Erschienen in: Limnologica ; 63 (2017). - S. 31-35
https://dx.doi.org/10.1016/j.limno.2016.12.001

Incorporation of dietary carotenoids into the fins of yellow- and red-finned Eurasian perch *Perca fluviatilis*

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A B S T R A C T

The colour of the ventral, anal and caudal fins of Eurasian perch can range from pale yellow to intense red. Within spatially separated populations, however, individuals are usually very uniform in colour. We fed astaxanthin- and canthaxanthin-enriched dry feed to juvenile perch from a yellow-finned and a red-finned population to compare the influence of dietary carotenoids on fin colour between these two populations. In this way we wanted to test whether fin colour in perch is predominantly a phenotypically plastic trait or whether differently coloured individuals represent colour morphs. The ventral fins of perch from the red-finned population always exhibited significantly more intense redness when their feed was supplemented with either one or both carotenoid additives. Perch from both populations deposited more canthaxanthin in their ventral fins than astaxanthin, red-finned perch in particular. Yellow-finned perch probably converted canthaxanthin into β-carotene, which was the dominant carotenoid in their ventral fins, whereas the fins of red-finned perch contained only trace amounts of β-carotene. We conclude that these two populations represent colour morphs that differ fundamentally in their ability to metabolise and deposit dietary carotenoids into their ventral fins. Considering the multiple physiological functions of carotenoids, these fundamental differences in carotenoid metabolism between perch colour morphs may have far-reaching consequences for the performance of different populations, for example in their response to parasite infections.

Keywords:
Astaxanthin
Canthaxanthin
Fin colour
HPLC analysis
Redness intensity

1. Introduction

Eurasian perch *Perca fluviatilis* L. 1758 (hereafter perch) can differ markedly in fin colour, ranging from pale yellow to intense red (Pimakhin, 2012). Perch with red fins seem to occur more frequently: in photos uploaded by Fishbase users (Froese and Pauly, 2013) red-finned perch outnumber yellow-finned perch by 13:3 and a Google search for Eurasian perch images yields a ratio of 6:1. Within populations, however, fin colour is often uniform. For example, in Lake Constance until recently, all perch had pale yellow fins while those in the small lakes north of Lake Constance have orange or bright red fins. In Swedish forest lakes and in the River Rhine near Basel, however, yellow- and red-finned perch co-occur (P.Hirsch, Univ.Basel, pers. comm.). It is not yet known whether fin colour in perch is predominantly a phenotypically plastic trait whose expression is influenced by environmental factors such as diet composition or water colour and turbidity, or whether differently coloured individuals represent colour morphs that differ even though they ingest the same type of dietary carotenoids.

Dietary carotenoids contribute to the yellow, orange and red colouration of the skin, fins and fillet in salmonids (Shahidi and Brown, 1998), but their influence on the fin colour of perch has not previously been studied. Water colour or turbidity can influence the coloration of perch as shown by Kekäläinen et al. (2010). In the least humic (i.e. most transparent) of four boreal Finnish lakes, perch had the lightest and less coloured belly and perch were more colourful in the littoral than in the pelagic habitat, suggesting divergent selection in littoral and pelagic zones. However the mechanism by which different fin coloration is obtained in the different habitats remains unknown. Consistent differences in caudal fin colour between perch from different water bodies have also been observed by Mairesse et al. (2005) who were able to discriminate perch from the River Rhine and Lake Geneva by caudal fin colour. However this study encountered a confounding factor in that caudal fin colour attributes, such as lightness, chroma and hue, were significantly related to fish size.

The existence of colour morphs in this species was suggested recently in a study by Roch et al. (2015) who compared age-0 perch

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with pale yellowish fins and perch with red fins sampled from the littoral of Lake Constance. During the few decades prior to 2010, yellow-finned perch were the sole phenotype in Lake Constance (professional fishermen and fishery scientists, pers. comm.), but in that year individuals with red fins began to be caught. Roch et al. (2015) showed by microsatellite analysis that the red-finned fish differed from the yellow-finned individuals and from fish with mixed fin colour, which are probably backcrosses between red- and yellow-finned individuals. These results suggest that red-finned fish are not a colour variant of the native perch population, but more likely the progeny of individuals that have invaded the lake from other water bodies in the drainage basin.

