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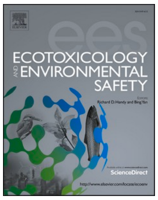

Correlation of skin color and plasma carotenoid-related metabolites of ornamental koi carp under temperature fluctuations

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ABSTRACT

The skin color of koi carp (*Cyprinus carpio* L.) is one of the traits that most influence their ornamental and economic values. The present study suggested the effects of temperature fluctuation on koi carp in terms of skin color and plasma carotenoids and related-metabolites. The main results were as follows. (1) The vulnerability of koi skin color to acute temperature stress was in the order of white koi> black koi> yellow koi. Both high- (25°C-30°C-25°C) and low-temperature (25°C-20°C-25°C) fluctuations tended to decrease the saturation of white koi. The temperature fluctuation had little effects on the skin color of black and yellow koi. (2) Targeted metabolomics analysis indicated that the effects of cooling stress on oxycarotenoids of all five koi varieties were reversible. The plasma oxycarotenoids in mirror koi with all colors were insensitive to acute heat stress. However, the cooling process from a high temperature (30°C-25°C) still made contributions to the increase of oxycarotenoids. (3) The principal component analysis confirmed the deviation of carotenoid-related metabolites after high temperature fluctuation and the reversibility after low temperature fluctuation. Finally, the correlation analysis revealed that koi skin brightness was negatively correlated with the plasma guanine content and that temperature fluctuations might change koi skin brightness via the L(-)-epinephrine-guanine pathway. The red hue and yellow hue were negatively correlated with the oxycarotenoids in plasma, suggesting that oxycarotenoids were favorable for enhancing koi skin color saturation. Overall, this study revealed the direct action of temperature fluctuations on the skin color and carotenoid-related metabolites of koi.

1. Introduction

Ornamental koi carp have bright colors, diverse patterns, vigorous swimming and extremely high ornamental and economic values. After about 200 years of artificial selection and cross-breeding, ornamental koi carp have developed into more than 100 varieties in 16 groups based on coloration, pattern, and scalation (data from Zen Nippon Airinkai). Ornamental koi carp are distributed in multiple countries in Southeast Asia, Europe, and North America. The global koi industry is currently growing rapidly. In 2019, the export value of Japanese freshwater ornamental fish (mainly koi) was 4.7 billion Japanese Yen, which was 2.5 times greater than in 2005 (https://www.nippon.com/en/japan-data/h00852/). According to the United States National Agricultural Statistics Service, 149 farms across 29 states in USA raised koi in 2018 with a gross production value of 8.14 million USD. Israel earns about 10 million USD annually from exporting koi to European countries (David et al., 2004).

Skin color is one of the main factors determining the ornamental and economic values of koi. Fish skin color is mainly dependent on pigment cells, which in koi include melanophore, xanthophore, erythrophore, and iridophore. Melanophore primarily contains melanin pigment, which is not a macromolecule with a clear sequence, but a random copolymer with a complex structure. Xanthophores and erythrophores, which contain several carotenoids and pteridines, are responsible for the yellow-orange-red skin color of fish skin (Junqueira et al., 1978; Dick et al., 2018; Tian et al., 2018). Koi carotenoids reported to date include astaxanthin, canthaxanthin, lutein, zeaxanthin and so on. Iridophores mainly contain guanine crystals, which can reflect light, are responsible for the formation of shiny skin colors, and interact with other pigment cells to form gold, silver, and blue colors (Zarnescu, 2007; Gur et al., 2020).

The skin color of koi is primarily determined by genetics (David et al., 2004). However, the breeding environment conditions, such as temperature, oxygen and salinity, can directly or indirectly affect

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pigment cells and pigment particles, causing changes in skin color (Vissio et al., 2021). These factors are environmental pressures that induce acute or chronic responses in the pigment cells of fish according to the time length of stress. For example, carotenoid deposition in the skin of Arctic charr (*Salvelinus alpinus* L) is associated with stress response (Backstrom et al., 2021). Additionally, changes in background color cause changes in the black color of the *Oreochromis mossambicus* skin (Van der Salm et al., 2005), while ultraviolet exposure can change the structural coloration of cichlid fish by acting on iridophores (Cahn et al., 2015). The skin color change caused by temperature fluctuation has been noticed as a common phenomenon for a long time in koi aquaculture. But nevertheless, little was reported about the detailed temperature-color correlation and the underlined regulation mechanism.

Elucidating the regulation mechanism of temperature on skin color will promote to maintain the ornamental-value stability and aquaculture sustainability of koi. In this study, the effects of acute temperature fluctuations on koi skin color via action in pigments (carotenoids and guanine) and related metabolites were investigated by the combination of quantitative evaluation of skin color and targeted metabolomics analysis.

2. Materials and methods

2.1. Koi temperature stress test

Koi carp (*Cyprinus carpio* L.) were purchased from Guangzhou Huadu Huasheng Koi Farm, domesticated in a breeding pond for one month, and then transferred to a recirculating aquaculture system for one week before stress test. The breeding conditions include water temperature 25°C, pH 7.5–8, light/dark cycle 12 h/12 h, and continuous introduction of air to the water. During the experiment, the koi were provided with as many feed pellets (Aqua Master, Wheat Germ) as they could eat (1.5% weight of the koi) within 5 min twice a day according to the instruction. Quantitative feeding at regular time was performed. The feed was suitable for low-temperature and could be fed normally during 20°C-30°C. The experimental fish included five varieties with different skin color phenotype: mirror (scaleless) koi covered with uniform black color (MB), full scaled koi covered with uniform white color (JW), shiny mirror koi covered with uniform white color (sMW), mirror koi covered with uniform yellow color (MY), and shiny mirror koi covered with uniform yellow color (sMY). There were five fish in each variety with an age of four months and a length of 20±2 cm. The fish were randomly placed into two 80 L experimental tanks. All experimental tanks shared one recirculating aquaculture system that was equipped with a temperature-control system. At the beginning of the experiment, the water temperature was set at 25°C, which was held for 48 h, then changed to 30°C (high temperature exposure) or 20°C (low temperature exposure), which was held for 48 h, after which returned to 25°C holding for 72 h. The heating or cooling process took about two hours. Before the end of each temperature treatment, all experimental fish were anesthetized with tricaine methanesulfonate (MS222, Sigma-Aldrich). Then the experimental data were collected, and experimental samples were collected. The anesthetized fish were resuscitated as soon as possible and returned to the breeding system.

