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Temperature and oxygen related ecophysiological traits of snow trout (*Schizothorax richardsonii*) are sensitive to seasonal changes in a Himalayan stream environment

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ABSTRACT

In this study, we investigated the seasonal changes in key eco-physiological traits of a wild population of snow trout, *Schizothorax richardsonii* from river Gola in the Indian Himalayan region over one year. Live specimens (5.8–31.4 g) were electro-fished from their natural habitat during representative months of four seasons with notable differences in water temperature, oxygen concentration and saturation. After 24–72 h of captive-acclimation, the fishes were examined for upper and lower critical thermal limits (CT_{max} and CT_{min}), incipient lethal oxygen thresholds (ILOC and ILOS), apparent routine and maximum oxygen consumption rates (M_{O2rout} and M_{O2max}), and blood haemoglobin-haematocrit. Across the seasons, mean CT_{min} and CT_{max} values ranged from ~0 to 34.6 °C, suggesting a relatively wide acute thermal tolerance range for this predominantly cold-water fish. Changes in the habitat's thermal condition during winter to summer was reflected in the CT_{min} (~0–2.4 °C) and CT_{max} (31.7–34.4 °C) estimates, while the highest thermal scope (CT_{max}-CT_{min}; 33.2 °C) was recorded in autumn. Concurrently, the incipient lethal hypoxia threshold observed in autumn (ILOS-2.6% and ILOC-0.19 mgO_2/L) was significantly lower than the other three seasons, possibly linked to warm-acclimation. The reduction in blood haemoglobin-haematocrit levels during winter could limit the oxygen carrying capacity, with possible reciprocations in thermal tolerance and aerobic metabolism. Concerning body mass corrected oxygen consumption, the apparent M_{O2rout} was found to increase in a temperature-dependent manner from 150.3 $mgO_2/kg/h$ at 12 °C to 315.2 $mgO_2/kg/h$ at 26 °C, with Q_{10} ranging from 1.6 to 2.2. Whereas, changes in M_{O2max} was not temperature sensitive (Q_{10} of 0.7–1.3), except during spring-summer (Q_{10}-2), with lowest and highest measurements in spring and autumn (934 and 1514 $mgO_2/kg/h$), respectively. Collectively, these data form the first information report on the seasonal plasticity in thermal and respiratory physiology of a Schizothoracine fish species, bearing significance for their conservation, aquaculture and habitat monitoring.

1. Introduction

The snow trout, *Schizothorax richardsonii* (Gray, 1832) is a rheophilic, demersal cyprinid fish species, which is widely distributed in the ecologically fragile freshwater streams and rivers of the Indian Himalayan region. It forms an integral part of the local subsistence fisheries and is slowly gaining attention as a potential candidate for aquaculture. The ecological factors that determine their natural occurrence and geographic distribution are water discharge, current velocity, temperature, dissolved oxygen, substratum and food availability (Sehgal, 1999; Sharma and Mehta, 2010). In stream ecosystems, most of the above factors are known to fluctuate with the annual cycle of seasons and to cope, resident fishes either adapt their physiology and behaviour or relocate to a more suitable environment (McCullough et al., 2009). Environmental extremities are known to drive up and downstream migration of *S. richardsonii* (Sehgal, 1999), but hitherto there is no comprehensive information on their ecophysiological adaptations.

As per the thermal primacy paradigm, especially in the case of ectotherms like fishes, changes in environmental temperature is the abiotic master factor which shapes all their life sustaining physiological processes and performance attributes (Angilletta et al., 2002; Somero, 2010). Nevertheless, thermal tolerance zone of a fish is known to

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reversibly or irreversibly change with diel temperature changes, natural seasons, acclimation regimes, life stages, spawning status and nutritional condition (Beitinger et al., 2000). The degree of this thermal plasticity is suggested to play a crucial role in the life history and evolutionary success of any fish (Ficke et al., 2007). The reported natural thermal range of occurrence of Indian snow trout is from near zero to 27 °C (Sharma and Mehta, 2010). Changes in ambient temperature are known to influence the ovulation status, spawning activity, body morphology and haematology of *S. richardsonii* (Qadri et al., 1983; Sehgal, 1999; Kapila et al., 2002; Rajput et al., 2013). Critical thermal maximum (CT_{max}) estimates have been reported to be in the range of 31–34.5 °C for geographically distinct and captive bred congeneric Schizothoracine fishes, *S. kozlovi* (He et al., 2014) and *S. prenanti* (Yang et al., 2018). However, sub-lethal temperature threshold of neither *S. richardsonii* nor any wild population of Schizothoracine fish has been investigated prior to this study, at both the ends of the temperature spectrum.

