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Anti-predator Adaptations in Aquatic Environments





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"I reject your reality and substitute my own!"

Doctor Who – *The deadly Assassin* (1974)

ABSTRACT

Predation is an important selective force that has led to the evolution of a variety of fascinating anti-predator adaptations, such as many types of protective coloration and prey behaviours. Because the evolution of life has begun in the aquatic environment and many anti-predator adaptations are found already in relative primitive taxa, it is likely that many of these adaptations evolved initially in the aquatic environment. Yet, there has been surprisingly little research on the mechanisms and function of anti-predator adaptations in aquatic systems. To understand the function of anti-predator adaptations and natural selection imposed on prey appearance and behaviour, I have investigated how protective coloration can be used, either as such or together with behavioural adaptations, to manipulate predator behaviour and decrease predation risk. To this end I conducted a series of behaviour ecological laboratory experiments in which I manipulated the visual appearance of artificial backgrounds and prey items.

In paper I of this thesis, I investigated background choice as an anti-predator strategy, by observing the habitat choice of the least killifish (*Heterandria formosa*) between pairs of artificial backgrounds, both in the presence and absence of predation threat. It has been suggested that prey could decrease their risk of being detected by predators either by preferring backgrounds into which they blend or by preferring visually complex backgrounds. The least killifish preferred a background that matched their patterning to a background that mismatched it, showing that they are able to respond to cues of visual similarity between their colour pattern and the surrounding environment. Interestingly however, in female least killifish visual complexity of the background was a more important cue for habitat safety and may override or act together with background matching when searching for a safe habitat. It is possible that in females, preference for visually complex backgrounds is associated with lower opportunity costs than preference for matching backgrounds would be. Generally, the

least killifish showed stronger preference while under predation threat, indicating that their background choice behaviour is an anti-predator adaptation.

Many aquatic prey species have eyespots, which are colour patterns that consist of roughly concentric rings and have received their name because they for humans often resemble the vertebrate eye. I investigated the anti-predator function of eyespots against predation by fish in papers **II**, **III** and **IV**. Some eyespots have been suggested to benefit prey by diverting the strikes of predators away from vital parts of the prey body or towards a direction that facilitates prey escape. Although proposed over a century ago, the divertive effect of eyespots has proven to be difficult to show experimentally. In papers **II** and **III**, I tested for divertive effect of eyespots towards attacking fish by presenting artificial prey with eyespots to laboratory reared three-spined sticklebacks (*Gasterosteus aculeatus*). I found that eyespots strongly influenced the behaviour of attacking sticklebacks and effectively drew their strikes towards the eyespots. To further investigate this divertive effect and whether the specific shape of eyespots is important for it, I tested in paper **III** the response of fish also to other markings than eyespots. I found that eyespots were generally more effective in diverting the first strikes of attacking fish compared to other prey markings. My findings suggest that the common occurrence of eyespots in aquatic prey species can at least partly be explained by the divertive effect of the eyespot shape, possibly together with the relative simple developmental mechanisms underlying circular colour patterns.

An eyebar is a stripe that runs through the eye, and this pattern has been suggested to obscure the real eyes of the prey by visually blending parts of the eyes and head of the prey and by creating false edges. In paper **III**, I show that an eyebar effectively disrupts an eyelike shape. This suggests that eyebars provide an effective way to conceal the eyes and consequently obstruct detection and recognition of prey. This experiment also demonstrates that through concealment of the eyes, eyebars could be used to enhance

the divertive effect of eyespots, which can explain the common occurrence of eyebars in many species of fish that have eyespots.

Larger eyespots have been shown to intimidate some terrestrial predators, such as passerine birds, either because they resemble the eyes of the predator's own enemy or because highly salient features may have an intimidating effect. In papers **II** and **IV**, I investigated whether the occurrence of eyespots in some aquatic prey could be explained by their intimidating effect predatory fish. In paper **IV**, I also investigated the reason for the intimidating effect of eyelike prey marks. In paper **II**, I found no clear intimidating effect of eyespots, whereas in paper **IV**, using a different approach, I found that sticklebacks hesitated to attack towards eyelike but not towards non-eyelike marks. Importantly, paper **IV** therefore presents the first rigorous evidence for the idea that eye mimicry, and not merely conspicuousness, underlies the intimidating effect. It also showed that the hesitation shown by fish towards eyelike marks is partly an innate response that is reinforced by encounters with predators.

Collectively, this thesis shows that prey colour pattern and the visual appearance of the habitat influence the behaviour of fish. The results demonstrate that protective coloration provides numerous distinctive ways for aquatic prey to escape predation. Thus, visual perception and behaviour of fish are important factors shaping the appearance and behaviours of aquatic prey.

SAMMANFATTNING

Predation är ett viktigt selektionstryck som har lett till evolutionen av en mängd olika fascinerande antipredator anpassningar såsom många typer av skyddsfärgteckningar och beteenden hos bytesdjur. Eftersom evolutionen av livsformer har sitt ursprung i den akvatiska miljön och många anpassningar mot predation upptäckts redan hos relativt primitiva djurtaxa, är det även troligt att många utav dessa anpassningar ursprungligen evolverat i akvatiska miljöer. Ändå har förvånansvärt lite forskning studerat mekanismerna och funktionerna av anpassningar mot predation i akvatiska miljöer. För att förstå funktionen av anpassningar mot

predation och den naturliga selektionen på bytesdjurs utseende och beteende, har jag undersökt hur skyddsfärgteckningar kan användas, antingen som sådana eller tillsammans med beteendemässiga anpassningar, för att manipulera predatorers beteende och på så vis minska predationsrisken. Med detta som målsättning utförde jag en serie av beteendekologiska laboratorieexperiment där jag manipulerade det visuella utseendet hos artificiella byten och bakgrunder.

I studie I undersökte jag bytesdjurs användning av bakgrunder som anpassning mot predation genom hur dvärgtandkarpar (*Heterandria formosa*) valde habitat mellan par av artificiella bakgrunder, både med och utan en predator närvarande. Det har tidigare föreslagits att bytesdjur kan minska sin upptäcktsrisk antingen genom att välja bakgrunder där deras färgteckning smälter in eller genom att välja bakgrunder som är visuellt komplexa. Dvärgtandkarparna föredrog bakgrunden med mönster som likade fiskarnas eget mönster framom en bakgrund med mönster som inte liknade det, vilket visar att omgivande miljöns visuella likhet med deras eget mönster påverkar tandkarpars habitatsval. En intressant upptäckt hos dvärgtandkarphonor var att visuell komplexitet hos bakgrunden var en viktigare signal för habitatstrygghet, och kan därför övertrumfa eller verka tillsammans med visuell likhet med bakgrunden i sökandet efter ett tryggt habitat. Det är möjligt att preferens för visuell komplexitet hos dvärgtandkarpshonor kan leda till lägre kostnader i form av förlorade tillfällen att delta i andra fitnesshöjande aktiviteter än vad preferens för visuell likhet med bakgrunden skulle göra. Generellt så visade dvärgtandkarparna starkare preferens för endera bakgrunden under predationshot, vilket tyder på att beteendet är en anpassning mot predation.

