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HISTOLOGICAL CHANGES IN *HORABAGRUS BRACHYSOMA* ACCLIMATED TO WARM TEMPERATURE AND EXPOSED TO CRITICAL THERMAL MAXIMA AND MINIMA

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Abstract. Escalating concerns about global climate change necessitate investigations into its consequences for the survival of poikilothermic animals like fish. The present study describes histological alterations in gill, skin, kidney, liver, and brain tissues of the juvenile *Horabagrus brachysoma* specimens acclimated to 26°C (control) and 36°C for 30 days, and those exposed to dynamic temperature changes (increased or decreased at the rate of 0.3°C/min from 26°C), the critical thermal maxima (CTMax) and critical thermal minima (CTMin). The fish acclimated to 36°C and those exposed to CTMax (40.23 ± 0.12°C) and CTMin (14.15 ± 0.10°C) showed severe histological aberrations in gill, skin, liver, kidney, and brain tissues. Histological alterations in gill tissues included loss of epithelial cells in the branchial arch, thinning of the primary lamellae, and loss of the secondary lamellae. Thickening of the epithelial layer (36°C), and desquamation of epithelial cells (CTMax) were the histological alterations detected in skin tissues. Alterations in liver tissues included severe congestion with vacuolization and the cloudy appearance of cells and extensive loss of cellular contour. Extensive vacuolization with complete flattening of the tubule epithelial cells, distorted appearance of tubular lamellae, and marked loss of glomerular tuft were the changes recorded in kidney tissues. Brain tissue alterations comprised increased cellularity and vacuolization in the cerebrum (36°C), and nodular masses of various sizes in the cerebrum (CTMin). The present study showed that acclimation to warm temperature (36°C) and exposure to dynamic temperature changes (CTMax and CTMin) cause histopathological alterations in the vital organs of *H. brachysoma*. The findings of the present study can help in monitoring the health of *H. brachysoma* in the natural environment and in culture systems under a climate change scenario.

INTRODUCTION

Temperature is an important physical environmental factor profoundly influencing lives of all poikilothermic animals, including fish. Unfavorable changes in the environmental temperature cause stress in fish, influencing their physiological homeostasis and affecting their optimum temperature for growth, reproduction, and disease resistance (Pörtner and Peck 2010; Alfonso et al. 2021; Islam et al. 2022a). Thermal extremes, both maximum and minimum, cause stress in fish altering their biochemical processes and cellular architecture (Das et al. 2014; Ern et al. 2023). Severe or irreversible cellular and histopathological changes in the vital organs can seriously affect their normal functions eventually leading to the death of fish. Tissue histology is a valuable tool for monitoring fish health in aquaculture systems and in the natural environment (Saraiva et al. 2015; Abbaszadeh and Şişman 2021). Therefore, histopathological investigations in relation to such environmental protuberances can help understand and correlate the physiological and behavioural changes observed in fish during stressful conditions (Wolf et al. 2015; Phrompanya et al. 2021).

Depending on emission scenarios, global mean surface temperatures are expected to rise by 0.9–5.4 °C by 2100, potentially causing an increase in water temperature (Pörtner et al. 2019). Climate change is a significant stressor in aquatic ecosystems, and freshwater ecosystems are highly vulnerable to global warming (Capon et al. 2021) affecting several aquaculture species (Mugwanya et al. 2022). Rising freshwater temperatures are predicted to affect species' geographical ranges, disease outbreaks, phenology, and survival of fishes (Reid et al. 2019). Climate change is expected to have a significant influence on tropical freshwater fish species (Ficke et al. 2007; Li et al. 2022; de Visser et al. 2023), with 74–81% of them declining and more than half of their range becoming endangered with a 2°C rise in global temperature (Barbarossa et al. 2021). It is also predicted that global climate change may also cause "surprises" or sudden ecological changes altering the limnological characteristics, which may lead to increased incidences of fish kills in the coming years (Ficke et al. 2007; Panikkar et al. 2022). Several researchers have reported fish kills due to rapid temperature changes in freshwater bodies (Nair et al. 2021; Phelps et al. 2019; Kangur et al. 2016). The present study was therefore undertaken to understand the effect of both acclimation to warm temperature (36 °C) and short-term dynamic changes in water temperature, i.e., critical thermal maxima (CTMax) and critical thermal minima (CTMin), on the histological alterations in rare and endemic catfish, *Horabagrus brachysoma* (Family: Bagridae).

We used the critical thermal methodology (CTM) to investigate the effect of dynamic temperature changes on the histology of the vital organs of *H. brachysoma*. CTM is widely used to determine the temperature tolerance in fish and to estimate lethal temperatures without killing the fish, wherein, fish are exposed to a linear and constant rate of temperature increase or decrease until a pre-death thermal point is reached, designated as a critical thermal maximum and critical thermal minimum, respectively (Beitinger et al. 2000). The endpoint is manifested by the disorganized locomotory movements of the fish, indicating the loss of ability to escape the situation that could ultimately result in its death (Beitinger et al. 2000; Desforges et al. 2023; Conte et al. 2023). It is suggested that this approach is more relevant to temperature variations occurring in natural conditions (Bennett and Judd 1992; Terblanche et al. 2011).