Further, the onset of thermal limitation of life sustaining processes during acute thermal challenges has been linked to a decrease in aerobic performance, which is characterized by a mismatch between the oxygen demand and supply to tissues (Pörtner, 2002; Pörtner and Knust, 2007; Pörtner and Farrell, 2008). Though the ecological relevance and universality of this relationship between thermal tolerance and aerobic performance is controversial (Jutfelt et al., 2018), it is agreed that episodes of hypoxia and high-temperature are likely to co-occur, and may interact in a variety of ways while impacting the cardiorespiratory and aerobic metabolic processes in fish (Farrell, 2009; McBryan et al., 2013). Measures of blood haemoglobin and haematocrit levels, routine and maximal rate of oxygen consumption per unit time (M_{O2rout} and M_{O2max}), and incipient lethal oxygen threshold (ILOC) are considered to be good indicators of environment induced changes in oxygen transport, aerobic metabolism and hypoxia tolerance in fish (Claireaux and Chabot, 2016; Killen et al., 2016; Muñoz et al., 2018). By definition, M_{O2rout} is the amount of oxygen consumed by a post-absorptive, undisturbed fish; M_{O2max} is the amount of oxygen consumed at a state of maximal sustained activity; and ILOC is the minimal oxygen level at which fish cannot maintain equilibrium (McBryan et al., 2013; Claireaux and Chabot, 2016). Concerning available information in congeneric Schizothorazine fishes, the oxygen consumption rate of *Schizothorax chongi* was estimated to range from 306.6 to 1294.1 $mgO_2\ kg^{-1}\ h^{-1}$ at 26 °C, with increasing flow rate in a flume-type swimming respirometer (Tu et al., 2011). Likewise in *S. prenanti*, the mean M_{O2rout} was found to vary from 292.4 $mgO_2\ kg^{-1}\ h^{-1}$ at 15 °C to 368.6 $mgO_2\ kg^{-1}\ h^{-1}$ at 23 °C, in a swim chamber (Cai et al., 2014). Whereas in a closed system, the body mass-specific oxygen consumption rate of *S. plagiostomus* was reported to be in a relatively lower range of 130–260 $mgO_2\ kg^{-1}\ h^{-1}$ at 14 ± 2 °C, depending on the fish strain and density (Kvigstad, 2013). Further, based on loss of equilibrium and aquatic surface respiration responses, the hypoxia tolerance threshold of *S. prenanti* was reported to be 0.4–0.6 mgO_2/L (Fu et al., 2014). Similar understanding of variability in these oxygen related indices is highly essential to predict future ecological impacts of climate change on *S. richardsonii* populations, as their performance and survival will depend on the interactions between their temperature and oxygen related ecophysiology (Pörtner, 2001; Pörtner and Farrell, 2008; Somero, 2010).

At present, *S. richardsonii* population in the wild is reported to be declining due to anthropogenic activities like habitat destruction and overexploitation, and as a result, this species is categorized as vulnerable in the IUCN red list (Vishwanath, 2010). Coupled to this, climate change driven environmental fluctuations in freshwater ecosystems could attenuate the performance of snow trout at various biological levels, as coldwater fishes are considered to have finite adaptive potential (McCullough et al., 2009; Woodward et al., 2010). In this context, the present study was undertaken to generate fundamental information about the physiological ability of *S. richardsonii* to cope with seasonal environmental changes, particularly in terms of its thermal tolerance, hypoxia limits and aerobic capacity.

2. Materials and methods

2.1. Fish collection and holding conditions

Live specimens of snow trout, *Schizothorax richardsonii* of mixed sexes were collected from the torrential Kalsa tributary of river Gola in Chaffi, Uttarakhand, India (29° 22′ N and 79° 34′ E at 1200 m elevation). Fishes were caught once during the representative month of each season in 2017-18 (September - autumn, December - winter, April - spring and July - summer), using a back-pack electrofisher (Safari Research 550-E, Smith-Root, Dublin) with pulsed direct current of 250 V. The minimum, maximum and average body mass and total length of the experimental fishes was 5.8, 31.4 and 12.7 ± 4.5 g (mean ± SD); and 9.4, 15.8 and 11.7 ± 1.4 cm, respectively. Within 3 h of capture, they were packed in oxygen filled plastic bags containing water from the sampling site and brought to the wet laboratory facility of ICAR-Directorate of Coldwater Fisheries Research at Bhimtal. They were then placed in 1000 L rectangular tanks with flow-through (2–3 $L\ min^{-1}$) freshwater, at an ambient temperature similar to their habitat, i.e., 13 ± 1 °C in winter, 21 ± 1 °C in spring, 24 ± 1 °C in summer and 21 ± 0.5 °C in autumn. In the acclimation tank, within 4–5 h, the captive specimens showed no visible signs of stress and started consuming the compounded dry feed pellets (40% protein and 7% lipid). Further, during the acclimation period, no abnormal behaviour or mortality was recorded and the fishes were fed twice a day. After 24–72 h of acclimation, the fishes were taken for experimentations.

2.2. In-situ water quality analyses

Water quality readings at the fish collection site such as temperature, dissolved oxygen, oxygen saturation, pressure, pH, conductivity, salinity, total dissolved solids, carbon dioxide, alkalinity and total hardness were recorded once during each sampling season. Temperature (°C) readings were taken using a precise digital thermometer (TP3001, China). Dissolved oxygen concentration (mg/L), oxygen saturation (%) and water pressure (hPa) were measured using a multi-parameter LDO probe (HQ40d, Hach, USA). pH, conductivity (mS) and salinity (ppt) were measured using a pocket digital combometer (Hanna, USA). Total dissolved solids (mg/L) was measured using a pocket TDS meter (Himedia, India). Carbon dioxide (mg/L), alkalinity (mg/L) and total hardness (mg $CaCO_3$/L) were colorimetrically estimated using commercially available drop titration kits (Himedia, India), following the manufacturer's protocol.

2.3. Determination of upper and lower critical thermal limits

The critical temperature maximum (CT_{max}) and minimum (CT_{min}) of *S. richardsonii* were determined using the standard critical thermal methodology (Beitinger et al., 2000), where the experimental fish is subjected to constant continuous linear increase or decrease in temperature until a near lethal (but sub-lethal) endpoint is reached. The equipment that we used for CT_{max} measurement was a 10 L laboratory water bath provided with a heating electrode, thermostat and water circulation (LabTech, UK), whereas for CT_{min} analysis, an aquarium chiller (Hailea HC-1000A, China) was connected to a 40 L circular tub to which ice was added at the final stages (below 3 °C). Both the setups ensured homogenous temperature within the entire unit. A factory calibrated digital thermometer with ± 0.1 °C accuracy (TP3001, China) and handheld multi-parameter probe (HQ40d, Hach, USA) were concurrently used to measure the water temperature in the experimental unit. On the day of analysis, fishes (a different set of 10 individuals for CT_{max} and CT_{min} tests, N = 10 per season) were randomly taken from the holding tank with minimum disturbance and placed in a meshed basket kept either inside the thermostatic water bath for CT_{max} study or in the chiller-circular tub for CT_{min} analysis. The initial temperature was set to 12, 21, 25 and 20 °C for winter, spring, summer and autumn measurements, respectively. Water was then heated or cooled at a constant rate of 0.3 °C per minute to allow the fish's body temperature to closely track water temperature and to prevent it from gaining tolerance (Becker and Genoway, 1979). Exceptionally, there was difficulty in achieving linearity in the rate of temperature decrease, when cooling was below 4 °C. Throughout the experiment, changes in the behaviour of the fishes and water temperature were continuously monitored to record the exact temperature at which they reach their thermal end points. Loss of equilibrium or righting response was considered the CT_{max} end point, whereas non-reaction to prodding was the CT_{min} end point (Ford and Beitinger, 2005). When an individual reached the thermal end point, the corresponding temperature, time taken and length-weight were measured, before placing the fish in the recovery tank. An oxygen concentration above 90% saturation was maintained throughout the test using aerators. For each season, critical temperature values were determined within 72 h of capturing the fish.