Många akvatiska bytesdjur har ögonfläckar, vilka är färgmönster som består av ungefärligt koncentriska ringar och som har fått sitt namn för att de, åtminstone för människor, liknar vertebratögon. Jag undersökte funktionen av ögonfläckar mot predation av fisk i studierna II, III och IV. Somliga ögonfläckar har föreslagits vara fördelaktiga för bytesdjur genom att rikta predatorers hugg bort

från vitala delar av kroppen eller i en riktning som underlättar flykt. Trots att denna riktande effekt föreslagits för över ett århundrade sedan, har den visat sig vara svårt att påvisa empiriskt. I studie **II** och **III** testade jag ögonfläckars riktande effekt genom att presentera artificiella byten med ögonfläckar till laboratorieuppfödda storspiggvar (*Gasterosteus aculeatus*). Mina resultat visade att ögonfläckar starkt påverkade spiggarnas beteende och riktade effektivt deras hugg mot sig. För att vidare undersöka denna riktande effekt och huruvida den specifika formen av en ögonfläck är viktig för effekten, testade jag i studie **III** även fiskarnas respons gentemot andra mönster. Resultaten visade att ögonfläckar var generellt mer effektiva i att rikta fiskarnas första attacker mot sig jämfört med andra bytesmönster. Mina resultat tyder på att den vanliga förekomsten av ögonfläckar hos akvatiska bytesdjur åtminstone delvis kan förklaras av den riktande effekten som den specifika formen hos ögonfläckar skapar, möjligtvis tillsammans med de relativt enkla utvecklingsmekanismerna som ligger till grund för cirkulära färgmönster.

Ett ögonstreck är en rand som löper igenom ögat, och detta mönster har föreslagits dölja bytesdjurets egna ögon genom att visuellt smälta samman delar av ögat och huvudet på bytesdjuret, samt genom att skapa falska kanter. I studie **III** visar jag att ett ögonstreck effektivt löser upp en ögonlik form. Detta tyder på att ögonstreck är ett effektivt sätt att dölja ögonen på, och på så vis hindra upptäckt och igenkännande av bytesdjur. Detta experiment visar även att genom att dölja de riktiga ögonen kan ögonstreck användas för att förstärka den riktande effekten av ögonfläckar, vilket kan förklara varför många fiskar som har ögonstreck även har ögonfläckar.

Stora ögonfläckar har visat sig fungera skrämmande mot vissa terrestra predatorer som tättingar, antingen för att de liknar ögonen hos predatorns egen predator, eller för att mycket iögonfallande mönster generellt kan ha en skrämmande effekt. I studie **II** och **IV** undersökte jag huruvida förekomsten av ögonfläckar hos åtskilliga akvatiska bytesdjur kan förklaras av deras skrämmande effekt mot rovfiskar. I studie **IV** undersökte jag även den bakomliggande

orsaken till den skrämmande effekten av ögonlika mönster. I studie **II** fann jag ingen tydlig skrämmande effekt av ögonfläckar, medan det i studie **IV** där jag använde en annan metod, visade sig att spiggarna tvekade att anfälla byten med ögonlika mönster men inte att anfälla byten med icke-ögonlika mönster. Resultaten i studie **IV** ger därför det första rigorösa stödet för idén att det är ögonlikhet, och inte enbart iögonfallande, som ligger till grund för den skrämmande effekten. Studien visar även att den tvekan som fiskarna visade mot de ögonlika mönstren är delvis en medfödd respons som också förstärks utav erfarenhet av predatorer.

Sammandraget visar denna avhandling att mönster hos bytesdjur och utseendet hos habitatet påverkar fiskars beteende. Resultaten demonstrerar att skyddsfärgteckning ger många, distinkta möjligheter för akvatiska bytesdjur att undkomma predation. Därmed är visuell perception och beteende hos fisk viktiga faktorer i utformandet av utseende och beteende hos akvatiska bytesdjur.

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LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their respective Roman numerals:

- I **Kjernsmo, K.** & Merilaita, S. (2012) Background choice as an anti-predator strategy: the roles of background matching and visual complexity in the habitat choice of the least killifish. *Proceedings of the Royal Society B* **279**: 4192-4198, doi:10.1098/rspb.2012.1547
- II **Kjernsmo, K.** & Merilaita, S. (2013) Eyespots divert attacks by fish. *Proceedings of the Royal Society B* **280**: 20131458, <http://dx.doi.org/10.1098/rspb.2013.1458>
- III **Kjernsmo, K.** Grönholm, M. & Merilaita, S. Eyespots, eyebars and diversion of attacks by fish. *Manuscript*
- IV **Kjernsmo, K.** & Merilaita, S. Intimidating eye-mimicry and eyespots: eyelike prey marks cause hesitation in attacking fish. *Manuscript*

© Royal Society Publishing (**papers I and II**)

1. INTRODUCTION

1.1 Anti-predator adaptations

Predation is omnipresent, and the most susceptible prey individuals are killed even before they have reproduced. Therefore, predation is a strong selective force in nature. Predator-mediated selection favours those individuals whose traits allow them to postpone death until they have managed to reproduce at least once (Alcock 2005). This has led to the evolution of a large variety of anti-predator adaptations in prey and also to the evolution of counter-adaptations in predators (e.g. Darwin 1859). Anti-predator adaptations can be morphological (such as exoskeletons, spines and various types of protective prey colorations) and behavioural (such as avoiding dangerous areas, group formation or hiding in safe refuges) (e.g. Poulton 1890; Cott 1940; Ruxton *et al.* 2004; Nonacs & Blumstein 2010). Obviously, selection pressures other than predation also can have an important impact in shaping the appearance and behaviour of animals (e.g. sexually selected traits, naturally selected traits involved in intraspecific communication, traits that are involved in other fitness enhancing processes (thermoregulation)), but these are outside the scope of this thesis.

In this thesis I have focused on anti-predator adaptations in aquatic environments. This because even though most anti-predator defences have evolved in the aquatic milieu, surprisingly little has been studied about the adaptive value and functions of anti-predator defences in aquatic systems. One of my main aims has been to investigate predator-mediated selection imposed on or by fish. More specifically, I have focused on protective coloration and related behavioural adaptations, mainly habitat choice. All these anti-predator adaptations target the visual sensory modality of predators. Despite my use of aquatic organisms, many of my results have a broader importance due to the generality of the evolutionary question that I have addressed.

1.2 Protective coloration and related anti-predator behaviours

Protective coloration has intrigued naturalists and biologists already for a long time, and the potential adaptive value of camouflage was discussed by Charles Darwin's grandfather Erasmus Darwin, who wrote that:

“The colours of many animals seem adapted to the purpose of concealing themselves, either to avoid danger, or to spring upon their prey.”

(Darwin 1794)

There are many forms of protective coloration in animals, and these are commonly divided into two major categories: 1) coloration that prevents detection or recognition (i.e. various types of camouflage) and 2) coloration that decreases for a detected prey the risk of an attack or the harm it may cause (i.e. anti-predator signals, such as warning colouration, mimicry and eyespots) (Poulton 1890; Thayer 1909; Cott 1940; Ruxton *et al.* 2004). These two categories of protective coloration are also often associated with some behavioural adaptations. For example, to increase the efficacy of camouflage a prey may decrease its mobility during the times it has an elevated risk of encountering a predator (Ruxton *et al.* 2004). By studying protective coloration we can gain important information about why animals look and behave the way they do, and also increase our understanding of questions regarding predator psychology.

In this thesis, I have studied how prey can use colouration either alone or in combination with a behavioural adaptation to manipulate the behaviour of predators in a way that would either reduce the probability of being detected, attacked or to reduce the likelihood of an attack to be successful. I have primarily focussed on three types of protective colouration: crypsis, eyespots, and eyebars and will therefore confine myself to these three and address them separately below.

