Effects of thermal stress responses in goldfish (Carassius auratus): Growth performance, total carotenoids and coloration, hematology, liver histology, and critical thermal maximum

Anurak Khieokhajonkhet (anurakk@nu.ac.th)
Naresuan University
Marisa Phoprakot
Naresuan University
Niran Aeksiri
Naresuan University
Gen Kaneko
University of Houston-Victoria
Wutiporn Phromkunthong
Prince of Songkla University

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Abstract

The present study aimed to investigate the effect of thermal stress on growth, feed utilization, coloration, hematology, liver histology, and critical thermal maximum (CTmax) in goldfish (*Carassius auratus*) cultured at three different acclimation temperatures including 27˚C, 30˚C, and 34˚C for 10 weeks. Goldfish were assigned randomly to tanks with a quadruplicate setup, accommodating 20 fish per tank. Fish were manually fed four times a day until satiation. The result showed that fish acclimated at 34˚C exhibited suppress growth indices and significantly decreased feed utilization with linear and quadratic effects on feed efficiency ratio and protein efficiency ratio. The coloration parameter (a* value) was significantly decreased in the trunk region and total serum carotenoids at week 5, as well as a decline in L*, a*, b* values and total serum carotenoids at week 10. Total carotenoid contents in muscle and skin also decreased with increasing temperature. Aspartate aminotransferase, alanine aminotransferase, and triglycerides significantly increased with increasing temperature. However, high-density lipoprotein cholesterol decreased linearly and quadratically. Glucose and cortisol levels linearly increased with increasing temperature with a quadratic effect observed only in glucose levels. Liver histology showed swollen hepatocytes, nuclei displacement, and infiltration of inflammation in fish cultured at 34˚C. Goldfish exposed to a temperature of 34˚C displayed a higher CTmax of 43.83˚C compared to the other groups. Taken together, increasing temperature slightly improves growth (up to 30˚C) but the temperature at 34˚C significantly suppresses feed utilization, coloration as well as stress response with liver histological damage in goldfish.

Introduction

In the current context of climate change, global warming poses significant threats to life due to increasing seasonal temperatures, larger thermal variances, and a higher frequency of intense heat waves (Collins, 2020; Frölicher et al. 2018). Global water temperature is anticipated to rise by 1 4 degrees Celsius by 2100 (IPPC, 2014). The persistent temperature rise and escalating occurrence of extreme heatwaves are already having an impact on animal populations worldwide (Alfonso et al. 2021) with significant influences on chemical processes, distribution, biological systems, behavior, and physiology (Gracey et al. 2004). These changes in environments and animals also directly affect human life recent global climate change has wreaked havoc on several commercial aquatic creatures, resulting in significant economic losses (Mangi et al. 2018). The ability to cope with temperature fluctuation is thought to be a key predictor of organisms' fitness and future under global change.

Temperature stress triggers an endocrine response in animals that can be both beneficial and harmful (Barton, 2002). An acute stress response is sometimes considered useful for promoting physiological and/or behavioral adaptations that reclaim homeostasis under challenging environments (Barton, 2002; Khieokhajonkhet et al. 2022c). Such changes, on the other hand, may be inappropriate when the stress is repeated or sustained, and animals cannot become accustomed to it (Barton, 2002). Poikilothermic animals like fish are especially vulnerable to thermal stresses because they are unable to maintain a constant internal temperature. The response to thermal stress in fish includes the cellular-level processes

to maintain the metabolism by changing gene and protein expressions as well as the systemic processes mainly controlled by the hypothalamus of the central nervous system (Qi et al. 2013). In the latter process, the stress factors first trigger the release of catecholamine and cortisol, which in turn regulates cellular, hematological, and immunological changes as the secondary responses (Barton, 2002; Qi et al. 2013; Khieokhajonkhet et al. 2022c). The final step to preserve their homeostasis, the tertiary responses, is the changes in behavior and development (Barton, 2002; Qi et al. 2013).

The production of ornamental fish is estimated to be approximately 15 30 billion US$ each year in the aquaculture industry, and about 55% of worldwide their market supply is produced in Asian counties, where the sector has a considerable impact on the economies of the countries (FAO, 2021). Along with body shape, fin shape, and size, which are mostly determined by carotenoids, skin pigmentation is typically a major factor in the marketability and pricing of ornamental fish (Gouveia et al. 2005; Ota et al. 2016). Interestingly, previous studies reported that carotenoids could also enhance growth and survival, acting as natural immunostimulants that can boost an organism's antioxidant capacity, immunological functions, and lipid peroxidation (Amar et al. 2001; Babin et al. 2015; Waagbø et al. 2003). The stress response of cortisol is also known to be enhanced by carotenoids (Amar et al. 2001; Waagbø et al. 2003). It is therefore valuable to investigate the effect of thermal stress on ornamental fishes with a special focus on carotenoids.

Ornamental goldfish (*Carassius auratus*) holds a prominent position as one of the most commercially important and popular exotic fish species worldwide. This is primarily attributed to their captivating colors, distinctive fin patterns, and unique body shapes (Khieokhajonkhet et al. 2022b; Ota and Abe, 2016). According to Freshwater Ornamental Fish (2021), Singapore, Thailand, and Indonesia stand out among Southeast Asian counties as the world's leading exporters of freshwater ornamental fishes. Goldfish, in particular, thrive within a temperature range of 20˚C to 28˚C (Kestemont, 1995). Nevertheless, they often encounter water temperatures surpassing 34.9˚C during the summer season in Thailand and other Southeast Asian countries (Jeamsripong et al. 2018; Whangchai et al. 2018). Such high temperatures might have increased susceptibility to opportunistic bacterial infections, resulting in severe economic losses. Indeed, a previous study reported that high temperature makes goldfish vulnerable to stress (Ford and Beitinger, 2005; Fry and Hart, 1948). The present study investigated the effect of high temperatures in goldfish with the aim to advance our understanding of the thermal stress response. Several parameters were determined including growth performance, feed utilization, total carotenoids, coloration, hematology, liver histology, and CTmax.

Materials and methods

Fish rearing conditions and feeding protocols

A total of 300 goldfish (*C. auratus*) with similar size (2.45 ± 0.57 g/fish) were purchased and transported from the ornamental fish market (Chatuchak, Bangkok, Thailand) to the fish nutrition laboratory of The University of Naresuan (Phitsanulok, Thailand). Goldfish were introduced in a 500 L plastic tank with

nonchlorinated water in the recirculation system under natural photoperiod. Fish were manually fed four times a day at 08:00, 11:00, 14:30, and 17:30 h. until visual satiation with a High Grade 9006T commercial diet containing 40.14% crude protein, 4.04% crude fat, 12.72% moisture, 4.48% crude ash, and 0.01 g/kg diet of total carotenoids (Samutsakhon, Thailand). Water temperature during the acclimation period ranged from 26.5˚C to 27.8˚C.

Prior to the thermal challenge, goldfish underwent a fasting period of 24 h. Thereafter fish were anesthetized consisting of 30 mg/L. A stock solution of clove oil anesthetizer was prepared by combining 1 ml of clove oil with 9 ml of ethanol. Goldfish, with an average weight of 6.69 ± 0.02 g/fish, were collectively weighed and then assigned randomly to 16 rectangular plastic tanks (90 L capacity, 0.6 × 0.4 × 0.4 m^3) with quadruplicates in each treatment group at the density of 20 fish/tank (total 320 fish). All experimental setups followed a previous publication from our laboratory (Khieokhajonkhet et al. 2022c). In brief, each tank was equipped with a pump, a filter, and a submerged digital thermostat heater. To ensure a uniform water temperature, each treatment group was connected to a central sump tank equipped with a pump for circulating water. This setup allowed for the distribution of water with consistent temperature throughout all the tanks. Water temperature was incrementally raised at a rate of approximately 1˚C per 5 hours until it reached three different target temperatures: 27˚C, 30˚C, and 34˚C. The temperature in the plastic tanks was double-checked using a mobile thermometer. Fish were acclimated to each temperature for 70 days under the same feeding regime as mentioned above. Approximately 35% of water was daily replenished using temperature-adjusted non-chlorinated water. Throughout the challenging trial, dissolved oxygen and pH were determined four times a week (5.9 ± 0.4 mg/L and 7.1 ± 0.4 throughout the experimental period, respectively), while water temperature was measured twice daily (27.3 ± 1.8˚C, 30.8 ± 0.8˚C, and 34.3 ± 1.1˚C, respectively).

Evaluation of growth performance and feed utilization

The goldfish were fasted for 24 h, following which were anesthetized using a solution composed of a mixture of clove oil with a concentration of 30 mg/L. All goldfish in each tank were counted, and their collective weight was measured to evaluate the somatic performance by following our previously described in Khieokhajonkhet et al. (2022c), including weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCR), and Feed efficiency ratio (FER). To determine protein utilization including protein efficiency ratio (PER) and protein productive value (PPV), crude protein of initial fish, final goldfish, and experimental diets were analyzed using a method described in section 2.5 by following our previously described in our laboratory (Khieokhajonkhet et al. 2022c).

After anesthetized with an overdose of clove oil mixture solution (50 mg/L), individual two fish from each tank (N = 8) were collected to determine the body physical indicators. Individual goldfish had their overall length and weight measurement to determine condition factor (K, g/cm^3), HSI (%), and VSI (%) using the following equations as previously described in Khieokhajonkhet et al. 2022c.

Colorimetric measurement

In the beginning of the thermal challenge, ten fish were randomly collected and anesthetized to determine color parameters. During temperature acclimation at weeks 5 and 10, three fish from each tank (n = 12) were randomly selected. After anesthetization, goldfish samples were determined on the trunk region at the dorsal section closed to the lateral line. Quantitative colorimeter assays were performed using a Hunter Lab MiniScan EZ 4500L (Reston, VA, USA). The values of color parameters were expressed using the International Commission on Illumination (CIE, 1976). The lightness parameter (L*) represents the perceived brightness, the redness or greenness dimension is represented by the a* value, and the yellowness or blueness dimension is represented by the b* value (Hunter and Harold, 1987).

