

Assessing Colouration in Ichthyology: Insights from the Melanophore Index Perspective

ABSTRACT

Fish colouration significantly influences various aspects of their biology, including communication, camouflage, and mate selection. Among the pigment cells responsible for generating colours, melanophores are particularly significant due to their ability to produce dark pigments called melanin. The Melanophore Index (MI) quantitatively evaluates melanophore density and distribution across different fish species. This comprehensive review delves into the significance of fish colouration, the biology of melanophores, methods for assessing MI, factors influencing MI variations, and the ecological and evolutionary implications of MI in fish populations. By synthesizing current research and highlighting gaps in knowledge, this review aims to provide a foundational understanding of assessing fish colouration through the lens of the Melanophore Index.

Key words: Fish colouration, pigment cells, melanin, melanophore, melanophore index

1. INTRODUCTION

1.1. Importance of Fish Colouration

The resplendent array of colours and intricate patterns adorning various species of fish not only captivates the eye but also serves as a cornerstone of their survival strategies and communication mechanisms. This mesmerizing spectacle of fish colouration has captured the attention of researchers and enthusiasts alike, prompting a profound exploration into the depths of these remarkable creatures. The colouration of fish plays a pivotal role in various aspects of evolutionary biology, ecology, and behaviour, making it a topic of significant interest and importance in scientific research.

The intricate colour patterns on vertebrate integuments epitomize the natural world's beauty and have captivated observers for generations. Over the past century, studying these pigment patterns has become essential for understanding pattern formation in biology. They demonstrate diverse patterns, rapid evolution, and significant ecological roles in mate selection, camouflage, and communication, captivating scientific interest[1-3].

1.2. Evolutionary Significance: Colouration patterns in fish, intricately woven by the forces of evolutionary dynamics, notably encompass natural selection, sexual selection, and genetic drift. These phenomena, acting as sculptors of biological diversity, have indelibly etched their mark upon the tapestry of piscine evolution. The elucidation of these patterns not only unveils the evolutionary saga and diversification of fish taxa but also reveals the intricate interplay between genotype and phenotype in shaping the evolutionary trajectory [4].

Delving deeper into the genetic underpinnings of colouration traits and their heritability unveils a rich trove of insights into the mechanisms propelling evolutionary transformations and the process of speciation within fish populations [5]. By deciphering the genetic code underlying these phenotypic

manifestations, we gain a nuanced understanding of the underlying molecular mechanisms steering evolutionary change, thereby unravelling the mysteries of adaptation and divergence in aquatic ecosystems [6, 7].

Beyond mere aesthetics, fish colouration serves as a potent visual signal, resonating across the murky depths of evolutionary discourse. It serves as a beacon in the vast expanse of mate choice, guiding individuals in their quest for reproductive partners and sculpting the intricate dance of courtship rituals [8]. Moreover, it functions as a shield against the lurking shadows of predation, with cryptic colouration offering a cloak of invisibility against the prying eyes of predators. Furthermore, it serves as a herald of kinship, facilitating species recognition and fostering the cohesion of social bonds within communities [9].

1.3. Ecological Implications: The chromatic manifestation of fish intricately intertwines with the ecological dynamics of aquatic habitats. Cryptic colouration and disruptive patterns, artfully inscribed upon piscine physiognomies, serve as clandestine guardians, enabling evasion from the predatory gaze and fostering seamless assimilation into the aquatic milieu. Such adaptive strategies, honed through evolutionary epochs, ensure piscine denizens remain veiled amidst the aqueous expanse, an epitome of nature's ingenuity in the realm of survival [10].

In stark contrast, conspicuous colouration emerges as a beacon amid the aqueous tapestry, transcending the liquid depths with its vibrant proclamation. Whether it serves as a herald of peril through the eloquence of aposematic or as a flamboyant expression amidst territorial disputes or amorous courtship rituals, conspicuous colouration unveils the intricate interplay between phenotype and behaviour [11]. Given its paramount importance in the context of reproductive fitness across numerous species, colouration may serve a fundamental role in species diversification and in the reinforcement of pre-zygotic isolation through mechanisms such as character displacement [12-19].

Nestled within the mosaic of fish populations, the phenomenon of colour polymorphism stands as a testament to nature's adaptive artistry. These variegated hues, forged within the crucible of ecological selection, mirror the multifaceted tapestry of habitat divergence and resource utilization. Through the lens of colour polymorphism, we are afforded profound insights into the nuanced intricacies of habitat selection, niche partitioning, and the structural fabric of aquatic communities [20].

1.4. Behavioural Insights: The utilization of colouration among fish transcends mere ornamentation, serving as a sophisticated medium for both intra- and interspecific communication [21]. From the intricate composition of courtship displays to the assertive pronouncements of territorial signaling, piscine hues orchestrate a symphony of interaction within aquatic realms. Each chromatic flourish, precisely crafted by evolutionary forces, conveys a narrative of affiliation, dominance, and reproductive prowess, shaping the very fabric of piscine societies.

Within the hidden realm of mimicry and mimicry facilities, fish assume the guise of other species or environmental elements, manipulating perception and altering the dynamics of predator-prey relationships and community structure. Through mimicry, fish engage in a delicate balancing act, oscillating between predator evasion and prey attraction, exemplifying the intricate play of survival amidst the ever-shifting currents of ecological selection [22].