2.4. Determination of oxygen consumption rates and hypoxia tolerance limits

Static respirometry is considered optimal for oxygen consumption measurements in fishes like *S. richardsonii,* which are inactive under resting conditions (Clark et al., 2013). So, our simple closed experimental set-up comprised a rectangular air-tight fish containing chamber and a high-precision optical sensor based dissolved oxygen probe (HQ40d, Hach, USA). The water holding capacity of the respirometer was 475 ml and the ratio of fish mass to water volume ranged from a minimum of 1:20 to a maximum of 1:80 (with an average of 1:37). The measurement protocol was similar to previous studies in freshwater carps (Chatterjee et al., 2004; Das et al., 2004; Akhtar et al., 2013; Sharma et al., 2015). Before the actual oxygen consumption trials, a blank run was carried out without fish, to quantify and ensure that the reduction in oxygen concentration due to microbial respiration was negligible in the respirometer. Same individual fishes (N = 8 per season) were used for measuring both maximum (M_{O2max}) and apparent routine (M_{O2rout}) rate of oxygen consumption. Firstly, a standard chase protocol was employed to measure M_{O2max} (Norin and Clark, 2016), where individual fishes were randomly selected, placed in a circular tub (for unimpeded swimming) and manually chased with a hand net for 3 min. Upon exhaustion, the fish was further exposed to air for 30 s by taking it out of water in a hand net, to maximally increase the metabolic demands. Then the exercised individual fish was immediately placed in the static respirometer set-up and oxygen readings were monitored for 10 min (in 2 min intervals). Within this period, a maximum peak in oxygen consumption rate was observed (for 95% of the fishes, M_{O2max} occurred within 6 min, across seasons). Soon after this procedure, the fish was individually housed in a respirometer chamber-like unit (475 ml rectangular box) with a perforated lid and placed in a water tub maintained at the corresponding seasonal temperature, with continuous oxygenation. In this arrangement, the fishes were left undisturbed for a minimum period of 24 h and were not fed to ensure a post-absorptive resting state. After the holding period, the fish containing unit was slowly taken out of the tub with minimum disturbance, oxygen probe was placed inside and the container was tightly sealed, allowing only the fish's respiration to decrease the oxygen concentration of water. After an initial stabilization period, changes in oxygen concentration recorded over 10 time intervals within a 30 min measurement segment (where variation was minimum) was chosen to calculate the oxygen consumption rates. The R^2 values for the slopes of decline in oxygen as a function of time varied from 0.938 to 0.995 in winter, 0.959–0.998 in spring, 0.982–0.996 in summer and 0.904–0.99 in autumn. During the measurement period, the fishes were continuously observed and found to be passive in the respirometer chamber, with occasional changes in orientation. But, as the M_{O2rout} measurements were based on short-term and less robust static respirometry, we express our observation as apparent M_{O2rout} of snow trout. The simple formula used for calculating oxygen consumption (applicable to both apparent M_{O2rout} and M_{O2max}) is as follows.

Rate of oxygen consumption (mg O_2/kg/h) = [(Final oxygen concentration × Respirometer volume) - (Initial oxygen concentration × Respirometer volume)]/(Metabolic weight of fish × ΔTime).

Subsequent to the M_{O2rout} procedure, incipient lethal oxygen level (ILOC/ILOS) were also measured in the same set of individual fishes, by holding them in the respirometer chamber (without flushing freshwater throughout the trial) and taking oxygen readings continuously until it lost its righting response or equilibrium. ILOC and ILOS were noted as the respective oxygen concentration and saturation at which each fish lost its equilibrium (Claireaux and Chabot, 2016). Once ILOC was reached, the fish was transferred to a recovery tank after quickly measuring its length and weight. In each season, all the oxygen consumption and hypoxia tolerance indices were estimated within one week of capturing the fish. The thermal coefficient (Q_{10}) values for apparent M_{O2rout} and M_{O2max} were calculated using the standard formula (Clarke and Johnston, 1999).

2.5. Analysis of blood haemoglobin-haematocrit and fish condition

Another set of snow trouts (N = 8 individuals per season) were mildly anaesthetized in clove oil (40 µl/L of water) and blood was collected from the caudal vein using a 27 gauge needle and 1 ml syringe, pre-rinsed with anticoagulant (2.7% EDTA) solution. 10 µl of the collected blood sample was immediately used to estimate the haemoglobin and haematocrit levels using a calibrated digital point of care testing device (Mission Hb, Acon, USA). The length (cm) and weight (g) measurements of individual fishes recorded after every analysis was finally used to estimate the physical body condition of the wild caught specimens (N = 36 per season), calculated as Fulton's condition factor using the following formula.

Fulton's condition factor (K) = 100 × [Weight of fish/(Total length of fish) ^{3}].

2.6. Statistical analyses

All the statistical analyses were performed using SPSS statistics software (version 25, IBM Corporation, USA) and results are presented as means ± SD. One-way analysis of covariance (ANCOVA) was used to find out the significant difference between body mass adjusted seasonal values of the analysed temperature, oxygen and blood indices, with season as fixed factor and body mass as covariant. This was followed by post-hoc multiple comparisons using the Bonferroni test. Differences were considered statistically significant when the *P* value was less than 0.05.

