You Are What You Eat
Or
How Did the Clownfish Get Brighter Stripes?

A classroom activity based on the senior project of
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Tank Raised Clownfish

The ever increasing trade in coral, reef fish, live rock, and other reef organisms contributes significantly to the decline and destruction of coral reefs. Many marine species marketed in “tropical fish” stores are collected by destructive methods, such as using dynamite to stun fish, collecting live rock, and using cyanide to poison fish, that are extremely harmful to their reef home. Wild fish captured by these methods also tend to have a very low survival rate after collection because they are stunned or harmed during collection which causes death during the transportation period.

A recent program (see link below) at the University of Maine (UMaine) is attempting to reduce the need for the wild harvest of tropical reef fish by raising fish in captivity. Fish coloration is important to the attractiveness and can affect the marketability of fish raised in captivity. Along with good vibrant color, good health and size are also key factors important to the sale of these fish. A large number of studies have shown that natural diets available to fish can impact their coloration and growth. A major challenge for aquarists interested in culturing ornamental reef fish as an alternative to wild harvesting is to produce fish that have the same vibrant colors as those found in the wild.

Carotenoids

Nearly all living things obtain their color from natural pigments. Some of the most common natural pigments are compounds known as carotenoids. Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Some familiar examples of carotenoid coloration are the oranges of carrots and citrus fruits, the reds of peppers and tomatoes, and the pinks of flamingoes and salmon.

Carotenoids are fat-soluble pigments often found in plants, algae, and some types of bacteria, where they are important to photosynthesis. Generally, animals cannot make their own carotenoids and instead obtain these pigments from their diet (you are what you eat). Thus, a flamingo’s feathers and a salmon’s flesh are only pink or reddish if they obtain carotenoids in their diet.

Carotenoids and Clownfish

The bright and intricate coloration of ornamental fish is the major reason these fish species are being exploited from tropical reefs around the world. High-quality coloration in the clownfish is very important to sales and trade of these fish. Typically, aquarists check to see that fish have good color (pigmentation), followed by good body shape, fin shape and body size. Clownfish, like other animals, cannot produce their own carotenoids and these compounds must be supplemented through their diet. Fish in their natural habitat tend to consume plants and organisms containing carotenoids. Captive raised fish must be fed diets that contain these carotenoids in order to develop bright colors.

The project you are embarking on asks what affect carotenoids have on the color of clownfish (species name *Amphiprion ocellaris*) by feeding juvenile clownfish diets that have very different nutritional profiles. Methods for raising clownfish in captivity are well-established and include a variety of diets that promote growth and survival. More information on the source of fish can be found at the UM website http://www.umainetoday.umaine.edu/issues/v7i5/clowns.html.

The Hypothesis

Scientific experiments are set up to test a specific hypothesis. A hypothesis is a prediction of what will happen when we run an experiment. In the case of our clownfish experiment we are testing the hypothesis (prediction):

Clownfish that are fed a diet with more carotenoids will develop skin color that is brighter and, in this case, a deeper orange.

Two different types of diets will be fed to two groups of clownfish in our experiment. The "control" or regular diet has a low amount of astaxanthin, a common carotenoid found in fish food. In contrast, the "experimental" diet has a higher amount of astaxanthin. Although the specific diet used in your experiment may differ from those in Table 1, notice how the *Cyclop-eeze* diet contains almost six times as much astaxanthin as the *Golden Pearl Diet*. The diets you will be using similarly have very different amounts of astaxanthin and another carotenoid canthaxanthin.

Table 1

Parameters	Golden Pearl Diet	Cyclop-eeze
Protein	60	60
Lipid	18	35
Ash	15	3
Carbonhydrate		2
Astaxanthin	500 ppm	2,867 ppm
Canthaxanthin		15 ppm

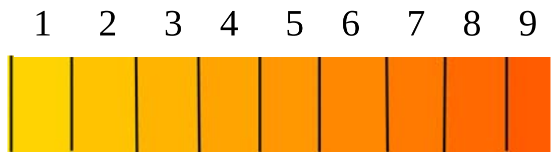
The Experiment

You have been provided a basic description of how to care for your clownfish in a separate document. What follows are the details for an experiment testing our hypothesis.