Total carotenoid analysis

At the 5th and 10th week of the experiment, four fish from each tank (pooled sample, n = 4) were randomly selected and then anesthetized using clove oil solution to determine total carotenoid content in serum (Barbosa et al. 1999). In brief, a volume of 50 µl of blood serum (see section 2.6) was thoroughly homogenized with a 0.4: 1.0 (v/v) combination of 95% ethanol and hexane. The mixture solution was then subjected to centrifugation at 4500 rpm at 4˚C for 10 min. The upper layer of the resulting solution was used for absorbance measurement at a wavelength of 450 nm using a UV-1800 spectrophotometer manufactured by Shimadzu (Kyoto, Japan). The total carotenoid content was determined using the method outlined by Barbosa et al. (1999). The calculated carotenoid content was then multiplied by the dilution factor to express the resulting in µg carotenoid per mL of the sample.

At week 10, the total carotenoid content of goldfish tissues including fin, muscle, skin, and liver as well as the experimental diets, was measured using a modified version of Torrissen and Naevdal (1988). Two goldfish were chosen from each tank, resulting in a total of 8 fish (n = 8). Tissue samples, weighing approximately 1 g each, were minced and subsequently mixed with 5 ml of cold acetone and 1 g anhydrous Na_2SO_4. The solution was collected in the fresh tube, and extracted with an identical volume until exhausted. The extracted solution was subjected to centrifuge at 3500 rpm at 4˚C for 5 min. The upper layer of the centrifuged solution was utilized for spectrophotometer analysis at a wavelength of 450 nm using a UV-1800 spectrophotometer manufactured (Shimadzu, Kyoto, Japan).

Chemical scrutiny

The proximate compositions of the initial whole-body goldfish (ten fish, data not shown), final goldfish (two fish per tank, pooled, n = 4), and the commercial diet were determined using the standard methods (AOAC, 1997). Moisture content was performed using the 920.85 method, utilizing the Memmert model UL50 air-dryer (Schwabach, Germany). The samples were dried at a temperature of 105˚C until constant weight was achieved. Crude fat was extracted with petroleum ether by following the 920.85 method using a conventional classical Soxhlet apparatus (Gerhardt, Germany). To determine crude protein content, the samples were digested using sulfuric acid (H_2SO_4) in a Kjeldatherm block heating system. Subsequently, distillation and fixation were performed using a semi-automatic Kjeldahl apparatus (Königswinter,

Germany). The ash content was determined using 942.05 method. The samples were subjected to a Carbolite ELF 11/14 muffle furnace (Hope Valley, England).

Hematological and biochemical parameters

At week 10, two groups of fish samples were randomly selected and subsequently anesthetized using a clove oil mixture solution with a concentration of 30 mg/L. In the first group, blood samples from four fish from each tank (pooled, n = 4) were collected from the caudal vein with heparin-containing syringe. The pooled blood samples were used to determine including hematocrit (Hct), hemoglobin (Hb), white blood cell count (WBC), and red blood cell count (RBC) by following the Hesser (1960). Blood glucose levels were carried out using an active glucometer, Roche (Mannheim, Germany). Serum biochemical parameters were determined as previously described (Khieokhajonkhet et al. 2022a) in our laboratory. Briefly, blood was withdrawn from another set of fish (pooled, n = 4) with a non-heparinized syringe. After allowing the collected blood samples to clot on ice for 1 h, blood samples were centrifuged at 3000 rpm for 10 min at 4˚C. The serum was immediately kept at a temperature of 80˚C until used for analysis. Serum albumin and total protein levels were measured using specific methods. Serum albumin was determined using the Bromocressol-green method, while the total protein was measured using the biuret method (Drupt et al. 1974; Scoffone and Fontana, 1975). To determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), globulin, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), the colorimetric method described by Coz-Rakovac et al. (2008) was employed. The measurements were performed using a Cobas C311 automated analyzer for chemistry, manufactured by Roche Diagnostics in Switzerland. Serum cortisol levels were analyzed using a hormonal analyzer manufactured by Tencan in Switzerland. The method described by Brown et al. (2004) was followed for the cortisol analysis.

Histological studies

At week 10, two fish (n = 8) were randomly collected from each tank for histological analyses. Prior to dissection, fish samples were briefly cleaned with distilled water and disinfected with 75% ethanol. Liver samples, approximately 0.5 cm^3, were collected and rinsed with cold phosphate-buffered saline (PBS) at a pH of 7.4. Tissue samples were immediately fixed with 10% neutral formalin solution containing 0.9% NaCl and 1.2% Na_2HPO_4. Fixed tissues were processed using standard histological procedures. Briefly, fixed tissues were undergone with a series of steps including dehydration, clearing, and embedding in paraffin wax. Tissue blocks were then sectioned with a thickness of approximately 6 7 µm. Subsequently, the tissue sections were stained with eosin and hematoxylin (H&E). Tissue sections were observed according to the previous described by Khieokhajonkhet et al. (2022c); (2021).

The CTmax analysis

For the critical thermal maxima (CTmax) analysis, two goldfish were randomly selected from each tank, resulting in a total of eight fish (n = 8). The experimental setup and procedures were followed by the

previously described by Khieokhajonkhet et al. (2022c). Briefly, fish were moved to a 70 L glass tank that was filled with water controlled by a thermostat. The water temperature was programmed to gradually increase by 1˚C h per 5 h until it reached a target temperature of 45˚C. During this period, their physiological reactions, e.g., experiencing loss of balance, sinking downwards, floating in an inverted position, and remaining motionless, were recorded.

Statistical analysis

All results are represented with mean ± standard error of the mean (SEM) values. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. All statistical analyses were conducted using a significant threshold of $P < 0.05$, and the results were evaluated accordingly. The statistical software SPSS for Windows, Version 17.0 (Chicago, IL, USA) was used for conducting all statistical analyses in this study. Furthermore, polynomial contrasts were computed to investigate the linear and quadratic effects of temperature levels ranging from 27˚C to 34˚C.

Results

Fish physiology, growth, and organosomatic indexes

During the culture with different temperatures for 70 days, no fish lost their equilibrium, and all fish appeared to be visually healthy with no body abnormalities or disease symptoms. The overall fish survival rate was found to be above 95%. The control group exhibited the highest survival rate, although the difference was not statistically significant ($P > 0.05$). At the of end thermal acclimation at 70-days, goldfish cultured at 27˚C showed normal physiology (Fig. 1A), while the fish cultured at 30˚C showed fading color at the head, trunk region, fins, and tails (Fig. 1B). Similarly, goldfish cultured at 34˚C showed more color fading in all parts of the goldfish body (Fig. 1C).

Temperature quadratically affected several growth indices (Table 1). Goldfish cultured at 30˚C exhibited higher FBW compared to the fish in the other treatment groups ($P < 0.05$). On the other hand, the fish cultured at 34˚C had the lowest FBW, and this decrease in FBW showed a significant quadratic effect ($P = 0.041$). Similarly, WG and SGR were also observed ($P = 0.041$ and 0.042, respectively). Feed utilization of goldfish cultured at 30˚C and 34˚C exhibited a linear and quadratic decrease in FER and PER ($P < 0.05$), and only a linear effect was observed in PPV ($P < 0.001$). The highest FER, PER, and PPV were observed in goldfish cultured at 27˚C, while the lowest values were found in the fish cultured at 34˚C. In contrast to FCR, the fish cultured at high temperatures linearly increased FCR ($P < 0.001$). Survival, K value, and HSI were not significantly influenced by temperature ($P > 0.05$). However, VSI showed a significant linear and quadratic increase with increasing temperature acclimation ($P < 0.001$, $P = 0.001$). The group of goldfish cultured at 34˚C exhibited the highest VSI value among all the temperature groups. (Table 1).

Table 1
Growth performance, feed utilization, survival, and organosomatic indexes of goldfish acclimated with three different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Growth						
IBW (g $fish^{-1}$)	6.69 ± 0.02	6.71 ± 0.01	6.69 ± 0.02	0.000	0.554	0.158
FBW (g $fish^{-1}$)	17.31 ± 0.57^{ab}	17.89 ± 0.86^{a}	16.34 ± 0.74^{b}	0.534	0.093	0.041
WG (%)	158.74 ± 7.80^{ab}	166.75 ± 12.55^{a}	144.34 ± 10.60^{b}	10.174	0.088	0.041
SGR (% day^{-1})	1.36 ± 0.04^{ab}	1.40 ± 0.07^{a}	1.28 ± 0.06^{b}	0.003	0.079	0.042
Feed utilization						
FCR	1.81 ± 0.03^{b}	2.18 ± 0.20^{a}	2.48 ± 0.17^{a}	0.025	< 0.001	0.088
FER	0.60 ± 0.01^{a}	0.57 ± 0.04^{a}	0.44 ± 0.03^{b}	0.001	< 0.001	0.017
PER	1.36 ± 0.04^{a}	1.29 ± 0.07^{a}	0.99 ± 0.08^{b}	0.004	< 0.001	0.013
PPV (%)	23.50 ± 0.48^{a}	22.28 ± 1.69^{a}	18.15 ± 1.42^{b}	1.698	< 0.001	0.103
Survival (%)	98.33 ± 3.33	96.67 ± 3.85	96.67 ± 3.85	13.080	0.357	0.589
Organosomatic indexes						
K (g/cm^3)	13.29 ± 0.83	13.31 ± 1.76	11.88 ± 1.93	2.163	0.061	0.212
HSI (%)	1.59 ± 0.14	1.73 ± 0.43	1.46 ± 0.27	0.096	0.372	0.106
VSI (%)	12.93 ± 0.81^{b}	15.58 ± 0.38^{a}	15.99 ± 1.00^{a}	0.608	< 0.001	0.001

All parameters represent the average values obtained from four tanks per treatment (20 fish per replicate tank for growth and feed utilization parameters and 2 fish per replicate tank for organosomatic indexes). Values are represented as mean ± SE. Data with alphabetical superscripts indicated significant differences between various groups ($P < 0.05$). Abbreviations: IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; FER, feed conversion efficiency; PER, protein efficiency ratio; PPV, protein productive value; K, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index.

