

Chapter 2

Color Control in Shrimp

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Introduction

The six experiments on crustacean color change presented here are relatively simple and inexpensive and are suitable for use in introductory biology or more advanced animal physiology or behavior classes. They introduce students to an interesting physiological phenomenon that can be measured quantitatively by assessing the degree of pigment dispersion in chromatophores. The large number of experiments described here would not all fit into a standard 3 hour laboratory period, but instructors can select experiments that are appropriate to their students needs and the available time.

The marine sand shrimp, *Crangon septemspinosus*, are used in these experiments as they are fairly abundant in the inter-tidal waters of Atlantic Canada. However, many other marine crustacean species could be used instead, such as isopods, shrimps, and crabs. Addresses of possible suppliers are given in Appendix A. Lower vertebrates such as small fish (perch, minnows, mummichogs (*Fundulus*) or tadpoles also exhibit color change and have easily visible chromatophores. The number of animals used in each experimental group can easily be altered depending on the availability of animals, time constraints, etc. You may wish to increase the number of shrimp per group if your students are going to statistically analyze the data.

These experiments lend themselves well to independent research projects as well as more structured formal laboratories. I give my third-year comparative animal physiology students a list of references and ask them to design experiments to investigate the answer to the hypotheses described in Parts A, B, and C, leaving them about 5 weeks to do the reading, experimental work, and submit a report. There are many variables that should be controlled or taken into consideration during the experimental design: including temperature, salinity, background color, light intensity and wavelength, circadian and tidal rhythms, sex, size, molt stage, and reciprocal effect of other animals. The following experiments will try to control for as many of these variables as possible.

In Part A, students examine the effect of several environmental factors (background color, light intensity, light wave-length, and temperature) on pigment dispersion. In Part B, students locate the site of the receptors that bring about color change to determine whether the control is primary (direct action on chromatophores) or secondary (involves other receptors). Finally, in Part C they determine the type of communication (hormonal or nervous) between the receptors and the effectors (the chromatophores). Ideas for additional experiments are also provided.

Materials

All materials given are per experimental group.

Experiment 1: Background Color

16 shrimp; 5 1-liter glass beakers; sand-colored, yellow, red, and white paper to cover sides and bottom of beakers; dissecting microscope; thermometer; petri dish; scissors, tape; sea-water.

Experiment 2: Light Intensity

16 shrimp pre-adapted to sand-colored background; 4 1-liter glass beakers; sand-colored paper to cover sides and bottom of 4 beakers; light meter (photographic type); dissecting microscope; thermometer; petri dish; scissors, tape; sea-water.

Experiment 3: Light Wavelength

16 shrimp pre-adapted to sand-colored background; 4 1-liter glass beakers; white paper; 4 dissecting microscopes with colored filters (transparent plastic, available from art suppliers, taped over the light source); dissecting microscope; 4 large boxes to cover dissecting microscopes; thermometer; 4 petri dishes; sea-water.

Experiment 4: Effect of Temperature

12 shrimp pre-adapted to sand-colored background; 3 1-liter glass beakers; clear plastic bags to surround 1-liter glass beakers and elastic bands; sand-colored paper; 3 water baths set for 5°, 15°, and 25°C (ice can be used for 5° and 15°C if cooling baths are unavailable; four groups can share 1 water bath); dissecting microscope; scissors, tape; 3 thermometers; petri dish; sea-water.

Experiment 5: Painting Eyes to Block Light Entry

16 shrimp pre-adapted to a sand-colored background; 3 1-liter beakers; white and black paper; white typewriter correction fluid (Liquid Paper); dissecting microscope; thermometer; scissors, tape; petri dishes; sea-water.