Investigations into the effect of fish acclimation to warm temperatures have revealed various histological alterations in different tissues. For example, the acclimation of the hybrid catfish (♂ *Clarias gariepinus* × ♀ *C. macrocephalus*) to 37°C caused gill shortening, severe hyperplasia of epithelial cells, and desquamation of the gills; hepatocyte vacuolization, nuclei displacement, and pyknotic hepatic cells in the liver; peripheral distribution of mucus cells and increased epithelial layer thickness of the skin (Khieokhajonkhet et al. 2022). The advanced fingerlings of *Labeo rohita* acclimated to 36, 38, and 40°C exhibited such histopathological changes in the gills as primary lamellar hyperplasia, lamellar fusion and clubbing of the secondary lamellae; in the liver- disarrangement of hepatic cells and vacuolar degeneration; in kidneys- degeneration and a complete loss of cellular organization, massive vacuolar degeneration of the renal tissue; and in the heart- degeneration of the myofibrillar layer (Dash et al. 2011). However, research on the effect of CTMax and CTMin on fish histology is sparse. Hernández-López et al. (2018) reported that exposure of *Sardinops sagax caeruleus* to CTMax caused severe histological alterations in liver (vacuolated or necrotic hepatocytes and infiltration of inflammatory blood cells) and kidney (degenerative glomeruli and renal tubules, and increased melanomacrophage centers) tissues.

The catfish, *H. brachysoma*, is propagated as a candidate species for aquaculture (Raghavan et al. 2016) and is valued as an ornamental fish in India and in international trade (Anvar Ali et al. 2007; Sureshkumar 2013). Its natural home range is reported in the rivers of southern India states, including Kerala (Anvar Ali et al. 2007), Karnataka (Kali and Aghanashini) (Bhat 2001), and Northern Western Ghats in Maharashtra (Katwate et al. 2012). In India, *H. brachysoma* is considered an emerging species for small-scale aquaculture and is also caught from the wild for local consumption. Techniques for its captive breeding (Padmakumar et al. 2011) and mass production (Sahoo et al. 2010, 2014, 2015) are successfully established. Earlier studies on *H. brachysoma* revealed its increased heat tolerance and increased metabolic rates with acclimation temperatures increasing from 15 to 36 °C, with CTMax ranging from 34.86 ± 0.09°C to 42.79 ± 0.02°C, and the final preferred temperature estimated from the Q_{10} value ranging between 31 and 33 °C (Dalvi et al. 2009); the correlation of its increased heat tolerance and HSP70 levels with increasing acclimation temperatures (20 and 30 °C) (Dalvi et al. 2012); and modulation of its metabolic enzymes and cellular stress response (Dalvi et al. 2017). In the present study, we investigated histological changes in the vital organs such as the skin and gills, (exposed directly to the external environment) and such internal organs as the liver, kidney, and brain (equilibrated to the external environment temperature through blood circulation) of the juvenile *H. brachysoma* specimens acclimated to 26 (Control) and 36 °C and those exposed to dynamic temperature changes (CTMax and CTMin).

MATERIALS AND METHODS

Experimental fish

Juvenile *H. brachysoma* (average weight 20.72 ± 2.06 g) were procured from a local vendor (Aquatic world, Mumbai, India) and transported with proper oxygenation to the wet laboratory of the Central Institute of Fisheries Education (CIFE), Mumbai, India. The fish were acclimated to the laboratory conditions (26 ± 1 °C) for 30 days in a circular plastic tank (dimensions: 0.9 m diameter × 0.6 m height, capacity 350 L, Plasticrafts Group, Mumbai, India), and were fed *ad libitum* daily with live *Tubifex* worms (Dalvi et al. 2009). The natural photoperiod of approximately 12 h light: 12 h dark was maintained during the acclimation. Water quality parameters such as dissolved oxygen (5.8 to 6.3 mg.L^{-1}) and temperature (25.8 to 26.1°C) (Merck, Germany), pH (6.9 to 7.4) (digital pH meter; Labindia, Mumbai, India), free carbon dioxide (negligible), total hardness (60–80 mg.L^{-1}), ammonia (0.014–0.06 mg.L^{-1}), nitrite (0.001 to 0.005 mg.L^{-1}) and nitrate (0.05 to 0.06 mg.L^{-1}) were recorded following standard procedures (APHA 1998), and water was exchanged (10%) manually every day. The experiment was conducted with the permission of CIFE's local committee for animal experimentation.

Acclimation and critical thermal studies

Acclimation and exposure to CTMax and CTMin temperatures were carried out as described earlier (Dalvi et al. 2009). Briefly, 24 juvenile *H. brachysoma* were equally distributed (6 fish per aquarium) in 4 digital thermostatic aquaria (dimensions: 45 cm length × 32 cm width × 40 cm height, capacity 52 L, sensitivity ± 0.2°C, Suan Scientific Instruments & Equipments, Kolkata, West Bengal, India) with the initial water temperature maintained at 26 °C. One of the aquaria maintained at 26 °C was used as the control. For warm acclimation, the water temperature in an aquarium was increased at a rate of 1°C per day over the ambient water temperature of 26°C until 36 °C. The fish were maintained at 36 °C for a period of another 30 days prior to sampling. During the acclimation period fish were fed daily with live *Tubifex* worms *ad libitum*. Excess worms were removed from the experimental aquaria 1 h post-feeding. One-tenth of water in aquaria was daily exchanged with the water having the temperature like the water in which fish of the respective treatment group were kept. Water quality parameters, namely, pH, oxygen, alkalinity, nitrogen, and ammonia, were measured once every four days in each tank. Dissolved oxygen concentration was maintained at 5.6 ± 0.5 mg.L^{-1} throughout the experimental period by continuous aeration using a 2 HP centralized air blower. For the critical temperature study, another set of the fish (6 fish per aquarium) acclimated to 26°C for 30 days in two separate thermostatic aquaria was exposed to a linear water temperature increase (for CTMax) or decrease (for CTMin) at a rate of 0.3°C min^{-1} until the loss of equilibrium occurred in the fish. The CTMax and CTMin of the fish acclimated to 26°C were 40.23 ± 0.12°C and 14.15 ± 0.10°C, respectively. At the end of CTMax and CTMin exposure, the fish were transferred to separate tanks with water maintained at 26°C and allowed to recover for 24 h prior to sampling for histology.