Adaptation takes on a dynamic dimension among select fish species endowed with the remarkable ability of colour change. These chromatic chameleons deftly adjust their appearance in response to environmental cues, social encounters, or internal physiological states, thereby affording them unparalleled behavioural flexibility and survivability. Such adaptive prowess, honed through millennia of evolutionary refinement, underscores the profound interconnectedness between phenotype and environment, unravelling the intricate tapestry of piscine adaptation and resilience in the face of environmental flux. Moreover, the physiological and endocrine mechanisms governing both immediate

and ultimate morphological colour changes further accentuate the nuanced dynamics of adaptation within these species [23].

The chromatic tapestry adorning fish is far from a superficial embellishment; rather, it stands as a dynamic and multifaceted facet of their biological essence, with far-reaching ramifications for their evolutionary odyssey, ecological interactions, and adaptive strategies. The study of fish colouration unveils a veritable trove of insights into the mechanisms propelling biodiversity, sculpting the ebb and flow of community dynamics, and underpinning the delicate balance of ecosystem functioning within aquatic realms. Indeed, piscine hues serve as eloquent storytellers, recounting tales of evolutionary triumphs and tribulations. They witness the unceasing ballet of adaptation and selection, inscribed upon the canvas of evolutionary trajectories with meticulous precision.

By examining fish colouration, we attain profound insights into the driving forces behind diversification and speciation, uncovering innovative adaptations within the vast expanse of aquatic environments. Fish colouration serves as a conduit for the orchestration of ecological interactions, weaving a complex web of relationships within aquatic ecosystems. Their vibrant hues serve as beacons of communication, heralding warnings, professing affiliations, and orchestrating the delicate ballet of survival amidst the fluid currents of ecological change. The study of fish colouration offers invaluable insights into the tapestry of ecosystem functioning within aquatic realms. By unravelling the nuances of chromatic adaptation and behavioural plasticity, we come to appreciate the interplay between phenotype and environment, shedding light on the mechanisms underpinning the resilience and stability of aquatic ecosystems in the face of environmental perturbations [24]. The study of fish colouration offers a gateway to understanding the essence of piscine existence, revealing the interconnectedness between form and function, adaptation and environment, and ultimately, the enduring legacy of life within aquatic ecosystems. This review aims to provide a deep understanding of evaluating fish colouration using the Melanophore Index (MI). It will cover the importance of fish colouration, melanophore biology intricacies, MI measurement techniques, factors influencing MI variations, and the ecological and evolutionary implications of MI in fish populations.

2.MELANOPHORES AND THEIR ROLE IN FISH PIGMENTATION

Melanophores, the most prevalent and studied chromatophores, are integral to vertebrate dorsal pigmentation[25]. These dendritic-shaped cells extend pigmented projections, known as melanosomes, parallel to the skin's plane [26, 27]. While primarily located in the dermis of teleosts' skin, melanophores are also present within the epidermis [26]. They facilitate the impressive array of hues seen in many fish, amphibians, reptiles, and invertebrates, capable of swift chromatic transformations, contrasting starkly with the more subdued variations in skin tone typically observed in mammals, including humans [28]. The rapid physiological colour changes, occurring within seconds to hours, are orchestrated by chromatophores, including melanophores, which mobilize pigments or nanostructures for this purpose [29, 30]. This remarkable ability underscores the fascinating complexity of vertebrate pigmentation and its evolutionary adaptations.

In fish, melanophores originate from the neural crest during embryonic development. This transient structure emerges at the junction of the neural plate and epidermis in early vertebrate development. Neural crest cells undergo epithelial-to-mesenchymal transition (EMT), detaching from the neural tube and migrating throughout the embryo. During this migration, these cells differentiate into various lineages, including melanocytes, which serve as precursors to melanophores [31, 32].

2.1. MelaninProduction

Melanocytes are specialized cells responsible for synthesizing melanin, the pigment that imparts dark colouration. During fish development, melanocytes undergo further differentiation into melanophores,

which play a primary role in producing dark pigmentation in the skin, imbuing fish with a spectrum of shades ranging from deep browns to velvety blacks across their scales, skin, and other anatomical features [33-41].

This differentiation process is regulated by a complex interplay of inherent genetic programming and external environmental cues. Critical regulators of melanocyte development include transcription factors such as the microphthalmia-associated transcription factor (MITF), which coordinates melanocyte specification, proliferation, and melanin biosynthesis. In fish, MITF tightly regulates the transformation from melanocytes to melanophores, responding to both genetic factors and environmental stimuli. MITF plays a crucial role in coordinating various aspects of melanocyte development, including lineage specification, proliferation, and melanin synthesis.

Melanocytes specialized in melanin production; undergo transition into melanophores, acting as reservoirs for melanin granules crucial in pigment distribution and camouflage. This progression relies on intricate molecular pathways [42, 43]. MITF, a pivotal transcription factor, regulates genes essential for melanocyte development and function, influencing lineage determination, cell proliferation, and melanin biosynthesis. External factors such as light exposure, temperature variations, and stressors modulate melanocyte activity and pigment distribution within fish integuments, precipitating the migration and dispersion of melanophores and altering **colouration** for enhanced concealment or communication.

3. MELANOPHORES: BIOLOGY AND FUNCTION

Melanophores intricately oversee the trafficking of melanosomes along cytoskeletal filaments, orchestrating a diverse array of striking **colour** patterns. The process responsible for the physiological change in **colour** mediated by these melanophores involves both the physical and biochemical aspects of melanosome dynamics.