2.2. Measurement of koi skin color

Anesthetized fish were washed with water, after which a towel was used to remove the water residue. With the head facing left, the fish was placed horizontally on a wet sponge, after which the lightness (L*), red hue (a*), and yellow hue (b*) of the skin below the dorsal fin and above the lateral line was measured using an Illuminance Spectrophotometer (CL-500A, Konica Minolta, Japan). The fish were put back into the breeding system as soon as possible at the end of the experiment. The color saturation (C) and hue angle (H) are calculated based on these three color spaces and then used to quantitatively and qualitatively describe mixed colors. The reduction formulas were given as follows.

$$H=ATAN[(a^{*}\cdot a^{*})/(b^{*}\cdot b^{*})]$$

$$C=[(a^{*}\cdot a^{*})/(b^{*}\cdot b^{*})]^{1/2}$$

2.3. Targeted metabolomics analysis

A targeted metabolomics procedure based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reactions monitoring (MRM) (LC-MS/MS-MRM) was constructed to analyze 15 carotenoids and related metabolites (CRMs) in the koi plasma samples (Table 1).

Caudal venous puncture was used to collect 500 μL of blood from each fish, after which the blood sample was placed in an EDTA-Na_2 anticoagulation tube, rapidly frozen in liquid nitrogen, then stored at −80°C. For analysis, the sample was stored at 4°C, after which 100 μL plasma was taken and mixed with 100 μL of pre-cooled water and 800 μL of pre-cooled acetonitrile/isopropanol (1:1, v/v). Next, the mixture was sonicated in an ice bath for 60 min and then incubated at −20°C for 1 h. After the protein was precipitated, the mixture was centrifuged at 16,000 *g* for 20 min at 4°C and the supernatant was collected. The same amount of internal standard L-Glutamate D5 was subsequently added to each sample, after which they were vacuum dried. For mass spectrometry analysis, the sample was reconstituted in 100 μL of acetonitrile-water solution (1:1, v/v), the solution was centrifuged at 16,000 *g* for 20 min at 4°C, and the supernatant was collected for analysis.

A Shimadzu Nexera X2 LC-30AD High-Performance Liquid Chromatography (HPLC) system was used for separation. The mobile phases were 0.1% formic acid-water solution (phase A) and isopropanol/acetonitrile (1:1) (phase B). The flow rate was 200 μL/min and the injection volume was 5 μL. Additionally, aliquots of all samples were mixed in equal amounts to serve as quality control (QC) samples, which were added to the sample queue to monitor and evaluate the stability of the system and the reliability of the experimental data.

A QTRAP5500 mass spectrometer (AB SCIEX) was used for mass spectrometry analysis under positive/negative ion mode. The parameters of the electrospray ionization (ESI) mode were as follows: 550°C, 40, 50, ±4500 V. MRM mode was used to detect the ion pairs to be measured.

The MultiQuant software was used to extract the chromatography peak areas and retention times. The retention times were calibrated using metabolites reference standards.

Table 1
The targeted carotenoids and related metabolites in koi serum.

Classification	Metabolite Name	Cas No.
Neurotransmitter	Acetylcholine	51–84–3
	Norepinephrine	51–41–2
	L(-)-Epinephrine	51–43–4
	Melatonin	73–31–4
	Progesterone	57–83–0
Signaling molecule	Cyclic adenosine monophosphate	86594–35–6
	Cyclic 3′,5′-guanosine monophosphate	7665–99–8
Pigment in iridophore	Guanine	73–40–5
Pigment in xanthophore, /erythrophore	alpha-Carotene	7488–99–5
	beta-Carotene	7235–40–7
	Lutein	127–40–2
	Astaxanthin	472–61–7
	Canthaxanthin	514–78–3
	Zeaxanthin	144–68–3
	Tunaxanthin	12738–95–3

2.4. Data analysis

The loading concentration of each metabolite was calculated based on its standard curve, after which the concentrations of metabolites in each sample were calculated. The contents of CRMs were the average values of samples (after Ln data conversion). All data were expressed as the sample mean ± standard error. The Principle Component Analysis (PCA) was performed using the OmicStudio tools (https://www.omicstudio.cn) with z-score normalization. The correlation heat mapping was performed using the OmicStudio tools after spearman correlation analysis. **indicated p value <0.01. * indicated p value≥ 0.01 & p value<0.05.

One-way analysis of variance (ANOVA) model and Tukey's honestly significant difference (HSD) post hoc test were used to identify significant differences among groups. The uppercase alphabetic characters indicates $\alpha = 0.01$. The lowercase alphabetic characters indicates $\alpha = 0.05$.

3. Results

3.1. Effects of temperature fluctuations on koi skin color

We measured the International Commission on Illumination (Commission Internationale de l'Eclairage, CIE) 1976-(L*a*b*) (CIELab) color space parameters on the skin above the lateral line and below the dorsal fin of one black koi variety (MB koi), two white koi varieties (JW koi and sMW koi), and two yellow koi varieties (MY koi and sMY koi). The CIE values of the black koi were [L*: 6.2 ± 0.2, a*: 2.5±0.2, b*: 3.3 ± 0.6, H: −0.1 ± 0.1, C: 6.3 ± 1.2], while those of the white koi were [L*: 8.6 ± 0.2, a*: 0.4 ± 1.0, b*: 4.3 ± 0.8, H: 0.4 ± 0.9, C: 7.4 ± 1.3] and those of the yellow koi were [L*: 8.1 ± 0.1, a*: 6.8 ± 0.3, b*: 7.9 ± 0.5, H: 0.1 ± 0.1, C: 15.1 ± 0.9] (Fig. 1). These results indicated that the CIELab parameters of koi of different colors had stable and characteristic value ranges. Moreover, there was no significant difference among individual koi carps of the same color.