1.2.1 Crypsis (paper I)

The term crypsis includes traits that decrease the risk of becoming detected when potentially perceivable to an observer (Stevens & Merilaita 2009a). Crypsis comprises both morphological features such as background matching coloration, (colour patterns which generally resemble the colour and lightness of one or several background types, Endler 1978; Merilaita 1999; Ruxton 2004; Merilaita & Stevens 2011), and behavioural traits that enhance crypsis (Stevens & Merilaita 2009a). In this thesis, I have studied how animals can use behaviour to enhance their crypsis. Several experimental studies have shown that background matching (i.e. the similarity in appearance between a prey and its visual

background) effectively reduces predation risk (e.g. predation by fishes: Feltmate & Williams 1989; Merilaita 2001; Johnsson & Källman-Eriksson 2008; and birds: Kettlewell 1955; Cooper 1994; Merilaita & Dimitrova 2014). Consequently, it has also been a common assumption that animals have been selected to actively choose visually matching backgrounds (Kettlewell 1955; Popham 1943; Sargent & Keiper 1969; Kettlewell & Conn 1977; Caro 2005). It is somewhat surprising therefore that experimental evidence for this idea is scarce (but see Boarman *et al.* 1974; Gillis 1982; Sandoval 1994). Furthermore, several studies have not found support for such behaviour (Lees 1975; Merilaita, & Jormalainen 1997; Garcia & Sih 2003). Thus, even though the idea that camouflaged prey has been selected to prefer matching backgrounds has become such a common assumption, support for the idea is not as strong as often believed.

It has been proposed that visual complexity of the background is another factor that enhances prey concealment (Merilaita 2003). This idea has received experimental support from Dimitrova & Merilaita (2010, 2012, 2014), who have shown that an increase in various aspects of visual complexity of the background (complexity and diversity of shapes as well as the density of the elements that constitute the visual background) increases prey search time by blue tits (*Cyanistes caeruleus*). Consequently, instead of preferring visually matching background, it could be beneficial to prey to prefer visually complex backgrounds to decrease its predation risk (Merilaita 2003; Dimitrova & Merilaita 2010). However, the importance of preference for complex backgrounds has thus far not been studied. Therefore, in paper I, I investigate the importance of preference for visually matching backgrounds and preference for visually complex backgrounds by studying the background choice of the least killifish *Heterandria formosa* (Girard, 1859).

1.2.2 Eyespots (papers II, III and IV)

A prominent example of protective coloration is eyespots (Poulton 1890, Blest 1957a). Eyespots are patterns consisting of roughly concentric rings of contrasting colours and have received their name because to humans they often resemble the vertebrate eye. Eyespots are common in many terrestrial taxa such as insects (particularly in the order *Lepidoptera*), birds and reptiles, and they are also widespread in many aquatic taxa such as molluscs, flatworms and fishes (Poulton 1890; Cott 1940; Blest

1957a; Neudecker 1989; Meadows 1993; Stevens 2005). Because of their salience and taxonomically wide occurrence, eyespots have intrigued naturalists and biologists for more than a century (e.g. Poulton 1890). The developmental pathway and genetic basis of eyespots particularly in butterflies are now well understood (e.g. Nijhout 1991; Brakefield, *et al.* 1996; Beldade & Brakefield 2002; Monteiro *et al.* 2003). The developmental pathway of eyespots seems to be similar in fish (Ohno & Otaki 2012).

Despite the knowledge of the developmental mechanism that generates eyespots, disentangling their adaptive and functional significance has proven to be quite a challenge. It has not been until this century that rigorous, empirical support for the anti-predator functions of eyespots has started to accumulate. There are two hypotheses that are most widely-known regarding the anti-predator utility of eyespots: 1) the diversion hypothesis (also called the deflection hypothesis) and 2) the intimidation hypothesis.

1.2.2.1 The divertive effect of eyespots

The common occurrence of (generally small) eyespots found on the wing margins of many Lepidoptera and eyespots located on the caudal area in some species of fishes has been suggested to depend on the divertive function of such eyespots (Poulton 1890; Blest 1957). According to the diversion hypothesis, eyespots serve to direct the strikes of attacking predators towards less vital or defended parts of the prey body, or towards a direction that would facilitate prey escape (Poulton 1890; Blest 1957, II). For example, in a study using yellow buntings (*Emberiza citrinella*) as predators, and mealworms (*Tenebrio molitor*) with a simple spot painted onto them, Blest (1957) argued that the birds directed their attacks towards the eyespots. However, his study suffered immensely from pseudo-replication, and thus his results were not statistically sound. Despite the fact that this divertive function of eyespots was proposed over a century ago (Poulton 1890), finding empirical support for this effect has been difficult and several studies have not found any clear support for a diversion hypothesis (e.g. against birds: Lytinen *et al.* 2003, 2004; lizards: Vliieger & Brakefield 2007; against fish Gagliano 2008).

During the last few years though, some support for the divertive function of eyespots has been provided in systems using passerine birds that attack butterflies or artificial prey that have marginal eyespots

(Vallin *et al.* 2011; Olofsson *et al.* 2010; 2013). However, to date, no direct, unambiguous behavioural tests have been conducted with aquatic taxa, despite the fact that eyespots are common in aquatic environments. As previously mentioned, many species of fish have eyespots located near of in the area of their caudal fins, and these spots have been suggested to misdirect predator attacks (Cott 1940; Kelley *et al.* 2013; II). In paper II, I therefore investigate the behavioural response of fish towards eyespots to test for their potential divertive effect. I did this in a series of experiments in which I presented artificial prey items with or without eyespots to laboratory-reared, predator-naïve three-spined sticklebacks (*Gasterosteus aculeatus*). Specifically, I tested whether eyespots smaller than the attacking fish's own eye could have a diverting effect on attacking fish, by observing where the fish directed their attacks.

Even though it has been shown that eyespots can draw the attacks of predators towards them (Olofsson 2010, 2013; Vallin *et al.* 2011; II), the mechanisms behind this divertive effect of eyespots remains unknown. It is still unclear whether the specific shape of eyespots is important for their function and whether eye-mimicry is involved in the divertive effect of eyespots, or could any contrasting marking have a similar effect. Paper III focuses on these questions by testing for the divertive effect of differently shaped markings that were controlled for size and contrast to the rest of the prey item. Also, this study is based on a series of laboratory experiments in which I observed where predator-naïve three-spined sticklebacks that were presented with artificial prey items directed their attacks.

1.2.2.2 The intimidating effect of eyespots

Another possible anti-predator function of eyespots that has been invoked to explain their existence in some prey is predator intimidation (Poulton 1890, Blest 1957). Generally large eyespots, for example, those found in many species of Lepidoptera, could serve to intimidate potential predators, subsequently thwart, delay or otherwise prevent an attack from being successful in favour of the prey. Recent studies have provided support for the intimidating effect of eyespots against passerine birds (Vallin *et al.* 2005; 2007; Stevens *et al.* 2007, 2008; Kodandaramaiah *et al.* 2009; Merilaita *et al.* 2011; Olofsson *et al.* 2013; Hossie & Sherratt 2012, 2013). However, the question why some eyespots intimidate predators has so far been unresolved and debated. Two main hypotheses for the intimidating nature of eyespots have been

proposed, one is that eyespots resemble the eyes of the predator's own enemy (Poulton 1890; Blest 1957; Janzen *et al.* 2010) and hence suggest the presence of a potential threat, and the other is that the high conspicuousness of eyespots is a property that intimidates (Blest 1957; Stevens 2005; Stevens *et al.* 2007, 2008, 2009).