Epidermal melanophores, responsible for the gradual process of morphological **colour** alteration, are commonly delineated as "spindle-shaped," featuring varying degrees of dendritic branching, and are situated at the basal stratum of the epidermis [28]. Dermal melanophores, frequently linked with the dermal chromatophore unit, reside in the dermis and feature a central cell body with dendritic extensions. These extensions can radiate outward or ascend towards the epidermis, resulting in a basket-like morphology of the cell [44]. Unlike other chromatophore types, dermal melanophores store all produced melanin and are unique to vertebrates capable of experiencing physiological alterations in **colouration**. Morphological changes in **colour** involve adjustments in both pigment synthesis rates within existing cells and alterations in the size and density of chromatophores. These shifts occur gradually over several days or weeks. Over the long term, these processes also affect the survival or programmed cell death of chromatophores, thereby contributing to morphological **colour** change, as elucidated by [45-47].

3.1. Types and Functions of Chromatophore

In fishes, the primary determinant of body pigmentation involves four types of pigment cells or chromatophores. These include melanophores, which are **dark-coloured** and house melanin; iridophores, displaying white or silver hues with grain or plate-shaped purine; xanthophores, imparting a yellow tone; and erythrophores, contributing a red hue with pteridines and carotenoids. These cells are responsible for pigment synthesis and storage and are characterized by numerous dendrites, giving them a star-like appearance [26,48]. Melanophores respond to neural stimulation triggered by increased potassium ion levels by relocating pigment particles, or melanosomes. This process relies on a complex system of Dynein- and Kinesin-motor proteins, directing melanosomes along the cytoskeleton [49,50]. Elevated potassium ion concentrations result in significant fluorosome aggregation, confirming that pigments aggregate in response to neuronal signals. Interestingly, fluorescent cells exhibit innervation patterns akin to melanophores [51].

The chromatophores of numerous fish species are primarily controlled by the sympathetic nervous system [26]. Essentially, the clustering of pigment cells is triggered by the activity of α -adrenoceptors found in the cell membrane, with adrenaline, one of the most common catecholamines, playing a significant role in this colour-changing process. Additionally, β -adrenoceptors have been identified in certain species, which facilitate the dispersion of pigment granules. However, since fish typically have pigment granules dispersed in their resting state, the aggregation mediated by α -adrenoceptors is predominant [26, 52, 53]. In certain fish species, acetylcholine (released from the parasympathetic nervous system) has also demonstrated efficacy in translocating pigment granules [54, 55].

3.2. Melanophore Development and Regulation

A higher quantity of organelles per cell or an increased amount of pigment per organelle leads to heightened intensity of black colouration in melanophores. Additionally, melanosomes exhibit the capability to aggregate within the cell body or disperse radially through microtubules upon stimulation by melanocyte-stimulating hormone, epinephrine, or melatonin, as evidenced by Nascimento et al., 2003 [56] and Odenthal et al., 1996 [57]. Microtubule-driven mobility is essential for transporting melanosomes to the actin-abundant apical domain, emphasizing the necessity of a collaborative mechanism involving both microtubule and actin motors in the capture process [58]. During dispersion, dynein deactivates, facilitating rapid pigment dispersal. Pigments pause at various positions, ensuring even distribution (**Fig. 1A**). Pigment aggregation and retention in the central pigment mass (CPM) rely on dynein and reduced intracellular cAMP levels. Dynein transports melanosomes toward the melanophore nucleus, while kinesin-like motors disperse pigment throughout the cell, aided by increased cAMP levels (**Fig. 1B**). This coordinated movement, involving dynein, kinesin, actin, and myosin V, orchestrates distribution. Coordination and maintenance of this dispersion are ongoing areas of investigation [27, 59–61].

3.3. Innervation Patterns and Motor Proteins in Pigment Movement

Although the specific regulatory factors governing melanosome motility may vary across taxa (as elaborated below), the fundamental processes of pigment translocation within melanophores seem to exhibit a remarkable degree of conservation among different groups. The rapid movement of melanin granules within melanophores, particularly considering their substantial size (up to several hundred microns in diameter; Bagnara and Hadley, 1973 [28]), prompts investigation into the mechanisms facilitating such swift transport. It is postulated that melanosome translocation is intricately coordinated by the cytoskeletal architecture, primarily composed of microtubules (MTs) and actin filaments, along with their associated molecular motors -see **Fig.1** [62-66]. Specifically, the motor protein kinesin drives melanin granules toward the cell periphery during pigment dispersion with subsequent transportation of melanosomes along randomly oriented actin myofilaments (see **Fig. 1A**). Conversely, upon stimulation inducing melanosome aggregation, the tubulin-based motor protein dynein is implicated in transporting melanin granules back towards the nucleus - see **Fig. 1B** [67].

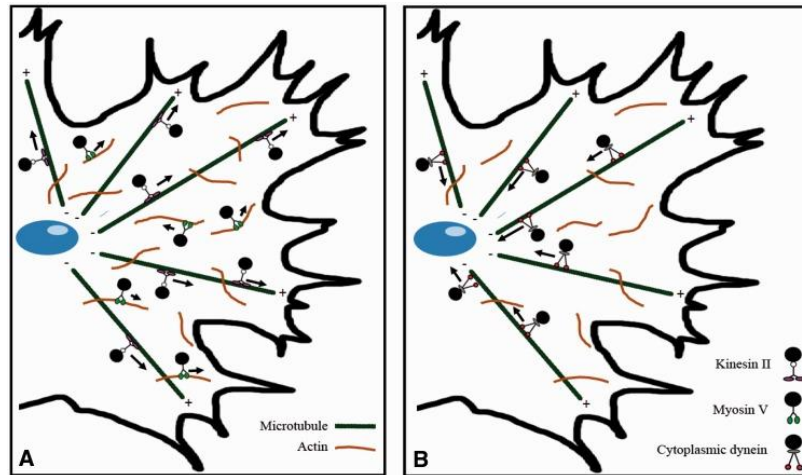


Fig.1. Model of melanosome transport during dispersion and aggregation [68].

