Thermal stress response of different age group of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) exposed to various temperature regimes

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Research Article

Abstract

Climate change and temperature variations are of paramount importance to aquaculture. Here, we investigated the thermal stress response of five life stages of tilapia viz. spawn, fry, fingerling, juveniles and adults exposed to different temperatures (28°C to 40°C). Stress response was assessed in terms of survival/mortality, thermal shock, changes in hematology, histopathology of liver and gonad. The spawn, fry and fingerlings died within 1 to 39 min at 40°C due to thermal shock. Thermal acclimation was observed in these stages till 34°C. Beyond 34°C, low feed intake and susceptibility were marked. Significant increments in the hematological parameters were noticed when the water temperature elevated from 28°C to 32°C and thereafter deteriorated. Hematological parameters, gonadosomatic index (GSI), digestive somatic index (DSI) and hepatosomatic index (HSI) showed ideal conditions at temperatures between 28°C to 30°C. However, a marked change in the liver and gonad histoarchitecture and decreased organ somatic indices of adult tilapia were also noticed in response to elevated temperatures. This study suggests that the developmental stages of tilapia are highly susceptible to thermal shock and a gradual temperature rise helped them acclimate to 34°C. Further temperature rise may adversely affect tilapia aquaculture.

Introduction

Climate change and temperature rise have received global attention due to their potential to alter ecosystems. An increase in environmental temperature of 1.5°C was reported in 2018, while the prediction shows a 6°C rise in global temperature by 2100 (IPCC 2018). A few degrees rise in water temperature affects aquatic ecosystems and organisms living there (Ito et al. 2008). The limited temperature regulation potential of fish is known and they regulate body temperature by moving between areas of different water temperatures (Pough et al. 2009). A slight temperature change may lead to population decline (Ficke et al. 2007; Vernberg et al. 1978). Similarly, a profound temperature change may alter the natural population (unbalanced sex ratio), gonadal development and fertility rate in fish (Al-Deghayem et al. 2017; Im et al. 2016; Strüssmann et al. 1998; Strüssmann and Nakamura 2002). For some organisms, their optimal temperature range can be as narrow as a few degrees or as large as 20°C (Dodson 2005). Elevated water temperature is known to affect physiology including metabolic rate, growth, biochemical parameters of blood and hormonal function (Abdulfatah et al. 2013; Chatterjee et al. 2004; Cho et al. 2015; Khalil et al. 2011; Lermen et al. 2004; Sayed and Moneeb 2015; Tun-Lin W et al. 2000; Pang et al. 2011). High ambient temperature may result in suppression of appetite, growth, nitrogen metabolism and decreases in net protein accretion primarily due to increased degradation rate (Morgan et al. 2001) despite having higher thermal tolerance.

Many studies have reported the effect of temperature on freshwater fish (IPCC, 2001; Jobling, 1981; Pörtner and Peck 2010). The thermal threshold limits (minimum and maximum tolerance) and preference range has been reported for *T. mossambica* (Ananthakrishnan and Srinivasan 1977; Badenhuizen 1967; Allanson and Noble 1964) and *O. niloticus* (Chervinski 1982). The elevated water temperature (> 36°C) is known to affect the gonadal maturation process by depleting the germ cells in *Labeo rohita* (Patra et al.

2015). These studies dealt with either the adult stage or juveniles and lack information about the early life stages of fish. The knowledge gap is partly due to scarce information for each developmental stage of teleosts in general and tilapia (*O. niloticus*) in particular.

In many fishes, it is not known how these elevated temperatures effect the hematological parameters and organs. Hence, it is imperative to know the changes in the organ indices, histopathology and blood parameters in a widely cultured species such as tilapia at elevated temperatures. Here, Nile tilapia was used as a test species because the farming and domestication of this species is one of the most ancient (Harache 2002; Teletchea 2019) and it has been introduced in numerous countries worldwide (Lazard 2009). It ranks fifth among the most produced aquatic species in the world (Bentsen 2014). According to some experts, tilapia can become the most important aquaculture species in the world in the coming years (Gjedrem 2012). The FAO State of World Fisheries and Aquaculture (2016) reports that global tilapia production has exceeded 5 million tonnes per year since 2014 with a steady growth rate of 5 to 8 percent per annum. The seed production and monosex tilapia culture is a fast-growing sector in India.

Keeping in view the impact of climate change on the environment, particularly the temperature rise, here we studied the effect of different temperature regimes on various life stages of tilapia, *Oreochromis niloticus.* Different parameters that were studied here included survival rate, organ indices, hematology, and histopathology of organs to understand the physiological and biochemical mechanisms underlying thermal tolerance patterns and stress in tilapia.

Materials And Methods

Collection of tilapia and experimental setup

The whole study was divided in to three experiments, i.e., Experiment-I: Effect of thermal tolerance and thermal stress on developmental stages (spawn, fry and fingerling) of tilapia; Experiment-II: Effect of temperature on hematology and histopathology of tilapia (fingerling, juvenile and adult); Experiment-III: Effect of temperature on organ indices of adult tilapia. The details of the study are depicted in Fig. 1 and described under separate sub-headings. All temperature-related experiments were conducted (in triplicate) using Nile tilapia collected from the hatchery facility of ICAR-CIFA, Bhubaneswar, India in 175L capacity glass tanks (39 tanks, dimension of each tank 4'x 1.5'x1.5'). Physico-chemical parameters of water such as pH, dissolved oxygen (mg/l), free CO_2 (mg/l), total alkalinity (mg/l), P_2O_5 (mg/l), NH_4-N (mg/l), NO_3-N (mg/l) and conductivity (mho/cm) were tested for each tank following APHA (1992).

The temperature of the water in the tanks was maintained between 28°C to 40°C using thermostat-controlled immersion heaters (Pacific Coast Inc. Life Tech Aquarium, 2009). The temperature of the control tank was maintained at 28°C. Different life stages of tilapia viz.5-day old post-hatch spawn, 30-day old fry, 60-day old fingerling, juveniles and adults were used for all experimental purposes.

Experiment-I: Effect of thermal tolerance and thermal stress on developmental stages (spawn, fry and fingerling) of tilapia

For the thermal tolerance test involving different life stages of tilapia, each time 100 spawn (5-day old post-hatch), 50 fry (30-day old) and 20 fingerlings (60-day old) were stocked in separate tanks (in triplicate). The temperature of water in each tank was raised by 1°C/3h interval to attain the desired temperature. The highest tolerance temperature used here was 40°C. The choice of this particular rate of increase in temperature (1°C/ 3h) is based on the fact that earlier 1°C/day has been reported (Bishai 1963) and here we wanted to check the response at a faster rate (1°C/3h). The rearing of fish was done in the desired constant temperature after gradual acclimation to the nearest low temperature. For instance, when the desired test temperature was 40°C, the nearest lowest acclimation temperature was 39°C. The survival of different developmental stages of tilapia were assessed at each temperature treatment as shown in Fig. 1. Aeration was provided in each tank with the help of an air blower (Hailea Hi-blow Air pump, hap-200, PRC). To check the feed intake (acceptability) at various water temperatures, they were fed (5% of body weight) with a commercial floating diet containing 32% protein and 4% fat (Abis feed, IB Corporate House, India).

To test the thermal shock effect, each time 100 spawn (5-day old hatchling), 50 fry (average size 3.3 ± 0.08g) and 20 fingerlings (average size 13 ± 1.1g) of tilapia were directly exposed to the desired temperatures (28°C to 40°C) without acclimation in triplicate tanks. The effect of thermal shock was studied up to 8 h or till 100% mortality, whichever was earlier. This time duration was selected because our preliminary studies (data not shown) indicated that tilapia larvae, fry and fingerlings could not sustain more than 8 hours in elevated temperatures (40°C). During this trial, continuous observation was recorded at every 10 min interval about their behavior, feeding pattern, fatigues and mortality including the symptoms of thermal shock viz. darting, gasping, thrashing and swimming near the water surface.

Experiment-II: Effect of temperature on hematology and histopathology

To test the effect of temperature on hematology and histopathology of tilapia, each time 20 fingerlings (mean weight 13 ± 1.1 g), 10 juveniles (mean weight 120 ± 8.3 g) and 10 adults (mean weight 215 ± 4.3 g) were reared at 28°C to 40°C for a maximum duration of 45 days. The temperature of the water in the tanks were maintained between 28°C to 40°C using thermostat-controlled immersion heaters (Pacific Coast Inc. Life Tech Aquarium, 2009). The temperature of the control tank was maintained at 28°C.

Assessment of hematological parameters

For hematological studies, each time 3–5 ml of blood samples were collected from the caudal vein of fingerlings (n = 20), juveniles (n = 10) and adults (n = 10) after exposure time of 40 h, 15 days and 15 days respectively using EDTA (ethylenediamine tetra-acetic acid) (Merck, Mumbai, India) rinsed syringe. To

avoid stress and ease of handling, fishes were anaesthetized by 2-phenoxyethanol (0.1 ml/l) (MP Biomedicals, LLC France) (Routray et al, 2002). The total number of RBCs were counted immediately using Neubauer's chamber with a dilution of 1:200 with diluting fluid and placed in hemocytometer for counting RBCs following Blaxhall and Daisley (1973). Four corner square of the hemocytometer was counted using a microscope and the values expressed as millions per cubic mm. Similarly, the white blood cells (WBCs) were counted after dilution (1:20) with a diluting fluid and the total number of WBCs expressed as thousands per cubic mm (Blaxhall and Daisley 1973). Sahli's acid haematin method was followed to assess the hemoglobin (Sahli 1909). The packed cell volume (PCV) or hematocrit value, red cells in the blood was examined by micro hematocrit method (McInroy 1954). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae of Dacie and Lewis, (1991).

$$\mathrm{MCHC(g/dl)} = \frac{\mathrm{Hb}}{\mathrm{PCV}} \times 100$$

$$\mathrm{MCH\,(pg)} = \frac{\mathrm{Hb}}{\mathrm{RBC}} \times 10$$

$$\mathrm{MCV\,(\mu m3)} = \frac{\mathrm{PCV}}{\mathrm{RBC}} \times 10$$

Histopathology

The liver and gonads were collected from five adult male and female tilapias from each treatment after 15 days exposure. The fish were anaesthetized (2-phenoxyethanol, 0.1 ml/l) and liver and gonads excised from them and fixed immediately in10% formalin to avoid autolysis. The samples were later dehydrated, embedded in paraffin wax and sectioned at 5 μm using a rotary microtome. The thin sections were stained with hematoxylin-eosin (HE) and analyzed under a histology microscope (Leica DM2000) at different magnifications and photomicrographs generated using a digital camera.

