Changes in metabolic rate, excretion, energy reserves, and starvation response of diploid and triploid Salvelinus fontinalis and diploid O. mykiss after long-term exposure to elevated temperature

Franz Lahnsteiner (✉ Franz.Lahnsteiner@baw.at)

Abstract

Routine metabolic rate (RMR), faeces composition, quantities of dissolved excretion, and energy reserves were investigated in diploid (2n) and triploid (3n) *Salvelinus fontinalis* and 2n *Oncorhynchus mykiss* exposed to 20°C for 32 d in comparison to fish acclimated to 9°C. At 20°C RMR of 2n *S. fontinalis* decreased with increasing exposure time and after 32 d it did not differ from 9°C. Also in *O. mykiss* RMR decreased with exposure time but remained 60% higher than at 9°C. In 3n *S. fontinalis* exposed to 20°C RMR was constantly increased for 120%. For all species/ploidy levels faeces dry weight and phosphorus concentration and quantities of excreted dissolved nitrogen and phosphate did not differ between 9°C and 20°C. Lipid concentration of faeces was decreased at 20°C. With exception of O. mykiss also protein of faeces was decreased at 20°C. In all species/ploidy levels liver glycogen and visceral fat was decreased at 20°C, while liver triglycerides and adenylate energy charge were not affected. In 2n and 3n *S. Salvelinus* temperature related responses to 5-d starvation were investigated. In 2n *S. fontinalis* RMR decreased in starving fish in comparison to fed ones at 9°C and increased at 20°C. RMR of 3n *S. fontinalis* did not change. For both ploidy levels, dissolved excretion was decreased due to starvation at 9°C and 20°C. Visceral fat was decreased due to starving at 9°C, liver glycogen and triglycerides at 20°C. The data are important to manage the impact of increased water temperature on salmonid aquaculture.

Introduction

Abrupt exposure of teleost fish species to a sudden temperature change leads to thermal shock reactions (Alfonso et al. 2021), while gradual exposure in combination with sufficient long exposure time induces acclimatization reactions (McKenzie et al.2021; Adams et al. 2022). Knowledge about acclimatization processes is important in ecology and fish culture to define the plasticity of teleost fish species to temperature changes (Adams et al. 2022). The acclimatization potential of economically valuable salmonid species is of particular interest in Austria as well as in many other temperate regions.

In fish culture oxygen consumption (= metabolic rate) and excretion of particular and dissolved waste products are important parameters limiting the production capacity and influencing the environment (Bureau and Hua K 2010). Data on their changes in response to elevated temperature are important for future management of fish farms. Standard metabolic rate of teleost fish increases, when they are abruptly exposed to elevated temperature (e.g. brown trout, *Salmo trutta*, – Auer et al. 2015

; brook trout, *Salvelinus fontinalis*, - Beamish 1964; Tang et al. 1995; Durhack et al. 2021; rainbow trout, *Oncorhynchus mykiss*, – Callaghan et al. 2016; Verhille et al. 2016; coho salmon, *O. kisutch*, - Lee et al. 2003; sockeye salmon, *O. nerka*, - Eliason et al. 2011). However, only little is known if and to which extent standard metabolic rate can be reregulated to acclimation conditions in form of a partial or total metabolic compensation. Standard metabolic rate of *O. mykiss* acclimated to 9°C and exposed to 20°C was partially re-regulated to acclimation conditions after 72–96 h (Evans 1990). Shorthorn sculpin (*Myoxocephalus scorpius*) showed total metabolic compensation following a temperature increase from

9°C to 16°C after eight weeks of acclimation (Sandblom et al. 2014). Similar results were also found in Atlantic halibut (*Hippoglossus hippoglossus*) after long-term exposure to 16°C (Gräns et al. 2014).

Only few data are available how the composition of particular excretion (faeces) and the quantity of dissolved excretion are affected by elevated temperature. Digestion processes decrease at temperatures outside the optimal range due to changes in enzyme activities and in gut transit time (walking catfish, *Clarias batrachus,* - Ahmad et al.2014; *Salvelinus fontinalis* - Amin et al. 2016; catla, *Catla catla,* - Sharma et al. 2017; Atlantic salmon, *Salmo salar*, *Salmo trutta*, Arctic charr, *Salvelinus alpinus*, - Elliott et al. 2010). Differences in food digestibility could lead to alterations in faeces composition. Excretion of dissolved ammonium depends on fish activity levels, diet, and protein catabolism (Bucking 2017; Kajimura et al. 2004). Quantities of excretion show a positive relation with temperature in several teleost fish species (*O. mykiss* - Kieffer et al. 1998; sea bass, *Dicentrarchus labrax,* - Person-Le Ruyet et al. 2004; Chinese large-mouth catfish, *Silurus meridionalis*, - Luo and Xie 2009; pike perch, *Sander lucioperca,* – Frisk et al. 2013; review Bucking 2017). In fed fish no relation was detected between ammonium excretion and temperature in *O. mykiss* (Medale et al. 1995; Azevedo et al. 1998), but it was positively correlated with temperature in walleye, *Stizostedion vitreum*, (Forsberg and Summerfelt 1992). Phosphorus excretion depends on the administered chemical form, the diet, the growth rate, and the body size (Milián-Sorribes et al. 2021). In a natural ecosystem, temperature had a minor influence on fish phosphorus excretion rates (Verant et al. 2007). In Silver perch, *Bidyanus bidyanus* (Mitchell 1838), the phosphorus excretion was significantly increased at elevated temperature (Kibria et al. 1997; Kibria et al. 1998).

The present study investigated if the routine metabolic rate (RMR), the composition of faeces (dry mass, protein, lipid, and phosphate content), and the quantities of excreted ammonium, total nitrogen bound, and phosphate of salmonid fish kept at 20°C for 32 d differed from fish acclimated to 9°C. To get an overall picture about fish physiology, energy reserves of liver (glycogen, triglycerides), visceral fat depots, and cell energy status (cellular ATP concentration, adenylate energy charge) were investigated, too. For the investigations diploid (2n) and triploid (3n) brook trout *Salvelinus fontinalis* and 2n *O. mykiss* were used as these species are of high economic value and as this study complements previous ones on adaptation processes to elevated temperature (Lahnsteiner 2022a, b). In fish culture it is a common practice to reduce or stop feeding at elevated temperature to decrease oxygen depletion and excretion load of water and therefore to maintain stable water conditions (Robb 2008; Poli 2010; Waagbø et al. 2017). Therefore, in 2n and 3n *S. Salvelinus* temperature related effects of a 5-d starving period on routine metabolic rate, quantities of dissolved excretion, and energy reserves were investigated in the end of the temperature exposure experiment.

Material And Method

The used experimental fish were 7 months old 2n *O. mykiss* (initial body mass 8 ± 3 g, n = 30, mean ± S.D.), 2n *S. fontinalis* (9 ± 3 g), and *3n S. fontinalis* (10 ± 4 g). The latter were produced by pressure shock. Experimental fish and experimental design were similar to a previously published study (Lahnsteiner 2022a). Briefly, experiments were conducted from July 12 – August 12 2021 in stream channels under

flow through conditions with a water supply of 0.2 l sec^{-1}. Four stream channels, respectively, were stocked with 2n rainbow trout, and 2n and 3n brook trout. Total stocked fish mass was 3 kg per stream channel resulting in approximately 200 fish. For each species/ploidy level two stream channel were maintained at 9°C (= controls). The other stream channels were gradually tempered to 20°C during a 7-d period. When the water temperature had reached 20°C, the experiment was started. The duration of the experiment was 37 d. Fish had a natural photoperiod and were fed a commercial trout diet (protein 39–41%, lipid 19–22%, raw fiber 1.0–2.0%, ash 4–8%, phosphorus 0.87%) at a ratio of 1.5% of the body weight using band feeders during daylight time. Similar quantities of feed were administered at both tested temperature regimes. Loss of equilibrium was used as an endpoint of critical thermal stress experiments (Lahnsteiner 2022b). The rate of fish maintaining equilibrium was calculated at the end of the experiments in relation to the total number of fish stocked in the stream channels. Hygienic concepts and screening methods for water quality and fish health status were described previously (Lahnsteiner 2022a). Experiments were carried out in accordance with Austrian regulations governing animal welfare and protection and with the EU directive 2010/63/EU for animal experiments.

Analysis design

The analysis design is illustrated in Fig. 1. After exposure periods of 8, 16, and 32 d to 20°C RMR was measured in 2n *O. mykiss* and 2n and *3n S. fontinalis* in comparison to control fish kept under similar conditions but at 9°C. Quantities of dissolved excretion, composition of particular excretion and fish energy reserves and energy status were measured on day 32 d. From day 33 to 37 feeding was stopped. On day 37 routine metabolic rate, concentration of dissolved excretion and fish energy reserves and energy status and were remeasured.

Analysis of particular and dissolved excretion

For analysis of particular excretion, 10 fish per stream channel were killed by prolonged exposure to 0.3% MS222 six hours after they had started feeding (i. e. 6 h after the belt feeders had been switched on). The abdominal cavity was opened and an amount of 20–25 mg faeces was collected from the rectum. The faeces samples were split in 2 subsamples and their mass was determined to the nearest 0.1 mg using an analytic balance. One subsample was used for determination of dry weight and total phosphate concentration, the other subsample for protein and lipid determination. For determination of dry mass, the subsample was heated to 100°C for 24 h and reweighed to the nearest 0.1 mg. Then the dry matter was digested in 200 µl 1 mol l^{-1} HCl in 1 ml screwed vials at 105°C for 4 h. The samples were cooled down and phosphate was determined according to standard methods (Murphy and Riley 1962). From the second subsample lipids were extracted with chloroform methanol (Bligh and Dyer 1959). In the chloroform methanol extract total lipid was determined with the sulphuric acid - vanillin method (Frings and Dunn 1970). The lipid free sample was used for total protein determination. Total protein was extracted with 500 µl of 0.1 mol l^{-1} NaOH in 3.5% NaCl at 60°C for 90 min (Fonkwe and Singh 1996). The

homogenates were centrifugated at 5000 g for 10 min to remove insoluble particles. Protein was analyzed with the Lowry method (Lowry et al. 1951).

For analysis of dissolved excretion 5 fish, respectively, were removed from the stream channels 6 h after they had started feeding and placed in a static 15 l tank of similar water temperature. Tanks were aerated with air stones using aquarium pumps. After 24 h the fish were removed from the tanks and their mass was determined to the nearest 0.1 g. 1-l water samples were taken according to standardized sampling procedures and frozen at -20°C until analysis. For each stream channel the experiment was performed in triplicate. Standardized methods were used for determination of NH_4-N (DIN 38406-5 1983), NO_2-N (OENORM EN 26777 1993), NO_3-N (DIN 38405-29 1988), and orthophosphate (DIN 38406-5 1983). Total nitrogen bound was calculated as the sum of NH_4-N, NO_2-N, and NO_3-N nitrogen. All analytes concentration were expressed in units kg $fish^{-1}$ h^{-1}.