Notes: A. Melanosomes disperse over long distances by kinesin along microtubules, and then shift to randomly oriented actin filaments at the cellular periphery, facilitated by myosin motor proteins. B. Melanosome aggregation, distinct from pigment dispersion, relies on dynein motors along microtubules.

Following their differentiation, melanophores undertake a migratory journey to establish themselves in their final destinations within the fish's integumentary system and other tissues. These cells serve essential functions in adaptive **colouration**, such as camouflage, communication, and thermoregulation, achieved through dynamic adjustments in pigment distribution in response to various stimuli, including light, temperature fluctuations, and hormonal **signalling** pathways.

In the fascinating realm of fish pigmentation, melanophores serve as the primary agents of darkness and contrast, contributing to various adaptive functions essential for survival and reproductive success. A significant role of melanophores lies in camouflage, enabling fish to seamlessly blend into their surroundings, thereby effectively concealing them from potential predators or prey [69]. Through the dynamic regulation of melanophore distribution and density, fish can adjust their **colouration** to match the background environment, thereby enhancing their chances of survival by avoiding detection. Beyond camouflage, melanophores also play essential roles in thermoregulation and protection against harmful ultraviolet (UV) radiation. The dark pigmentation provided by melanin absorbs and dissipates heat, helping fish maintain optimal body temperatures in diverse aquatic environments. Additionally, melanin acts as a natural sunscreen, shielding fish tissues from the damaging effects of UV radiation, which can otherwise cause cellular damage and impair physiological functions. The principal purpose of melanosome dispersal has evolved as a defensive adaptation aimed at mitigating UV damage, a role subsequently co-opted for camouflage purposes [70, 71]. In addition to their adaptive functions, melanophores are intricately involved in social and reproductive **behaviours** among fish species.

The intensity and distribution of melanin-based **colouration** often serve as visual cues during courtship displays, mate selection, and territorial interactions. Vibrant melanophore patterns may signify genetic fitness, reproductive readiness, or dominance status, influencing mate choice and social hierarchies within fish populations. Furthermore, the regulation of melanophore activity and pigment production is subject to complex genetic, hormonal, and environmental factors. Environmental stimuli such as light, temperature, water quality, and social interactions can influence melanophore proliferation, migration, and **colour** expression in fish. Understanding the molecular mechanisms underlying melanophore

development and regulation provides insights into the genetic basis of colour variation and adaptation in response to changing environmental conditions.

In summary, melanophores are central players in the intricate tapestry of fish pigmentation, contributing to both the aesthetic beauty and functional adaptability of these fascinating aquatic organisms. Their multifaceted roles in camouflage, thermoregulation, UV protection, social signaling, and reproductive behaviours highlight the remarkable evolutionary adaptations that have shaped the diversity of fish colouration patterns observed in nature. By unravelling the complexities of melanophore biology, researchers gain deeper insights into the mechanisms driving colour diversity and adaptation in fish, ultimately enriching our understanding of the evolutionary processes shaping life in aquatic ecosystems.

4. DECIPHERING THE MELANOPHORE INDEX: QUANTIFYING DENSITY AND DISTRIBUTION

The melanophore index, essential in biological research, objectively measures melanophore density and distribution, aiding in understanding colouration and patterning in species like fish and amphibians. This method involves systematically examining melanophore-rich areas using microscopy and image analysis for precise quantification. Researchers select standardized regions, observe them under high-resolution microscopy, and measure melanophore density, often expressed as cells per unit area.

These standardized regions are carefully chosen within the organism's body to ensure consistency across samples. They are then thoroughly examined under high-resolution microscopy to visualize individual melanophores and count their numbers accurately, quantifying melanophore density within a defined unit area. Beyond quantification, researchers analyse melanophore distribution patterns to understand camouflage, thermoregulation, and signalling behaviours, providing insights into genetic, environmental, and physiological influences on pigment distribution and adaptation.

The data obtained from melanophore index analyses are invaluable for researchers across disciplines like pigment biology, evolutionary ecology, and developmental biology. This quantitative information elucidates how genetic, environmental, and physiological factors interact to influence pigment distribution and expression. Moreover, the melanophore index facilitates comparative studies across different species or populations, exploring variation and adaptation in melanophore distribution and offering insights into evolutionary processes shaping biodiversity and phenotypic diversity.

In essence, the melanophore index is a crucial tool in pigment biology, providing a quantitative framework to understand melanophore density and distribution patterns and their evolutionary significance. It is versatile and applicable, aiding in deciphering colouration patterns and their role in the natural world.

The melanophores constitute a highly responsive system, wherein alterations in their environment can induce either the dispersion or aggregation of melanin granules, resulting in a corresponding darkening or blanching of the skin and a subsequent increase or decrease in the MI [60]. These factors exert their influence not directly on pigment cells but indirectly through neural and/or hormonal mechanisms. The activity of melanophores is subject to regulation by hormones such as melanocyte-stimulating hormone (MSH), melatonin, and MSH-release-inhibiting factor -MIF. The highly responsive melanophore system, influenced by environmental factors and hormonal regulation, modulates the melanophore index. Pharmaceutical agents can alter hormone release, affecting melanophore activity [72].

