Annotated Bibliographies

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There are 9 annotated bibliographies. These were ordered by first providing overall context from general to specific so the readers would understand what the behaviour is and what external influences would affect the body patterning behaviour of squids from more general to specific. The following articles describes the mechanisms behind the overall behaviour then, transitioning from chromatophore control to iridophore control.

Barbato, M., Bernard, M., Borelli, L., & Fiorito, G. (2007). Body patterns in cephalopods: "Polyphenism" as a way of information exchange. *Pattern Recognition Letters, 28*(14), 1854-1864. https://doi.org/10.1016/j.patrec.2006.12.023

Article Summary: This article reviews research that describes the interaction and integration of communication in cephalopod body patterning. Through elaborate neuromuscular mechanisms, squids can change colour and texture as they move. The body patterning of cephalopods is complex as it is produced through a combination of chromatic, textural, postural, and locomotor components. Further, these parts make a whole in terms of body patterning as the combination of those components is by-products of the morphology of the basics of their skin, including chromatophore (pigment organs) and iridophores (reflective cells), and internal organs. The duration of specific body patterns is also classified as either "chronic" patterns that last a long time (up to hours) without varying or "acute" patterns that are short (seconds to minutes).

Body pattern change is dependent on the organism's surrounding environment (background or social partner) and motivations (defense, aggression, or reproduction). Cephalopod body patterns function for two different reasons: concealment and communication. There are four different mechanisms of concealment: conforming to the brightness, colour pattern, and texture of the background, countershading (darker colouration on the upper side of the body and lighter on the underside), disruptive colouration (to break up the wholeness of the body) and imitation (to resemble inanimate objects in the environment).

Using body patterning to communicate has been highly debated as many studies have contradicting results. Some argue, however, that communication through body pattern change is dishonest signaling as other organisms perceive the signaller as something they are not.

Different species do range in body patterns and chromatic components, which are dependent on the characteristics of the environment. Since chromatophores are neural products, there is also a potential correlation of neural control and organization with body patterning; however, these studies are only preliminary and would require further research.

Article Contribution: This article was chosen to be a part of my literature review as it provided an overview of how cephalopods utilized their mutable skin to function in camouflage or information exchange or, rather, to impede and to signal dishonestly. It offered different examples of species-specific interactions and how the body pattern changes in response. It outlined the variability of the chromatic components in different species and the different behavioural observations found in many studies. One significant contribution that this article provided was the limitations in knowledge of the communication aspect and exact mechanism of body patterning behaviour in squids.

Hanlon, R.T., Maxwell, M.R., Shashar, N., Loew, E.R., & Boyle, K.-L. (1999). An ethogram of body patterning behavior in the biomedically and commercially valuable squid *Loligo pealei* off cape cod, Massachesetts. *Biological Bulletin*, *197*(1), 49-62. https://doi.org/10.2307/1542996

Article Summary: Squids change their body patterning through neural control of hundreds of thousands of chromatophores on their skin. This control allows them to change the colouration and patterning of their appearance almost instantaneously, in which the change could last from a couple of seconds to hours. Due to their ability to change into distinct patterns, researchers can categorize these patterns to associate with certain behaviours like crypsis, camouflage, instar-specific signaling, and alarm signals. The objective of this study is to create a catalog of squid patterns in association with distinctive behaviours as an effort to further study their reproductive behaviour.

Laboratory trials from May to October 1996-1998 and observations of squid spawning grounds off the southern arm of Cape Cod, Massachusetts in May of 1996-1998 were recorded and reviewed multiple times to categorize the chromatic components of the squids and the associated behaviours. It was found that squids employ different behavioural strategies in terms of body pattern and colouration depending on whether they want to be conspicuous or not. Banded patterns were the most common during camouflage as they broke up the longitudinal aspect of the squid's body. Squids also employ different patterns depending on the behavioural function, such as male guarding, aggression, and even showing a difference when a squid is paired or unpaired with a mate. The significance of these findings illustrates that these body patterns could be a method to signal and communicate during reproduction since some patterns do not function cryptically. Many changes in body patterns were seen in intraspecific communication showing just how social squids are. However, some patterns were often seen, such as all an all-dark body pattern in which the function is unknown and could be further explored in future studies.

