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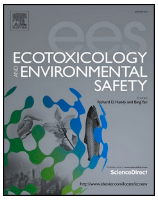

Targeted metabolomics revealed the seasonal plasticity of skin color and pigment metabolites in ornamental koi carp

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ABSTRACT

The koi carp is an ornamental fish that was obtained through artificial selection from the common carp (*Cyprinus carpio* L.). The most economically important traits of koi are their beautiful skin patterns in bright colors. As seasonality is an important factor in the biology and ecology of fish, we thus assumed that seasonal changes are involved in regulating the formation of skin color and patterns of koi carp. The white, red, cyan, and black skin colors from four varieties of scaleless koi carp (Doitsu Shiromuji (W), Doitsu Kohaku (W*R*), Doitsu Showa Sanke (WRI), and Kumonryu (WI)) were evaluated using the CIELab color space (lightness, redness, and yellowness) in different seasons. Compared to winter, the yellowness of the white color in all koi varieties decreased in summer and autumn. The black skin color areas in WRI and WI koi increased in summer and autumn compared to winter. The yellowness of the red color decreased only in WRI koi, while no changes were observed in W*R* koi. Targeted metabolomics analysis revealed that the levels of the structural pigment guanine of all koi varieties showed significant seasonal variation. Of seven detected carotenoids, the zeaxanthin and tunaxanthin contents in W, WI, and WRI koi changed with the seasons, while none of the carotenoids in W*R* koi were altered. Of the seven potential regulatory metabolites, epinephrine, melatonin, and cyclic adenosine monophosphate (cAMP) in all four koi varieties showed the highest levels in winter. A correlation analysis suggested that the seasonal changes in white skin color occurred through the epinephrine-cAMP pathway; melanin-dependent and carotenoid-dependent skin color changes occurred through melatonin in koi carp. This study demonstrated the seasonal plasticity of skin color in koi carp regulated by melatonin and epinephrine, associating with variety and color specificity.

1. Introduction

Seasonality is one of the most important factors in the biology and ecology of fish. Seasonal changes in food supply and abiotic factors, such as temperature and daylight length, together affect the development and maturity levels of fish. The seasonal variation of daylight length is the principal determining factor affecting the maturity of the major intensively farmed fish species. The maturation and spawning of cyprinid fishes are triggered by temperature and rainfall as well as the length of the photoperiod (Bromage et al., 2001). The ability to alter physiological states according to environmental rhythms gives fish significant advantages in adaptability. Numerous studies have focused on the effects of seasonality on fish physiology. The seasonal variation of photoperiod, temperature, water quality, dissolved oxygen, salinity, and food consumption rate may alter hematologic parameters (Courtois, 1976) as well as the immune responses of fish species (Zapata et al., 1992).

The ornamental koi carp was artificially selected from the common carp (*Cyprinus carpio* L.) approximately 200 years ago (Ikuta and Yamaguchi, 2005). Due to its bright skin colors and beautiful patterns, koi have become a popular ornamental aquarium fish worldwide. At present, the commercial koi trade has spread to North America, Europe, the Asia-Pacific region, South America, the Middle East, and Africa. Koi aquaculture has gained momentum, booming in South China, North China, and Northeast China. It is estimated that the global market value for koi in 2023 was 2.3 billion USD. The global market is expected to grow at an average rate of 12.2 % during 2023–2030 (Sneha Mali, Koi Market Report 2023 (Global Edition). Congnitive Market Research.2023–11–14.).

Their bright skin color and beautiful color patterns are the most economically important traits of koi. However, the mechanisms

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underlying the formation and regulation of these bright skin colors have not yet been elucidated. The changes in skin color in teleost fishes are caused by the diffusion or aggregation of pigments in chromatophores controlled by the endocrine and sympathetic nervous systems. The process is regulated by a variety of environmental factors. Previously, we investigated the effects of temperature fluctuation on the skin color of monochromatic koi and reported that the lightness of the skin color could be affected by temperature fluctuations through the epinephrine-guanine pathway (Liu et al., 2024). Environmental stressors and light wavelength may also change the skin color of fish by acting on pigmentation (Backstrom et al., 2021) or pigment arrangement (Cahn et al., 2015). In addition, fish skin color is affected by seasonal variation of daylight, dissolved oxygen, salinity, and other aquaculture conditions (Vissio et al., 2021). Due to the seasonality of fish hormone levels (Lucon-Xiccato et al., 2022), we hypothesized that seasonal changes may regulate the movement of pigments via hormones, thus changing the skin colors of koi carp.

2. Materials and methods

2.1. Experimental design and fish collection

Six-month-old ornamental scaleless koi (Doitsu koi) carp were provided by the Beijing Yayue Koi Farm. In October 2019, the koi carp were transferred from the outdoor fishpond to a cement pond in a greenhouse for 1 month of acclimation. The koi were then transferred to independent transparent glass tanks and injected with animal ID microchips (RBC-Z00, Raybaca) in the dorsal muscle for follow-up experiments. Commercial feed pellets (1.5 % of the weight of the koi) were given twice each day.

The experimental fish comprised 8 Doitsu Shiromuji koi (W, with an entirely white body); 8 Doitsu Kohaku koi (W*R*, with a white body adorned with vibrant red markings); 16 Doitsu Showa Sanke (WRI) koi, including 4 normal fish (WRIn, with a black base color overlaid with vibrant red and white markings); 12 cyan koi (WRIc, with a black and cyan base color overlaid with vibrant red and white markings); 15 Kumonryu koi, including 8 normal fish (WIn, with a deep black pattern on top of a white skin); and 7 cyan fish (WIc, with a black and cyan pattern on top of a white skin). The average body length of all experimental fish was 22.7±2.5 cm. The average body weight was 180±20 g.