The first clue that fin coloration in perch is influenced by diet came from a preliminary feeding experiment in our laboratory in which juveniles of a red-finned population received diets comprising either chironomid larvae or lake zooplankton for eight weeks (Eckmann, unpubl. data). Fish feeding on chironomid larvae had pale-orange fins whereas the fins of those feeding on zooplankton were intensely orange-red, the anal and ventral fins in particular. Following this preliminary study, two experiments were designed to compare the influence of dietary carotenoids on fin coloration between a red- and a yellow-finned population of perch. As astaxanthin and canthaxanthin are the main chromophore compounds present in perch fins (Czeczuga, 1979b), we used these two carotenoids as feed additives. In the first experiment, fish were fed a dry feed supplemented with both carotenoids, and in the second experiment different treatment groups received dry feeds supplemented with either astaxanthin or canthaxanthin. It was the aim of our study to test the null hypothesis that perch from yellow- and red-finned populations develop the same ventral fin coloration when feeding on diets with identical carotenoid content.

2. Material and methods

The red-finned perch used in this experiment originated from Lake Karsee (K_perch), a small (3 ha) lake north of Lake Constance. The yellow-finned perch originated from Lake Constance (C_perch), the second-largest (536 km^2) perialpine lake north of the European Alps. Spawners from both populations were kept separately in outdoor mesocosms where they spawned voluntarily from mid-April to mid-May. Egg strands were collected from the mesocosms and incubated in the laboratory. Larvae were reared on *Artemia* nauplii for four weeks and then weaned to a dry diet (Inicio 917, BioMar) which they received until the experiments started.

2.1. Experiment 1

In October 2013, two aquaria of 85 L volume were stocked with 30 age-0 K_perch each and a further two aquaria with 30 age-0 C_perch each. All four groups received a formulated diet supplemented with 50 mg each of astaxanthin and canthaxanthin (Lucantin™Pink and Lucantin™Red, BASF) per 1000 g of pellets. This is a concentration of carotenoids commonly used in feeding studies with salmonids (Shahidi and Brown, 1998). As ours is the first study in which carotenoids are added to the diet of perch, we adopted these values as a guideline. The additives were ground in a mortar with a small quantity of pellets, and the powder mixed with 1000 g of feed. The mixture was wetted with water, mixed thoroughly to a pulp, passed through a meat mincer with 2 mm bore diameter, and dried. The fish lived at $20 \pm 0.2\,^{\circ}C$ with 14 h light per day. The aquaria were supplied with tap water from the City of Constance waterworks at a rate implying a theoretical water renewal time of 2–3 h, and food was provided *ad libitum* twice per day. After three months, one tank group of each population was switched onto a non-supplemented dry feed (codes: K_Car/df and C_Car/df, respectively) while the second group of each population continued to receive the supplemented diet (codes: K_Car and C_Car, respectively). The experiments ended after five months.

Sampling took place at the start of the experiment, after three months and at the end of the experiment, with ten fish removed from each aquarium, euthanized with an overdose of MS 222 and photographed in ventral aspect with a colour reference chart in each picture. Photos were taken under a white canvas soft box illuminated with video floodlights to eliminate surface reflections and hard shadows. The ventral fins were cut off at the bases, blotted dry, weighed to 0.1 mg and stored at $-20\,^{\circ}C$ until further processing.

2.2. Experiment 2

In April 2014, a second experiment began using fish from the same source batches as those used in experiment 1. Four 85 L aquaria were each stocked with 30 K_perch and a further four aquaria with 30 C_perch each. Two tank-groups from each population received a formulated diet supplemented with 100 mg of astaxanthin per 1000 g of pellets (codes: K_Ast1/2 and C_Ast1/2, respectively), and the other two groups received a diet supplemented with 100 mg of canthaxanthin per 1000 g (codes: K_Can1/2 and C_Can1/2, respectively). The experimental conditions were the same as in experiment 1. Photos and fin samples of ten fish from each aquarium were taken at the start of the experiment and after nine weeks at the end.

2.3. Redness intensity

Photos were evaluated with Image J (http://rsbweb.nih.gov/ij/) using the RGB colour model. Luminosity, i.e. the overall brightness of a photo, was calculated by converting each pixel to grayscale using the weighted RGB conversion. The brightness of all photos was auto-adjusted so that the average grey value (range 0–255) of the white reference chart exceeded 240. An elliptical selection of the most intensely coloured part of each ventral fin was marked, and the average red and grey values of this selection noted along with those of the white and red standard charts. Fin colour was scored according to the redness intensity, I, of the selected fin region, which was calculated as $I = 100^{*}(F\text{-}W)/(R\text{-}W)$ according to Villafuerte and Negro (1998), where F, W and R are the quotients of average red and grey values of the fin area, the white standard and the red standard chart, respectively. This gives a standardized redness value ranging from 0 to 100. In both experiments, right and left ventral fins of all sampled fish matched in terms of redness intensity (*t*-test for paired values, $p > 0.05$ in all cases). Therefore, mean values for each fin pair were used in further analyses.