Experiment 6: Injection of Crude Extracts of Color Control Hormones

8 intact shrimp (pre-adapted to sand-colored background); 8 shrimp with eyes covered with Liquid Paper; 2 1-liter beakers; sand-colored paper; 8 1-ml disposable syringes (25-gauge needle); crustacean saline (see Appendix B); crude eyestalk extract from shrimp (see Appendix B); dissecting microscope; thermometer; scissors, tape; petri dish.

Student Outline

Introduction

Crustaceans are able to change their color or shading in response to numerous environmental changes, and can exhibit a great variety of pigment colors (for reviews see Fingerman, 1970; Florey, 1966; Hoar, 1983; Prosser, 1973). This ability to match body color with their background environment provides a useful mechanism for avoiding predators. We will be studying color change in the marine sand shrimp, *Crangon septemspinus*, as it is fairly abundant in the inter-tidal waters of Atlantic Canada and its color change mechanisms have been well studied. However, many other marine crustacean species could be used instead.

The ability to change body color to match environmental changes (physiological color change) is brought about by pigment movements within the chromatophores in crustaceans. There are four primary pigment colors (yellow, red, silver, and black) observable in the chromatophores of *Crangon septemspinus*, but the predominant and easiest to stage are the black-brown ommochromes. We will use a 5-stage chromatophore index to quantify the degree of brown-black pigment dispersion in the ommochromes which produces the changes in color and shading (Figure 2.1).

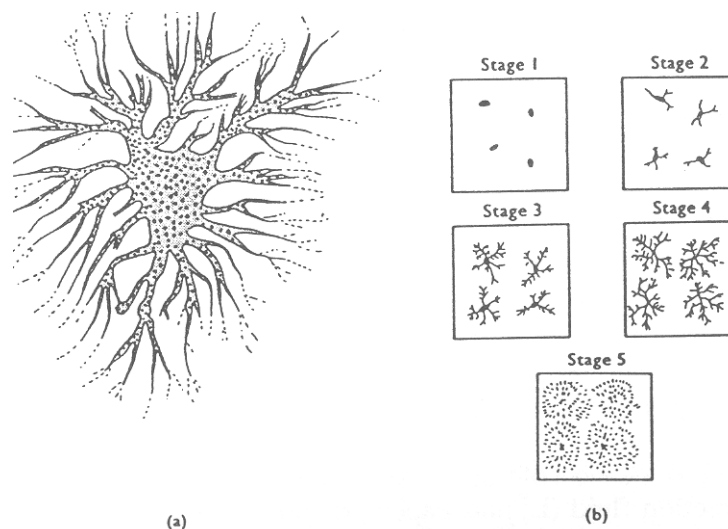


Figure 2.1. (A) Diagram of a chromatophore from the uropod of the prawn, *Leander serratus*, with the pigment fully dispersed. (B) The 5-stage chromatophore index showing the stages of pigment dispersion, from Stage 1 (fully concentrated) to Stage 5 (fully dispersed) (Highnam and Hill, 1977). Reprinted with permission.

In the following experiments we will examine the effect of several environmental factors (background color, light intensity, light wave length, and temperature) on pigment dispersion or color change (Part A). In Part B we will perform an experiment to locate the site of the receptors that bring about color change to determine whether the control is primary (direct action on chromatophores) or secondary (involves visual receptors). Finally, in Part C we will perform an experiment to determine the type of communication (hormonal or nervous) between the receptors and the effectors (the chromatophores).

There are many variables that should be controlled in these experiments: including temperature and salinity of sea-water, background color, light intensity and wavelength, circadian and tidal rhythms, sex, size, molt stage, and reciprocal effect of other animals. We will try to control for as many of these variables as possible during the following experiments. By doing the experiments at the same time of day circadian and tidal rhythms should be negated. Any changes in temperature should be noted during the experiments, but they will probably be minimal. It is best to use animals of approximately the same size as larger animals have more dispersed chromatophores. Determining sex and molt stage is too time consuming, but avoid using egg-bearing females and keep records of shrimp that molt during the experiments.

