Chromatophores in crustaceans and fish

- To identify the type, shape and abundance of chromatophores present in Crustacea subjected to different colour backgrounds
- To identify the shape, type and abundance of chrmatophores on the scales of fish subjected to different colour backgrounds

The ability of an animal to change their coloration, according to their background, allows protection from predators through camouflage and mimicking. Chromatophores possess numerous granules in which pigments are stored. The different types of pigments account for the different colouration seen in the animal.

Melanophores are chromatophores that store the black pigment melanin, thus this allows the animal to appear black. By storing carotenoids instead of the melanin, a yellow pigment is obtained. This pigment is now known as a Xanthophore. Erythrophores store the red pigments, pteridine, while Leucophores lack pigments. Iridophores store guanine or other purine crystals which reflect light giving an iridescent or shimmering appearance. (Gelfond & Rogers, 2006)

If the pigment granules in the chromatophore migrate out from the cell centre an increased coloration of the animal is seen. Alternatively these pigment granules may aggregate in the cell centre and the animal appears less coloured. Whether or not these granules move, the speed at which they move, and the manner in which this movement is controlled varies in different animals and between species. (Nyquist & Toner, 1997)

In the practical below the chromatophores of crustaceans, fish and cephalopods are to be studied and compared. During the practical the type and distribution of chromatophores on the different parts of the body of Ligia italic are to be observed with respect to the different coloured backgrounds. The cephalopod octopus is also subjected to differently coloured backgrounds and the change in the animals' colour is observed.

In the fish, the types of chromatophores present on the scales were observed and the effect of different chemicals such as acetylcholine and sodium chloride on the shape of the scale chromatophores is to be observed.

Apparatus

Stereomicroscope Adrenalin (1mg/ml of sea water)

Light microscope Acetylcholine (100mg/mL of sea water)

2 microscope slides 0. 1 M NaCl

Blu-tac 0. 2 M NaCl

Petri dish 0. 1 KCl

Red box 0. 2 M KCl

Yellow box 0. 1 M CaCl2

White box 0. 2 M CaCl2

Black box red rocks

Fish scales Blue rocks

Pipette White rocks

Aquarium

Ligia italic

Vulgaris

Method:

Refer to attached sheet

Precautions:

- Care was taken when handling the animals to prevent injury and stress to the animal
- The animals were left for some time in their appropriate background to allow them to acclimatise and adapt to the new environment.
- The same species were used in the different coloured background to obtain fair comparable results. This due to the fact that different organisms may adapt differently.
- Scales were cleaned before addition of a different chemical.
- The least possible interference was inflicted on the octopus when changing the coloured rocks.

Sources of error:

The animal was not studied in its natural environment and thus was inevitably stressed.

More than one animal in each background should have been studied for comparable fairer results

Due to inevitable weather circumstances the octopus was not left overnight to acclimatise to its environment. This thus must have stressed the animal.

The octopus was inevitably subjected to stress due to the constantly changing environment

Results

Discussion:

Crustacean

The isopod crustacean Ligia italica is a monochromatic crustacean that shows only a limited range of colour change with dark and pale as its two extremes. Pigment dispersal is seen to change in different background colours. This is also known as the albedo response. Colour change depends on the ration 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. (O'HAlloran, 1986)

An increase in light intensity will increase the amount of incident and reflected light and will cause an aggregation of the dark pigments as the increased light will cause the background to appear lighter.

As seen in the results, yellow Xantophores are seen to have a punctuate shape and are seen to be distributed all over the body at different densities. These are seen to be present at high density all over the body, when the isopod was subjected to a red background. However, it was present at very low density when the animal was subjected to a black background.

Erythrophores are seen to have a stellate or reticulostellate shape. This pigment was seen to be absent when the isopod was subjected to red or yellow light. Also it was found in relatively high densities in the white and black backgrounds. The black melanophores having a reticulate, reticulostellate, punctate or punctostellate shapes are seen at high density in all the isopods studied.

The pigments, although distributed all over the body, are seen to be at highest density in the abdomen and thorax region. The results, however, did not take into account temperature change which could have significantly affected the results obtained.