Chip scanning and color measurements were performed for all fish, and photographs were taken. Blood samples were collected from all koi in the middle of winter (January), spring (April), summer (July), and autumn (October) of 2020. During the experiment, the water temperature, the hydrogen potential, and the concentration of dissolved oxygen were recorded as winter (3.3±0.7, 7.0±0.2, 14.3±0.9), spring (20.4 ±0.8, 7.2±0.3, 9.6±0.3), summer (28.0±0.4, 7.6±0.2, 8.1±0.5), autumn (16.7±0.3, 7.5±0.4, 10.1±1.1). The values were presented as means±the standard error of the mean (SEM).

2.2. Color measurement

The koi were anesthetized with tricaine methanesulfonate (MS-222, Sigma-Aldrich). Scanning and recording from the microchips in the fish were performed with a Wireless RFID Reader (RBC-S03, Raybaca). A clean towel was saturated, wrung out, and used to gently absorb moisture from the fish skin. The head of the koi was positioned left horizontally to measure the length and weight of the body. Each fish was evaluated for the white, red, black, and cyan skin colors using an Illuminance Spectrophotometer (CL-500A, Konica Minolta) for the CIELab color space parameters of lightness (L), redness (a*), and yellowness (b*). Before measurement, the spectrophotometer was calibrated using a standard white color card.

2.3. LC-MS/MS

Blood (500 μL from each fish) was sampled by caudal venous puncture and placed in ethylene diamine tetraacetic acid blood collection tubes (68784, BD Medicine), frozen with liquid nitrogen, and stored at −80°C. Liquid chromatography-tandem mass spectrometry with multiple reaction monitoring (LC-MS/MS-MRM) was adopted to quantify the pigments and regulatory metabolites (PRMs). The LC-MS/MS-MRM methods were the same as that described previously (Liu et al., 2024). Briefly, the metabolites were extracted by acetonitrile/isopropanol (1:1, v/v) and separated by Shimadzu Nexera X2 LC-30AD High-Performance Liquid Chromatography (HPLC) system. Mobile phase A: 0.1 % formic acid-water solution; Mobile phase B: isopropanol/acetonitrile (1:1). The mass spectrometry analysis was conducted by QTRAP5500 mass spectrometer (AB SCIEX) under with multiple reactions monitoring mode. The PRMs included β-carotene (CAS: 7235–40–7), α-carotene (CAS: 7488–99–5), lutein (CAS: 127–40–2), astaxanthin (CAS: 472–61–7), canthaxanthin (CAS: 514–78–3), zeaxanthin (CAS: 144–68–3), tunaxanthin (CAS: 12738–95–3), guanine (CAS: 73–40–5), acetylcholine (CAS: 51–84–3), norepinephrine (CAS: 51–41–2), L(-)-Epinephrine (CAS: 51–43–4), progesterone (CAS: 57–83–0), cyclic adenosine monophosphate (CAS: 86594–35–6), and cyclic 3′,5′-guanosine monophosphate (CAS: 7665–99–8). The Shimadzu Nexera X2 LC-30AD High-Performance Liquid Chromatography (HPLC) system was used for the separation of targeted metabolites. A QTRAP5500 mass spectrometer (AB SCIEX) was used for mass spectrometry analysis under positive/negative ion mode. The MultiQuant software was used to extract the chromatography peak areas and retention times.

2.4. Statistical analysis

All data were assessed for normal distributions using Kolmogorov–Smirnov tests, and homogeneity of variance was assessed using Levene's test.

Changes in the CIELab color space parameters (L, a*, and b*) and targeted metabolites data obtained from different skin colors of different varieties in the four seasons were analyzed using two-way analysis of variance (ANOVA). Means were compared using Duncan's multiple range test. The final data were presented as mean ± SEM. The principle component analysis (PCA), partial least squares discriminant analysis (PLS-DA), correlation network, and clustering correlation heatmap with signs between CIELab color space parameters of different skin colors and targeted metabolites in the plasma of koi were performed using the OmicStudio tools (https://www.omicstudio.cn). The uppercase alphabetic characters indicated $\alpha = 0.01$. The lowercase alphabetic characters indicated $\alpha = 0.05$. ** indicated p value <0.01. * indicated p value≥ 0.01 & p value<0.05.

3. Results

3.1. Seasonal changes in skin colors of koi

To quantitatively evaluate the seasonal changes in skin color of koi, the skins of all varieties (W, W*R*, WI, WRI) were measured for the CIELab color space parameters (L, a*, and b*) in winter (Win), spring (Spr), summer (Sum), and autumn (Aut).

The white skin in all varieties of koi showed significant seasonal variation. The skin was whiter in Sum/Aut than in Win/Spr (Fig. 1 A-F). The CIELab color space data further showed that the b* value of white skin in all varieties of koi was the highest in Win, up to 13.6±2.5 (WIc group); it gradually decreased in Spr/Sum and became the lowest in Aut, only 0.6±3.8 (W*R* group) (Fig. 1 G–L). The L value of the white color in W koi was 84.6±2.5 in Win and decreased to 77.6±2.5 in Spr. (Fig. 1C, I). The results suggested that the white skin of the koi was whiter in Sum/Aut than in Win/Spr.