Article Contribution: This article was chosen because it almost fully described every pattern that the longfin inshore squid (*Loligo pealei*) has been observed to display and included the associated behaviours of each specific pattern. It included figures to visualize each pattern easily and described how their pigment organs expanded or retracted to produce each variety of patterns. Additionally, it provided an ethogram of the type of body patterning seen in this squid along with the number of occurrences displaying how common each pattern was used, agreeing with previous studies that banded pattern was most common for crypsis.

Rosen, H., Gilly, W., Bell, L., Abernathy, K., & Marshall, G. (2015). Chromogenic behaviors of the humboldt squid (*Dosidicus gigas*) studied in situ with an animal-borne video package. *Journal of Experimental Biology*, *218*(2), 265-275. https://doi.org/10.1242/jeb.114157

Article Summary: Little knowledge on the pattern-changing behaviour is known about the Humboldt squid (*Dosidicus gigas*), which inhabit the oxygen minimum zone. Most of the knowledge on colour-changing cephalopods is based on the pencil squids (Loliginidae family), which mainly inhabits the coastal or shelf environments. Pigment structures in the Loliginidae family differ from the Humboldt squid as the former has three classes of pigment structure, red, yellow, and brown. In contrast, the ladder only has reddish-brown pigment structures. Therefore, the objective of this study is to analyze the chromogenic behaviour of *D. gigas* in their natural habitat.

Humboldt squid colour-generating behaviours were studied through video packages. A spectral analysis was done to investigate the frequency at which these squids would display flickering and flashing behaviour through these recordings. It was found that these squids commonly employed three different types of pattern-changing behaviour, static, dynamic flashing, and flickering. Static patterns shared similarities with other squids because they could be maintained for several seconds. However, flashing and flickering were specific to a few species of squids. Flashing from one squid was never observed without the presence of a conspecific, whereas flickering in body pattern occurs during periods of camouflage. The findings illustrate the high degree of neural control these squids have as they can vary the amplitude, frequency, and phase of their dynamic flashing body pattern. This finding further indicates the importance of the flashing pattern regarding intra-specific signaling. Flickering behaviour acts as a form of dynamic crypsis since the Humboldt squid often travels to better-illuminate depths; it is more beneficial to mimic sunlight hitting the body of water than to mimic benthic objects like the Loliginids. Future studies could include the effectiveness of flickering in different water depths and how well predators perceive the squids.

Article contribution: This article was chosen because it outlined how differences in pigment structures between different species of squids can change how they display their body patterns. Since the Loliginid family has three different classes of pigment structures, they control their body pattern spatially whereas, *D. gigas* have one type of pigment structure and therefore control their body pattern on a time domain. Further, this article advances knowledge in the field as it is one of the first to study body patterning in the Humboldt squid under natural conditions. In contrast, most studies on body patterning were observed in laboratory settings.

Staudinger, M.D., Hanlon, R.T., & Juanes, F. (2011). Primary and secondary defences of squid to cruising and ambush fish predators: Variable tactics and their survival value. *Animal Behaviour, 81*(3), 585-594. https://doi.org/10.1016/j.anbehav.2010.12.002

Article Summary: As squids have soft bodies and lack defensive structures like spines and shells, they must employ a variety of defense behaviours to avoid or evade predators. These behaviours can be classified into primary defense (avoiding detection by predators) or secondary defense (delaying or escaping the predator's approach). As different predators display different approaches to attacking a squid, the anti-predatory behaviours employed by squids change accordingly. This study aims to answer four questions. (1) When threatened by a predator, is a squid's initial response to flee or stay? (2) What is the best sequence of behaviour in the presence of each predator? (3) Are primary or secondary behaviours better for a squid's survival? (4) Does the squid's behaviour change in response to the type of predator they encounter?