Part A: The Effect of Environmental Factors on Color Change

Many environmental factors are known to affect pigment dispersion in the chromatophores of shrimp. Background color, light intensity, light wave-length, and temperature will be studied in these experiments. Alterations in pigment dispersion with changes in background color or shading are known as albedo responses. They depend on the ratio of incident to reflected light, so that the dark pigments disperse on a dark background as it reflects less light, while the reverse happens on white backgrounds. Decreases in light intensity will decrease the amount of incident and reflected light and will probably cause dispersion of the dark pigments as the reduced light will make the background appear darker. Light of different wavelengths should have no effect on the albedo response, but red and yellow light causes red and yellow pigments to appear in the center of each chromatophore. At higher temperatures the dark pigments usually concentrate in the center of the chromatophore so the shrimp appear lighter and will reflect more light from their body surface and therefore absorb less heat.

Experiment 1: Background Color

1. Surround the sides and bottom of a 1-liter glass beaker with sand-colored paper.
2. Cover the remaining four beakers with yellow, red, white, and black paper.
3. Fill all beakers with sea-water and record water temperature.
4. Place 16 shrimp in the sand-colored beaker. These shrimp must have been pre-adapted to a neutral (sand-colored) background for 30 minutes to ensure uniform chromatophore dispersion.
5. Stage 20 body chromatophores on each shrimp by placing each shrimp on a petri dish under a dissecting microscope. Recording the degree of black pigment dispersion using the 5-point chromatophore index given in Figure 2.1. Place a piece of sand-colored paper under the petri dish while staging to ensure constant color. Stage chromatophores from the same body region each time to eliminate any differences. Note any red- or yellow-colored inclusions in the center of each chromatophore. This will be the time zero staging.
6. Now place four of these staged shrimp in the yellow-covered beaker, and four in each of the red-, white-, and black-covered containers. Note the temperature of the water in each container. Allow the shrimp to adapt to their new background colors for 30 minutes. Record changes in water temperature.
7. After 30 minutes stage 20 chromatophores on each shrimp using a dissecting microscope. Note any colored inclusions. Remember to place colored paper under the petri dish during staging to match the background beakers.

- Calculate a total chromatophore number for each shrimp by multiplying the number of chromatophores at each stage by the stage number, and totaling these numbers, as shown in Table 2.1.

Table 2.1. Calculation of total chromatophore number. The total chromatophore number for 20 chromatophores is 35.

<i>Shrimp</i>	<i>Chromatophore Stage</i>		<i>Number of Chromatophores</i>		
#1	1	X	10	=	10
	2	X	5	=	10
	3	X	5	=	15
	4	X	0	=	0
	5	X	0	=	0
		Total =	20		35

- The mean chromatophore numbers can then be determined for each group of shrimp on the different background colors. The observed differences can be statistically analyzed. Generally, the darker the background color then the greater the pigment dispersion within the chromatophores, and thus a higher chromatophore index is obtained.

Experiment 2: Light Intensity

- Take four 1-liter glass beakers and cover the bottom and sides with sand-colored paper.
- Make a round paper lid to cover the top of one beaker, and then make paper lids to cover three-quarters and one-half of two beakers, and leave one with the top uncovered.
- Measure the light intensity inside each beaker using the light meter provided. The light meter measures either in foot-candles or lux (metric).
- Fill each beaker with sea-water and note the temperature.
- Take 16 shrimp that have been pre-adapted on a sand-colored background for 30 minutes and place four in each beaker. in the same body region as described in Expt. 1.
- Put the lid and partial lids on the beakers leaving the control exposed to the room light.
- Let stand for 30 minutes and then re-stage the chromatophores. Record the water temperature.
- Calculate the chromatophore numbers for each shrimp, and determine the means for each group. The shrimp in the completely covered beaker should darken the most (have the highest chromatophore number) and the partially covered shrimp should show the least pigment dispersion.

