

Understanding eyespots underwater

Madicken Åkerman



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Abstract

Avoiding predation is a fundamental challenge for most animals, driving the evolution of diverse defensive strategies, including body colouration. Eyespots, characterised by concentric rings of contrasting colours, are a prominent example, having evolved repeatedly across distantly related taxa. Extensive experimental work has established a compelling case for their protective function in terrestrial systems and has increasingly examined what perceptual features drive predator responses. However, empirical evidence for comparable protective benefits in marine animals remains scarce, with conclusions still largely extrapolated from terrestrial systems.

In this thesis, I evaluate the adaptive significance of eyespots as visual antipredator signals in marine systems by integrating macroevolutionary analyses with experiments on predator behaviour and prey survival. Using a phylogenetic comparative approach, **Paper I** shows that eyespots and robust physical defences follow alternative evolutionary trajectories in skates and rays. Species with caudal stings or electric discharges rarely evolved eyespots, whereas species lacking such defences were more likely to evolve eyespots when small-bodied and inhabiting shallower depths. These findings indicate that eyespots are associated with ecological contexts characterised by elevated predation risk and conditions favourable for visual signalling, consistent with the hypothesis that eyespots function as antipredator signals. To test this hypothesis, **Paper II** examines whether marine predators respond to eyespots in the same way as terrestrial predators. While red junglefowl (*Gallus gallus*) delayed attacks on eyespot-bearing prey, consistent with an intimidation function, both lab-reared bamboo sharks (*Chiloscyllium punctatum*) and wild-caught lemon sharks (*Negaprion brevirostris*) showed the opposite response, attacking eyespot-bearing prey faster than controls. These contrasting responses indicate that eyespots do not elicit uniform behavioural effects across predator taxa, raising questions about their functional role. Because predator responses to eyespots may be context-dependent, **Paper III** tests whether ambient light conditions modulate behavioural responses to eyespots. However, eyespots did not influence attack latency in three-spined sticklebacks (*Gasterosteus aculeatus*) under either high or low light conditions. They did, however, pause more frequently when approaching eyespot-bearing prey, thereby altering approach behaviour without deterring attacks. To test whether eyespots confer survival benefits, **Paper IV** conducts a field predation experiment in the Bahamas. Artificial prey with eyespots survived no longer than control prey, providing no evidence that eyespots reduce predation risk in marine systems.

Overall, this thesis reveals a divergence between evolutionary pattern and functional outcome. Although the macroevolutionary distribution of eyespots in skates and rays aligns with a visual antipredator hypothesis, experimental tests provide no evidence that they deter marine predators or improve survival. Even so, the thesis provides rare empirical evidence from marine systems, in a field dominated by terrestrial studies, broadening our understanding of an iconic antipredator defence.

Keywords: *Ocelli, eye-like markings, defensive colouration, deceptive signals, batoids.*

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- III** Juntorp, E.*, Åkerman, M.*, & Fitzpatrick, J. L. (2022). Are behavioral responses to eyespots in sticklebacks influenced by the visual environment? An experimental examination. *Ecology and Evolution*, 12, e9089. *equal contributions
- IV** Åkerman, M., Balogh, A., Gotthard, K., & Fitzpatrick, J.L. Testing the protective function of eyespots in a marine field experiment. *Manuscript in preparation*.

Candidate contributions to thesis articles*

	I	II	III	IV
Conceived the study	Significant	Significant	Significant	Significant
Designed the study	Significant	Significant	Significant	Substantial
Collected the data	Substantial	Significant	Minor	Minor
Analysed the data	Minor	Substantial	Significant	Substantial
Manuscript preparation	Significant	Substantial	Significant	Substantial

*** Contribution Explanation**

Minor: contributed in some way, but contribution was limited.

Significant: provided a significant contribution to the work.

Substantial: took the lead role and performed the majority of the work.

This thesis builds partly on Madicken Åkerman's licentiate thesis (defended on June 15th, 2023). Of the four papers included in this doctoral thesis, one paper (Paper III) was part of the licentiate thesis in its published form. Papers I and II are based on a single study presented in the licentiate thesis, which has since been divided into two separate papers and substantially revised. Paper IV is new and was not part of the licentiate thesis.

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Introduction

One of the greatest challenges in an animal's life is avoiding being eaten. Survival can hinge on a single encounter, and given the severe consequences of predation, it is no wonder that prey have evolved a variety of defences. Antipredator strategies take many forms, including staying hidden from sight, fleeing or seeking cover, and fighting back vigorously when attacked. Sharp spines, thorns, protective shells, and other physical structures can deter or even harm pursuing predators (Barnard, 1983). This diversity of adaptations reflects the pervasive role of predation in shaping animal evolution, with defensive colouration providing some of the most compelling examples (Ruxton et al., 2019; Stevens, 2015).

Prey can employ body colouration and patterning to reduce the likelihood of predation by influencing how predators perceive and respond to prey (Caro & Koneeru, 2021). For instance, some animals blend in so closely with their surroundings that predators fail to notice them (crypsis, Stevens & Merilaita, 2011). Others are readily detected but are not recognized as prey because they resemble objects that predators routinely ignore, such as leaves, twigs, or bird droppings (masquerade, Skelhorn et al., 2010). Rather than preventing detection or recognition, some prey are conspicuous in appearance and signal that they are toxic, distasteful, or otherwise unprofitable to predators (aposematism, Mappes et al., 2005). Such warning signals can also be exploited by species lacking the advertised defence, thereby gaining protection by resembling defended species (Batesian mimicry, Mappes & Alatalo, 1997). Despite the risk of attracting attention, some prey possess conspicuous markings that do not function as warning signals. Eyespots often have a striking appearance

with concentric rings of contrasting colours that are likely to draw a predator's attention. Yet they are not threatening or unappealing in any obvious way, so predators have no clear reason to avoid them. Intriguingly, even such seemingly counterintuitive adaptations can reduce predation by deceiving predators.

Eyespots have attracted sustained scientific attention for over a century and are now widely recognized as an effective defence against bird predation (Blest, 1957; Cott, 1940; Mizuno et al., 2024; Poulton, 1890; Wallace, 1877). However, in marine systems, conclusions regarding their protective benefits largely rely on terrestrial analogies (Kodandaramaiah, 2011). Whether eyespots have similar protective value in marine environments therefore remains unclear. To avoid adaptive storytelling, such interpretations should be supported by empirical evidence, particularly given that visual conditions, predator sensory systems, and ecological interactions differ fundamentally from those in terrestrial systems (Wilkins et al., 2016).