Although empirical support for the intimidating function of eyespots has been provided in terrestrial systems, surprisingly little is known about whether eyespots can have an intimidating function towards fish, despite the fact that eyespots are common also in many aquatic taxa. For this reason, in paper **II**, I tested whether eyespots larger than the fish's own eye would intimidate attacking fish, by presenting three-spined sticklebacks with both spotted and spotless prey items and comparing attack latencies between these two treatment groups. Furthermore, to disentangle the importance of eye-mimicry for the intimidating function of eyespots, I studied the response of both predator-naïve, and predator-experienced three-spined sticklebacks towards prey with more or less eyelike patterns in paper **IV**.

1.2.3 Eyebars (paper III)

Interestingly, many species of fish, of which many also have eyespots, have a distinct stripe running vertically through their actual eye (Fig 1). Such eye stripe (or eyebar) has been suggested to serve to obscure the eyes (Thayer 1909; Cott 1940; Barlow 1972; Kelley *et al.* 2013). Eye stripes have been suggested to disrupt the appearance of the eye by visually merging the adjacent, discontinuous surfaces of parts of the eye and the head. This particular type of disruptive coloration, which visually joins together separate and potentially revealing parts of an animal body, is called "coincident disruptive coloration" (Cott 1940; Stevens & Merilaita 2009b; Cuthill & Székely 2009). This means of concealment has received empirical support from an experiment conducted by Cuthill and Székely (2009), who pinned artificial "moths" on tree trunks and found that moths that had coincident disruptive coloration merging their body with wings survived predation by wild birds better than did moths that simply matched the background. However, there exist no experimental studies that would have demonstrated the benefit of eye-stripes. In paper **III**, I investigated whether an eyebar could (a) effectively disrupt an eyelike shape, (b) influence predators' response to eyelike targets and (c) facilitate the divertive function of eyespots. I did this by observing whether an eyebar influenced where the three-spined sticklebacks

directed their attacks when presented with a prey item that had both an intact eyespot and an eyespot disrupted by a stripe.



Figure 1. Copperband butterflyfish (*Chelmon rostratus*) with a posteriorly located eyespot and a distinct, vertical eye stripe running through its real eye. (Photo: Karin Kjærnsmo)

2. MATERIALS AND METHODS

2.1 General methods

As my main study species I used the least killifish (*Heterandria formosa*) in paper I and the three-spined stickleback (*Gasterosteus aculeatus*) in papers II-IV. All individuals were laboratory-reared descendants of wild caught fish (one or two generations) and a more detailed description of these fish are given below. All experiments were conducted in the aquatic laboratory at the Department of Biosciences, Åbo Akademi University, Turku, Finland. I have taken utmost care to provide good attendance and environment for all the fish through the course of these studies, and all experiments were performed with permission from the Regional State Administrative Agency for Southern Finland. In each experiment of all the four studies of this thesis (papers I – IV), I have presented individual fish in 12-30-l aquaria with either

artificial backgrounds, prey items or both, and observed the behavioural responses of the fish to address my specific study questions. The artificial backgrounds and prey items used in these experiments enabled easy manipulation of their visual features, while keeping other conditions constant. Moreover, by using laboratory reared fish I could control for how much prior experience they had of predators or the stimuli they were presented with in the experiments. This would obviously have been impossible to control for, if I had used wild-caught animals.

All artificial backgrounds and prey items were created with several purpose-written programmes using the software MATLAB R2008b (The MathWorks, Inc., Natick, MA, USA), and then printed with a laser printer (HP LaserJet P4015x with 1200 dpi resolution) on water resistant paper ("Rite in the rain", J. L. Darling Corporation, Tacoma, WA, USA). The behavioural observations of all test individuals were recorded in real time using the event-recording software J-watcher (version 1.0, available at: <http://www.jwatcher.ucla.edu>). Because different methodological approaches were required for each study, I below describe the methods of each study separately.

2.2 Study species

2.2.1 *Heterandria formosa* (paper I)

In paper I, I used the least killifish (*Heterandria formosa*) as my study species. This species can reach a body length of up to 3.5 cm, which makes it the smallest member of the Poeciliidae family. The Poeciliidae family is mainly characterized by females being livebearers, and by males having a gonopodium (modified anal fin) used for internal fertilization. The colour pattern of the least killifish consists of a light ground colour and a distinct black lateral, longitudinal stripe both in males and in females (Fig. 2). The least killifish commonly occurs in heterogeneous algal beds, where their colour patterns (i.e. the longitudinal stripe) are thought to have a concealing effect (Cox *et al.* 2009), which made them a suitable species for this study. The least killifish live in slowly moving fresh water streams or ponds, but also occur in brackish waters in southeastern parts of the USA. All least killifishes used in paper I were laboratory reared descendants from a wild population in Otter creek, Florida, USA. They were kept in 200-l (l x w h: 100 x 50 x 40 cm) aquaria, with a water temperature varying

between 24-28°C (due to weekly water changes), and held on a light/dark schedule of 16:8 hours. The least killifishes were fed 1-3 times daily *ad libitum* with live newly hatched brine shrimp (*Artemia* spp.) or commercial flake food.



Figure 2. The study species that were used in paper I, the least killifish (*Heterandria formosa*).

2.2.2 *Gasterosteus aculeatus* (papers II, III, IV)

For the experiments in paper II, III and IV, I used the three-spined stickleback (*Gasterosteus aculeatus*) as my study species (Fig. 3). I chose the three-spined stickleback because it is considered to be primarily a visual predator (Wootton 1976; Ohguchi 1978; Litvak & Leggett 1992; Hart & Gill 1994; Rowe *et al.* 2004), distributed over almost the entire northern hemisphere and is fairly easy to maintain in high numbers in the laboratory. To avoid any profound effects of parasitism on the behaviour of the sticklebacks, I derived laboratory populations from parental fish caught outside Tvärminne zoological field station in South-western Finland (59° 50' N, 23° 12' E) through artificial fertilization. It has been shown that for example the common tapeworm, *Schistocephalus solidus*, can affect their behaviour drastically (eg. Giles 1987, Godin & Sproul 1988, Barber & Huntingford 1995). Also, another benefit of using laboratory reared fish is that they are certain to be naïve to both predators and to prey with eyespots, which is why I have been able to study their innate behavioural response towards the prey items.



Figure 3. The three-spined stickleback (*Gasterosteus aculeatus*) that was used as study species in papers II-IV.

2.3 Background choice as an anti-predator strategy (paper I)

As mentioned in the introduction, it has traditionally been assumed that to decrease its predation risk, a prey should prefer habitats that are visually similar to its colour pattern. This hypothesis has been challenged by a hypothesis according to which a preference for visually complex backgrounds provides another way to decrease predation risk and that this could be even more beneficial than preference for matching backgrounds. Visual background complexity has been shown to decrease the risk of being detected by predators (Dimitrova & Merilaita 2010, 2012). Moreover, it may be cognitively more demanding to identify visually similarity (that requires a comparison) than visual complexity. To study the relative importance of background matching and visual complexity for the background choice of the least killifish, I therefore conducted a series of controlled laboratory experiments and presented the fish with artificial backgrounds that had varying patterns. I also manipulated the levels of predation threat (predator present or absent) to establish whether the observed behaviour is an anti-predator response. To simulate predation risk, I used convict cichlids (*Amatitlania siquia*) as predators. Convict cichlids are substantially larger than the least killifish, and can reach a total length of 12 cm.