Tissue preparation and histology

Following the 30-day acclimation period, three fish specimens from the control (26°C) and warm acclimation (36°C) groups, and those exposed to CTMax (40.23 ± 0.12 °C) and CTMin (14.15 ± 0.10 °C) were anaesthetized individually with clove oil (50 µL.L^{-1}). The gill, skin, liver, kidney, and brain tissues were carefully dissected, blotted on blotting paper to remove traces of blood clots, fixed in 10% neutral buffered formalin (HiMedia, India), dehydrated in different grades of alcohol, cleared in xylene, and embedded in paraffin wax. Tissues (brain: cerebrum area; kidney: posterior or trunk region; skin: dorsal trunk region; gill: vertical sections; and liver: whole organ sections including both right and left lobes) were sliced into thin sections (7 µm), and stained with hematoxylin and eosin, as described earlier (Kumar et al. 2016). The stained sections were examined using a binocular research microscope (Olympus CX-31, Japan) equipped with Nikon FX-35DX camera and photographs were taken wherever necessary.

RESULTS AND DISCUSSION

Suitable environmental conditions are essential for sustaining optimal physiological functions in poikilothermic animals. Long-term exposure of fish to extreme temperature that lies within their tolerance range causes stress and brings about physiological and biochemical reorganization leading to adaptation (Wendelaar Bonga 1997; Alfonso et al. 2021; Islam et al. 2022a). However, exposure to drastic thermal changes may cause pathological changes in the vital organs by distorting their structural integrity and functional processes that may prove detrimental for the species survival (Phrompanya et al. 2021). In the present study, the fish acclimated to 26°C (Control) showed normal histoarchitecture of gill (Figure 1a), skin (Figure 2a), liver (Figure 3a), kidney (Figure 4a), and brain (Figure 5a) tissues. However, the fish acclimated to 36°C and those exposed to CTMax and CTMin showed alterations in the histoarchitecture of the vital organs. During the 24 h recovery period following CTMax and CTMin exposure, the fish displayed behavioral changes such as cessation of feeding, erratic and sluggish swimming with occasional abrupt swimming hitting the walls of the aquaria, and increased surface activity and air gulping.

In our study, the gills of the fish acclimated to 36°C showed loss of epithelial cells in the branchial arch;

