ACHIEVING NATURAL COLORATION IN FISH UNDER CULTURE

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ABSTRACT

Fish that are colored in nature often acquire faded coloration under intensive culture conditions. Experiments adding top-coated algae to the diets of ornamental fish have resulted in color enhancement. Freshwater red velvet swordtails *Xiphophorus helleri,* rainbowfish *Pseudomugil furcatus*, and topaz cichlids *Cichlasoma myrnae* became significantly more intensely colored when fed a diet containing 1.5-2.0% of a carotenoid-rich strain of *Spirulina platensis* and 1.0% of a specially grown *Haematococcus pluvialis* for 3 wk. Though color enhancement was apparent after only a wk, when the fish consumed these doses of algae, lower doses (0.5% and 0.4%, respectively) were not significantly different for kissing gouramis *Helostoma temmincki*, 24 K mollies *Pachouli latipinna*, and rosy barbs *Barbus chunkiness*, were examined after the 3-wk feeding period. Both treatments were significantly more effective than control treatments with no added carotenoid, and better than treatments with traditional carotenoid sources. Color enhancement appeared to occur via natural carotenoid receptors. Thus, color intensity diminished when fish were stressed, coloration appeared only in males in species where only the males are normally colored, and between rosy barbs and topaz cichlids color enhancement was environment-sensitive. Topaz cichlid color developed only after the aquaria were divided into territories and rosy barb color intensified when floating substrate was present. It is concluded that ornamental fishes are good models for color enhancement through diet and that this enhancement may be achieved using products made by marine biotechnology companies.

INTRODUCTION

The fledgling freshwater ornamental fish industry in Hawai‘i has experienced the problem of faded coloration in fish, especially those grown in clear water. These fish are traditionally rejected by ornamental fish wholesalers, leaving the growers with fish that can’t be sold or fish sold at a very reduced price. For this reason it was decided to investigate fish coloration. The current work was based on prior studies using cultured clownfish *Amphiprion ocellaris* and *Premnas biaculeatus* which only achieved color patterns and intensities seen in fish in the wild when treated for 2 wk by incorporating astaxanthin into the diet (Ako and Tamaru 1999). Several species of fish at the Waikiki Aquarium and Sea Life Park on O‘ahu, Hawai‘i, USA, responded similarly, and maintenance of the bright coloration appears achievable with incorporation of 25 mg/kg astaxanthin into the various diets on a continual basis. However, chemically synthesized astaxanthin is a different stereoisomer from natural astaxanthin (Ako and Tamaru 1999) and is difficult for the small grower to incorporate into fish diets. Fortunately, carotenoid sources were available from a marine biotechnology company in Hawai'i in the form of carotenoid-rich strains of *Spirulina platensis* and specially grown *Haematococcus pluvialis*. Incorporation of these algae into the diets of ornamental fish is the subject of this report.

MATERIALS AND METHODS

All fish were treated (fed enhanced diets) in clear water aquaria equipped with biofilters. They were fed three times/d with meal sizes adjusted so that very few feed particles (<5% of the applied dose) remained in the aquarium after 10 min. All treatment periods were a duration of 3 wk.

Treatment algae containing carotenoids were obtained from Cyanotech Corporation (Keahole, Hawai'i, USA) and were added to the high palatability salmon fry feeds we are recommending to ornamental fish farmers (Tamaru and Ako 1997; Ako et al. 1997; Tamaru et al. 1998; Ako et al. 1999). The algae easily adhered to the 14% lipid feed particles.

Swordtail fish of the red velvet variety were obtained from grazed down (clear water) ponds. They were treated with the feed described above ± 1.5% *Spirulina* and 1% *Haematococcus*. Rainbowfish were obtained from clear water, indoor aquaria and treated with the feed containing 2% *Spirulina* and 1% *Haematococcus*. Control fish of each species were fed a flake feed which reported no source of carotenoids in the listed ingredients. Topaz cichlids were obtained as juveniles and were reared in the same way as the rainbowfish. Treated cichlids were fed as described above but control fish animals were fed a commercial cichlid feed containing alfalfa meal as the carotenoid source. Among the cichlids, no color changes were observed after 3 wk and the experiment was continued for an additional 3 wk after bottles were put into the tanks to provide territories.

The rosy barbs used in the experiment were obtained from a grower who had reared the fish in green water. Because the fish has bright coloration when obtained, they were held for 1 mo. in shaded, algae-free aquaria before the trials began. The rosy barbs were treated with one of four diets: a flake with no added carotenoid source, a flake with traditional carotenoids, a flake incorporating 0.5% *Spirulina* and 0.4% *Haematococcus*, and a flake with 1.5% *Spirulina* and 1.0% *Haematococcus*. Kissing gouramis and 24K mollies were also obtained from growers but were from shaded aquaria containing no algae and they received the same diets as the rosy barbs.