Squid interactions with two different types of predators were recorded with 35 trials for squids and bluefish (chase predator) and 29 trials for squid and flounder (ambush predator). Results found that the most common initial response of squids to bluefish was to drop to the substrate, remain motionless, and display a disruptive body pattern by showing a banded body pattern. However, this was not seen during squid interactions with flounder as the squid's initial reaction was, instead, to flee. This response was significant as it showed that the squid's defense behaviour varied, corresponding to the type of predator that it interacted with. Squids would use camouflage and crypsis tactics for predators that chased prey, relying more on body pattern changes. Crypsis was used to avoid movement, which would attract chase predators. For predators that primarily used ambush as their primary tactic, squids did not rely on body pattern changes and, instead, relied on fleeing. A further question this study poses would be how these behaviours may differ between natural versus laboratory conditions.

Article Contribution: This article was chosen because it shows how effectiveness varies in the body pattern-changing behaviour in response to the different types of predators that it faces, highlighting variation in body patterning in response to external stimuli. This study further advanced knowledge in the field as it was one of the first to evaluate how different types of predators may affect squid body patterning behaviour (when they choose to use it as a defense tactic). A new question this article poses is how previous experience may affect a squid's decision in when and what body pattern is displayed.

York, C.A., & Bartol, I.K. (2016). Anti-predator behavior of squid throughout ontogeny. *Journal of Experimental Marine Biology and Ecology, 480*, 26-35. https://doi.org/10.1016/j.jembe.2016.03.011

Article Summary: As squids go through different morphological stages in their lifetime, this study focuses on how anti-predator behaviours are employed throughout each major life stage (paralarva, juvenile, and adult). Anti-predator behaviours and strategies such as jet-driven escapes, camouflage, quick body pattern and colour change, postural displays, and inking are used to confuse predators and allow the squid the time to escape. There are two objectives of this study. The first was to document how chromatic patterning, posturing, and inking in squid change in response to predators as they develop into adults. The second is to better understand behavioural cues that trigger anti-predator responses by measuring the body's motion associated with squid-predator interactions.

Squid-predator interaction trials were held for 60 paralarvae squids, nine juvenile squids, and eleven adult squids, where each trial lasted 10 minutes and was video recorded. For each trial, a single squid was placed in an arena with two summer flounders, and their different anti-predatory behaviours were recorded. What was found was that different body patterns were observed for paralarvae and juveniles/adults. Paralarvae used clear body patterns significantly more than intermediate and dark body patterns. Juveniles and adults, on the other hand, were observed to have a banded pattern significantly more than dark body, dark arms with clear body or clear body patterns. These findings highlight why squids at different life stages demonstrate a preference for certain body patterning behaviour. As paralarvae are small and do not have well-developed neural and motor controls, they opt for clear body patterning to make them difficult to detect. Banded body patterning for adults and juvenile squids makes it difficult for predators to detect them as prey. Further questions for this study could look further into the effectiveness of each category of body-patterning behaviour in deterring predators.

Article contribution: This article was chosen because it highlighted how body pattern changes in response to a specific behaviour (predator avoidance). This article advanced knowledge in the field as it was the first to show how body patterning behaviour changed to deter predators through different life stages (paralarvae, juvenile to adult). This is important as it ties the differences in morphology and ecological niches to explain why we see different body patterning behaviour in the paralarvae squids and the juvenile/adult squids. Lastly, it provided important context to the importance of neural and motor control to body patterning change.

Liu, T.H. & Chiao, C.C. (2017). Mosaic organization of body pattern control in the optic lobe of squids. *Journal of Neuroscience, 37*(4), 768-780. https://doi.org/10.1523/JNEUROSCI.0768-16.2016

Article Summary: Cephalopod's visual system and brain organization are the most sophisticated among all invertebrates. They have a hierarchically organized set of lobes within the brain consisting at the highest level, the optic lobe, lateral basal lobes and at the lowest level, the anterior/posterior chromatophore lobes. Chromatophores (pigment structures) are supplied by one or more motor neurons in which these lobes control the expansion/contraction to produce specific body patterns for intraspecific communication and camouflage. There is little knowledge surrounding the neural organization of body pattern generation. Therefore, the objective of this study is to explore how motor units are spaced in relation to the expression of body pattern components.