2.4. High-performance liquid chromatography (HPLC)

For the analysis of carotenoids in ventral fins by HPLC, deep-frozen fins were ground in liquid nitrogen in a mortar. Carotenoids were extracted with acetone, dried over Na_2SO_4 and the samples were centrifuged for 6–12 min at 13000 rpm. The supernatants were transferred into HPLC vials, evaporated under a nitrogen flow, and the vials were stored at $-20\,^{\circ}C$. For HPLC analysis carotenoids were re-dissolved in 0.2 mL acetone and separated on an Agilent Zorbax Eclipse XDB-C8 column (150 mm × 4,6 mm, 5 μm) using an Agilent 1100 HPLC system with a diode array detector. HPLC conditions were: solvent A: H_2O 0.1% acetic acid, solvent B: acetonitrile; HPLC programme: gradient elution from 70% B to 100% B in 10 min, 100% B for 15 min; flow rate: 1 mL/min, diode detector range 280–550 nm; the UV trace of 462 nm was used for quantitative analysis. Injection volumes ranged from 5 to 50 μL. Astaxanthin (retention time: 8.3 min), canthaxanthin (retention time: 11.5 min) and β-carotene (retention time: 21.5 min) (BASF) were used as

Table 1
Total length (cm) and redness (cf. Materials and methods for definition of redness) of the ventral fins of juvenile Eurasian perch from Lake Constance (C_) and Karsee (K_) after three and five months of *ad libitum* feeding on dry feed enriched with 50 mg/kg of astaxanthin and canthaxanthin (10 fish analysed per treatment). The fish received the carotenoid-enriched feed for five months (_Car) or for three months followed by two months on non-enriched dry feed (_Car/df). Values with the same superscript indicate non-significant differences within columns. Significance levels were set at $P < 0.05$ in all comparisons.

Treatment	Total length (cm)		Redness	
	3 mo	5 mo	3 mo	5 mo
C_Car	10.1 ± 0.7^{b}	13.2 ± 0.7^{bc}	14.5 ± 5.9^{b}	23.1 ± 5.1^{bc}
C_Car/df	10.3 ± 0.8^{b}	13.1 ± 1.2^{c}	12.5 ± 2.9^{b}	13.9 ± 2.1^{c}
K_Car	11.4 ± 1.3^{a}	14.5 ± 1.2^{a}	57.8 ± 22.1^{a}	74.5 ± 14.6^{a}
K_Car/df	11.1 ± 1.3^{ab}	14.1 ± 1.3^{ab}	60.5 ± 17.1^{a}	55.1 ± 12.2^{ab}

standards and to generate calibration curves for the quantitative analysis of extracted carotenoids.

2.5. Data analysis

Start values of total length and of redness intensity were compared between populations by *t*-test. Treatment means were compared with ANOVA when test assumptions of normality (Shapiro-Wilk W test) and homoscedasticity (Bartlett test) were fulfilled, otherwise Kruskal-Wallis-ANOVA (KW-ANOVA) was used. Multiple comparisons of means were conducted with Student's t on the difference across every pair of levels or with Nemenyi-test, respectively. Results were considered statistically significant at $P < 0.05$. Tests were calculated using JMP (SAS Institute Inc. Cary, North Carolina, USA) version 7.0.1. or STATeasy (Wissenschaftliche Auswertungen, Hamburg, Germany), version 2009.

3. Results

3.1. Experiment 1

At the start of the experiment, K_perch were significantly larger than C_perch (7.0 ± 0.7 *vs.* 6.4 ± 0.6 cm total length; *t*-test, $P < 0.05$) probably because K_perch spawned and hatched about three weeks earlier. After three months K_perch continued to be larger than C_perch, and the same was true after five months when one treatment group per morph had been switched to a non-enriched feed (Table 1). These differences however, could only partly be confirmed statistically (ANOVA, $F_{3,34} = 3.02$, $P = 0.043$ and $F_{3,36} = 3.67$, $P = 0.021$, respectively).