In this thesis, I address the functional and evolutionary significance of eyespots as antipredator signals underwater. The following sections provide an overview of the theoretical and empirical background that forms the basis of this thesis. The subsequent chapters summarise the included papers and discuss how their results expand our understanding of eyespots as antipredator signals.

The role of eyespots as anti-predator mechanisms

Defining an eyespot

The term eyespot derives from the widely held impression that circular markings with a contrasting outer ring resemble a typical vertebrate eye (Stevens & Ruxton, 2014). Such markings are also referred to as “ocelli” (Latin ocellus, meaning “little eye”), “false heads”, “false eyes”, “false eyespots”, or more generally “eye-like marks” (Alfaro et al., 2019; Caro & Koneru, 2021; Cott, 1940). However, what constitutes an eyespot is defined inconsistently across the literature. Some authors restrict the term to markings enclosed by a complete or nearly complete contrasting ring (e.g. Hemingson et al., 2020), whereas others apply it more broadly to include markings both with and without such a ring. In the latter case, unringed forms (i.e. simple coloured spots) are sometimes termed “simple eyespots”, while “ocellus” is reserved for ringed variants (Karan et al., 2025; Meadows, 1993).

Throughout this thesis, I follow Kjærnsmo (2017) and define eyespots as *animal colour patterns consisting of roughly concentric rings of contrasting colours* (Figure 1). While the boundary between eyespots and similar structures is somewhat subjective, and other markings may serve similar functions (Mukherjee & Kodandaramaiah, 2015), I maintain a terminological distinction between eyespots and other conspicuous markings, the latter referring to markings that diverge from the remaining body pattern.

The antipredator function of eyespots

The idea that eyespots protect prey from predators is not new. Poulton (1890) described eye-like marks in his book *The Colours of Animals*, a work widely regarded as foundational to the functional interpretation of animal colour patterns, as the most striking examples of protective mimicry. In his view, a harmless species could exploit the warning signals of a dangerous animal from a different taxonomic group. He interpreted paired spots on caterpillars as deceptive imitations of predator eyes, representing impressive snake mimics that terrify their enemies. This interpretation rests on the assumption that predators mistake these markings for the eyes of a dangerous animal and withdraw accordingly. Decades later, Cott (1940) further expanded the functional interpretation of eyespots in his book *Adaptive Coloration in Animals*, proposing that such markings may also function through mechanisms related to attack placement. In particular, he described caudal eyespots as deflective marks that steer predator strikes away from the head. Later work began to question whether eyespots function primarily through mimicry, and subsequent research has proposed several non-mutually exclusive mechanisms to explain their antipredator function (Stevens, 2005, Figure 2).

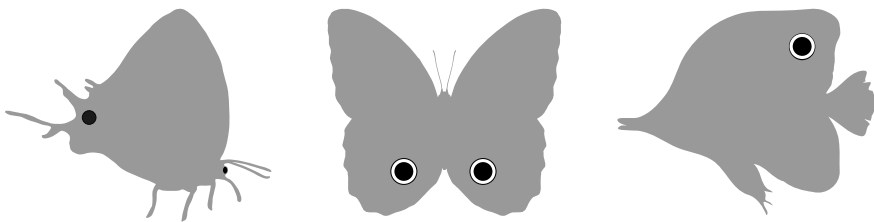
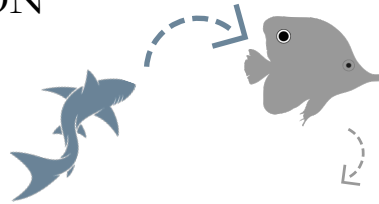


Figure 1: Schematic silhouettes showing spot and eyespot patterns. Butterfly with spot (left), butterfly with paired eyespots (middle), and fish with single eyespot (right). Eyespots are characterized by concentric rings of contrasting colours

Antipredator mechanisms and predicted predator responses

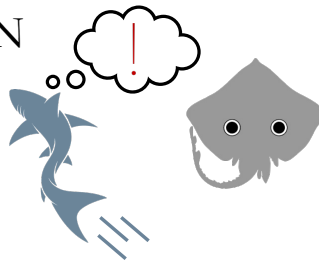
DEFLECTION

Marginal eyespots
redirect attacks,
predator misses



INTIMIDATION

Paired central eyespots
deter predators,
that abort attacks



DETECTION

Paired central eyespots
deter predator attacks
as surprise is lost

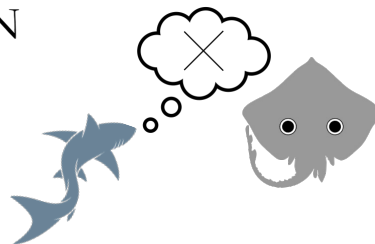


Figure 2: Schematic representation of three proposed antipredator mechanisms for eyespots. Deflection redirects attacks (top), while intimidation (middle) and detection (bottom) deter attacks.

Intimidation hypothesis

Eyespots can hinder an attack by eliciting a brief hesitation, a startle response, or complete avoidance by the predator, any of which can interrupt or delay the attack and provide the prey with an opportunity to flee (Stevens, 2005). This intimidation effect may arise because eyespots resemble the eyes of a larger or more dangerous organism, thereby triggering innate or learned fear responses in predators that perceive the eyes as belonging to their own enemies (eye-mimicry hypothesis). An alternative explanation emphasizes conspicuousness rather than eye mimicry. Under this view, it is the high-contrast visual salience of eyespots, rather than their resemblance to eyes, that triggers fear responses through sensory bias, neophobia, or sensory overload, with similar responses potentially elicited by other high-contrast markings (conspicuous signal hypothesis). Eyespots may also function as part of startle displays in which normally concealed markings are suddenly revealed (Drinkwater et al., 2022; Nakayama et al., 2025; Vallin et al., 2005). While the mechanisms underlying intimidation have been the subject of long-standing debate, their ability to reduce predation risk and enhance prey survival is widely accepted.