This study was investigated by electrically stimulating various areas in the optic lobe on oval squids for 10 seconds. Video was recorded to analyze and record the dynamic changes of the body patterns that occurred. It was found that when an electrical stimulus was applied to the medulla of the optic lobe, there was a frequent expression of body patterns and observed expansion of chromatophores. Further, the stimulation of the optic lobe resulted in more expression of the chromatophores on the mantle than the head and arms. Most of the body patterns observed could be induced by the stimulation of multiple sites of the optic lobe. The significance of these findings suggests that body pattern expression on the mantle showed ipsilateral control (on the same side of the brain). In contrast, the head and arms showed bilateral control (coordination of both sides of the body). Further, the induction of individual components by multiple sites suggests that motor units of chromatophores are organized in a mosaic manner. However, further research consisting of temporal analysis (rather than just spatial) on body patterning components would be required for further evidence for the mosaic model.

Article Contribution: This article was chosen because it described the mechanism behind body pattern change in squid through neural control. It advanced knowledge in the field as the article also illustrated the neural organization of body patterns in squids, highlighting different forms of control of the optic lobe on specific patterns. Further, their experiment also showed that certain patterns were induced more often than others in young adult squids. This indicates that motor components related to pattern changes during reproduction may be added on later in life, further demonstrating age as a proximate influence on the behaviour.

Mäthger, L.M., & Hanlon, R.T. (2007). Malleable skin coloration in cephalopods: Selective reflectance, transmission and absorbance of light by chromatophores and iridophores. *Cell and Tissue Research*, *329*(1), 179-186. https://doi.org/10.1007/s00441-007-0384-8

Article Summary: Cephalopods can change colours by a combination of their pigment organs and reflective cells. There are three classes of pigment organs, red, yellow, and brown, which they expand and contract to reflect and transmit different wavelengths of visible light. Additionally, they have colourless light-reflecting cells underlying the pigment structures and can reflect almost any wavelength of the visible light spectrum.

This study aims to quantify different wavelengths that can be observed on the squid's skin to further obtain a detailed document of the nature in which pigment and structure of the squid's skin interact. A fiber optic spectrometer was used to obtain spectral reflectance and transmission measurements on small samples of mantle tissue with intact skin of *Loligo paelaeii* (no sample size was given as it was not relevant). A major finding was that yellow and red pigment organs transmitted more light than brown. Additionally, all classes of expanded pigment organs reflected less incoming light in the long-wavelength band and absorbed shorter wavelengths. Different colour combinations are produced through the interaction of pigment and structure, which would not be produced alone.

Reflectance and transmission of wavelengths through pigment and structure allow squids to change their colours almost instantaneously, which is unique as body pattern change in other animals happens in the duration of several seconds to days. The amount of transmittance of chromatophores is significant as they function to control iridescence. The significance of this study was that it was the first to examine at a microscopic level, pigment, and structural reflector interaction of any animal which gives a key insight into the malleability of cephalopod skin. This study presents a future question of how body colour/patterning changing is mediated through species of squids when cephalopods are essentially colour blind.

Article contribution: This article was chosen to be part of my literature review as it describes the mechanism of how colour change in squids is possible. It is essential to know how colour change in squids can be so instantaneous as it gives insight into their behaviour of how they would use their body patterns to either signal or camouflage. Understanding how both the squids' pigment organs and light-reflecting cells interact to transmit and reflect light also gives insight into the limitations of their body pattern change in what colours or patterns they can produce.

