Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 32, 245-258, 2011

Fundulus Melanophores: from Physiology to Cell Biology

Seung M. Hong and Gary Laverty

Department of Biological Sciences, University of Delaware, Newark DE 19716 USA
(**smhong@udel.edu**;**laverty@udel.edu**)

Background color adaptation in poikilotherms is a great subject for investigative physiology course. The physiological color change is relatively fast and reversible. Moreover, the control mechanisms are complex and diverse among species, providing many follow-up studies for research projects. Dermal melanophores in *Fundulus heteroclitus* are responsible for darkening in black background and easily identified for indexing under low magnifications. To further explore organismal observations, isolated scales can be studied *in vitro* addressing questions concerning cell signaling and cytoskeletal motor proteins. This following study presents introductory experiments and proposes a model that integrates physiology and cell biology for investigative biology labs.

Keywords: *Fundulus* melanophores, melanosome movement, microtubules, investigative lab, background color adaptation, isolated fish scale, perfusion chamber, adrenergic mediation, imaging

Introduction

The experiments described for this session were originally designed for two separate courses taught in our department, BISC316 (Experimental Physiology) and BISC315 (Experimental Cell Biology). Both courses, along with 3 other investigative lab courses (Molecular Biology, Ecology, Genetics) have provided upper level biology majors with intensive, faculty-taught and stand-alone laboratory courses that focus on the scientific process and methods in several biology disciplines. Students in the BA degree program are required to take one of these laboratory courses, usually during their senior year. As of fall 2009, all of these courses have been enhanced to 3 credits, and are also now designated as Discovery Learning Experience (DLE) courses, a new university requirement. For both BISC316 and BISC315, the basic format is that students spend the first half of the semester doing a core series of "prescribed" laboratory exercises, followed by small group independent projects during the second half of the semester.

Developing a new curriculum for Experimental Physiology required a model system that can be explored with experimental research questions concerning a whole animal. *Fundulus heteroclitus*, sometimes called mummichog or killifish, live in salt marshes in Delaware and exhibit physiological color changes when exposed to various background colors (1-3). This adaptation process is reversible and relatively fast compared to other teleosts (4, 5), which fits well for a 3-hour classroom investigation. Among other vertebrates, and even in other teleosts the neural and endocrine control mechanisms that regulate color change are complex and diverse (6-8). For example, many teleosts are neuronally regulated, while amphibians and reptiles are predominantly controlled by circulating hormones. Moreover, the ultimate shade (paling versus darkening) is determined by the specific receptors located in the pigments cells. This complex nature of the color change in animals in turn provides many follow-up studies for independent research projects. Since *Fundulus heteroclitus* resides in a temperate zone in the field, environmental factors, such as ambient temperature, salinity, and photoperiod, have also been of much interest to students as they design independent research projects. For students in Experimental Cell Biology, the focus of a number of student projects has been on cell signaling associated with neuronal control mechanisms, or on visualization and pharmacological manipulation of the cytoskeleton of dermal melanophores, which underlie the adaptive color changes.

The dermal melanophores in *Fundulus heteroclitus* are responsible for darkening and paling of the dorsal skin when subjected to a black or white background respectively. Animals darken as melanosomes (melanin containing vesicles) migrate from the cell center toward the periphery, whereas they become pale as melanosomes relocate toward the nucleus of the melanophore. The degree of pigment distribution can be easily detected under low magnification of fish scales collected from animals adapted to a light or dark background color, and these distributions can be scored from 1-5 as a melanophore index (MI) (9). The melanophores in *Fundulus* fish scales are large (around 100 μm in diameter), flattened and stellate in shape so that often times the length of the pigment trail within a cell process is measured to score a dispersal status. In fact, the *Fundulus* melanophore was one of the first model systems used in early studies on cytoskeletal biology, demonstrating the critical role of microtubules, for example, in intracellular migration of pigments (10-12). Until the time of this discovery in *Fundulus*, the pigment move-

ment inside teleost chromatophores was thought to take place in response to contraction and relaxation of the radial muscles anchored on the cell membrane as shown in cephalapods.