The pattern of the least killifish (Fig. 1) enabled me to create artificial backgrounds with black elements that approximately matched the shape and the area of the lateral stripe of the fish. The stripes in the backgrounds had the same average size as the least killifish's stripes. A total of four differently patterned backgrounds were produced: one with matching pattern, one with a mismatching pattern, and two different types of complex patterns (Fig. 4), and all of them had identical black to white ratio, consisting of 22% black.

Two of these four backgrounds at time were used over the course of three different experiments. First, the fish was given a choice between the matching (horizontal) and mismatching (vertical) stripes (Fig. 3a and b, respectively). In the second experiment, the matching stripes was presented together with a background consisting of the size- and shape-matching stripes, but now with added complexity through random orientation and by allowing overlap between the randomly distributed stripes (Fig. 3c). This overlap decreased the match between the prey and this background pattern, but it also increased the visual complexity (i.e.,

the perimeter-to- $\sqrt{\text{area}}$ ratio, Dimitrova & Merilaita 2012) of the shape of the background pattern. In the third experiment of this study, the matching background was presented together with a background consisting of non-overlapping elements with increased complexity of shape compared to the stripes (Fig. 3d). In each aquarium, the two backgrounds covered exactly 50% each of the sides.

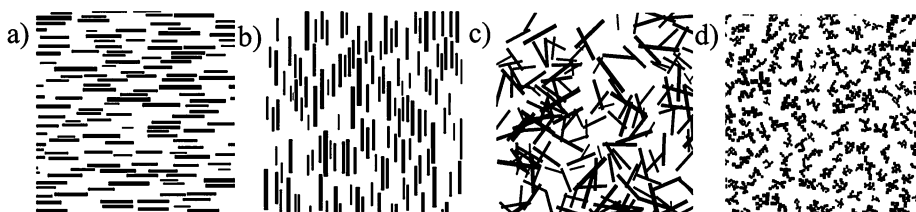


Figure 4. Sample of the four different background patterns used in the background choice experiment. a) The background pattern with matching, horizontally oriented stripes, b) otherwise similar stripes but with a mismatching, vertical orientation, c) otherwise similar stripes but with complex orientation and overlap allowed and d) the background consisting of the complex shapes.

In the beginning of each replicate, I placed a randomly chosen killifish in the middle of the experimental 12-l aquaria. I ran half of the replicates with simulated predation threat. For the predation treatment, I put a convict cichlid in a cylindrical container made of transparent plastic and mesh (diameter 10 cm, height 15 cm), and placed it in the middle of the aquarium. After the focal fish had had two minutes to calm down, I observed each replicate for 15 minutes, recording the background choice of the fish. An equal number of females and males were used in each treatment group, and each individual were only tested once.

2.4 Divertive eyespots (papers II, III)

With these studies, I investigated the anti-predator function of eyespots and tested for the importance of the divertive effect in aquatic environments. More specifically, I studied if eyespots could be used to manipulate where attacking fish direct their strikes, and whether this divertive effect is affected by the visual properties, such as the eyelike shape of the marking. In these experiments I presented predator-naïve, laboratory reared three-spined sticklebacks (*Gasterosteus aculeatus*) with artificial prey items and recorded their response towards prey that had different markings.

I first trained individual three-spined sticklebacks to attack the artificial prey items and then studied their response to the patterning of the experimental prey items (Fig. 5). All prey items consisted of two components, a rectangular piece of printed paper that covered a thawed red mosquito larva (*Chironomidae* sp.) which served as a reward and ensured that the fish would be motivated to attack the artificial prey items. Depending on the experiment, each rectangular prey item had either: one eyelike or non-eyelike mark on only one half of its upper surface, two different marks (i.e. one on either half), or no mark if it was a control prey (Fig. 5). Each prey item was produced by printing different black patterns onto white paper, and consisted of different conspicuous markings that were controlled for size and contrast to the rest of the prey item. In all experiments, I used eyespots as well as non-eyelike marks and tested if they diverted the attacks of the sticklebacks by observing where the fish directed their attacks. In the last experiment of paper III, I studied if an eyebar could be used to effectively disrupt an eyelike shape and thus also influence predators' response to the shape. In the latter experiment, I presented the fish with prey items that had both an intact eyespot and an eyespot disrupted by an eyebar (Fig. 4).

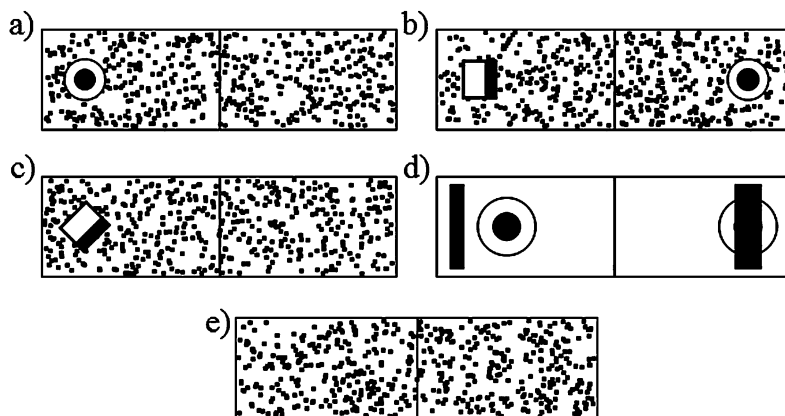


Figure 5. The prey items used to study the divertive effect of prey markings. a) A single eyespot, b) an eyespot together with a square-shaped element, c) a single square, d) an intact eyelike mark and an eyelike mark disrupted by a bar, e) the control prey with only the mottled background pattern.

Training and experiments were conducted in 30-l aquaria that were lit from above by natural light simulating fluorescent lamps. Each aquarium consisted of three main zones, the start zone that was separated from the mid zone by a removable opaque plastic divider, and

the foraging zone where the foraging plate (9.5 × 7 cm in size) and prey items was placed (Fig. 6).

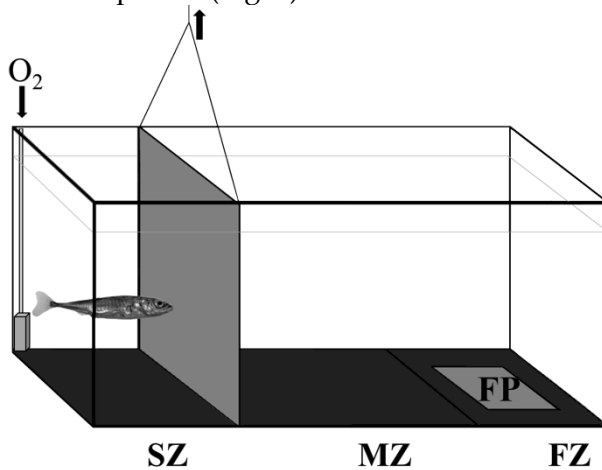


Figure 6. Schematic figure of the experimental aquarium. The fish was initially put in the start zone (SZ), separated by an opaque divider from the mid zone (MZ) and the foraging zone (FZ). FP is the foraging plate where the artificial prey item was placed.

There was only one fish in each experimental aquarium. However, each individual was tested four times to establish how repeated encounters with the prey influenced the behaviour of the fish. At the beginning of an experimental trial, the fish was in the start zone, and a prey item was placed in the foraging zone two minutes before the trial started. When a trial started, the divider was lifted to give the fish access to the foraging zone. A trial lasted until a fish attacked the prey item or the maximum observation time of 15 minutes had passed.