Deflection hypothesis

An alternative, not mutually exclusive mechanism is deflection, an antipredator strategy in which eyespots influence the point of a predator's initial contact with the prey's body (Humphreys & Ruxton, 2018). Under this mechanism, eyespots redirect strikes away from vital body regions toward less vulnerable areas such as hindwings or fins, reducing the likelihood of lethal injury. Eyespots may also create the illusion of a false head at the posterior end of the animal, causing predators to misjudge the prey's orientation or direction of movement and initiate an attack in the wrong direction (Yumnam & Kodandaramaiah, 2025). In contrast to the intimidation hypothesis, eyespots associated with deflection are typically small and marginally positioned, and may occur in combination with markings that obscure the true eyes.

Detection hypothesis

While intimidation and deflection have dominated empirical research on eyespots, an additional mechanism that has been proposed is deceptive detection. Under this hypothesis, originally proposed in the context of interactions between raptors and mobbing birds, eyespots are suggested to mimic forward-facing eyes, causing approaching predators to perceive that they have been detected (Negro et al., 2007). By removing the advantage of surprise, such signals may reduce the likelihood of attack, similar to pursuit-deterrent behaviours in predator-prey interactions (Hasson, 1991). Field experiments showing that eyespots painted on cattle reduce ambush predation demonstrate that eye-like markings can function as detection signals (Radford et al., 2020), with similar deterrent effects reported in racing pigeons (Stamenov et al., 2025). However, whether prey animals exploit eyespots to mimic pursuit-deterrent signalling has not been extensively examined.

Empirical support

Terrestrial eyespots

Experimental studies in terrestrial systems have provided robust evidence that eyespots reduce predation by birds (Mizuno et al., 2024). Artificial prey bearing eyespots experience lower attack rates than controls in field experiments, with effects demonstrated across multiple prey types including butterflies, moths, and pastry baits (De Bona et al., 2015; Stevens, 2005). Laboratory studies further show that eyespots can trigger avoidance behaviours and startle responses in bird predators, particularly when suddenly revealed (Vallin et al., 2005). The deflection hypothesis has also gained empirical support where butterflies with marginal eyespots show a higher frequency of non-lethal wing damage compared to species lacking such markings, suggesting that eyespots successfully redirect strikes away from the body (Olofsson et al., 2010a; Prudic et al., 2015).

Although experimental studies provide evidence that visual defensive signals can influence predator behaviour and reduce preda-

tion risk (Mizuno et al., 2024), comparative analyses indicate that their effectiveness is context-dependent, with their adaptive value likely depending on body size and ecological conditions (Ho et al., 2016; Hossie et al., 2015), suggesting that eyespots evolve depending on the conditions under which they are perceived, and are selected against when they become disadvantageous.

Marine eyespots

Eyespots in marine fishes have long been interpreted as a case of functional convergence with butterfly eyespots (Figure 3), although comparative evidence supporting analogous antipredator mechanisms has only recently emerged (Hemingson et al., 2020; Karan et al., 2025; Kelley et al., 2013). In coral reef fishes, species that spend more time swimming in the water column typically bear eyespots in the posterior dorsal region, consistent with the hypothesis that marginal eyespots may facilitate escape by deflecting predator attacks (Hemingson et al., 2021). This strategy is likely to be most effective in species capable of rapid escape once attacked and in which eyespots are positioned on body regions that can sustain non-lethal damage, as predicted by deflection theory (Humphreys & Ruxton, 2018). In contrast, slower benthic species that remain close to the substrate often display eyespots near the head or mid-body (Hemingson et al., 2021). This placement is less consistent with a divertive function and may instead reflect a deterrent role, such as startling or intimidating predators. These correlational patterns suggest that eyespots may function analogously across environments, yet such patterns alone cannot confirm functional effects on predator behaviour.

The strongest experimental evidence of an antipredator function comes from studies on three-spined sticklebacks, which preferentially target small eyespots on artificial prey (Kjernsmo et al., 2016, 2019; Kjernsmo & Merilaita, 2013), consistent with a deflection function. These findings demonstrate that at least one mechanism, deflection, may operate in aquatic predator-prey interactions. Demonstrating the intimidation function has proven more challenging. Although sticklebacks show hesitancy when approaching stim-

uli that resemble eyes, studies have not been able to demonstrate the same response to typical eyespot patterns characterized by concentric rings (Kjernsmo & Merilaita, 2013, 2017). Thus, whether eyespots can deter marine predators remains unresolved.

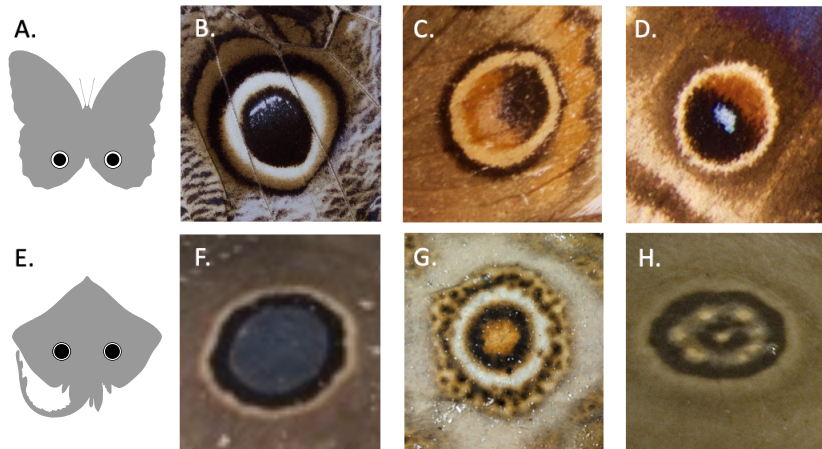


Figure 3: . Eyespots in A. Lepidoptera (B. *Caligo brasiliensis*, C. *Junonia coenia*, D. *Salamis temora*) and in E. batoids (F. *Raja miraletus*, G. *Diplobatis ommata*, H. *Rostroraja velezi*, I. *Caliraja rhina*, J. *Raja radula*, K. *Caliraja stellulata*, L. *Beringraja binoculata*). Photo credit: John Fitzpatrick (B-D), Amy Rowley (F), Andy Murch (G-H).

Aim

This thesis evaluates the adaptive significance of eyespots as visual antipredator signals in marine systems. To achieve this aim, the research was structured around the following objectives:

- Characterise the macroevolutionary patterns and drivers of eyespot evolution (**Paper I**)
- Assess predator behavioural responses to eyespots (**Paper II–III**)
- Evaluate whether eyespots confer survival advantages (**Paper IV**)

Together, these objectives examine the adaptive significance of eyespots at multiple levels. First, by testing whether the macroevolutionary distribution of eyespots is consistent with an antipredator function and then evaluates this hypothesis by assessing behavioural responses and survival consequences.

