blood Physiology

The temperature rearing conditions had only a minor effect on measured blood parameters (Table 2). In July 2005, blood pH was significantly lower and partial pressure of CO_2 (pCO_2) significantly higher in the T-step 19-16 group (Student–Newman–Keuls test, $P < 0.05$). Notably, plasma glucose was lowest in the T-step 19-16 group, followed by the T-step 22-19-16 group and highest in the C16 group at both measurements (Student–Newman–Keuls test, $P < 0.05$).

Discussion

Our study indicates that even at near-optimal temperature for a given size, the thermal history of the fish may influence growth potential, so that a short rearing period at high temperature may give a long-term growth advantage. Such long-term advantage of short-term heating was

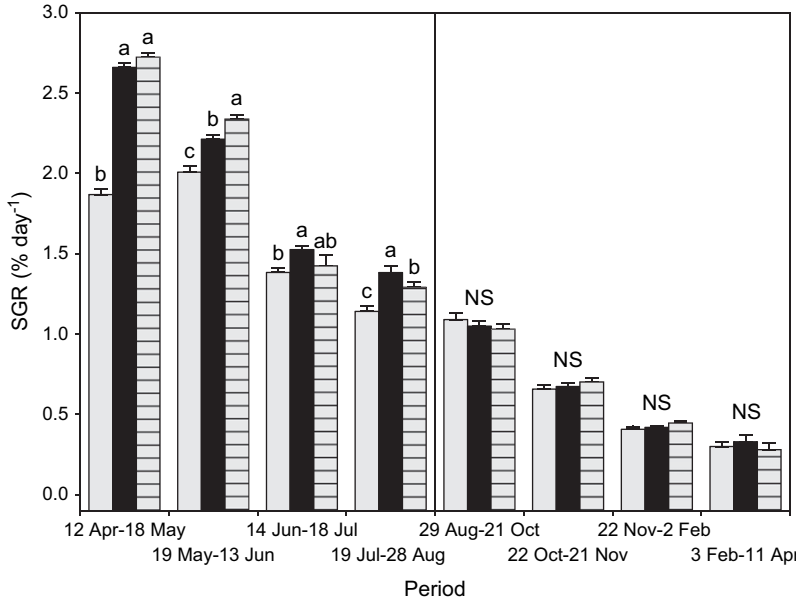

FIGURE 2. *Mean specific growth rates of individually tagged turbot reared at three temperature regimes during the experimental period. Vertical whiskers indicate standard error. Different letters denote significant differences (Student–Newman–Keuls test,* $P < 0.05$*) between treatments in each period. The black line indicates transfer from three different temperatures to one common rearing temperature. Symbols – C16, white bars; T step 19-16, black bars; T step 22-19-16, hatched bars.* $N = 88–110$ *for each mean value; NS, not significant.*

TABLE 2. *Measured and calculated blood parameters in juvenile turbot reared at three different temperature regimes and later at one common temperature regime.*[1]

	July 2005			April 2006		
	C16	T-step 19-16	T-step 22-19-16	C16	T-step 19-16	T-step 22-19-16
Na^+ (mmol/L)	150.14 (1.20)	153.00 (1.09)	149.86 (1.49)	154.20 (1.53)	155.62 (0.78)	154.57 (1.56)
K^+ (mmol/L)	3.84 (0.20)	4.20 (0.20)	3.89 (0.20)	3.48 (0.13)	3.55 (0.12)	3.76 (0.27)
Glucose (mmol/L)	2.33 (0.14)^{a}	1.89 (0.07)^{b}	2.18 (0.13)^{ab}	3.53 (0.23)^{a}	2.87 (0.18)^{b}	3.22 (0.11)^{ab}
Hematocrit (%)	13.00 (0.87)	15.60 (1.69)	13.00 (0.89)	22.00 (1.76)	21.31 (0.64)	21.86 (0.99)
pH	7.42 (0.03)^{ab}	7.38 (0.01)^{b}	7.49 (0.04)^{a}	7.58 (0.03)	7.57 (0.03)	7.54 (0.05)
pCO_2 (mmHg)	13.77 (0.29)^{b}	15.92 (0.83)^{a}	12.88 (0.50)^{b}	7.15 (0.28)	7.58 (0.32)	7.69 (0.31)
TCO_2 (mmol/L)	9.03 (0.44)	9.42 (0.28)	9.86 (0.61)	6.78 (0.53)	7.05 (0.41)	6.70 (0.49)
HCO_3^- (mmol/L)	8.98 (0.44)	9.36 (0.28)	9.81 (0.61)	6.75 (0.53)	7.02 (0.41)	6.67 (0.49)
Hemoglobin (g/dL)	4.41 (0.29)	5.30 (0.56)	4.44 (0.31)	7.50 (0.59)	7.25 (0.22)	7.27 (0.30)

[1] Values are given as mean (SD). Different letters denote significant differences (Student–Newman–Keuls multiple comparisons, $P < 0.05$) between treatments.

found for Atlantic cod, *Gadus morhua* (L.) (Imsland et al. 2005), where cod reared at declining temperature regime was 7–12% larger than fish reared at constant temperatures. The authors concluded that environment-related growth differences in 0 group of fish are mirrored in size differences at harvesting. Combined with the current data, these findings are important for commercial rearing of finfishes as prior growth advantages can be used at later stages of the production. In a few other studies, the positive effect of $T_{opt}G$ rearing scheme has been noted. In Atlantic halibut, *Hippoglossus hippoglossus* (L.), (size range 160–400 g) reared at constant (11 and 14 C) or switched (14 C moved to 11 C and vice versa) temperature regimes, Aune et al. (1997) found that growth rate was highest in fish transferred from 14 to 11 C, mimicking the drop in $T_{opt}G$ seen for Atlantic halibut in this size range. Similarly, Imsland et al. (2007) found that juvenile turbot (size range 72–205 g) transferred from a high (20 C) to a low (16 C) temperature showed higher growth than groups reared at constant (16 and 20 C) or upward switched (from 16 to 20 C) temperature regime. Our findings are in line with these results and the finding on plaice, *Pleuronectes platessa* (L.), and flounder, *Platichthys flesus* (L.) (Fonds et al. 1992), Atlantic cod (Björnsson et al. 2001), and the modeling results for several finfish (Cuenco et al. 1985), demonstrating the gain of rearing fish at stepwise regimes instead of constant temperatures.