Experiment-III: Effect of temperature on organ indices of adult tilapia

The effect of temperatures (28°C, 30°C, 32°C, 34°C) on the organ indices was studied separately in adult tilapia for a duration of 45 days in triplicate. For this study, each experimental tank was stocked with 10 adult tilapia (mean weight 215 ± 4.3 g) and 30−40% of water exchanged per week during the entire period to maintain ideal water quality in tanks. The gonad, liver and gut were excised from adults after sacrificing the fish humanely at the end of the experiment. To avoid the unwarranted water temperature fluctuation in tanks during water exchange, a separate reserve tank with a 200-litre capacity was maintained at 40°C and water from this tank was used to maintain specific temperatures after dilution with tap water (28°C) and temperature normalization. Fish were fed with floating pellet feed (30% crude protein) two rations per day at 5% of their body weight. The feed was prepared at ICAR-CIFA, Bhubaneswar having ingredients such as groundnut oil cake (40%), rice bran (25%), fish meal (10%),

roasted soybean meal (20%), vegetable and fish oil (3%) and vitamin-mineral premix (2%) (Nandi et al., 2007). The excised organs (gonad including both ovary and testis, liver, gut) were used for estimation of gonadosomatic index (GSI) and hepatosomatic index (HSI) and digestive somatic index (DSI) following the equations:

$$HSI\,(\%) = \frac{Weight of liver}{Weight of body} \times 100$$

$$GSI\,(\%) = \frac{Weight of gonad}{Weight of body} \times 100$$

$$DSI\,(\%) = \frac{Weight of intestine}{Weight of body} \times 100$$

Statistical analysis

Data were tested for normality by Kolmogorov–Smirnov and homogeneity of variance. Differences due to water temperature treatments were detected by one-way analysis of variance (ANOVA). A probability value $p < 0.05$ was taken as statistically significant. Statistical analyses were performed in SPSS 20.0 package (SPSS Inc., Chicago, USA).

Results

Effect of temperature on survival/mortality of different developmental stages

All the three evaluated fish stages viz. spawn, fry and fingerlings survived the exposure temperatures of 34°C, 36°C and 37°C respectively. However, thermal threshold point (100% mortality) was observed in spawn, fry and fingerlings at 37°C, 39°C and 40°C respectively. The survival rate of exposed fish decreased when the temperature was raised from 34°C to 40°C (Table 1). The temperature at which all the exposed fish (spawn, fry and fingerlings) died here was considered as the thermal threshold value beyond which "fish kill" phenomena occurred. The feed intake by tested groups of fish (spawn, fry and fingerlings) was normal (as all the supplied feed was consumed) within the temperature range of 28°C to 35°C and after that reduced drastically in spawn. Similar observation was marked in fry and fingerlings at 37°C and 38°C respectively. The assessment of feed intake was based on the consumption by fish and visual observation only.

The survival and mortality pattern of spawn, fry and fingerlings are shown in Fig. 2–4. It was observed that more than 50% spawn survived at 35°C for more than 24 h. However, no spawn survived at 37°C after 24 h and more than 50% died after 9 h exposure. The fry of tilapia could not tolerate a temperature of 39°C beyond 36 h and complete mortality occurred, whereas more than 60% survived at 37°C for more

than 48 h. Similarly, fingerlings could not survive at 40°C and all the tested animals died by 49 h. Nevertheless, it was noticed that all the fingerlings could sustain a temperature of 38°C till 49 h.

Thermal shock response at different temperatures

It was observed that the spawn has a lower thermal shock tolerance capacity than the fry and fingerlings. The five-day-old spawn could not sustain for more than 2.2 h (132 min) at 37 ° C (Fig. 5). Similarly, the spawn could not survive beyond 0.1h (6 min) at a temperature of 38°C. It was further recorded that all the fish died in minutes after being released into the rearing tanks having water temperatures of 39°C and 40°C. In case of fry and fingerlings more than 50% survived for 0.4 h (24 min) and 4 h respectively at 38°C. No fingerlings survived beyond 8 h at 38°C. Further, it was noticed that more than 80% of the fingerlings could survive for more than 2 h at 39°C. It was also observed that no fry survived beyond 0.58 h (34.8 min), 0.32 h (19.2 min), 0.14 h (8.4 min) at rearing temperatures of 38°C, 39°C, and 40°C respectively (Fig. 6). Similarly, fingerlings could not survive beyond 8 h, 3 h and 0.65 h (39 min) at temperatures of 38°C, 39°C and 40°C (Fig. 7).

Effect of temperature on hematological parameters

It was observed that the effect of rearing water temperature on hematological parameters was significantly different among stages. It is pertinent to mention that the hematological parameters of different stages of tilapia viz. fingerlings, juveniles and adults showed clear differences in values (Hb, RBC, WBC, PCV, MCV, MCH) at different temperatures (Table 2–4). The hemoglobin, RBC, WBC and PCV were significantly higher at 35°C in all stages of tilapia as compared to other temperatures (Fig. 8). All the parameters (Hb, RBC, WBC, PCV, MCV, MCH) were significantly affected in different stages of tilapia when the rearing water temperature was raised beyond 35°C.

Histopathology of gonad and liver

Male and female gonad histopathology revealed normal development at 28 °C to 30 °C. Several changes in the histoarchitecture of gonads of both sexes were noticed at higher temperatures. A clear sloughing off germ cells was seen in testis of fish reared at 34 °C upwards. Other changes that were noticed as inflammation in the seminiferous epithelium, enlarged and degenerative tubules and vacuolation. The arrest of spermatogenesis was marked at a rearing temperature of 40 °C (Fig. 9). Similarly, there were fragmented oocytes and irregular shrinkage of oocytes in the ovary at 34 °C treatment group. Further increase in temperature (40 °C) lead to changes in melano-macrophage centers and increased fragmentation of oocytes also (Fig. 10). Normal polygonal hepatocytes with round nucleus and prominent nucleolus was observed in the liver of male and female fishes reared in the temperature regimes of 28 °C to 30 °C. There were considerable changes in the histopathological observations in the liver of both sexes beyond 34 °C. When the rearing temperature increased (36 °C to 38 °C), hepatic vacuolation, hepatocyte nuclei migration, nuclear pyknosis, hydropic degeneration and varying degrees of steatosis were seen. At 40 °C, the histoarchitecture of livers from both sexes showed necrotic changes (Figs. 11 and 12).

Effect of temperature on GSI, HSI and DSI

Highest GSI was observed in both sexes of tilapia reared at 30°C, as the size of the gonads were more prominent than the fish reared at other temperatures. The GSI was reduced to a minimum level in both males and females as the rearing water temperature increased to 34°C (Fig. 13). The effect of rearing water temperatures (28°C, 30°C, 32°C, and 34°C) on the liver of both sexes of tilapia was assessed and presented in Fig. 14. A prominent liver was seen in the fish acclimatized at water temperature of 28°C as evident from the HSI. Comparatively lower HSI was observed in females than males in all tested temperatures. The HSI of fish acclimatized in water temperature of 32°C and 34°C had lower values than fish acclimatized at 28°C. The effect of rearing water temperatures on the digestive somatic index (DSI) is shown in Fig. 15. The highest DSI was observed in tilapia at a rearing water temperature of 34°C.

Discussion

This study is the first in-depth report about the impact of elevated water temperature on different life-history stages of *O. niloticus* and the endurance patterns in response to short-term and prolonged exposures. Thermal threshold, thermal shock patterns, survival, feed intake behavior, organ indices and hematological parameters of five different life stages of Nile tilapia are reported here along with changes in histoarchitecture of liver and gonads. In the first set of experiment, the spawn, fry and fingerlings survived at 34°C, 36°C and 38°C water temperatures respectively. However, a thermal threshold point (100% mortality) was observed in these three stages at rearing water temperatures of 37°C, 39°C and 40°C respectively. The temperature at which all the exposed stages of fish (spawn, fry and fingerlings) died here is considered as the thermal threshold value beyond which "fish kill" phenomena (i.e., a localized die-off of fish populations) occurred (Gainesville 2005). The most common cause of fish kill is reduced oxygen in the water, where the sustained increase in water temperature is believed to be the cause. The majority of aquaculture takes place in waters that are not thermo-regulated and undergoes more or less pronounced daily variations (Azaza et al. 2010). Similar events also occur in tropical water bodies in the summer season.

It is important to mention that the temperature tolerance and mortality rates vary from species to species and even among developmental stages of the same species (Jobling 1981). The survival and mortality pattern of spawn reported here is believed to be a distinct one where a wide range of temperatures with and without acclimation was tested. The survival rate of spawn and fry decreased with increasing water temperature in the present study, which is similar to that reported for 9-days and 50-days post-hatch tilapias by Pandit and Nakamura (2010). However, they reported a higher survival of fry (80%) in comparison to ours (65%) at a rearing temperature of 37°C. The discrepancies observed may be due to the micro variations in the tank environment and differences in handling. Better spawn survival has been reported in other species (*C. carpio* and *Silurus glanis*) at lower temperatures (15°C to 26°C) (Sapkale et al. 2011; Teletchea et al. 2009; Schiemer et al. 2002; Schiemer et al. 2004).

Unlike the spawn and fry, no mortality of fingerlings of tilapia was noticed at 37°C. However, the fingerling survival rate dropped to 25% at 39°C and none survived at 40°C. Here a greater thermal tolerance in tilapia fingerlings were noticed and more than 60% survived till 28 h at a temperature of 40°C but 100%

mortality occurred around 50 h. Thus, we noticed that the thermal tolerance limit increased as the developed stage of tilapia was used. The thermal threshold temperature of tilapia is reported at 41°C with the optimum oxygen consumption and respiration at 30°C and 37°C respectively (Nitithamyong 1988). This may be attributed to the variety/strain of tilapia used by them. Here the recorded mortality rate was 100% at 37°C in spawn and correspondingly 39°C for fry and 40°C for fingerlings. It showed that the size and developmental stage were inversely proportional to the mortality rate, which is in line with the results reported by others (Sifa et al. 2002). The resistance of fish to elevated temperatures has long been reported to vary with species, acclimation temperature, size, age, season, thermal history and duration of exposure to high temperatures (Bishai 1960). Chatterjee et al. (2004) studied the thermal threshold point in early fingerlings of *Labeo rohita* and *Cyprinus carpio* and reported inter-species-specific variation between them at 30°C and 35°C respectively. A wide distribution of *O. niloticus* and their ability to live under varied ecological conditions, especially tolerance to high temperatures and other conditions have been reported (Lowe-Mcconnell 1958). There are scanty reports about upper lethal temperatures and heat resistance of different life stages of tilapia that are further elaborated here. The thermal tolerance capacity and survival of spawn, fry and fingerlings increased by gradually increasing temperature (1°C/ 3h) as the fish were allowed to acclimatize. The choice of this particular rate of increase in temperature is comparable with earlier reports (Bishai 1963). Higher thermal tolerance has been reported in sheep head minnow, *Cyprinodon variegatus* that survived at 45.1°C by gradually rising the water temperature 1°C by every hour (Bennett and Beitinger 1997).

The spawn reared between 37°C to 40°C without gradual acclimation resulted in 100% mortality within a time range of 0.9 min to 2.2 h due to thermal shock. Similar event of 100% mortality was recorded in the fry reared at 38°C to 40°C in a time span of 8 min to 34 min and the same happened in fingerlings after an exposure time of 39 min to 8h between at temperatures between 38°C to 40°C. A temperature tolerance trend was marked in different developmental stages of tilapia without gradual acclimation. As the development progressed from spawn (hatchlings) to fingerlings, the adaptability to higher water temperature was evident. To the best of our knowledge, there are no reports about the temperature effects (thermal shock due to non-acclimation) on different developmental stages of tilapia. Temperature shock with an abrupt thermal change of more than 4°C has been reported in *S. sagax caeruleus* where more than 50% died (Hernández-López et al. 2018).