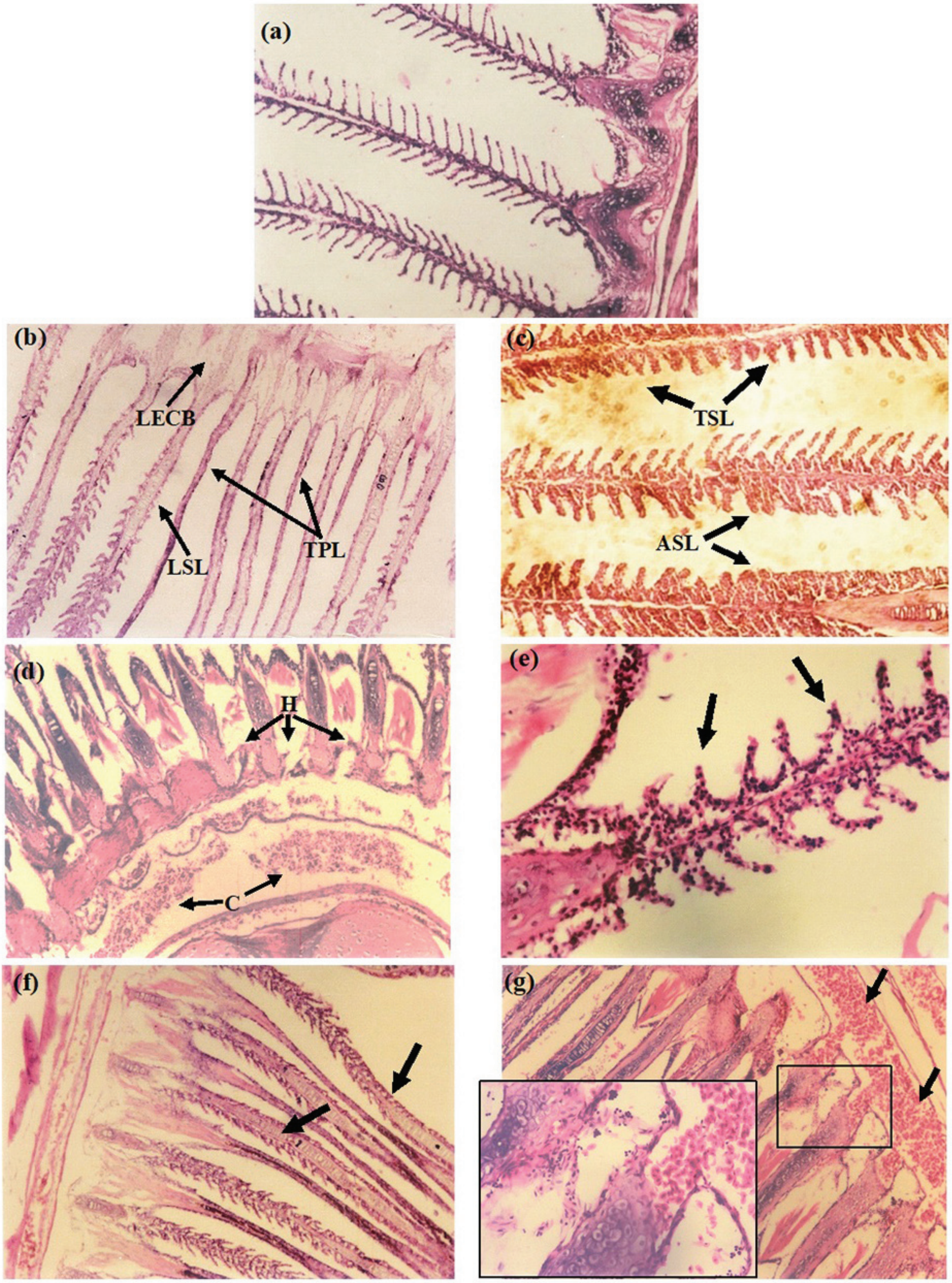

Figure 1. Histology of the gill of *H. brachysoma* (H and E staining). (a) acclimated to 26°C: normal histoarchitecture (40X); (b) acclimated to 36°C: loss of epithelial cells in the branchial arch (LECB), loss of the secondary lamellae (LSL); thinning of the the primary gill lamellae (TPL) (20X); (c) acclimated to 36°C: thickening (TSL) and atrophy (ASL) of the secondary gill lamellae (80X); (d) exposed to CTMax: congestions (C) and hemorrhages (H) in the branchial arch (40X); (e) exposed to CTMax: the damaged secondary lamellae (arrow) (160X); (f) exposed to CTMin: congestion in the primary gill filament (arrow) (40X); and (g) exposed to CTMin: congestion in the branchial arch (arrow) (40X) and the primary lamellae (box and insert) (160X).

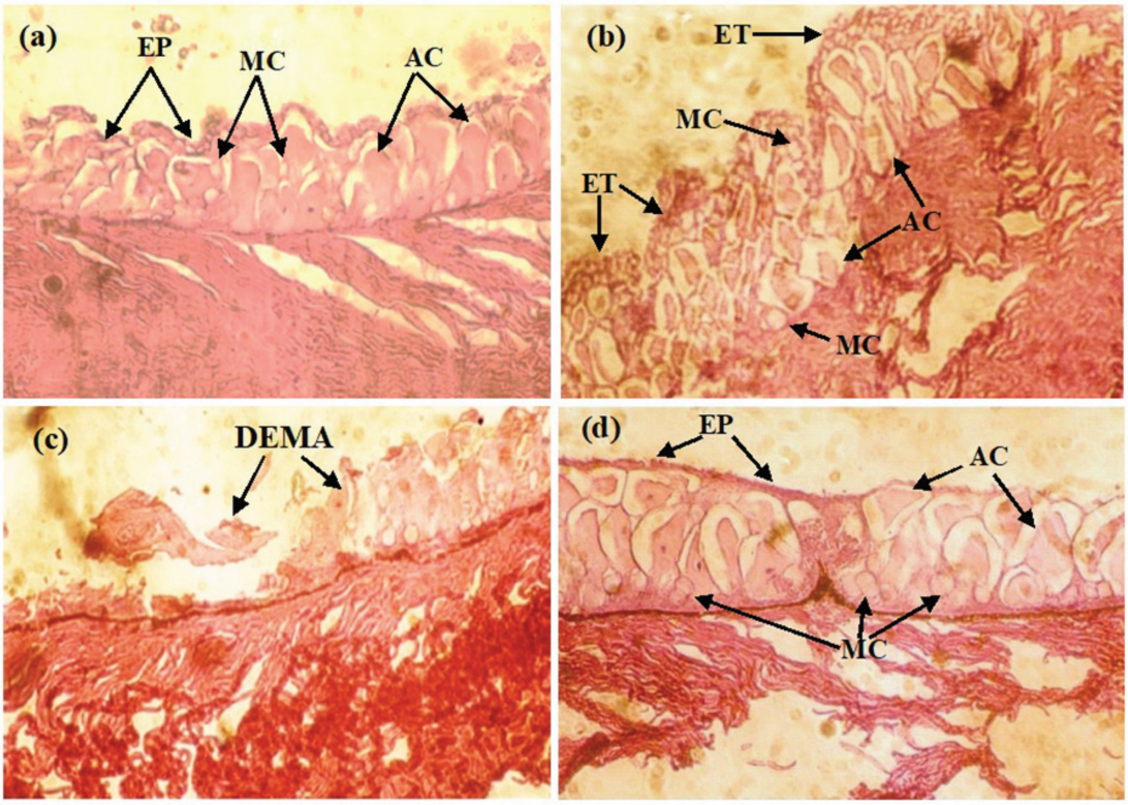

Figure 2. Histology of the skin of *H. brachysoma* (H and E staining). (a) acclimated to 26°C: normal histoarchitecture (80X) showing alarm / cuboid cell (AC), mucus cells (MC) and epithelium (EP); (b) acclimated to 36°C: epithelial thickening (ET) (80X); (c) exposed to CTMax: desquamation of epithelial, mucus and alarm cells (DEMA); and (d) exposed to CTMin: appeared normal (H and E; 80X).

