



Clownfish Astaxanthin Study

Executive Summary

This independent study was conducted by Virginia Tech – Virginia Seafood Agricultural Research and Extension Center (AREC) as part of an ongoing, integrated collaborative research program between AREC, Mid-Atlantic AquaTech, and Reed Mariculture.

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Key Findings

According to both objective and subjective parameters, the most desirable market coloration in the *Amphirion ocellaris* ("false *Percula* clownfish") can be achieved by a formulation where high-quality food is 'top-dressed' with the marine microalgae *Haematococcus Pluvialis* in a quantity sufficient to result in 400 ppm of the red carotenoid astaxanthin. Ideal coloration is achieved with the right balance of hue, saturation and luminosity.



**0 ppm
Astaxanthin**

Test Tank 12a



**250 ppm
Astaxanthin**

Test Tank 23a



**400 ppm
Astaxanthin**

Test Tank 3b

Research Note

The fish in these photos are alive. According to the researchers, "A thin film of water was placed in the weigh boat along with the fish to ensure their health and safety."



Background

A study of the effects on clownfish coloration when different levels of astaxanthin top-dressing of TDO were utilized, was performed at the Virginia Tech – Virginia Seafood Agricultural Research and Extension Center (AREC) under the mentorship of Steve Urick, Lab and Research Specialist. The research was carried out by two students from the Governor's School for Science and Technology (Virginia), Cassidy Clark and Lauren Cook, graduating class 2016.

"In their senior year, students design and conduct a year-long research project under the direction of a scientific professional in their field of interest. The field component is supported by an in-school course which guides students through the entire process, from the selection of a problem to the final presentation. Major aspects of the mentorship experience include: preparation of a formal written proposal for their project, oral presentation of the proposal and a status report at mid year to GSST faculty, a final research document, and presentation of final results to a panel of professionals in appropriate fields at the GSST Spring Symposium."

Reference

<http://nhrec.org/~shelley.sykes/sciencejournal2016.pdf>

Each student wrote a "final research document" with the title "Determining an Optimum Level of Dietary Astaxanthin to Improve Coloration of *Amphiprion ocellaris*." This is a summary of their findings.



Methodology

The study tested *Amphiprion ocellaris* (popularly called “false Percula Clownfish”) fed Reed Mariculture’s TDO Chroma Boost™ fish feed with the red carotenoid astaxanthin added at levels of 0, 150, 250, 400, 640, or 1020 ppm (parts per million, dry weight basis) for seven weeks.

There were 40 juvenile fish per astaxanthin level, ten fish in each of four tanks for scientific replication for a total of 240 fish.

Results were judged objectively by color analysis (hue, luminosity, and saturation) of photographs of the fish using an HSL color algorithm from WorkWithColor.com (Ho et al 2014).

Subjective evaluations were made by a panel of six local pet store owners/managers who were experienced in commercial sale of clownfish and were familiar with customer color preferences.

All panel members were first tested to confirm that they could visually discriminate between different hues and luminance of colors by means of triangle tests. Then they assigned a color preference score ranging from 0 (lowest preference) to 9 (highest preference) to fish at each astaxanthin level.



Triangle Test Setup

Three sets of tanks, composed of three tanks each, were used to determine panel members' ability to distinguish the difference in hue and luminance. The contents in one tank in each group was different from the other two.



Six Tank Setup

A six tank setup was used for judging subjective preference.





Results

The objective color analysis demonstrated a consistent trend: as the astaxanthin level in the feed increased the color of the fish shifted from orange toward red (hue), and the color became more intense (increased saturation) and darker (reduced luminosity).

In the subjective evaluations, the clear favorite was 400 ppm with an average score of 8.3. All other scores were considerably lower.

Astaxanthin Level	Average Preference Score
0 ppm	2.8
150 ppm	4.8
250 ppm	5.3
400 ppm	8.3
640 ppm	5.2
1020 ppm	4.8

It is noteworthy that although the highest astaxanthin levels resulted in a more intense red color, this was not the most preferred. This is probably because as the astaxanthin level in the skin of the fish becomes very high, although the color appears more intense (more saturated) it is also darker (lower luminosity), resulting in a less “colorful” visual impression.

For more information on aquaculture, please visit the [Reed Mariculture website](#).