3. Results

3.1. In-situ water quality parameters

Among the different physicochemical properties examined in the water flowing at the fish collection site, remarkable seasonal differences were observed only in water temperature, dissolved oxygen concentration and saturation (Table 1). During the study period, water temperature showed a clear annual cyclic pattern of increase from winter (12.2 °C) to spring (21.8 °C) to summer (26.1 °C) and a subsequent decrease in autumn (20.9 °C). At the same time, the highest concentration of dissolved oxygen was observed in spring (11.2 mg/L), followed by winter, summer and autumn (9.9, 8.4 and 8.1 mg/L, respectively); and oxygen saturation percentage showed a decreasing

Table 1
In situ water temperature and oxygen content at the time of fish collection during the different seasons.

Parameter	Winter	Spring	Summer	Autumn
Temperature (°C)	12.2 ± 0.2	21.8 ± 0.6	26.1 ± 0.7	20.9 ± 0.3
Dissolved oxygen (mg/L)	9.91 ± 0.07	11.21 ± 0.5	8.36 ± 0.2	8.14 ± 0.06
Oxygen saturation (%)	106.8 ± 0.3	151.8 ± 3.4	120.7 ± 1.6	108.1 ± 0.3

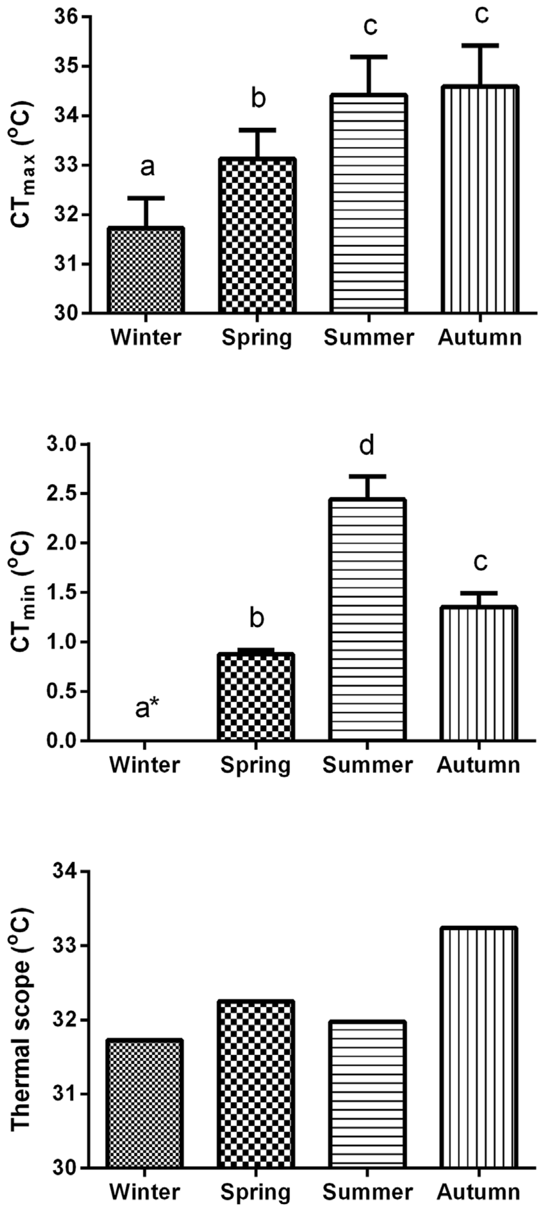

Fig. 1. CT_{max}, CT_{min} and thermal scope values of *S. richardsonii* during different seasons. CT values are expressed as means ± SD (N = 10 fish per season). Different superscript letters (a, b, c, d) in the same series indicate significant difference (ANCOVA, $p < 0.05$). * Fishes did not show end-point (i.e., non-reaction to prodding) even at 0 °C in winter.

Table 2
Intra-annual transitions in the major *in situ* water quality indices and related ecophysiological variables in *S. richardsonii*.

Parameter	Δ Winter-Spring	Δ Spring-Summer	Δ Summer-Autumn	Δ Autumn-Winter
In situ water temperature (°C)	+9.6	+4.3	- 5.2	- 8.7
In situ dissolved oxygen content (mg/L)	+1.3	- 2.9	- 0.2	+1.8
In situ oxygen saturation (%)	+45.0	- 31.1	- 12.6	- 1.3
Mean CT_{max} (°C)	+1.4	+1.3	+0.2	- 2.9
Mean CT_{min} (°C)	+0.9	+1.6	- 1.1	- 1.4
Thermal scope (°C)	+0.5	- 0.3	+1.3	- 1.5
Mean ILOS (%)	- 0.3	- 1.5	- 2.2	+4.0
Mean ILOC (mg/L)	- 0.11	- 0.14	- 0.15	+0.4
Mean M_{O2rout} (mgO2/kg/h)	+89.6	+75.3	- 19.0	- 145.9
Mean M_{O2max} (mgO2/kg/h)	- 303.9	+316.4	+258.7	- 271.2
Mean haemoglobin (g/dL)	+1.7	+0.5	- 0.3	- 2.0
Mean haematocrit (%)	+5.0	+1.8	- 0.9	- 5.9
Mean condition factor	- 0.08	- 0.005	+0.06	+0.02

trend from spring to winter (151.8–106.8%). Apart from these, the observed values of pH, 7.7–8.2; alkalinity, 30–50 mg/L; total hardness, 15–40 mg $CaCO_3$/L; carbon dioxide, 2–6 mg/L; total dissolved solids, 40–60 mg/L; conductivity, 0.07–0.11 mS; salinity, 0.03–0.07 g/L; and pressure, 870–885 hPa showed no notable difference between seasons and fell within the acceptable limits for cyprinid fishes.