Temperature fluctuations exhibited the most apparent impact on skin color parameters of white koi (JW, sMW). The a* values of JW koi in the high-temperature group and low-temperature group varied significantly with temperature changes. In the high-temperature group, the a* values of the JW koi increased significantly while the b* and C decreased significantly, demonstrating that high-temperature stress significantly changed the skin color of JW koi. The a* values of JW koi in the low-temperature group increased first and decreased after recovery from low temperature, indicating that the effect of low-temperature stress on the red hue of white koi was reversible. Additionally, great individual varieties of color space were observed in sMW koi responding to temperature while consistence in JW koi, indicating instability in the skin color of white mirror koi under thermal stress (Fig. 2).

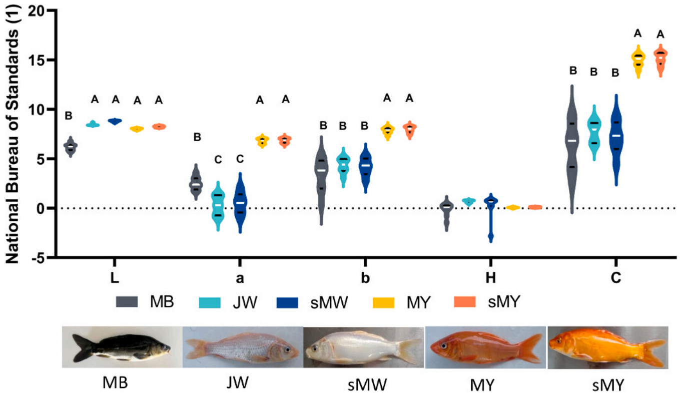

Fig. 1. CIELab color space parameter range of different color koi skin. The left dorsal skin below the dorsal fin is measured by CIELab color space. Eight fish were measured in each variety. The uppercase alphabetic characters indicates $\alpha = 0.01$.

Temperature fluctuations had little significant effect on the color of black and yellow koi. The C value of MB koi after recovery from high-temperature exposure was considerably higher than pre-exposure. There was a rise in color saturation of MB koi after recovery exposure, indicating the skin color faded visually (Fig. 2).

3.2. Effects of temperature fluctuations on plasma CRM levels of koi

A LC-MS/MS-MRM based targeted metabolomics assay procedure was constructed for quantitative detection of the total 15 plasma CRMs (Supplementary Fig. 1). The results indicated that the following carotenoids were observed in the pre-exposure koi of all five varieties: beta-carotene > alpha-carotene > lutein > canthaxanthin > zeaxanthin > tunaxanthin > astaxanthin. The plasma lutein and canthaxanthin levels of koi with different skin color differed. The plasma levels of lutein and canthaxanthin in black koi (MB) were significantly higher than in yellow koi (MY, sMY) ($p<0.01$). The plasma levels of lutein and canthaxanthin in white koi (JW, sMW) tended to be lower than in black koi. Additionally, there was no difference in guanine levels among koi with different skin colors (Fig. 3).

In the high-temperature group, the plasma levels of oxycarotenoids (lutein, canthaxanthin, zeaxanthin, tunaxanthin, and astaxanthin) in the koi were changed by temperature fluctuation, while beta-carotene and alpha-carotene (lacking oxygen atom) showed no change in levels. The levels of canthaxanthin, zeaxanthin and tunaxanthin of JW koi under high-temperature and high-temperature-recovery periods were higher significantly than pre-exposure ($p<0.01$). For all mirror koi varieties (MB, sMW, MY and sMY), the levels of oxycarotenoids tended to increase in high-temperature-recovery periods when compared to pre-exposure. Multiple neurotransmitters and hormones in the plasma of koi from the high-temperature group were affected. The levels of melatonin and cAMP from all varieties of koi decreased significantly in recovery periods, while the levels of progesterone increased ($p<0.01$). The L(-)-epinephrine levels of MB, sMW and sMY koi varieties in high temperature period were higher than pre-exposure (Fig. 4).

In the low-temperature group, the plasma levels of oxycarotenoids (lutein, canthaxanthin, zeaxanthin, tunaxanthin, and astaxanthin) changed while beta-carotene and alpha-carotene showed no change in levels. Compared to pre-exposure, the levels of oxycarotenoids in all five koi varieties showed an increasing tendency in low temperature periods and retreated in low-temperature-recovery periods. The levels of melatonin and progesterone in JW and sMY koi increased significantly in low temperature periods and retreated in recovery periods ($p<0.01$, Fig. 4).

Compared to pre-exposure, the guanine levels of sMW, MY koi were significant higher in high temperature periods. In low temperature periods, the guanine levels of sMW koi decreased significantly and retreated in low-temperature-recovery periods when compared to pre-exposure ($p<0.01$). (Fig. 4).

Principal component analysis (PCA) was used to analyze changes in the level of plasma CRMs of koi under temperature fluctuations. We found an apparent separation between the samples before and after low-temperature exposure, as well as an overlap between samples from the low-temperature recovery and the pre-exposure periods, suggesting that the impact of a certain degree of acute low-temperature stress on the levels of CRMs of koi was reversible. There was an overlap in the levels of samples from the high-temperature and the pre-exposure periods, as well as an apparent separation between the high-temperature recovery exposure and the control (Fig. 5). These findings indicated that a certain degree of heat stress did not have a significant impact on the CRMs levels of koi, while the acute cooling during the high-temperature recovery period could affect their CRM levels.