Determination of routine metabolic rate

Routine metabolic rate is the rate of metabolism when the fish is undergoing behaviors normal to fish farm conditions. A closed rectangular respirometer chamber with a volume of 4 l was used which could be operated under flow through conditions by connecting it via valves to the water supply of the fish farm and under recirculation conditions by connecting it to a recirculation pump. The whole recirculation system had a volume of 8.5 l. The flow volume was 0.4 l sec^{-1} resulting in velocities of flow of 0.01 m sec^{-1} in all operation modes. Flow through conditions were used during the acclimation phase and for water renewal after the measurements. The recirculation mode was used during the oxygen measurements. Oxygen concentration was measured using an optic oxygen sensor (WTW FDO 925) sealed within the chamber. It was connected to a central control unit logging the oxygen concentration. Five measurements per stream channel were performed. The operation procedure was as follows: The respiratory chamber was switched to flow through conditions, and stocked with 3 fish. After 20 min acclimation under flow through conditions the respiration chamber was switched to recirculation conditions and oxygen measurements were started. Oxygen consumption was recorded for 30 min in 5 min intervals. Only measurements were used where the decrease in oxygen consumption was linear which was considered as an indication that results were not falsified by fish related short-term stress responses or activity changes. After the experiments were finished, fish mass was weighed to the nearest 0.01 g. Total oxygen consumption in the respirometer chamber per time unit was calculated and extrapolated to express the oxygen consumption in mg O_2 h^{-1} kg $fish^{-1}$. For measurement of standard metabolic rates reintermittent-flow respirometry persisting over 24−48 h is an alternative to provide absolutely stressless conditions (Svendsen et al. 2016; Snyder et al. 2016). However, these procedures were not applicable in combination with the used experimental design. Long measurement periods would have interacted with feeding regimes and photoperiod and could have induced uncontrollable microbiological growth resulting in falsified oxygen values. Moreover, RMR may better reflect the conditions of fish farms, where fish cannot be maintained in stressless environment.

Analysis of fish energy reserves and cell energy status

Liver and viscera (digestive tract without liver) mass was determined. Liver subsamples were weighed in 1.5-mL microcentrifuge tubes with an analytical balance to obtain a reference unit for the metabolic measurements. Metabolites were extracted into 3 mol l^{-1} perchloric acid. Samples were homogenized and kept in extraction solution for 15 min under constant agitation, centrifuged at 1500 g for 10 min and the supernatants were collected. Finally, supernatants were neutralized using 1 mol l^{-1} potassium carbonate. Glycogen, triglycerides, ATP, AMP, and ATP were measured UV-spectrophotometrically with methods of Bergmeyer (1985). Adenylate energy charge (AEC) was calculated according to the formula

$$AEC = \frac{Conc_{ATP} + 0.5 * Conc_{ADP}}{Conc_{ATP} + Conc_{ADP} + Conc_{AMP}}$$

From the viscera lipids were extracted according to the procedure of Bligh and Dyer (1959). The solvent was evaporated at 70°C and the extracted fat was determined gravimetrically. Liver glycogen and triglycerides and visceral fat are energy resources for the fish. Therefore, to compensate for differences in organ and body mass, the total concentration in the organ was calculated and referred to 100 g body mass. Liver ATP levels and adenylate energy charge were used to characterize the energy status of cells and referred to a defined organ mass.

Statistics

Data are presented in form of boxplots. The solid lines represent the medians, boxes represent lower and upper quartiles, and whiskers the minima and maxima, circles the outliers. Percentage data (hepatosomatic index) were transformed by angular transformation (arcsin√p). For statistical analysis continuous data were tested on normal distribution by Shapiro-Wilk test and transformed by log transformation when necessary. Data were analyzed by two-way ANOVA with treatment and species as independent variables and analytes values as dependent variables. Tukey test was used as posthoc test at a significance level of $P \leq 0.05$. Statistical analysis was performed with JASP software (JASP Team, 2022).

Results

Fish equilibrium rate and growth

A percentage of $\geq 95\%$ of 2n and 3n *S. fontinalis* and 2n *O. mykiss* exposed to 20°C for 32d and a percentage of $\geq 96\%$ of control fish acclimated to 9°C maintained equilibrium. Control fish ingested the total amount of administered food during the whole duration of the experiment. Fish exposed to 20°C leftover some part of the food during the first 8 d of the experiments, thereafter the total amount of administered food was ingested, too. In 2n *S. fontinalis* kept at 9°C body mass increased for 164.0 ± 7.2% during the duration of the experiment, at 20°C for 214.8 ± 8.8%. In 3n *S. fontinalis* it increased for 181.3 ± 8.8% at 9°C and for 203.3 ± 7.8% at 20°C. In *O. mykiss* the increase in body mass amounted 176.2 ± 6.5% at 9°C and 139.5 ± 7.9% at 20°C. 2n and 3 n *S. fontinalis* tolerated also a 5-d starving period at both

investigated temperatures (percentage of fish maintaining equilibrium > 98%). In 2n *S. fontinalis* the loss in body mass was 3.5 ± 1.4% at 9°C and 4.5 ± 2.1% at 20°C after the 5-d starving period. In 3n *S. fontinalis* it was 4.3 ± 1.7% and 3.5 ± 2.6% at 9°C and at 20°C. The differences were not statistically significant (P > 0.05).

Routine metabolic rate (RMR)

Differences between 9°C and 20°C (Fig. 2): After 8 d exposure to 20°C RMR of 2n *S. fontinalis* was significantly higher (for 50%, P ≤ 0.05) than at 9°C. It decreased with increasing exposure time and after 32 d it did not differ any more from 9°C (P > 0.05). At 9°C RMR of 3n *S. fontinalis* was significantly lower than that of 2n *S. fontinalis*. In 3n *S. fontinalis* exposed to 20°C RMR was significantly higher (for 120%) than at 9°C and it did not change during the exposure period. Also, in *O. mykiss* RMR was significantly higher (P ≤ 0.05) at 20°C than at 9°C (for 110% after 8d). It decreased with exposure time but remained higher (for 60%) than in 9°C fish after 32 d (P > 0.05).

Differences between fed and starving fish (Fig. 3): In 2n *S. fontinalis* RMR differed significantly between fed and starving fish. At 9°C it was decreased in starving fish in comparison to fed ones, at 20°C it was increased. RMR of 3n *S. fontinalis* did not differ (P > 0.05) between fed and starving fish neither at 9°C nor at 20°C.

Particular and dissolved excretion

Differences in particular excretion between 9°C and 20°C (Fig. 4):

Dry weight and phosphorus concentration of faeces did not differ between the species/ploidy levels and between the temperature regimes (P > 0.05). Protein concentration of faeces was similar in 2n and 3n *S. fontinalis* and in *O. mykiss* (P > 0.05) at 9°C. After 32 d exposure to 20°C protein concentration of faeces of 2n and 3n *S. fontinalis* was significantly (P ≤ 0.05) lower than at 9°C, that of *O. mykiss* did not differ between 9°C and 20°C. The lipid concentration of faeces of *2n S. fontinalis* and 2n *O. mykiss* acclimated to 9°C was similar, that of 3n *S. fontinalis* was significantly lower. At 20°C lipid content of faeces of 2n and 3 n *S. fontinalis* and of *O. mykiss* was significantly decreased in comparison to 9°C.

Differences in dissolved excretion between 9°C and 20°C (Fig. 5): NH_4-N, total nitrogen bound (tNB), and o-PO_4-P concentration of ground water were ≤ 0.005 mg l^{-1}. The quantities of NH_4-N, t-ON-N, and o-PO_4-P excreted into the water did not differ (P > 0.05) between the species/ploidy levels and between the temperature regimes.

Differences in dissolved excretion between fed and starving fish (Fig. 6): The quantities of NH_4-N, tNB, and o-PO_4-P excreted into the water were significantly (P > 0.05) decreased in starving 2n and 3n *S. fontinalis.* For the nitrogen compounds the decrease was greater at 9°C than at 20°C. This was similar for both ploidy levels.

Fish energy reserves and cell energy status

Differences between 9°C and 20°C (Fig. 7)

Liver glycogen and triglycerides concentrations in relation to total body mass, liver tissue ATP concentration and adenylate energy charge, and visceral fat concentration in relation to total body mass did not differ between 2n and 3n *S. fontinalis* and 2n *O. mykiss* acclimated to 9°C. In fish exposed to 20°C for 32 d liver glycogen and visceral fat were significantly ($P \leq 0.05$) decreased, while liver triglycerides were not affected. ATP levels of 2n and 3n *S. fontinalis* did not differ between 9°C and 20°C, ATP levels of *O. mykiss* were significantly increased at 20°C. Adenylate energy charge did not differ between 9°C and 20°C in all investigated species and ploidy levels.

Differences between fed and starving fish (Figs. 8, 9)

ATP concentration, and adenylate energy charge were similar ($P > 0.05$) in fed and starving 2n and 3n *S. fontinalis* acclimated to 9°C and exposed to 20°C. Liver glycogen and triglycerides concentrations were similar between fed and starving 2n and 3n *S. fontinalis* at 9°C but they were significantly decreased in starving fish in comparison to fed ones at 20°C ($P \leq 0.05$). Visceral fat was significantly decreased due in starving 2n and 3n *S. fontinalis* at 9°C. At 20°C no significant differences were detectable between fed and starving fish.

Discussion

Routine metabolic rate (RMR)

Different types of metabolic thermal compensation occurred in the investigated Salmonidae when exposed to 20°C for 32 d. In 2n *S. fontinalis* RMR was re-regulated to values similar to acclimation temperature which represents a total metabolic thermal compensation. Therefore, it can be concluded that 2n *S. fontinalis* is able of adjusting to a wide range of thermal regimes, which is conform to previous studies (Chadwick and McCormick 2017; Durhack et al. 2021). Also, in *O. mykiss* metabolic thermal compensation occurred. However, it was only partial as RMR of fish exposed to 20°C remained 50% over the values measured at 9°C. 3n *S. fontinalis* had lower RMR than 2n *S. fontinalis* under acclimation conditions. This is in accordance to previous data of Stillwell and Benfey (1996) and Lahnsteiner et al. (2019). During a 32d exposure to 20°C 3n *S. fontinalis* did not re-regulate the RMR to acclimatization conditions, as it was constantly increased for > 110%. This could indicate decreased thermal plasticity of 3n *S. fontinalis*. Inability of 3n *S. fontinalis* for metabolic thermal compensation did not influence performance of fish at 20°C, as the equilibrium rate was 100% and the growth rate was higher than at 9°C. Previous data on temperature tolerance 3n *S. fontinalis* are contradictory. Benfey et al. (1996) found no ploidy specific effect on critical thermal maxima in this species. According to a study of Atkins and Benfey (2008) 3n *S. fontinalis* had higher metabolic rates than diploids at lower temperature, and lower

metabolic rates than diploids at higher temperature and it was suggested that this fact might lead to lower thermal tolerance in triploids.