3.2. Maximum and minimum critical thermal tolerance limits

The season-wise critical thermal tolerance limits of *S. richardsonii* are presented in Fig. 1. During the study period, individual CT_{max} readings of snow trout ranged from 30.4 to 35.8 °C. The mean CT_{max} value in winter (31.7 ± 0.6 °C) was 2.7–2.9 °C lower than in summer and autumn (34.4 and 34.6 °C, respectively), whereas spring CT_{max} was in the intermediate range (33.1 °C). At the other end of the temperature spectrum, mean values of CT_{min} differed season to season in the following order: summer (2.4 °C) > autumn (1.4 °C) > spring (0.9 °C) > winter (~0 °C). Individual CT_{min} response ranged from ~0 to 3 °C across seasons. Particularly in winter, none of the examined fish reached the expected CT_{min} end-point (non-reaction to prodding), even at freezing conditions (~0 °C). Across seasons, the highest and lowest thermal scope (CT_{max} - CT_{min}) of snow trout was observed in autumn (33.1 °C) and winter (31.7 °C), respectively, with intermediate values in spring (32.2 °C) and summer (32 °C). Further, the transitional changes in CT_{max}, CT_{min} and thermal scope in relation to increasing and decreasing trends in environmental temperature are presented in Table 2. The maximum rise in thermal scope occurred during the summer-autumn phase, as CT_{max} remained high and CT_{min} decreased with decreasing water temperature.

3.3. Incipient lethal thresholds of hypoxia

Seasonal variations in incipient lethal oxygen threshold indices of *S. richardsonii* are shown in Fig. 2. From winter to autumn, a decreasing trend was observed for ILOC and ILOS values, with significant lower readings (0.19 mgO_2/L, 2.6%) only in autumn. Across the seasons, individual ILOC and ILOS values ranged from 0.1 to 1.0 mgO_2/L and 1.4–11.2%, respectively. The annual transition in ILOC showed a gradual decrease from winter to autumn and a sudden increase during autumn to winter phase, without any clear-cut relationship with the environmental changes in temperature and dissolved oxygen (Table 2).

3.4. Apparent routine and maximum oxygen consumption rates

With respect to metabolic oxygen consumption corrected for body mass, the apparent M_{O2rout} was significantly lower in winter than the other three seasons (Fig. 3). Corresponding to the seasonal changes in water temperature, M_{O2rout} showed an increasing trend from 150.3 ± 48.2 mgO_2/kg/h in winter to 315.2 ± 14.8 mgO_2/kg/h in

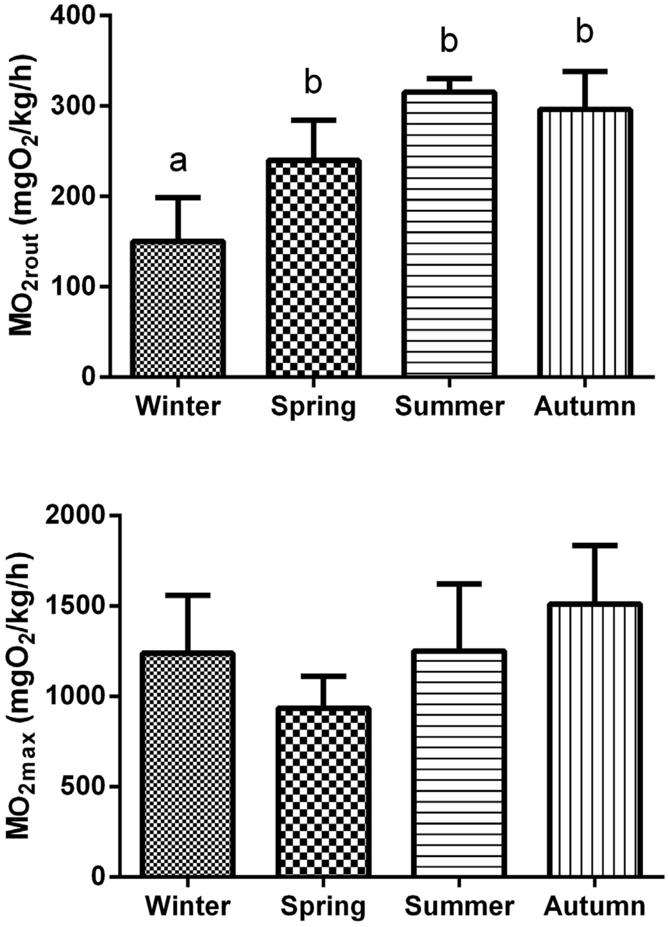

Fig. 2. Apparent routine (M_{O2rout}) and maximum (M_{O2max}) oxygen consumption rates of *S. richardsonii* during different seasons. Values are expressed as means ± SD (N = 8 fish per season). Different superscript letters (a, b) in the same series indicate significant difference (ANCOVA, $p < 0.05$).

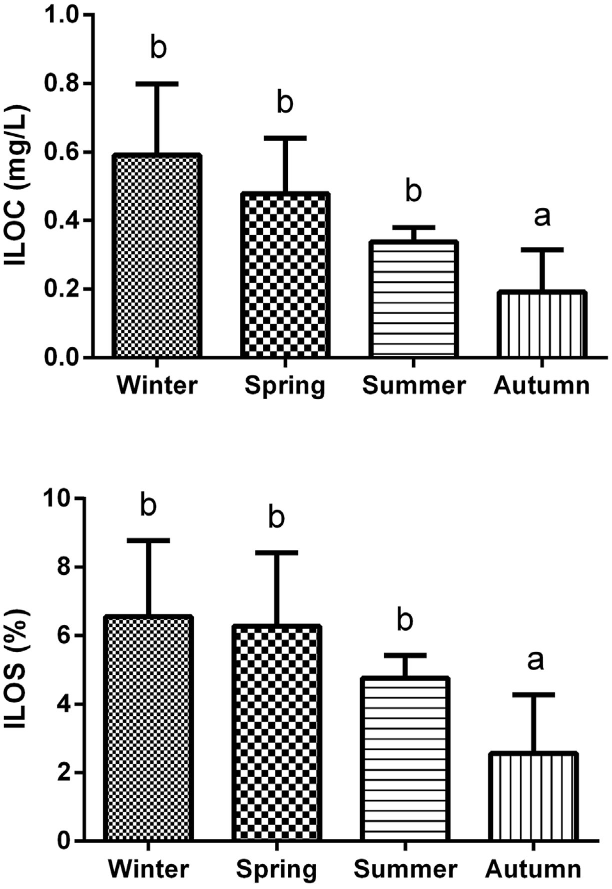

Fig. 3. Incipient lethal oxygen limits (ILOC/ILOS) of *S. richardsonii* during different seasons. Values are expressed as means ± SD (N = 8 fish per season). Different superscript letters (a, b) in the same series indicate significant difference (ANCOVA, $p < 0.05$).

