

## Retention of Fillet Coloration in Rainbow Trout After Dietary Astaxanthin Cessation

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### Abstract

This study was conducted to determine the retention time of rainbow trout (*Oncorhynchus mykiss*) fillet coloration, as indicated by hue, chroma, and entire color index (ECI) values, after the cessation of dietary astaxanthin. After 12 weeks of receiving a diet containing astaxanthin, rainbow trout [mean (SD) length 240 (26) mm, weight 194 (65) g] were either switched to a non-astaxanthin diet or continued to receive the same astaxanthin diet for 55 d. In addition, a control group was fed a non-astaxanthin diet for the entire duration of the study. Digital coloration measurements ( $L^*$ ,  $a^*$ , and  $b^*$ ) were recorded on fillets and used to calculate hue, chroma, and ECI values. Hue, chroma, and ECI did not significantly change within any of the treatments over the course of the study. Hue, chroma, and ECI were also not significantly different between the fillets of those fish that either continued to receive astaxanthin-supplemented feed compared to those that were switched from an astaxanthin diet to diet free of astaxanthin. However, ECI, hue, and chroma were significantly different in fillets from the fish that had never received dietary astaxanthin compared to fillets from the other two treatments. These results indicate that catchable-sized rainbow trout stocked into recreational fishing waters with minimal natural feed should retain fillet coloration for at least 55 d after stocking, making astaxanthin supplementation in recreational hatcheries a viable tool to improve angler satisfaction.

**Keywords:** Rainbow trout; Astaxanthin; *Oncorhynchus mykiss*; Coloration

### Introduction

Consumer and angler satisfaction is of great importance for both commercial and recreational aquaculture. The work of Forsberg and Guttormsen stated that with salmon, the appearance of wild characteristics is the most important criteria of perceived quality by consumers [1]. Fillet coloration is particularly important. In rainbow trout (*Oncorhynchus mykiss*), fillets containing red pigmentation are deemed much more desirable than non-pigmented, white fillets [2]. A 2009 study also found a positive relationship between color and consumer demand in snapper (*Pagrus auratus*) [3]. In recreational fisheries, Simpson reported that anglers overwhelmingly preferred more brightly colored rainbow trout, as well as more red-colored trout fillets [4].

The red fillet color preferred by both consumers and recreational angler's results primarily from the consumption of astaxanthin, a carotenoid pigment that fish cannot synthesize de novo [5]. Astaxanthin occurs in many prey items consumed by wild fish, while in cultured fish this pigment must be provided in the artificial diets [5]. Including the synthetic or natural forms of astaxanthin in formulated feeds increases production costs, but this is typically more than compensated by increases in pricing power [1], or in the case of natural resource agencies, by increases in angler satisfaction [4]. However, anglers in the Simpson survey were not told that such coloration in hatchery fish would require the addition of supplemental astaxanthin [4].

The time and amount of astaxanthin supplementation required to produce red-colored fillets in salmonids is well documented [1,6-8]. However, unlike trout produced for direct human consumption by commercial aquaculture which are slaughtered at peak coloration, trout produced for stocking into water bodies by recreational hatcheries may be harvested months after stocking [9].

A 1998 study reported few differences in the color of frozen fillets

from Atlantic salmon *Salmo salar* starved for up to 86 days after being fed a diet containing astaxanthin [10]. Except for the very short duration experiment of López-Luna, we are not aware of any studies that have examined long-term coloration depletion rates and subsequent changes in fillet coloration following the cessation of feeding dietary astaxanthin in rainbow trout [11]. This information is needed by resource agencies and other organizations to determine the effectiveness and utility of stocking of catchable-sized rainbow trout with red-colored fillets due to supplemental astaxanthin. Thus, the objective of this study was to examine the long-term effects on rainbow trout fillet coloration after the removal of supplemental dietary astaxanthin.

### Methods

This study was conducted at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, USA, using degassed and aerated spring water (11°C, total hardness as CaCO<sub>3</sub>, 36 mg L<sup>-1</sup>; alkalinity as CaCO<sub>3</sub>, 210 mg L<sup>-1</sup>; p<sup>H</sup>-7.6; total dissolved solids, 390 mg L<sup>-1</sup>). Four circular production tanks (6.1 m diameter, 0.9 m operating depth), each containing approximately 5,500 Erwin-Arlee strain rainbow trout [mean (SD) length 240 (26) mm, weight 194 (65) g] were initially used in this experiment. Beginning on June 4, 2008, two of the tanks were fed a 4.5 mm classic trout diet (Skretting, Tooele, Utah, USA), while

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the other two tanks were fed the same diet with the inclusion of 40 mg/L synthetic astaxanthin. Feeding rates were based on a projected growth rate of 0.065 cm/day and an anticipated feed conversion ratio of 1.1. Because the fish were part of normal hatchery production, small numbers of fish were periodically removed from each tank for stocking into recreational fishing waters. As the number of fish in each tank changed, feeding amounts were adjusted correspondingly.