NOTE: If you have access to a darkened room, this experiment can be performed using a bench lamp as a light source and placing glass tanks containing shrimp at different distances from the light source to give variations in light intensity. If larger containers are used the water will not warm up as much. A tank containing water only can be placed immediately in front of the lamp to absorb radiated heat.

Experiment 3: Light Wavelength

1. Tape colored transparent plastic (red, yellow, blue) over the lights of three dissecting microscopes. One microscope should have no plastic filter.
2. Place a 1-liter glass beaker near each microscope so that the light will be pointing at it. Place a piece of white paper under each beaker. Fill the beakers with sea-water and note the temperature.
3. Place four shrimp in each beaker. These shrimp should have been pre-adapted to a sand-colored background for 30 minutes.
4. Stage 20 chromatophores from each shrimp as described in Experiment 1.
5. Turn on the lights of each dissecting microscope and cover the microscope and beaker with a large box to block out room light. Let stand for 30 minutes.
6. After 30 minutes record the water temperature. Re-stage the chromatophores while shining the respective light color on the microscope stage. Note any colored pigments in the centers of the chromatophore.
7. Calculate the chromatophore number for each shrimp, determine the mean values for each group, and look for differences caused by wavelength color.

NOTE: If you have access to a dark room this experiment can be set-up easily with all four dissecting microscope lights set up around the room, as there will be little interference from these low intensity bulbs. The sea-water temperature will not increase as much as when the lights are enclosed in a box.

Experiment 4: Effect Of Temperature

1. Cover the sides and bottom of three 1-liter glass beakers with sand-colored paper. Place a clear plastic bag around each and secure tightly with a rubber band to keep water from soaking the paper. Be sure to leave the top of the beaker uncovered.
2. Fill each beaker with sea-water and place in either a 5°, 15°, or 25°C water bath. Place a thermometer in each beaker.
3. Stage 20 chromatophores from 12 shrimp (that have been pre-adapted to a sand-background for 30 minutes) as described in Experiment 1.
4. When the beakers of sea-water have reached the correct temperature in each water bath, add four of the staged shrimp to each beaker. Let stand for 30 minutes, checking occasionally that the temperature remains constant.
5. Re-stage 20 chromatophores from each shrimp and calculate the total chromatophore number. Determine the mean value of each group and look for differences caused by temperature. One would expect the shrimp at higher temperatures to have less dispersed pigment and to be lighter in color as they would be more effective at reflecting heat from sunlight.

Additional Experiments on Environmental Factors and Color Change

The albedo response can also be studied easily by comparing the degree of pigment dispersion of shrimp that have been kept in containers whose interiors have been painted with (a) shiny, gloss, reflective paint of different colors or (b) matte, flat, non-gloss paint, as this will vary the amount of light that is reflected off the background onto the lower surface of the eye.

Circadian rhythms can be examined by keeping groups of shrimp for 5 days under different light cycles and staging the chromatophores at regular 6 hour intervals. Studies have found that the chromatophores have a diurnal rhythm of darkening at night and lightening in the day (Fingerman and Lowe, 1970). Different groups of shrimp could be kept in total darkness, total light, reversed photoperiod (dark in the day, light at night), or other abnormal light/day cycles and compared with shrimp kept under normal photoperiods. Since *Crangon septemspinus* survive longer at temperatures below 15°C, glass tanks should be set up in a temperature controlled room if possible. Lamps set on timers that automatically turn on and off are convenient. All of the experimental groups can be set up in one temperature controlled room if they are partitioned from each other using clamp stands and black plastic sheets.

Part B: Location of Receptors Responsible for Color Change

The eyes are known to be the receptors responsible for color change in shrimp, but various experiments can be done to prove this and to check whether other areas of the body are also involved. Chromatophores that respond directly to changes in illumination are classified as **primary responses**. Chromatophore responses that involve visual receptors and pathways are classified as **secondary responses**. The following experiment involves painting the eyes to block light entry and then placing the shrimp on different color backgrounds to see if the ability to change color ceases.