Methodological overview

Phylogenetic comparative analyses

If eyespots provide antipredator benefits, a key question is why they occur in some species but not others. Examining trait distributions across species can help identify the ecological and evolutionary conditions under which they arise (Cornwallis & Griffin, 2024). Comparative phylogenetic approaches allow such questions to be investigated by analysing patterns of trait evolution across many species while accounting for shared ancestry (Weber & Agrawal, 2012).

Paper I investigates the macroevolutionary distribution of conspicuous markings (including eyespots) in skates and rays using a large comparative dataset covering the vast majority of extant batoid species. Ancestral state reconstruction and models of evolutionary transitions were used to infer how conspicuous marking types evolved across the batoid phylogeny. Phylogenetic logistic regressions were then used to test whether the presence of conspicuous markings was associated with ecological variables or alternative antipredator defences.

Experimental tests of predator responses

Comparative analyses can identify evolutionary contexts in which traits arise, but they cannot provide strong causal inference about their functional role (Kikuchi et al., 2023). Direct experimental tests are therefore necessary to evaluate mechanistic hypotheses derived from phylogenetic patterns and determine whether eyespots function as antipredator defences (Hemingson et al., 2021; Weber &

Agrawal, 2012).

While Paper I identifies the evolutionary contexts associated with eyespot evolution, Papers II–III use experiments to test whether predators respond to eyespots in ways consistent with an antipredator function. Paper II tests whether eyespots influence predator attack latency using red junglefowl, brown-banded bamboo sharks, and lemon sharks attacking artificial prey. Because predator responses to eyespots may depend on the conditions under which they are encountered, Paper III tests whether predator responses vary with ambient light by measuring attack latency in three-spined sticklebacks under high- and low-light conditions (Olofsson et al., 2010b).

Field experiment on prey survival

Whether eyespots function as antipredator signals ultimately depends on whether they reduce predation risk. Although laboratory experiments can reveal how predators perceive and respond to signals, such experiments may not accurately predict outcomes in nature, where free-ranging predators encounter prey in complex and variable environments. Field experiments therefore provide an important approach for testing whether eyespots reduce predation risk (Irschick et al., 2009).

Paper IV tested whether eyespots influence the survival times of artificial prey deployed in shallow coastal waters. Continuous video monitoring allowed attack events to be verified directly and predator identities to be recorded, overcoming a limitation of artificial prey experiments in which predation events are typically inferred from prey disappearance (Akcali et al., 2019; Seimandi-Corda et al., 2025).

Artificial prey

The experimental studies in Papers II–IV used artificial prey, which are widely employed in studies of predator–prey interactions because they allow specific prey traits to be isolated experimentally and provide a practical way to estimate predation pressure under

field conditions (Lövei & Ferrante, 2017; Nagy et al., 2020). Artificial prey models have also been used to test how visual prey markings influence predation risk, including studies examining the effects of eyespots on predator attack rates using paper prey models (e.g. Stevens et al., 2007). By controlling visual features while standardising other prey traits, researchers can directly test how particular signals influence predator behaviour. A limitation of artificial prey is that they reduce ecological realism compared with live prey (Lövei & Ferrante, 2017). However, they provide the experimental control needed to isolate the effects of specific visual signals. Artificial prey have therefore been widely used both to estimate predation risk in field experiments and to test the function of visual signals (Cuthill et al., 2005; Lövei & Ferrante, 2017).

In Papers II–IV, predator responses were tested using artificial prey targets made from patterned paper and baited with food. Although prey designs differed among species and experimental settings, all experiments compared responses to prey bearing a pair of eyespots with responses to otherwise identical control prey lacking eyespots. This design isolated the effect of the eyespot pattern while controlling for other prey traits.

Study systems

Skates and rays (superorder Batoidea)

Skates and rays (superorder Batoidea) are a diverse group of cartilaginous fishes distributed across waters worldwide (Last et al., 2016). They are preyed upon by a wide range of predators, including sharks, marine mammals, birds of prey, and teleost fishes, suggesting that predation may be an important selective force shaping defensive traits (Flowers et al., 2021; Langerhans, 2007). Batoids vary substantially in the types of defences they deploy (Last et al., 2016). Some taxa possess robust defences: stingrays and their allies (Order Myliobatiformes) bear caudal stings capable of injuring predators, while electric rays (Order Torpediniformes) can produce electric discharges that deter or incapacitate them (Dean et al., 2017; Papastamatiou et al., 2025). In contrast, skates (Order Rajiformes) and

shovelnose rays and their allies (Order Rhinopristiformes) lack these defences. Instead, they rely on dorsal thorns and denticles, crypsis, burial in the substrate, and flight responses (Heithaus et al., 2022). This contrast between species with robust active defences and those lacking them, distributed across a well-resolved phylogeny comprising more than 600 extant species, provides the comparative leverage needed to test whether eyespots evolve as alternatives to more robust forms of defence (Kikuchi et al., 2023; Naylor, 2018).

Batoids also exhibit extensive variation in body size and depth distribution. The smallest species are only about 10 cm long, whereas the largest reach approximately 7 m in length, and species occupy habitats ranging from intertidal shallows to depths exceeding 1000 m (Last et al., 2016). Variation in depth corresponds to substantial differences in the visual environment, as light availability decreases steeply with depth and is largely absent below approximately 200 m (Lythgoe, 1988). Because the effectiveness of antipredator signals may depend on ecological conditions influencing both predator detection and prey vulnerability, this ecological diversity provides an additional axis along which selection on conspicuous markings such as eyespots may vary (Endler, 1993).

Sharks (*Chiloscyllium punctatum* and *Negaprion brevirostris*)

Two shark species were used to examine marine predator responses to eyespots. Bamboo sharks (*Chiloscyllium punctatum*) are small benthic sharks that can be maintained in laboratory aquaria, allowing for controlled behavioural experiments with standardized visual stimuli. Lemon sharks (*Negaprion brevirostris*) are larger and more active predators that can be studied in semi-captive field pens around Bimini, Bahamas, where established research programmes provide the infrastructure necessary for experimental work with wild individuals. Both species have been used in behavioural studies of visual discrimination and learning, making them appropriate models for experiments investigating predator responses to visual stimuli. Furthermore, both species lack colour vision and instead rely primarily on contrast sensitivity, making them particularly suitable for

testing responses to high-contrast markings in underwater environments, where lighting conditions can alter colour perception (Brown & Schluessel, 2023; Ryan et al., 2016).