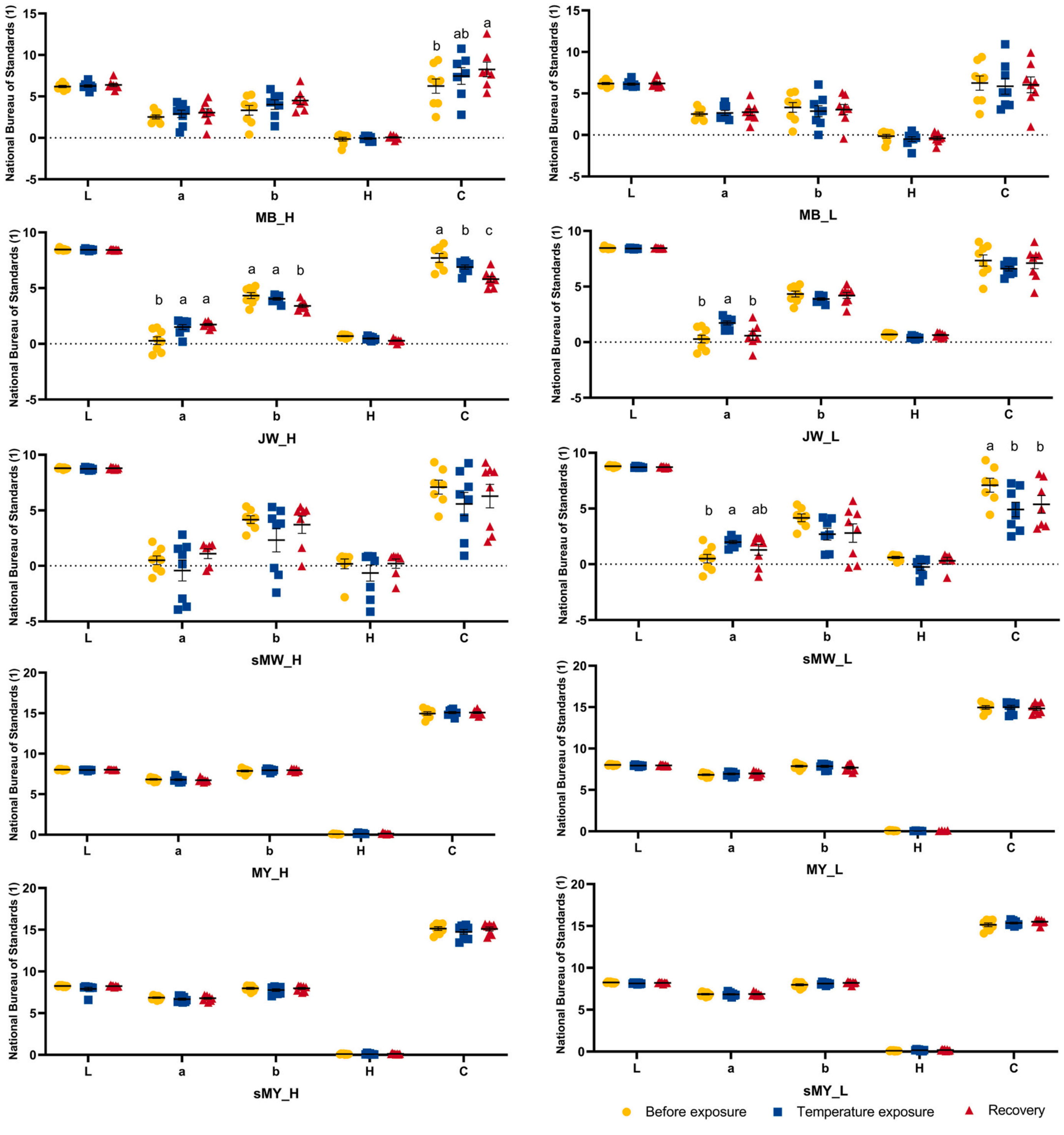

Fig. 2. Effects of temperature on CIELab color space parameters of different koi varieties (n=8). The lowercase alphabetic characters indicates α = 0.05.

3.3. *Correlation analysis of skin color and metabolites*

Correlation analysis of CIELab color space values of koi skin color and plasma CRMs content revealed that the L* value was negatively correlated with the levels of L(-)-epinephrine, guanine ($p < 0.01$) and astaxanthin ($p < 0.05$). Both the a* and b* value of koi skin color were negatively correlated with lutein, zeaxanthin or tunaxanthin levels ($p < 0.01$) (Fig. 6).

4. Discussion

4.1. *Quantitative evaluation of skin color and plasma CRMs of koi*

Establishment of a standardized quantitative analysis method for determination of color quality would facilitate precise evaluation of the ornamental value of koi. There are several methods reported in evaluating skin colors (Nurhadi et al., 2019; Olier et al., 2023). The CIELab color space is an international standard recommended by the CIE. L* represents brightness and has a range of 0–100 (black to white). While

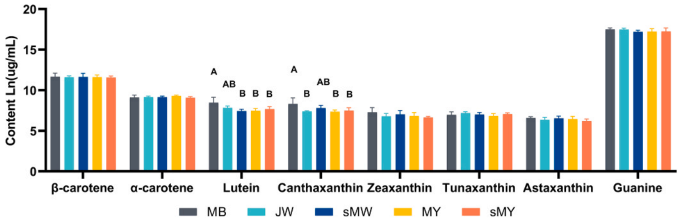

Fig. 3. Plasma carotenoid metabolite levels in different varieties of koi before temperature exposure based on liquid chromatography-tandem mass spectrometry (n=5). The uppercase alphabetic characters indicates α = 0.01.

a* and b* characterize color tone, representing red hue (from green to red) and yellow hue (from blue to yellow), respectively. The color capture based on the CIELab color space is similar to the attributes of the human eye, but the accuracy and degree of quantitation are considerably much higher than those of the human eye. This technique has been widely used in evaluation of the color and quality of fruits (Salvador et al., 2007; Rodriguez-Pulido et al., 2012) and meat (Sarriés and Beriain, 2006), and is increasingly being used for detection of changes in fish skin color (Gouveia et al., 2003; John et al., 2021).

In present study, the CIELab color space evaluation method was applied to measure the skin color of koi with uniform color pattern. The tested five koi varieties sharing genetic similarity were mainly distinguished by different skin color phenotypes. White, black and yellow/red skin colors are the most popular colors in ornamental koi market. The average body length of koi was long enough to make sure sufficient area for detecting CIELab color space. Thus the samples were detected with homogeneity and stability.