summer, and then slightly decreased in autumn. The temperature coefficient (Q_{10}) of M_{O2rout} ranged from 1.6 to 2.2 for the different increasing temperature phases (Table 3). On the other hand, M_{O2max} was found to be highest in autumn (1513.6 ± 355.7 $mgO_2/kg/h$), lowest in spring (934 ± 174.9 $mgO_2/kg/h$) and intermediate in winter and summer (1237.9–1250.4 $mgO_2/kg/h$), but with no significant difference between the seasons. Q_{10} estimates of M_{O2max} ranged between 0.7 and 1.3, except for the sharp increase (Q_{10} of 2) during spring-summer. While seasonal transitions in M_{O2rout} showed a linear relationship with changes in the surrounding water temperature (Table 2), changes in M_{O2max} was inversely related to the increasing and decreasing trends in *in situ* dissolved oxygen concentration.

3.5. Blood indices and fish condition

In the blood of the experimental fishes, the average haemoglobin concentration was found to vary from 7.2 to 9.4 g/dl and haematocrit values ranged from 21.1 to 27.9% during the different seasons (Fig. 4). Winter measurements of haemoglobin and haematocrit were significantly lower than the other three seasons. The intra-annual transitions in haemoglobin and haematocrit was a clear reflection of the seasonal changes in water temperature (Table 2). Concerning body morphology, as per the calculated Fulton's condition factor (K), body condition of all the experimental fishes was in the mean range of 0.73–0.82. Nevertheless, season-wise, the K value of winter caught fishes were significantly higher than the spring and summer specimens. Annually, the K value shows a decreasing phase during winter to summer and increasing phase during summer to winter, inverse to the change in water temperature.

Table 3
Temperature co-efficient (Q_{10}) for oxygen consumption rates of *S. richardsonii.*

Oxygen consumption index	Winter-Spring	Spring-Summer	Winter-Summer	Winter-Autumn
M_{O2rout}	1.6	1.9	1.7	2.2
M_{O2max}	0.7	2.0	1.0	1.3

4. Discussion

Lotic freshwater systems show marked seasonality in hydrological regimes, which includes changes in water flow, discharge volume, temperature, dissolved oxygen and turbidity (Malmqvist and Rundle, 2002; Woodward et al., 2010). As observed in this study, there is an annual cycle of increasing and decreasing stages of temperature with the highest and lowest peaks in summer and winter. The water temperature range that we recorded (12.2–26.1 °C) was consistent with previous reports from Himalayan streams at similar altitudes (Sehgal, 1999; Sharma and Mehta, 2010; Sharma et al., 2015). With seasonal variation in temperature, the solubility of oxygen is known to be inversely affected (Boyd, 2015), and this was evident in the oxygen concentrations that we measured in winter (9.9 mg/L) and summer (8.4 mg/L). However, unexpectedly, the highest oxygen concentration (11.2 mg/L) was observed in spring (21.8 °C), at super-saturation levels (151.8%). Though the exact reason is not known, this could be a