5. ROLE OF THE MELANOPHORE INDEX IN ASSESSING ENVIRONMENTAL STRESS IN AQUATIC SPECIES

The MI assesses melanosome dispersion on a five-point scale, offering quantitative insights into pigment dispersal [73]. This approach is vital for understanding stress pathways triggered by

environmental contaminants in aquatic species, highlighting the correlation between MI and genomic stress indicators. This methodology delineates five distinct stages, each denoting varying degrees of pigment dispersal within melanophores. Stage 1 represents the most aggregated state, while stage 5 signifies the utmost dispersion, with stages 2, 3, and 4 (see **Fig. 2**) embodying intermediary levels of pigment dispersal, this system facilitates the derivation of a quantitative index indicative of the relative melanosome dispersal, suitable for both numerical analysis and graphical representation, allowing for precise determination of the degree of melanosome dispersion.

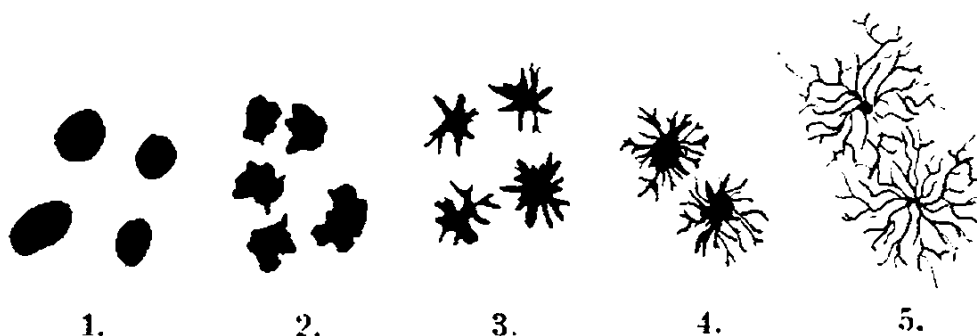


Fig.2. Hogben Melanophore Index

Roggen, 1962 [74] introduced the concept of the melanophore index, originally conceived by Hogben and Slome in 1931, which remains a prevalent morphological metric in scientific inquiry. Although its prevalence makes a detailed recounting unnecessary, a brief explanation of its merits and limitations is warranted. The Hogben-Slome index offers advantages such as expedience, simplicity, and enhanced precision in quantifying morphological attributes.

Establishing a correlation between the primary stress indicator, the MI, and a set of genomic stress indicators is crucial for understanding the cellular stress pathway induced by xenobiotics in aquatic species [75]. Given melanophores' heightened sensitivity to environmental stressors, it is essential to substantiate the connection between the MI and arrays of functional stress indicators during exposure to environmental contaminants.

In *Fundulus heteroclitus*, dermal melanophores play a crucial role in regulating the pigmentation dynamics of the dorsal skin in response to environmental cues. Exposure to a black background triggers darkening, while a white background prompts lightening. The cell orchestrates the migration of melanosomes—vesicles containing melanin—from its core towards the periphery to induce darkening or, conversely, towards the nucleus to evoke a lighter hue [76]. The resultant pigment distribution can be easily discerned through basic observation under modest magnification of fish scales obtained from individuals acclimatized to different background colours. This assessment is quantified using a scale ranging from 1 to 5, denoted as the MI, as delineated by Fujii and Oshima, 1986 [77]. The colour change mechanism in *Clarias fariatus* entails the coordinated action of both, the nervous system, through pigment-aggregating fibers, and the hormonal system, involving a melanophore-aggregating hormone. Throughout the process, these systems synergistically regulate the chromatic response, a pattern reminiscent of observations in *Rasbora elanga* [78] and *Nandus nandus* [79]. The rate of colour changes resulting from adaptation to different backgrounds, i.e., white and black, suggests a biphasic control mechanism initiated by neural control and completed through simultaneous hormonal regulation in the fish [80].

Measurements of pigment movements acquired through this automated, objective method exhibited strong agreement with measurements obtained through manual tracing of melanophore images and subsequent calculation of areas and perimeters. Moreover, the continuous variables obtained through these digital image processing techniques were suitable for statistical analyses of melanophore

dynamics, enabling the computation of parameters such as population mean and standard deviation. This stands in contrast to traditional ranked variable measurements, exemplified by the widely recognized melanophore index, which lacks the precision necessary for such statistical analyses [81].

In the past, *P. melodella spelaea* and *Pimelodella kronei* exhibited paler skin due to a reduction in both melanophore number and size. Their caudal fins contained about half the number of melanophores compared to the epigeal species *Pimelodella transitoria*. Additionally, *P. spelaea* showed approximately half the chromatophore density in equivalent dorsal areas compared to epigeal *Pimelodella* species from the Upper Tocantins river basin [82]. This reduction in melanophore density aligns with observations in cave-dwelling Astyanax species with variable eyes and pigmentation [83, 84].

Sita, 2016 [85] studied colour changes in fish responding to backgrounds, highlighting neural and hormonal coordination. They found rapid melanosome aggregation in isolated scale melanophores of three Puntius fish species when exposed to K⁺ ions, indicating neural regulation via the sympathetic nervous system. K⁺ ions induced melanosome aggregation in dispersed melanophores in saline, with quick dispersion upon K⁺ withdrawal, revealing direct innervation and efficient bidirectional melanosome movement within scale melanophores. Concentration-dependent effects of K⁺ ions on melanophores support these findings [86].