In many teleosts, including *Fundulus heteroclitus*, dermal melanophores are innervated by postganglionic sympathetic fibers (13-17) and sympathetic stimulation, mediated by α_2-adrenergic receptors, induces aggregation of the melanosomes (18-23). Any agonist that enhances the cAMP levels in the cell causes the dispersion of melanosomes (24), while antagonists that decrease the cAMP levels result in melanosome aggregation. Other agents, such as melanophore stimulating hormone (MSH) or melanin concentrating hormone (MCH), have been consistently shown to mediate background color changes in other teleosts (25, 26) and amphibians (9, 27, 28). However, the hormonal mediation for physiological color changes in *Fundulus* is yet to be determined (29, 30).

Going Further

A number of additional experiments, appropriate for either Experimental Physiology or Experimental Cell Biology, have been designed by students in these classes. Both courses require that students, working in small teams, propose and carry out 5-6 week independent projects during the last half of the semester. In general, the focus of these studies has been on the neuronal regulation of melanosome movements and on the role of the cytoskeleton in these movements. Because these are not part of the "prescribed" core of experiments, detailed protocols are not included in the Student Outline below. However, the following summary should provide a good starting point for those interested in expanding these studies.

Essentially, we use a variety of microscope types (stereo, standard upright compound, inverted compound) to view single isolated scales during different kinds of treatments. Some of our microscopes are equipped with digital cameras or video cameras, so that images can be used for data analysis. To capture rapid changes in pigment distribution we use different approaches to manipulate solution changes. With inverted microscopes, multi-well culture plates work very well. Solutions can be aspirated and replaced quickly in 96-well plates without removing the scales; while the scale may move out of focus, it can usually be relocated quickly to follow the time course of pigment granule movements. For more complete exchange or washout, aspiration and replacement can be repeated. Alternatively, scales can be moved from one well to another with fine forceps. This approach is easier with larger well formats (e.g., 24-well plates). Individual wells can be pre-filled with the various test solutions to be used.

With standard upright microscopes chamber slides can be constructed to fit the microscope stage (since scales are derived from a heterotherm, a heated stage is not necessary). One approach is to use stopcock grease to build a shallow well on the slide, with the scale contained in the well under a coverslip. A cleaner chamber can be made using CoverWell perfusion chambers available from Molecular Probes Inc. (now part of InVitrogen, Inc). These chambers are silicon gaskets that are simply pressed on to a glass microscope slide to form a chamber. They are available in several sizes and in multi-well formats and have two ports that can be used to perfuse solutions through the chamber. We have found that these chambers can be re-used several times. To exchange solutions a pipettor can be used to add solution to one port with a Kimwipe or other absorbent paper positioned over the opposite port to wick away the old solution. A more elaborate perfusion system can be constructed with blunted syringe needles positioned over the ports with modeling clay.

Under the 20x or 40x objective, scales can be viewed to monitor multiple or single melanophores. Students have routinely conducted several experiments to demonstrate the neural regulation of melanosome movement. A 1-5 numerical scale can be used to quantify the extent of dispersal with full aggregation corresponding to 1 and full dispersal to 5. The experiments include addition of exogenous norepinephrine (10^{-8} to 10^{-6} M) and substitution of the bathing solution with a high potassium solution, which causes depolarization and release of endogenous neurotransmitter. Both of these treatments cause pigment granule aggregation within minutes, due to the stimulation of alpha-type adrenergic receptors. In contrast, the phosphodiesterase inhibitor caffeine (10 mM), or other treatments that raise intracelluar levels of cAMP, will cause dispersal of the granules. Other, more complex experiments can be designed to demonstrate the specific function of alpha-adrenergic receptors (eg, alpha versus beta agonists or receptor antagonists).

Another line of experiments can be designed to investigate the role of cytoskeletal elements in pigment granule movements. Colchicine or related compounds (demecolcine) disrupt the microtubule network and cytochalasins similarly disrupt actin microfilaments. Thus, these compounds can be used to test the relative role of microtubules and actin filaments in dispersal or aggregation. Generally, one can predisperse or pre-aggregate melanophores before applying the inhibitor, then test for responses to high potassium or caffeine as above. Finally, some student teams have complemented these functional studies with attempts to image the cytoskeleton, using immunohistological methods. This has proven difficult, however, because of the overlying epithelial cell layer and because the pigment granules themselves will tend to obscure the underlying cytoskeletal tracts.

We believe that the *Fundulus* melanophore system provides a very useful model for student independent investigation and experimental design, and also provides an excellent way to integrate physiological principles (adaptive color change, neuronal regulation) with cell biology (membrane receptors and channels, cytoskeletal motors, etc).