2.5 Intimidating effect of eyespots and the importance of eye mimicry

Because it has been shown that eyespots have an intimidating function in terrestrial systems, and large eyespots are common in the aquatic environment as well, I tested whether eyespots could have an intimidating function against attacking fish. More specifically, I investigated whether an eyespot larger than the fish's own eye would intimidate potential predators, by presenting the fish with either a prey item that had a large eyespot, or a spotless prey item, and compare attack latencies between these two (paper II). The experimental

procedure followed that of paper **II** and **III**, i.e. the fish was first trained to search for artificial prey items, and then I observed the response of the fish towards the experimental prey items.

Although there is convincing support for the intimidating effect of eyespots, it has been unclear what causes this effect. So far no direct evidence has been provided for the idea that predators do associate eyelike shapes with a threat from their enemies, despite the fact that this has been the most popular hypothesis invoked to explain this effect. To investigate the importance of eye mimicry for the intimidating effect of eyespots, I therefore tested the behavioural response of both predator-naïve and predator-experienced three-spined sticklebacks (*Gasterosteus aculeatus*) to artificial prey items that had marks that were either eyelike, being either similar to or different from the typical eyespot shape, or non-eyelike (paper **IV**). One key assumption for the approach I chose for this study is that if eye mimicry is important for the intimidating effect of eyespots, then not only displays based on the orthogonal projections of the eye, resembling the typical shape of eyespots, but also displays based on other projections of the eye should intimidate predators.

Following the procedure of paper **II** and **III**, I derived stickleback fry from wild-caught parental fish through artificial fertilization (Kjernsmo & Merilaita 2013). To manipulate predator-experience, I then randomly divided the stickleback fry into two long term pre-experiment treatment groups. One group was subjected to visual and olfactory cues of perch (*Perca fluviatilis*), which is a natural predator of three-spined sticklebacks. The other group reared in the absence of any cues of predators.

After the sticklebacks had been exposed to the pre-experiment treatment for at least half a year, I tested their behavioural response to the different prey items. Each prey item consisted of two components, a rectangular piece of paper (Fig. 7), and a frozen red chironomid larva that was placed on the middle of the prey item. Four different types of prey items were created containing either: 1) one mark mimicking the lateral view of a perch eye, 2) two marks mimicking the frontal view of a perch eyes, 3) a non-eyelike rectangular mark that had equally large black and white areas as the single eyelike mark to control for the level of contrast and conspicuousness or 4) only the mottled background patterning (Fig. 7). The eye-mimicking patterns were reproduced from photographs of perches' eyes.

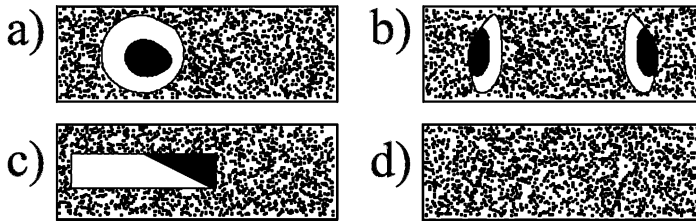


Figure 7. The four different prey types that were used to study the importance of eye mimicry. The prey had either a) one mark mimicking the lateral view of a perch eye, b) two marks mimicking the frontal view of perch eyes, c) a rectangular, non-eyelike mark that was equally large and conspicuous compared to the eyelike mark in a) and d) a control prey without any additional mark.

In the experiment, a randomly chosen fish from the pre-experiment treatment were placed in the start zone of a 30-l experimental aquarium, behind a removable opaque plastic divider that separated the start zone from the rest of the aquarium. One out of four different prey items were then placed on a foraging plate in the foraging zone of the aquarium (Fig. 6), and the observation of each fish then started by lifting the divider. I recorded the latency to prey attack (defined as the time it took for a fish to attack the prey item from initiating prey approach i.e. when positioned above the foraging plate face down towards the prey) by using the same event-recording software as in papers I, II and III.

3. RESULTS AND DISCUSSION

3.1 Background choice as an anti-predator strategy (paper I)

3.1.1 Predator induced preference for background-matching pattern

When testing for choice between matching and mismatching background pattern, neither male nor female least killifish showed any background preference in the absence of a predator (Fig. 8a). Interestingly, however, under simulated predation threat both males and females a significant preference for the matching background before the mismatching background (Fig. 8b). These results provide empirical support for the idea that prey could be selected to prefer habitats that yield protection through improved background matching (Kettlewell 1955). Furthermore, the results also suggests that the least killifish are able to assess and respond to some cues related to visual similarity

between their body pattern and the background, and that those cues serve as indicators for habitat safety.

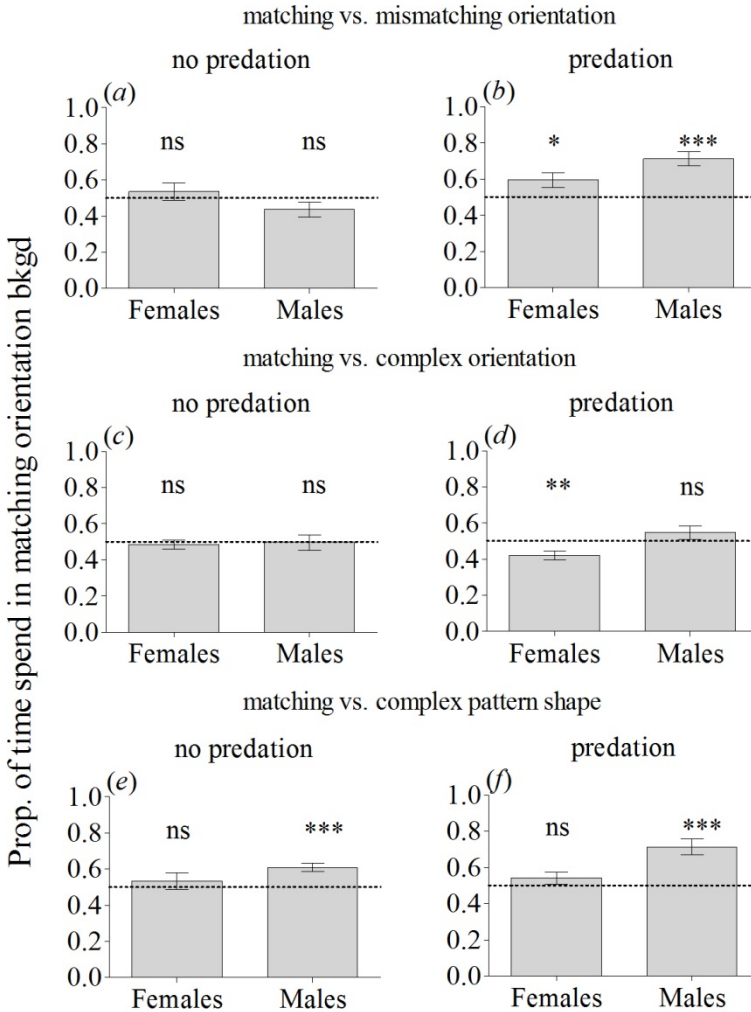


Figure 8. Proportion of time (mean \pm s.e.) spent on the matching background (bkgd) in both males and females. Panels a, c and e show background choice without predation threat and panels b, d and f show background choice under simulated predation threat. $N = 15$ in each group for the matching vs. mismatching treatment, and $N = 20$ each for all the other groups. The dashed line indicates the expected no choice value of 50%. For each treatment, I tested whether time spent in the matching background deviated from the 50% expectation using the t-test (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; n.s.: non-significant).