The second set of experiment focussed on the physiological changes that occurs due to elevated temperatures. This includes the hematological parameters that are considered as reliable indicators of the health status and condition of the fish (Akinrotimi et al. 2012; Katalay and Parlak 2004; Clauss et al. 2008; Adeyemo et al. 2009). These parameters are reported to be affected by elevated temperatures in tilapia (Mali and Chavan 2014). Among the hematological parameters; hemaglobin, RBC and hematocrit are used to assay the functional status of oxygen transportation capacity of the bloodstream (Shah and Altindag 2004) and physiological status of fish (Fernandez and Mazon 2003). Similarly, RBCs and WBCs are the first hematological parameters affected by stress. Here, the hemaglobin, RBC, WBC and hematocrit values of fingerlings, juveniles and adults showed an increasing trend as the temperature was raised from 28°C to 31°C. Decreased level of Hb, RBC and hematocrit were detected after 35°C, which is

believed to have altered the oxygen-carrying capacity of RBC and other functional activities (Fig. 8). It is natural that the oxygen-carrying capacity of blood may have reduced when these blood parameters deteriorated resulting in severe stress and death of fish.

Here, Hb decreased in fingerlings, juveniles and adults as the temperature was elevated and fish died may be due to less oxygen carrying capacity of blood but not due to the non-availability of dissolved oxygen in water as it was maintained above 3ppm through aeration (Table 5). Although, it is important to mention that the DO level falls in water when the temperature is raised (Sollid 2005). Hedayati and Tarkhani (2014) reported that the concentration of RBC, Hb and hematocrit increases to increase the blood's carrying capacity under stressful conditions. The effect of thermal stress on blood parameters of *Oreochromis mossambicus* (Peters 1852) was evaluated based on the critical thermal maximum (CTMax) and the upper incipient lethal temperature (UILT). The CTMax and UILT responses modified the blood parameters of *O. mossambicus* acclimated at different temperatures. Sayed and Moneeb (2015) reported the hematological and biochemical characteristics of different stock of monosex tilapia and the results were similar to this study. However, they have not studied these parameters in different developmental stages of tilapia. Pörtner and Knust (2007) in their historic paper has shown that thermally limited oxygen delivery closely matches environmental temperatures beyond which growth performance and abundance decreases in the eelpout, *Zoarces viviparus*. Therefore, the observed decline in the hematological parameter indicates an inadequate supply of oxygen to the tissues of the fishes beyond 35°C. As a result, it is plausible that energy metabolism in these fishes is considerably affected. These studies suggest that the blood parameters and metabolism are affected significantly when fishes are reared at temperatures beyond their thermal optimum.

It has been found in previous studies that elevated water temperature significantly affect gonad development (Dorts et al. 2012). Here, histopathology of testis showed spermatogenesis at various developmental stages in the control group. However, significant histopathological changes were observed in the testis of the fishes exposed to stressfully high temperatures that included degeneration of the spermatozoa with enlarged and degenerative seminiferous tubules and arrest of spermatogenesis. The highest frequency of alterations occurred at 38 °C and 40 °C treatment groups. A significant depletion in the number of spermatozoa of *Odontesthes bonariensis* at 23 °C and 27 °C has been reported earlier by Soria et al. (2008). Similarly, reduced and fragmented oocytes, irregular shrinkage, and development of melano macrophage centres were observed in the ovary of fish reared at elevated water temperatures. It is reported that the termination factor of the spawning season of red sea bream in early summer was due to an increase in water temperature (Okuzawa et al. 2003). Histopathological findings of gonads of tilapia reared at elevated temperatures beyond their thermal optimum are scanty and it is believed that this study will help in understanding the changes in the gonad architecture due to temperature changes in the environment.

The liver histopathology of tilapia studied here reveals adverse effects of higher temperatures. The most common alterations observed in the liver tissues of the fishes reared at higher water temperature groups included MMC aggregation, steatosis and cytoplasmic vacuolization. Fournie et al. (2001) reported that

the higher water temperature (32 °C) resulted in an increase in MMC in liver tissues which is a prime indicator of stress-induced change. Here, liver tissues of both the sexes of tilapia reared at a temperature of 40 °C developed necrosis that implies that the liver could not withstand prolonged exposure to a higher temperature. Chinabut et al. (1995) reported an increase in MMCs in liver tissue as a response to counter pathogens during high-temperature exposure, which supports our results. A similar report by Dash et al. (2011) in *Labeo rohita* suggests liver inflammation and hepatocyte damage at higher temperatures also.

The third set of experiment focussed on the effect of elevated temperatures on the organ indices (GSI, HSI and DSI) of tilapia. The organ indices were significantly affected at higher temperatures. The size of the gonads was significantly prominent and highest GSI value marked in both sex of fish reared at 30°C. The shrunken gonads and lower GSI were marked in fish reared at temperatures above 30°C. It has been reported that the water temperature influences the development of gonads (Hermelink et al. 2011; Miranda et al. 2013) and elevated temperature adversely affects the size and development of oocytes (King et al., 2003). Further, implications of higher temperatures are reported to curtail the hormonal level in the brain-pituitary-gonad axis and impairs the spawning activity (Im et al. 2016; Miranda et al. 2013). In small fish like minnows, the water temperature and seasonal changes altered the GSI (Im et al. 2016). Al-Deghayem et al. (2017) exhibited that the GSI declines at 32°C in African catfish. In the current study, the GSI showed a typical response to the temperature ranges between 28°C to 34°C, but 30°C was found to be optimal for gonadal growth.

It was observed that the size of the liver was significantly prominent in fish reared at 28°C which was evident from the HSI. A decreasing trend of HSI was observed in both males and females at tested temperatures (28°C to 34°C). The HSI is an indicator of the status of energy reserve in fish. Here, HSI declined with elevated water temperatures except for 34°C. Jacquin et al. (2019) reported that high temperature alone causes behavioural and physiological changes in goldfish. Here, HSI decreased initially but when the temperature was elevated to 34°C, it showed an abrupt increase and the reasons for this could not be established in this study. One possible reason could be related to that the dual function of the liver in females (lipid metabolism and yolk precursor synthesis). However, more studies are needed to elucidate this.

Here, the DSI of tilapia reared at elevated temperature was also assessed. The DSI is the ratio of the intestine to the mass of the fish body and considered as an indicator of high digestive capability of fish. The highest DSI of 2.4 and 2.8 was observed in female and male tilapia respectively exposed to 34°C. This DSI value is similar to low when compared to other in freshwater fish viz. *Puntioplites proctozysron, Hemibagrus spilopterus, Ompok bimaculatus*, and *Kryptopterus geminus* but lower than *Puntius gonionotus, Oreochromis niloticus*, (Champasri et al. 2021). In the present study, no significant differences in the DSI of adult tilapia were observed in three temperatures (28°C, 30°C and 32°C). At 34°C, the DSI was higher may be due to the higher feed consumption and metabolism. At this temperature, earlier HSI was also found to be higher, which may be attributed to the storage of more glycogen in the liver due to enhanced metabolic activity.

Conclusion

Overall, these results indicate that the rise of water temperature due to global warming is anticipated to affect the physiological activities of fish. Here, we documented the effect of exposure to a higher temperature regime as a model to mimic the extreme water temperature during summer peaks in tropical climates due to global warming and climate change, particularly in stagnant waters. The results suggest that the hatchlings/spawn are more susceptible to elevated water temperature than the fry and fingerlings. When the temperature goes beyond 35°C and the acclimation period persists, it affects normal activities such as feed consumption, growth, reproduction and survival. The simulated water temperature experiment showed that 28°C to 30°C is ideal for tilapia acclimation and breeding. Further, this study may help to develop climate resilient aquaculture.

Declarations

Funding

Conflicts of interest/Competing interests

The above authors declare that No actual or potential conflict of interest, including any financial, personal or other relationships with other people or organization, exist, concerning the submitted work.

Ethics approval/declarations

All experimental procedures carried out in this study were approved by the institutional ethics committee of ICAR- Central Institute of Freshwater Aquaculture.

Consent to participate

Consent was obtained from individual participants.

Consent for publication

All the authors agree to publish the manuscript as it is

Data availability statement

The data that support the findings of this study are available with the corresponding author and can be provided upon request.

Code availability

Not Applicable

Authors' contributions

BP, LS and PR conceived and designed the experiments. BP, SN, GM, SP and OGK performed the experiments and curation of data. GM and SP analyzed and interpreted the hematological parameters. BP and OGK were involved in the dissection of samples and histopathology slide preparation. BP and KRK wrote the first draft of manuscript and formal analysis of data. DKV and SA participated in data analysis and revising of the manuscript. LS and PR involved in collection of sample, supervision, writing-review & editing. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

References

1. Abdulfatah A, Fontaine P, Kestemont P, Milla S, Marie M (2013) Effects of the thermal threshold and the timing of temperature reduction on the initiation and course of oocyte development in cultured female of Eurasian perch *Perca fluviatilis*. Aquaculture 376:90–96. https://doi.org/10.1016/j.aquaculture.2012.11.010

2. Adeyemo OK, Okwilagwe OO, Ajani F (2009) Comparative assessment of sodium EDTA and heparin as anticoagulants for the evaluation of haematological parameters in cultured and feral African cathfish (*Clarias gariepinus*). Braz J Aquat Sci Tech Sci 13:19–24. https://doi.org/10.14210/bjast.v13n1.p19-24

3. Akinrotimi OA, Agokei EO, Aranyo AA (2012) Changes in haematological parameters of *Tilapia guineensis* exposed to different salinity levels.J. Environ Sci Eng 1:4–12

4. Al-Deghayem WA, Al-Balawi HF, Kandeal SA, Suliman EAM (2017) Gonadosomatic index and some hematological parameters in African catfish *Clarias gariepinus* (Burchell, 1822) as affected by feed type and temperature level. Braz Arch Biol Technol 60. https://doi.org/10.1590/1678-4324-2017160157

5. Allanson BR, Noble RG (1964) The tolerance of *Tilapia mossambica* (Peters) to high temperature. Trans Am Fish Soc 93:323–332. https://doi.org/10.1577/1548-8659

6. Ananthakrishnan KR, Srinivasan K(1977) Effect of dilutions of sea-water on the upper lethal temperature of the cichlid fish, *Tilapia mossambica*.Comp.Biochem.Physiol.£co/2,154–157

7. APHA, (American Pubic Health Association) (1992) Standard methods for the examination of water and waste water (18th Edition). APHA (American Water Works Association and water Pollution Control Federation), Washington DC. (in USA)

8. Azaza MS, Legendre M, Kraiem MM, Baras E (2010) Size-dependent effects of daily thermal fluctuations on the growth and size heterogeneity of Nile tilapia *Oreochromis niloticus*. J Fish Biol 76:669–683. doi:10.1111/j.1095-8649.2009.02524.x

9. Badenhuizen TR (1967) Temperatures selected by *Tilapia mossambica* (Peters) in a test tank with a horizontal temperature gradient. Hydrobiologia 30:541–554. https://doi.org/10.1007/BF00964032

10. Bennett WA, Beitinger TL (1997) Temperature Tolerance of the Sheepshead Minnow *Cyprinodon variegatus* Published by: American Society of Ichthyologists and Herpetologists Temperature Tolerance of the Sheepshead Minnow, Cyprinodon variegatus. Socy 1:77–87. doi: 10.2307/1447842

11. Bentsen NS, Felby C, Thorsen BJ (2014) Agricultural residue production and potentials for energy and materials services.Prog. Energy Combust Sci 40:59–73. doi:10.1016/j.pecs.2013.09.003

12. Bishai HM (1960) The effect of water currents on the survival and distribution of fish larvae.ICES. J Mar Sci 25:134–146. https://doi.org/10.1093/icesjms/25.2.134

13. Bishai HM(1963) Fish farming in Equatorial Province. Khartoum, Hydrogiol. Resol. Uni. Spec. Publi. p.1:39

14. Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5:771–781. doi:10.1111/j.1095-8649.1973.tb04510.x