Red junglefowl (*Gallus gallus*)

Red junglefowl (*Gallus gallus*), the wild ancestor of the domestic chicken, is a well-established model species in behavioural research. As terrestrial foraging birds that rely heavily on visual cues to locate and evaluate food items, junglefowl are well suited for studies of visual signal perception and predator decision-making (Garnham & Løvlie, 2018). Although they are not natural predators of marine prey, they provide a useful comparative reference system because the deterrent effects of eyespots are well documented in birds (Mizuno et al., 2024). Responses in junglefowl therefore offer a benchmark against which predator responses observed in marine species can be interpreted.

Three-spined stickleback (*Gasterosteus aculeatus*)

Sticklebacks are small teleost fish that can be readily maintained in laboratory aquaria, allowing controlled behavioural experiments. They are visually oriented predators that feed on small invertebrates and fish larvae, making them suitable model organisms for studying responses to prey visual signals. Sticklebacks have previously been used in studies of eyespot function, particularly in experiments where predators attack artificial prey bearing eyespots and control patterns, providing a basis for comparison with the results of Paper III (Kjernsmo et al., 2016; Kjernsmo & Merilaita, 2013).

Paper summaries

Paper I - The evolution of eyespots in skates and rays

To identify the drivers of eyespot evolution and test whether their distribution matches predictions from antipredator theory, Paper I examines the occurrence and evolutionary dynamics of eyespots and other conspicuous markings across skates and rays.

Conspicuous markings—including eyespots and other markings—were scored from dorsal body patterns of 580 batoid species, representing >90% of extant skates and rays. Eyespots were defined as circular markings with concentric rings of contrasting colors, while other markings included circular markings without rings and irregularly shaped markings. For each species, data were compiled on antipredator defence type (standard vs. robust, with robust defences including caudal stings or electric organs), adult body size, and depth profiles (as a proxy for the visual environment). Phylogenetic comparative methods reconstructed ancestral states, modeled evolutionary transitions among marking types, and tested whether conspicuous markings co-evolved with ecological and defensive traits.

Conspicuous markings were found in 83 of 580 species (14%). Of these, 25 had eyespots and 58 had other markings. Most conspicuous markings were positioned centrally on the pectoral fins and arranged as bilaterally symmetrical pairs, with 76% of species displaying a single pair. Conspicuous markings were more prevalent in skates, occurring in 23% of species compared to 6% in rays. Eyespots were found almost exclusively in skates, with 23 of 25 eyespot-

bearing species belonging to this order.

The presence of conspicuous markings was shaped by interactions between body size and defence type, and between body size and depth. Across most body sizes, species lacking robust defences were more likely to possess conspicuous markings. However, among the smallest species, this pattern reversed: those with robust defences were more likely to exhibit conspicuous markings than those without such defences. Smaller body size was also associated with a higher probability of conspicuous markings, but only in species inhabiting shallower depths.

When eyespots and other markings were analysed separately, distinct patterns emerged. Eyespots were more common in species that lacked robust defences, had small body sizes, and lived at shallower depths. Other markings, by comparison, were more likely to be found in small-bodied species with robust defences.

Ancestral state reconstructions indicated that the common ancestor of skates and rays lacked conspicuous markings. Conspicuous markings evolved multiple times but were lost approximately nine times more frequently than they were gained. When conspicuous markings did arise, other markings evolved more readily than eyespots. Eyespots most often evolved from pre-existing other markings rather than originating directly in lineages lacking conspicuous markings. Transitions in marking number typically involved initial gains of multiple markings, followed by reductions to bilaterally symmetrical pairs. Conspicuous markings evolved primarily in species lacking robust defences and inhabiting shallow-water habitats. Combinations involving robust defences or deep-water habitats were evolutionarily unstable.

These findings demonstrate that skates and rays follow alternative evolutionary trajectories in anti-predator defence. The evolution of conspicuous markings is associated with defence type, body size, and depth, reflecting functional trade-offs with physical defences. Conspicuous markings evolved primarily in smaller species lacking robust defences and inhabiting shallow water, a pattern consistent with expectations under size-dependent predation risk and with the greater effectiveness of visual signals in well-lit environments.

Paper II - Behavioural responses to eyespots

If eyespots serve analogous antipredator functions across environments, similar deterrent effects would be expected in marine predators as in terrestrial predators. To test this prediction, Paper II examined whether sharks show behavioural responses to large, paired eyespots comparable to those observed in birds.

Red junglefowl (*Gallus gallus*), brown-banded bamboo sharks (*Chiloscyllium punctatum*), and lemon sharks (*Negaprion brevirostris*) were trained to approach and attack baited artificial prey in experimental arenas. Each individual completed multiple trials with control-patterned prey, followed by a trial with eyespot-patterned prey. Differences in attack latency between prey types were used to evaluate responses to eyespots within individuals. This within-individual design allowed behavioural differences to be attributed to prey pattern rather than to individual variation in attack behaviour.

If large, paired eyespots function as deterrent signals, they should increase attack latencies. Consistent with this expectation, red junglefowl delayed attacks on eyespot-patterned prey. In contrast, both shark species attacked eyespot-patterned prey faster. This pattern was consistent in lab-reared brown-banded bamboo sharks and wild-caught lemon sharks, indicating that the response occurred regardless of prior predator experience. Thus, unlike in birds, large eyespots did not deter sharks but instead elicited faster attacks.

Paper III - Influence of the visual environment on responses to eyespots

Eyespot effectiveness may be context-dependent, varying with the conditions under which predators encounter them. Consequently, previous null results for large eyespots in aquatic predators may reflect testing conditions rather than an inherent lack of function. Therefore, Paper III tests whether ambient light conditions influence behavioural responses to eyespots.

Three-spined sticklebacks (*Gasterosteus aculeatus*) were assigned to either low-light or high-light conditions and trained to approach a feeding plate. Each fish completed a baseline trial in which it approached food on a plain plate, followed several hours later by a test trial in which food was presented on artificial prey bearing either large, paired eyespots or a control pattern of scattered dots. Attack latency was measured in both trials, and differences between baseline and test trials were compared between prey types and light conditions. This within-individual design ensured that behavioural changes could be attributed to prey pattern and light conditions rather than individual differences in motivation or approach.