Chemical scrutiny of whole-body fish

Data on the whole-body composition of goldfish cultured at different temperatures are shown in Table 2. No significant linear or quadratic effects of temperature acclimation were observed on dry matter, crude protein, crude fat, and ash content in any of the treatment groups ($P > 0.05$).

Table 2
Chemical composition of initial and final whole-body of goldfish

Parameters	Initial	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Dry matter	19.49 ± 0.04	27.34 ± 0.84	28.05 ± 1.05	28.77 ± 1.42	1.288	0.071	0.994
Crude protein	9.15 ± 0.04	12.55 ± 0.13	12.48 ± 0.25	12.74 ± 0.27	0.054	0.275	0.283
Crude fat	5.25 ± 0.01	11.10 ± 0.08	11.19 ± 0.06	11.38 ± 0.13	0.010	0.139	0.631
Ash	2.39 ± 0.00	2.90 ± 0.01	2.79 ± 0.08	2.92 ± 0.04	0.246	0.053	0.565
All parameters represent the average values obtained from four tanks per treatment (two fish per replicate tank were pooled, n = 4). Values are represented as mean ± SEM.							

Coloration

The body color of the fish was assessed immediately after they were removed from the tank, and no discernible alterations in their body color were observed as a result of the handling stress. Temperature significantly affected skin coloration (Table 3). At the start of the trial, the L*, a*, and b* color parameters had values of 60.45 ± 1.24, 24.50 ± 1.31, and 39.10 ± 1.43, respectively (Table 3). At week 5, the a* value demonstrated a linear and quadratic decrease with increasing water temperature ($P < 0.001$, $P < 0.001$). Similarly, L* demonstrated results with a significant linear effect observed ($P < 0.001$). However, no significant differences were found in the b* value ($P > 0.05$) at week 5. Overall, as the temperature increased, the color values (L*, a*, and b* values) demonstrated a significant linear and quadratic decrease ($P < 0.05$, Table 3) at week 10.

Table 3
Color parameters on trunk region of goldfish acclimated with different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters		Initial	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Luminosity (L*)	Initial	60.45 ± 1.24						
	Week5		63.95 ± 1.51^{a}	55.87 ± 1.76^{b}	48.39 ± 2.40^{c}	3.734	< 0.001	0.664
	Week10		64.18 ± 1.40^{a}	59.57 ± 1.61^{b}	57.95 ± 1.47^{c}	2.256	< 0.001	0.008
Redness (a*)	Initial	24.50 ± 1.31						
	Week5		25.00 ± 1.36^{a}	17.27 ± 1.51^{b}	14.83 ± 0.65^{c}	1.535	< 0.001	< 0.001
	Week10		25.95 ± 1.25^{a}	12.72 ± 0.57^{b}	10.97 ± 1.87^{c}	1.810	< 0.001	< 0.001
Yellowness (b*)	Initial	39.10 ± 1.43						
	Week5		36.78 ± 1.58	36.38 ± 2.88	35.98 ± 1.91	4.833	0.378	0.992
	Week10		43.59 ± 1.72^{a}	31.01 ± 1.10^{b}	29.83 ± 1.73^{b}	2.403	< 0.001	< 0.001

All parameters represent the average values obtained from four tanks per treatment (ten fish initial, n = 10; three fish per tank, n = 12 used to determine color parameters at week 5 and week 10). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups (P < 0.05).

Total carotenoid content

At week 5, the total carotenoid content in serum was found to be linearly and quadratically (P < 0.001, P = 0.07) influenced by the increasing water temperature. Among the treatment groups, those cultured at 27˚C exhibited the highest serum carotenoid content, followed by the groups cultured at 30˚C and 34˚C. Similar results were observed in the goldfish cultured for 10 weeks (Table 4).

Table 4
Total carotenoid content (μg carotenoid/mL of sample) in serum of goldfish acclimated with different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Week 5	1.64 ± 0.14^{a}	1.14 ± 0.13^{b}	1.13 ± 0.05^{b}	0.013	< 0.001	0.007
Week 10	2.24 ± 0.38^{a}	0.65 ± 0.03^{b}	0.53 ± 0.09^{b}	0.055	< 0.001	0.001

All parameters represent the average values obtained from four tanks per treatment (four fish per tank was pooled, n = 4). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups (P < 0.05).

The increasing water temperature had an impact on the total carotenoid content in various organs of goldfish, including the fin, muscle, and skin (Table 5). Goldfish cultured at 27˚C exhibited a higher total carotenoid content in the fin compared to the other groups (P < 0.05). In addition, linear and quadratic decreases were observed in muscle and skin (P < 0.05). However, total carotenoid content did not have a significant effect on the total carotenoid content in the liver (P > 0.05, Table 5).

Table 5
Total carotenoids content (μg carotenoid/g of sample) in several parts of goldfish acclimated with three different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Fin	21.63 ± 0.85^{a}	14.54 ± 3.97^{b}	11.03 ± 1.07^{b}	5.893	0.002	0.335
Muscle	42.42 ± 5.32^{a}	18.63 ± 3.65^{b}	19.46 ± 2.86^{b}	5.682	0.003	0.027
Skin	137.96 ± 7.91^{a}	81.69 ± 2.00^{b}	76.06 ± 2.62^{b}	7.207	< 0.001	0.005
Liver	36.41 ± 2.44	40.26 ± 2.80	37.60 ± 6.20	17.429	0.738	0.313

All parameters represent the average values obtained from four tanks per treatment (two fish per replicate tank, n = 8). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups (P < 0.05).

Blood hematology and biochemistry

Increasing temperature demonstrated both linear and quadratic impact on RBC (P < 0.001, P = 0.018), as well as the Hct levels, with only a linear trend observed (P < 0.001, Table 6). However, no significant effects of temperature were observed for WBC and Hb (P > 0.05). Increasing temperature affected all tested serum biochemical parameters in goldfish (P < 0.05), except for A:G ratio and LDL-c levels (P > 0.05). Albumin and globulin levels were linearly deceased (P = 0.002). In line with these results, total protein content exhibited a quadratic decrease as the water temperature increased (P = 0.020). The levels of AST, ALT, and triglycerides demonstrated both linear and quadratic increases with increasing water temperatures, while the levels of HDL-c exhibited a linear and quadratic decrease (P < 0.001, P = 0.009). ALP and total cholesterol levels exhibited a linear increase with increasing temperature (P < 0.05, Table 6).

Table 6
Hematological characteristics of goldfish acclimated with different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Hematological parameters						
RBC ($\times 10^6$)	0.77 ± 0.15^b	0.79 ± 0.10^b	1.30 ± 0.16^a	0.019	< 0.001	0.018
WBC ($\times 10^3$)	1.54 ± 0.25	1.33 ± 0.18	1.26 ± 0.09	0.035	0.065	0.537
Hct (%)	22.53 ± 0.22^c	24.90 ± 0.61^b	27.69 ± 0.56^a	0.243	< 0.001	0.560
Hb (g/dL)	11.40 ± 1.07	10.50 ± 0.39	10.46 ± 1.62	1.317	0.279	0.554
Biochemical parameters						
Total protein (g/dL)	3.08 ± 0.08^a	2.75 ± 0.05^{ab}	2.88 ± 0.16^b	0.011	0.062	0.020
Albumin (g/dL)	1.45 ± 0.05^a	1.30 ± 0.04^b	1.28 ± 0.04^b	0.002	0.002	0.070
Globulin (g/dL)	1.67 ± 0.05^a	1.48 ± 0.06^b	1.45 ± 0.05^b	0.003	0.002	0.055
A:G ratio	0.86 ± 0.05	0.88 ± 0.01	0.88 ± 0.05	0.002	0.695	0.854
AST (U/L)	102.02 ± 15.33^c	127.43 ± 4.78^b	191.86 ± 4.08^a	90.498	< 0.001	0.028
ALT (U/L)	25.58 ± 0.62^b	23.29 ± 1.12^b	47.06 ± 4.35^a	6.866	< 0.001	0.002
ALP (U/L)	16.17 ± 1.04^b	19.28 ± 3.04^{ab}	24.19 ± 2.03^a	4.811	0.004	0.585
Total cholesterol (mg/dL)	245.15 ± 6.75^b	257.64 ± 17.48^b	295.01 ± 6.41^a	30.719	0.002	0.175
Triglycerides (mg/dL)	220.84 ± 0.61^b	233.06 ± 5.24^b	273.10 ± 6.76^a	24.494	< 0.001	0.007

All parameters represent the average values obtained from four tanks per treatment (four fish were pooled per replicate tank, n = 4). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups ($P < 0.05$). Abbreviations: RBC, red blood cell; WBC, white blood cell; Hct, hematocrit; Hb, hemoglobin; A:G, ratio of albumin and globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
HDL-c (mg/dL)	92.41 ± 5.57[a]	66.38 ± 0.71[b]	58.97 ± 2.12[b]	12.019	< 0.001	0.009
LDL-c (mg/dL)	149.28 ± 2.06	152.82 ± 10.10	156.18 ± 10.49	72.058	0.358	0.989

All parameters represent the average values obtained from four tanks per treatment (four fish were pooled per replicate tank, n = 4). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups (P < 0.05). Abbreviations: RBC, red blood cell; WBC, white blood cell; Hct, hematocrit; Hb, hemoglobin; A:G, ratio of albumin and globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol.