The Student Outline presented below details exemplary experiments used for introductory investigations of physiological color changes in lower vertebrate (teleosts and lizards) and invertebrates (fiddler crabs). These two experiments are part of 6 "prescribed" lab exercises that students in Experimental Physiology perform at the beginning of the semester.

Student Outline

Chromatophores and Color Changes: I. Background Color Adaptation

Key Concepts

Chromatophores in poikilotherms
Physiological vs. morphological color change
Translocation of cargos in cells

Materials

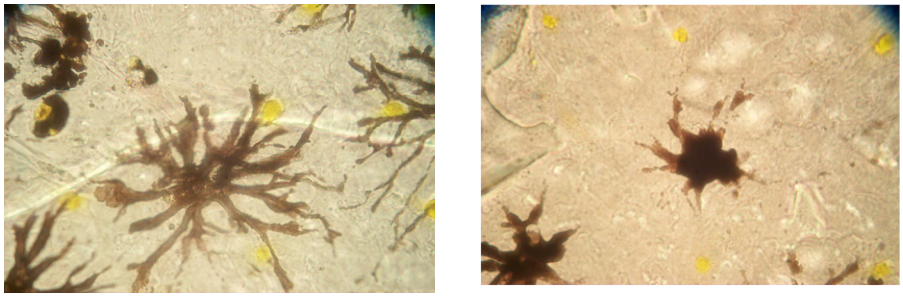
Killifish
Lizard
Small dishpan (black and white) with a clear lid
Air pump fitted with aerators
Small fish net
Small clear container with a lid

Background

Many lower vertebrates and invertebrates exhibit integumentary color changes in response to changes in background color (Fig. 1). This ability to adapt to an environmental factor (i.e., substrate color) is a primary attribute of a "living" organism, and is often used for various reasons, such as camouflage, advertising, protection from harmful radiations and thermoregulation.

Figure 1. The killifish (*Fundulus heteroclitus*) exposed to white vs. black background for 1 hour. (Courtesy of Dr. Gary Laverty)

There are two types of integumentary color changes; physiological and morphological. Whereas morphological color change is a long-term process, in weeks or months, resulting from a change in pigment amount and/or pigment cell number (proliferation or apoptosis), physiological color change takes place rather rapidly caused by translocation of existing pigments within the cell. During a fast background color adaptation, the pigment vesicles containing motor proteins (i.e., kinesin, dynein, myosin) travel along the intracellular tracts, microtubules and actin filaments. As pigment vesicles move toward the cell periphery (dispersal state, Fig. 2a), they impart the pigment color to the animal. For example, when melanosomes (black pigments) move toward the cell periphery, the skin becomes dark brown or black. Then as they relocate toward the nucleus (aggregation state, Fig. 2b), the animal appears pale.



(a) (b)

Figure 2. Phase contrast images of dermal melanophores in the killifish, *Fundulus heteroclitus*: (a) with melanosomes dispersed throughout the cell; (b) with melanosomes concentrated in the center of the cell. (Courtesy of Dr. Gary Laverty)

In most cases, dermal chromatophores are responsible for physiological color change. Three types are well defined in poikilotherms; melanophore, xanthophores, and iridophores. Each chromatophore synthesizes and stores different pigments in pigment organelles; 1) melanophores – melanin (brown to black pigments), 2) xanthophores – carotenoid, pteridine (yellow or orange pigments), 3) iridophores – guanine, purine in reflecting platelets.

Melanophores are richly populated in the dorsal skin of the *Fundulus heteroclitus*, giving a darker appearance than other areas. Dermal chromatophores in lizards are relatively large and arranged into "dermal chromatophore units", a basket-like form; each unit has three types of chromatophores (xanthophores, iridophores, melanophores). These three distinct pigment cells are arranged in layers, xanthophores being most superficial undergirded by iridophores that lie above large melanophores. As melanosomes in melanophores aggregate, the color of iridophores and xanthophores represent the primary color for the skin.

In the following experiment we will define the time course of the physiological color change in animals subjected to black or white background. The temporal patterns will be compared between darkening vs. paling process as well as between animal species (fish vs. lizard).