If eyespots function as intimidating signals primarily under low-light conditions, longer attack latencies were expected for fish approaching eyespots in the low-light treatment only. However, eyespots did not affect attack latency, and although fish were slightly slower to attack under low-light conditions, this effect was not specific to eyespot prey. Eyespots did influence other aspects of behaviour though, as sticklebacks stopped more frequently when approaching eyespot prey and remained motionless except for pectoral fin movements regardless of light treatment. Thus, light conditions did not modify eyespot responses as predicted.

Paper IV – Field test of the protective value of eyespots in a marine environment

Because behavioural responses do not necessarily translate into reduced predation risk, Paper IV tests whether eyespots influence survival in a field-based predation experiment using artificial prey.

Pairs of artificial prey were deployed in shallow coastal waters around Bimini, The Bahamas. Each pair consisted of two baited prey items. One was printed with large, centrally located eyespots and the other was a plain white control. Both were placed simultaneously on the substrate and monitored using continuous underwater video recording. Analysis of the recordings identified which prey item was attacked first and how long each remained unattacked, allowing an assessment of whether eyespots altered survival risk.

If eyespots function as protective signals, eyespot-bearing prey should survive longer than controls. However, fish did not preferentially attack either prey type first, and eyespots did not reduce the likelihood of attack or delay attacks compared with control prey. This outcome was consistent across the two dominant species, mojarra and schoolmaster. Thus, large, centrally located eyespots did not confer detectable survival benefits in this marine field setting.

Synthesis

This thesis evaluates the adaptive significance of eyespots as visual antipredator signals in marine systems. Across four papers, I integrate macroevolutionary analyses, behavioural experiments, and field-based tests to test this hypothesis. Macroevolutionary analyses of skates and rays show that eyespots evolve preferentially in small species lacking robust defences and inhabiting shallow waters, consistent with predictions of selection for visual antipredator signalling (Paper I). By contrast, behavioural experiments provide no evidence that eyespots deter attacks (Papers II–III), and a field experiment detects no survival advantage (Paper IV). Collectively, these findings reveal a paradox: eyespots show the evolutionary signature of adaptive antipredator coloration, yet experimental tests provide little evidence of a protective effect and in some cases suggest the opposite.

Adaptive evolution of eyespots

The evolutionary concentration of eyespots in small, shallow-water batoids lacking robust defences is consistent with selection for visual antipredator signalling. Small body size increases vulnerability to a wider range of predators because they fall within the gape size of many predators, and those lacking robust defensive weapons pose little threat during encounters. For species combining these vulnerabilities, eyespots may offer an alternative defence, but only where predators can perceive the signal. Thus, the restriction to shallow waters, where light penetration supports visual detection, rather than deeper environments where visual conditions deteriorate, is consistent with a visual antipredator function. Together, these pat-

terns suggest that eyespots evolve under conditions combining high predation risk and effective signal transmission.

Eyespots were evolutionarily unstable in species inhabiting deep water or possessing robust defences, with these combinations repeatedly lost (Paper I), suggesting that selection constrains eyespot evolution to specific ecological contexts. This constraint is not unique to batoids. In coral reef fishes, eyespots show a similar concentration in small-bodied species and are often lost as body size increases, consistent with reduced predation pressure on larger individuals (Hemingson et al., 2020). Collectively, these macroevolutionary patterns across independent marine lineages suggest convergent evolution driven by predator–prey interactions.

While macroevolutionary patterns suggest that eyespots evolved under selection driven by predator–prey interactions, comparative evidence alone cannot confirm that eyespots affect predator behaviour. The critical question is therefore whether eyespots function as antipredator signals, which requires experimental assessment of predator responses and their consequences for prey survival.

The experimental contradiction

If eyespots evolved as visual antipredator signals, they should elicit behavioural responses in predators that translate into survival benefits. Because eyespots in batoids are predominantly located centrally on the body rather than marginally (Paper I), misdirecting strikes through deflection seems unlikely to be the primary mechanism. Instead, centrally located eyespots should deter predators. Consistent with this expectation, red junglefowl delayed attacks on eyespot prey, replicating effects widely documented in terrestrial systems (Paper II, Mizuno et al., 2024). However, marine predators showed little evidence of deterrent responses. Sharks attacked eyespot prey faster (Paper II), sticklebacks did not delay attacks (Paper III), and eyespot-bearing prey experienced no survival advantage in field experiments (Paper IV).

Sharks attacked eyespot prey faster in both naive lab-reared

bamboo sharks and wild-caught lemon sharks (Paper II), regardless of prior experience, suggesting this response is unlikely to reflect an experimental artefact. These faster attacks run counter to the prediction that eyespots deter predators, and instead suggest that eyespots may increase prey conspicuousness during the search phase of predation. Similar effects have been reported in caterpillars, where eyespots enhance detectability and impose costs when prey are too small for effective deterrence or when the markings compromise camouflage Hossie & Sherratt (2012). If the protective effect of eyespots is conditional, such that the costs of increased conspicuousness sometimes outweigh deterrent benefits, this trade-off may help explain the evolutionary lability of eyespots observed in batoids (Paper I) and coral reef fishes (Hemingson et al., 2020).

Sticklebacks did not delay attacks on eyespot prey, and responses did not differ between high and low light conditions, contrary to predictions that reduced visual resolution in dim light would prevent predators from recognizing eyespots as harmless body patterns and thereby deter attacks (Paper III). However, sticklebacks stopped more frequently during their approach to eyespot prey. In fishes, such stop–start behaviour is associated with predator inspection, in which individuals pause and orient toward a stimulus to assess potential threats (Kelley & Magurran, 2011; Näslund et al., 2016). The increased stop frequency indicates that eyespots trigger predator inspection behaviour, suggesting these markings are perceived as predator-like or threat-associated cues. Despite this, inspection did not translate into delayed attacks, revealing that predators respond to eyespots without this translating into a deterrent effect.

If eyespots confer antipredator benefits, eyespot-bearing prey should survive longer than control prey. However, in paired field deployment trials, fish did not preferentially attack either prey type first, and eyespots did not delay attacks relative to control prey (Paper IV). Thus, large, centrally located eyespots did not confer detectable survival benefits under field conditions.

Eyespots are associated with ecological conditions consistent with predation-driven selection, but experiments fail to detect the predicted behavioural or survival benefits. What might explain this

discrepancy?