15. Champasri C, Phetlum S, Pornchoo C (2021) Diverse activities and biochemical properties of amylase and proteases from six freshwater fish species. Sci Rep 11:1–11. doi:10.1038/s41598-021-85258-7

16. Chatterjee N, Pal AK, Manush SM, Das T, Mukherjee SC (2004) Thermal tolerance and oxygen consumption of *Labeo rohita* and *Cyprinus carpio* early fingerlings acclimated to three different temperatures. J Ther Biol 29:265–270. doi: 10.1016/j.jtherbio.2004.05.001

17. Chervinski J(1982) Environmental physiology of tilapias, In R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The biology and culture of tilapias. ICIJARM Conference Proceedings 7, 432 p.ICLARM. Manila, p. 119–128. (In Philippines)

18. Chinabut S, Roberts RJ, Willoughby GR, Pearson MD (1995) Histopathology of snakehead, Channa striatus (Bloch), experimentally infected with the specific Aphanomyces fungus associated with epizootic ulcerative syndrome (EUS) at different temperatures. J Fish Dis 18:41–47. doi:10.1111/j.1365-2761.1995.tb01264.x

19. Cho HC, Kim JE, Kim HB, Baek HJ (2015) Effects of water temperature change on the hematological responses and plasma cortisol levels in growing of Red Spotted Grouper, *Epinephelus akaara*. Dev Reprod 19:19–24. doi: 10.12717/devrep.2015.19.1.019

20. Clauss TM, Alistair DM, Arnold JE (2008) Hematologic disorders of fish. 11:445–462. 10.1016/j.cvex.2008.03.007

21. Dacie JV, Lewis SM (1991) Practical Hematology. Churchill Livingstone. Edinburgh, London, Melbourne and New York, pp 521–524

22. Dash G, Yonzone P, Chanda M, Paul M (2011) Histopathological changes in *Labeo rohita* (Hamilton) fingerlings to various acclimation temperatures. 2:29–36. 10.4103/2229-5186.79347

23. Dodson S (2005) Aquatic ecosystems and physiology energy flow. Introduction to limnology. McGraw-Hill, New York, NY, pp 221–222

24. Dorts J, Grenouillet G, Douxfils J, Mandiki SN, Milla S, Silvestre F, Kestemont P (2012) Evidence that elevated water temperature affects the reproductive physiology of the European bullhead Cottus gobio. Fish Physiol Biochem 38:389–399. doi: 10.1007/s10695-011-9515-y

25. FAO., The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.Rome.200pp

26. Fernandez MN, Mazon AF (2003) Environmental pollution and fish gill morphology. In: Val AL, Kappor BG (eds) Fish Adaptations. Scientific Publishers, Inc;, Enfield, NH, pp 203–231

27. Ficke AD, Myrick CA, Hansen LJ (2007) Potential impacts of global climate change on freshwater fisheries. Rev Fish Biol Fish 17:581–613. https://doi.org/10.1007/s11160-007-9059-5

28. Fournie JW, Summers JK, Courtney LA, Engle VD (2001) Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. J Aquat Anim Health 13:105–116

29. Gainesville FL (2005) University of Florida. "Fish kill." Archived 24 September 2008 at the Wayback. Machine Plant Management in Florida's Waters

30. Gjedrem T (2012) Genetic improvement for the development of efficient global aquaculture: A personal opinion review. Aquaculture 344–349:12–22. doi: 10.1016/j.aquaculture.2012.03.003

31. Harache Y (2002) Development and diversification issues in aquaculture. A historical and dynamic view of fish culture diversification. Cah Options Mediterr 59:15–23. http://om.ciheam.org/om/pdf/c59/02600076

32. Hedayati A, Tarkhani R (2014) Hematological and gill histopathological changes in iridescent shark, *Pangasius hypophthalmus* (Sauvage, 1878) exposed to sublethal diazinon and deltamethrin concentrations.Fish Physiol. Biochem 40:715–720. doi:https://doi.org/10.1007/s10695-013-9878-3

33. Hermelink B, Wuertz S, Trubiroha A, Rennert B, Kloas W, Schulz C (2011) Influence of temperature on puberty and maturation of pikeperch, *Sander lucioperca*. Gen Comp Endocrinol 172:282–292. https://doi.org/10.1016/j.ygcen.2011.03.013

34. Hernández-López JR, Hernández-Rodríguez M, Rivas-Manzano P, Bückle-Ramirez LF (2018) Thermal Effect of Acute and Chronic Stress on Hepatic and Renal Tissue of the Pacific Sardine, Sardinops sagax caeruleus (Jenyns, 1842). Inter J Morphol 36:212–220. doi:10.4067/s0717-95022018000100212

35. Im J, Kong D, Ghil S (2016) Effects of water temperature on gonad development in the cold-water fish, kumgang fat minnow *Rhynchocypris kumgangensis*. Cytologia 81:311–317. doi:10.1508/cytologia.81.311

36. IPCC., : Climate Change 2001: Synthesis Report. A Contribution of Working Groups I, II, and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change Watson, R.T. and the Core Writing Team (eds.). Cambridge University Press, Cambridge, (pp. 398) United Kingdom, and New York, NY, (In USA)

37. IPCC (2018) : Summary for Policymakers. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty (Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (eds.). World Meteorological Organization, pp 32. (in Geneva, Switzerland)

38. Ito LS, Cornejo AM, Yamashita M, Strüssmann CA (2008) Thermal Threshold and Histological Process of Heat-Induced Sterility in Adult Pejerrey (*Odontesthes bonariensis*): A Comparative Analysis of Laboratory and Wild Specimens.Physiol. Biochem Zool 81:775–784. https://doi.org/10.1086/591035

39. Jacquin L, Gandar A, Aguirre-Smith M, Perrault A, Hénaff MLe, Jong L, Paris-Palacios, Laffaille S. and Jean PS(2019) High temperature aggravates the effects of pesticides in goldfish. Ecotoxicol. Environ. Saf, Elsevier. 172: 255–264. 10.1016/j.ecoenv.2019.01.085

40. Jobling M (1981) Temperature tolerance and the final preferendum rapid methods for the assessment of optimum growth temperatures. J Fish Biol 19:439–455

41. Katalay S, Parlak H(2004) The Effects of water Pollution on Haematological Parameters of Black Goby (Gobius niger L.,1758) in Foca and Aliaga Bays.E.U. JFAS.21:113–117. doi: 10.12714/egejfas.2002.19.1.5000157130

42. Khalil WKB, Hasheesh WS, Mohamed-Assem SM, Abbas HH, Zahran EA (2011) Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus*. Life Sci J 8:329–343

43. King HR, Pankhurst NW, Watts M, Pankhurst PM (2003) Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. J Fish Biol 63:153–167. https://doi.org/10.1046/j.1095-8649.2003.00137.x

44. Lazard J (2009) La pisciculture des tilapias. Cah Agric 18:174–182

45. Lermen CL, Lappe R, Crestani M, Vieira VP, Gioda CR, Schetinger MRC, Baldisserotto B, Moraes G, Morsch VM (2004) Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. Aquaculture 239:497–507. https://doi.org/10.1016/j.aquaculture.2004.06.021

46. Lowe-Mc Connell RH (1958) Observations on the biology of *Tilapia nilotica* Linne in East African waters. Rev J Afr Zool Bot 57:129–170

47. Mali RP, Chavan PN (2014) Temperature dependent haematological alterations of freshwater cultivable fish, *Oreochromis mossambicus* from Nanded District (Ms) India. ISSN:2319–875311

48. McInroy RA (1954) A Micro-haematocrit for Determining the Packed Cell Volume & Haemoglobin Concentration on Capillary Blood. J Clini Pathol 7:32–36. https://doi.org/10.1136/jcp.7.1.32

49. Miranda LA, Chalde T, Elisio M, Strüssmann CA (2013) Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*. Gen Comp Endocrinol 192:45–54. https://doi.org/10.1016/j.ygcen.2013.02.034

50. Morgan IJ, Donald M, Wood CM (2001) The cost of living for freshwater fish in a warmer, more polluted world. Glob Change Biol 7:345–355

51. Nandi S, Routray P, Gupta SD, Rath SC, Dasgupta S, Meher PK, Mukhopadhyay PK (2007) Reproductive performance of carp, *Catla catla* (Ham.), reared on a formulated diet with PUFA supplementation. J Appl Ichthyol 23:684–691. doi:10.1111/j.1439-0426.2007.00874.x

52. Nitithamyong C(1988) Bioenergetics approach to the study of anabolic steroids effects of 17 alpha-methyltestosterone in blue tilapia, *Oreochromis aureus*. Ph.D. Thesis, University of, pp 124. (in Wisconsin-Madison)

53. Okuzawa K, Kumakura N, Gen K, Yamaguchi S, Lim BS, Kagawa H (2003) Effect of high-water temperature on brain-pituitary-gonad axis of the red seabream during its spawning season. Fish Physiol Biochem 28:439–440. https://doi.org/10.1023/B:Fish.0000030621.31336.91

54. Pandit N, Nakamura M (2010) Effect of high temperature on survival, growth and feed conversion ratio of Nile tilapia, *Oreochromis niloticus*. Our Natl 8:219–224. https://doi.org/10.3126/on.v8i1.4331

55. Pang X, Cao ZD, Fu SJ (2011) The effects of temperature on metabolic interaction between digestion and locomotion in juveniles of three cyprinid fish (*Carassius auratus*, *Cyprinus carpio*, and *Spinibarbus sinensis*). Comp Biochem Physiol 159:253–260. https://doi.org/10.1016/j.cbpa.2011.03.013

56. Patra S, Mishra G, Panda B, Verma DK, Dash SK, Nandi S, Routray P(2015) Reproductive characteristics and Germ status of Indian Major Carp, *Labeo rohita* reared in elevated water temperature regime.J. Aqua.23

57. Peters WCH (1852) Diagnoses of new river fish from *Mossambique*. Monthly Prus Acad sci 275–276:681–685

58. Pörtner HO, Peck MA (2010) Climate change effects on fishes and fisheries: towards a cause and effect understanding. J fish Biol 77:1745–1779. https://doi.org/10.1111/j.1095-8649.2010.02783.x

59. Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–97. https://doi.org/10.1126/science.1135471

60. Pough F, Harvey CM, Janis. and John BH(2009) Vertebrate Life (8th edition). San Francisco, CA: Pearson Education, Inc. 380

61. Routray P, Suzuki T, Strüssmann CA, Takai R (2002) Factors affecting the uptake of DMSO by the eggs and embryos of medaka, *Oryzias latipes*. Theriogenology 58:1483–1496. https://doi.org/10.1016/S0093-691X(02)01076-2

62. Sahli H(1909) Lehrbuch d. klin. Untersuchungen Methode, (5th edition.), p. 846, (in Leipzig)

63. Sapkale PH, Singh RK, Desai AS (2011) Optimal water temperature and pH for development of eggs and growth of spawn of common carp (*Cyprinus carpio*). J Appl Anim Res 39:339–345. https://doi.org/10.1080/09712119.2011.620269

64. Sayed AEDH, Moneeb RH (2015) Hematological and biochemical characters of monosex tilapia (*Oreochromis niloticus*, Linnaeus, 1758) cultivated using methyltestosterone. JOBAZ 72:36–42 https://doi.org/10.1016/j.jobaz.2015.03.002

65. Schiemer F, Keckeis H, Kamler E(2002) The early life history stages of riverine fish: Ecophysiological and environmental bottlenecks, in: Comp. Biochem. Physiol. A Mol. Integr. Physiol. pp.439–449. https://doi.org/10.1016/S1095-6433(02)00246-5