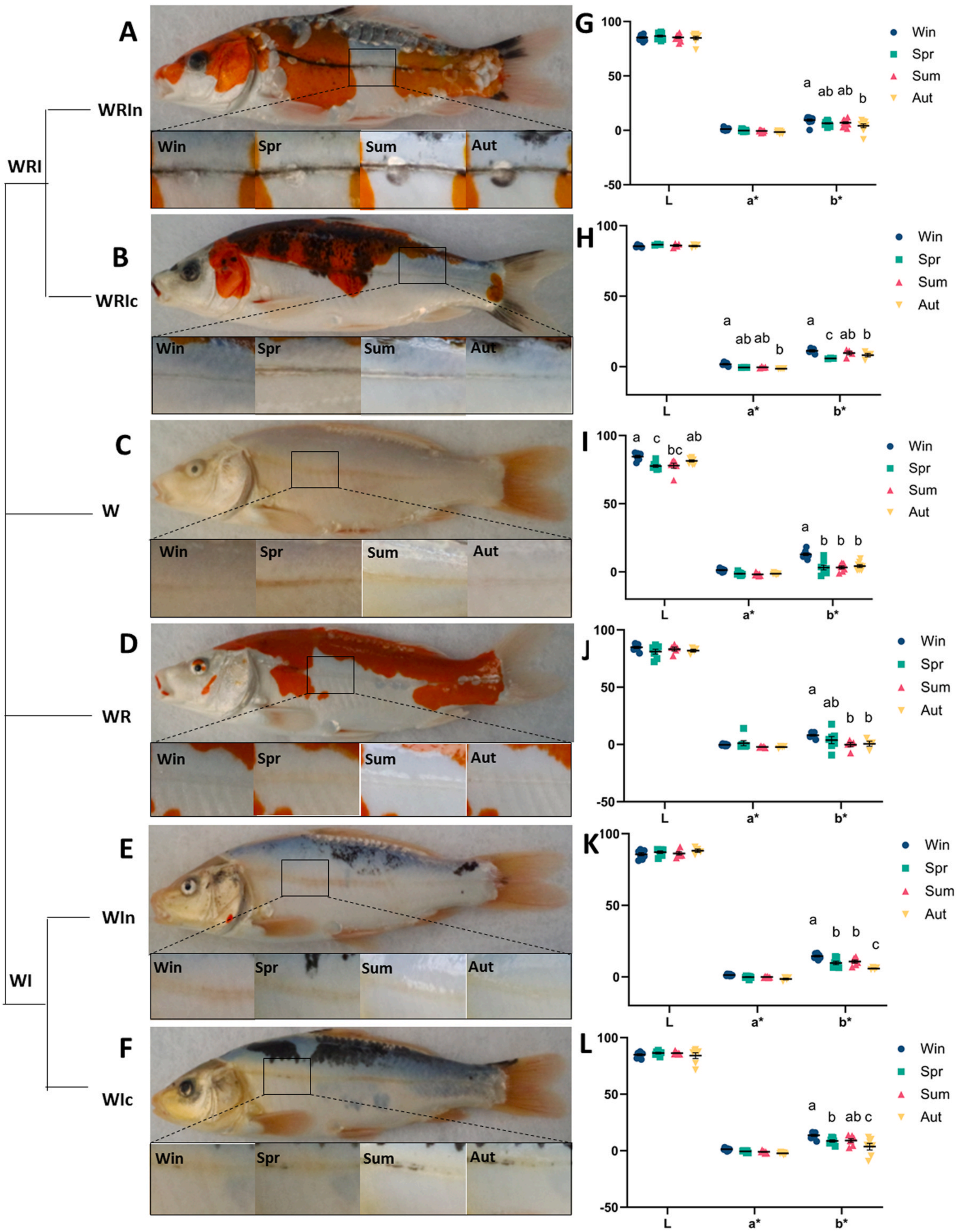

Fig. 1. Seasonal changes of white skin in koi varieties. W: Doitsu Shiromuji koi; *WR*: Doitsu Kohaku koi; WRIn: normal Doitsu Showa Sanke koi; WRIc: cyan Doitsu Showa Sanke koi; WIn: normal Kumonryu koi; WIc: cyan Kumonryu koi. Win: Winter; Spr: Spring; Sum: Summer; Aut: Autumn. The lowercase alphabetic characters indicated α = 0.05.

The red skin areas of WRI and *WR* koi with irregular patches did not change significantly with the seasons (Fig. 2A, B). The red skin color of WRI koi showed seasonal changes. Compared to Win/Spr, the b* value of red skin in WRI koi decreased 18.5 % in Sum/Aut; meanwhile, the L and a* values did not change with the seasons (Fig. 2C). Each of the CIELab parameters of the red skin in WRI koi was relatively discrete in the four seasons of the year (Fig. 2C), suggesting strong individual differences in the red skin of WRI koi. Nonetheless, the CIELab parameters [51.6±1.0,42.8±0.7,57.3±1.6] of the red skin in *WR* koi showed no changes with the seasons. Moreover, the CIELab parameters were stable at the same time point, indicating homogeneity of the red skin of *WR* koi (Fig. 2D).

The skin of the WRI and WI koi exhibited various black patches accompanied at times by cyan skin, which was generally observed in Win/Spr (Fig. 2 E-G). In Sum/Aut, most cyan skin turned black (Fig. 2E, F) with 60 % decrease of L value (Fig. 2 H,I). The cyan skin in some koi