Histological assessments

Liver histological analysis of the goldfish cultured at 27˚C showed no observable effects from temperature acclimation. The hepatocytes exhibited a normal structure with a small number of lipid droplets and round nuclei centrally located in the cytoplasm (Fig. 2A). However, fish cultured at 30˚C group showed huge dispersed cytoplasmic vacuoles on swelling hepatocytes, and endothelial cells with abnormalities in the major hepatic vein, nuclei displacement, and partly hepatic hypertrophy and infiltration of inflammatory cells (Fig. 2Ba, 2Bb). In addition, these pathological differences were found in fish cultured at 34˚C with more severe hepatic hypertrophy, irregular hepatocyte cells, enlargement of the cytoplasmic vacuole, nuclear displacement and disappearance, and infiltration of inflammatory cells (Fig. 2Ca, 2Cb).

Cortisol and glucose levels

The glucose levels exhibited both linear and quadratic increases in response to temperature increases (P < 0.001), while the cortisol levels showed a linear increase with increasing temperature (P = 0.010). The goldfish cultured in the 27˚C group exhibited the lowest cortisol level compared to the other groups, while the highest was found in the 34˚C group (Table 7).

Table 7
Serum glucose and cortisol levels of goldfish acclimated with different temperatures (27˚C, 30˚C, and 34˚C) for 70 days.

Parameters	27 ˚C	30˚C	34˚C	*SEM*	Linear	Quadratic
Glucose (nmol/L)	60.34 ± 1.28[b]	72.66 ± 1.15[a]	74.66 ± 0.57[a]	1.333	< 0.001	< 0.001
Cortisol (ng/mL)	14.39 ± 0.61[c]	16.50 ± 0.74[ab]	19.83 ± 2.94[a]	3.183	0.010	0.644

All parameters represent the average values obtained from four tanks per treatment (four fish were pooled per replicate tank, n = 4). Values are represented as mean ± SEM. Data with alphabetical superscripts indicated significant differences between various groups (P < 0.05).

CTmax analysis

The CTmax of goldfish challenged with increasing temperature showed a significantly increased (Fig. 3). CTmax value showed the highest value in the 35˚C group, followed by the 30˚C and 27˚C groups ($P < 0.05$).

Discussion

Temperature changes, as is well documented, can have a suppressive influence on teleost physiology and metabolic processes (Cheng et al. 2015; Qi et al. 2013). Understanding the physiological changes that occur as a result of heat stress is critical for strategy adjustment in the era of global warming. However, the impact of high temperature on growth, feed utilization, hematology, and color content in ornamental goldfish species is poorly understood. In the present study, goldfish cultured at high temperatures exhibited quadratic increases in FBW, WG, and SGR ($P < 0.05$). These findings suggested that high temperatures might enhance the efficiency of intestinal digestion, absorption, and metabolic processes (Ashaf-Ud-Doulah et al. 2020; Ma et al. 2015). Several previous studies also showed an increase in growth performance with increasing water temperature in various fish species, including European seabass (*Dicentrachus labrax*) (Peres and Oliva-Tele, 1999); Thai pangas (*Pangasianodon hypophthalmus*) (Islam et al. 2019); yellowtail kingfish (*Seriola lalandi*) (Abbink et al. 2012; Bowyer et al. 2012); greater amberjack (*Seriola dumerili* Risso 1810) (Fernández-Montero et al. 2018); Nile tilapia (Ma et al. 2015), rohu, (*Labeo rohita*) (Ashaf-Ud-Doulah et al. 2020; Das et al. 2005), and hybrid catfish (*Clarias gariepinus* × *C. macrocephalus*) (Khieokhajonkhet et al. 2022c). In the case of goldfish, the culture within the temperature range of 22˚C to 30˚C did not yield significant differences in terms of growth and feed utilization (Gouveia and Rema, 2005). Moreover, goldfish cultured at 34˚C showed poor growth performance and appetite compared to the 27˚C- and 30˚C-acclimated groups. Previous studies also found that temperatures between 31˚C to 37˚C had reduced growth and feed utilization in rohu (*Labeo rohita*), striped catfish (*Pangasianodon hypophthalmus*), and hybrid catfish (*Clarias gariepinus* ×*C. macrocephalus*) (Ashaf-Ud-Doulah et al. 2020; Das et al. 2005; Islam et al. 2019; Khieokhajonkhet et al. 2022c). It has been known that high temperature significantly declines dissolved oxygen levels, which is known as a hypoxia condition. Especially, the combination of these two factors, high temperature and oxygen level lower than 35% of saturated dissolved oxygen level, induces stress, poor feed intake and feed utilization, growth retardation, and physiological status in fish (Das et al. 2005).

Goldfish cultured at different temperatures did not alter whole-body composition in this study ($P > 0.05$). A similar result was reported for pikeperch juvenile (*Sander lucioperca*), in which acclimation to 20˚C to 28˚C did not affect whole body composition (Wang et al. 2009). However, the opposite results are likely more common. Hybrid catfish acclimated to 27˚C 37˚C significantly affected whole-body crude protein, moisture, crude fat, and fat content in the liver, visceral muscle, and skin (Khieokhajonkhet et al. 2022c); similar effects were also found in barramundi (*Lates calcarifer*) (Katersky and Carter, 2007), cobia (*Rachycentron canadum*) (Sun and Chen, 2014), and European seabass (*Dicentrarchus labrax*) (Peres and Oliva-Teles, 1999). These inconsistencies may be caused by temperature, nutrition, and different animal used in the studies.

Color is a vital factor in determining the market appeal of ornamental fish, and carotenoids also play a crucial role in achieving vibrant hues (Khieokhajonkhet et al. 2022b; Ota and Abe, 2016). Several factors that affect the brightly colorful ornamental fish such as sunlight, chemical residue, starvation, water quality, background color, and stress (Dharmaraj and Dhevendaran, 2011; Eslamloo et al. 2015). In the present study, high temperature led to a linear and/or quadratic decrease in the total carotenoid content in various tissues, including muscle, skin, fin, and serum (Tables 4 and 5; $P < 0.05$). In week 5, the a* color exhibited linear and quadratic decreases, while the L* value demonstrated only a linear effect. Furthermore, L*, a*, and b* values at week 10 showed the same tendencies ($P < 0.05$). The supportive evidence of the whole-body fading color was also found in Fig. 1 in the present study. In line with our results, Thai mahseer (*Tor tambrodides*), cultured at upper thermal limits for a week exhibited fading color, heavy breathing and gasping for air, and loss equivalent (Do et al. 2019). Flesh pigmentation of rainbow trout shows seasonal fluctuations with low muscle carotenoid content during spring and summer (Mørkøre and Rørvik, 2001; Nguyen et al. 2020; Nordgarden et al. 2003; Torrissen et al. 1995). Goldfish cultured at temperatures from 22˚C 28˚C significantly increased a* value as temperature increased, but higher than 28˚C was significantly decreased (Gouveia and Rema, 2005). The relationship between temperature, carotenoid content, and skin color is not so simple. The previous studies showed that hybrid catfish and red porgy (*Pagrus pagrus*) cultured at high temperatures showed higher melanin accumulation in the skin (Khieokhajonkhet et al. 2022c; Pavlidis et al. 2008). High temperature generally decreases nutrient digestibility and increases gut evacuation rate, which may negatively affect carotenoid absorption (Bergot and Breque, 1983; Jobling, 1994; Pfeffer et al. 1991). However, further studies are required to fully understand the underlying mechanisms that govern the relationship between increasing temperature and its impact on carotenoid content.

Blood hematology provides useful key markers for diagnosing pathological and physiological states in fish. Fish hematological parameters are influenced by several factors; e.g., water pollution, nutritional state, environmental changes, and stressors (Khieokhajonkhet et al. 2021; Stoskopf, 1993; Wendelaar Bonga, 1997). In this context, the observed linear and/or quadratic increase in RBC and Hct with the increase in water temperature may indicate an enhanced capacity of oxygen transport to meet their metabolic requirement in goldfish which must have been also enhanced by high temperature to cope with the adverse environmental condition (Talpur et al. 2012). Khosravi-Katuli et al. (2021) also reported an increase in RBC in fish fed with- and without-symbiotic after being challenged with a temperature from 27.1˚C to 34.0˚C for 10 days in gilthead seabream. However, rohu (*Labeo rohita*) exposure in high temperatures (up to 36˚C) caused thermal stress and failure of the hematopoietic system by reducing RBC content (Ashaf-Ud-Doulah et al. 2020; Ashaf-Ud-Doulah et al. 2019). These discrepancies could be attributable to a variety of variables such as fish species, stress conditions, nutrition, range of temperature challenges, and environmental factors (Ahmed et al. 2020).

Blood indices are indeed commonly used to assess the physiological and immunological condition of fish, providing insights into their overall health status. These indices are also regarded as relevant stress indicators, capable of detecting even minor cellular damage (Farag et al. 2022; Khieokhajonkhet et al. 2022c). In the present study, it was observed that the levels of total serum protein, albumin, and globulin

demonstrated a significant decrease when compared to the control group ($P < 0.05$), while the A:G ratio showed a slightly increased with increasing water temperature. Similar observations were made by Kumar et al. (2018) and Verma et al. (2007) in an Indian major carp (*Labro rohita*) and common carp (*Cyprinus carpio*), respectively, in which increasing temperature reduced total protein, albumin, and globulin. In contrast, hybrid striped bass (*Monrone chrysops* × *Morone saxatilis*) showed increased total protein, album, and globulin acclimated with the different temperatures at 10˚C – 29˚C (Hrubec et al. 1997). Among these total serum protein, albumin, and globulin indices have a role in the immunological response in teleost fish (Kumar et al. 2007). Wiegertjes et al. (1996) suggested that a higher level of total protein, albumin, globulin, and the lowest level of A:G ratio indicate that fish are strong immunity and are healthy.