1. Introduce approximately 5 clownfish to each of two 10 gallon aquaria. The fish should be allowed to adjust (acclimate) to the aquaria for up to two weeks before the experiment is started. During this time both groups of fish should be provided with the standard, low astaxanthin control diet.

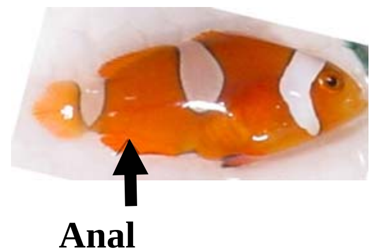
2. After the adjustment period, the fish in the "experimental" tank should be switched to a diet containing 50% cyclop-eeze diet/50% control diet. However, both tanks should be fed the same total amount of food at each feeding.

3. After the experimental tank has been switched to the high astaxanthin diet, the color of the fish in both tanks should be monitored using the accompanying clownfish color index chart.



Color Index

We recommend that the color of the fish be determined in the tank. That is, do not remove the fish from the tank as this may cause undue stress. However, try to ensure that the background color for the tank is the same for both tanks as different "backgrounds" will affect the students' ability to discern color differences for the fish. For example, you might place a white piece of paper over the back of each tank when determining fin color.



Have several students judge the color of the anal fin for each fish in each tank to see how consistent the scores are. Record the average value for each fish on each day after the new diets have been introduced.

4. Depending on grade level, you can graph the color for each fish in each tank on the Y axis with day on the X axis. Alternatively, students can calculate and graph the mean color and standard error for each tank during each day of the experiment.

5. Any differences should be evident in 7 to 9 days, although if interest level is high the experiment can be continued for 28 days or more.

6. An example from Gordon Lorenson's project is given in Figure 1.

Where does this project fit within a science curriculum?

There are any number of places where you might be able to incorporate this activity into what you already teach. We have identified several potential links (along with appropriate Website links) and we will probably think of more during the workshop. Our goal is to build text and web resources to support you so let us know where you think this activity fits in your classroom if you are thinking of adopting it. We have also included several examples of follow-up information for some of these links for your consideration.