Recently, several methodologies have emerged to detect and quantify environmental contaminants in aquatic ecosystems. However, these techniques have limitations in correlating the toxicity induction and stress with contaminant concentrations in biological systems. The development of appropriate biosensors and cytosensors shows promise for measuring oxidative stress induced by environmental chemicals in aquatic organisms, as suggested by Tine et al., 2010 [87], Holt and Miller, 2011 [88], and Kaur and Dua, 2012 [89].

The sensitivity of many aquatic species to changes in water parameters, mediated by biological molecules like chromatophores, appears well-aligned with environmental stress levels. This alignment indicates that chromatophores could be valuable bio-indicators for assessing aquatic pollution. Kaur and Dua, 2012 [89] demonstrated significant alterations in the distribution and dispersion of chromatophores (including melanophores, xanthophores, and iridophores) in *Channa punctatus* due to wastewater exposure. They proposed fish scales as effective indicators of wastewater pollution. The current understanding of how chromatophores are expressed, their functional genomics, and their effectiveness as stress indicators due to chemical exposure.

Daiwile et al., 2015 [75] investigated the correlation between the MI and genomic stress indicators in *Oreochromis mossambicus* exposed to lead nitrate, phenol, and hexachlorocyclohexane (HCH). Their study assessed fish responses to sub-lethal concentrations of these chemicals over varying exposure periods using established protocols. The study concluded that changes in MI, along with functional stress indicators, provide a more precise assessment of environmental stress from contaminants.

The recommendation is to use fish scales as indicators of wastewater toxicity from the international water channel, Tung Dhab drain, due to their extensive exposure to external environmental conditions. The observed alterations in scale structural integrity, such as displaced and damaged lepidonts, along with chromatophore dispersion, provide compelling evidence supporting the reliability of fish scales as effective indicators of wastewater pollution [89].

Chaplen et al., 2002 [90] concluded that different classes of agents elicit varying responses from chromatophores. Some agents cause hyperdispersion of pigment granules; others induce only partial aggregation, while some have no visible effects. Elevated levels of cAMP and melanocyte-stimulating hormone (MSH) result in the dispersion of chromatophores.

Allen et al., 2004 [91] investigated to examine the impact of arsenic on the chromatophores of *C. punctatus*. Their study involved observing reticulated chromatophores in both control fish and those exposed to arsenic for 30, 60, and 90 days. The findings from their research led to the conclusion that chromatophores can serve as a swift and dependable biomarker for detecting pollution caused by aquatic metals. Notable super dispersion phenomenon in melanophores treated with cytochalasin B at the concentrations of 1 and 10 µg/ml of 60 minutes. Notably, there was an absence of pigment concentration in the centrosphere, as the pigment dispersed throughout the branch extremities [92].

Frisch, 1911 [93] and Parker, 1948 [94] have advocated the notion that individual chromatophores possess the ability to "aggregate or disperse" in response to suitable external stimuli. However, the mechanism underlying this remarkably coordinated and integrated response remained unexplained until the middle of the century. The predominant view emphasizes the role of the nervous system and direct innervations of chromatophores, David et al., 2004 [95] and Pouchet's (1876) [96] research on teleost fish showed that sectioning peripheral nerves or electrically stimulating spinal nerves resulted in localized darkening or paling.

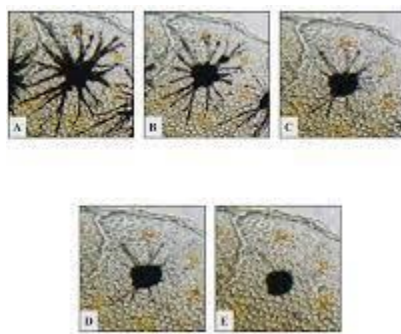
Pigment-laden cells known as melanophores are abundant within the scales and ocular structures of numerous fish species. Within these cells, mobile pigment granules possess the capacity to aggregate, imparting a pallid hue, or disperse, resulting in a darker appearance. To scrutinize the enduring impacts of medetomidine on piscine pigmentation and melanophore functionality in vivo, rainbow trout were subjected to aqueous exposure to three varying concentrations of medetomidine (0.05, 0.5, and 5.0 nM). The findings of this investigation intimate that protracted exposure to medetomidine at these concentrations may induce modifications in fish pigmentation and potentially impede melanophore efficacy [97].

The chromatophores showed alteration in the shape, density, and dimension after fluoride exposure which indicates that fluoride has a definite accelerative effect on the dispersion of the melanin pigments and its effect was duration and dose - dependent [98]. Tripathi et al., 2005 [99] who have reported similar alterations in chromatophores of freshwater fish *Channa punctatus* after fluoride exposure [100], after cadmium exposure in catfish *Heteropneustes fossilis* [101] and after methyl parathion exposure in *Oreochromis mossambica*. A decrease in the chromatophore size and number indicates the impairment of pigment cells [102]. Chromatophores especially melanophores have been shown to differentiate and to die by apoptosis under the influence of factors that regulate motile responses. These factors are likely to utilize common intracellular signalling pathways used in part to regulate both types of changes [27].