Experiment 5: Painting Eyes To Block Light Entry

1. Obtain 16 shrimp that have been pre-adapted to a neutral (sand-colored) background for 30 minutes to ensure their chromatophores are uniformly dispersed. Stage 20 chromatophores on the body as described in Experiment 1 (Part A).
2. Paint the eyes of eight shrimp with white typewriter correction fluid (Liquid Paper). Blot the shrimp dry using tissues before painting the eyes. Allow to dry.
3. Prepare two beakers: one covered (bottom and sides) with white paper and the other with black paper. Fill both beakers with sea-water and record the temperature.
4. Place four shrimp with painted eyes in each beaker, and add four intact shrimp to each beaker to serve as controls. Let stand for 30 minutes. Note the changes in water temperature.
5. Re-stage the chromatophores from each shrimp. Calculate the mean chromatophore numbers for each of the four experimental groups and note any differences. The shrimp with painted eyes will probably show no change in pigment dispersion when put on a different background as the entry of light to the eyes is blocked. The intact shrimp should darken on the black background, and lighten on the white background.

Additional Experiments on Receptor Location

Various parts of the body can be painted or shielded from light and the shrimp placed on different backgrounds to observe whether the chromatophores in the covered region have the same amount of pigment dispersion as the ones on the uncovered regions. Any differences would indicate that light reacts directly on the chromatophores (primary receptors) rather than through the eyes (secondary receptors). To shield parts of the body from light the shrimp can be placed in shrimp-sized black tubing, or placed in narrow glass vials that have one half covered with black tape. The shrimp can be easily removed from the tubing or vials to enable counting of the chromatophores from the covered regions. The chromatophores in the tail region should be studied independently since they are thought to be controlled by different hormones than the body region.

Part C:

Determining Whether Color Change is Under Hormonal or Nervous Control

Color change in shrimp is brought about by hormones (Carlisle and Knowles, 1959; Fernlund, 1970; Fernlund and Josefsson, 1972; Fingerman, 1985) and there are thought to be one set of hormones responsible for body lightening and darkening, and another set for the tail region. Color change in other species (cephalopods) is nervous and brought about by the contraction of muscles surrounding an elastic sac. The sinus gland at the base of the eyestalk is known to be the immediate source of chromatophore hormones (chromatophorotropins) in crustaceans. The hormones are thought to arise from the neurons of the x-organ, brain, or ventral ganglia and are then stored in the sinus gland at the base of the eyestalk (Figure 2.2). In the following experiment crude hormone extracts (prepared by grinding eyestalks in crustacean saline; see Appendix B) will be injected into shrimp to see if any changes in pigment dispersion occur in the chromatophores. More sophisticated solvent extractions can be performed to further purify these peptide hormones (Fernlund and Josefsson, 1972; Fingerman, 1985). Commercially prepared hormones are not available.

Experiment 6: Injection of Crude Extracts of Color Control Hormones

1. Stage 20 chromatophores from the body region of the eight intact shrimp that were pre-adapted to a sand-colored background for 30 minutes and eight shrimp with eyes painted with Liquid Paper (from Experiment 5 in Part B).
2. Cover the sides and bottom of two 1-liter glass beakers with sand-colored paper. Fill with seawater and note the temperature.
3. Inject four intact shrimp and four shrimp with painted eyes with 0.05 ml of the crude eyestalk extract. Inject on the ventral side of the posterior body region using the 25-gauge 1-ml syringes provided. Place these eight shrimp into one of the sand-colored beakers.
4. Inject (in the same body region) four intact shrimp and four shrimp with painted eyes with 0.05 ml of crustacean saline as above. Place in a second sand-colored beaker. These will serve as your controls.
5. Let all shrimp stand for 30 minutes. Occasionally record changes in water temperature.
6. Re-stage 20 chromatophores from the body of each shrimp. Determine the total chromatophore number for each shrimp and then calculate the mean for both groups and note any differences. Were the hormone-injected shrimp darker or lighter than the control shrimp?