66. Schiemer F, Keckeis H, Nemeschkal H, Schludermann E, Winkler G, Zweimüller I (2004) Ontogenetic patterns in thermal adaptation of fish vs. long-term temperature trends in large rivers. Int Congr 1275:209–217. https://doi.org/10.1016/j.ics.2004.09.039

67. Shah SL, Altindag A (2004) Hematological parameters of tench (Tinca tinca L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull Environ Contam Toxicol 73:911–918. https://doi.org/10.1007/s00128-004-0513-y

68. Sifa L, Chenhong L, Dey M, Gagalac F, Dunham R (2002) Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China. Aquaculture 213:123–129. https://doi.org/10.1016/S0044-8486(02)00068-6

69. Sollid J (2005) Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. J Exp Biol 208:1109–1116. https://doi.org/10.1242/jeb.01505

70. Soria FN, Strüssmann CA, Miranda LA (2008) High water temperatures impair the reproductive ability of the pejerrey fish *Odontesthes bonariensis*: effects on the hypophyseal-gonadal axis. Physiol Biochem Zool 81:898–905. https://doi.org/10.1086/588178

71. Strüssmann CA, Nakamura M (2002) Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. Fish Physiol Biochem 26:13–29. https://doi.org/10.1023/A:1023343023556

72. Strüssmann CA, Saito T, Takashima F (1998) Heat-induced germ cell deficiency in the teleosts *Odontesthes bonariensis* and *Patagonina hatcheri*. Comp Biochem Physiol A Mol Int Physiol 119:637–644. https://doi.org/10.1016/S1095-6433(97)00477-7

73. Teletchea F(2019) Fish domestication: An overview. In: Teletchea F, editorial Animal Domestication. doi: 10.5772/intechopen.79628

74. Teletchea F, Fostier A, Kamler E, Gardeur JN, Bail L, Jalabert PY, Fontaine P (2009) Comparative analysis of reproductive traits in 65 freshwater fish species: application to the domestication of new fish species. Rev in Fish Biol Fisheries 19:403–430. https://doi.org/10.1007/s11160-008-9102-1

75. Tun-Lin W, Burkot TR, Kay BH (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med Vet Entomol 14:31–37. https://doi.org/10.1046/j.1365-2915.2000.00207.x

76. Vernberg WB, Mckellar H, Vernberg FJ (1978) Toxicity Studies and Environmental Impact Assessment. Environ Manage 2:239–243. https://doi.org/10.1007/BF01866552

Tables

Table 1 to 5 are available in the Supplementary Files section.

Figures
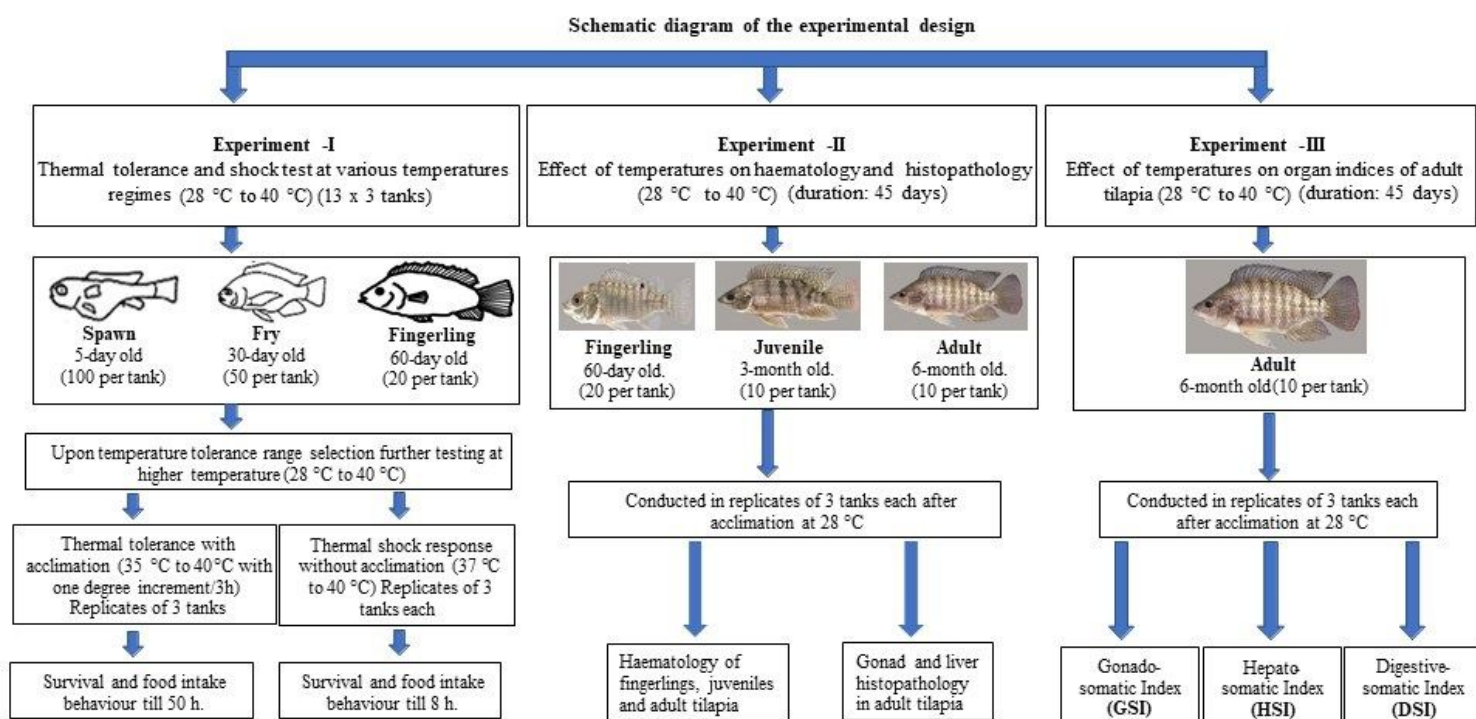
Figure 1

Schematic depiction of the experimental design to study the thermal acclimation/threshold and response of tilapia, *O. niloticus*. All treatments were conducted in triplicate and 28 °C served as control
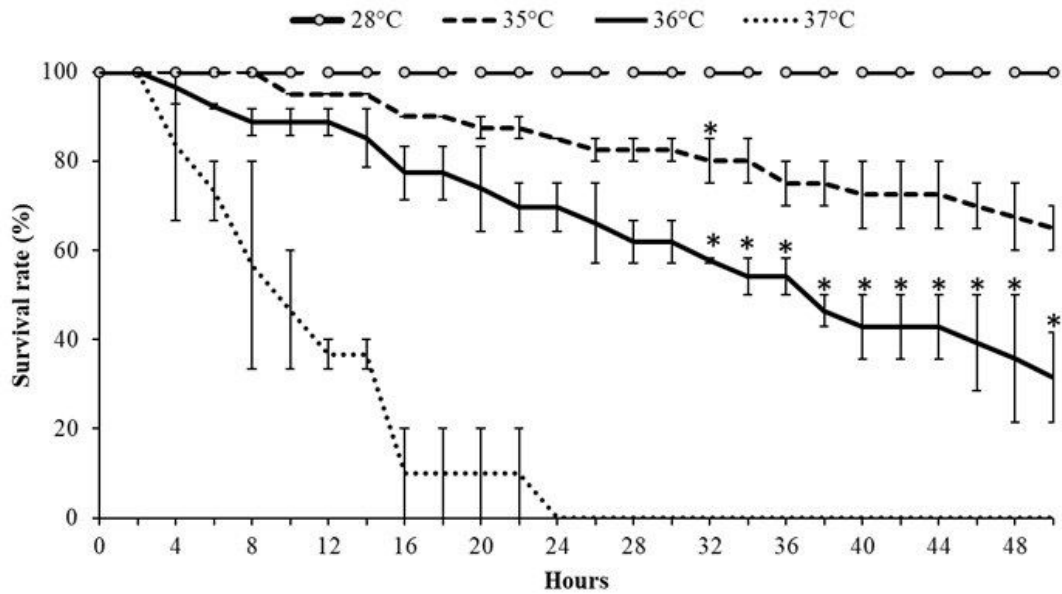
Figure 2