Experimental Protocol

I. Time course for color change in fish (*Fundulus heteroclitus*) (Trial #1-3)

- Darkening process (from white to black pan)
  - Take 8 fishes from the main tank and place them in white dishpan fitted with aerator for 20 min, keeping the handling stress at minimum.
  - Transfer 4 fishes to black dishpan at time "0". Observe the color change of the dorsal skin and determine the color index (1-5, 5 being the darkest) every 30 seconds for 5 minutes or until you obtain 3 identical readings. The animals in white background will serve as "negative" control (Fig. 3).

- Paling process (from black to white pan)
  - First, return 4 pale fishes (negative control) in the white dishpan back to the main tank.
  - Transfer the darkened fishes from the black dishpan into the white pan, and check the color index every 30 seconds until everyone appears pale (fully recovered; Index "1").

** Repeat this experiment twice in order to minimize subjectivity in reading.*

Figure 3. Skin color index 1. (Courtesy of Kyle Bruffy)

II. Time course for color change in lizard (*Anolis carolinensis*)

- Darkening process (from white to black pan)
  - Take one lizard from the main terrarium and place in a small clear plastic container housed in white dishpan for 20 min in order to establish the most paled color (skin color index 1).
  - Transfer the clear tank to black background; this is time 0.
  - Observe the "dorsal" skin color of the lizard and determine the color index (1-5, 5 being the darkest) every 5 minutes for one hours or until you obtain 3 identical readings. The lizard in the white pan will serve as a negative control ("green" hue). Make sure not to "disturb" the animals while observing

Table 1. Time course for integumental color changes in *Fundulus heteroclitus*. Animals were subjected to black background, and were transferred to white background when (*) fully adapted to the black background.

Trial #1

Time (min)	Skin Color Index						
	Fish 1	Fish 2	Fish 3	Fish 4	Mean	SD	SEM
0.0							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0* (0)							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0							

Trial #2

Time (min)	Skin Color Index						
	Fish 1	Fish 2	Fish 3	Fish 4	Mean	SD	SEM
0.0							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0* (0)							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0							

Trial #3

Time (min)	Skin Color Index						
	Fish 1	Fish 2	Fish 3	Fish 4	Mean	SD	SEM
0.0							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0* (0)							
0.5							
1.0							
1.5							
2.0							
2.5							
3.0							
3.5							
4.0							
4.5							
5.0							

Table 2. Time course for integumentary color changes in *Anolis carolinesis* subjected to black background.

Time (min)	Skin Color Index						
	Lizard 1	Lizard 2	Lizard 3	Lizard 4	Mean	SD	SEM
0							
5							
10							
15							
20							
25							
30							
35							
40							
45							
50							
55							
60							

Related Articles

Connolly, C.J. 1925. Adaptive changes in shades and color of *Fundulus*. *Biological Bulletin* 48:56-77.
Fingerman, M. 1970. Comparative physiology: Chromatophores. *Annual Review in Physiology* 32:345-372.
Hadley, M.E., and J.M. Goldman. 1969. Physiological color changes in reptiles. *American Zoologist* 9:489-504.
Hill, A.V., J.L. Parkinson, and D.Y. Solandt. 1935. Photo-electric records of the colour change in *Fundulus heteroclitus*. *Journal of Experimental Biology* 12:397-399.
Parker, G.H. 1943. Coloration of animals and their ability to change their tints. *The Scientific Monthly* 56:197-210.
Parker, G.H. 1944. The time factor in chromatophore responses. *Proceedings of the American Philosophical Society* 87:429-434.
Parker, G.H. 1955. Background adaptations. *The Quarterly Review of Biology* 30:105-115.

Chromatophores and Color Changes: III. Neurohormonal control of dermal melanophores

Key Concepts

Dermal chromatophores
Signal transduction pathway for peptide hormones
Actions of melanocyte stimulating hormone (MSH)
Sympathetic neurotransmitter/adrenergic receptors

Materials

Killifish
Lizard
Small dishpan (black and white) with a clear lid
Air pump fitted with aerators
Small fish net
Dissecting scope
96-well culture plate
Pipetman for 200 µl with tips

Kimwipes
Fine pair of forceps
Fish saline
Amphibian Ringer
Caffeine (10 mM)
MSH (0.1 g/ml)

Background

The major agents mediating the physiological color change in animals are hormones and neurotransmitters. Several peptide hormones have been identified to induce dispersion or aggregation in dermal melanophores in lower vertebrates. Melanocyte stimulating hormone (MSH), which is released from the intermediate lobe of the pituitary gland, seems to be the major hormone responsible for darkening the skin in most vertebrates. In addition, melanin-concentrating hormone (MCH) was proposed to mediate the skin paling process in some teleosts. In crustaceans, however, pigment dispersing or aggregating (or concentrating) hormones are produced and released from the sinus gland in the eyestalks upon stimulation by neurotransmitters, such as norepinephrine, serotonin or dopamine.