Redness intensity was already significantly greater for K_perch than for C_perch at the beginning of the experiment (4.3 ± 1.4 vs. 3.3 ± 0.8; *t*-test, $P < 0.05$). After three months, redness intensity was significantly greater for K_perch than for C_perch (KW-ANOVA, $H = 27.5 > \chi^2 = 7.81$; Table 1). After five months, redness intensity had increased further in the two groups receiving the carotenoid-enriched feed but not in the groups that fed on non-enriched feed for the last two months. Even so, K_perch still exhibited significantly more intense redness than C_perch that received the same type of feed (KW-ANOVA, $H = 33.6 > \chi^2 = 7.81$; Table 1; Fig. 1a + b).

Astaxanthin was only found sporadically in the ventral fins of both morphs, except K_perch that had fed for five months on carotenoid-enriched feed, whose fins consistently contained low amounts of astaxanthin. Canthaxanthin was detected in C_perch in very small amounts, but was the dominant carotenoid in ventral fins of K_perch (Table 2). After switching to non-enriched feed, the concentration of canthaxanthin in the fins of K_perch did not change significantly but in the group that continued to feed on a carotenoid-enriched diet, the concentration increased about threefold (ANOVA, $F_{3,16} = 3.64$, $P = 0.036$; Table 2). Beta-carotene was not found in K_perch fins but was the dominant carotenoid in the ventral fins of C_perch. In both C_perch groups the concentrations of β-carotene found after five months did not differ significantly from those measured after three months (Table 2), even though the concentration in the group that continued to feed on a carotenoid-enriched diet was about fourfold higher than in the group that fed the non-enriched diet during the last two months (KW-ANOVA, $H = 8.42 > \chi^2 = 7.81$).

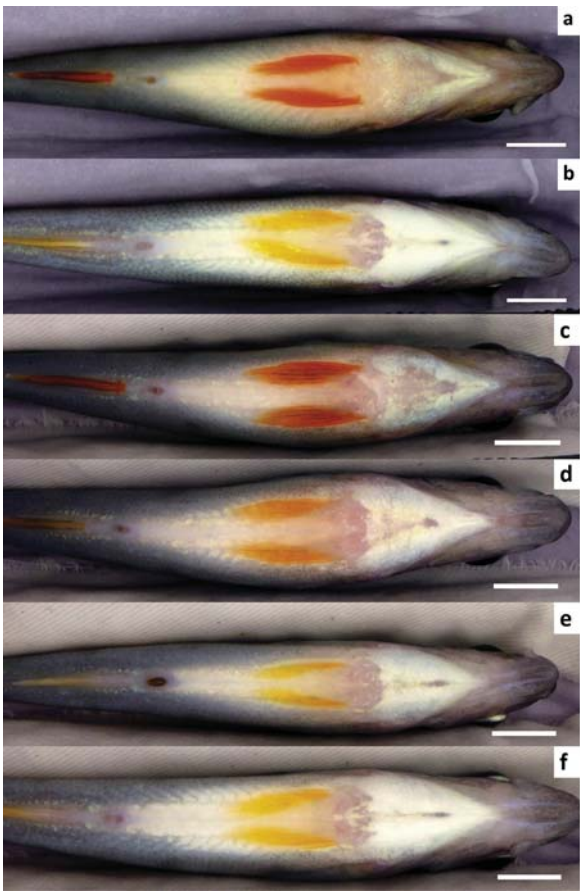

Fig. 1. Ventral views of juvenile *Perca fluviatilis* from two spatially separated populations fed with dry feeds supplemented with carotenoids. a) Karsee perch fed for five months with 50 mg/kg astaxanthin- and 50 mg/kg canthaxanthin-supplemented feed. b) Lake Constance perch receiving the same treatment. c) Karsee perch fed for nine weeks with 100 mg/kg astaxanthin-supplemented feed. d) Karsee perch fed for nine weeks with 100 mg/kg canthaxanthin-supplemented feed. e) Lake Constance perch fed for nine weeks with 100 mg/kg astaxanthin-supplemented feed. f) Lake Constance perch fed for nine weeks with 100 mg/kg canthaxanthin-supplemented feed. Bars: 10 mm.