3.1.2 Preference for visual complexity differs between males and females

In the second experiment, in which the fish were presented with a choice between the matching and the more complex background with matching but randomly-rotated, overlapping stripes, neither females nor males showed any preference for either background in the absence of predator (Fig. 8c). In the presence of a predator, females did, however, prefer the complex background before the matching background, whereas males showed no preference (Fig. 8d). For males, this could be either because they found both backgrounds equally protective, or because they found them equally unprotective. The first alternative seems more likely because males displayed a strong preference for the matching background when given a choice between the matching- and the mismatching pattern.

In the third experiment where the fish were presented with a choice between the matching background and the background consisting of complex-mismatching pattern elements, males and females again behaved differently. Males spent significantly more time in the matching background than in the complex background, regardless of predator presence (Fig. 8e, f). Females, on the other hand, did not show a preference for either background (Fig. 8e, f). This may indicate that the males experience that the complex background deviated too much from the males' own pattern or that the level or type of complexity was not protective enough, whereas the females experienced them equally protective.

Both a visual match between prey colour pattern and the background and visual complexity of the background are known to decrease predation risk (Kettlewell 1955; Feltmate & Williams 1989; Merilaita 2001; Dimitrova & Merilaita 2010, 2012). Collectively, my results show that in the least killifish, background matching is an important aspect in background choice, but also some aspects of complexity are important and may even override background matching. Particularly in visually patchy or variable habitats, visual complexity may often be a less specific requirement and therefore more likely to come across than a visual match between a specific pattern and background. Visual complexity could therefore serve in many habitats as a simpler and more straightforward cue of a protective background.

Background choice differed to some extent between sexes, such that in general females preferred the more complex backgrounds more than

males did. This suggests that male and female least killifishes have different background choice or habitat use strategies, as previously suggested in many other species (Shine 1986; Asakura 1995; Merilaita & Jormalainen 1997, 2000). Since female least killifishes are larger than males, they probably also have higher resource needs (Alcock 2005), which suggest that opportunity costs for background matching (Ruxton *et al.* 2004) could be higher for females than males. To decrease this cost, females could therefore benefit from preferring visual complexity over background matching if it expands the range of microhabitats where they can forage safely. Similarly, the smaller size in males suggests that males could acquire their necessary resources by utilizing a smaller range of microhabitats than females, which results in lower opportunity costs for background matching in males and could explain why males generally showed preference for the matching background.

3.2 Divertive eyespots (papers II, III)

3.2.1 Eyespots divert attacks by naïve fish, whereas less eyelike marking shapes do not

When presented with a prey item that had one small eyespot on one half of the prey, significantly more of the sticklebacks directed their first attacks towards the eyespot compared to the spotless side of the prey ($\chi^2 = 11.64$, d.f. = 1, $p < 0.001$). This is an important finding, because it provides empirical support for the idea that eyespots can draw the attacks of predators towards them and thus, manipulate predator behaviour. Compared to previous studies, this effect seems strikingly strong, and to my knowledge, this is also the first time that unambiguous, empirical support for divertive effect of eyespots has been shown in aquatic taxa.

Interestingly, when the eyespot was replaced with an equally conspicuous, square-shaped mark, there was no divertive effect on the first attack of the fish ($\chi^2 = 0.20$, d.f. = 1, $p = 0.65$). Also, when the prey had both an eyespot and the square-shaped marking, the markings did not influence to which half of the prey the fish directed their first attack. However, when the prey had both an intact eyespot, and an eyespot disrupted by a bar, significantly more of the sticklebacks directed their first attack towards the intact eyespot ($\chi^2 = 11.27$, d.f. = 1, $p < 0.001$).

These results are in accordance with some other recent studies that have shown that eyespots can be used to divert attacks by predators

(Olofsson 2010, 2013, Vallin *et al.* 2011). Particularly the result of the last experiment suggests that eyelike shapes are especially effective divertive marks. The finding that divertive effect of an eyelike shape could be manipulated with an eyebar supports the principle of coincident disruptive colouration (Cott 1940; Cuthill & Székely 2009). In addition, this result suggests that the relatively common occurrence of eyebars in fishes could be explained by increased camouflage or, in species that have eyespots, amplified divertive effect through concealment of the eye.

3.3 Intimidating eyespots and the importance of eye-mimicry (papers II, IV)

The experiment in paper II did not provide any clear support for intimidating effect of eyespots towards attacking fish. When comparing attack latencies between the spotless prey and the prey that had a large eyespot, there was no significant difference in any of the four trials (Cox regression, trial 1: Wald = 0, $n = 41$, $p = 0.98$; trial 2: Wald = 2.91, $n = 41$, $p = 0.09$; trial 3: Wald = 1.98, $n = 41$, $p = 0.16$; trial 4: Wald = 1.03, $n = 41$, $p = 0.31$). This result was somewhat surprising, considering the strong support the intimidating function of eyespots has received in terrestrial studies using passerine predators and butterflies as prey (Vallin *et al.* 2005, 2006, 2007, Kodandaramaiah *et al.* 2009, Merilaita *et al.* 2011), and that eyespots have been suggested to have an intimidating effect in fish as well (Coss 1979; Neudecker 1989).

As opposed to the lack of support in paper II, the setup of paper IV provided clear support for intimidating effect of eyelike patterns towards attacking fish by demonstrating that eyelike prey patterns induced hesitant behaviour in the sticklebacks whereas non-eyelike patterns did not have such effect (Fig.9). Both the predator-naïve sticklebacks (two-sample *t*-test: $t = 4.20$, d.f. = 28, $p < 0.001$) and the sticklebacks that had been subjected to visual and olfactory cues of predators ($t = 5.25$, d.f. = 28, $p < 0.001$) were more hesitant to attack the prey that had an eyelike mark than the prey that had a non-eyelike mark. Moreover, sticklebacks that had experience on predator cues took significantly longer to attack the prey item that had the single eyelike mark that resembled a typical eyespot, than the prey item that had the equally conspicuous, but non-eyelike rectangular marking ($t = 5.25$, d.f. = 28, $p < 0.001$). Importantly, predator-experienced fish also took longer to

attack the prey that had two eyelike marks (that imitated the frontal view of perch eyes and that did not resemble the typical eyespot shape) compared the prey that had a rectangular, non-eyelike marking ($t = 4.72$, $d.f. = 28$, $p < 0.001$).

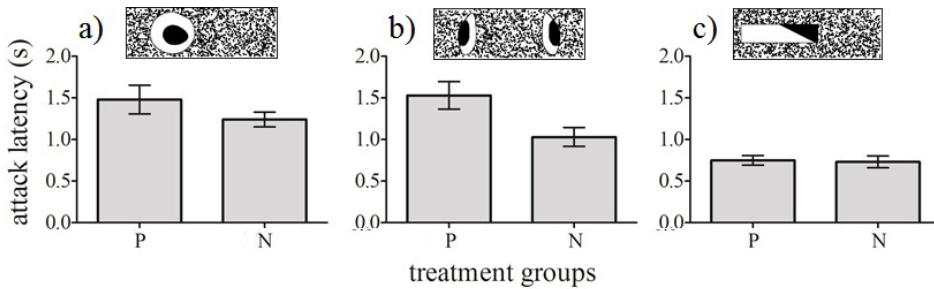


Figure 9. Mean attack latencies (\pm s.e.) for fish that attacked the eyelike patterns are shown in a) and b), and c) show the results from the non-eyelike, rectangular element. P is the pre-experimental “predation” treatment and N represents “no predation”. $N = 15$ for each group.