thinning of the primary gill lamellae, and occasional loss of the secondary gill lamellae (Figure 1b); and thickening and atrophy of the secondary gill lamellae (Figure 1c). The gills of the fish exposed to CTMax showed congestions and hemorrhages in the branchial arch (Figure 1d), and damage to the primary gill filament with massive destruction of the secondary lamellae (Figure 1e), while the gills of those exposed to CTMin showed congestions in the primary filament (Figure 1f and 1g) and the branchial arch (Figure 1g). Along with its respiratory functions, the gills also act as heat exchangers for heat conduction between the environment and the fish body and facilitate the exchange of ions across the membrane. Drastic and rapid changes in water temperature cause the gills to undergo significant histological changes, which impact their primary functions (Wendelaar Bonga 1997), leading to passive ion effluxes and water intake in freshwater fishes inhibiting ion exchange (Wendelaar Bonga and Lock 1992). Higher water temperatures also affect the capacity of fish to maintain osmotic balance because they change the lipids in their gill cells, which causes cell leakage and lowers the effectiveness of salt excretion and osmotic balance (Pörtner et al. 2005; Das et al. 2014). Compensatory responses of the gills to changes in the external environment include hypertrophy and hyperplasia of the epithelium (Mallatt 1985). Although the present study revealed structural alterations in the gill tissue of the *H. brachysoma* specimens acclimated to 36°C, in an earlier study we observed an increase in the oxygen consumption rate in the fish acclimated to 36°C (Dalvi et al. 2009). Such increased oxygen demand during stress compromises the hydromineral balance in fish, with high levels of catecholamine and reduction in the vascular resistance in the gills by circulating epinephrine (Wendelaar Bonga 1997). The increased blood flow together with the branchial lamellae distention increases the effective respiratory surface area of the gills, thereby increasing the surface area for diffusion and, also the diffusion rate of water, ions, and even larger organic molecules, the process known as the osmorespiratory compromise (Wendelaar Bonga 1997; Onukwufor and Wood 2020; Wood and Eom 2021). Our results agree with those reported earlier for chronic exposure to increased temperatures in *L. rohita* (Dash et al. 2011; Islam et al. 2020), *Cyprinus carpio* (Saber 2011), *Paralichthys olivaceus* (Liu et al. 2014), killifish (*Aphanius dispar*) (Akbarzadeh et al. 2014), Nile tilapia (Islam et al. 2022b), hybrid catfish (♂*C. gariepinus* ×♀*C. macrocephalus*) (Khieokhajonkhet et al. 2022); and for acute thermal exposure in *Oreochromis niloticus* (Phrompanya et al. 2021).