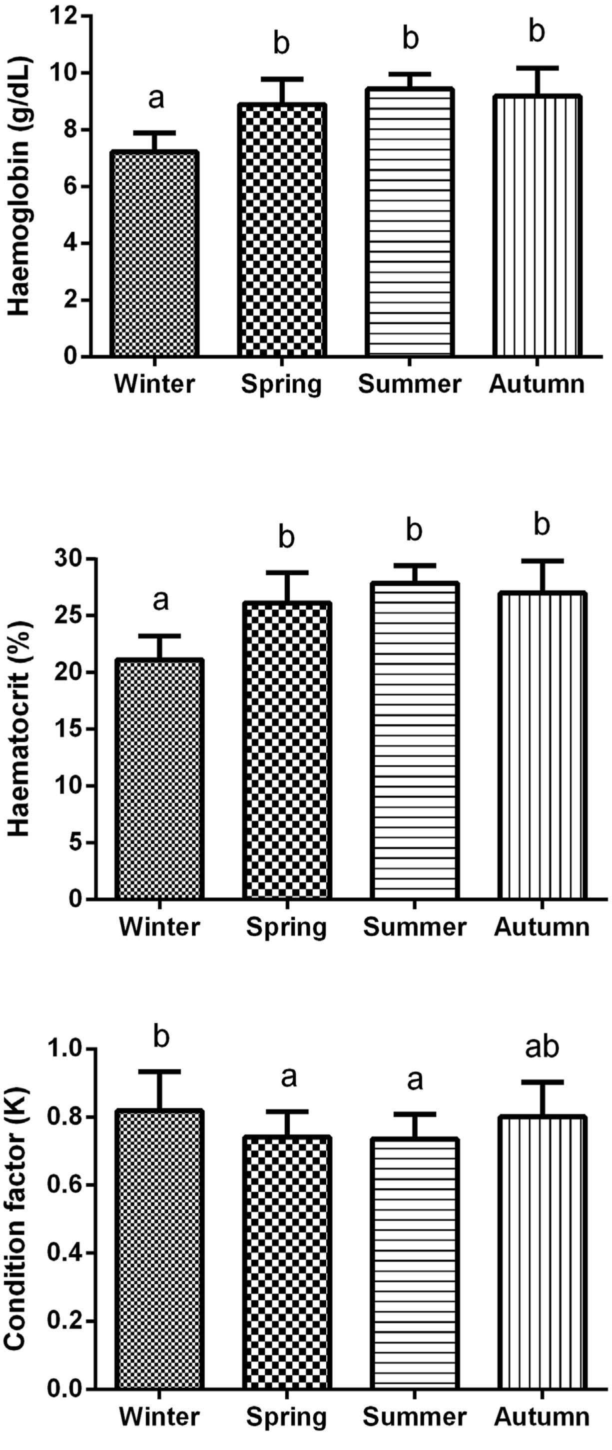

Fig. 4. Seasonal variation in blood haemoglobin-haematocrit levels and body condition factor of *S. richardsonii*. Values are expressed as means ± SD (N = 8 and 36 fish per season for blood indices and fish condition, respectively). Different superscript letters (a, b) in the same series indicate significant difference (ANCOVA, $p < 0.05$).

distinct characteristic of this torrential stream linked to turbulent shallow drainage in spring. In fact, oxygen saturation was found to be above 100% during all the four seasons at the site of fish collection, indicating continuous oxygen diffusion and minimal use of oxygen for aquatic respiration and decomposition (Boyd, 2015). Together, all these seasonal water quality changes remarkably influence the life history traits of the inhabitant fishes. In other words, the physiological capacity of a fish to adapt to these environmental fluctuations will decide their survival, distribution and abundance (Somero, 2010). Examining the degree of this physiological plasticity in *S. richardsonii*, with respect to temperature and hypoxia tolerance and aerobic metabolic capacity was the primary objective of this study.

4.1. *Temperature tolerance capacity of S. richardsonii*

Being ectotherms, fishes are very sensitive to changes in their environmental temperature and exhibit distinct thermal preferences, critical tolerance limits and distribution boundaries (Beitinger et al., 2000; Isaak et al., 2017). Especially, fishes inhabiting streams and small rivers are said to be more vulnerable to short-term (seasonal) and long-term (climate change) changes in water temperature (Kowalski et al., 1978; McCullough et al., 2009). In agreement, stream dwelling population of *S. richardsonii* showed significant seasonal variation (≤ 3 °C) in their critical tolerance limits at both ends of the temperature spectrum (CT_{min} and CT_{max}). The observed range of CT_{max} (31.7–34.6 °C) concurred with previous reports in captive congeneric Schizothoracine fishes (He et al., 2014; Yang et al., 2018), suggesting that the upper thermal limits of these species do not vary substantially with geographic separation or domestication. However, at the community level, *S. richardsonii* had the lowest CT_{max} (34.6 °C) among co-existing cyprinids, namely *Barilius bendelisis* (36 °C; Sharma et al., 2015), *Tor putitora* (41.8 °C; Akhtar et al., 2013) and *Neolissochilus hexagonolepis* (36 °C; Majhi et al., 2013). Therefore, they can serve as a sentinel taxon for climate change or in other words, changes in the natural distribution and abundance of snow trout could be a proxy indicator of the impact of global warming on Himalayan stream ecosystems (Izzo et al., 2016). Physiologically, the lowest CT_{max} observation in winter correlates with a corresponding reduction in blood haemoglobin and haematocrit, giving an indication of agreement with the oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis (Farrell, 2009; Beers and Sidell, 2011). According to this hypothesis, decreased oxygen carrying capacity (haemoglobin and haematocrit) of the blood could limit the upper thermal tolerance in fish, as insufficient supply of oxygen to tissues could impair locomotor capacity and cell membrane fluidity, and cause early LOE during an acute thermal challenge (Muñoz et al., 2018). With respect to CT_{min}, this is the first experimental investigation of the lower temperature threshold in Schizothoracine fishes and our observation (~0–2.4 °C) nearly coincided with the documented natural range of occurrence (Sehgal, 1999). Compared to CT_{max}, seasonal differences in CT_{min} manifested a stronger reflection of their thermal exposure history or annual changes in habitat condition. The thermal tolerance scope (CT_{max} - CT_{min}; 31.7–33.1 °C) of *S. richardsonii* was higher than that of the co-existing Himalayan cyprinid, *Barilius bendelisis* (28.9–30.5 °C; Sharma et al., 2015). Concerning seasonal variations, the maximum thermal scope of snow trout was recorded in autumn, whereas in *Barilius bendelisis*, the thermal scope was equally higher in both spring and autumn (Sharma et al., 2015). Such species specific seasonal variation in thermal scope in co-existing fishes has been previously reported in coastal fish communities (Shultz et al., 2016). On a broader species scale, the observed sub-lethal thermal window of *S. richardsonii* was better than that of the temperate fish rainbow trout (~0–29.8 °C; Currie et al., 1998), but was well short of the eurythermal sheepshead minnow (0.6–44.2 °C; Bennett and Beitinger, 1997) and gold fish (0.3–43.6 °C; Ford and Beitinger, 2005).

4.2. *Hypoxia tolerance threshold of S. richardsonii*

In aquatic systems, changes in ambient temperature are usually accompanied by temporal gradients in dissolved oxygen, with potential interactive effects on the temperature and hypoxia tolerance of the resident fishes (Pörtner, 2001; McBryan et al., 2013). On the one hand, higher water temperatures is known to challenge the oxygen needs of a fish and reduce their hypoxia tolerance (Nilsson et al., 2010; Yang et al., 2015), but on the other hand, chronic warm acclimation is known to improve hypoxia tolerance in fish (Sollid et al., 2005; McBryan et al., 2016). In agreement to the latter, we observed a decreasing trend for ILOC and ILOS values of snow trout from winter to autumn, with a significant reduction in autumn, concurrent to their maximum thermal tolerance scope. We postulate that this gradual increase in hypoxia tolerance from winter to autumn could be possibly related to habitat-specific cumulative changes in oxygen uptake (remodelling of gills), oxygen transport (haemoglobin and haematocrit) and metabolic capacity (Mandic et al., 2008). For instance, higher ILOC values of the winter caught fish could be partly due to the decreased oxygen transport capacity of blood in winter, as evidenced by the reduction in haemoglobin-haematocrit values; and partly because of subdued anaerobic metabolic capacity with continuous exposure to high ambient oxygen concentration. Such links between environmental oxygen concentration, oxygen carrying capacity of the blood, anaerobic performance and hypoxia tolerance is well documented in fish (Richards, 2011; Claireaux and Chabot, 2016). Species-wise, *S. richardsonii* displayed ILOC values (0.2–0.6 mg O_2/L; 3.3–8.8 Torr) similar to *S. prenanti* (Fu et al., 2014) and relatively higher than other cyprinids (Dhillon et al., 2013). This can be attributed to their natural adaptation to a highly oxygenated habitat, where exposure to hypoxic conditions is rare.