Survival and mortality pattern of spawn (5 day old hatchling) tested at four different temperatures (28 °C control, 35 °C, 36 °C, 37 °C). Data shown as mean ± SEM (n=100 for each treatment). Asterisks indicate significant differences between treatments (P<0.05)
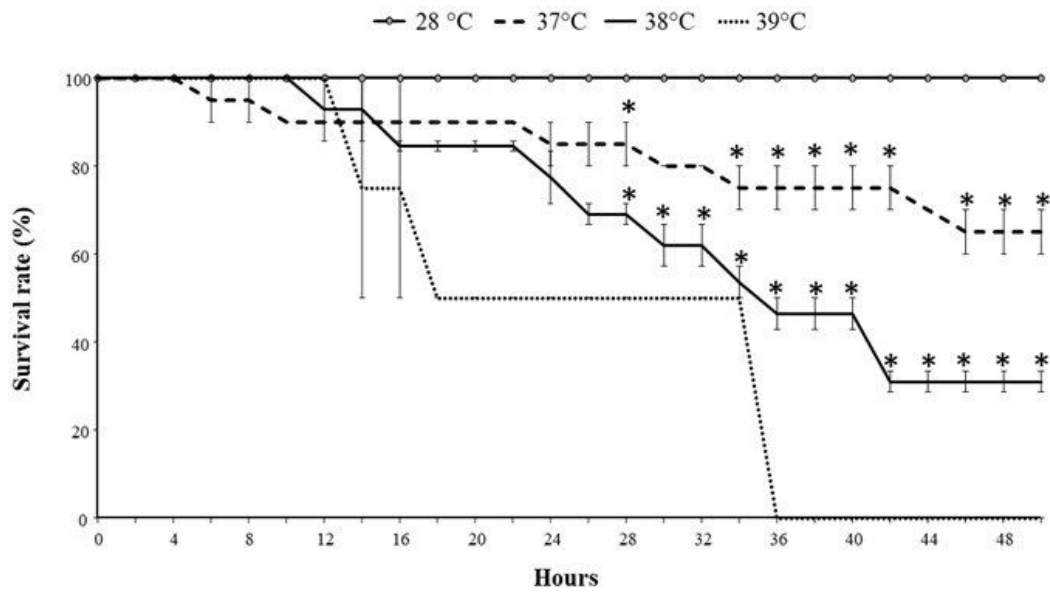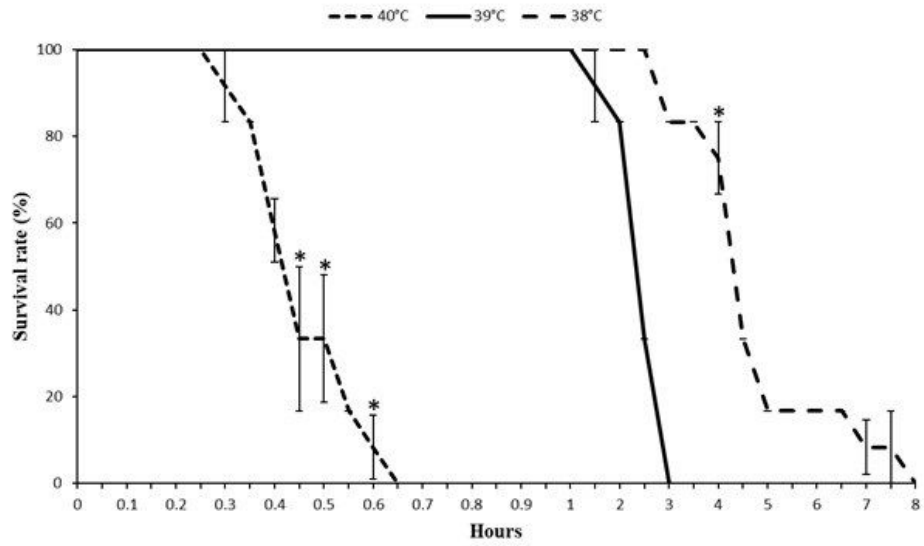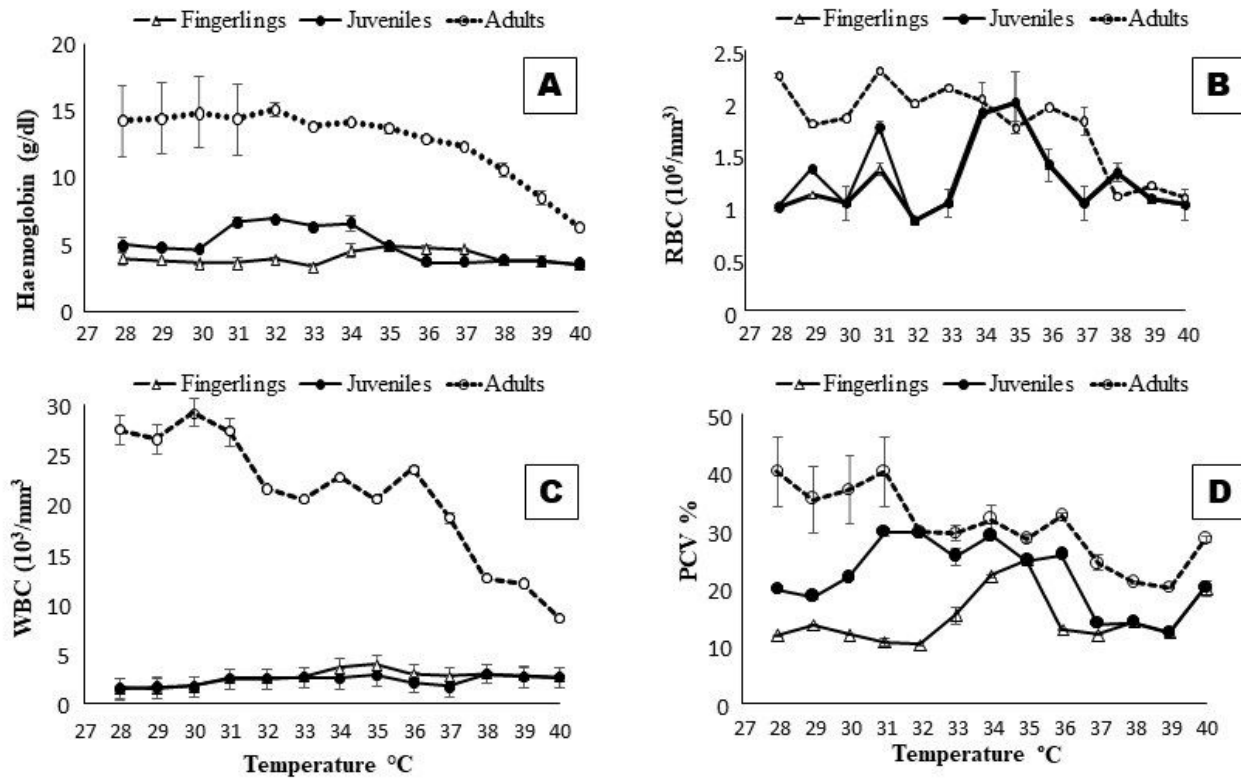
Figure 3

Survival and mortality pattern of fry tested at four different temperatures (28 °C control, 37 °C, 38 °C, 39 °C). Data shown as mean ± SEM (n=50 for each treatment). Asterisks indicate significant differences between treatments (P<0.05)
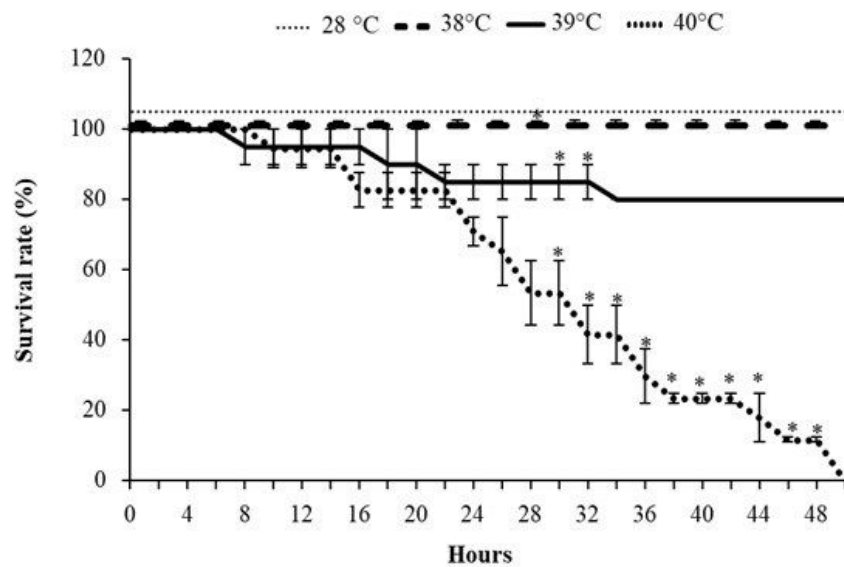
Figure 4

Survival and mortality pattern of fingerlings tested at four different temperatures (28 °C control, 38 °C, 39 °C, 40 °C). To differentiate overlapping lines between control and 38 °C, the control line is slightly moved from its original position. Data shown as mean ± SEM (n=20 for each treatment). Asterisks indicate significant differences between treatments (P<0.05)
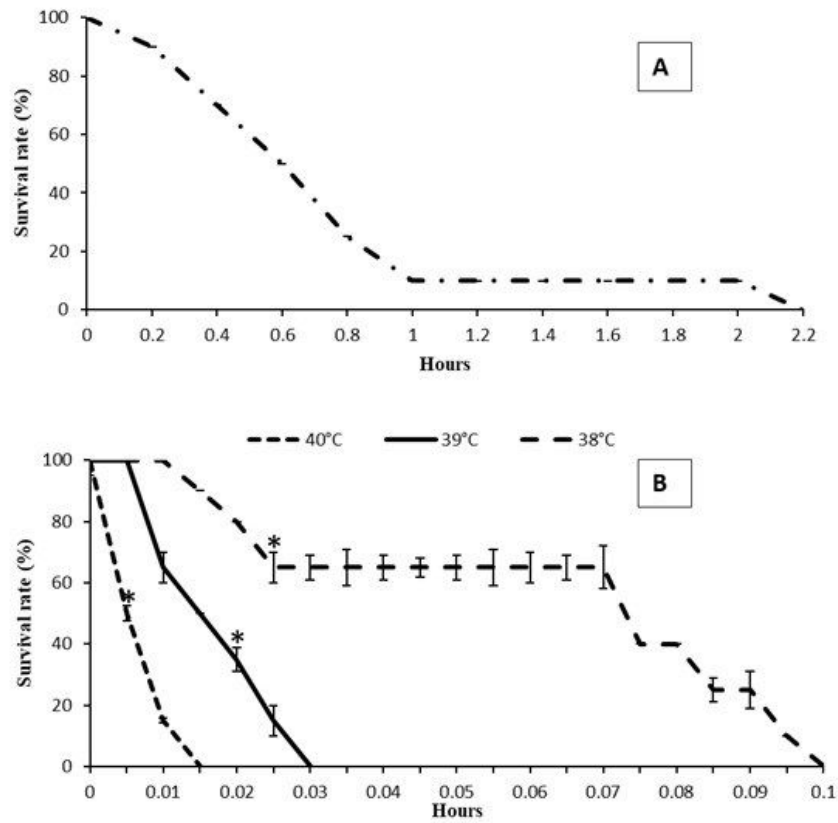
Figure 5

Thermal shock response of tilapia spawn (5 day old hatchling) exposed directly (without acclimation) to test temperatures, A: 37 °C and B: 38 °C to 40 °C. Data shown as mean ± SEM (n=100 for each treatment). Asterisks indicate significant differences between treatments (P<0.05)
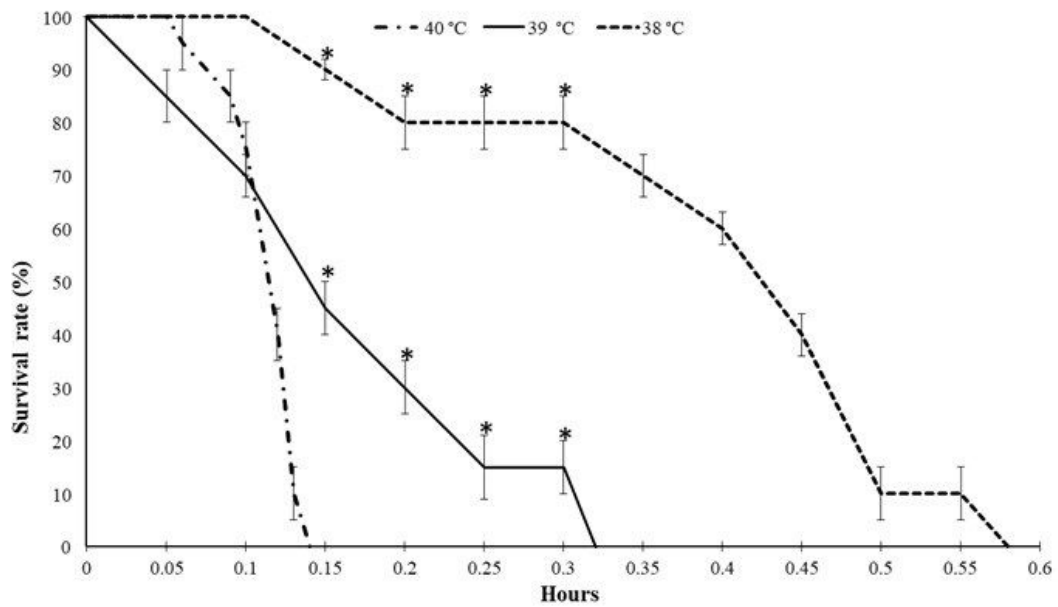
Figure 6