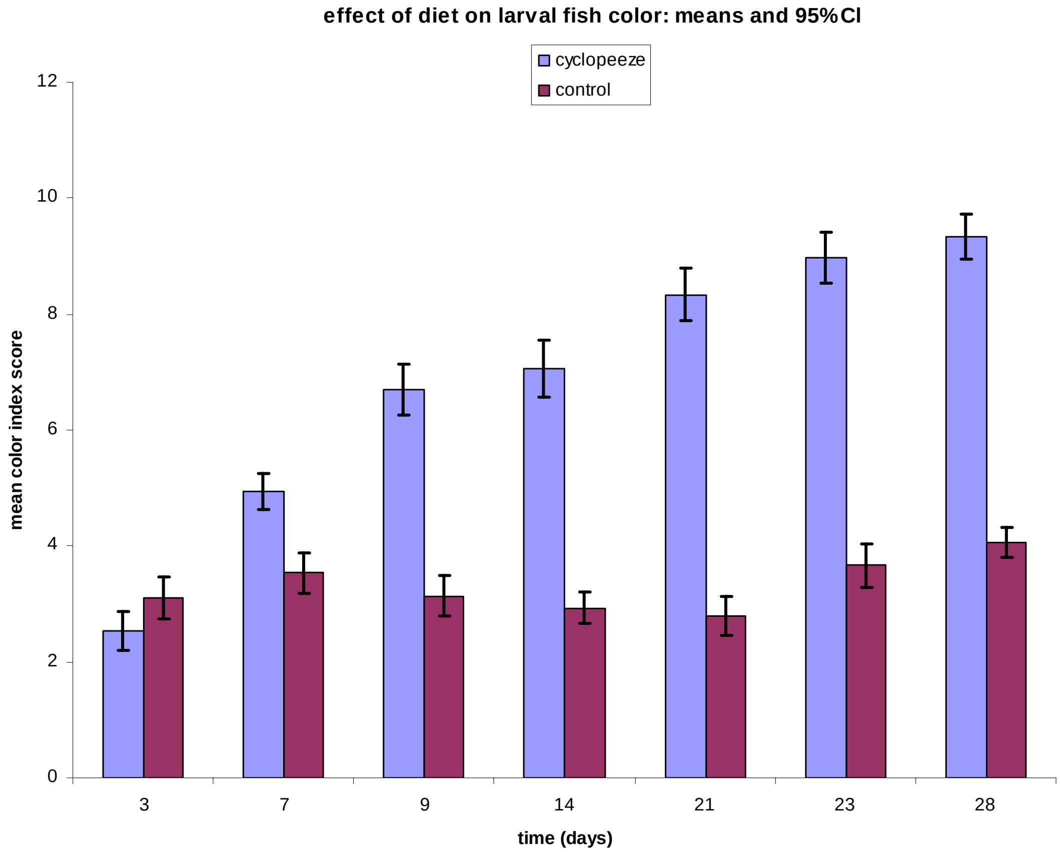


Figure 1. The effect of diet on coloration of juvenile *Amphiprion ocellaris.* Fish fed the cyclop-eeze diet had significantly higher color scores. Mean color scores are plotted; error bars indicate 95 % confidence intervals (N= 30 fish per treatment per time point).

Clownfish Activity "Links"

I. General Information on Clownfish from the Tree of Life project

- http://www.tolweb.org/treehouses/?treehouse_id=3390

II. Links to Ecology and the Environment

Overfishing and Destruction. For a more in-depth discussion of how fishing activities, including those for collecting fish for the aquarium trade, are having a toll on reef ecosystems:

- http://www.cotf.edu/ete/modules/coralreef/CRanthro.html
- http://oceanworld.tamu.edu/students/coral/coral5.htm

Symbiosis. There are many symbiotic relationships between coral reef inhabitants of which the clownfish and the anemone is only one example.

- http://www.tolweb.org/treehouses/?treehouse_id=3390 (tree of life)
- http://www.ms-starship.com/sciencenew/symbiosis.htm

Coral Reef Food Web. Clown fish are only one part of a much larger food web. You can build on the need for clown fish to get pigments from primary producers as a lead in to broader discussion of how material and energy flows through a food web.

- http://www.teachersdomain.org/resources/hew06/sci/life/reg/foodweb/index.html

III. Pigments (Chemistry)

Leaf Chromatography. Pigments abound in plants which are the primary source for pigments in animals. One can link the clownfish activity with a related exercise examining pigments in leaves.

- http://scidiv.bcc.ctc.edu/rkr/Biology101/labs/pdfs/Chromatography101.pdf (a copy is included in this writeup)

Other examples include the oyster and salmon examples that are also included in this section.

- http://www.oysters.us/green-oysters.html

IV. Carotenoids and Human Nutrition

Carotenoids are an important part of human nutrition (e.g., role as antioxidants) and like clown fish we derive them from the food we eat. The following link provides more background on the topic.

- http://www.astaxanthin.org/carotenoids.htm

V. Marketing

The experiment that our clownfish exercise was based on also examined whether fish that are more vibrant might be in higher demand and therefore 1) reduce demand for wild caught fish and 2) fetch a better price and in this way help support development of the captive production of clownfish. The oyster and salmon links also examine how color of product affects marketability. Thus, the exercise can also be used as a lead-in or tie-in to other topics.

Follow-Up Information

I. Green Oysters Anyone?

What might sound to some of us like something right out of "Green Eggs and Ham" by Dr. Seuss, is actually a prized specialty: green oysters from Marennes-Oléron! They are known as "*Les Vertes*" ("the green ones"). Hence, the oysters offered in Marennes-Oléron can be labeled either "*blanche*" (or not at all in terms of a color), which usually describes any oyster with the typical light "brownish or grayish" meat color tone, or be labeled "*verte*", which tells the oyster lover that the meat will definitely reveal plenty of green coloring.