This study established and optimized the process and parameters of targeted metabolomics LC-MS/MS detection using MRM mode for targeted detection of 15 CRMs. Seven types of carotenoids were found in the plasma of all the five koi varieties. Beta-carotene and alpha-carotene, which contain no oxygen atoms, were found in the highest

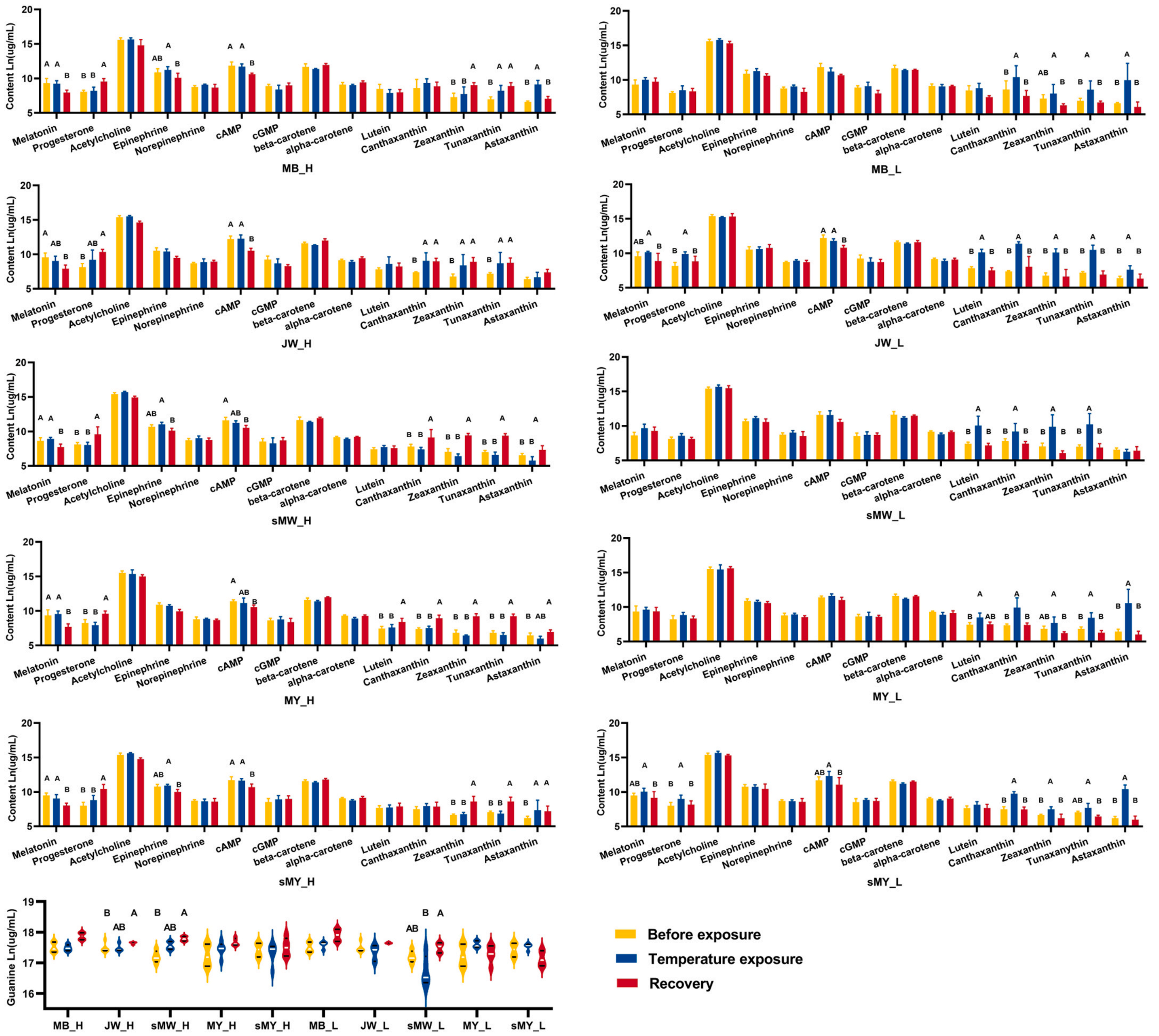

Fig. 4. Effects of temperature fluctuations on plasma plasma CRMs levels based on liquid chromatography-tandem mass spectrometry (n=5). H: High-temperature exposure group; L: Low-temperature exposure group.