Thermal shock response of tilapia fry exposed directly (without acclimation) to test temperatures 38 °C, 39 °C and 40 °C. Data shown as mean ± SEM (n=50 for each treatment in triplicate). Asterisks indicate significant differences between treatments (P<0.05)
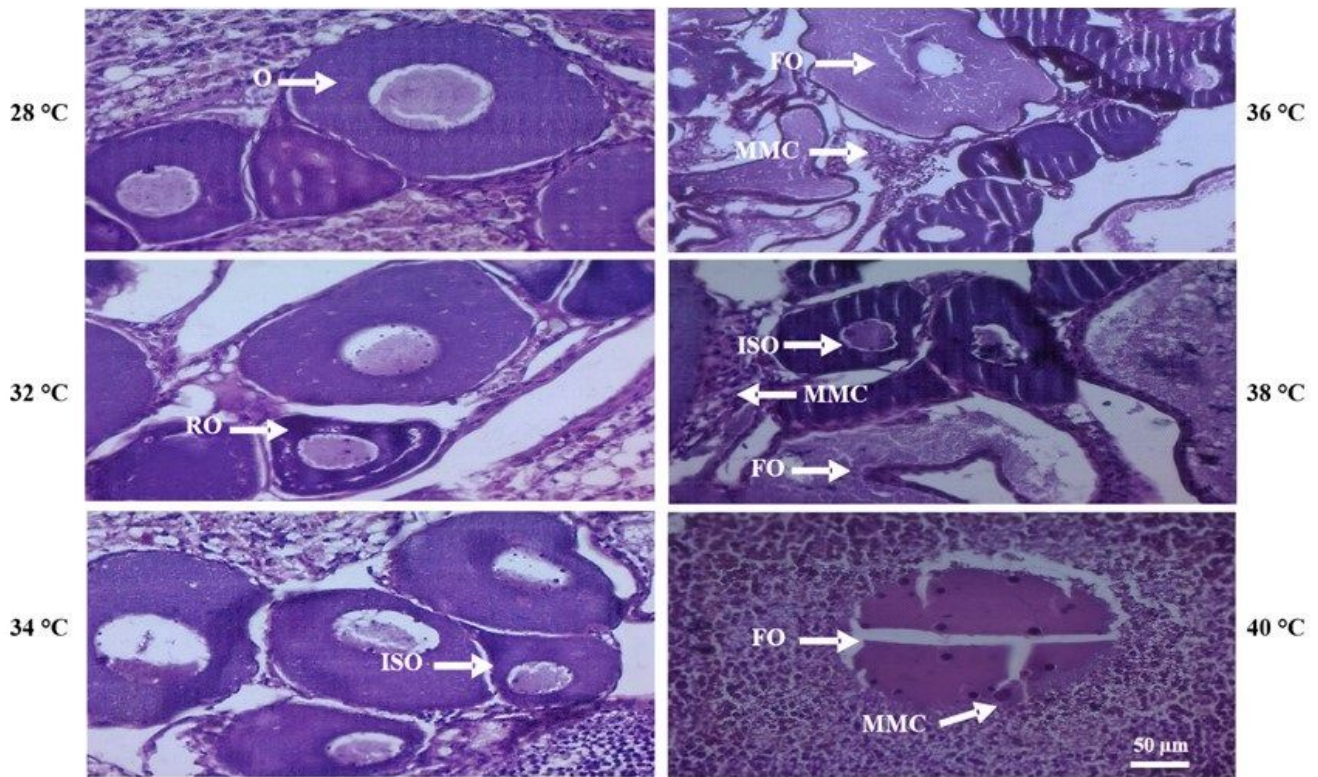
Figure 7

Thermal shock response of tilapia fingerlings exposed directly (without acclimation) to test temperatures 38 °C, 39 °C to 40 °C. Data shown as mean ± SEM (n=20 for each treatment in triplicate). Asterisks indicate significant differences between treatments (P<0.05)

Figure 8

Hematological parameters of three stages of tilapia (fingerlings, juveniles and adults) treated with different temperature regimes ((Control) 28°C to 40°C), **A**: Haemoglobin; **B**: RBC; **C**: WBC; **D**: PCV. Data shown as mean ± SEM
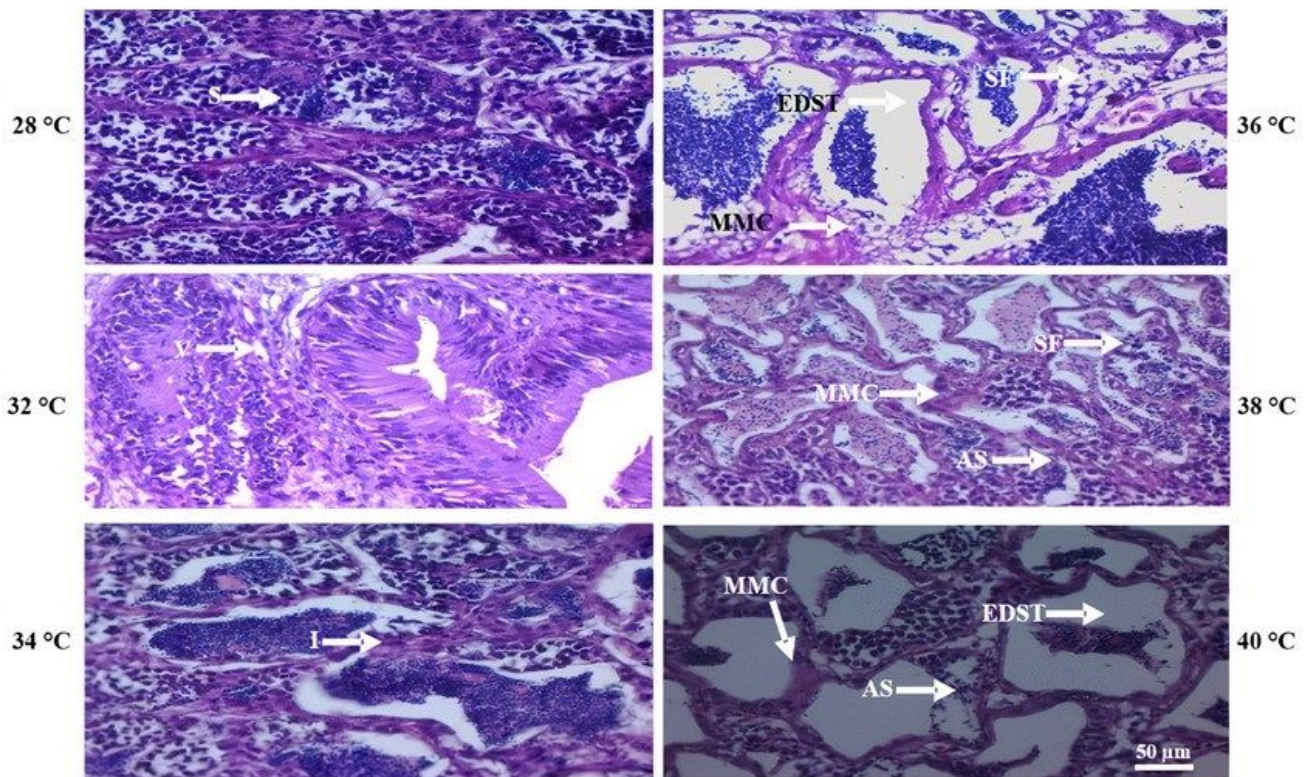
Figure 9

Histopathological changes in the testis of adult tilapia after exposure to 28 °C (control), 32 °C, 34 °C, 36 °C, 38 °C, 40 °C. (stain: H& E; magnification: 40X). Abbreviations: Male gonad (testis); S: active spermatogenesis; I: inflammation in seminiferous epithelium; EDST: enlarged and degenerative seminiferous tubules; V: vacuolation; MMC: melano-macrophage centers; SF: severe fragmentation; AS: arrested spermatogenesis. Scale bar = 50 µm
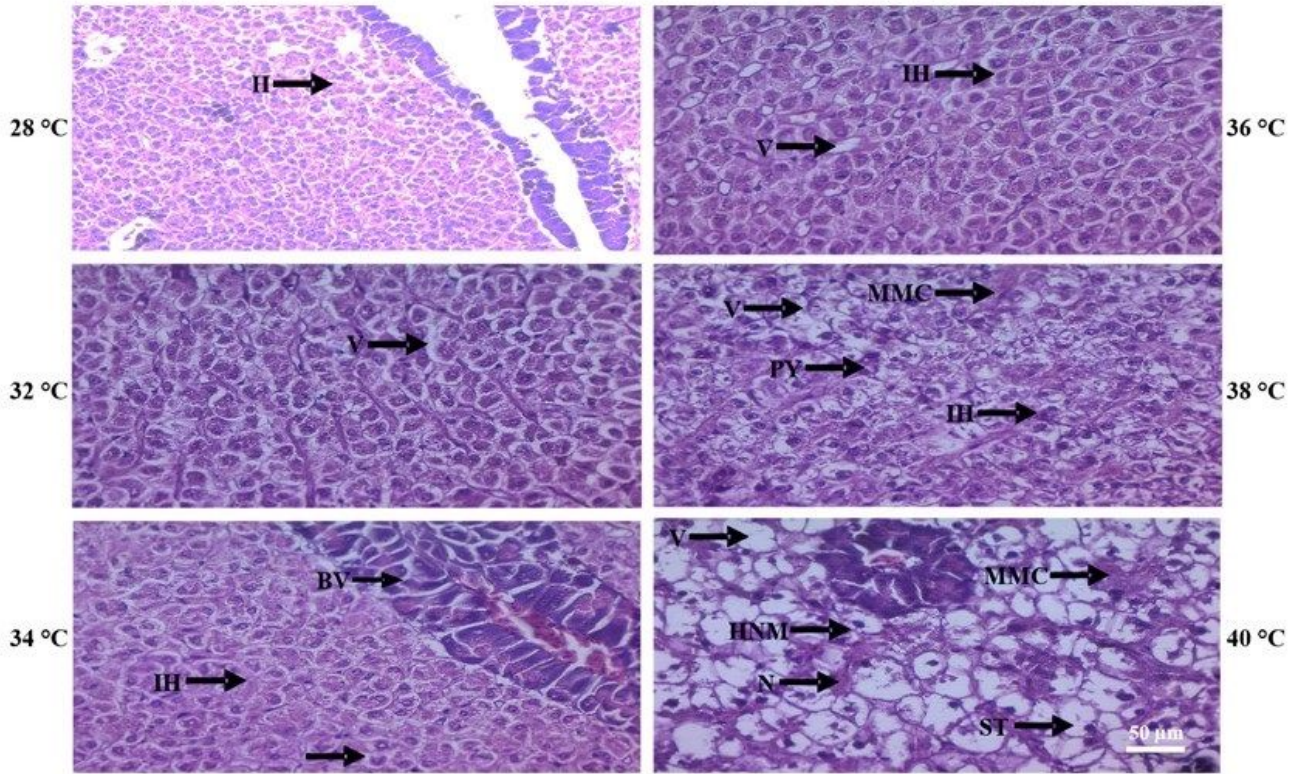
Figure 10