In many bony fishes, sympathetic nerves innervate dermal melanophores. Denervation of sympathetic branches to dermal scales blocks the induction of aggregation in melanophores by norepinephrine. While many amphibians and reptiles are devoid of sympathetic inputs to dermal chromatophores, the cells respond to adrenergic agonists with melanosome aggregation or dispersion depending on the location in skin.

These hormones and neurotransmitters (peptides, catecholamines) serve as the first messengers working on the cell surface. Upon binding to its own membrane receptors the signal is transferred to the second messengers (i.e., cAMP), resulting in the translocation of pigment vesicles as the ultimate cellular response. While factors that increase the level of cAMP induce dispersion of melanosomes, ones that reduce the cAMP level cause the aggregation of pigment vesicles. For example, forskolin, which increases activity of the adenyly cyclase, induces melanosome dispersion. Caffeine (or IBMX) also causes melanosome dispersion by decreasing the phosphodiesterase activity, which results in increase in the cAMP level. The process of translocation is in turn coordinated by simultaneous activation or deactivation of the motor proteins (kinesin and myosin for dispersion, dynein for aggregation).

In the following experiment, we will first examine various dermal chromatophores in 3 animal species then explore possible control mechanisms for peptide hormones and neurotransmitters mediating physiological color change in lower vertebrates.

Experimental Protocol

I. Observation of dermal melanophores in dorsal scales of killifish (*Fundulus heteroclitus*) subjected to black vs. white background

- Prepare one fully darkened and one fully paled fish according to the time course obtained in the previous experiment.
- Add 100 µl fish saline to 8 wells (A4 → D4 for "B" scales, A7 → D7 for "W" scales) on a 96-well tissue culture plate.
- When fishes are fully adapted to the background color, wrap the fish with a wet paper towel while exposing the dorsal skin. Collect with a pair of fine forceps four scales from the dorsum between the pectoral fin and pelvic fin then transfer them into the designated wells previously loaded with fish saline.
- Place the tissue culture plate under the dissecting scope and observe the appearance of the whole scale. These fish scales are bony plates derived from the dermis, and covered superficially by epidermal cell layer (mucus cells, keratocytes).
- Draw a simple sketch of a scale. What type of scale is this (cycloid or ctenoid)? Label dermal melanophores as well as other prominent structures (radus, circulus, focus). Make a note of which area of the scale is covered by dermal melanophores.
- Count the total number of melanophores, and classify the cells into three categories;
  - Punctate (fully aggregated, Stage 1)
  - Intermediate (Stage 2-3)
  - Stellate (fully dispersed, Stage 4-5)
- Compare the distribution of the cells at each category between pale and dark fish.
- Repeat the observation of scales 1 hour post "denervation" and make a note whether the melanophore index remained constant or not.

Q1. If the punctate melanophores became dispersed, then what does this implicate?
Q2. If the stellate melanophores became punctate, then what does this suggest?
Q3. If the melanophore index remained the same, then what does this mean?

II. Observation of dermal chromatophores in sand fiddler crabs (*Uca pugilator*)

- Take one male crab from the main tank, and place it in a small (or medium) Petri dish.
- Examine under the dissecting scope ALL chromatophores seen through the cuticle on the anteroventral surface of the second walking leg.

Q1. How many different types of chromatophores can be detected under the dissecting scope?
Q2. How are they different in tint, shape, size and number?

III. Effects of drugs on dermal melanophores in lizards (*Anolis carolinensis*).

- Preparation of skin pieces: the instructor will harvest skin tissue from a lizard. Groups will be given strips of the isolated skin. Place them in Amphibian Ringer in a Petri dish. The skin piece should float on the solution and spread out evenly (dermal side is hydrophilic whereas the epidermal side is hydrophobic). Cut the strip lengthwise to prepare 3x3 mm squares. Each group will need 12 squares.
- Preparation of culture plate

  Fill the following wells with 200 µl of the following solutions:

  A1 → D1 No drugs (Ringer solution as "negative" control group)
  A3 → D3 MSH (0.1 µg/ml) (experimental group)
  A4 → D4 No drugs (Ringer solution, for reversal action)
  A6 → D6 Caffeine (10 mM) (experimental group)
  A7 → D7 No drugs (Ringer solution, for reversal action)

  Place skin pieces in A1→D1 and observe the chromatophores under the dissecting scope. Make a note of the locations for the epidermal vs. dermal melanophores. The skin piece should float with the superficial side (outside) up.