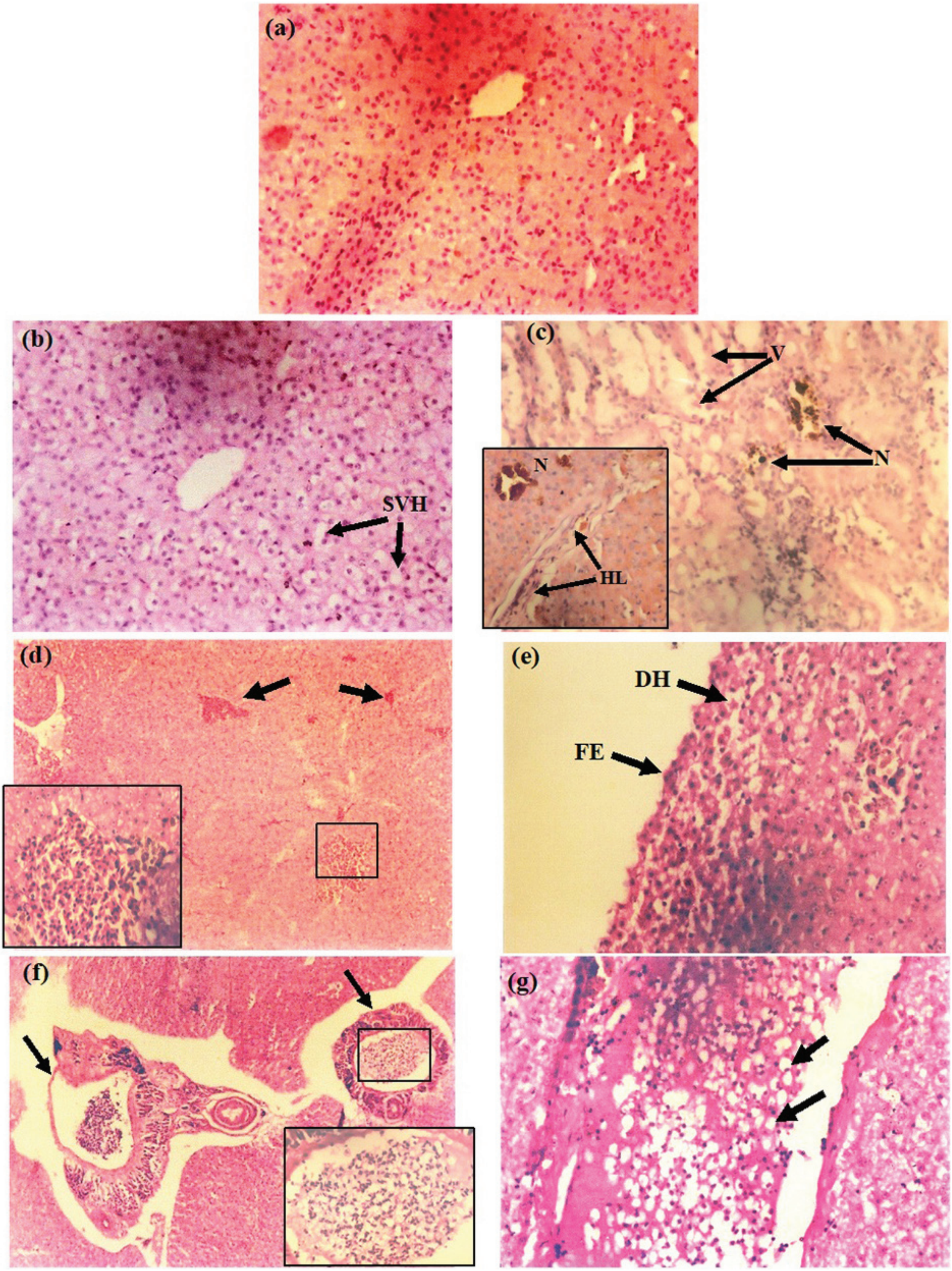

Figure 3. Histology of the liver of *H. brachysoma* (H and E staining). (a) acclimated to 26°C: normal histoarchitecture (160X); (b) acclimated to 36°C: swelling and vacuolation of hepatocytes (SVH) (160X); (c) acclimated to 36°C: occasional necrosis (N) and vacuoles (arrow) (160X), and occasional hemolysis (HL) and necrosis (N) (insert, 80X); (d) exposed to CTMax: severe congestion (arrow, 40X; box and insert, 160X); (e) exposed to CTMax: fragmented edges (FE) and degenerated hepatocytes (DH) (arrow) (160X); (f) exposed to CTMin: dilated blood vessels (arrow) (40X) with haemolysis (box and insert) (160X); and (g) exposed to CTMin: swollen hepatocytes (arrow) (160X).

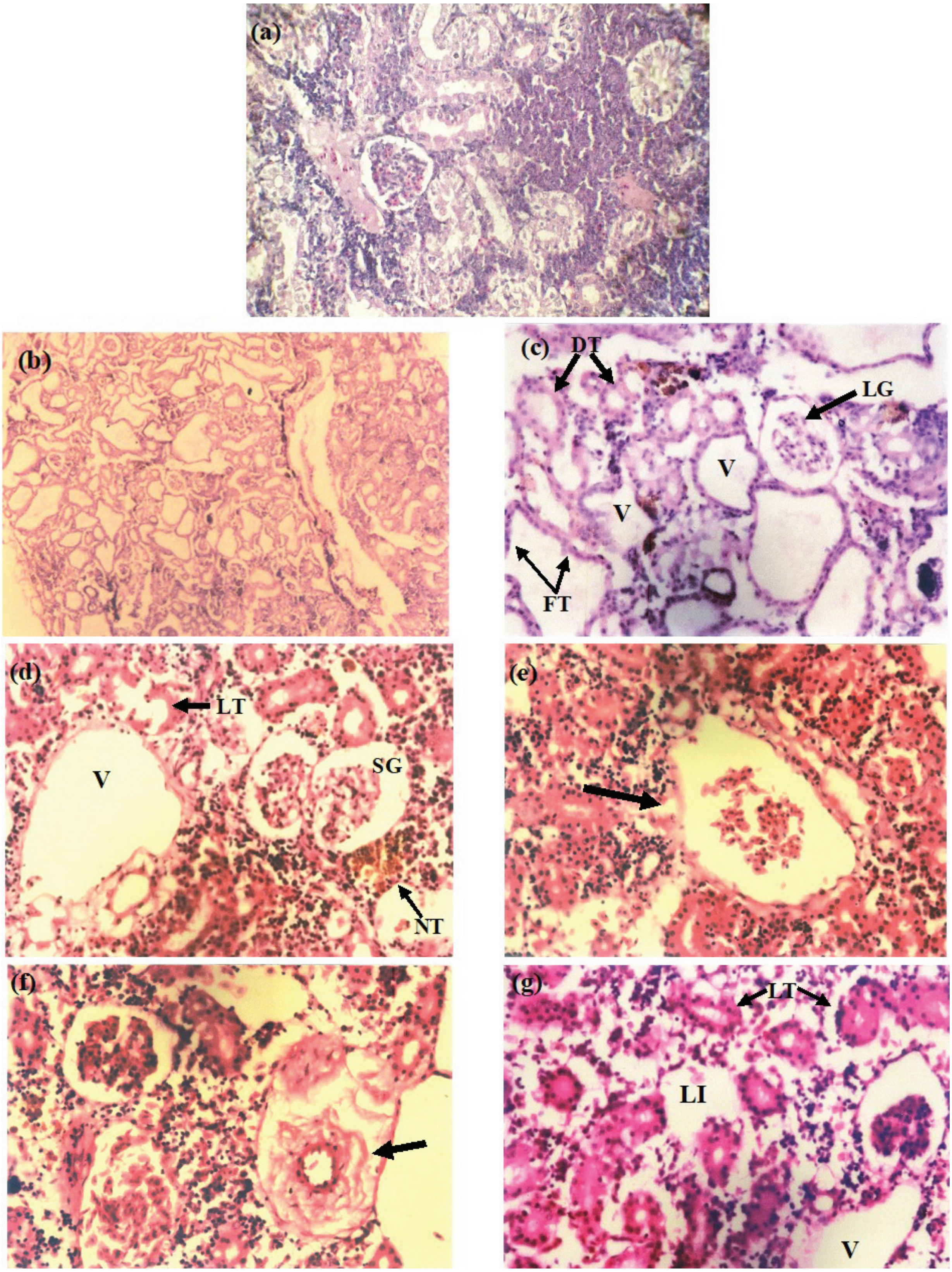

Figure 4. Histology of the kidney of *H. brachysoma* (H and E staining). (a) acclimated to 26°C: normal histoarchitecture (160X); (b) acclimated to 36°C: extensive vacuolation (40X); (c) acclimated to 36°C: severe vacuolation (V), flattening of the tubule epithelial cell (FT) and loss of glomerular tuft (LG) (160X); (d) exposed to CTMax: vacuolation (V), loss of tubules (LT), necrosis of tubules (NT), and glomeruli shrinkage (SG) (160X); (e) exposed to CTMax: invariably dilated blood vessels (160X) (f) exposed to CTMin: ballooning of the blood vessels and markedly thin empty tunica-interna detached from the tunica-media (arrow) (160X); (g) exposed to CTMin: vacuolation (V), loss of interstitium (LI), and loss of tubules (LT) (160X).