RMR of 5-day starving 2n *S. fontinalis* was decreased in comparison to fed ones at acclimation conditions. In teleost fish, a decrease of the metabolic rate in response to food withdrawal is a mechanism to preserve energy reserves (Martin et al. 2010; Auer et al. 2015; Zeng et al. 2017). At acclimation temperature standard metabolic rate was reduced in starving *S. salar* (Cook et al. 2000; O'Connor et al. 2000; Stien and Oppedal 2020) similar as in *S. trutta* (Auer et al. 2015; Archer et al. 2020), in qingbo, *Spinibarbus sinensis*, (Zeng et al. 2017) and in largemouth bass, *Micropterus salmoides*, (Gingerich et al. 2010). However, RMR of 2n *S. fontinalis* exposed to 20°C increased due to starvation. This might indicate the activation of oxidative metabolism to generate sufficient energy to maintain cell homeostasis. RMR of 3n *S. fontinalis* did not respond to a 5-day starving period, neither at 9°C nor at 20°C. This could be interpreted as an inflexibility of 3 n *S. fontinalis* to respond to changing physiological situations. The present data demonstrate that the concept of metabolic rate reduction due to food deprivation cannot be generalized but depends on the ploidy level and on the temperature. Therefore, also the common aquaculture practice of food withdrawal at elevated temperature to counteract oxygen depletion might be only of limited value.

Particular and dissolved excretion

In 2n and 3n *S. fontinalis* and in 2n *O. mykiss* exposure to 20°C had no negative on the composition of faeces and the quantities of excreted nitrogen and phosphate. Faeces dry weight did not differ between fish acclimated to 9°C and exposed to 20°C for 32 d. This is an indication that water absorption rate in the intestine was similar for the two temperature regimes and that faeces consistence was not affected. In addition, phosphorus concentration of faeces and the quantities of excreted dissolved phosphorus did not differ between 9°C and 20°C. This is conformed to an earlier study on *O. mykiss* where phosphorus waste outputs were similar in a temperature range from 6–15°C (Azevedo et al. 1998). In 2n and 3n *S. fontinalis* exposed to 20°C the protein and lipid concentration of faeces was lower than at 9°, in *O mykiss* only the lipid concentration indicating better feed digestibility at elevated temperature. The data on 2n *S. fontinalis* agree to those of Durhack et al. (2021). A positive relationship between nutrient digestibility and temperature has also been observed in studies on *S. salar* (Bogevik et al. 2010; Huguet et al. 2015) while other studies noted no effect (*Salmo salar* - Ng et al. 2004; yellowtail kingfish, *Seriola lalandi*, - Miegel et al. 2010; review Rosenfeld et al. 2015). Discrepancies between the cited studies may be related to the tested temperature range. Digestibility depends on gut transit time and activities of digestive enzymes (Volkoff and Rønnestad 2020). Gut transit time decreases with temperature (*S. salar* - Handeland et al. 2008; Mock et al. 2022; *Seriola lalandi* - Miegel et al. 2010; *O. mykiss* - Fauconneau et al. 1983), while temperature optima of digestive enzymes are type and species-specific (Gelman et al. 2008). The excretion of dissolved ammonium (NH_4-N) and total nitrogen bound did not differ between 2n and 3n *S. fontinalis* and 2n *O. mykiss* acclimated to 9°C and exposed to 20°C for 32d. Increased ammonium excretion would be an indication for increased protein catabolism either due to higher amount of feed uptake or due to catabolism of muscle tissue (Bucking 2017). Also, other studies on *O. mykiss* found no

differences in ammonium excretion in relation to temperature (Forsberg and Summerfelt 1992; Azevedo et al. 1998). Under practical considerations the present data demonstrate, that excretion load of water was not increased by elevated temperature and that the species and ploidy specific differences were only minor. A 5-d starving period decreased the quantities of excreted dissolved ammonium, total nitrogen bound, and phosphate in both ploidy levels of *S. fontinalis* and at both tested temperature regimes. In starving fish, the excretion of ammonium and total nitrogen bound was higher at 20°C than at 9°C which might be an indication for increased metabolic activity and probably also for a higher rate of protein catabolism at elevated temperature. The data are in contrast to previous ones demonstrating a positive relation between ammonium excretion of non-fed teleost fish species and temperature (*O. mykiss* - Kieffer et al. 1998; *Dicentrarchus labrax* - Person-Le Ruyet et al. 2004; *Silurus meridionalis* - Luo and Xie 2009; *Sander lucioperca* – Frisk et al. 2013). Differences may be due to the tested temperature and exposure time and the period of starving. Lauff and Wood (1996) observed that ammonium excretion increased with the duration of the starving period when muscle protein was catabolized.

Fish energy reserves and cell energy status

Exposure to 20°C was an energetic stress for 2n and 3n *S. fontinalis* and 2n *O. mykiss* as the organismic energy reserves (visceral body fat, liver glycogen) were decreased. Perivisceral lipid is a main energy depot located around the digestive tract (Wang et al. 2017). Glycogen and triglycerides are the main hepatic storage forms of energy (Bruslé & Anadon 1996). Exhaustion of energy levels might impede a response to additional stress factors. Cellular ATP concentration and cellular adenylate energy charge were not affected by temperature indicating that cell energy status and homeostasis could be maintained. Ploidy and species-specific differences in energy status were only minor. Glycogen depletion in response to thermal stress has been described in previous studies for *O. mykiss* (Viant et al. 2003)d *salar* (Corey et al. 2017).

Responses of energy metabolism in relation to starving were temperature dependent. At 9°C, starving 2n and 3n *S. fontinalis* used visceral lipids as energy resources as their amount significantly decreased. This is conformed to previous studies (*O. mykiss* - Jezierska et al. 1982; Furné et al. 2012; review McCue 2010). At 20°C, for both ploidy levels liver glycogen and triglycerides decreased demonstrating their use as energy resources. This is probably due to the fact, that visceral fat depots were already depleted before the starving period began. Therefore, food deprivation at elevated water temperature should be considered critical. It can lead to complete exhaustion of energy resources and to a loss of cell homeostasis. Also, Lauff and Wood (1996) observed, that lipid catabolism was high during the initial period of starving and was followed by carbohydrate utilization when lipid reserves were depleted. In the present study there was no indication for metabolization of muscle protein, as body mass of fed and starving fish did not reveal significant differences and ammonium excretion was not increased.

Conclusions

In Salmonidae different types of metabolic thermal compensation occur after 32 d exposure to 20°C, a full thermal compensation in 2n *S. fontinalis*, a partial one in 2n *O. mykiss* and none at all in 3n *S.*

fontinalis. When offering similar feed quantities, temperature related effects on excretion of waste products were minor for all investigated species and ploidy levels. Exposure to 20°C represented an energetic stress situation for 2n and 3n *S. fontinalis* and 2n *O. mykiss* as the visceral fat and liver glycogen depots were depleted. The temperature related effects of a 5-d starving period were investigated in 2n and 3n *S. Salvelinus* at the end of the 32d lasting experiment. The effect on the metabolic rate was temperature and ploidy level specific (2n *S. fontinalis*: decrease at 9°C, increase at 20°C; 3n *S. fontinalis*: no changes). After 5-d starvation quantities of excreted dissolved nitrogen and phosphorus were decreased in comparison to fed fish at 9°C and 20°C. At elevated temperature starving can result in a critical energetic stress situation which might lead to complete exhaustion of energy resources.

Declarations

Ethics Approval and Consent to participate

The project was evaluated and approved by a committee of the Federal Agency for Water Management and of the Federal Ministry for Agriculture, Regions and Tourism. It was classified as project "exposing fish to conditions not significantly exceeding stress situations occurring in fish farms". It is officially listed as institutional project 3143.

Human and Animal Ethics

Experiments were carried out in *accordance* with Austrian regulations governing *animal welfare* and protection (Tierversuchsgesetz, BGBl. I Nr. 114/2012) and with the EU directive 2010/63/EU for animal experiments. All practices and procedures for the care and management of animals based on current best practice under the supervision of skilled workers of the fish farm Kreuzstein and of the responsible veterinarian.

Consent for publication

Not applicable

Availability of supporting data

The datasets of the current study are available from the corresponding author on reasonable request.

Competing interests

The author declares that he has no competing interests.

Funding

The author declares that no funds, grants, or other support were received for the conducted study and for the preparation of this manuscript.

Authors' contributions

As sole author *F. Lahnsteiner designed the study, performed the experiments and analysis. He* wrote the manuscript text and prepared the figures. He reviewed the manuscript *and approved the final version.*

Authors' information

F. Lahnsteiner is the head of the research laboratory and of the experimental fish farm of Kreuzstein. This institution is part of the Federal Agency of Water Management and is the Austrian Research and Competence Center for Aquaculture.

References

1. Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB (2015) Flexibility in metabolic rate confers a growth advantage under changing food availability. J Anim Ecol 84:1405–1411. https://doi:10.1111/1365-2656.12384

2. Adams O A, Zhang Y, MH Gilbert, CS Lawrence, M Snow, AP Farrell (2022) An unusually high upper thermal acclimation potential for rainbow trout. *Cons Physiol* 10: coab101. https://doi.org/10.1093/conphys/coab101

3. Ahmad T, Singh SP, Khangembam BK, Sharma JG, Chakrabarti R (2014) Food consumption and digestive enzyme activity of *Clarias batrachus* exposed to various temperatures. Aquacult Nutr 20:265-272. https://doi.org/10.1111/anu.12072

4. Amin MN, Carter CG, Katersky Barnes RS, Adams LR (2016) Protein and energy nutrition of brook trout (*Salvelinus fontinalis*) at optimal and elevated temperatures. Aquacult Nutr 22:527-540. https://doi.org/10.1111/anu.12274

5. Alfonso S, Gesto M, Sadoul B (2021) Temperature increase and its effects on fish stress physiology in the context of global warming. J Fish Biol 98:1496-1508. https://doi.org/10.1111/jfb.14599

6. Archer LC, Hutton SA, Harman L, Poole W R, Gargan P, McGinnity P, Reed T E (2020). Metabolic traits in brown trout (*Salmo trutta*) vary in response to food restriction and intrinsic factors. *Cons Physiol* 8:coaa096. https://doi.org/10.1093/conphys/coaa096

7. Atkins, ME, Benfey TJ (2008) Effect of acclimation temperature on routine metabolic rate in triploid salmonids. Comp Biochem Physiol - Part A: Mol & Integr Physiol 149:157-161. https://doi:10.1016/j.cbpa.2007.11.004

8. Azevedo P A, Cho C Y, Leeson S, Bureau D P (1998) Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*).

Aquat Liv Res 11:227-238. https://doi.org/10.1016/S0990-7440(98)89005-0.