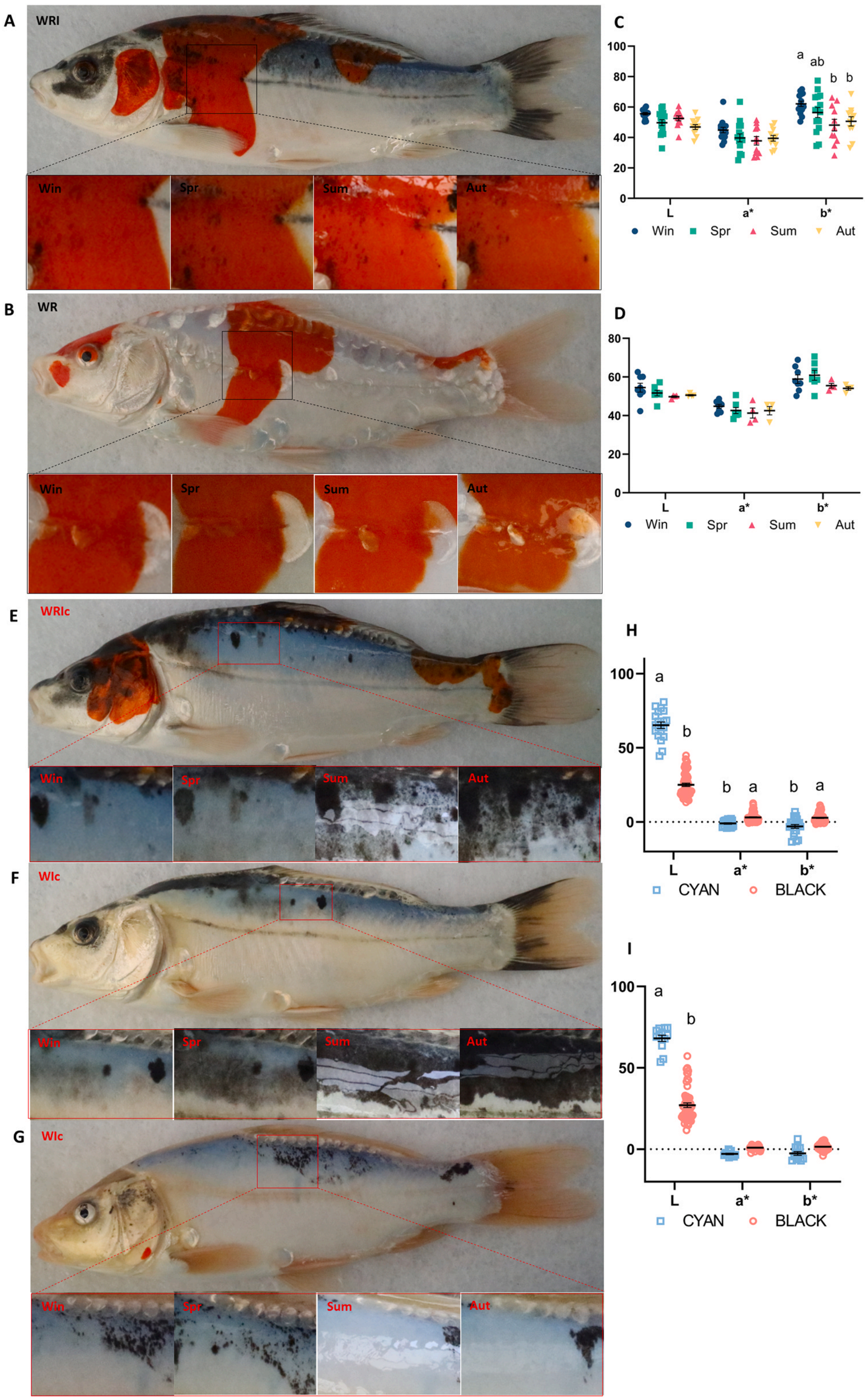

Fig. 2. Seasonal changes of red and black skin colors in koi varieties. WRI: Doitsu Showa Sanke koi; WR: Doitsu Kohaku koi; WRIc: cyan Doitsu Showa Sanke koi; WIc: cyan Kumonryu koi. Win: Winter; Spr: Spring; Sum: Summer; Aut: Autumn. The lowercase alphabetic characters indicated α = 0.05.

turned white in Sum/Aut, accompanied by a decreasing area of black skin (Fig. 2G). These results indicated that the black skin varied with the seasons.

3.2. Seasonal changes in plasma PRMs in koi

Targeted metabolomics technology was used to quantify the plasma PRMs, including carotenoids such as β-carotene, α-carotene, lutein, canthaxanthin, zeaxanthin, tunaxanthin, and astaxanthin. The levels of related regulatory hormones such as progesterone, acetylcholine, norepinephrine, and epinephrine, as well as the small signal molecules cAMP and cGMP, were measured.

We applied PCA to the plasma PRM datasets of W, *WR*, Win, WIc, WRIn, and WRIc koi in winter. The first principal component (PC1) captured 98.6 % of the total variance. W, WRIn, and WRIc were clustered together and were generally separated from *WR* by PC1. Large individual variation was found in WIn and WIc koi (Fig. 3A). Subgroups in the same variety (WRIc and WRIn, WIc and WIn) did not differ significantly in Win (Fig. 3A). The difference in PRM levels between WRIn and WRIc was observed only in Sum (Fig. 3B), while a difference between WIn and WIc was observed in Aut (Fig. 3C).

All varieties of koi carp contained seven carotenoids and guanine. Of the seven carotenoids, β-carotene accounted for the highest proportion (Fig. 3D). The main carotenoids that differed among the four varieties of koi were lutein, zeaxanthin, tunaxanthin, and astaxanthin. The levels of lutein, zeaxanthin, and tunaxanthin in W koi were significantly higher than those of the others ($p < 0.05$). Meanwhile, there was no significant difference in the levels of β-carotene or α-carotene among all four koi varieties (Fig. 3D). The levels of progesterone, norepinephrine, and melatonin differed among koi varieties (Fig. 3E).

PLS-DA of plasma PRMs in four seasons showed that the PC1 and PC2 together separated Win/Spr from Sum/Aut. Win and Spr were clustered for the W variety, while Sum and Aut were clustered for the WRI koi variety. Win, Spr, and Sum were separated from Win; meanwhile, Aut and Sum overlapped (Fig. 4).

The levels of epinephrine in all four varieties of koi reached their highest values in Win, decreased significantly in Spr, and remained low in Sum and Aut. The levels of melatonin in WI and WRI koi had the same trend as epinephrine. cAMP reached its highest level in Spr. The levels of guanine remained low in Win and Spr, increased significantly ($p < 0.05$) in Sum, and remained high in Aut (Fig. 4).