Table 2
The content of astaxanthin, canthaxanthin and β-carotene in the ventral fins of Eurasian perch after three and five months of *ad libitum* feeding on a carotenoid-enriched dry feed. Values are given in μg carotenoid per g fresh weight and are presented as means ± S.D. for five fish. When a value without S.D. is given in a table cell, then only one out of five fish contained that particular carotenoid. Values with the same superscript indicate non-significant differences between canthaxanthin values for K_perch after three and five months, and between β-carotene values for C_perch after three and five months. Significance levels were set at $P < 0.05$ in both comparisons. For treatment codes refer to Table 1.

Treatment	Astaxanthin		Canthaxanthin		β-carotene	
	3 mo	5 mo	3 mo	5 mo	3 mo	5 mo
C_Car	0	0	0.1 ± 0.1	0.1 ± 0.1	2.2 ± 0.8^{a}	4.7 ± 2.7^{a}
C_Car/df	0.3	0.2		0	3.0 ± 1.5^{a}	1.2 ± 0.5^{a}
K_Car	0.8	0.4 ± 0.3	4.8 ± 6.1^{b}	12.1 ± 6.8^{a}	0	0
K_Car/df	0.4	0.2	4.1 ± 2.9^{b}	2.9 ± 1.7^{b}	0	0

Table 3
Total length (cm) and redness (cf. Materials and methods for definition of redness) of the ventral fins of juvenile Eurasian perch from Lake Constance (C_) and Karsee (K_) after nine weeks of *ad libitum* feeding on carotenoid-enriched dry feeds. The dry feed was supplemented with either 100 mg/kg of astaxanthin (_Ast) or canthaxanthin (_Can). There were two replicates per treatment (10 fish analysed per replicate) and the results are given as means ± S.D. Values with the same superscript indicate non-significant differences. For each variable the groups receiving the canthaxanthin-enriched feed are compared in the first column below the variable name, those receiving the astaxanthin-enriched feed are compared in the second column, and the four groups of Lake Constance or Karsee perch are compared with each other in the third column. Significance levels were set at $P < 0.05$ in all comparisons.

Treatment	Total length (cm)			Redness		
C_Ast1		10.4 ± 0.7^{a}	b		17.4 ± 3.6^{b}	b
C_Ast2		10.6 ± 0.7^{a}	b		16.4 ± 2.5^{b}	b
C_Can1	12.2 ± 0.5^{a}		a	25.9 ± 1.9^{b}		a
C_Can2	10.6 ± 0.7^{b}		b	23.3 ± 3.7^{b}		a
K_Ast1		10.5 ± 0.8^{a}	b		57.9 ± 8.8^{a}	a
K_Ast2		9.6 ± 0.6^{b}	c		42.7 ± 9.0^{a}	b
K_Can1	10.9 ± 0.5^{b}		b	33.8 ± 8.6^{a}		c
K_Can2	12.4 ± 0.6^{a}		a	33.4 ± 7.9^{a}		c

3.2. Experiment 2

At the start of the experiment, total body length did not differ between K_perch and C_perch (7.3 ± 0.5 *vs.* 7.2 ± 0.9 cm; *t*-test, $P > 0.05$). After nine weeks total body length showed no consistent pattern between perch morphs feeding on canthaxanthin (ANOVA, $F_{3,36} = 22.68$, $P < 0.0001$) or astaxanthin (ANOVA, $F_{3,36} = 4.13$, $P = 0.0129$) as well as among perch morphs feeding on two different enriched feeds (C_perch: ANOVA, $F_{3,36} = 15.45$, $P < 0.0001$; K_perch: ANOVA, $F_{3,36} = 33.55$, $P < 0.0001$). For both perch morphs, however, the largest fish were found in groups feeding on canthaxanthin-enriched diets (cf. second column in Table 3).

Redness intensity (I) was significantly higher for K_perch than for C_perch at the beginning of the experiment (6.5 ± 2.2 vs. 4.6 ± 1.4; *t*-test, $P < 0.05$). After nine weeks, K_perch exhibited significantly greater I values than C_perch feeding on both astaxanthin- and canthaxanthin-enriched feed (KW-ANOVA, $H = 31.60 > \chi^2 = 7.81$ and $H = 15.11 > \chi^2 = 7.81$, respectively; Table 3). Among C_perch, fish feeding on canthaxanthin-enriched feed exhibited significantly greater redness intensity than those feeding on astaxanthin-enriched feed (ANOVA, $F_{3,36} = 23.34$, $P < 0.0001$) whereas in K_perch fins were redder in fish feeding on astaxanthin-enriched feed, (ANOVA, $F_{3,36} = 17.93$, $P < 0.0001$; Fig. 1, c–f).