The ability of teleost to convert amino acids to glucose is well documented. As a result of increasing AST and ALT activities indicates aspartate and alanine are mobilized for glucose synthesis via gluconeogenesis in response to stress and hepatic damage (Chatterjee et al. 2006; Khieokhajonkhet et al. 2022a, b). Increased feeding of ketoacids into the TCA cycle during stress might impact oxidative metabolism (Chatterjee et al. 2006; Knox and Greengard, 1965). In the present study, goldfish cultured at high water temperature linearly and quadratically increased AST and ALT levels, suggesting hepatocyte damage. This is also supported by the higher glucose levels and liver histological integrity in the present study. Alkaline phosphatase is a lysosomal enzyme that can protect fish during the early stages of wound healing, infection of parasites, and stress responses (Palaksha et al. 2008; Zhou et al. 2012). In this study, increased ALP activity with increasing water temperature was observed, which is similar to spotted seabass (*Lateolabrax maculatus*) (Cai et al. 2020) and large yellow croaker (*Larimichthys crocea*) (Cai et al. 2020). While Nile tilapia exposed to winter thermal stress showed decreased ALP activities (Hassaan et al. 2019). In this study, we found that increasing water temperature enhanced lipid metabolism and reduced HDL-c levels. Consistent with our finding, a previous study showed that hybrid catfish acclimated to a high-temperature significantly increased in total cholesterol, triglycerides, and LDL-c, while there was a significant decrease in HDL-c (Khieokhajonkhet et al. 2022c). However, another study on pikeperch (*Sander lucioperca*) showed the opposite result that culture at high temperature from 23˚C to 36˚C significantly decreased serum cholesterol, triglycerides, HDL, and LDL levels. In turbot exposed to high temperature stress showed significantly increased triglyceride, total cholesterol, LDL, and HDL levels, however, severe exposure to high temperature inhibited lipid metabolism (Li et al. 2019; Zhao et al. 2021).

The most prevalent stress markers in fish are glucose and cortisol, which rise during times of stress and increase energy consumption. To compensate for the increased energy expenditure, secondary physiological reactions are rising in glucose levels in the plasma (Sun et al. 2020). In the present study, it was observed that glucose levels showed both a linear and quadratic increase as the temperature increased ($P < 0.001$). This suggests a relationship between temperature and glucose metabolism, indicating that higher temperatures may affect glucose regulation in the goldfish. During the early stages of thermal stress, glucose is the predominant source of energy. However, as stress levels increase, lipids might become the primary energy source if the stress is prolonged or severe enough to stabilize

metabolic homeostasis (Li et al. 2018; Sun et al. 2020; Zhao et al. 2021). Cortisol levels in the blood linearly increased with increasing temperature in the current study, which aligns with previous studies on various fish species during stress circumstances (Barton, 2002; Cai et al. 2020; Khieokhajonkhet et al. 2022c). The fluctuations in blood glucose and cortisol levels represent adaptive responses aimed at supplying the necessary energy during a stressful situation. Cortisol, on the other hand, has a deleterious impact on the immunological system of fish during exposure to stress circumstances (Barton, 2002).

The liver, being the primary organ responsible for detoxification, is highly vulnerable to damage. Changes occurring in the liver can potentially act as early indicators of environmental stress. It has been documented that the liver is a sentinel organ by increasing temperature stress (Hall et al. 2000). In the present study, goldfish cultured at high temperatures showed swollen hepatocytes, vacuole enlargement infiltration of inflammatory cells, and progressively worsen over the 34˚C group. These findings align with previous studies conducted on fish, which have reported similar results (Khieokhajonkhet et al. 2022c; Li et al. 2019; Liu et al. 2016) and other terrestrial species (Hall et al. 2000; Liu et al. 2018; Zeng et al. 2014). The pathological differences might indicate liver deleterious effects, which is supportive evidence in AST and ALT activities. These enzymes are expected to be released from cells as a result of damage to the cell membrane and, in certain circumstances, injury to organelles, with subsequent discharge from the cytosol to the cell membrane.

CTmax assays are commonly used to determine acute temperature tolerance and are considered ecologically important (Beitinger et al. 2000). In the present study, goldfish cultured at 27˚C exhibited a CTmax value of 43.10˚C. Previous studies also found that the CTmax of goldfish was approximately 37.28˚C – 43.60˚C (Ford and Beitinger, 2005; Yanar et al. 2019). Yanar et al. (2019) investigated the CTmax in twelve ornamental fish species cultured at 20˚C ranging from 33.91˚C to 39.71˚C. Goldfish cultured at high temperatures showed higher CTmax as observed in this study. These results are consistent with findings from other studies conducted on various ornamental species (Yanar et al. 2019) as well as other fish species (Dalvi et al. 2009; Das et al. 2005; Khieokhajonkhet et al. 2022c; Sarma et al. 2010), indicating that fish acclimated to high temperatures tend to exhibit increased tolerance to higher temperatures.

Conclusion

Overall, the present study revealed that increasing temperature had an effect on growth and feed utilization in goldfish. Although the culture at 30˚C has the potential to enhance growth performance of goldfish, feed conversion ratio, liver histology, serum biochemistries, and cortisol levels were all negatively affected by increasing temperature. In addition, goldfish cultured higher than 30˚C significantly decreased coloration and total carotenoid in muscle, skin, and serum at week 10. In the goldfish aquaculture scenario, the temperature should keep below 30˚C to avoid the suppression of growth, coloration, total carotenoids, histological pathology, and stress responses. In addition, carotenoid supplementation in diet or natural carotenoid must be fed during increased temperature.

Declarations

Ethics approval

All fish handling and experimental protocols were inspected and approved by The Faculty of Agriculture, Natural Resources and Environment, Naresuan University (Approval number: AG-AQ0002/2564). In addition, all experimental animal was also conducted in accordance with the guidelines of the Institute of Animals for Scientific Purpose Development (IAD), the National Research Council of Thailand's Ethic of Animal Experimentation (reference number: U1/00704/2558).

Competing interests

The authors declare no competing interests.

Author's contributions

Anurak Khieokhajonkhet: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review and editing, Supervision; Marisa Phoprakot: Performed experiments, Data collection; Niran Akesiri: Hematological analysis; Gen Kaneko and Wutiporn Phromkunthong: Review and editing the manuscript. The final manuscript was examined and accepted by all contributors.

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Availability of data and materials

The data that support the finding of this study are available from the corresponding author upon reasonable request.

References

1. Abbink W, Blanco Garcia A, Roques JAC, Partridge GJ, Kloet K, Schneider O (2012) The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. Aquaculture 330–333: 130–135. https://doi.org/10.1016/j.aquaculture.2011.11.043
2. Alfonso S, Gesto M, Sadoul B (2021) Temperature increase and its effects on fish stress physiology in the context of global warming. J Fish Biol 98(6): 1496–1508. https://doi.org/10.1111/jfb.14599

3. Ahmed I, Reshi QM, Fazio F (2020) The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquac Int 28: 869–899. https://doi.org/10.1007/s10499-019-00501-3
4. Amar E, Kiron V, Satoh S, Watanabe T (2001) Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac Res 32: 162–173. https://doi.org/10.1046/j.1355-557x.2001.00051.x
5. AOAC (1997). Official methods of analysis of AOAC International, 16th Ed. Association of Official Analytical Chemists, Washington 1997. (Arlington, VA: AOAC International) (In P.A. Cunniff (Ed.)).
6. Ashaf-Ud-Doulah M, Mamun AA, Rahman ML, Islam SMM, Jannat R, Hossain MAR, Shahjahan M (2020) High temperature acclimation alters upper thermal limits and growth performance of Indian major carp, rohu, *Labeo rohita* (Hamilton, 1822). J Therm Biol 93: 102738.https://doi.org/10.1016/j.jtherbio.2020.102738
7. Ashaf-Ud-Doulah M, Shahjahan Md, Islam SMM, Emran MA, Rahman MS, Hossain MAR (2019) Thermal stress causes nuclear and cellular abnormalities of peripheral erythrocytes in Indian major carp, rohu *Labeo rohita*. J Therm Biol 86: 102450. https://doi.org/10.1016/j.jtherbio.2019.102450
8. Babin A, Saciat C, Teixeira M, Troussard JP, Motreuil S, Moreau J, Moret Y (2015) Limiting immunopathology: Interaction between carotenoids and enzymatic antioxidant defences. Dev Comp Immunol 49(2): 278–281. https://doi.org/10.1016/j.dci.2014.12.007
9. Barbosa MJ, Morais R, Choubert G (1999) Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 176(3): 331–341. https://doi.org/10.1016/S0044-8486(99)00115-5
10. Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42(3): 517–525. https://doi:10.1093/icb/42.3.517
11. Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of north American freshwater fishes exposed to dynamic changes in temperature. Environ Biol Fishes 58(3): 237–275. https://doi:10.1023/A:1007676325825
12. Bergot F, Breque J (1983) Digestibility of starch by rainbow trout: Effects of the physical state of starch and of the intake level. Aquaculture 34(3): 203–212. https://doi.org/10.1016/0044-8486(83)90203-X
13. Bowyer JN, Qin JG, Smullen RP, Stone DAJ (2012) Replacement of fish oil by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal and suboptimal temperatures. Aquaculture 356–357: 211–222. https://doi.org/10.1016/j.aquaculture.2012.05.014
14. Brown J, Walker S, Steinman K (2004) Endocrine manual for the reproductive assessment of domestic and non-domestic species. Endocrine research laboratory, Department of reproductive sciences, Conservation and research center, National zoological park, Smithsonian institution, Handbook. 1–93.
15. Cai LS, Wang L, Song K, Lu K-L, Zhang CX, Rahimnejad S (2020) Evaluation of protein requirement of spotted seabass (*Lateolabrax maculatus*) under two temperatures, and the liver transcriptome

response to thermal stress. Aquaculture 516: 734615. https://doi.org/10.1016/j.aquaculture.2019.734615