Explaining the paradox

What may first come to mind is that eyespots no longer serve an antipredator function and instead represent evolutionary relics maintained despite having lost their protective value. However, the evolutionary dynamics in batoids (Paper I) argue against this. The repeated and directional loss of eyespots indicates active selection against eyespots in contexts where they impose costs, rather than the gradual erosion of a non-functional trait. Comparative analyses of batoids further show that conspicuous markings other than eyespots are evolutionarily unstable, whereas eyespots tend to evolve following the appearance of other conspicuous markings and then persist. This selective retention of eyespots suggests that they are maintained by selection in a way that other conspicuous markings are not. The persistent presence of eyespots in small, shallow-water species lacking robust defences therefore indicates ongoing selection.

Given that macroevolutionary evidence suggests an antipredator function, the lack of support from marine predator experiments may reflect that selection is driven by seabirds attacking from above. Although junglefowl are not natural predators of marine prey, deterrent responses to eyespots appear widespread across avian predators (Mizuno et al., 2024), suggesting that the response observed in Paper II reflects a general aversion among birds. However, eyespot-bearing fish face predation from diverse marine taxa, and if eyespots increase detectability, as suggested by sharks attacking faster in Paper II, they may attract some marine predators even while deterring seabirds. For such a signal to be maintained, deterrent effects against seabirds would need to outweigh the costs of increased vulnerability to marine predators. Given the evolutionary persistence of eyespots across diverse lineages, this seems an unlikely explanation for their maintenance.

Even if the relevant predators were represented in the experiments, the shark trials may not provide a clear test of visually medi-

ated responses. Sharks rely on multiple sensory systems, and if they recognized the artificial targets as non-living, they may have had little incentive to abort attacks in response to eyespots, since stimuli not perceived as alive are unlikely to be treated as threatening. Under such circumstances, predator responses may not reflect how sharks would respond to visual antipredator signals during natural predation encounters. Beyond experimental limitations, the discrepancy may also reflect problems with the mechanism typically proposed to explain eyespot function.

One explanation for this discrepancy is that eyespots may not operate through a single perceptual mechanism. Different predator species may interpret the same visual pattern in different ways. Intimidation, startle, detection, and deflection are often treated as alternative mechanisms, but they are not necessarily mutually exclusive. The same body pattern may therefore elicit different behavioural responses depending on predator identity, hunting strategy, and ecological context. This complicates experimental design because, without knowing which mechanisms operate for which predators, it is difficult to test the appropriate predictions. However, these mechanisms may not be mutually compatible.

The incompatibility of intimidation and deflection mechanisms

During foraging, predators encounter diverse stimuli and rely on specific cues to distinguish prey from non-prey and to identify potentially dangerous prey (Pie, 2005; Stevens, 2013). Misinterpreting these cues can have significant consequences, as predators may miss feeding opportunities or, worse, risk injury or death by approaching a genuine threat. Because the costs of failing to avoid a threat exceed those of missing a meal, animals may evolve built-in aversions to cues associated with danger, allowing them to avoid potentially harmful prey without relying on prior experience (Johnson et al., 2013). However, such aversions can impose a trade-off if the same cues also interfere with prey recognition, underscoring the importance of distinct signals for prey and threats (Pie, 2005).

This framework raises a potential conflict for intimidation signals in marine systems. In fish predation, recognition of the head is critical for successful attacks (Karplus & Algom, 1981). If predators rely on the head and potentially the eyes as key orienting cues when attacking prey, the deflection hypothesis holds that marginal eyespots exploit this reliance. The predator is attracted to the eye-like cue, strikes the wrong end, and the prey escapes in the opposite direction. The intimidation hypothesis, by contrast, requires that the same eye-like cues trigger avoidance rather than attraction by predators. Thus, cues that predators use to guide attacks may also function to deter them. If paired central eyespots primarily function as intimidation signals, predators would be expected to exhibit aversion to these cues, which could interfere with their reliance on eyes when orienting attacks. This potential conflict may explain why intimidation signals function in some predation systems but not others. In marine systems where head recognition is critical, the evolution of eyespots may therefore be better explained by alternative mechanisms.

Future perspectives

The work presented in this thesis reveals a mismatch between the evolution and proposed defensive function of eyespots in batoids. The adaptive function of what appear to be intimidating eyespots remains unresolved, potentially due to limitations in current experimental approaches and their underlying assumptions about how eyespots operate in marine environments. Progress may therefore depend not only on refining existing methods, but also on reconsidering the contexts, mechanisms, and predator–prey interactions underlying eyespot function.

One approach is to examine ontogenetic changes in eyespot appearance, which may reveal when eyespots are most effective and against which predator types. In many coral reef fish, eyespots disappear as body size increases (Hemingson et al., 2020). Although Paper I did not systematically test ontogenetic patterns in batoids, photographic observations suggest a similar pattern in the big skate

(*Raja binoculata*), where eyespots are prominent in juveniles but less pronounced in adults. This raises the question of whether eyespots function primarily during vulnerable early life stages or provide protection across the entire lifespan. Comparing ontogenetic patterns across multiple eyespot-bearing species could reveal whether eyespots consistently fade at similar body size thresholds, which could suggest they are effective against a particular range of predators.

Another approach is to examine ontogenetic changes in predator behaviour. Video recordings from the field experiment in Paper IV suggested that smaller and larger fish interacted differently with artificial prey, although the experimental design did not allow this observation to be formally tested. Many fish undergo ontogenetic shifts toward piscivory as they grow, transitioning from invertebrate or planktonic prey to fish, which could influence responses to eyespots if eyes serve as targeting cues during predation. Testing whether fish change their responses to eyespots before and after transitioning to piscivory could provide clues about the mechanisms underlying predator responses. If behavioural responses remain similar before and after the dietary shift, this would be more consistent with an intimidation mechanism, which should deter predators regardless of diet. In contrast, if responses change after the transition to piscivory, this could indicate that eyespots interact with cues used during fish predation, as only these predators would benefit from assessing whether their prey has detected them.

Beyond refining experimental designs, understanding eyespot function may ultimately require observations of natural predator-prey encounters. Direct observations in natural predation contexts may reveal whether eyespot-bearing prey remain immobile during predator inspection, present their eyespots suddenly as a startle display before fleeing, or employ other behavioural components not captured by static experimental trials. Such observations would help identify the mechanisms involved and inform more realistic experimental designs. Batoids may be particularly tractable for such observations, as their relatively large body size, slower movement, and non-schooling behaviour make predator-prey interactions easier to document than in many schooling reef fishes. This thesis establishes the evolutionary context and tests predator responses under con-

trolled conditions, but observing predator–batoid interactions in the wild remains a critical gap and an important next step.