9. Beamish FWH (1964) Respiration of fishes with special emphasis on standard oxygen consumption: ii. influence of weight and temperature on respiration of several species. Can J Zoology 42:355-366. https://doi.org/10.1139/z64-016

10. Bergmeyer HU (1985) Methods of Enzymatic Analysis. Weinheim, VCH Verlagsgesellschaft.

11. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917. https://doi.org/10.1139/o59-099

12. Bogevik A S, Henderson R J, Mundheim H, Waagbø R, Tocher DR, Olsen RE (2010) The influence of temperature on the apparent lipid digestibility in Atlantic salmon (*Salmo salar*) fed *Calanus finmarchicus* oil at two dietary levels. Aquaculture 309:143– 151. https://doi.org/10.1016/j.aquaculture.2010.08.016

13. Bucking C (2017) A broader look at ammonium production, excretion, and transport in fish: a review of impacts of feeding and the environment. J Comp Physiol B Biochem Syst Environ Physiol 187:1– 18. https://doi.org/10.1007/s00360-016-1026-9

14. Bureau DP, Hua K (2010) Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. Aquacult Res 41:777-792. https://doi:10.1111/j.1365-2109.2009.02431.x

15. Bruslé, J, Anadon GG (1996) The structure and function of fish liver. In: Munshi JSD, Dutta HM (eds.) Fish Morphology. CRC Press, Boca Raton, pp 77-93.

16. Callaghan NI, Tunnah L, Currie S, MacCormack TJ (2016) Metabolic Adjustments to Short-Term Diurnal Temperature Fluctuation in the Rainbow Trout (Oncorhynchus mykiss). Physiol Biochem Zool 89:498-510. https://doi.10.1086/688680.

17. Chadwick JG, McCormick SD (2017) Upper thermal limits of growth in brook trout and their relationship to stress physiology. J Exp Biol 220:3976-3987. https://doi.org/10.1242/jeb.161224

18. Cook JT, Sutterlin AM, McNiven MA (2000) Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). Aquaculture 188:47-63. https://doi.10.1016/s0044-8486(00)00333-1.

19. Corey E, Linnansaari T, Cunjak RA, Currie S (2017) Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). Conserv Physiol 27:cox014. https://doi.10.1093/conphys/cox014.

20. DIN 38405-11 (1983) German standard methods for the examination of water, waste water and sludge; Anions (group D); determination of phosphorus compounds (D 11)

21. DIN 38406-5 (1983) German standard methods for the examination of water, waste water and sludge; cations (group E); determination of ammonium-nitrogen (E 5)

22. DIN 38405-29 (1988) German standard methods for the examination of water, waste water and sludge - Anions (group D) - Part 29: Spectrometric determination of nitrate with sulfosalicylic acid (D 29)

23. Durhack TC, Mochnacz NJ, Macnaughton CJ, Enders EC, Treberg JR (2021) Life through a wider scope: Brook Trout (*Salvelinus fontinalis*) exhibit similar aerobic scope across a broad temperature range. J Thermal Biol 99:102929. https://doi.org/10.1016/j.jtherbio.2021.102929

24. Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK (2011) Differences in thermal tolerance among sockeye salmon populations. Science 332:109-112. https://doi: 10.1126/science.1199158.

25. Elliott JM, Elliott JA (2010) Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. J Fish Biol:77:1793-1817. https://doi.org/10.1111/j.1095-8649.2010.02762.x

26. Evans DO (1990) Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. Trans Am Fish Soc 119:585-600. https://doi.org/10.1577/1548-8659(1990)119<0585:MTCBRT>2.3.CO;2

27. Fauconneau B, Choubert G, Blanc D, Breque J, Luquet P (1983) Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. Aquaculture 34:27– 39. https://doi.org/10.1016/0044-8486(83)90289-2

28. Forsberg JA, Summerfelt RC (1992) Effect of temperature on diel ammonium excretion of fingerling walleye. Aquaculture 102:115-126. https://doi.org/10.1016/0044-8486(92)90294-U

29. Fonkwe lG, Singh RK (1996) Characterization of alkali-extracted protein prepared from deboned turkey residue. J Food Proc Pres 20:359-378. https://doi.org/10.1111/j.1745-4549.1996.tb00753.x

30. Frings CS, Dunn RT (1970) A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. Am J Clin Pathol 53:89-91. https://doi.10.1093/ajcp/53.1.89. PMID: 5410040.

31. Frisk M, Steffensen JF, Skov PV (2013) The effects of temperature on specific dynamic action and ammonium excretion in pikeperch (*Sander lucioperca*). Aquaculture 404–405:65–70. https://doi.org/10.1016/j.aquaculture.2013.04.005

32. Furné M, Morales AE, Trenzado CE, García-Gallego M, Carmen Hidalgo M, Domezain A, Sanz Rus A (2012) The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. J Comp Physiol B Biochem Syst Environ Physiol 182:63-76. https://doi.10.1007/s00360-011-0596-9

33. Gelman A, Kuz'mina V, Drabkin V, Glatman L (2008) Temperature adaptation of digestive enzymes in fish. In: Cyrino JEP, Bureau DP, Kapoor BG (eds). Feeding and digestive functions in fishes. Science Publishers, Enfield, NH, USA, p. 155–225.

34. Gingeric, AJ, Philipp DP, Suski CD (2010) Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. J Comp Physiol B Biochem Syst Environ Physiol 180:371–384. https://doi.org/10.1007/s00360-009-0419-4

35. Gräns A, Jutfelt F, Sandblom E, Jönsson E, Wiklander K, Seth H, Olsson C, Dupont S, Ortega-Martinez O, Einarsdottir I, Björnsson BT, Sundell K, Axelsson M (2014) Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. J Exp Biol 217:711–717. https://doi:10.1242/jeb.096743

36. Handeland SO, Imsland AK, Stefansson SO (2008) The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture 283:36– 42. https://doi.org/10.1016/j.aquaculture.2008.06.042

37. Huguet CT, Norambuena F, Emery JA, Hermon K, Turchini GM (2015) Dietary n-6/n-3 LC-PUFA ratio, temperature and time interactions on nutrients and fatty acids digestibility in Atlantic salmon. Aquaculture 436:160–166. https://doi.org/10.1016/j.aquaculture.2014.11.011

38. *Jezierska B, Hazel JR, Gerking SD (1982) Lipid mobilization during starvation in the rainbow trout, Salmo gairdneri Richardson, with attention to fatty acids. J Fish Biol 21:681-692.* https://doi.org/10.1111/j.1095-8649.1982.tb02872.x

39. Kajimura M, Croke SJ, Glover CN, Wood CM (2004) Dogmas and controversies in the handling of nitrogenous wastes: The effect of feeding and fasting on the excretion of ammonium, urea and other nitrogenous waste products in rainbow trout. J Exp Biol 207: 1993–2002. https://doi.org/10.1242/jeb.00901

40. Kibria G, Nugegoda D, Fairclough R, Lam P (1997) The nutrient content and the release of nutrients from fish food and faeces. Hydrobiologia 357:165–171. https://doi.org/10.1023/A:1003147122847

41. Kibria G, Nugegoda D, Fairclough R, Lam P (1998) Effect of temperature on phosphorus losses and phosphorus retention in silver perch, *Bidyanus bidyanus* (Mitchell 1838), (Teraponidae) fed on artificial diets. Aquacult Res 29:259-266. https://doi.org/10.1111/are.1998.29.4.259

42. Kieffer JD, Alsop D, Wood CM (1998) A respirometric analysis of fuel use during aerobic swimming at different temperatures in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 201:3123-3133. doi: 10.1242/jeb.201.22.3123.

43. Lahnsteiner F, Lahnsteiner E, Kletzl M (2019) Differences in metabolism of triploid and diploid *Salmo trutta f. lacustris* under acclimation conditions and after exposure to stress situations. Aquacult Res 50:2444-2459. https://doi.org/10.1111/are.14198

44. Lahnsteiner F (2022a) Hematological adaptations in diploid and triploid *Salvelinus fontinalis* and diploid *Oncorhynchus mykiss* (Salmonidae, Teleostei) in response to long-term exposure to elevated temperature. J Thermal Biol 106:103256. https://doi.org/10.1016/j.jtherbio.2022.103256

45. Lahnsteiner F (2022b) Seasonal differences in thermal stress susceptibility of diploid and triploid brook trout, *Salvelinus fontinalis* (Teleostei, Pisces). J Fish Biol 101:276-288. https://doi.10.1111/jfb.15118.

46. Lauff RF, Wood CH (1996) Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic swimming in juvenile rainbow trout. J Comp Physiol B Biochem Syst Environ Physiol 166:501–509. https://doi.org/10.1007/BF02338293

47. Lee C, Farrell A, Lotto A, MacNutt M, Hinch S, Healey M (2003) The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J Exp Biol 206:3239–3251. https://doi.org/10.1242/jeb.00547

48. Luo Y, Xie X (2009) The effect of temperature on post-feeding ammonium excretion and oxygen consumption in the southern catfish. J Comp Physiol B Syst Environ Physiol 179:681–689.

https://doi.10.1007/s00360-009-0351-7

49. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265-275.

50. McCue MD (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comp Biochem Physiol A Mol Integ Physiol, 156:1-18. https://doi.org/10.1016/j.cbpa.2010.01.002

51. Martin SA, Douglas A, Houlihan DF, Secombes CJ (2010) Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (*Salmo salar*). *BMC Genomics* 11**:**418. https://doi.org/10.1186/1471-2164-11-418

52. McKenzie DJ, Zhang Y, Eliason EJ , Schulte PM , Claireaux G, Blasco FR, Nati JJH, Farrell AP (2021) Intraspecific variation in tolerance of warming in fishes. J Fish Biol 98: 1536 – 1555. https://doi.org/10.1111/jfb.14620.