16. Cai X, Zhang J, Lin L, Li Y, Liu X, Wang Z (2020) Study of a noninvasive detection method for the high-temperature stress response of the large yellow croaker (*Larimichthys crocea*). Aquac Rep 18: 100514. https://doi.org/10.1016/j.aqrep.2020.100514
17. Chatterjee N, Pal AK, Das T, Mohammed MS, Sarma K, Venkateshwarlu G, Mukherjee SC (2006) Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. Aquac Res 37(5): 472–476. https://doi.org/10.1111/j.1365-2109.2006.01469.x
18. Cheng CH, Yang FF, Liao SA, Miao YT, Ye CX, Wang AL, Tan JW, Chen XY (2015) High temperature induces apoptosis and oxidative stress in pufferfish (*Takifugu obscurus*) blood cells. J Therm Biol 53: 172–179. https://doi.org/10.1016/j.jtherbio.2015.08.002
19. CIE (1976) International commission on illumination. Recommendations on uniform color spaces, color difference equations, psychometric color terms. Supplement No. 2 to CIE Publication No 15, Colorimetry. Bureau Central de la CIE, Paris.
20. Collins M (2020) Extremes, abrupt changes and managing risks. Paper presented at the ocean sciences meeting 2020.
21. Coz-Rakovac R, Smuc T, Topic Popovic N, Strunjak-Perovic I, Hacmanjek M, Jadan M (2008) Novel methods for assessing fish blood biochemical data. J Appl Ichthyol 24(1): 77–80. https://doi.org/10.1111/j.1439-0426.2007.01041.x
22. Dalvi RS, Pal AK, Tiwari LR, Das T, Baruah K (2009) Thermal tolerance and oxygen consumption rates of the catfish *Horabagrus brachysoma* (Günther) acclimated to different temperatures. Aquaculture 295(1): 116–119. https://doi.org/10.1016/j.aquaculture.2009.06.034
23. Das T, Pal AK, Chakraborty SK, Manush SM, Sahu NP, Mukherjee SC (2005) Thermal tolerance, growth and oxygen consumption of *Labeo rohita* fry (Hamilton, 1822) acclimated to four temperatures. J Therm Biol 30(5): 378–383. https://doi.org/10.1016/j.jtherbio.2005.03.001
24. Dharmaraj S, Dhevendaran K (2011) Application of microbial carotenoids as a source of colouration and growth of ornamental fish *Xiphophorus helleri*. WJFMS 137: 137–144.
25. Do TD, Thi Mai N, Duy Khoa TN, Abol-Munafi AB, Liew HJ, Kim CB, Wong LL (2019) Molecular characterization and gene expression of glutathione peroxidase 1 in *Tor tambroides* exposed to temperature stress. Evol Bioinform 15: 1–8. https://doi:10.1177/1176934319853580
26. Drupt F, Paris M, Frydman A, Leclerc M (1974) Serum albumin assay by bromocresol green method: application to different automatic apparatus. Ann Pharm Fr 32(5): 249–256.
27. Eslamloo K, Akhavan SR, Eslamifar A, Henry MA (2015) Effects of background colour on growth performance, skin pigmentation, physiological condition and innate immune responses of goldfish, *Carassius auratus*. Aquac Res 46(1): 202–215. https://doi.org/10.1111/are.12177
28. FAO (2021) 3rd international ornamental fish trade and technical conference. https://www.fao.org/in-action/globefish/news-events/details-news/en/c/1373555/

29. Farag MR, Alagawany M, Khalil SR, El-Aziz RM, Zaglool AW, Moselhy AAA, Abou-Zeid SM (2022) Effect of parsley essential oil on digestive enzymes, intestinal morphometry, blood chemistry and stress-related genes in liver of Nile tilapia fish exposed to Bifenthrin. Aquaculture 546: 737322. https://doi.org/10.1016/j.aquaculture.2021.737322
30. Fernández-Montero A, Caballero MJ, Torrecillas S, Tuset VM, Lombarte A, Ginés RR, Izquierdo M, Robaina L, Montero D (2018) Effect of temperature on growth performance of greater amberjack (*Seriola Dumberili* Risso 1810) Juveniles. Aquac Res 49(2): 908–918. https://doi.org/10.1111/are.13537
31. Ford T, Beitinger TL (2005) Temperature tolerance in the goldfish, *Carassius auratus*. J Therm Biol 30(2): 147–152. https://doi.org/10.1016/j.jtherbio.2004.09.004
32. Freshwater Ornamental Fish (2021). Exporter and importers, Trade by country 2021. Available at:
33. https://oec.world/en (accessed 29 May 2023).
34. Frölicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. Nature 560(7718): 360–364.
35. Fry FEJ, Hart JS (1948) The relation of temperature to oxygen consumption in the goldfish. Biol Bull 94(1): 66–77. doi:10.2307/1538211
36. Gouveia L, Rema P (2005) Effect of microalgal biomass concentration and temperature on ornamental goldfish (*Carassius auratus*) skin pigmentation. Aquac Nutr 11(1): 19–23. https://doi.org/10.1111/j.1365-2095.2004.00319.x
37. Gracey AY, Fraser EJ, Li W, Fang Y, Taylor RR, Rogers J, Brass A, Cossins AR (2004) Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. Proc Natl Acad Sciences 101(48): 16970–16975. https://doi.org/10.1073/pnas.0403627101
38. Hall DM, Xu L, Drake VJ, Oberley LW, Oberley TD, Moseley PL, Kregel KC (2000) Aging reduces adaptive capacity and stress protein expression in the liver after heat stress. J Appl Physio 89(2): 749–759. https://doi:10.1152/jappl.2000.89.2.749
39. Hassaan MS, El Nagar AG, Salim HS, Fitzsimmons K, El-Haroun ER (2019) Nutritional mitigation of winter thermal stress in Nile tilapia by propolis-extract: Associated indicators of nutritional status, physiological responses and transcriptional response of delta-9-desaturase gene. Aquaculture 511: 734256. doi:https://doi.org/10.1016/j.aquaculture.2019.734256
40. Hesser EF (1960) Methods for routine fish hematology. The Progressive Fish-Culturist 22(4): 164–171. https://doi:10.1577/1548-8659(1960)22[164:MFRFH]2.0.CO;2
41. Hrubec TC, Robertson JL, Smith SA (1997) Effects of temperature on hematologic and serum biochemical profiles of hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Am J Vet Res 58(2): 126–130.
42. Hunter RS, Harold RW (1987) The Measurement of Appearance, the, 2nd ed. (pp. 411). New York, NY: John Wiley & Sons.
43. IPPC (2014) Climate change 2014. Synthesis Report. Contribution of working Groups I, II, and II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 151. IPCC, Geneva,

Switzerland. Core-Writing Team. https://www.weadapt

44. .org/knowledge-base/national-adaptation-planning/climate-change-2014-synthesis-report.
45. Islam MA, Uddin MH, Uddin MJ, Shahjahan M (2019) Temperature changes influenced the growth performance and physiological functions of Thai pangas *Pangasianodon hypophthalmus*. Aquac Rep 13: 100179. https://doi.org/10.1016/j.aqrep.2019.100179
46. Jeamsripong S, Chuanchuen R, Atwill ER (2018) Assessment of bacterial accumulation and environmental factors in sentinel oysters and estuarine water quality from the Phang Nga estuary area in Thailand. Int J Environ Res Public Health 15(9): 1970.
47. Jobling M (1994) Ingestion, absorption and excretion. In: Jobling, M. (Ed.), Fish Bioenergetics. Chapman Hall, London, 99–119.
48. Katersky RS, Carter CG (2007) A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer* at different temperatures. Aquaculture 267(1–4): 157–164.
49. Kestemont P (1995) Influence of feed supply, temperature and body size on the growth of goldfish *Carassius auratus* larvae. Aquaculture 136(3): 341–349. https://doi.org/10.1016/0044-8486(95)00060-7
50. Khieokhajonkhet A, Aeksiri N, Rojtinnakorn J, Van Doan H, Kaneko G (2022a) Sacha inchi meal as a fish-meal replacer in red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) feeds: effects on dietary digestibility, growth metrics, hematology, and liver and intestinal histology. Aquac Int 30: 677–698. https://doi:10.1007/s10499-022-00833-7
51. Khieokhajonkhet A, Uanlam P, Ruttarattanamongkol K, Aeksiri N, Tatsapong P, Kaneko G. (2022b). Replacement of fish meal by black soldier fly larvae meal in diet for goldfish *Carassius auratus*: Growth performance, hematology, histology, total carotenoids, and coloration. Aquaculture 561: 738618. https://doi.org/10.1016/j.aquaculture.2022.738618
52. Khieokhajonkhet A, Sangphrom S, Aeksiri N, Tatsapong P, Wuthijaree K, Kaneko G (2022c) Effects of long-term exposure to high temperature on growth performance, chemical composition, hematological and histological changes, and physiological responses in hybrid catfish [*Clarias gariepinus* (Burchell, 1822) × *C. macrocephalus* (Günther, 1864)]. J. Therm. Biol. 105: 103226. https://doi.org/10.1016/j.jtherbio.2022.103226
53. Khieokhajonkhet A, Muichanta S, Aeksiri N, Ruttarattanamongkol K, Rojtinnakorn J, Kaneko G (2021) Evaluation of sacha inchi meal as a novel alternative plant protein ingredient for red hybrid tilapia (*Oreochromis niloticus*×*O. mossambicus*): Growth performance, feed utilization, blood biochemistry, and histological changes. Anim Feed Sci Tech 278: 115004. https://doi.org/10.1016/j.anifeedsci.2021.115004
54. Khosravi-Katuli K, Mohammadi Y, Ranjbaran M, Ghanaatian H, Khazaali A, Paknejad H, Santander J, (2021) Effects of mannan oligosaccharide and synbiotic supplementation on growth performance and immune response of gilthead sea bream (*Sparus aurata*) before and after thermal stress. Aquac Res 52: 3745–3756. https://doi.org/10.1111/are.15220