Weekly measurements of fillet color were obtained from fish euthanized by a lethal dose of 250 mg/L MS-222 using a HunterLab MiniScan XE Plus Colorimeter (HunterLab, Reston, Virginia, USA). Measurements occurred mid-fillet, below the dorsal fin and above the lateral line. Color was measured from whole fillets, rather than ground tissue, because this coloration would be most evident to the recreational angler. The colorimeter measured color along the CIELAB color scale (CIE 1976), which includes L\*, a\*, and b\* values. The a\* score indicates red-green chromaticness and b\* indicates yellow-blue chromaticness [7]. L\* indicates white-to-black values [12]. At 12 weeks, after which a\* values were no longer increasing in the tanks receiving supplemental astaxanthin, one-half of these fish were switched to a non-astaxanthin diet and moved into two additional tanks. Fillet color data was then collected at approximately 14 day intervals for the next 55 days. The focus of this study was solely on changes in coloration, not growth or any other rearing variable. Thus, only coloration data was collected.

The L\*, a\*, and b\* values were used to calculate Hue, Chroma, and the Total Color Index using the following formulas:

$$\text{Hue} = \arctan(b^*/a^*)$$

$$\text{Chroma} = [(a^*^2 + b^*^2)^{1/2}]$$

$$\text{Entire Color Index}(ECI_i) = C_i * \cos(H_i - H_{mean}),$$

Where  $H_{mean}$  = mean hue and  $C_i$ ,  $H_i$  = the chroma and hue values for each measurement [13].

Statistical analysis was done using Minitab 16 software (Minitab Inc., State College, Pennsylvania, USA). Color depletion data was analyzed using multiple regression models. Models were done using the overall means of the treatments at each measurement to determine significance in changes of color variables. Statistical significance was predetermined at  $P < 0.05$ .

## Results

Hue, chroma, and ECI did not significantly change in any of the treatments over the course of the study (Figures 1-3). Hue, chroma, and ECI were also not significantly different between the fillets of those fish that either continued to receive astaxanthin-supplemented feed (Color) compared to those that were switched from an astaxanthin diet to diet free of astaxanthin (Depletion). However, ECI, hue, and chroma were significantly different in fillets from the fish that had never received dietary astaxanthin (No Color), compared to fillets from both Color and Depletion treatment fish.

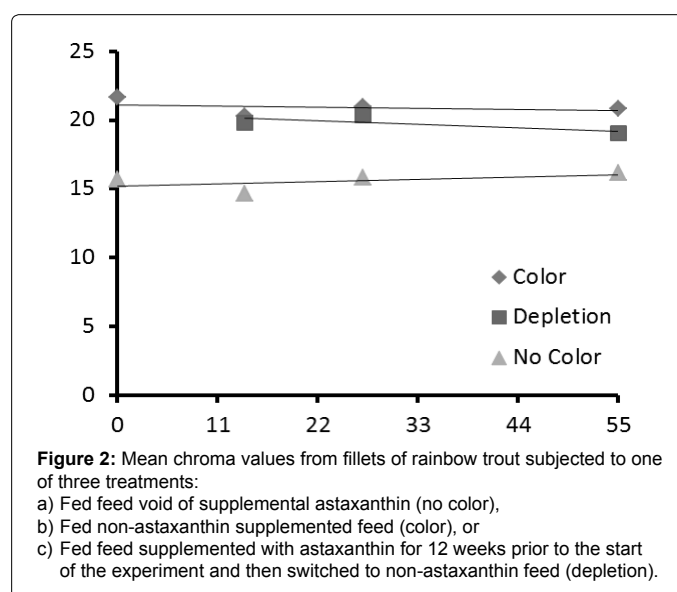
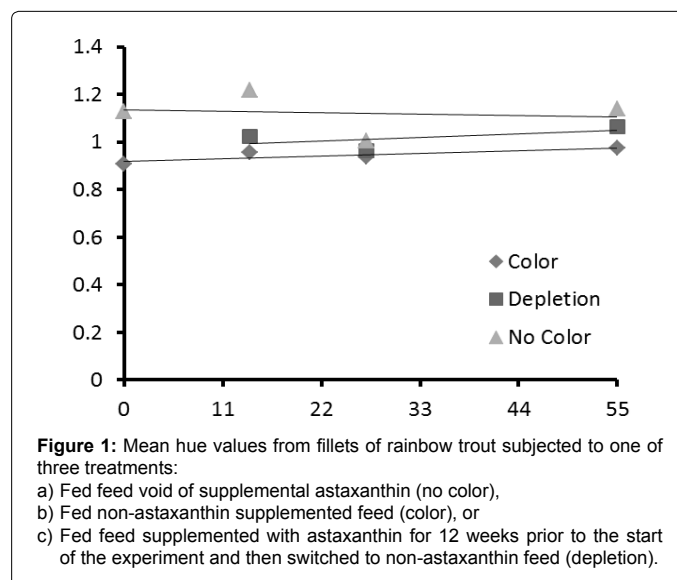
Best fitted models for each color variable are as follows:

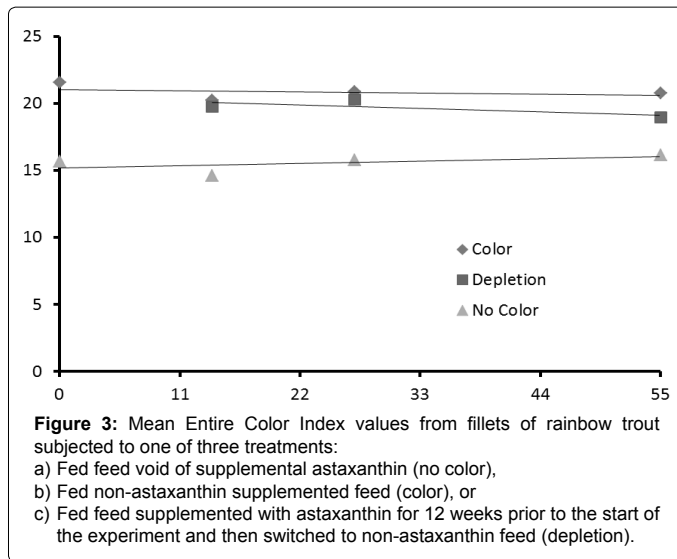
- Hue:** No Color =  $-27.75 - 0.00073 (\text{Date})$ ; Color and Depletion =  $-27.9 - 0.00073 (\text{Date})$   $R^2 = 60.1$ ,  $d.f. = 2, 10$ .
- Chroma:** No Color =  $261.15 - 0.0062 (\text{Date})$ ; Color and Depletion =  $268 - 0.0062 (\text{Date})$   $R^2 = 91.8$ ,  $d.f. = 2, 10$ .
- ECI:** No Color =  $258.18 - 0.0061 (\text{Date})$ ; Color and Depletion =  $263 - 0.0061 (\text{Date})$   $R^2 = 91.8$ ,  $d.f. = 2, 10$ .

## Discussion

These results indicate that, at least for the 55 d duration of the study, there was no loss in fillet coloration after the cessation of feeding dietary astaxanthin. This timing is important in recreational fisheries sustained by the stocking of catchable-sized rainbow trout into waters with minimal natural feed. In a study conducted by Barnes, most of the rainbow trout stocked into two public fishing lakes were caught within the first few weeks after stocking, and nearly all of the fish were harvested within the first 55 days [9]. Thus, natural resource agencies can feed supplemental astaxanthin to catchable rainbow trout without concern that the internal and external benefits of pigmentation will disappear before the fish are caught, thereby meeting angler preferences for more colorful fish and fillets [4].

The results from this study also have application to commercial trout hatcheries, where fillet coloration influences customer perceptions of quality, customer demand, and pricing power [1-3,13,14]. Rather than incur the expense of feeding supplemental astaxanthin until the trout





are ready for market, producers could discontinue feeding astaxanthin at 55 d pre-harvest and still maintain the fillet color so desired by consumers. With astaxanthin accounting for as much as 10%-15% of total feed costs, the cessation of feeding astaxanthin for nearly two months would produce considerable cost-savings [3].

López-Luna noted no difference in fillet  $L^*$ ,  $a^*$ , and  $b^*$  values of rainbow trout previously fed astaxanthin after three days of starvation at 11.5°C (34.1 degree-days, whereby one degree-day equals 24 hours at 1°C) [11]. Our study extends this timeframe out to 605 degree-days (55 days at 11°C). In frozen fillets from Atlantic salmon starved for up to 86 days after being fed a diet containing astaxanthin, no differences in hue or red ( $a^*$ ) values were observed by Einen and Thomassen [10]. The lack of decrease in fillet coloration over 55 days likely reflects the ability of rainbow trout muscle to store relatively large amounts of astaxanthin [15].

The color values are well within the range reported for rainbow trout in other studies involving astaxanthin [6,11,12,16,17]. Although the results from this study are likely applicable to other strains of rainbow trout, the influence of other dietary factors on astaxanthin absorption and ultimately fillet coloration could potentially influence the results if different diets are used [18].

This study only examined the loss of coloration in trout fillets. It does not address any potential issues that recreational anglers or consumers may have about the use of synthetic astaxanthin in hatchery diets [19,20].

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