Concluding remarks

Believed to protect prey by deceiving predators, eyespots have captivated scientists for more than a century. While their antipredator function has been well studied in butterflies, whether marine eyespots serve the same role has remained largely speculative, inferred from resemblance rather than empirical evidence. This thesis provides an integrated evaluation of the adaptive significance of eyespots in marine systems, integrating evolutionary, behavioural, and survival evidence.

By examining the evolution of eyespots in skates and rays, the work presented here brings one of the oldest extant jawed vertebrate lineages into a research tradition from which they have been conspicuously absent. It also broadens experimental work on eyespot function by testing intimidation-based predictions in sharks and assessing survival consequences underwater. Taken together, the results reveal a divergence between evolutionary pattern and functional outcome. While the macroevolutionary distribution of eyespots aligns with a visual antipredator hypothesis, the behavioural and field evidence fail to demonstrate the deterrent or survival effects observed in terrestrial systems, raising questions about their functional role.

For a signal that evolved to be seen, eyespots in skates and rays spent a remarkable century being overlooked. Even species exceeding two meters in length remained absent from the literature devoted to such markings. Despite the body of work conducted over the course of this thesis, what function they serve is a question this thesis addresses but does not resolve. Thus, how eyespots benefit what is probably the largest eyespot-bearing species remains a question that

will continue to intrigue me.

Svensk sammanfattning

En av de största utmaningarna i ett djurs liv är att inte bli uppätet. Ett enda möte kan vara skillnaden mellan liv och död, och därför är det inte konstigt att djur har utvecklat olika försvarsstrategier. Ett exempel är färgteckning. Kroppsfärg och mönster kan hjälpa djur att smälta in i sin omgivning så att de inte upptäcks. Andra färgteckningar är istället iögonfallande för att tydligt signalera att de är giftiga, osmakliga eller på annat sätt inte värda att äta. Ibland har dock djur färgteckning som kan dra till sig rovdjurs uppmärksamhet, trots att de inte är farliga eller oätliga. Ett sådant exempel är ögonfläckar.

Ögonfläckar har ofta ett slående utseende bestående av koncentriska ringar av kontrasterande färger. Dessa cirkulära markeringar som liknar stirrande ögon har länge fascinerat forskare såväl som allmänheten på grund av deras förmåga att vilseleda rovdjur. Ögonfläckar tros skydda djur genom att likna ögon hos rovdjurets egna fiender eller genom att ge ett så starkt visuellt intryck att rovdjuret tvekar inför eller avstår från en attack. Mycket forskning visar att ögonfläckar hos fjärilar är ett effektivt skydd mot fåglar. Men fungerar de likadant i havet? Ögonfläckar finns hos både fiskar och bläckfiskar, men deras funktion har till stor del förblivit spekulativ och bygger främst på likheter med fjärilar snarare än på direkta bevis. Denna avhandling undersöker ögonfläckar i havet genom en kombination av evolutionära analyser, beteendestudier och överlevnadsexperiment.

Om ögonfläckar verkligen är ett bra försvar, varför har då vissa arter dem medan andra inte? Genom att jämföra många arter kan vi fastställa vilka faktorer som samvarierar med ögonfläckar och

därmed bygga en förståelse för när de är gynnsamma och varför de har utvecklats. Därför kartlade den första studien ögonfläckar hos 580 arter av rockor, platta broskfiskar nära släkt med hajar. Resultaten visar att arter med robusta försvar, som giftgaddar eller elektriska organ som ger stötar, sällan har ögonfläckar. Istället utvecklar små arter utan sådana försvar oftare ögonfläckar, särskilt om de lever på grunt vatten där ljusförhållandena är bra. Detta mönster tyder på att ögonfläckar fungerar som ett visuellt försvar mot rovdjur som kompenserar för brist på andra försvar, men bara där rovdjur faktiskt kan se dem, alltså i grunt vatten.

Om ögonfläckar ska fungera som försvar måste de påverka rovdjurens beteende på ett sätt som ökar överlevnaden. I den andra studien undersöktes därför om hajar avskräcks av ögonfläckar på samma sätt som fåglar. Röd djungelhöna var långsammare att attackera ett konstgjort byte när det hade ögonfläckar. Båda hajarterna däremot attackerade snabbare när ögonfläckar fanns. Ögonfläckar hade därmed olika effekt på olika djurgrupper, vilket väcker frågor om hur de fungerar i havet.

Den andra studien visade att hajar inte avskräcktes av ögonfläckar. Detta kan bero på att experimenten gjordes under fel förhållanden. I den tredje studien testades därför om ljusförhållanden påverkar hur ögonfläckar fungerar. Spigg attackerade dock konstgjorda bytesdjur lika snabbt oavsett om de hade ögonfläckar eller inte, både i starkt och svagt ljus. Ljusförhållanden påverkade alltså inte hur snabbt bytet attackerades.

Beteendexperiment kan visa vad rovdjur gör när de ser ögonfläckar, men inte om ögonfläckar faktiskt ökar överlevnaden. I den fjärde studien testades därför om ögonfläckar ger överlevnadsfördelar i det vilda. Par av konstgjorda byten placerades ut i grunt vatten på Bahamas, det ena med två stora ögonfläckar och det andra utan mönster. Videokameror filmade hur länge varje byte "överlevde" innan det åts av fisk. Resultaten visade att båda bytena attackerades lika snabbt. Ögonfläckar gav därmed inga mätbara överlevnadsfördelar i havet.

Sammantaget visar denna avhandling en kontrast mellan evolution och funktion. Mönstret av vilka rockarter som har ögonfläckar tyder på att de fungerar som visuellt försvar mot rovdjur. Men

experimenten kunde inte visa att de avskräcker marina rovdjur eller ökar överlevnaden. Trots detta klargör avhandlingen inte vilken funktion ögonfläckar har. Hur ögonfläckar gynnar rockor, som troligen är de största ögonfläckbärande arterna på jorden, förblir därför oklart. Avhandlingen bidrar samtidigt med unika data från havet i ett forskningsområde som har dominerats av studier på land och breddar därmed vår förståelse av en ikonisk färgteckning.

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