53. Medale F, Brauge C, Vallee F, Kaush SJ (1995) Effects of dietary protein energy ratio, ration size, dietary energy source and water temperature on nitrogen excretion in rainbow trout. Wat Sci Tech 31:185-194. https://doi.org/10.1016/0273-1223(95)00438-S

54. Milián-Sorribes MC, Tomás-Vidal A, Peñaranda DS, Carpintero L, Mesa JS, Dupuy J, Donadeu A, Macías-Vidal J, Martínez-Llorens S (2021) Estimation of phosphorus and nitrogen waste in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) diets including different inorganic phosphorus sources. Animals 11:1700. https://doi.10.3390/ani11061700

55. Miegel RP, Pain SJ, van Wettere WHEJ, Howarth GS, Stone DAJ (2010) Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (*Seriola lalandi*). Aquaculture 308:145–151. https://doi.org/10.1016/j.aquaculture.2010.07.036

56. Mock TS, Alkhabbaz ZHRAA, Rocker MM, Lewis MJ, Cumming EE, Smullen RP, Turchini GM, Francis DS (2022) **Gut transit rate in Atlantic salmon (*Salmo salar*) exposed to optimal and suboptimally high water temperatures.** *Aquacult Res* 53:4858-4868.https://doi.org/10.1111/are.15979

57. Murphy J, Riley JP (1962) A modified single solution method for determination of phosphate in natural waters. Analyt Chim Acta 27:31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

58. Ng WK, Sigholt T, Bell JG (2004) The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquacult Res 35:1228– 1237. https://doi.org/10.1111/j.1365-2109.2004.01131.x

59. O'Connor KI, Taylor AC, Metcalfe NB (2000) The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *J Fish Biol* 57:41-51. https://doi.org/10.1111/j.1095-8649.2000.tb00774.x

60. OENORM EN 26777 (1993) Water quality - Determination of nitrite - Molecular absorption spectrometric method.

61. Person-Le Ruyet J, Mahe K, Le Bayon N, Le Delliou H (2004) Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass. Aquaculture 237:269–280.

https://doi.10.1016/j.aquaculture.2004.04.021

62. Poli B (2010) Farmed fish welfare-suffering assessment and impact on product quality. Ital J Anim Sci 8: 139-160. https://doi.org/10.4081/ijas.2009.s1.139

63. Robb DHF (2008) Welfare of fish at harvest. In: Branson EJ (ed) Fish welfare. Blackwell Publishing Ltd; Oxford, pp 217–241.

64. *Rosenfeld JS, Van Leeuwen TE, Richards JG, Allen D (2015) Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. J Anim Ecol 84:4–20.* https://doi.org/10.1111/1365-2656.12260

65. Sharma J, Singh SP, Chakrabarti R (2017) Effect of temperature on digestive physiology, immune-modulatory parameters, and expression level of Hsp and LDH genes in *Catla catla* (Hamilton, 1822). Aquaculture 479:134-141. https://doi.10.1016/j.aquaculture.2017.05.031

66. Snyder S, Nadler L E, Bayley J S, Svendsen MBS, Johansen JL, Domenici P, Steffensen JF (2016) Effect of closed v. intermittent-low respirometry on hypoxia tolerance in the shiner perch *Cymatogaster* aggregate. J Fish Biol 88:252–264. https://doi.10.1111/jfb.12837

67. Stillwell EJ, Benfey TJ (1996) Hemoglobin level, metabolic rate, opercular abduction rate and swimming efficiency in female triploid. Fish Physiol Biochem 15:377–383. https://doi.org/10.1007/BF01875580

68. Svendsen MBS, Bushnell PG, Steffensen JF (2016) Design and setup of intermittent-flow respirometry system. for aquatic organisms J Fish Biol 88:26–50. doi:10.1111/jfb.12797

69. Sandblom E, Gräns A, Axelsson M, Seth H (2014) Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. Proc Biol Sci. 281:20141490. https://doi:10.1098/rspb.2014.1490

70. Stien LH, Oppedal F (2020) The metabolic rate response to feed withdrawal in Atlantic salmon post-smolts. Aquaculture 529:735690. https://doi.org/10.1016/j.aquaculture.2020.735690

71. Tang M, Boisclair D (1995) Relationship between respiration rate of juvenile brook trout (*Salvelinus fontinalis*), water temperature, and swimming characteristics. Can J Fish Aquat Sci 52:2138-2145. https://doi.org/10.1139/f95-806

72. Verant ML, Konsti MK, Zimmer KD, Deans CA (2007) Factors influencing nitrogen and phosphorus excretion rates of fish in a shallow lake. Fresh Water Biol 52:1968-1981. https://doi.org/10.1111/j.1365-2427.2007.01820.x

73. Verhille CE, English KK, Cocherell DE, Farrell AP, Fangue NA (2016) High thermal tolerance of a rainbow trout population near its southern range limit suggests local thermal adjustment. Conserv Physiol. 9:cow057. https://doi.10.1093/conphys/cow057.

74. Viant MR, Werner I, Rosenblum ES, Gantner AS, Tjeerdema RS, Johnson ML (2003) Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout (*Oncorhynchus mykiss*) chronically exposed to elevated temperature. Fish Physiol Biochem 29:159–171. https://doi.org/10.1023/B:FISH.0000035938.92027.81

75. Volkoff H, Rønnestad I (2020) Effects of temperature on feeding and digestive processes in fish. Temperature 7:307-320. https://doi.10.1080/23328940.2020.1765950

76. Zeng L-Q, Wang L, Wang G-N, Zeng Y, Fu S-J (2017) The relationship between growth performance and metabolic rate flexibility varies with food availability in juvenile qingbo (*Spinibarbus sinensis*). Comp Biochem Physiol A: Mol & Integr Physiol 212:56–63. https://doi.10.1016/j.cbpa.2017.07.005)

77. Waagbø R, Jørgensen SM, Timmerhaus G, Breck O, Olsvik PA (2017) Short-term starvation at low temperature prior to harvest does not impact the health and acute stress response of adult Atlantic salmon. PeerJ. 2017 27:e3273:1-22. https://doi.10.7717/peerj.3273.

78. Wang, YW, Zhang, J, Jiao, JG, Du, X, Limbu, S, Qiao, F, Zhang, ML, Li, DL, & Du, ZY (2017). Physiological and metabolic differences between visceral and subcutaneous adipose tissues in Nile tilapia (*Oreochromis niloticus*). Am J Physiol - Reg Integ Comp Physiol 313:R608-R619. https://doi.org/10.1152/ajpregu.00071.2017
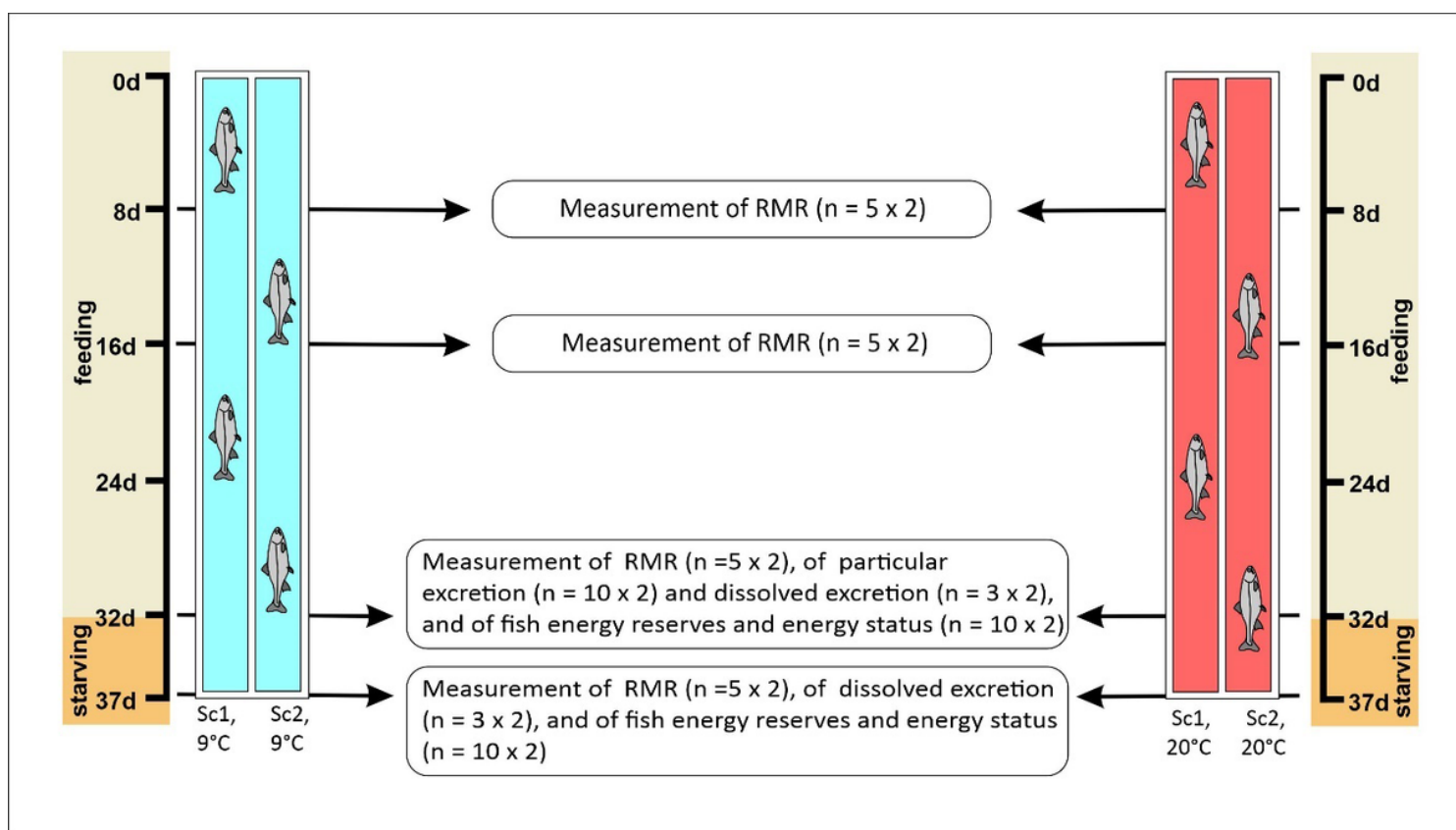
Figures