In WI koi, the levels of lutein, canthaxanthin, zeaxanthin, and tunaxanthin were the highest in Sum. In W koi, the levels of lutein, zeaxanthin, and tunaxanthin were the highest in Win. In WRI koi, the

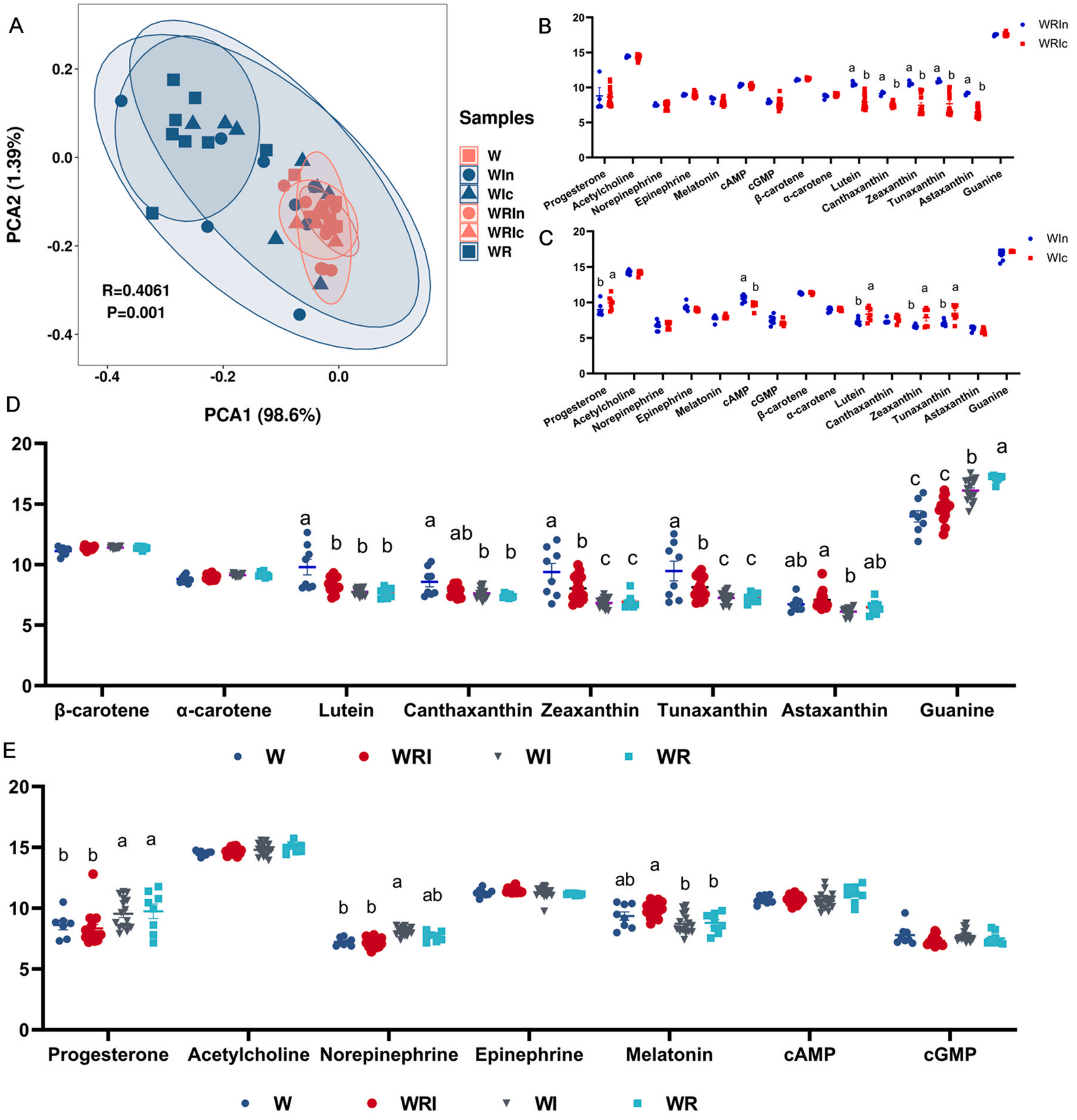

Fig. 3. Quantification of pigments and related metabolites (PRMs) of koi varieties. Principal component analysis of PRMs in different koi varieties. (B) PRM levels between WRIn and WRIc in summer. (C) PRM levels between WIn and WIc in autumn. (D) Pigments levels of W, WRI, WI, and *WR* koi carp in winter. (E) Related metabolites of W, WRI, WI, and *WR* koi carp in winter. W: Doitsu Shiromuji koi; *WR*: Doitsu Kohaku koi; WRIn: normal Doitsu Showa Sanke koi; WRIc: cyan Doitsu Showa Sanke koi; WIn: normal Kumonryu koi; WIc: cyan Kumonryu koi. The lowercase alphabetic characters indicated α = 0.05.