Like the gills, the skin is also susceptible to changes in environmental parameters because of their proximity to the external environment. We observed mild thickening in the skin epithelial layers of the fish acclimated to 36°C (Figure 2b). The skin of the fish exposed to CTMax appeared markedly odious because of the desquamation of epithelial cells (Figure 2c), while the skin of those exposed to CTMin did not exhibit any discernible changes (Figure 2d). It is reported that there is hypersecretion of mucus followed by depletion of mucus cells with differentiation of new mucus cells in the branchial region of fishes as a compensatory response to various environmental stressors (Mallatt 1985). Khieokhajonkhet et al. (2022) reported that the hybrid catfish (♂*C. gariepinus* ×♀*C. macrocephalus*) acclimated to 37°C had increased peripheral distribution of mucus cells in the subcutaneous skin, and enlarged club cells and melanophores in the skin resulting in increased epithelial layer thickness. Similarly, increased cellularity in the epithelial layer observed in our study indicates the differentiation of the new mucus and other cells in the skin of the fish acclimated to 36°C. However, acute increase in temperature (CTMax) had a detrimental effect on the skin of the tested *H. brachysoma* specimens. Hernández-López et al. (2018) reported that external responses of the skin of the Pacific sardines (*S. sagax caeruleus*) exposed to acute (CTMax) and chronic heat stress (warm acclimation) were desquamation and mucus production. Yang et al. (2022) reported that exposure to mild (24°C) and high (28°C) heat stress for one week caused skin damage in Siberian sturgeon (*Acipenser baerii*), including exfoliation and necrotization of the epidermis with raised cell debris, a partially broken epidermal layer with slight dissociation and epidermal shriveling, and a decrease in the number of mucous cells.

Unlike the gills and skin, the liver, kidney, and brain are not in direct contact with water. The complete equilibrium of the blood and environment temperatures at the gills leads to an equally complete thermal equilibrium between these essential organs through the circulating blood because thermal diffusion is considerably faster than molecular diffusion (Hazel and Prosser 1974; Pörtner et al. 2005). In our study, the liver of the *H. brachysoma* specimens acclimated to 36°C showed moderate swelling and vacuolization of the hepatocytes (Figure 3b), and occasional hemolysis and necrosis (Figure 3c). The liver of the fish exposed to CTMax showed severe congestion (Fig 3d) with fragmented edges, the hepatocytes were markedly degenerated with the cloudy appearance of the cells and the extensive loss of cellular contour (Figure 3e), while the fish exposed to CTMin showed highly dilated blood vessels with haemolysed RBCs (Figure 3f) and swollen hepatocytes (Figure 3g). Our results agree with those reported earlier for chronic exposure of *L. rohita* (Dash et al. 2011) and hybrid catfish (♂*C. gariepinus* ×♀*C. macrocephalus*) (Khieokhajonkhet et al. 2022) to increased temperatures and acute heat exposure of juvenile blunt snout bream (*Megalobrama amblycephala*) (Liu et al. 2016; Li et al. 2019), and *S. sagax caeruleus* (Hernández-López et al. 2018). Changes in the liver may act as indicators of earlier exposure to environmental stressors because the liver is the primary organ responsible for detoxification and is particularly vulnerable to damage from stress (Velmurugan et al. 2007). Hepatocyte vacuolization is suggested to be a sign of the imbalance between the rate of substance production in parenchymal cells and the rate of these compounds release into the circulatory system (Gingerich 1982). Exposure of *M. amblycephala* to acute temperatures has been reported to damage mitochondria, leading to the leaking of reactive oxygen species into the cytosol causing oxidative stress, suggesting that the imbalance between lipid peroxides and the antioxidant system may cause cell dysfunction and cell damage (Liu et al. 2016).

In our study, the kidney of the *H. brachysoma* specimens acclimated to 36°C showed extensive vacuolation with complete flattening of the tubule epithelial cells (Figure 4b & 4c), distorted appearance of tubular lamellae and marked loss of glomerular tuft / capillaries (Figure 4c). The kidney of the fish exposed to CTMax appeared markedly vacuolar with loss of tubules, occasional areas of necrosis of the tubules, and scattered leucocytic infiltration with occasional shrinkage in the glomeruli (Figure 4d), and invariably dilated blood vessels (Figure 4e). The fish exposed to CTMin exhibited ballooning of the blood vessels and markedly thin empty tunica-interna detached from the tunica-media (Figure 4f), vacuolation with large empty spaces and with loss of interstitium and tubules, however, glomeruli appeared normal (Figure 4g). Our results agree with those reported for *P. olivaceus* (Liu et al. 2015) and Pacific sardine (*S. sagax caeruleus*) (Hernández-López et al. 2018) exposed to acute and chronic heat stress, and for *L. rohita* acclimated to increased temperatures (Dash et al. 2011). Histological changes in kidneys are suggested to be good indicators of environmental contamination because the renal tissue is close to the adrenal tissue that synthesizes stress hormones such as adrenaline, noradrenaline and glucocorticoids that are released during stress conditions in animals (Hinton 1993).