Color was judged by test panels of persons randomly recruited from around the biochemistry laboratory at the University of Hawai'i. The treatments were not revealed to the individuals who were asked to rank the fish according to intensity of color. Color ranking was by a score of 1-4 (one being the lowest) for fish with four treatment groups. Ties were allowed but did not often occur. Color rankings were either one or zero for fish of two treatment groups with the score of one relating to more intensely colored fish. Scores were subjected to ANOVA or *t*-test. In all cases, pairs of control and treatment fishes were photographed and can be seen at http://www.sandersbshrimp.com.

RESULTS

Panel members clearly identified the most intensely colored swordtails, rainbowfish and topaz cichlids. Those treated with *Spirulina* and *Haematococcus* (biotech algae) received significantly higher scores than control fish fed no carotenoid (Table 1).

Table 1. Mean scores of treated and control fish. Different alphabetical designations indicate significantly ($P<0.05$) different scores.

Fish	Number of panelists	SCORES Control	Experimental
Swordtails	8	0.0b	1.0a
Rainbowfish	15	0.1b	0.9a
Topaz cichlids	13	0.0b	1.0a

While the panelists convened after the 3-wk treatments, differences were already noticeable between treated and control swordtails after only 1 wk. It should be emphasized that, unlike some of the other fishes tested, only male rainbowfish and topaz cichlids acquired color.

Rosy barbs fed the *Spirulina* and *Haematococcus* were judged to be significantly (P<0.05) more intensely colored than rosy barbs given feed containing no carotenoids (control) or those given control feeds containing traditional carotenoid sources (traditional) (Table 2). There was a dose effect of low (low biotech) or medium (med biotech) levels of *Spirulina* and *Haematococcus* in the feeds but the effect was opposite of expectations. The 24K mollies fed *Spirulina* and *Haematococcus* were significantly (P<0.05) more intensely colored than those fed no carotenoids or traditional sources of carotenoid though there was no detectable dose effect among fish fed *Spirulina* and *Haematococcus*. Kissing gouramis fed carotenoids were significantly (P<0.05) more intensely colored than those fed no carotenoids.

Table 2. Mean scores of treated and control fish. Different alphabetical designations indicate significant (P<0.05) differences.

Fish	Number of panelists	SCORES Control	Traditional	Low biotech	Med biotech
Rosy barbs	16	1.7d	1.9d	3.9a	3.0b
24K mollies	16	1.9c	1.4c	2.8a	3.6a
Kissing gouramis	16	1.0cd	3.0a	2.6a	2.8a

DISCUSSION

The treatments reported here are efficacious. Since they use natural algae that mimic the absorption of carotenoid that occurs in the wild, they should be more acceptable to consumers who may have concerns about the use of chemicals or hormones to enhance color.

A “cocktail” of algae supplying natural stereoisomers of ß-carotene, zeaxanthin, lutein, canthaxanthin, and astaxanthin was used in the current work. The cocktail approach was taken because fish sometimes seem to metabolize carotenoids before depositing them onto natural receptors in the skin in a species dependent way (Miki et al. 1985; Matsuno et al. 1985; Katsuyama et al. 1987; Katsuyama and Matsuno 1988). This was observed in preliminary results whereby the blue-green fluorescent colors in discus fish *Symphysodon* var. seemed to be enhanced by feeding sources of ß-carotene and red colors seemed to be enhanced by feeding sources of canthaxanthin and astaxanthin. However, the red color of red swordtails and tinfoil barbs *Barbus schwanenfeldi* seemed to be enhanced by ß-carotene sources.

The test panels used to judge color intensity in the current work were modeled after panels used to judge taste and texture of tropical fruits. Panelists were blinded as to treatments and did not interact with those who recorded results, as the latter stood physically behind the panel members. Chemical extraction (Miki et al. 1985; Matsuno et al. 1985; Katsuyama et al. 1987; Katsuyama and Matsuno 1988) and fiber optic reflectance spectrophotometry (Wallat and Lazur 1999) are other methods of quantifying color intensity. The test panel method, however, offers the advantages of low cost and convenience but color photographs (which are expensive to produce) have an immediate and long-lasting impact. Photographs of control and experimental swordtails and rainbowfish may be viewed on the Internet at http://www.sandersbshrimp.com.

The current work should be of immediate interest to pet feed manufacturers who service customers who keep their fish in clear water aquaria. The coloration of pet fish will fade if the fish are not provided with carotenoids. Traditionally, manufacturers have used complex formulations which may result in reduced palatability when introducing carotenoids into their feeds. The current work may also be of interest to ornamental fish feeds manufacturers who service growers with grazed down or otherwise clearwater systems, but it should be noted that ornamental fish growers do not use large amounts of feed because their fish are small. The largest market for the enhancement formulas may be food fish growers who wish to color the flesh of their fish or who grow fish such as the red sea bream that lose their coloration under culture. The algae described here work well to provide color and also offer advantages in application. They may be used to topcoat other feeds, which is appealing in simplicity, reducing losses during extrusion. Because the algae used in this study are natural products, their use would negate possible hesitation of the public to consume food fish grown on chemically produced algae and satisfy those who want to feed more "organic" feeds to their pets.

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