But what is the production value of the current results? To try to answer this, a cost/benefit analysis for one of the farms (Silfurstjarnan) participating in this study was performed. The aim was to investigate whether faster growth, leading to greater biomass turnover, may be expected to outweigh the cost associated with the initial temperature control. The bioeconomic model developed for intensive aquaculture production (Kamstra 2003) was used for this purpose. When analyzing initial costs, it was found that the most important contributors to the cost price for this farm were company costs (i.e., interest, depreciation, maintenance, insurance, measure costs, and general costs, 25%), labor (19%), and feed (24%). The capital costs (depreciation and interest) are an intrinsic part of the farm and their contribution to the cost price can only be reduced by increasing the production of fish at the farm. The effect of production (ton/year) on the cost price (€/kg) of turbot produced at Silfurstjarnan was given as:

$$\text{Cost price} = 144.79 \times \text{Production}^{-0.6872},$$
$$r^2 = 0.99.$$

This means that that applying the current T-step rearing and, thus, imposing a 20% increase in production at the farm would lead to a cost price reduction of 15%. For Silfurstjarnan, which has access to all-year-round temperatures in the range of 10–20 C (geothermal heat), there are

no obvious costs related to the temperature control of the T-step rearing method, and hence, the benefit is around 15% reduction in production costs. For farms that need to heat or cool seawater to maintain the T-steps, the situation is more complex. However, our data demonstrate that heating (from 16 to 19 C is adequate) is only needed for a limited period (here 4 mo) of the early juvenile period to achieve long-term benefits.

The changes that occur during thermal acclimation involve a series of adaptations at the enzymatic level (i.e., digestive and metabolic enzymes) that may lead to higher feed efficiency. There is some evidence that downward thermal acclimation (as applied in the present study) may result in increased activities in enzymes involved in aerobic energy liberation and ion transport in muscle and increased digestive enzyme activity (Kuzmina et al. 2003). Savoie et al. (2007) investigated the relationship between temperature, growth, and metabolic and digestive enzyme activities in spotted wolffish, *Anarhichas minor*. They found that trypsin showed a positive compensation (higher activity at lower temperature), whereas glycolytic enzymes (pyruvate kinase and lactate dehydrogenase) and aspartate aminotransferase showed a negative compensation (lower activity at lower temperature). Lamarre et al. (2007), working with common wolffish, *Anarhichas lupus*, observed the same trend but this time in periods of restricted feeding. Both studies give a clear indication that in order to achieve high growth rates, fish rely heavily on trypsin activity. Optimal temperature for enzymatic activity can vary with fish size (Luzkovich and Stellwag 1993). Accordingly, the fish in the T-step groups in the present trial may have been reared closer to optimal temperatures for enzymatic activity during the early juvenile period than the fish in the constant-temperature group. Alternatively, enzymatic activity in key digestive enzymes (e.g., trypsin) could increase as a result of stepwise-declined temperatures applied in the T-step groups. In both cases, growth and FCE would improve. Imsland et al. (2005) showed that FCE in Atlantic cod was improved in line with reduced temperature in a T-step group as FCE increased from 1.0 to 1.2 and 1.35 when cod were reared at 16, 13, and 10 C, respectively. Similarly, in the present trial, FCE increased from 1.17 to 1.54 when juvenile turbot were transferred from 22 to 19 C.

Growth and plasma glucose levels were inversely correlated in our study. The reason why a decrease in plasma glucose levels was seen in the T-step 19-16 group may be related to an increased need for energy substrate to fuel the higher growth seen in this group. An increased energy demand of cells may cause a faster uptake of glucose from blood and cause the observed depletion in plasma glucose levels (Iwama 1998). Higher plasma glucose levels at low temperatures have earlier been observed (Gabillard et al. 2005). It has been postulated that glucose might be involved in growth control through regulation of the growth hormone/insulin-like growth factor I (the GH/IGF-I system, Gabillard et al. 2005). Gabillard et al. (2005) found a negative correlation between glucose and GH levels, which might indicate partial inhibition of GH secretion by glucose, thereby affecting growth through the GH/IGF-I axis. Our data fit this model as the group showing the highest growth (T-step 19-16 group) had the lowest glucose levels, whereas the group showing the lowest growth (C16 group) had the highest glucose level.

Conclusions

Juvenile turbot transferred from a high (22 and 19 C) to a lower (16 C) temperature showed higher growth than fish reared at a constant (16 C) temperature regime. The T-step 19-16 showed the highest overall growth. This indicates that even at near-optimal temperature for a given size, the temperature history of the fish may influence future growth. From a practical viewpoint, our results demonstrate the production advantage of rearing the fish at elevated temperatures during the juvenile period as size differences established at this stage will lead to a shortening of the total rearing time to market size.

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