- Skin color index in *Anolis carolinensis*

  As you observe the skin color under the dissecting scope, quantify melanosome dispersal using a "skin color index" which has a 5-point system; 5 being fully darkened, 1 being green. Since we are examining a piece of skin consisting of multiple "scales" (small bumps), go through the scales one by one and determine the degree of dispersal from 1-5. Then determine the "overall" dispersal for the whole piece.

  In the microscope field you will see several rows of scales. When melanophores are fully aggregated, colors from other chromatophores become more noticeable (green or orange). Some scales may show black pigment cells on the surface (epidermal melanophores). Disregard them for MI evaluation, as they are irresponsive to drugs.

- Hormones and drug treatment

  Effects of MSH (0.1 µg/ml) on skin color index

  Place a skin piece in each of the wells (A3→D3). Observe the color change for 15 min or until the color no longer changes. Record the skin color index every 30 seconds. Once the color is established, transfer them to the Ringer solution (A4→D4) and continue to examine the recovery for 15 min. Compare the temporal pattern between dispersal vs. aggregation.

  Effects of caffeine (10 mM) on skin color index

  Follow the directions for MSH treatment. Use the wells (A6→D6) for the caffeine treatment, and A7→D7 for the recovery process.

Related Articles

Burton, D. 2005. In vitro pattern-related effects of forskolin on melanophore adrenoceptor-mediated responses of winter flounder. *Journal of Fish Biology* 66:230-241.

Fingerman, M., N.C. Jackson, and R. Nagabhushanam. 1998. Hormonally-regulated functions in crustaceans as biomakers of environmental pollution. *Comparative Biochemistry and Physiology Part C* 120:345-350.

Greenberg, N. 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. *Integrative and Comparative Biology* 42:526-540.

Gross, S.P., M.C. Tuma, S.W. Deacon, A.S. Serpinskaya, A.R. Reilein, and V.I. Gelfand. 2002. Interactions and regulation of molecular motors in *Xenopus* melanophores. *The Journal of Cell Biology* 156:855-865.

Hogben, L., and D. Slome. 1931. The pigmentary effector system – VI. The dual character of endocrine co-ordination in amphibian colour change. *Proceedings of the Royal Society of London B* 108:10-53.

Ryan, R.W.J., J.I. Post, M. Sole, P.V. Hodson, and G.M. Ross. 2002. Catecholaminergic neuronal degeneration in rainbow trout assessed by skin color change: a model system for identification of environmental risk factors. *NeuroToxicology* 23:545-551.

Sugimoto, M., H. Nagamori, H. Yasiu, and N. Oshima. 1997. Regulation of melanophore responsiveness in the background-adapted medaka, *Oryzias latipes*: Change in the intracellular signaling system. *Comparative Biochemistry and Physiology* 117C:259-265.

Van der Salm, A.L., J.R. Metz, S.E.W. Bonga, and G. Flik. 2005. Alpha-MSH, the melanocortin-1 receptor and background adaptation in the Mozambique tilapia, *Oreochromis mossambicus*. *General and Comparative Endocrinology* 144:140-149.

Vaughan, G.L., and N. Greenberg. 1987. Propranolol, a beta-adrenergic antagonist, retards response to MSH in skin of *Anolis carolinensis*. *Physiology and Behavior* 40:555-558.

Zhu, Y., and P. Thomas. 1997. Effects of somatolactin on melanosome aggregation in the melanophores of red drum (*Sciaenops ocellatus*) scales. *General and Comparative Endocrinology* 105:127-133.

Acknowledgements

This study was supported by an Undergraduate Biological Sciences Education Program Grant from Howard Hughes Medical Institute.

Literature Cited

Connolly, C.J. 1925. Adaptive changes in shades and color of *Fundulus*. *Biological Bulletin* 48:56-77.