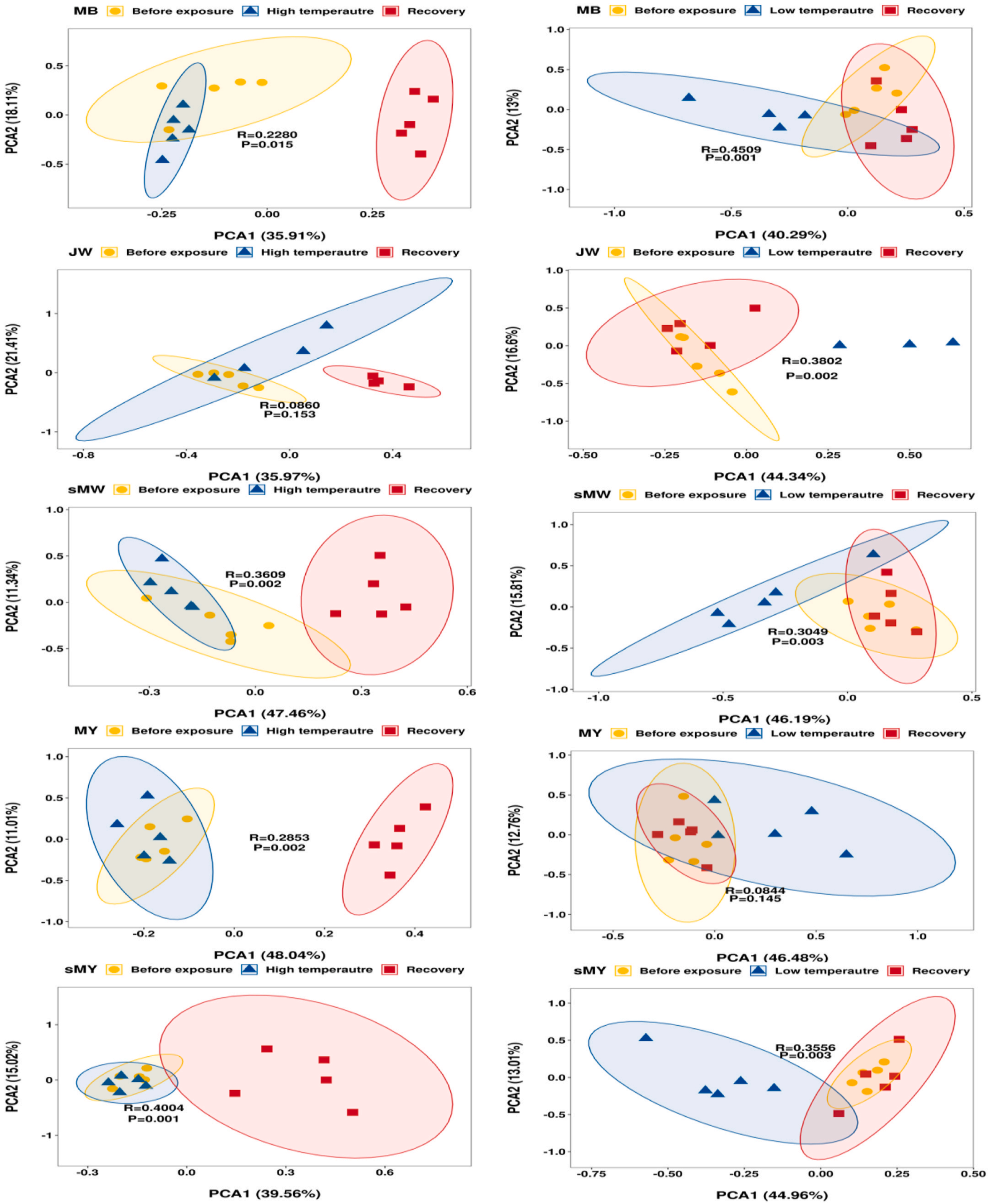

Fig. 5. PCA analysis of plasma plasma CRMs of koi after high- or low-temperature exposure (n=5).

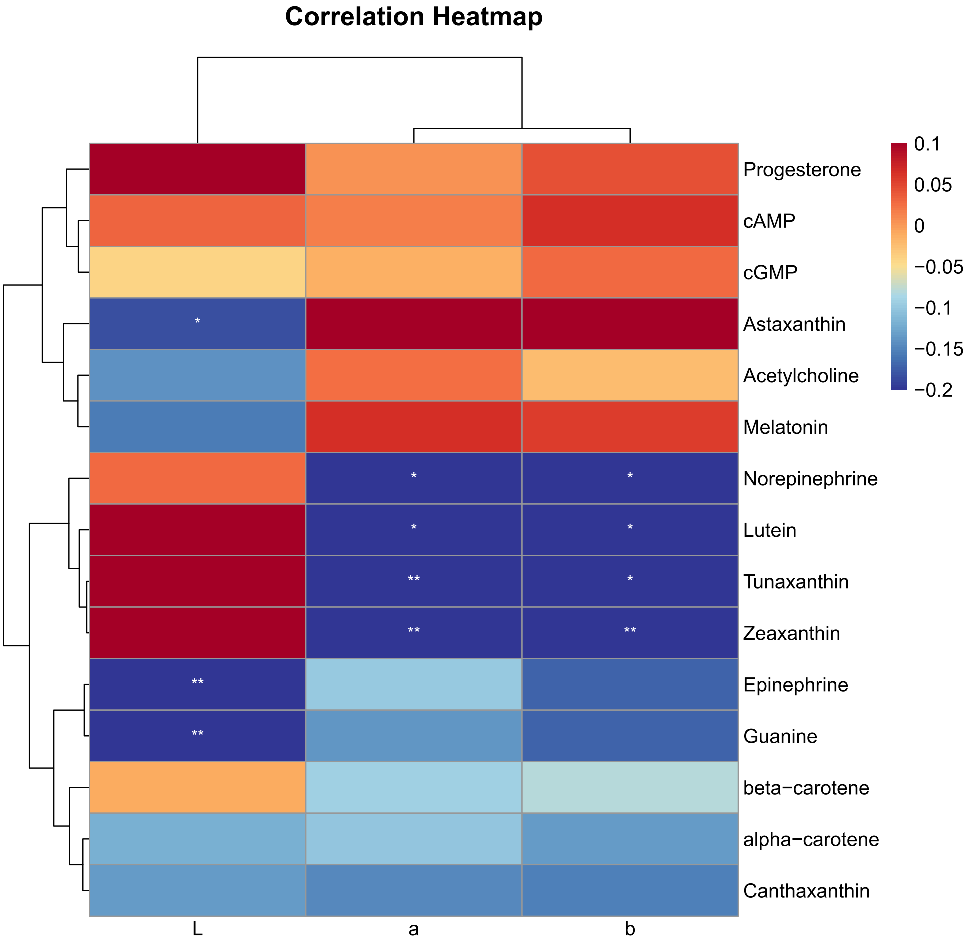

Fig. 6. Correlation heat mapping of koi skin color and plasma CRMs. The L*, a*, b* value and the CRMs levels were calculated for the correlation (n=125). The color indicates spearman correlation coefficient (r) between skin color CIELab color space and plasma contents of CRMs.

levels, accounting for 84%–87% of the total. In a previous study, beta-carotene in the skin of Arctic charr, a fish with black skin covered with orange patches, accounted for 48% of the total skin pigment contents (Metusalach et al., 1996). The levels of beta-carotene and alpha-carotene did not differ significantly among different koi varieties. Conversely, skin color of black, white and yellow koi was found to be associated with lutein and canthaxanthin. Similarly, lutein is also one of the main factors responsible for the difference in color between red and white Oujiang carp (Du et al., 2021). Overall, the results of this study were consistent with those of previous studies.

Astaxanthin is the carotenoid that has been most widely used and effective for improving skin color in koi, goldfish, and other ornamental fish species (Swain et al., 2020). In this study, the plasma level astaxanthin was present at the lowest levels among the seven carotenoids identified in all five investigated koi varieties. As the typical general pathway of carotenoid metabolism and conversion does not exist in fish, astaxanthin *in vivo* can only be converted from zeaxanthin and lutein in the body to via oxidation of the 4 and 4' positions of the ionone ring (Gouveia et al., 2003). Thus the amount of astaxanthin formed from the conversion is limited. Most of the astaxanthin in koi came from the feed additive. The study suggested that different varieties of koi absorbed the astaxanthin alike without distinction.