Melanophores exhibit the translocation of pigment particles, known as melanosomes, after neural stimulation induced by heightened levels of potassium ions (K⁺). This intricate mechanism involves a sophisticated network of Dynein- and Kinesin-motor proteins, which facilitate the transportation of melanosomes along the cytoskeleton, from dendrites towards the perikaryon and conversely [102, 103]. Upon treatment with elevated concentrations of potassium ions, a notable aggregation of melanosomes was observed, thereby validating the hypothesis that pigments can indeed aggregate in response to neuronal stimuli. Notably, fluorescent cells undergo innervation in a similar manner to melanophores [105]. The degree of melanosome dispersion was determined through the comparison of individual cells against a standardized chart designed for *Labeo rohita*, illustrating five distinct stages of melanophore index (refer to Fig. 1). This methodology is currently employed by numerous researchers worldwide to investigate the melanophore responses of teleosts, amphibians, and reptiles to various chemical stimuli, including drugs and melanotropic peptides. Teleostean melanophores reach a state of equilibrium characterized by nearly complete dispersion of melanosomes (MI = 5) when they are not subject to any stimulation and are placed in an isotonic physiological saline solution (in vitro). Norepinephrine and melanin-concentrating hormones induce a centripetal movement of the melanosomes towards the cell centre, resulting in a lower melanophore index, (MI = 1) [106].

Fuhrmann 2011, [107] articulated that to quantify the chromatophore response evident in the images, a Chromatophore Index (CI) was devised following the framework established by Hogben and Slome (1931), utilizing a scale ranging from 1 (indicating maximum pigment concentration) to 5 indicating maximum pigment dispersion; (see **Fig.3**).

Fig.3. Melanophore index (MI) from 1 to 5 of representatively selected pigment cells from the Fish *Belantiochelios melanopterus* ×100[108]



- A. MI = 5 (Fully dispersed state)
- B. MI = 4 (Intermediate state)
- C. MI = 3 (Intermediate state)
- D. MI = 2 (Intermediate state)
- E. MI = 1 (Fully aggregated state)

Upon the investigation of chromatophores in freshwater fishes procured from the local market of Amravati, it was observed that melanophores constituted the most abundant chromatophore type [109].

The impact of drugs on melanophore response was examined using a light microscope and assessed through the MI, a metric originally devised by Hogben and Slome in 1931 for categorizing amphibian melanophores. This index has since been conveniently adapted for use with fish pigment cells and occasionally even for chromatophores beyond melanophores. The method entails dividing the entire spectrum between maximum dispersion and maximum aggregation into five distinct stages or units. Stage 1 signifies maximal dispersion, with each subsequent numerical increment indicating progressively greater dispersion, culminating in stage 5 representing complete dispersion [85].

In Lennquist's [110] research, the effects of prolonged exposure to medetomidine on rainbow trout melanophores were examined. The study involved quantifying the paleness of fish and conducting further *in vitro* investigations into melanophore function. Specifically, the responses to melanophore stimulating hormone and subsequent addition of medetomidine were explored. Additionally, the quantity of melanophores was assessed using the Hogben–Slome index. This index employs a five-graded scale, where a score of 1 signifies maximum pigment aggregation, and a score of 5 denotes maximum pigment dispersal. The melanophore index and the melanophore potential at the cell centrosphere were assessed in fish that had adapted to both black and white backgrounds, as well as during background reversal [111].

Gempel et al., 2020 [112] conducted an assessment on pigment cell regression and potential differences in cellular responses to hormones and neurotransmitters. They achieved this by comparing three catfish species within the monophyletic genus *Pimelodella* Eigenmann, Eigenmann, 1888 (Siluriformes: Heptapteridae). The species examined were the troglobitic *P. spelaea* from central Brazil, along with the sympatric troglobitic *P. kronei* and epigeal *P. transitoria* from the southeast. The comparison utilized Hogben-and-Slome's chromatophorotropic index, a method extensively employed

in studies concerning the response of melanophores in teleosts, amphibians, and reptiles to various chemical stimuli, including drugs and melanotropic peptides.

When administered to tilapia melanophores ex-vivo, Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) induces significant pigment aggregation, marking a newly discovered function for this peptide in fish. Skin melanophore assays were conducted using scales obtained from six different fish in four independent assays, each employing 2-4 scales per treatment. The response of melanin translocation (aggregation or dispersion) was assessed using the MI. All assays were conducted using fish skin containing over 95% MI5 melanophores and were conducted in darkness to mitigate the influence of light, which typically promotes melanin pigment aggregation [113].

Acharya and Ovais, 2007 [114] investigated the effects of specific and non-specific adrenoceptor agonists and antagonists on isolated scale melanophores of *O. mossambicus* in a physiological Ringer solution. The responses were recorded using the melanophore size index. The aggregation or dispersion of pigments affects their visibility, as they cover less or more surface area, respectively [115, 116]. These researchers described alterations in pigment distribution in the frog *Xenopus laevis* by categorizing chromatophores into five classes and applying a MI for melanophores. This index is also known more broadly as the Chromatophore Index (CI) for chromatophores containing pigments other than melanin [117]. Despite its widespread usage (see Table 1 for recent examples), concerns have been voiced regarding its subjectivity, statistical validity, and labour intensiveness [118].

Table 1. Selected publications of fish applying the MI of Hogben and Slome (1931).