Parker, G.H., and A.J. Lanchner. 1922. The response of Fundulus to white, black and darkness. *American Journal of Physiology* 61:548-550.

Parker, G.H. 1943. Coloration of animals and their ability to change their tints. *The Scientific Monthly* 56:197-210.

Parker, G.H. 1944. The time factor in chromatophore responses. *Proceedings of the American Philosophical Society* 87:429-434.

Hill, A.V., J.L. Parkinson, and D.Y. Solandt. 1935. Photoelectric records of the colour change in Fundulus heteroclitus. *Journal of Experimental Biology* 12:397-399.

Fingerman, M. 1970. Comparative physiology: Chromatophores. *Annual Review in Physiology* 32:345-372.

Hadley, M.E., and J.M. Goldman. 1969. Physiological color changes in reptiles. *American Zoologist* 9:489-504.

Fujii, R., and N. Oshima. 1986. Control of chromatophore movements in teleost fishes. *Zoological Science* 3:13-47.

Hogben, L., and D. Slome. 1931. The pigmentary effector system – VI. The dual character of endocrine co-ordination in amphibian colour change. *Proceedings of the Royal Society of London B* 108:10-53.

Bikle, D, L.G. Tilney, and K.R. Porter. 1966. Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus* L. *Protoplasma* 61:322-345.

Green, L. 1968. Mechanism of movements of granules in melanocytes of *Fundulus heteroclitus*. *Proceedings of National Academy of Science USA* 59:1179-1186.

Murphy, D.B., and L.G. Tilney. 1974. The role of microtubules in the movement of pigment granules in teleost melanophores. *Journal of Cell Biology* 61:757-779.

Healey, E.G. 1951. The colour change of the minnow (*Phoximus laevis* Ag). I. Effects of spinal section between vertebrae 5 and 12 on the response of the melanophores. *Journal of Experimental Biology* 28:297-319.

Pye, J.D. 1964. Nervous control of chromatophores in teleost fishes III. Local temperature responses in the minnow (*Phoxinus phoxinus* L.). *Journal of Experimental Biology* 41:543-531.

Scott, G. 1965. Physiology and pharmacology of color change in the sand flounder *Scopthalamus aquosus*. *Limnology and Oceanography* 10: supplement: Alfred C. Redfield 75th Anniversary volume 230-246.

Fujii R., and R.R. Novales. 1969. The nervous mechanism controlling pigment aggregation in *Fundulus* melanophores. *Comparative Biochemistry and Physiology* 29:109-124.

Yamada, T., and R. Fujii. 2002. An increase in extracellular Ca^{2+} concentration induces pigment aggregation in teleostean melanophores. *Zoological Science* 19:829-839.

Andersson R.G.G., J.O. Karsson, and N. Grundstrom. 1984. Adrenergic nerves and the alpha$_2$-adrenoceptor system regulating melanosome aggregation within fish melanophores. *Acta Physiologica Scandinavica* 121:173-179.

Morishita F. 1987. Respponses of the melanophores of the medaka, *Oryzias latipes*, to adrenergic drugs: Evidence for involvement of alpha$_2$-adrenergic receptors mediating melanin aggregation. *Comparative Biochemistry and Physiology Part C* 88:69-74.

Morishita F., A. Shimada, M. Fujimoto, H. Katayama, and K. Yamada. 1993. Inhibition of adenylate cyclase activity in the goldfish melanophore is mediated by α_2-adrenoceptors and a pertussis toxin-sensitive GTP-binding protein. *Journal of Comparative Physiology B* 163:533-540.

Martensson L.G.E., and R.G.G. Andersson. 1996. A melatonin binding site modulates the α_2-adrenoceptor. *Life Sciences* 58:525-533.

Amiri, M.H. 2009. Postsynaptic alpha 2 – adrenoceptors mediate melanosome aggregation in melanophores of the white-spotted rabbitfish (*Siganus canaliculatus*). *Pakistan Journal of Biological Sciences* 12:1-10.

Mayo, D.J., and D. Burton. 1998. The in vitro physiology of melanophores associated with integumentary patterns in winter flounder (*Pleuronectes americanus*). *Comparative Biochemistry and Physiology Part A* 121:241-247.

Sammak, P.J., S.R. Adams, A.T. Haarootunian, M. Schliwa, and R.Y. Tsien. 1992. Intracellular cyclic AMP, not calcium, determines the direction of vesicle movement in melanophores: Direct measurement by fluorescence ratio imaging. *The Journal of Cell Biology* 117:57-72.