In order to adapt to increasing temperatures the darker pigments in the animal's body concentrate in the centre and thus would appear lighter. This would allow the isopod to reflect more light and therefore absorb less heat. (O'HAlloran, 1986)

The eyes are known to be the receptors responsible for colour change in isopods and other crustaceans. 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. (Oguro, 1962)

Also hormones one set of hormones are seen to be responsible for body lightening and darkening, and another set for the tail region. The sinus gland at the base of the eyestalk is known to be the immediate source of chromatophore hormones called chromatophorotropins. 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. (Parker, 1940)

Fish

Fish chromatophores possess a dendritic cell body in which the granules of pigments are able to move accordingly. Fish mainly possess melanophores and erythrophores that occur in association with microtubules and also involve the use of motor nucleotide triphosphate, ATP. The hydrolysis of ATP drives the displacement of the granules to their adjacent filaments. This mechanism is seen to involve both neural and endocrine mechanisms. (Parker, 1940)

Neural regulation is via the sympathetic component of the autonomic nervous system. This possesses both a pre and post ganglionic component which forms part of the efferent nervous system. Kinesin motors, which are responsible for the movement of pigments from the positive end of the microtubule, bring about dispersion of the melanophore pigments. Dynein motors, on the other hand cause an aggregation. (Parker, 1940)

Melaophores in fish are seen to have neural connections in which their presynaptic regions contain dense-cored granules with synapses. These appear to be of the alpha adrenergic type. Stimulation of the a1-adrenergic system results in aggregation of the pigment granules, and the resultant lightening of the fish scale coloration; whereas stimulation via the 2-adrenergic system inhibits aggregation. Also, on the surface of the melanophore, the adenosine receptor is also present. (Currie, 2004)

Pigment dispersion is activated by an increase in cAMP levels while aggregation occurs when cAMP levels are reduced. In fish, Adrenalin binds to a cell-surface receptor, which interacts with a G-protein. G-proteins have GTP binding and would cause the hydrolysis of GTP to GDP. When this hydrolysis occurs the G-protein is activated. This activation causes the inhibition of adenylate cyclase. The Function of this activated enzyme is to convert ATP to cAMP. The cAMP activates cAMP dependent protein kinase which can phosphorylate many targets. (Currie, 2004)

Although not seen in the results, when treating the fish scales with isotonic sodium chloride, melanophores disperse and appear to increase. This reaction is obtained when treating the black scale to 2M NaCl. Treatment of potassium ions should increase pigment dispersion in xanthophores and decrease it in melanophores. (Messenger, 2001)

Thus adrenalin causes the melanophores in fish to inhibit adenylate cyclase. This in turn will cause the cAMP levels to drop, protein kinase will be inhibited, and the pigment granules aggregate. Acetylcholine causes the opposite to occur and thus dispersion of the granules.

During the experiment the addition of acetylcholine to black and red scales caused an increase in the melanophores possessing a reticulate and reticulostellate shape. This appeared increase was due to the dispersal of the granules in the chromatophore. However, no change was seen with the white scales. Adrenalin caused the reticulate and stelloreticulate melanophores to appear in smaller densities due to the aggregation of the granules. This is present in also present in all the coloured scales.

Potassium ions act on the melanophores, aggregating nerve endings and causing the pigment granules to aggregate. The potassium during the experiment was present as potassium chloride.

Hormonal control is also responsible for changes in the chromatophores in fish. The Melanocyte- stimulating hormone (MSH) is produced in the intermediate lobe of the pituitary and known to target the melanophore where it causes pigment dispersion. This peptide hormone is specific to receptors present on the surface of melanocytes. For this hormone to be present calcium ions must be present. (Nyquist & Toner, 1997)

In fact as can be seen in the results, the addition of calcium ions, due to the addition of calcium chloride caused the reticulate melanophores to appear denser. The density increasing with additional concentration of the calcium ion. This, however, was only present in the fish with scales exposed to red and white colouration. The black scales appeared to show a decrease.