Astaxanthin was found in the ventral fins of all analysed K_perch receiving the astaxanthin-enriched feed, whereas in all other treatment groups this carotenoid was only found occasionally (Table 4). Canthaxanthin was found consistently in all groups receiving the canthaxanthin-enriched diet, and the average content of K_perch ventral fins was nearly tenfold greater than in C_perch (KW-ANOVA, $H = 15.46 > \chi^2 = 7.81$). In the groups receiving astaxanthin-enriched feed, canthaxanthin was found in only a few fish (Table 4). Beta-carotene was not detected in either C_Ast or in K_Ast groups, nor was it found in seven out of ten K_Can fish. C_perch feeding on canthaxanthin-enriched feed, however, had consistently high concentrations of β-carotene in their ventral fins (Table 4).

4. Discussion

The perch populations compared in these two experiments differed markedly in ventral fin coloration when feeding on the same carotenoid-enriched dry feeds, in that K_perch always exhibited more intense redness than C_perch. The null hypothesis that perch from yellow- and red-finned populations develop the same ventral fin coloration when feeding on diets with identical carotenoid content must therefore be rejected. This result suggests that the difference in ventral fin coloration and carotenoid deposition between yellow- and red-finned perch has a genetic basis, perhaps similar to that previously shown to influence variable pigmentation and coloration in salmonids (Bjerkeng, 2000; Choubert et al., 1995; Rye and Gjerde, 1996; Withler, 1986; Withler and Beacham, 1994). In contrast to salmonids, dietary carotenoids apparently do not influence flesh colour in perch. When adult yellow-finned perch were fed on broodstock feed for trout with a high carotenoid content for several months, we did not observe any change in the fish's pale-white flesh colour (Eckmann, unpublished data), and in the present study we did not observe by gross inspection any difference in flesh colour between perch from different treatments either.

Earlier work has suggested that growth rate, as a proxy for uptake of dietary carotenoids, could explain differences in ventral fin coloration. Mairesse et al. (2005) reported that caudal fin colour in Eurasian perch was significantly related to fish size, and a similar positive relationship between body size and carotenoid deposition was reported for salmonids by Storebakken and No (1992). At the end of our experiments, however, body lengths of K_perch and C_perch differed by only around 1 cm in experiment 1 (Table 1), and showed no consistent pattern in experiment 2 (Table 3), whereas in the study of Mairesse et al. (2005), fish differed by up to 20 cm in standard length. Furthermore, in experiment 1 after five months of feeding C_perch were around 2 cm longer than K_perch after three months, yet the smaller K_perch had higher redness intensity than the larger C_perch (Table 1). Fish size, therefore, is not a confounding factor in our study, suggesting that our yellow- and red-finned populations represent colour morphs with marked differences in carotenoid metabolism and/or incorporation into their fins.

The analysis of fin carotenoid content revealed pronounced differences between the two populations. When the dry feed contained canthaxanthin, K_perch deposited considerable amounts of this carotenoid in their ventral fins, between one (experiment 2) and two (experiment 1) orders of magnitude more than C_perch. Supplementary astaxanthin was incorporated consistently, albeit in small quantities by K_perch, after nine weeks in experiment 2 and after five months in experiment 1. In C_perch fins, astaxanthin was found only sporadically. Atlantic salmon and rainbow trout are known to utilize astaxanthin more efficiently than canthaxanthin for flesh pigmentation (Storebakken and No, 1992). Both colour morphs of perch, however, utilize these carotenoids for fin coloration in a different way, favouring canthaxanthin over astaxanthin.

The most striking difference between the two populations is the β-carotene content of the ventral fins. While this carotenoid was completely absent in K_perch, it was present in large amounts in C_perch consuming dry feed with canthaxanthin. Hence, C_perch likely converted canthaxanthin in β-carotene, probably using a metabolic pathway that has previously been detected in the skin and liver of rainbow trout (Guillou et al., 1989). Feeding labelled carotenoids to perch might help to substantiate the suggested conversion of canthaxanthin in β-carotene.