4.2. *Effects of temperature fluctuations on carotenoids- and guanine-based body color*

Our results revealed that koi skin color was affected by temperature fluctuations and the degree of impact was related to koi skin color, cover of scales, and the mode of temperature fluctuations. The vulnerability of koi skin color to acute temperature stress was in the order of white koi> black koi> yellow koi. The skin color of white koi (JW, sMW) suffered from both high- and low-temperature exposure. In yellow koi (MY and sMY), the CIELab color space parameters did not change significantly during thermal stress and the values within the same treatment groups were highly concentrated, suggesting that the skin color of yellow koi was stable under temperature fluctuations. Additionally, the skin color of mirror white koi (sMW) exhibited larger individual differences, poor stability and much more susceptibility to temperature stress compared to those with scales (JW).

Targeted metabolomics analysis indicated that the effects of low-temperature stress on the CRMs of koi were reversible. The plasma levels of most oxycarotenoids increased when the water temperature dropped to 20 °C and retreated in recovery periods (25 °C). The plasma oxycarotenoids in mirror koi were insensitive to acute heat stress. Nevertheless, the cooling process from a high temperature (30°C-25°C) still made contributions to the increase of oxycarotenoids. The whole stress exposure was conducted in a temperature window (20°C-25°C −30°C) suitable for koi growth.

Correlation analysis revealed that koi skin brightness was negatively correlated with plasma L(-)-epinephrine and guanine levels. Guanine crystals are the main structural component in the iridophores of koi skin. Large quantities of guanine crystals are arranged in specific azimuth angles and arrays with an extremely high reflectance, which are the cytological basis of the formation of shiny skin color (Funt et al., 2017). The regulation of arrangement and spacing of guanine crystals in teleost fish has not been yet fully clarified (Gur et al., 2020). Earlier, the platelet spacing and orientation of guanine crystals in iridophores are reported regulating by epinephrine (Mathger et al., 2003; Goda, 2017). In chromatophores of teleost fish, L(-)-epinephrine acts on alpha-adrenergic receptors and recruit the melanin and red pigment granules to the nuclear region(Oshima et al., 1986; Franco-Belussi et al., 2018). The skin brightness of koi was negatively correlated with plasma guanine content, indicating temperature fluctuations may change their skin brightness through the L(-)-epinephrine-guanine pathway.

For the white JW koi, the red hue/yellow hue of koi skin was negatively correlated with levels of oxycarotenoids, including lutein, zeaxanthin, and tunaxanthin. During the cooling process, the levels of oxycarotenoids in the plasma decreased, while those of oxycarotenoids increased, and the red and yellow hues of the skin increased. Dietary additive experiments have shown that providing natural astaxanthin (Boonyapakdee et al., 2015)and lutein (Yuangsoi et al., 2011) enhances the red hue of koi skin. We thus speculated one step further that the oxycarotenoids contributed to the color saturation in koi skin (both red hue and yellow hue).

The change of oxycarotenoids in koi might be related to the temperature-induced metabolism and conversion of oxycarotenoids. In bacteria (Seel et al., 2020), fungi (Guo et al., 2022), plants (Wang et al., 2022), and post-harvest foods (Hammaz et al., 2021), the degradation and metabolic pathways of oxycarotenoids are commonly found to be affected by temperature. The concentrations of lutein and zeaxanthin in *Ictalurus punctatus* declined faster under high temperature than low temperature (Li et al., 2011). In present study, the heat process made no difference on all carotenoids. The cooling process might result in the decreasing of metabolism rates and conversion of plasma oxycarotenoids in koi, thereby causing an increase of their levels. However, the effects of acute temperature fluctuations on the metabolism, degradation, and clearance pathways of oxycarotenoids in the body of koi need to be further explored.

5. Conclusions

This study evaluated the effects of temperature fluctuations on the skin color of koi and the levels of plasma carotenoids and related metabolites. Temperature fluctuations might change the brightness through the L(-)-epinephrine-guanine pathway in koi. Additionally, acute low temperature might enhance the color saturation of white koi skin via the utilization of oxycarotenoids. Overall, this study revealed the effects of temperature fluctuations on koi with white, black and yellow skin color in terms of temperature-hormones -pigments-color.

CRediT authorship contribution statement

Hua Zhu: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Huijuan Li:** Investigation, Validation. **Rong Zhang:** Conceptualization, Investigation, Validation. **Xiaowen Wang:** Investigation, Validation. **Lili Liu:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116165.

References

Backstrom, T., Brannas, E., Nilsson, J., Carlberg, H., Johansson, K., Magnhagen, C., 2021. Proof of concept: visual categorization of carotenoid pigmentation in Arctic charr (*Salvelinus alpinus* L) can predict stress response. J. Fish Biol. 98, 1192–1195.

Boonyapakdee, A., Pootangon, Y., Laudadio, V., Tufarelli, V., 2015. Astaxanthin extraction from golden apple snail (*Pomacea canaliculata*) eggs to enhance colours in fancy carp (*Cyprinus carpio*). J. Appl. Anim. Res. 43, 291–294.

Cahn, M.D., Brown, A.C., Clotfelter, E.D., 2015. Guanine-based structural coloration as an indicator of oxidative stress in a cichlid fish. J. Exp. Zool. Part A 323, 9.

David, L., Rothbard, S., Rubinstein, I., Katzman, H., Hulata, G., Hillel, J., Lavi, U., 2004. Aspects of red and black color inheritance in the Japanese ornamental (Koi) carp (*Cyprinus carpio* L.). Aquaculture 233, 19.

Dick, C., Arendt, J., Reznick, D.N., Hayashi, C.Y., 2018. The developmental and genetic trajectory of coloration in the guppy (*Poecilia reticulata*). Evol. Dev. 20, 207–218.

Du, J., Chen, H., Mandal, B.K., Wang, J., Shi, Z., Lu, G., Wang, C., 2021. HDL receptor / Scavenger receptor B1-Scarb1 and Scarb1-like mediate the carotenoid-based red coloration in fish. Aquaculture 545, 737208.

Franco-Belussi, L., De Oliveira, C., Skold, H.N., 2018. Regulation of eye and jaw colouration in three-spined stickleback *Gasterosteus aculeatus*. J. Fish Biol. 92, 1788–1804.