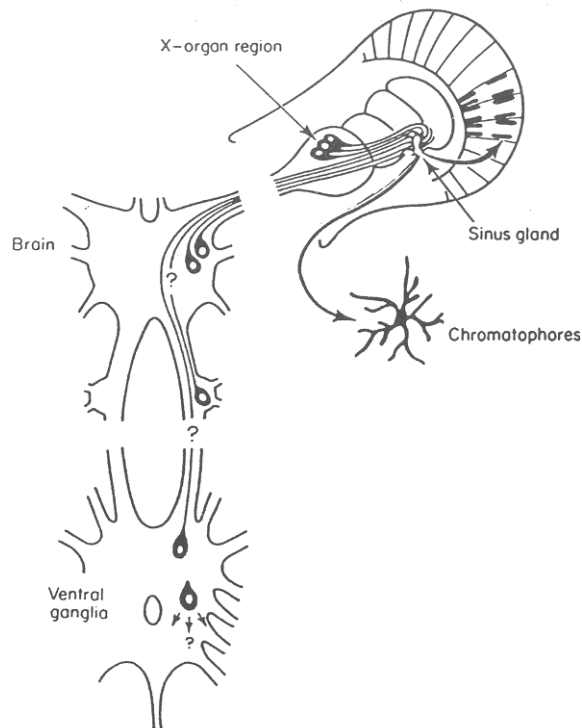


Figure 2.2. Diagram of the crustacean sinus gland neurosecretory system at the base of the eyestalk (from Waterman, 1961). Reprinted with permission.

Additional Experiments to Determine Type of Control

Classical experiments first reported by Koller (1929) can be performed but often result in mortalities as it is difficult to extract blood from small shrimp. Koller extracted blood from shrimp adapted to black backgrounds and injected it into shrimp on white backgrounds and darkening occurred. The ventral nerve cord can be cut to see if this prevents color change when shrimp are placed on a different background. However, a major blood vessel lies next to the ventral nerve cord making the operation very difficult. Different concentrations of possible crustacean neurotransmitters (5-hydroxy-tryptamine, adrenaline, acetylcholine) can be injected into shrimp to see if they play a part in relaying light changes received by the receptors in the eyes to the sinus gland x-organ complex at the base of the eyestalk.

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APPENDIX A
Addresses of Suppliers of Live Shrimp

Canada

Atlantic Biological Company
R.R. #2
St. Andrews, New Brunswick E0G 2X0
(*Crangon septemspinus* listed in catalogue for \$8.05 CDN per 12 shrimp)

USA

Gulf Specimen Company
P.O. Box 237
Panacea, Florida

Woods Hole Marine Biological Laboratory
Supply Department
Woods Hole, Massachusetts 02543

APPENDIX B
Preparation of Chemicals for Experiment 6

Marine Crustacean Saline

	g/liter
NaCl	26.7
KCl	1.11
MgCl ₂	0.36
MgSO ₄	0.62
CaSO ₄	3.4

Also add buffer (17.6 ml of 0.5 M boric acid and 0.956 ml of 0.5 M NaOH) per liter of solution.

Crude Hormone Extract

Quantity required per experimental group = 0.5 ml (injecting eight shrimp with 0.05 ml each).
Anaesthetize eight (8) shrimp in a solution of MS-222 (tricaine methanesulphonate; approximately 0.1 g/liter) until movement ceases. Place the shrimp on a petri dish under and dissecting microscope and remove the eyes as close to the base as possible with fine forceps or a scalpel. Place the eyes plus eyestalk into a small petri dish, add 0.5 ml of crustacean saline, and grind for a few minutes until a smooth homogenous solution is obtained.