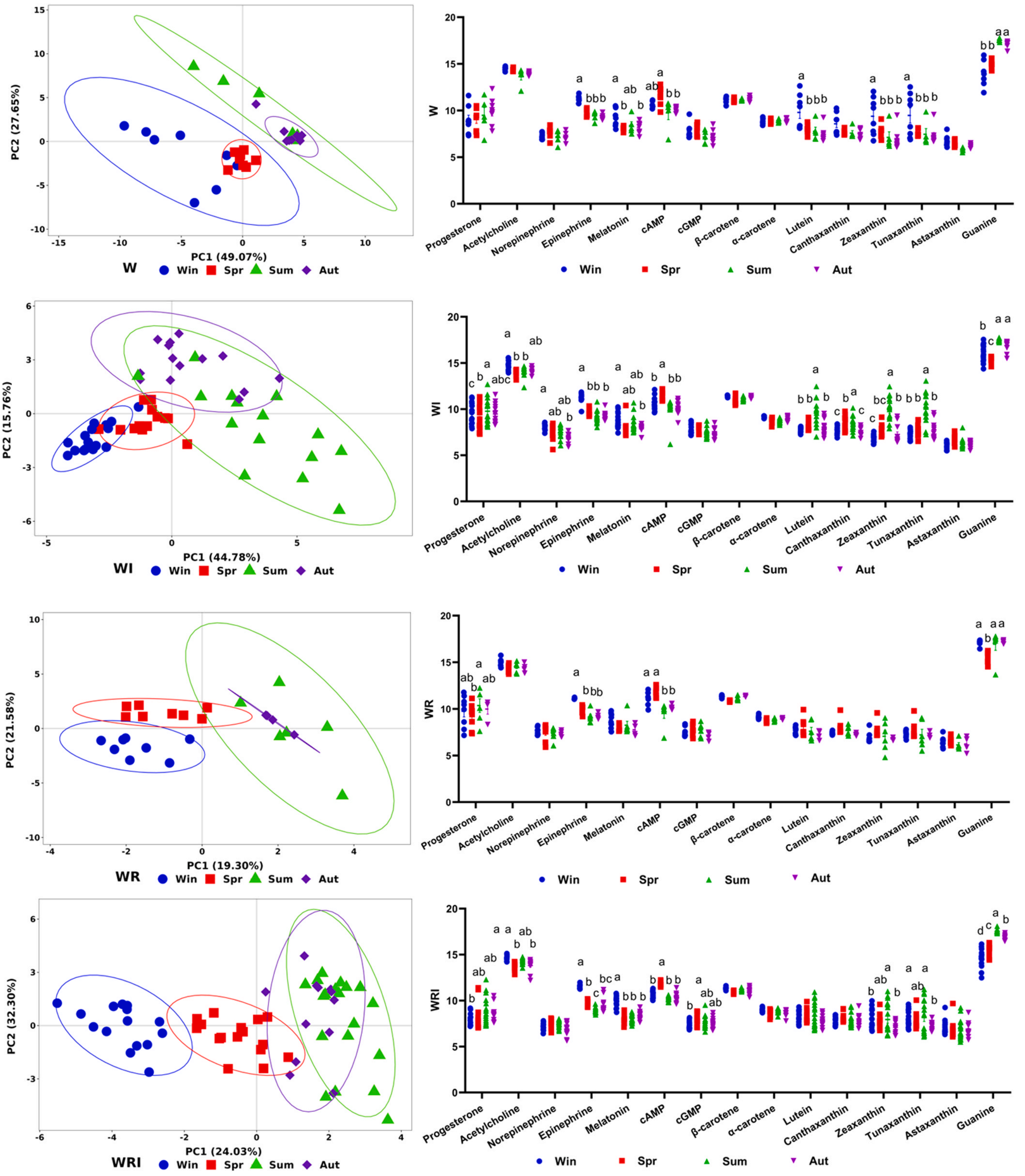

Fig. 4. Seasonal changes of pigments and related metabolites of W, WI, W*R*, and WRI koi carp. W: Doitsu Shiromuji koi; WI: Kumonryu koi; W*R*: Doitsu Kohaku koi; WRI: Doitsu Showa Sanke koi. Win: Winter; Spr: Spring; Sum: Summer; Aut: Autumn. The lowercase alphabetic characters indicated $\alpha = 0.05$.

levels of zeaxanthin and tunaxanthin decreased significantly in Aut ($p < 0.05$). There were no changes in the levels of carotenoids in W*R* koi, a pattern that was consistent with the color quantification results for red skin in W*R* koi (Fig. 4).

3.3. *Correlation analysis between skin color and PRMs*

The correlation network showed that the red and white skin colors of koi carp were correlated with PRMs (Suppl. Table 1). The L value of red skin was positively correlated with acetylcholine, adrenaline, and lutein, and the b* value of the white body color was positively correlated with cAMP, guanine, and melatonin. The correlation between black or cyan skin color and PRMs was relatively weak (Fig. 5A).

A heat map was used to further analyze the correlations between the CIELab color space of red/white skin colors and the contents of PRMs. The contents of guanine and melatonin were significantly positively correlated with red skin color, while cGMP, anthaxanthin, zeaxanthin, and α-carotenoid were negatively correlated ($p < 0.05$). The contents of β-carotenoid, guanine, cAMP, acetylcholine, and epinephrine were significantly positively correlated with the saturation (a* and b*) of white skin color, while the contents of astaxanthin, zeaxanthin, caramanthin, and cGMP were negatively correlated ($p < 0.05$, Fig. 5B).

4. Discussion

4.1. Seasonal plasticity of koi skin color

The ornamental aquarium fish market has grown significantly in the past decade. To improve their value and stabilize desired traits, it is necessary to understand how phenotypic traits are affected by the aquaculture environment, especially the seasonal changes in the color patterns of ornamental koi carp. In this study, the CIELab color space of skin and plasma PRMs of koi carp in different seasons were analyzed to elucidate the effects of seasonal changes on the skin colors of koi carp.

The skin colors of different varieties of koi carp were tracked through all four seasons, confirming the seasonal rhythms. For all varieties of koi in summer, the white skin became more visibly porcelain-white, with decreased yellowness. Melanin-based skin color appeared black or cyan in winter and spring; in summer and autumn, the black patches increased in area and decreased in lightness. Carotenoid-based skin color varied among seasons and varieties of koi. The red skin of W*R* koi remained stable in all seasons. The red skin of the WRI koi appeared orange-red visually with decreasing yellowness in summer/autumn. Excessive white light exposure led to the loss of yellow pigmentation in zebrafish embryos (Ustundag et al., 2019), suggesting that the prolongation of daylight length inhibits the development of xanthophores in fish skin. Considering the high similarity of pigment composition and regulatory pathways in erythrophores and xanthophores (Ligon and McCartney, 2016), the long light duration in summer may be the reason for the decrease in yellowness of WRI koi red skin. The seasonal changes in red skin occurred exclusively in WRI koi, indicating that the effects of seasonality on carotenoid-dependent skin color of koi were variety-specific.