Mäthger, L.M., Denton, E.J., Marshall, N.J., & Hanlon R.T. (2009). Mechanisms and behavioural functions of structural coloration in cephalopods. *Journal of the Royal Society Interface, 6*(2), 149-163. https://doi.org/10.1098/rsif.2008.0366.focus

Article Summary: This article reviews research that describes evidence regarding the reflection or diffraction of light through different cell types on squids and the underlying mechanism behind iridescence production. Despite the apparent colour blindness in squids, they can display an arrangement of body patterns that functions for camouflage and communication. These colour and pattern changes are almost instantaneous as they are mediated by the interaction of their pigment structures (chromatophores), which contain up to three classes of pigments depending on the species. As well, their reflector cells (iridophore) which depend on the incoming light and are what produces iridescence. Physiological control of these two components on a squid allows them to display a wide range of optical effects.

Evidence has shown that the iridescence of a squid's skin is mediated through multilayer reflectance and there has been no evidence for iridescence mediated through diffraction. Squids have iridophores on many parts of their body, underlying their chromatophores, and have precise arrangements indicating a behavioural function for them. Functions for iridescence on squid skin were found to be prominent in agnostic encounters. Iridescence can also be induced by acetylcholine (Ach) as many others have found that after application of Ach, the squid skin changes from non-reflective to red and orange. Another way of changing the reflectance of iridophores is through the expansion and retraction of chromatophores overlaying on top.

Further, squid pattern changes in relation to the direction and movement of the squid. This is important as squids are commonly found in large schools. Therefore, squids can use this colour change mediated by movement to communicate and coordinate themselves within the school to maintain integrity.

Further questions this article poses would be how iridescent of a squid's skin mediate communication and what the specific displays would be.

Article Contribution: This article was chosen to be part of my literature review because, unlike most articles that focused on the mechanism of colour change of squids mediated by chromatophores, this article focused on other structures such as the iridophores and how that affects the patterning of the squid. This article advanced knowledge in the field as it compiled different colours reflected off the squid's skin depending on the species and their environment. It also reviewed research that agrees with the information I have found in other primary articles such as the induction of iridophore iridescence through the application of Ach.

Hanlon, R.T., Cooper, K.M., Budelmann, B.U., & Pappas, T.C. (1990). Physiological color change in squid iridophores. *Cell and Tissue Research, 259*(1), 3-14.
https://doi.org/10.1007/BF00571424

Article Summary: It was previously thought that squids did not have control of the optical appearance in their colour reflecting cells (iridophore), but rather, the appearance of these cells was dependant on the light in the environment and expansion of their overlaying pigment organs. A handful of previous studies, however, have found evidence of active control of these colour-reflecting cells during intraspecific agnostic behavioural interactions. Otherwise, little is known on how squid colour reflecting cells contribute to their body patterning behaviour. The objectives of this study are to provide behavioural observations in a controlled environment regarding iridescence in the Atlantic brief squid (*Lolliguncula brevis*), compare the optical properties of active and passive light-reflecting cells, and test the hypothesis that light reflecting cells may be under the nervous system control.

Adult squids were observed in real-time along with photographs and videotapes to analyze for iridescence. The reflected wavelength of a decapitated iridophore dermal layer was measured through a microscope with room light. It was found that in *L. brevis*, passive iridophore cells only produced pearly or silvery iridescence, while active iridophore cells can show a wide range of colours depending on the behaviour that the animal is carrying out. Iridophores become iridescent in the presence of acetylcholine (Ach). There was also no evidence found for synaptic structures being associated with iridophores as fiber stains were not seen running to or in the iridophore layer. These findings are significant as a control in iridescence in squids allows them to use it in a variety of body patterns associated behaviours such as countershading, intraspecific interactions, and disruptive colouration. Further, the elimination of direct nerve control of iridophores could potentially suggest that active iridophores are activated through a hormone-like system by Ach. However, this hypothesis would need to be explored further.

Article Contribution: This article was selected as it was one of the few that studied the role of iridophore iridescent in body patterning when squids displayed different behaviours whereas most studies were about the overlaying pigment cells (chromatophores). This article advanced knowledge in the field as it contrasted previous studies that stated that iridophores were static cells. Rather, squids control these cells which are correlated to different types of behaviour. A new question this article provides to be explored is if the behaviour associated with different iridophore activation/iridescence differs in a laboratory setting versus in the animal’s natural environment.