Nagai M., N. Oshima, and R. Fujii. 1986. A comparative study of melanin-concentating hormone (MCH) action on teleost melanophores. *Biological Bulletin* 171:360-370.

Kawauchi H. 2006. Functions of melanin-concentrating hormone in fish. *Journal of Experimental Zoology* 305A:751-760.

Novales R.R., and W.J. Davis. 1969. Cellular aspects of the control of physiological color changes in amphibians. *American Zoologist* 9:479-488.

Rollag, M.D. 1988. Response of amphibian melanophores to melatonin. *Pineal Research Reviews* 6:67-93.

Abramowitz, A.A. 1937. The role of the hypophyseal melanophore hormone in the chromatic physiology of *Fundulus*. *Biological Bulletin* 73:134-142.

Abbott, F.S. 1970. The response of melanophores in isolated scales of *Fundulus heteroclitus* to melanophores-stimulating hormone (MSH). *Canadian Journal of Zoology* 48:581-584.

About the Authors

Seung Mook Hong received her B.S. in biology from the Yonsei University in Seoul, Korea and her Ph.D. in biological sciences from the University of Delaware. She has coordinated multi-sectioned lab courses in elementary human physiology, animal physiology and human anatomy and physiology, serving over 600 undergraduate students and 40 graduate and undergraduate TAs annually. In the newly adopted curriculum for biology majors, she developed an upper-level physiology lab course, a 2-credit, stand-alone and primary literature-based investigative course. This new course replaced the multi-sectioned lab taught by graduate TAs, and she has been teaching this course since. Her teaching interest also includes human anatomy and physiology, reproductive biology and chronobiology. Her research interest is in promoting integrative thinking practice in teaching and learning across all life experiences and the core value in her teaching is that *any* teaching activity is a means to student learning. She is a HHMI teaching fellow, a member of the Society for Neuroscience and the American Physiological Society, and currently serving as physiology lab coordinator and assistant professor in the Department of Biological Sciences at the University of Delaware.

Gary Laverty received his B.S. degree from Lowell Technological Institute (now UMass Lowell) and his Ph.D. degree from the University of Connecticut. He was a postdoctoral fellow at the University of Arizona Health Sciences Center, and came to the University of Delaware as an Assistant Professor in 1983. Dr. Laverty is currently an Associate Professor of Biological Sciences at the University of Delaware. He has received a Fulbright Research Fellowship and has received funding for his research over the year from the National Science Foundation. His main research interest is with renal and intestinal transport physiology in birds and endocrine regulation of epithelial transport. Current work is focused on a chloride transport system, studied in primary cell cultures of the chick renal proximal tubule. Dr. Laverty teaches lecture classes in General Physiology and Cell Physiology and also teaches the inquiry-based laboratory course in Experimental Cell Biology that is mentioned above.

Appendix

1. Fish saline (marine teleost saline, Foster & Hong)

   In one liter of deionized water, dissolve the following chemicals.

	Normal fish saline	**K^+-rich fish saline**
NaCl	7.8 g (134 mM)	3.9 g (67 mM)
KCl	0.18 g (2.4 mM)	5.25 g (70 mM)
$MgCl_2$	0.095	0.095 g
$NaHCO_3$	0.084 g	0.084 g
NaH_2PO_4	0.006 g	0.006 g
$CaCl_2$	0.166 g (add this last while stirring)	0.166 g (add this last while stirring)

2. K^+-rich fish saline - replace 50 % NaCl with KCl

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/**

Papers published in *Tested Studies for Laboratory Teaching: Proceedings of the Conference of the Association for Biology Laboratory Education* are ev*Alu*ated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Although the laboratory exercises in this proceedings volume have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibilities for risk. ABLE disclaims any liability with regards to safety in connection with the use of the exercises in this volume.

Citing This Article

Hong, S.M., and G. Laverty. 2011. *Fundulus* Melanophores: from Physiology to Cell Biology. Pages 245-258, in *Tested Studies for Laboratory Teaching,* Volume 32 (K. McMahon, Editor). Proceedings of the 32nd Conference of the Association for Biology Laboratory Education (ABLE), 445 pages. **http://www.ableweb.org/volumes/vol-32/?art=20**