Histopathological changes in the ovary of adult female tilapia after exposure to 28 °C (control), 32 °C, 34 °C, 36 °C, 38 °C, 40 °C. (stain: H & E, magnification: 40X). Abbreviations: RO: reduced oocyte; FO: fragmented oocyte;; ISO: irregular shrinkage of oocyte; FO: fragmented oocytes; MMC: melano-macrophage centers. Scale bar = 50 μm
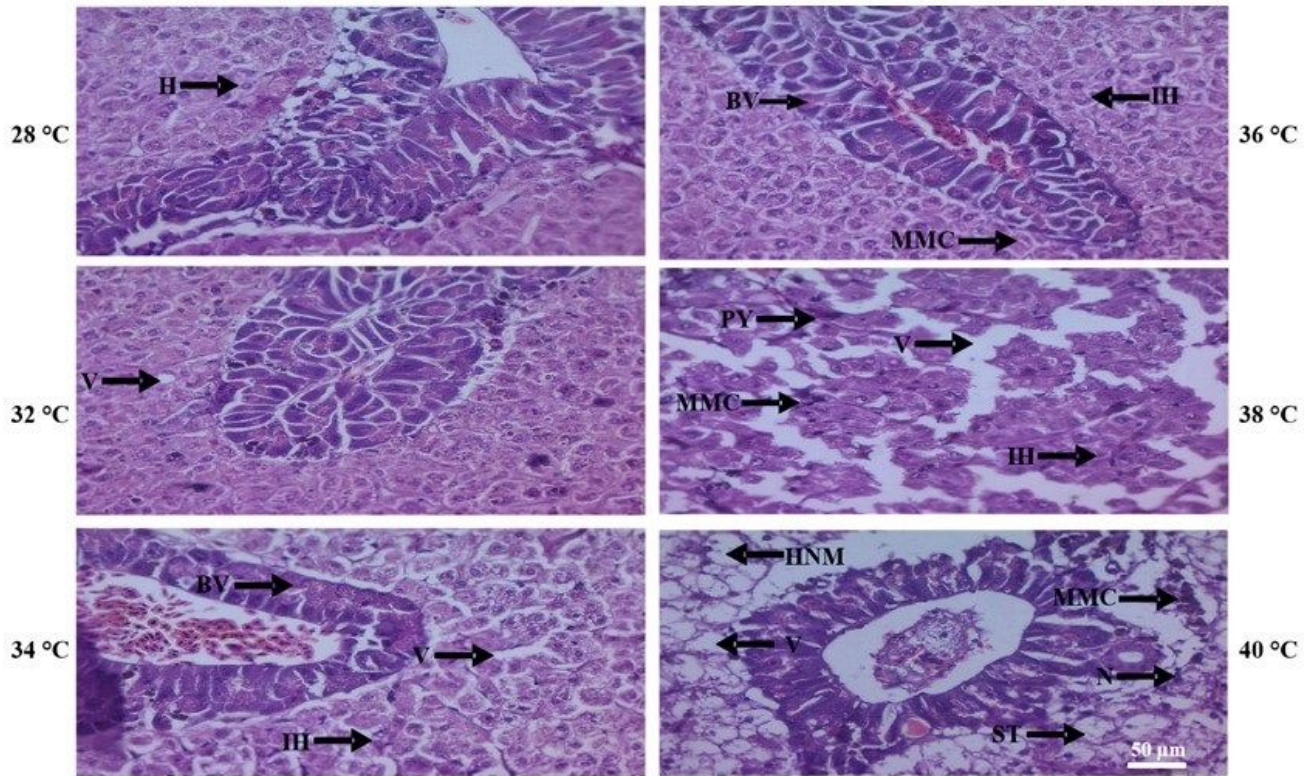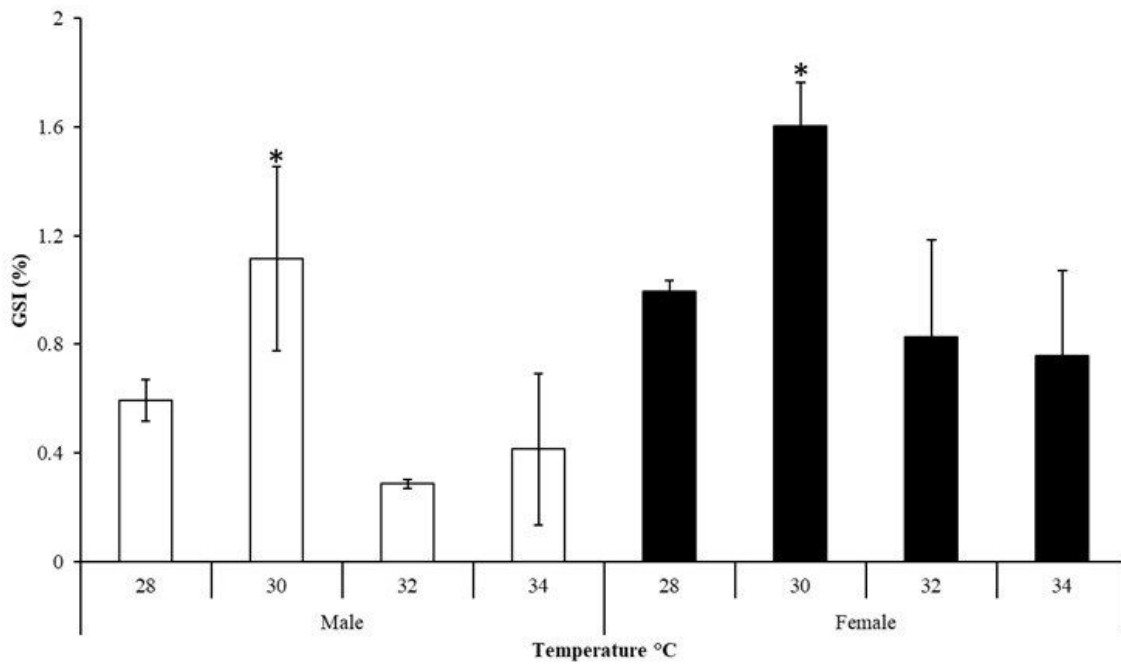
Figure 11

Histopathological changes in liver of adult male tilapia after exposure to 28 °C (control), 32 °C, 34 °C, 36 °C, 38 °C, 40 °C. (stain: H& E; magnification: 40X). Abbreviations: H: hepatocytes; V: cytoplasmic vacuolization; NP: nuclear pyknosis; BV: intravascular hemolysis in hepatoportal blood vessel; MMC: melano-macrophage centers; IH: irregular hepatocytes; N: necrosis of hepatocytes; HNM: hepatocytes nuclear migration and ST: steatosis. Scale bar = 50 µm
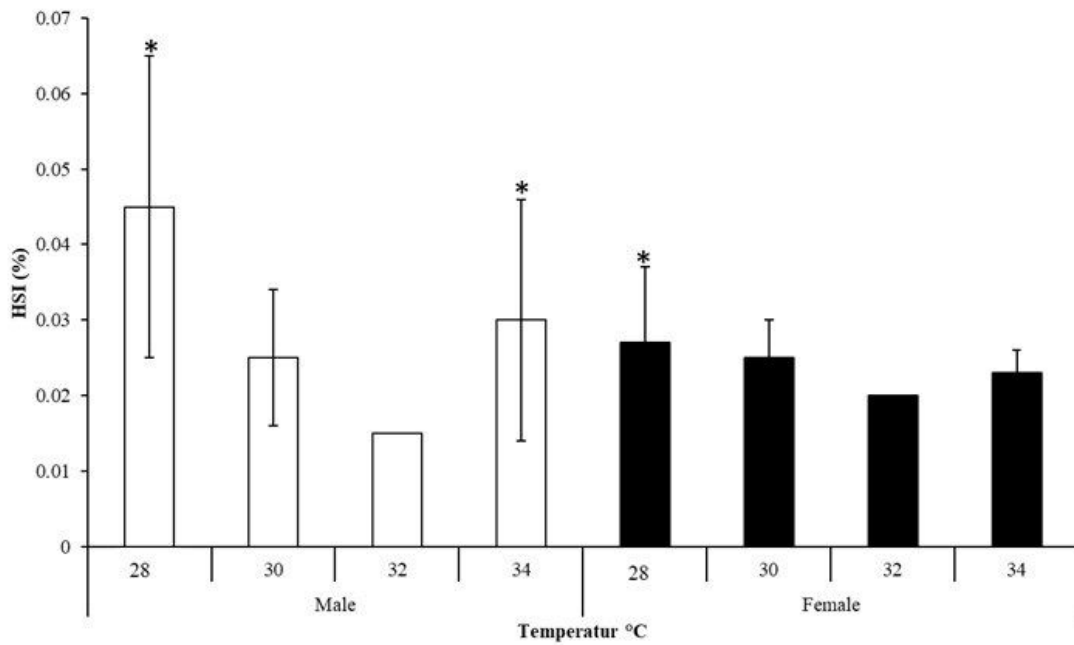
Figure 12

Histopathological changes in liver of adult female tilapia after exposure to 28 °C (control), 32 °C, 34 °C, 36 °C, 38 °C, 40 °C. (stain: H& E; magnification: 40X). Abbreviations: H: hepatocytes; V: cytoplasmic vacuolization; NP: nuclear pyknosis; BV: intravascular hemolysis in hepatoportal blood vessel; MMC: melano-macrophage centers; IH: irregular hepatocytes; N: necrosis of hepatocytes; HNM: hepatocytes nuclear migration and ST: steatosis. Scale bar = 50 µm
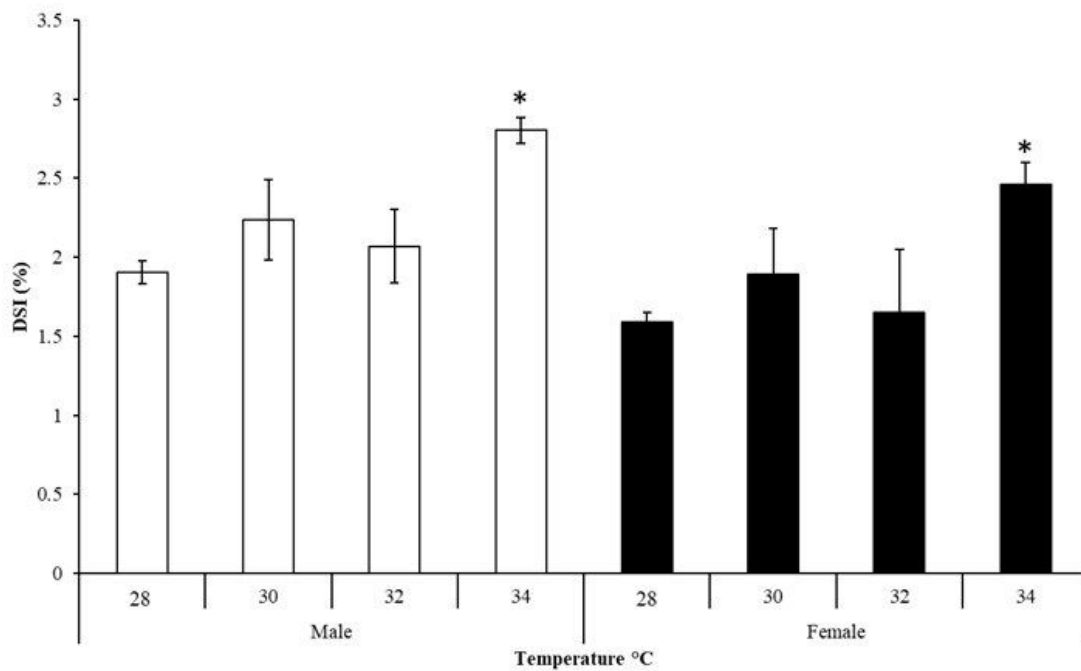
Figure 13

Changes in the gonado-somatic index (GSI) of male and female tilapia acclimatized at four at different temperatures (28 °C Control, 30 °C, 32 °C, 34 °C). Data shown as mean ± SEM (n=10 for each treatment from each tank). Asterisks indicate significant differences between treatments (P<0.05)

Figure 14

Changes in the hepato-somatic index (HSI) of male and female tilapia acclimated at four different temperatures (28 °C Control, 30 °C, 32 °C, 34 °C). Data shown as mean ± SEM (n=10 for each treatment from each tank). Asterisks indicate significant differences between treatments (P<0.05)

Figure 15

Changes in the digestive-somatic index (DSI) of male and female tilapia acclimated at four different temperatures (28 °C Control, 30 °C, 32 °C, 34 °C). Data shown as mean ± SEM (n = 10 for each treatment from each tank). Asterisks indicate significant differences between treatments (P<0.05)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS.pptx