4.3. Aerobic metabolic capacity of S. richardsonii

The rate of metabolic processes or biochemical reactions through which energy and materials are transformed within an organism is generally a function of its temperature (Gillooly et al., 2001; Schulte, 2015). Adhering to this concept of the universal temperature dependence of biological processes, fishes are known to exponentially increase their metabolic rate with temperature, to meet their increasing energy requirements (Killen et al., 2016). Employing the common method of oxygen consumption based indirect calorimetry in aquatic ectotherms (Nelson, 2016), we observed a corresponding linear trend of temperature-dependent increase in apparent M_{O2rout} of *S. richardsonii* from 150.3 ± 48.2 mg O_2/kg/h at 12 °C to 315.2 ± 14.8 mg O_2/kg/h at 26 °C. When compared to the congeneric *S. prenanti*, where mean M_{O2rout} was reported to vary from 292.4 mg O_2/kg/h at 15 °C to 359.9 mg O_2/kg/h at 27 °C (Cai et al., 2014), *S. richardsonii* displayed a more pronounced change in M_{O2rout} with increasing ambient temperature and this difference in temperature sensitivity is clearly reflected in the Q_{10} estimates (1.6–2.2 in *S. richardsonii* and 1.1–1.7 in *S. prenanti*). Moreover, the annual transitions in apparent M_{O2rout} showed a linear trend with the changes in blood haemoglobin-haematocrit and environmental temperature (Table 2), which indicates the temperature sensitivity of both blood oxygen transport capacity (respiratory oxygen cascade) and metabolic oxygen demand in snow trout, as observed in other fishes (Farrell, 2009). On the contrary, the annual changes in condition factor of the snow trout specimens were inversely related to the increasing and decreasing phases of ambient temperature and apparent M_{O2rout}. This is possibly due to the changes in the rate of energy utilization, energy allocation strategies and energy stores over the annual cycle (Encina and Granado-Lorencio, 1997). The mean range of apparent M_{O2rout} (corrected for body mass) of *S. richardsonii* was similar to that of the previous observations in *S. prenanti*, *S. chongi* and *S. plagiostomus* (Tu et al., 2011; Kvigstad, 2013; Cai et al., 2014), but was higher than *S. wangchiachii* (Yan et al., 2013). The possible influence of different respirometry methods (static closed *versus* swimming tunnel) on these species-specific measurements should be clarified in future studies. Unlike M_{O2rout}, the rate of maximum metabolic oxygen consumption (M_{O2max}) of *S. richardsonii* did not change in tandem with seasonal temperature changes, as evident in the Q_{10} estimates. This might potentially limit their aerobic scope at higher temperatures, as observed in a few previous studies (Nilsson et al., 2009; Donelson and Munday, 2012; Chretien and Chapman, 2016). Though there was no significant difference between the seasonal M_{O2max} observations, the highest values in autumn (1513.6 mg O_2/kg/h) and lowest values in spring (934 mg O_2/kg/h) caught fishes could be reciprocally associated with the annual changes in ambient oxygen concentration, and particularly the oxygen super-saturation levels in spring season (151.8%). With continuous exposure to hyperoxia, there are reports of a decrease in the ventilation frequency, volume and requirement of fishes (Dejours et al., 1977; Dong et al., 2011; Wu et al., 2016). But to the best of our knowledge, this is the first report of hyperoxia linked reduction in M_{O2max} of a fish species, which is contrary to earlier reports of positive or no correlation (Lefrancois and Claireaux, 2003; McArley et al., 2018). To understand the exact biological reasons behind this observation, further studies must be carried out under experimentally controlled oxygen conditions. Leaving aside this finding, the mean range of M_{O2max} that we observed in this study was similar to the maximum M_{O2} values reported in size-matched *S. chongi* in a swimming tunnel respirometer (Tu et al., 2011).

4.4. Conclusion

In an era of palpable climatic changes, it is increasingly imperative for fisheries and aquaculture research to focus on understanding the ability of fishes to cope with the expected environmental fluctuations. Particularly, knowledge of physiological traits that are related to temperature and oxygen is a fundamental requirement for all conservation and management measures. Therefore, through this study, we have generated the following base-line information of seasonal variability in temperature and hypoxia threshold and aerobic metabolic capacity of a natural population of *S. richardsonii* in the Indian Himalayan region. 1) The sub-lethal thermal tolerance window of snow trout (~0–34.6 °C) is relatively broad for a coldwater fish species. 2) Changes in CT_{min} gave a better reflection of the fish's thermal exposure history when compared to CT_{max}. 3) Corresponding changes in blood haemoglobin-haematocrit and CT_{max} (in winter) concurred with the OCLTT hypothesis. 4) Incipient lethal oxygen limits (hypoxia threshold) was possibly influenced by warm acclimation and limitation in blood oxygen transport. 5) While apparent M_{O2rout} increased in a temperature dependent manner to meet the higher metabolic energy demands, M_{O2max} did not change accordingly. In hindsight, coherent observations of high CT_{max}, thermal scope, M_{O2max} and low ILOC in autumn indicates a potential environmental optimum for *S. richardsonii* near that temperature (21 °C) and this needs to be validated in further investigations.

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