55. Knox WE, Greengard O (1965) The regulation of some enzymes of nitrogen metabolism - an introduction to enzyme physiology. Adv Enzyme Regul 3: 247–313. https://doi.org/10.1016/0065-2571(65)90059-2
56. Kumar P, Pal AK, Sahu NP, Jha AK, Kumar N, Christina L, Priya P (2018) Dietary L-Tryptophan potentiates non-specific immunity in *Labeo rohita* fingerlings reared under elevated temperature. J Therm Biol 74: 55–62. https://doi.org/10.1016/j.jtherbio.2018.03.010
57. Kumar V, Sahu NP, Pal AK, Kumar S (2007) Immunomodulation of *Labeo rohita* juveniles due to dietary gelatinized and non-gelatinized starch. Fish Shellfish Immunol 23(2): 341–353. https://doi:10.1016/j.fsi.2006.11.008
58. Li B, Sun S, Zhu J, Yanli S, Wuxiao Z, Ge X (2019) Transcriptome profiling and histology changes in juvenile blunt snout bream (*Megalobrama amblycephala*) liver tissue in response to acute thermal stress. Genomics 111(3): 242–250. https://doi.org/10.1016/j.ygeno.2018.11.011
59. Li C, Wang Y, Wang G, Chen Y, Guo J, Pan C, Liu E, Ling Q (2019) Physicochemical changes in liver and Hsc70 expression in pikeperch *Sander lucioperca* under heat stress. Ecotoxicol Environ Saf 181: 130–137. https://doi.org/10.1016/j.ecoenv.2019.05.083
60. Li M, Wang X, Qi C, Li E, Du Z, Qin JG, Chen L (2018) Metabolic response of Nile tilapia (*Oreochromis niloticus*) to acute and chronic hypoxia stress. Aquaculture 495: 187–195. https://doi.org/10.1016/j.aquaculture.2018.05.031
61. Liu B, Xu P, Brown PB, Xie J, Ge X, Miao L, Zhou Q, Ren M, Pan L (2016) The effect of hyperthermia on liver histology, oxidative stress and disease resistance of the Wuchang bream, *Megalobrama amblycephala*. Fish Shellfish Immunol 52: 317–324. https://doi.org/10.1016/j.fsi.2016.03.018
62. Liu ZP, Gu WB, Tu DD, Zhu QH, Zhou YL, Wang C, Wang LZ, Shu MA (2018) Effects of both cold and heat stress on the liver of the giant spiny frog (*Quasipaa spinosa*): stress response and histological changes. J Exp Biol 221(21). https//doi:10.1242/jeb.186379
63. Ma XY, Qiang J, He J, Gabriel NN, Xu P (2015) Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (*Oreochromis niloticus*) reared at three different temperatures. Fish Physiol Biochem 41(4): 937–950. https//doi:10.1007/s10695-015-0059-4
64. Mangi SC, Lee J, Pinnegar JK, Law R, Tyllianakis E, Birchenough SNR (2018) The economic impacts of ocean acidification on shellfish fisheries and aquaculture in the United Kingdom. Environ Sci Policy 86: 95–105. https://doi.org/10.1016/j.envsci.2018.05.008
65. Mørkøre T, Rørvik KA (2001) Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0 + smolts or 1 + smolts. Aquaculture 199(1): 145–157. https://doi.org/10.1016/S0044-8486(01)00524-5
66. Nguyen CDH, Amoroso G, Ventura T, Minich JJ, Elizur A (2020) Atlantic salmon (*Salmo salar* L., 1758) gut microbiota profile correlates with flesh pigmentation: Cause or Effect? Mar Biotechnol 22(6): 786–804. https//doi:10.1007/s10126-019-09939-1

67. Nordgarden U, Ørnsrud R, Hansen T, Hemre GI (2003) Seasonal changes in selected muscle quality parameters in Atlantic salmon (*Salmo salar* L.) reared under natural and continuous light. Aquac Nutr 9(3): 161–168. https://doi.org/10.1046/j.1365-2095.2003.00236.x
68. Ota KG, Abe G (2016) Goldfish morphology as a model for evolutionary developmental biology. Wiley Interdisciplinary Reviews: Dev Biol 5(3): 272–295.
69. Palaksha KJ, Shin GW, Kim YR, Jung TS (2008) Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol 24(4): 479–488. https://doi.org/10.1016/j.fsi.2008.01.005
70. Pavlidis M, Karkana M, Fanouraki E, Papandroulakis N (2008) Environmental control of skin colour in the red porgy, *Pagrus pagrus*. Aquac Res 39(8): 837–849. https://doi.org/10.1111/j.1365-2109.2008.01937.x
71. Peres H, Oliva-Teles A (1999) Influence of temperature on protein utilization in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture 170(3): 337–348. https://doi.org/10.1016/S0044-8486(98)00422-0
72. Pfeffer E, Beckmann-Toussaint J, Henrichfreise B, Jansen HD (1991) Effect of extrusion on efficiency of utilization of maize starch by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 96(3): 293–303. https://doi.org/10.1016/0044-8486(91)90159-5
73. Qi ZH, Liu YF, Luo SW, Chen CX, Liu Y, Wang WN (2013) Molecular cloning, characterization and expression analysis of tumor suppressor protein p53 from orange-spotted grouper, *Epinephelus coioides* in response to temperature stress. Fish Shellfish Immunol 35(5): 1466–1476. https://doi.org/10.1016/j.fsi.2013.08.011
74. Sarma K, Pal AK, Ayyappan S, Das T, Manush SM, Debnath D, Baruah K (2010) Acclimation of *Anabas testudineus* (Bloch) to three test temperatures influences thermal tolerance and oxygen consumption. Fish Physiol Biochem 36(1): 85–90. https//doi:10.1007/s10695-008-9293-3
75. Scoffone E, Fontana A (1975) Proteins analysis. In: Needleman, S.B. (ed.), Protein sequence determination: a source book of methods and techniques springer-verlag, New York. 162–203.
76. Stoskopf MK (1993) Clinical physiology. M.K. Stoskopf (Ed.), Fish Medicine, W.B. Saunders, Philadelphia.
77. Sun JL, Zhao LL, Wu H, Liu Q, Liao L, Luo J, Lian WQ, Cui C, Jin L, Ma JD, Li MZ, Yang S (2020) Acute hypoxia changes the mode of glucose and lipid utilization in the liver of the largemouth bass (*Micropterus salmoides*). Sci Total Environ 713: 135157. https://doi.org/10.1016/j.scitotenv.2019.135157
78. Sun L, Chen H (2014) Effects of water temperature and fish size on growth and bioenergetics of cobia (*Rachycentron canadum*). Aquaculture 426: 172–180. https://doi.org/10.1016/j.aquaculture.2014.02.001
79. Talpur AD, Ikhwanuddin MH (2012) Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection

in Asian sea bass, *Lates calcarifer* (Bloch). Aquaculture 364: 6–12. https://doi.org/10.1016/j.aquaculture.2014.02.001

80. Torrissen OJ, Christiansen RR, Struksnæs G, Estermann R (1995) Astaxanthin deposition in the flesh of Atlantic Salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. Aquac Nutr 1: 77–84. https://doi.org/10.1111/j.1365-2095.1995.tb00022.x
81. Torrissen OJ, Naevdal G (1988) Pigmentation of salmonids - Variation in flesh carotenoids of Atlantic salmon. Aquaculture 68(4): 305–310. https://doi.org/10.1016/0044-8486(88)90244-X
82. Verma AK, Pal AK, Manush SM, Das T, Dalvi RS, Chandrachoodan PP, Ravi PM, Apte SK (2007) Persistent sub-lethal chlorine exposure augments temperature induced immunosuppression in *Cyprinus carpio* advanced fingerlings. Fish Shellfish Immunol 22(5): 547–555. https://doi.org/10.1016/j.fsi.2006.08.001
83. Waagbø R, Hamre K, Bjerkås E, Berge R, Wathne E, Lie Ø, Torstensen B (2003) Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro-and antioxidants and lipid level. J Fish Dis 26(4): 213–229. https://doi.org/10.1046/j.1365-2761.2003.00449.x
84. Wang N, Xu X, Kestemont P (2009) Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*). Aquaculture 289(1): 70–73. https://doi.org/10.1016/j.aquaculture.2009.01.002
85. Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77, 591–625.
86. Whangchai N, Chitmanat C, Ramarai R, Itayama T (2018) Effects of water flow rate and water quality on tilapia culture in the Mae ping river, Thailand. Chiang Mai J Sci 45(3): 1318–1322.
87. Wiegertjes GF, Stet RJ, Parmentier HK, van Muiswinkel WB (1996) Immunogenetics of disease resistance in fish: a comparative approach. Dev Comp Immunol 20(6): 365–381. https://doi:10.1016/s0145-305x(96)00032-8
88. Yanar M, Erdoğan E, Kumlu M (2019) Thermal tolerance of thirteen popular ornamental fish species. Aquaculture 501: 382–386. https://doi.org/10.1016/j.aquaculture.2018.11.041
89. Zeng T, Li Jj, Wang Dq, Li Gq, Wang Gl, Lu, Lz(2014) Effects of heat stress on antioxidant defense system, inflammatory injury, and heat shock proteins of muscovy and pekin ducks: evidence for differential thermal sensitivities. Cell Stress and Chaperones 19(6): 895–901. https://doi:10.1007/s12192-014-0514-7
90. Zhao T, Ma A, Huang Z, Liu Z, Sun Z, Zhu C, Yang J, Li Y, Wang Q, Qiao X, Chen Z (2021) Transcriptome analysis reveals that high temperatures alter modes of lipid metabolism in juvenile turbot (*Scophthalmus maximus*) liver. Comp Biochem Physiol Part D Genomics Proteomics 40: 100887. https://doi.org/10.1016/j.cbd.2021.100887
91. Zhou Q, Wang L, Wang H, Xie F, Wang T (2012) Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (*Rachycentron canadum*). Fish Shellfish Immunol 32(6): 969–975. https://doi.org/10.1016/j.fsi.2012.01.024