S.No	Species	Area of interest	Topic	Method	Source
1.	<i>Amphiprion ocellaris</i>	Skin	Toxicology	CI	2024 [119]
2.	<i>Labio rohita</i> , <i>Catla catla</i> , <i>Cyprinus carpio</i>	Scales	Toxicology	MI	2021 [120]
3.	<i>Pimelodella spelaean</i> , <i>P.kronei</i> , <i>P.ransitoria</i>	Scale and Caudal fin	Physiology	MI	2020 [109]
4.	<i>Salmo trutta m. trutta</i> Linnaeus, 1758	Scales and Embryo	Developmental Biology	MI	2019 [121]
5.	<i>Heteropneustes fossilis</i>	Skin	Ecotoxicology	CI	2018 [122]
6.	<i>Catla catla</i>	Skin	Ecotoxicology	MI	2017 [123]
7.	<i>Channa punctatus</i>	Scales	Ecotoxicology	MI	2016 [124]
8.	<i>Puntius sophore</i> , <i>P.conchoni</i> and <i>P.ticto</i>	Scale	Neurology and Endocrinology	MI	2016 [85]
9.	<i>Labeo rohita</i> (Ham.)	Scale	Ecotoxicology	MI	2015 [125]
10.	<i>Oreochromis mossambica</i>	Scale	Ecotoxicology	MI	2015 [75]
11.	<i>Clarias geripinus</i>	Skin	Physiology	MI	2015 [80]
12.	<i>Channa punctatus</i>	Scale	Ecotoxicology	CI	2014

					[126]
13.	<i>Ctenopharyngodon idellus</i>	Scale	Physiology	MI	2013 [127]
14.	<i>Verasper moseri</i>	Base of caudal fin	Developmental biology	MI	2013 [128]
15.	<i>Balantiocheilos melanopterus</i>	Scales		MI	2013 [108]
16.	<i>Betta splendens</i>	Scales	Physiology	MI	2012 [129]
17.	<i>Heteropneustes fossilis</i> (Bloch).	Scales	Ecotoxicology	CI	2012 [98]
18.	<i>Channa punctatus</i>	Scales	Ecotoxicology	CI	2012 [89]
19.	<i>Channa punctatus</i>	Scales	Physiology	MI	2011 [130]
20.	<i>Labrus bimaculatus</i>	Skin	Cell physiology	MI	2011 [60]
21.	<i>Fundulus heteroclitus</i>	Scales	Physiology	MI	2011 [76]
22.	<i>Danio rerio</i>	Scale and embryo	Physiology	MI	2011 [131]
23.	<i>Apherusa glacialis</i>	Scales	Physiology	MI	2011 [107]
24.	<i>Oncorhynchus mykiss</i>	Scales	Ecotoxicology	MI	2010 [110]
25.	<i>Siganus canaliculatus</i>	Scales	Physiology	MI	2009 [132]
26.	<i>Oreochromis mossambica</i> (Peters)	Scales (in vitro)	Physiology	MI	2007 [114]
27.	<i>Oreochromis mossambica</i> (Peters)	Scales (in vitro)	Toxicology	MI	2007 [101]
28.	<i>Heteropneustes fossilis</i> (Bloch)	Scales	Toxicology	MI	2006 [100]
29.	<i>Channa punctatus</i>	Scales	Toxicology	MI	2005 [99]
30.	<i>Channa punctatus</i>	Scales	Neurotoxicology	CI	2004 [91]
31.	<i>Betta splendens</i>	Scales	Neurotoxicology	CI	2002 [90]
32.	<i>Channa striatus</i> (Bloch)	Skin	Ecotoxicology	MI	2000 [133]

MI, Melanophore Index ; CI, Chromatophore Index

6. CONCLUSION

The review has provided valuable insights into the mechanisms and functions of fish colouration, particularly focusing on the role of melanophores and the Melanophore Index (MI). It highlights the importance of considering genetic and environmental influences on colouration patterns and discusses the variations in MI can indicate adaptation to different ecological niches and predator-prey dynamics.

Research on MI has significant implications for understanding the evolution and ecology of fish colouration. By quantifying melanophore density, researchers can gain insights into the selective

pressures acting on fish populations and how colouration patterns contribute to survival and reproductive success. Moreover, MI research can elucidate the role of colouration in communication, camouflage, and thermoregulation in aquatic environments. Understanding these aspects of fish colouration is crucial for comprehending ecosystem dynamics and predicting responses to environmental change.

To advance the field of fish colouration research, future studies should explore additional factors influencing MI, such as diet, social interactions, and habitat complexity. Moreover, incorporating molecular techniques could provide a deeper understanding of the genetic basis of colouration variation within and between species. Additionally, researchers should investigate the relationship between MI and other physiological parameters, such as stress response and immune function. Methodologically, developing non-invasive techniques for MI measurement and standardizing protocols across studies would enhance the comparability and reproducibility of results.

In conclusion, the Melanophore Index (MI) is a pivotal instrument for probing the adaptive significance of fish colouration in response to environmental pressures and comprehending the underlying mechanisms propelling pigment production in fish. Through the meticulous quantification of melanophore abundance and dispersion, researchers can glean invaluable insights into the intricate tapestry of colouration evolution and ecology within aquatic ecosystems and the pivotal ecological functions that fish colouration fulfills. This on-going pursuit and amalgamation of interdisciplinary methodologies, alongside the progression of methodological innovations, harbour the potential to untangle these intricacies and make meaningful contributions to conservation endeavours safeguarding biodiversity in freshwater and marine realms. Consequently, further exploration in this domain is paramount for conservation initiatives and the enduring preservation of fish species in the face of persistent environmental adversities.

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