When the fish were switched from carotenoid-enriched to non-enriched feed in experiment 1, there was no change in redness intensity in either K_ or C_perch. By contrast, carotenoid content decreased after two months, most notably for canthaxanthin and β-carotene, but unfortunately these differences could not be confirmed statistically as variances were rather high. In rainbow trout muscle carotenoid concentration had not decreased after 85 days of starvation (Choubert, 1985). The marginal decrease of carotenoid concentration in perch ventral fins suggests that carotenoids deposited into perch ventral fins are not permanently incorporated and might be at least partly remobilized at a slow rate. Longer periods of carotenoid deprivation are needed to gain better information on the possible remobilization of carotenoids from perch ventral fins.

Table 4
The content of astaxanthin, canthaxanthin and β-carotene in the ventral fins of Eurasian perch after nine weeks of *ad libitum* feeding on carotenoid-enriched dry feed. Values are given in μg carotenoid per g fresh weight and are presented as means ± S.D. for five fish per replicate. When less than five fish per replicate contained the respective carotenoid, replicates were merged and the combined number of fish containing the carotenoid is indicated. These treatments were omitted from statistical comparisons. Values with the same superscript indicate non-significant differences within columns. Significance levels were set at $P < 0.05$ in all comparisons. For treatment codes refer to Table 3.

Treatment	Astaxanthin	Canthaxanthin	β-carotene
C_Ast1	0.2 ± 0.1 (n = 3/10)	0.1 ± 0.2 (n = 4/10)	0
C_Ast2			0
C_Can1	0.1 ± 0.1 (n = 5/10)	0.7 ± 0.2 [b]	4.1 ± 1.6 [a]
C_Can2		1.2 ± 0.5 [b]	5.8 ± 2.2 [a]
K_Ast1	0.5 ± 0.1 [a]	0.1 ± 0.1 (n = 3/10)	0
K_Ast2	0.2 ± 0.1 [b]		0
K_Can1	0.1 ± 0.1 (n = 4/10)	12.2 ± 5.3 [a]	0.8 ± 2.1 (n = 3/10)
K_Can2		6.4 ± 4.3 [ab]	

Astaxanthin seems to provide more intense redness than canthaxanthin in K_perch. In experiment 2, astaxanthin-supplemented feed led to greater redness intensity than canthaxanthin-supplemented feed (Table 3) even though the canthaxanthin content of K_perch ventral fins was more than an order of magnitude greater than their content of astaxanthin (Table 4). Similar results have been obtained in trout, where astaxanthin led to more intense redness of the flesh than canthaxanthin at comparable concentrations (Skrede and Storebakken, 1986; Skrede et al., 1989).

Beta-carotene has full vitamin A activity in fish, while both astaxanthin and canthaxanthin are vitamin A precursors (Gross and Budowski, 1966). However details of the further biological roles of carotenoids in fish are only partly understood (Shahidi and Brown, 1998). Other physiological functions commonly attributed to carotenoids include their ability to absorb potentially damaging radiation and to quench singlet oxygen and thus serve as antioxidants (Tacon, 1981). High levels of carotenoids have been linked to better resistance to bacterial and fungal diseases in salmonids (Czeczuga, 1979a), and to incubation-survival and disease resistance in eggs of *Oncorhynchus tshawytscha* (Tyndale et al., 2008). Given these potentially beneficial effects of carotenoids, the question arises whether yellow and red colour morphs of perch differ in physiological performance as well as fin colour. In a recent study, Roch et al. (2015) showed that the two colour morphs now resident in Lake Constance differ in their susceptibility to macroparasite infection. Red-finned specimens and fish with mixed fin colour showed better resilience to the pike tapeworm *Triaenophorus nodulosus* and the gill worm *Ancyrocephalus percae*. These results suggest that parasite susceptibility may be related to differences in carotenoid metabolism between perch colour morphs.

In conclusion, fin colour is not simply a phenotypically plastic trait that is influenced by environmental factors such as diet composition or water colour and turbidity, but yellow- and red-finned Eurasian perch represent colour morphs that differ fundamentally in their ability to metabolise and deposit dietary carotenoids into their ventral fins. Differences in carotenoid metabolism may have unexplored but far-reaching consequences for perch performance, for example in resisting parasite infection.

Acknowledgements

Dr. W. Pelletier, BASF, kindly provided the feed carotenoids. H. Thiele took care of the perch spawners and successfully weaned perch fry to dry feed. M. Schmid took care of the juvenile fish stock and helped during the entire course of the experiments. We thank A.-J. Beer for language correction and improvement of the manuscript and two anonymous referees for their helpful comments.

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