Figure 1

Scheme of the experimental design. SC – stream channel, RMR -routine metabolic rate.
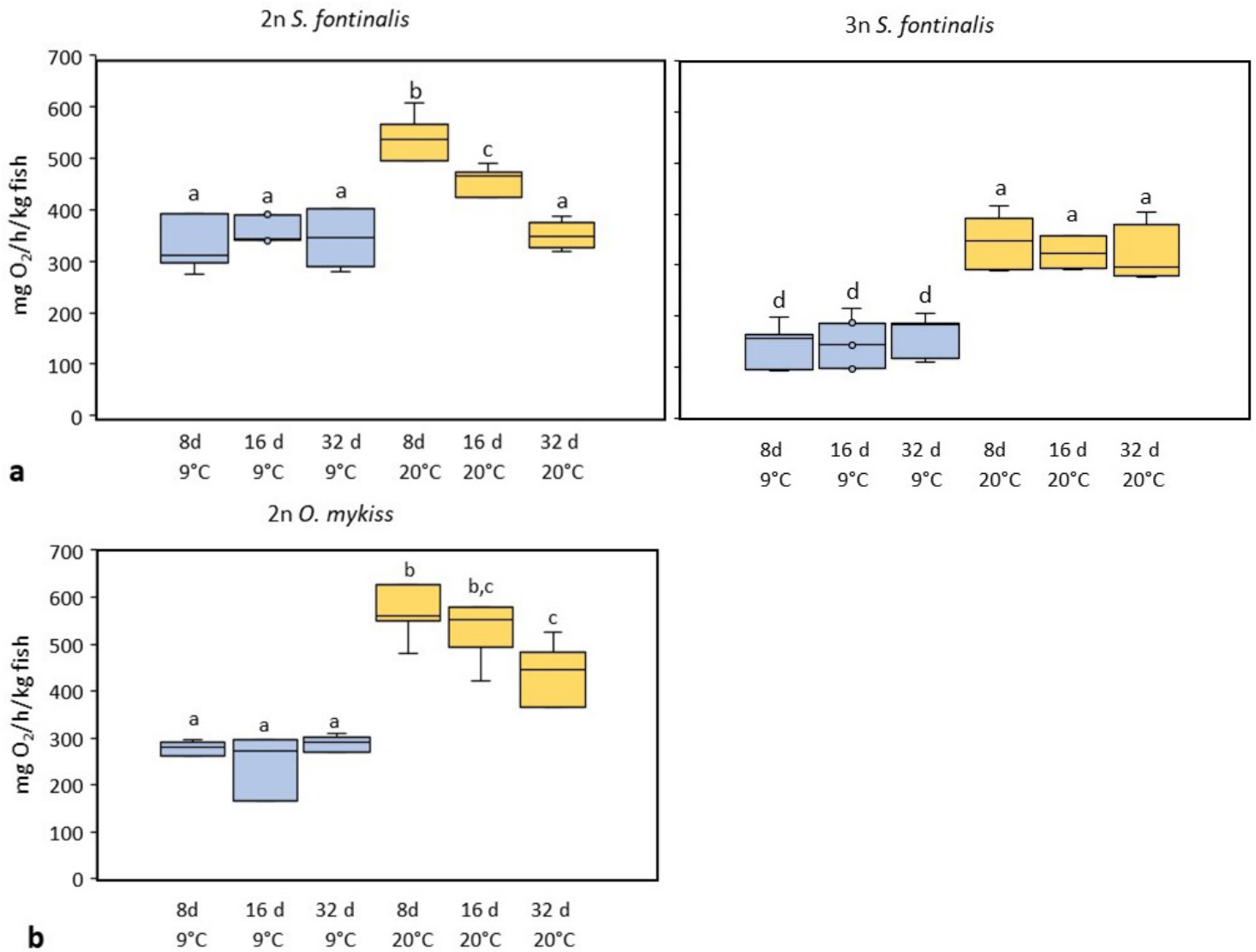
Figure 2

Boxplots of routine metabolic rate of 2n and 3n *S. fontinalis* (a, b) and 2n *O. mykiss* (c) after exposure to 20°C. Blue boxes: 9°C, orange boxes: 20°C. Data (n = 10) were compared between all species/ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).
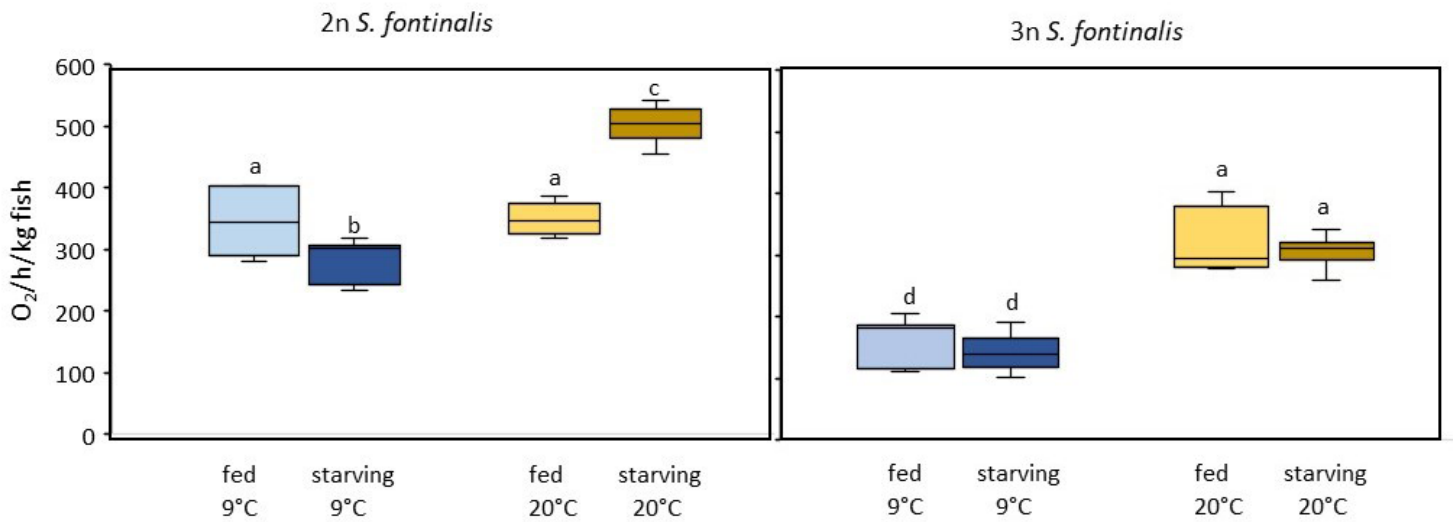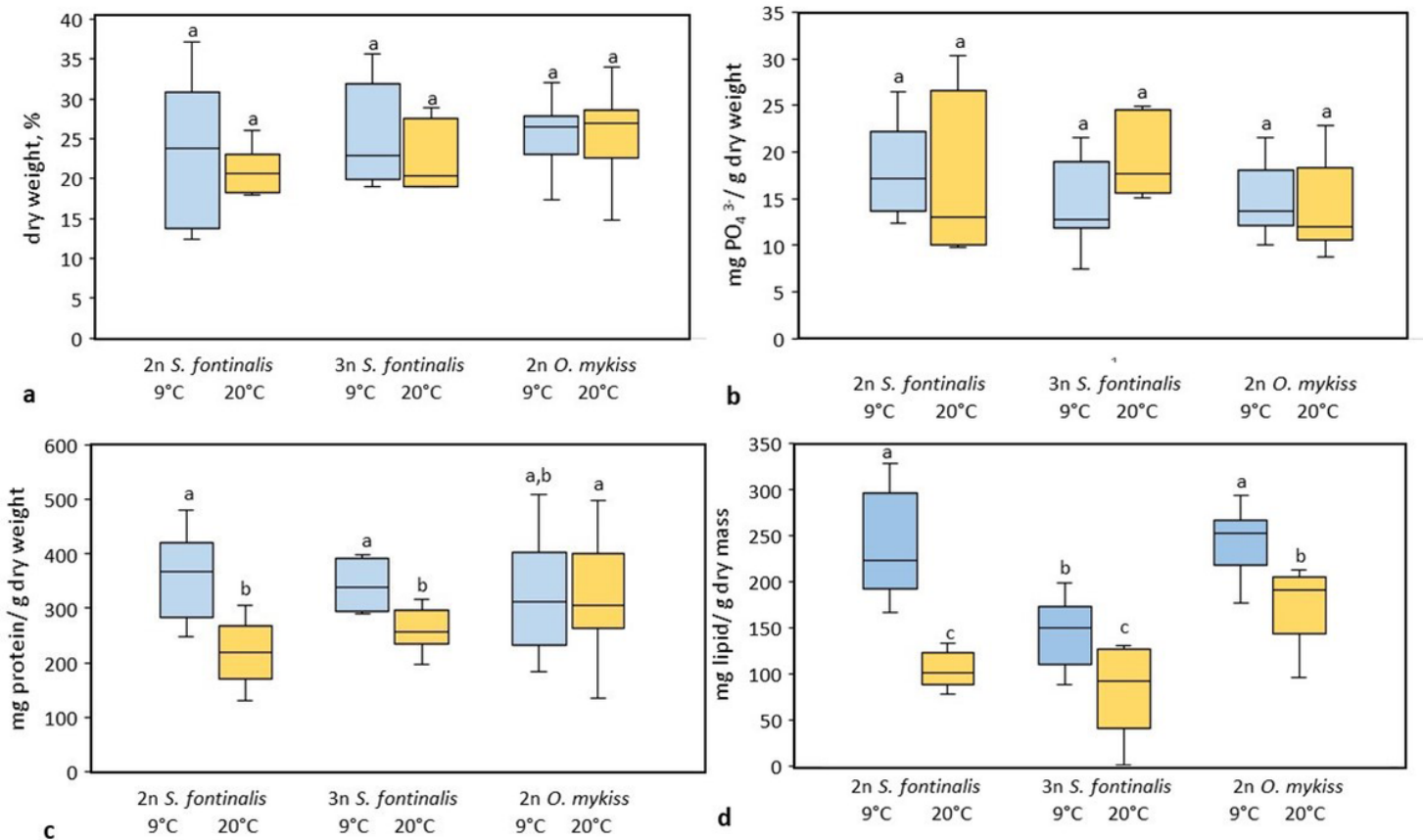
Figure 3

Routine metabolic rate of fed and 5d starving 2n and 3n *S. fontinalis* acclimated to 9°C and exposed to 20°C. Light blue boxes: fed fish at 9°C, dark blue boxes: starving fish at 9°C, light orange boxes: fed fish at 20°C, dark orange boxes: starving fish at 20°C. Data (n = 10) were compared between all ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).