Another hormone causing a lightening of the fish scales is the Melanin contracting hormone. This is produced in the posterior lobe of the pituitary and causes the aggregation of the pigment granules. The last hormone to target the melanocyte is Melatonin. This is synthesised in the pineal gland and aggregates pigment granules. (Currie, 2004)

Cephalopod:

Octopus macropus is primarily found in the Mediterranean and Caribbean Seas, as well as shallow temperate tropical western and eastern Atlantic Ocean. It is dark brown-red in colour with large white spots over its entire body and paired white spots down the arms. O. macropus is a nocturnal animal and has a narrow range of prey species. (Wiston & Wood, 2006)

Octopus vulgaris lives in tropical and semitropical waters in oceans around the world; from the Atlantic, Indian, Pacific Oceans and the Mediterranean Sea. They inhabit shallow waters and can be seen up to 200 meters, but the common octopus is generally found in the near shore zone. (Wiston & Wood, 2006)

Octopus vulgaris unlike O. macropus, is diurnal and thus active during the day. O. macropus is much less active and aggressive than O. vulgaris. O. macropus also has a narrower range of prey due to its activity during the night. These differences may reduce competition through temporal spacing of activity since these species live in the same habitat.

Light penetration into the sea is of utmost importance for photosynthesis to occur, and thus the production of oxygen and removal of carbon dioxide. Water scatters most of the visible light entering the ocean and absorbs certain wavelengths. Light rays with a long wavelength, such as red, orange and yellow, are seen to be the first to be absorbed. Yellow, having the smallest wavelength of the three can only penetrate approximately 50 meters. This depends, however, on how clean the water is. Murky waters allow less penetration. Blue is seen to have the longest wavelength and thus is able to penetrate the furthest into the ocean. Thus this is why the deep into the ocean everything appears blue. (Bernol, 2006)

Unlike the chromatophores other animals, those of the cephalopods are not controlled hormonally. Instead they are seen to be controlled by neuromuscular organs which have motor systems that operate automatically to the environment and do not need to apply any force for its colour change. They constitute a unique motor system that operates upon the environment without applying any force to it. (Anonymous, 2007)

Neural control of the chromatophores enables a cephalopod to change its appearance almost instantaneously, a key feature in some escape behaviours and during agonistic signalling. When excited the muscles contract, expanding the chromatophore when they relax, energy stored in the elastic sacculus retracts it. The size and density of the chromatophores varies according to habit and lifestyle of the octopus. (Messenger, 2001)

Cephalopods are able to change their colour as a result of colour cells called chromatophores. Each chromatophore organ comprises an elastic sacculus containing pigment, to which is attached a set of obliquely striated radial muscles, each with its nerves and glia. The colours they produce range from reddish brown to yellow-orange. They contain pigment granules and are surrounded by radial muscles. With the contraction and expansion of these muscles, the pigmentation of the chromatophores changes. These muscles are independently controlled by the central nervous system. When excited the muscles contract, expanding the chromatophore, when they relax, energy stored in the elastic sacculus retracts it. While some chromatophores expand others may contract. This allows cephalopods to change colour almost instantaneously

The chromatophores are complemented by reflecting cells. These cells produce various colours by refracting light, and white by reflecting light.

There are three types of reflecting cells. The first typeare the Iridophores that are able to reflect mainly pinks, yellows, greens, blues and silvers. Reflector cells, which are known only in octopuses, reflect blues and greens, and produce these colours by interference or diffraction. The third type are the Leucophores which are broad-band reflectors. These are able to reflect white light or the wavelength that is most prevalent in the environment. (Anonymous, 2007)

Octopus vulgaris possess dim light chromatophores that are present on the uppermost layer of their dermis. In the presence of dim light they are able to expand and act as a neutral density screen to match the brightness of the background. The ambient light rays do not reach the deeper-lying leucophores that are also present in this species. In bright light, however, the chromatophores retract allowing the ambient light to reach leucophores. These then allows for accurate reflection of spectral characteristics.

During the experiment O. macropus was seen to change to the background colour quicker and closer to the background colour. Time may have been so accurate since the octopus was subject to many stresses. The fact that O. macropus obtained a colour very close to the background could be due to the fact that it is present in shallower waters. This this means that this animal may be used to living in a larger selection of wavelengths including the longer wavelength.

Conclusion

Through the experinemnt above one may note that different animals respond differently to diferent colour backgrounds. One may also note that the time taken for the change in the animal also varies. For example, colour change in the isopod Ligia italic can take up to 24 hours, while that of the octopus takes only a few minutes. These adaptations are all important in the animal's respective environment for successful escape from prey together with communication.