Figures

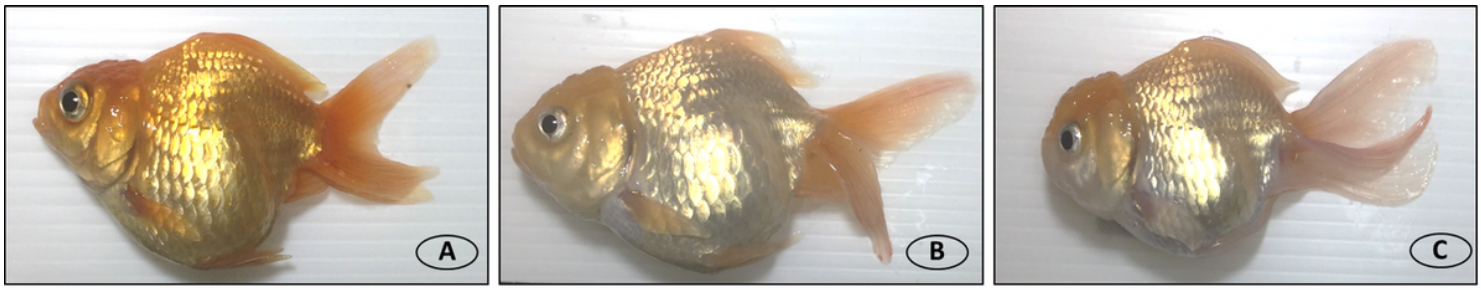

Figure 1

The phenotypic variation of skin pigmentation of goldfish acclimated at 27˚C, 30˚C, and 34˚C for 10 weeks.

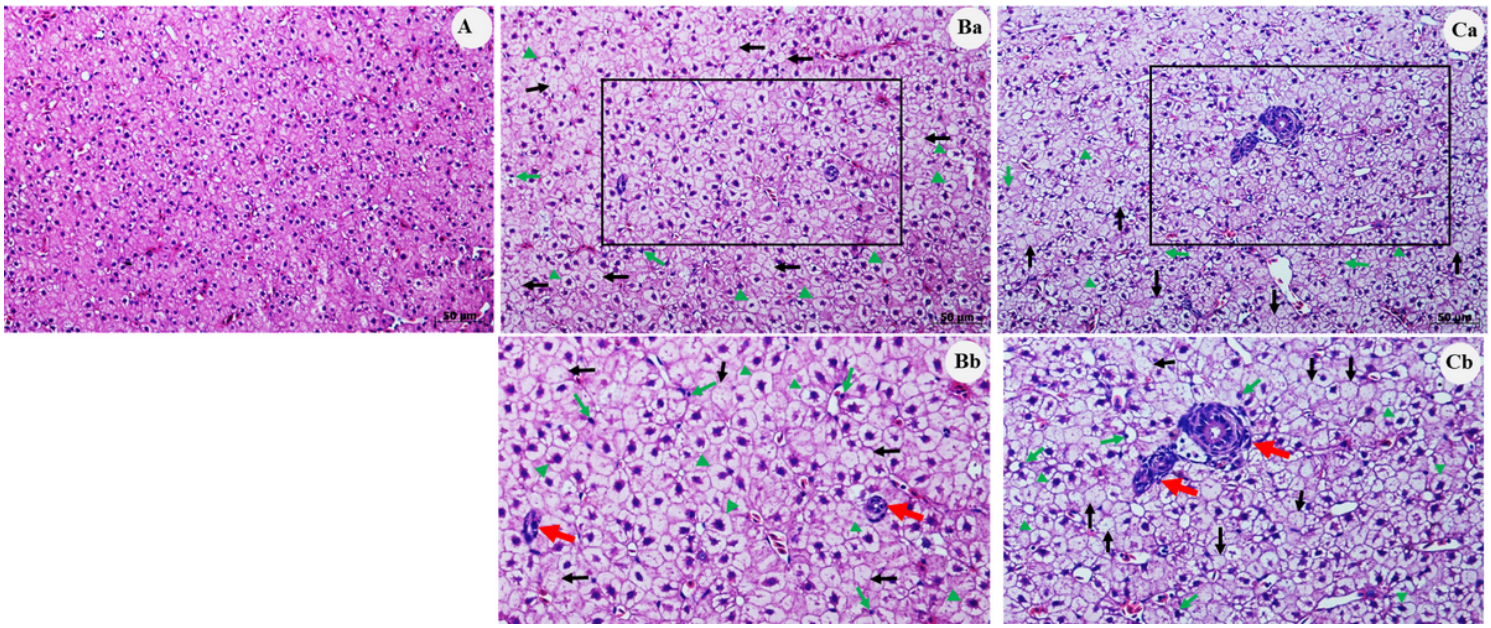

Figure 2

Photomicrographs of representative liver sections stained with hematoxylin and eosin (H&E; original magnification 40×) of goldfish acclimated at 27˚C (A), 30˚C (B), and 34˚C (C) for 10 weeks. Fig. Bb and Cb displayed a portion of the enlargement from the portion that was framed in Fig. Ba and Ca, respectively. Black arrows indicate an irregular shape and nuclear disappearance. Green arrows exhibited nuclear displacement of hepatocyte. Green arrowheads show cytoplasmic vacuole and swollen hepatocytes with vacuolization. Red arrows show infiltration of inflammatory cells.

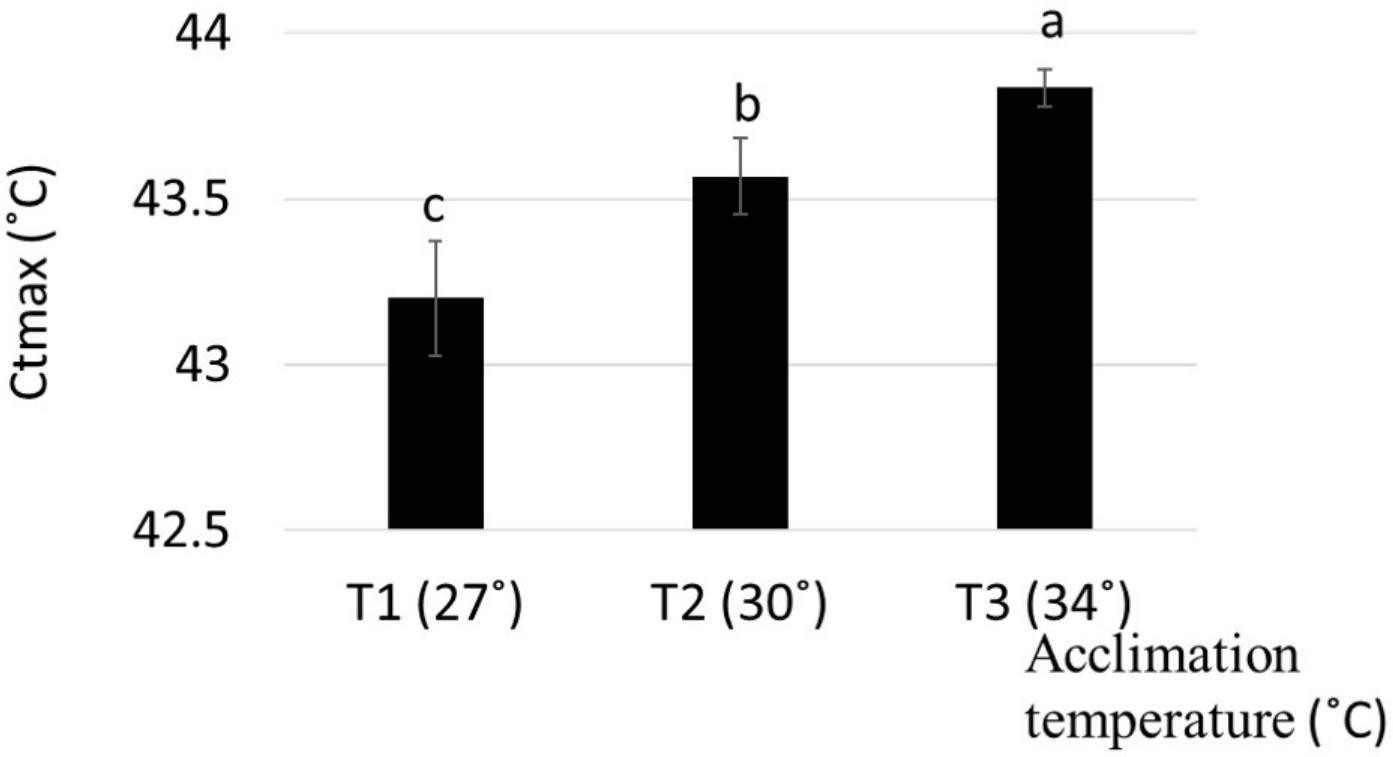

Figure 3

The critical thermal maximum (CTmax) of goldfish cultured at different temperatures (27, 30, and 35˚C).