If oysters are given the opportunity to feast on a particular micro-algae species called the "*Navicule bleue*" ("*Blue Navicula*"), scientifically referred to as "*Navicula ostrea*" or "*Haslea ostrearia*", a good portion of their meats will gain an emerald green color. Marennes-Oléron is most famous for its "green oysters". Long ago, as the story goes, the sun king Louis XIV, ordered a big batch of oysters from Marennes-Oléron. His new bride at the time, Madame de

Maintenon, upon visiting the royal kitchen, discovered that the flesh of the oysters was partially green. Horrified, and certain that they were an obvious attempt to poison the king, she ordered all the oysters to be discarded at once. She then immediately informed the king of the thwarted attempt on his life. The king, likely cringing at the thought of the tragic loss of these delicious oysters, put her at ease, explaining that these had been his beloved green oysters from Marennes-Oléron.

Although this special micro-algae species grows naturally in the claires, private marine laboratories also produce this type of algae and sell lots of it to French oyster cultivators.

http://www.oysters.us/green-oysters.html

John McCabe

II. Coloration in Salmon

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McGill

The Color of Salmon

You really can't judge a fish by its color. Especially when it comes to salmon. Wild salmon get their pink flesh from astaxanthin and canthaxanthin, compounds that are found in plankton, algae and crustaceans, foods that foraging salmon feast on. Chemically, these belong to the family of carotenoids, substances that are also responsible for the color of carrots, tomatoes and watermelon. Most of the salmon consumed today, however, come from fish farms and have never seen a pink crustacean. What they see are dyed pellets of fish food. Now that isn't as bad as it sounds because the dyes used are the same ones that dye the flesh of wild salmon. Fish farmers can have their food pellets custom colored to produce whatever hue of salmon they desire. The astaxanthin and canthaxanthin can be produced synthetically, or can be isolated from yeasts or algae. Canthaxanthin is also added to chicken feed to make egg yolks and carcasses more yellow. So, is there a concern here? Well, there may be an ethical issue because coloring allows farmed salmon to pose as wild in the supermarket and some people prefer wild salmon because it is less likely to be polluted by PCBs. These substances come from the food pellets that farmed salmon are fed, which often are made from fish caught in polluted waters. But what about the astaxanthin and canthaxanthin themselves? Do they pose a risk?.

It turns out that a high intake of canthaxanthin can cause something called "canthaxanthin retinopathy". Canthaxanthin and its metabolites can cause crystallization around or on the retina, blocking nerve signals and causing 'white flashes' and other such visual problems. Though usually this occurs at higher doses, the condition develops sooner in those with already diseased retinas. For this reason, canthaxanthin content of animal feed is being carefully regulated. Presently, the acceptable daily intake of canthaxanthin for a human is 0.03 mg/kg of body weight, yet the average intake from just one serving of fish can be over twice that amount. For this reason, the Scientific Committee on Animal Nutrition lobbied the European Commission of Health and Consumer Protection for the lowering of the accepted maximum content of canthaxanthin in food for fish and chickens to 25 mg/kg of feed. This would bring a human's daily intake down to the accepted level. The risk of retinal problems from eating fish is very slight, but canthaxanthin retinopathy has been observed in people who have tried to dye themselves from the inside out. Sunless tanning pills called Canthorex have been marketed to people who wanted to look as if they had a healthy tan but did not want to risk lying in the sun. These capsules did the same for humans as for salmon. It increased their chance of success in the market place. But it also increased their chance of eye problems. The daily intake of canthaxanthin in the pills recommended by the manufacturer was 4 mg/kg of body weight, over one hundred times more then the acceptable daily intake in Europe. No surprise that cases of canthaxanthin retinopathy have been observed in long-term users of oral tanning agents.

by Joe Schwarcz

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