In our study, the brain of the fish acclimated to 36°C showed increased cellularity and vacuolation in the cerebrum (Figure 5b). The fish exposed to CTMax showed empty spaces in the cerebrum (Figure 5c) and blood vessels appeared highly dilated (Figure 5d), while those exposed to CTMin showed nodular masses of various sizes in the cerebrum (Figure 5e), indicating capillary thrombosis in the brain tissue. Our results agree with those reported for *O. niloticus* exposed to acute temperature shocks (Phrompanya et al. 2021). It is suggested

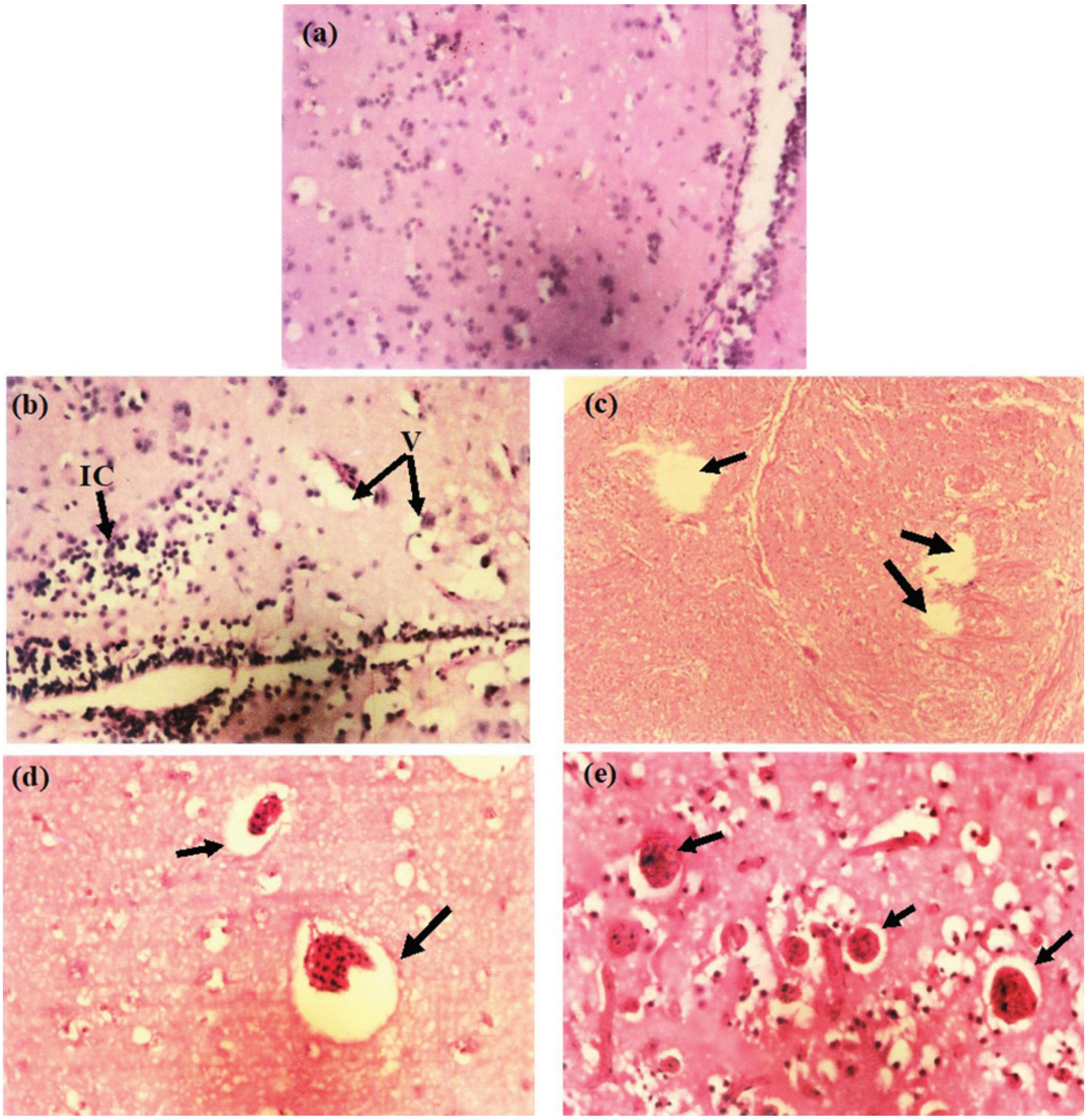

Figure 5. Histology of the brain tissue of *H. brachysoma* (H and E staining). (a) acclimated to 26, 31 and 33°C: normal histoarchitecture (80X); (b) acclimated to 36°C: increased cellularity (IC) and vacuolization (V) (160X); (c) exposed to CTMax: empty spaces in the cerebrum (arrow) (40X); (d) exposed to CTMax: dilated blood vessels (arrow) (160X); (e) exposed to CTMin: nodular thrombotic masses of various sizes (arrow) (160X).

that tissue deterioration in specific brain regions may cause impairment of specific brain functions as well as changes in the physiological and behavioral processes of the fish, resulting in such behavioral changes as respiratory dysfunctions, loss of equilibrium, and erratic swimming (Lakshmaiah 2017).

CONCLUSION

Overall, the observations of the present study suggest that acclimation to 36°C and exposure to CTMax and CTMin profoundly affects the histoarchitecture of the vital organs of *H. brachysoma*. The histopathological alterations in different vital organs of *H. brachysoma* after exposure to CTMax and CTMin temperatures indicate that the aberrations are more apparent during exposure to dynamic heat stress than during exposure to dynamic cold stress. The alterations observed in the histology of the vital organs suggest their contributory role in the aberrant behavior of the fish observed during exposure to dynamic temperature changes.

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Authors' contributions

RSD designed and executed the experiment, and drafted the manuscript, TD helped in experimentation and commented on the manuscript; AKP designed and supervised the experiment and discussed the results and commented on the manuscript, DD commented and proofread the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare that they do not have any conflict of interest.

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