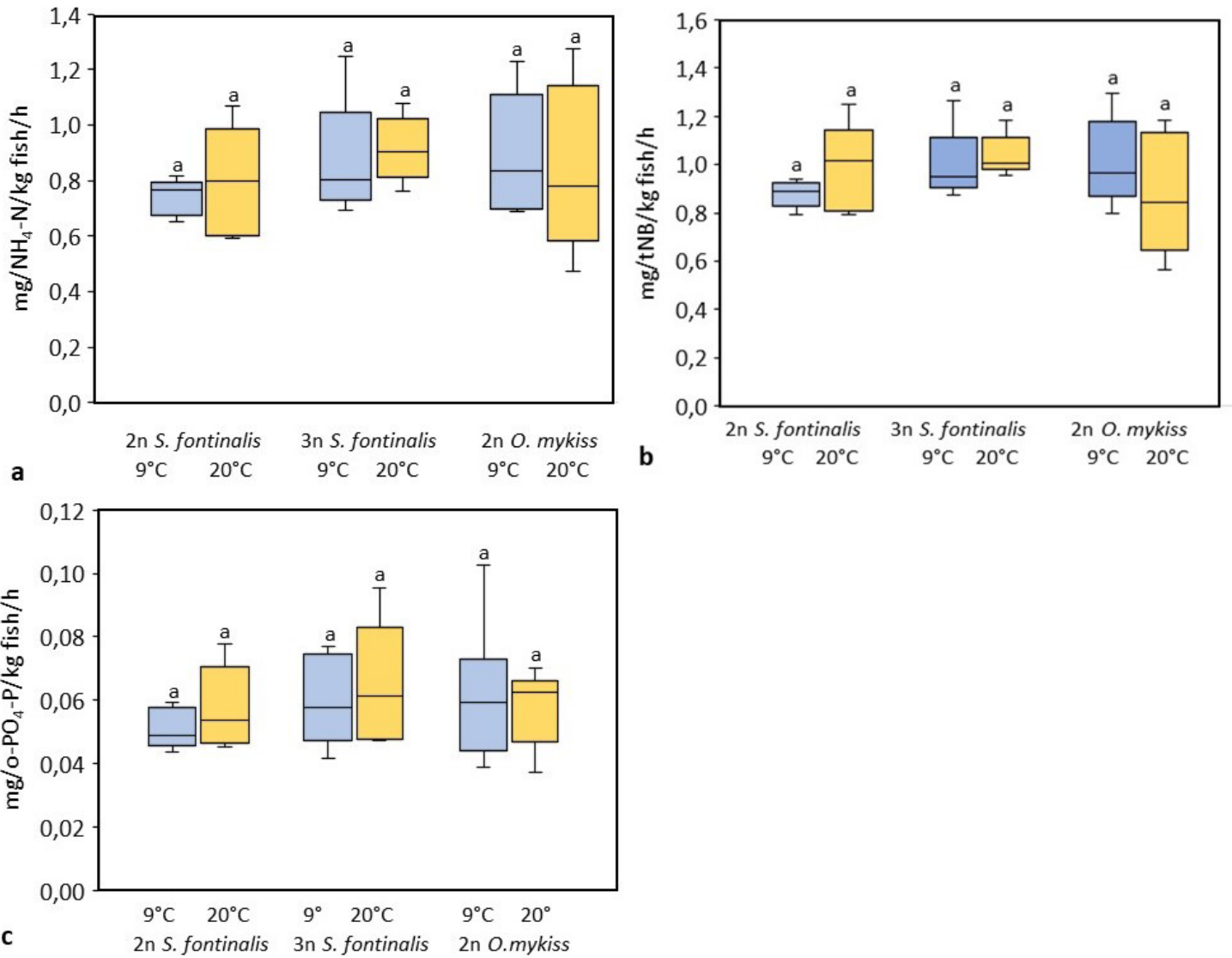**Figure 4**

Faeces dry weight (a) and phosphate (b), protein (c), and lipid (d) content of 2n and 3n *S. fontinalis* and *O. mykiss* acclimated to 9°C (blue boxes) and exposed to 20°C for 32d (orange boxes). Data (n = 20) were compared between all species/ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).
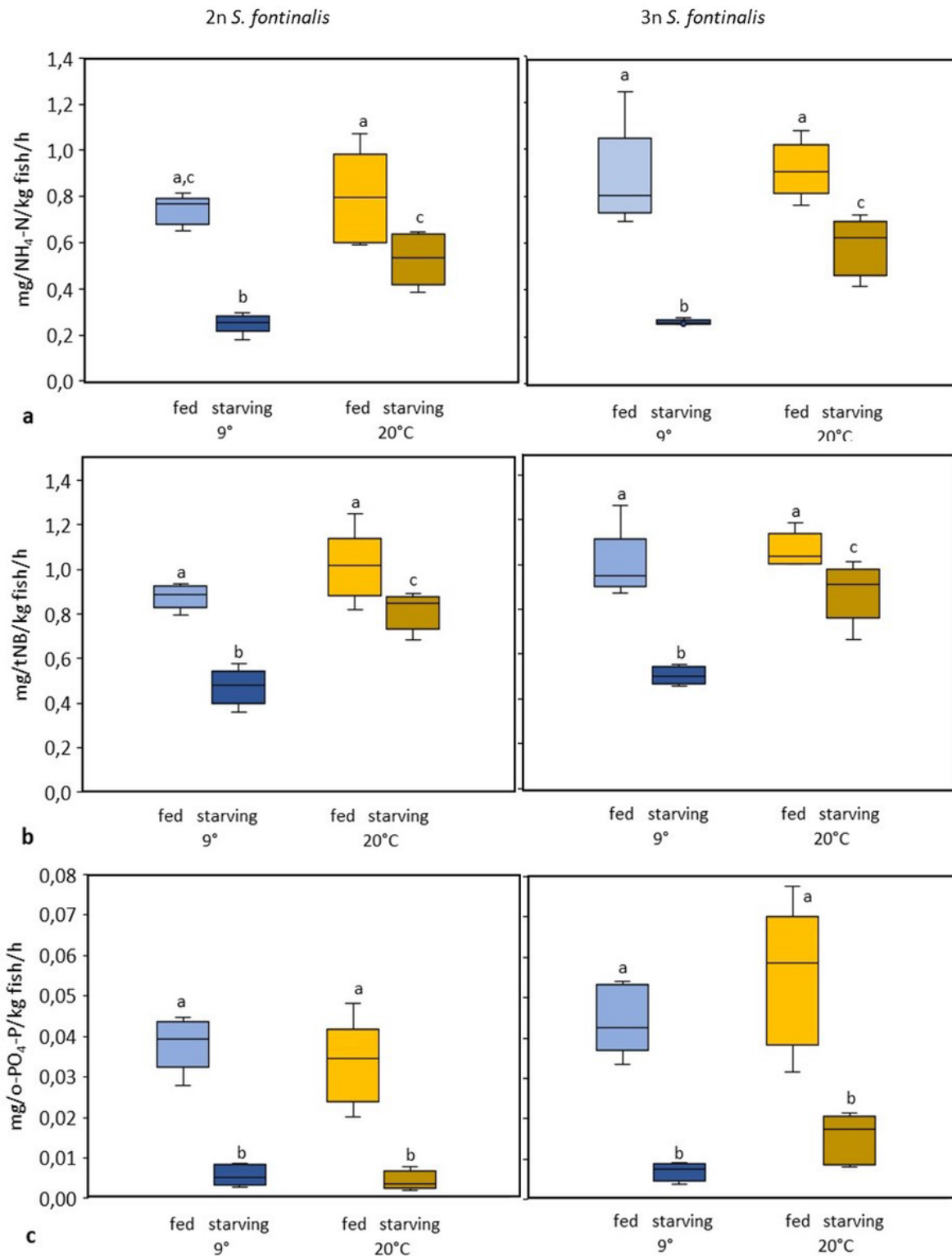
Figure 5

Ammonium (NH_4-N [a], tNB [total nitrogen bound] [b], and orthophosphate (c) excretion of 2n and 3n *S. fontinalis* and of *O. mykiss* acclimated to 9°C (blue boxes) and exposed for 32d to 20°C (orange boxes). Data (n = 10) were compared between species/ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).

Figure 6

Ammonium (NH$_4$-N [a]), tNB (total nitrogen bound [b]), and orthophosphate (c) excretion of fed and 5d starving 2n and 3n *S. fontinalis* acclimated to 9°C and exposed to 20°C. Light blue boxes: fed fish at 9°C, dark blue boxes: starving fish at 9°C, light orange boxes: fed fish at 20°C, dark orange boxes: starving fish at 20°C. Data (n = 10) were compared between all ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).
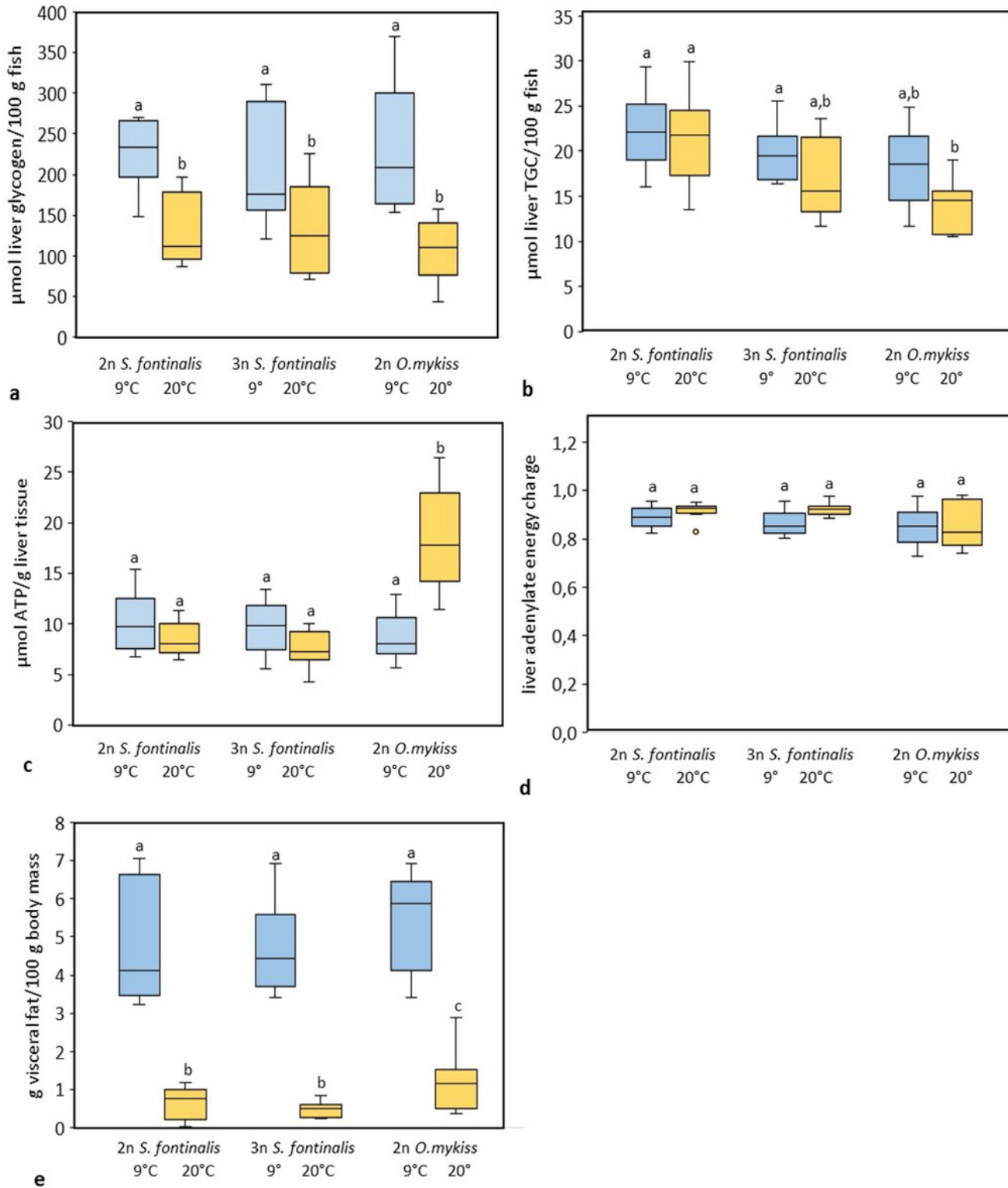
Figure 7