Funt, N., Palmer, B.A., Weiner, S., Addadi, L., 2017. Koi fish-scale iridophore cells orient guanine crystals to maximize light reflection. ChemPlusChem 82, 914–923.

Goda, M., 2017. Rapid integumental color changes due to novel iridophores in the chameleon sand tilefish *Hoplolatilus chlupatyi*. Pigment Cell Melanoma Res. 30, 368–371.

Gouveia, L., Rema, P., Pereira, O., Empis, J., 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquac. Nutr. 9, 123–129.

Guo, R., Liu, T., Guo, C., Chen, G., Fan, J., Zhang, Q., 2022. Carotenoid biosynthesis is associated with low-temperature adaptation in *Rhodosporidium kratochvilovae*. BMC Microbiol. 22, 319.

Gur, D., Bain, E.J., Johnson, K.R., Aman, A.J., Pasolli, H.A., Flynn, J.D., Allen, M.C., Deheyn, D.D., Lee, J.C., Lippincott-Schwartz, J., Parichy, D.M., 2020. *In situ* differentiation of iridophore crystallotypes underlies zebrafish stripe patterning. Nat. Commun. 11, 6391.

Hammaz, F., Charles, F., Kopec, R.E., Halimi, C., Fgaier, S., Aarrouf, J., Urban, L., Borel, P., 2021. Temperature and storage time increase provitamin A carotenoid concentrations and bioaccessibility in post-harvest carrots. Food Chem. 338, 128004.

John, L., Rick, I.P., Vitt, S., Thuenken, T., 2021. Body coloration as a dynamic signal during intrasexual communication in a cichlid fish. BMC Zool. 6, 13.

Junqueira, L.C., Lima, M.H., Farias, E.C., 1978. Carotenoid and pterin pigment localization in fish chromatophores. Stain Technol. 53, 91–94.

Li, M.H., Robinson, E.H., Oberle, D.F., Lucas, P.M., Peterson, B.C., Bates, T.D., 2011. Clearance of yellow pigments lutein and zeathanxin in channel catfish, *Ictalurus punctatus*, reared at different water temperatures. J. World Aquac. Soc. 42, 105–110.

Mathger, L.M., Land, M.F., Siebeck, U.E., Marshall, N.J., 2003. Rapid colour changes in multilayer reflecting stripes in the paradise whiptail, *Pentapodus paradiseus*. J. Exp. Biol. 206, 3607–3613.

Metusalach, Synowiecki, J., Brown, J., Shahidi, F., 1996. Deposition and metabolism of dietary canthaxanthin in different organs of Arctic charr (*Salvelinus alpinus* L.). Aquaculture 142, 99–106.

Nurhadi, T., Lili, W., Pratama, R.I., Haetami, K., 2019. Effects of astaxanthin and canthaxanthin addition to Ranchu goldfish (*Carassius auratus*) diet related to rate of color quality enhancement. World News of Nat. Sci. 178–183.

Olier, B.S., De Sousa, O.M., Sanches, E.G., 2023. Can canthaxanthin intensify the colour of the blood swordtail *Xiphophorus helleri*? J. Fish Biol. 103, 13–21.

Oshima, N., Kasukawa, H., Fujii, R., Wilkes, B.C., Hruby, V.J., Hadley, M.E., 1986. Action of melanin-concentrating hormone (MCH) on teleost chromatophores. Gen. Comp. Endocrinol. 64, 381–388.

Rodriguez-Pulido, F.J., Ferrer-Gallego, R., Gonzalez-Miret, M.L., Rivas-Gonzalo, J.C., Escribano-Bailon, M.T., Heredia, F.J., 2012. Preliminary study to determine the phenolic maturity stage of grape seeds by computer vision. Anal. Chim. Acta 732, 78–82.

Salvador, A., Sanz, T., Fiszman, S.M., 2007. Changes in colour and texture and their relationship with eating quality during storage of two different dessert bananas. Postharvest Biol. Technol. 43, 319–325.

Sarriés, M.V., Beriain, M.J., 2006. Colour and texture characteristics in meat of male and female foals. Meat Sci. 74, 738–745.

Seel, W., Baust, D., Sons, D., Albers, M., Etzbach, L., Fuss, J., Lipski, A., 2020. Carotenoids are used as regulators for membrane fluidity by *Staphylococcus xylosus*. Sci. Rep. 10, 330.

Swain, S., Kim, H., Acharya, A.P., Mohanty, B., 2020. Use of carotenoid supplementation for enhancement of pigmentation in ornamental fishes. J. Entomol. Zool. Stud. 6, 636–640.

Tian, X., Pang, X., Wang, L., Li, M., Dong, C., Ma, X., Wang, L., Song, D., Feng, J., Xu, P., Li, X., 2018. Dynamic regulation of mRNA and miRNA associated with the developmental stages of skin pigmentation in Japanese ornamental carp. Gene 666, 32–43.

Van der Salm, A.L., Spanings, F.A., Gresnigt, R., Bonga, S.E., Flik, G., 2005. Background adaptation and water acidification affect pigmentation and stress physiology of tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol. 144, 51–59.

Vissio, P.G., Darias, M.J., Di Yorio, M.P., Sirkin, D.P., Delgadin, T.H., 2021. Fish skin pigmentation in aquaculture: the influence of rearing conditions and its neuroendocrine regulation. Gen. Comp. Endocrinol. 301, 12.

Wang, Y., Zhang, C., Xu, B., Fu, J., Du, Y., Fang, Q., Dong, B., Zhao, H., 2022. Temperature regulation of carotenoid accumulation in the petals of sweet osmanthus via modulating expression of carotenoid biosynthesis and degradation genes. BMC Genom. 23, 418.

Yuangsoi, B., Jintasataporn, O., Areechon, N., Tabthipwon, P., 2011. The pigmenting effect of different carotenoids on fancy carp (*Cyprinus carpio*). Aquac. Nutr. 17, 306–316.

Zarnescu, O., 2007. Ultrastructure of the skin melanophores and iridophores in paddlefish, *Polyodon spathula*. Micron 38, 81–84.