Seasonality is a complex event that includes changes in temperature and day length (Bowden et al., 2007). The Beijing area has a typical northern temperate monsoon climate. Therefore, the seasonality observed in the present study included changes in atmospheric pressure, dissolved oxygen, and light intensity. The koi is a completely captive-bred ornamental fish, and thus its food supply is unaffected by seasonal changes. Therefore, the seasonality affecting the skin color of koi in this study included changes in water temperature, daylight length, atmospheric pressure, dissolved oxygen, and light intensity, but not food supply.

4.2. Seasonal regulation of skin color through epinephrine in koi

The melanophores, erythrophores, xanthophores, and iridophores individually or together form the various skin colors of koi carp. The formation and regulation of skin color in teleost fishes are primarily controlled by the endocrine and sympathetic nervous systems. To clarify the mechanism of seasonality in the formation of koi skin color, we conducted targeted metabolomics analysis of pigments, regulatory hormones, and neurotransmitters in koi plasma.

The catecholamines epinephrine and norepinephrine in fish are affected by seasonal variation, age, and sex (Suzuki et al., 2012; Rodriguez et al., 2013). In the present study, the norepinephrine levels in koi plasma were stable across seasons, while the epinephrine levels were higher in winter than in other seasons. The epinephrine levels in different organs of the eel *Anguilla anguilla* L. within 1 year were unchanged (Le Bras, 1979). A following three-year study found seasonal differences in plasma catecholamine levels that were largely determined by the night/day cycle and the annual cycle (Le Bras, 1984). Changes in catecholamine levels are also associated with the reproductive cycle. Norepinephrine levels in female catfish *Clarias batrachus* are higher during the spawning period than at other times of the year (Manickam and Joy, 1990).

The effects of catecholamines on the movement of pigment granules

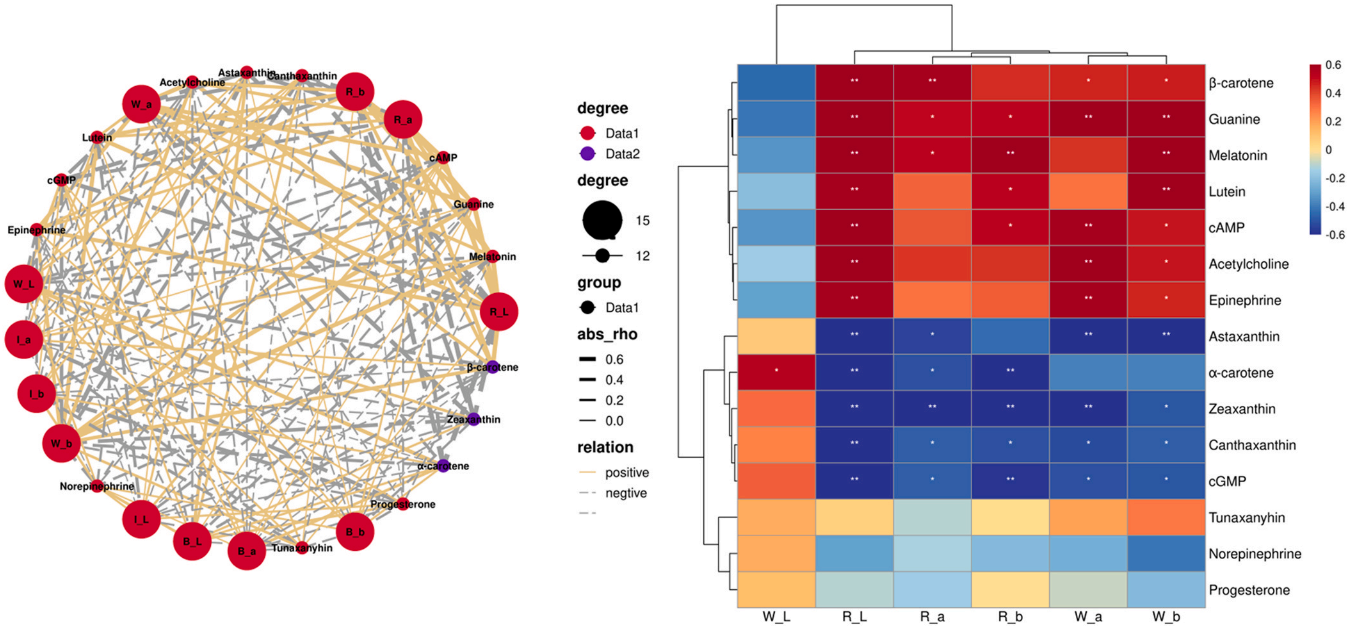

Fig. 5. Correlation analysis between skin color and pigments and related metabolites in koi carp. W_L: Lightness value of white skin color; W_a*: Redness value of white skin color; W_b*: Yellowness value of white skin color; R_L: Lightness value of red skin color; R_a*: Redness value of red skin color; R_b*: Yellowness value of red skin color. ** indicated p value <0.01. * indicated p value≥0.01 & p value<0.05.

in ectotherms have been extensively studied. Epinephrine and norepinephrine act on alpha-adrenergic receptors on chromatophore membranes of teleost fishes; the intracellular cAMP levels are reduced, and melanin and red pigment granules are recruited to the nuclear region (Oshima et al., 1986; Ligon and McCartney, 2016; Franco-Belussi et al., 2018). Injection or oral administration of epinephrine can induce rapid changes in skin color from brown to yellow in male stony creek frogs *Litoria wilcoxii* through the sympathetic nervous system (Kindermann et al., 2014). In summer and autumn, epinephrine and the yellowness of red skin were decreased in WRI koi. *In vitro* experiments (Shinohara et al., 2022) showed that epinephrine did not induce melanin aggregation in Showa koi scales, suggesting that melanophores of Showa Koi scales were weakly sensitive to epinephrine. In the present study, the area of melanin-based pigmentation of WRI koi increased in Sum/Aut. However, there was no correlation between black skin and epinephrine due to the limitations of sample size and detection methods.