Liver glycogen (a), triglycerides (TGC (b), ATP (c), adenylate energy charge (d), and visceral fat (e) of 2n and 3n *S. fontinalis* and *O. mykiss* acclimated to 9°C (blue boxes) and exposed for 32d to 20°C (orange

boxes). Data (n = 20) were compared between all species/ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).
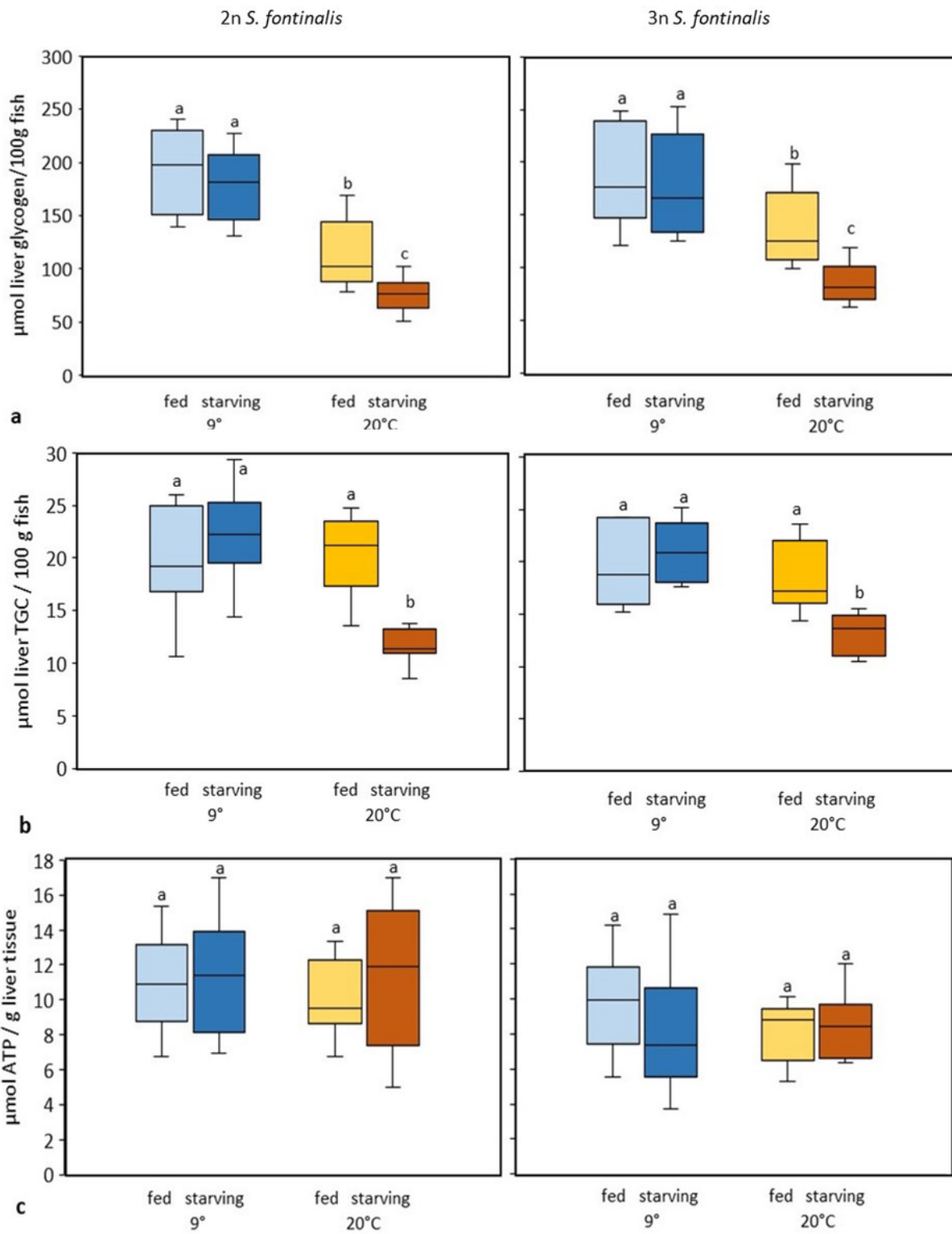

Figure 8

Liver glycogen (a), triglycerides (TGC) (b), and ATP concentrations (c) of fed and 5d starving 2n and 3n *S. fontinalis* acclimated to 9°C and exposed to 20°C. Light blue boxes: fed fish at 9°C, dark blue boxes: starving fish at 9°C, light orange boxes: fed fish at 20°C, dark orange boxes: starving fish at 20°C. Data (n = 20) were compared between all ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).
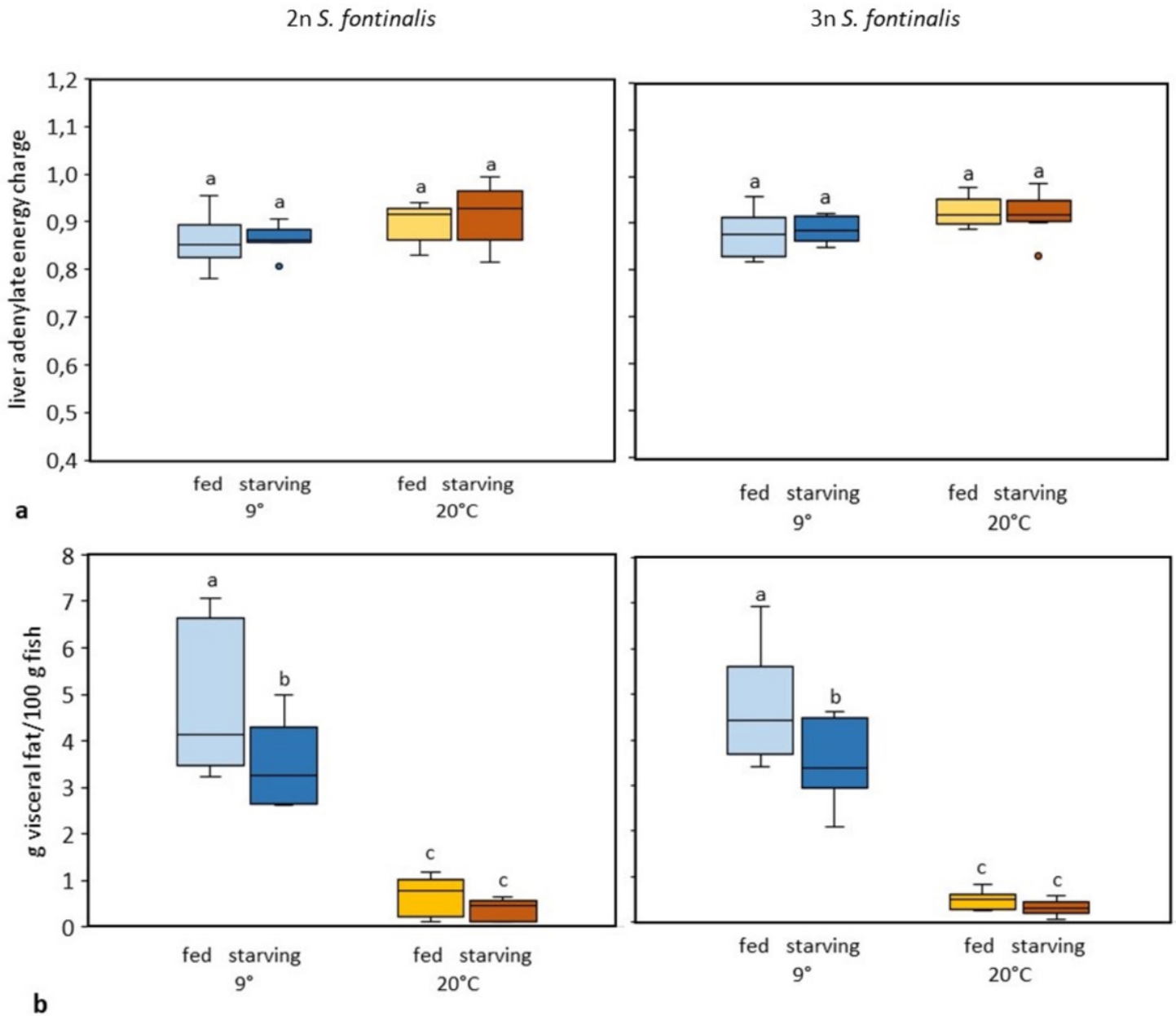

Figure 9

Liver adenylate energy charge (a) and visceral fat in relation to total body mass (b) of fed and 5d starving 2n and 3n *S. fontinalis* acclimated to 9°C and exposed to 20°C. Light blue boxes: fed fish at 9°C, dark blue boxes: starving fish at 9°C, light orange boxes: fed fish at 20°C, dark orange boxes: starving fish at 20°C. Data (n = 20) were compared between all ploidy levels and temperatures. Those superscripted by different letters are significantly different (ANOVA with subsequent Tukey posthoc test).