Little is known about the regulation of catecholamines on iridophores. The light reflection of iridophores depends on the thickness of guanine crystal platelets and the packing distance (Funt et al., 2017). The platelet spacing and orientation of guanine crystals in iridophores are regulated by epinephrine in the paradise whiptail *Pentapodus paradiseus* (Mathger et al., 2003) and chameleon sand tilefish *Hoplolatilus chlupatyi* (Goda, 2017).The regulation of arrangement and spacing of guanine crystals in teleost fishes has not yet been fully clarified (Gur et al., 2020).

In this study, the levels of epinephrine, cAMP, and guanine in koi changed significantly with season and were significantly positively correlated with the color saturation of white skin. As iridophores are the only chromatophores that exist in the white skin of koi carp, we inferred that seasonal changes may regulate the guanine in iridophores through the epinephrine-cAMP pathway, thereby affecting the change in white skin color (Fig. 6).

In the freshwater goby (Maeno and Iga, 1992), common surgeonfish (Goda and Fujii, 1998), and many other fishes, norepinephrine shares similar biological function with epinephrine via binding to α-adrenergic receptors to promote the dispersion of guanine crystal structures in iridophores. However, the norepinephrine in koi carp showed little seasonal variation and no significant association with guanine crystals. This may be related to the specificity of adrenergic receptor type and the distribution on koi chromatophores. The mechanisms thus deserve further exploration.

4.3. Seasonal regulation of skin color through melatonin

Melatonin secreted by the pineal gland is a photoperiod transducer. Melatonin is often considered to be an aggregator of melansomes in ectotherms, promoting the aggregation of pigment granules by indirectly inhibiting adenylate cyclase (Filadelfi and Castrucci, 1996). In *Xenopus laevis*, the pineal gland releases melatonin at night, inducing melanin aggregation and lightening the skin color (Bertolesi et al., 2020). Some studies suggest that melatonin's regulation of melanosome movement is bidirectional and dose-dependent (Ligon and McCartney, 2016; Fujii, 2000). In the present study, compared to winter, the area of melanin-dependent skin color increased along with decreased melatonin levels of WRI koi and WI koi in summer and autumn. Given the function conservation of melatonin, we hypothesized that there was seasonal regulation of melanin-dependent skin color through the melatonin in koi fish (Fig. 6). However, the direct action of melatonin on melanin in koi fish need further validation.

Melatonin induces pigment migration in erythrophores, as in melanophores (Satake, 1980; Oshima et al., 1986). However, there are fewer studies concerning their effects on erythrophores in teleost fishes (Ligon and McCartney, 2016). Melatonin is observed as an aggregation agonist of xanthophores in goldfish *Carassius auratus* (Satake, 1980). The seasonal changes of melatonin and the yellowness of red skin color in WRI koi, together with the positive correlation, suggest that seasonality may regulate the red skin color through melatonin (Fig. 6). In this process, the increase in plasma carotenoid levels may be due to the lack of synthesizing carotenoids *in vivo* (Gouveia et al., 2003). When the recruitment of carotenoids by skin chromatophores is reduced, the carotenoids in plasma are increased. There were no seasonal changes in melatonin, carotenoids, or red skin color in WR koi. This may be due to the variation in the sensitivity of different koi varieties to melatonin (Fujii and Novales, 1969; Nery and Castrucci, 1997; Fujii, 2000).

5. Conclusions

The study examined the seasonal plasticity of skin color in several varieties of koi. Compared to winter, in other seasons, the area of black skin color increased, and the yellowness of white/red skin color decreased, accompanied by seasonal changes in canthaxanthin, zeaxanthin, and guanine. Seasonal plasticity of white skin color relies on the epinephrine-cAMP pathway. The seasonal plasticity of melanin-based and carotenoid-based skin color relies on melatonin, with variety and chromatophore specificity.

CRediT authorship contribution statement

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

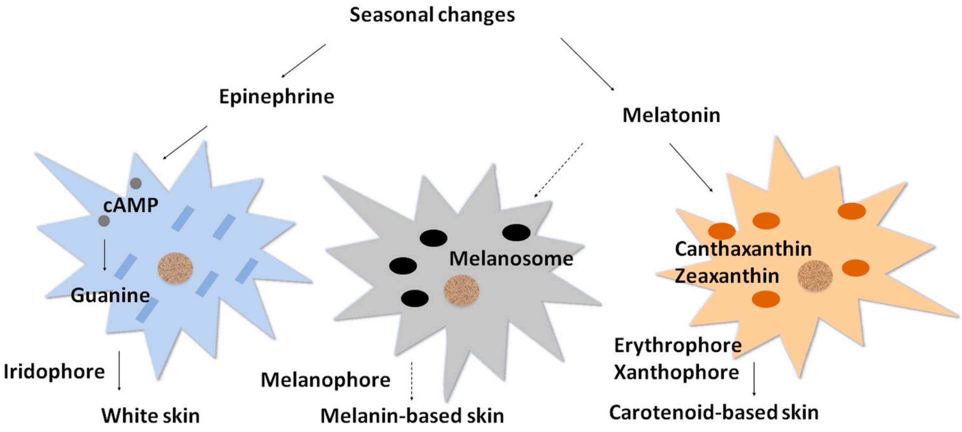

Fig. 6. Potential regulation pathways of seasonal changes on skin color of koi carp.

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.2024.116595.

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