These results support the suggestion that eye mimicry is important for the intimidating effect of eyespots for two reasons. 1) The sticklebacks took significantly longer time to attack the prey that had eyelike markings than the prey that had the equally conspicuous, rectangular marking. 2) Previous experience of predators only affected the response of the sticklebacks to the eyelike markings but not to the non-eyelike markings, suggesting that predator cues induced an association between the markings and a threat. These results contradict previous studies arguing that it is merely the conspicuousness, and not eye mimicry that is important for the intimidating function of eyespots (Stevens *et al.* 2007, 2008, 2009).

Considering that both the eyelike markings resembling the typical eyespot shape and the eyelike markings that deviated from the concentric, circular shape induced an intimidating effect towards attacking fish, it is interesting that most eyespots found in many fishes are more or less circular (e.g. Kelley *et al.* 2013). The appearance of eyespots could be explained by the developmental mechanisms that can relatively easily produce circular patterns that consist of concentric rings (Beldade & Brakefield 2002; Dilão & Sainhas 2004).

4. GENERAL CONCLUSIONS

The adaptive value and function of both cryptic colouration and eyespots have received much attention and have been studied particularly in terrestrial systems (e.g. Stevens & Merilaita 2011; Vallin *et al.* 2005, 2006; Kodandaramaiah *et al.* 2009), but far less is known about them in aquatic systems, despite the fact that probably all types of protective coloration found in terrestrial environments also exist in the aquatic environment and have initially evolved there. In this thesis, I have used aquatic experimental setups to study specific questions about the functions and adaptive value of protective coloration and related anti-predator behaviour in aquatic environments, and showed that the visual appearance of backgrounds and prey colour patterns indeed strongly affects the behaviour of fishes.

More specifically, in paper I, I have shown that certain aspects of the visual appearance of the available backgrounds are important for the habitat choice of fish and that this is related to predator avoidance. Because it has been shown that both a visual match in the appearance of prey colour pattern and background, and visual complexity of the background can decrease the risk of predation (Feltmate & Williams 1989; Kettlewell 1955; Dimitrova & Merilaita 2010, 2012), my finding that fish showed a preference for these cues when under predation risk suggests that this behaviour is indeed an anti-predator adaptation. Intriguingly, the latter finding also suggest that prey are able to assess and respond to some cues of visual similarity between their body pattern and the surrounding environment, but also that some aspects of visual complexity could be important and may even override background matching. Because visual complexity might be an easier and more straightforward cue than background matching to find in heterogeneous environments, future studies should investigate whether increased visual variability of the habitat leads to an increased preference for visual complexity, and try to pinpoint which aspects of visual complexity are important for habitat choice of prey.

In papers II, III and IV, I have shown that eyelike prey markings can be used to manipulate predator behaviour in a way that reduces the likelihood of a successful attack, hence providing adaptive explanations for the wide occurrence of eyespots in many prey species. The results from papers II and III show that eyespots very effectively draw the attacks of fish towards them, providing strong support for Cott's (1940)

suggestion that the existence of eyespots in many fishes can be explained by their divertive function. Moreover, the finding that circular, possibly eyelike marks drew the initial attacks of fish towards them more effectively than did marks of other shapes may at least partly, probably together with the relatively simple developmental process underlying circular marks, explain why divertive eyespots have their circular appearance. It is possible that eyelike shape is an important factor for the divertive effect due to eye mimicry in aquatic prey (Winemiller 1990). Although it is often assumed that divertive eyespots, particularly in Lepidoptera, draw attacks away from vital parts of the body, in many aquatic prey eyespots could instead serve as a false indicator of expected escape direction. This could explain why many species of fish have eyespots located near, or on their caudal fins. A posteriorly-located eye-mimicking mark may deceive a predator to attack towards that direction, and that way enhance the probability of a successful escape.

Paper **III** confirms the anti-predator utility of another common prey pattern, eyebar. My results show that a bar effectively disrupts an eyelike shape. Also the disruption of the eyelike shape resulted in a significant amount of attacks being directed towards the intact eyelike shape. This result therefore suggests functionally important things. First, eyebars provide an effective way to conceal the eye, which may be an important means of camouflage as the eye can serve as a cue for detection and recognition of potential prey. Moreover, in prey that has a divertive eyespot, the eyebar can strengthen the divertive effect of the eyespot, which might explain the joint occurrence of eyebars and eyespots in many species of fish.

I found no clear support for an intimidating effect of eyespots in paper **II**. It might be that the methodological approach I used to test for the intimidating effect of eyespots was not properly designed for testing this. Because I only measured the latency to attack from the fish's last entry in the foraging zone, and that some individuals swam around more than others before attacking the prey item, it might be that I failed to measure some important behavioural response that occurred after the fish's entry in the foraging zone, but before the prey attack.

On the contrary however, I found strong support for an intimidating effect of eyelike prey markings towards attacking fish in paper **IV**. Importantly, I have provided empirical evidence for the idea that eye mimicry, and not merely conspicuousness, is an important reason of this

intimidating effect. This experiment also revealed that the intimidating effect is partly innate and partly acquired through previous experience of predators.

To conclude, I have in this thesis presented adaptive explanations for the existence of eyespots, eyebars and background choice in prey, and that way increased our understanding of how natural selection imposed by predation can shape the appearance and anti-predator behaviour of prey over evolutionary time. Also, my finding that manipulating the visual aspects of prey appearance strongly influenced the behavioural response of predators has provided important knowledge in questions regarding predator psychology.

ACKNOWLEDGEMENTS

About six years ago, when I was still an undergraduate at the University of Gothenburg, I attended a seminar where this fantastic scientist presented his fascinating research on protective coloration in animals. I remember taking notes so enthusiastically, that it eventually made my hand cramp! How lucky am I, that the scientist in question a year later accepted me as his PhD student?! Therefore, I would first like to express my deepest gratitude to my fantastic supervisor, **Sami Merilaita**. Not only have you been a huge inspiration and encouraging mentor along the way, but also a wonderful friend who has made life for me here in Finland very easy from day one, and most importantly, joyful as well. **Sami**, thank you for giving me the opportunity to fulfil at least two of my lifelong dreams: study animal coloration and cuddle with koalas in Australia☺! Thank you also for your thorough comments, constructive criticism and stimulating scientific discussions that have truly helped me become a better scientist, and for the very existence of this thesis! I would also like to thank my co-supervisor, **Jörgen I. Johnsson**, who encouraged me to apply for this PhD position in the first place, because without you, none of this would have been possible. Thank you for teaching me how to plan and conduct excellent laboratory experiments using fish as study species, and for providing constructive criticism and comments that has helped me improve the quality of this thesis.

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REFERENCES

- Alcock J. 2005. *Animal behavior: an evolutionary approach*, 8th edn. Sunderland, MA: Sinauer Associates, Inc.
- Asakura A. 1995. Sexual differences in life history and resource utilization by the hermit crab. *Ecology*. **76**: 2295-2313.
- Barber I. & Huntingford FA. 1995. The effect of *Schistocephalus solidus* (Cestoda: *Pseudophyllidae*) on the foraging and shoaling behaviour of three-spined sticklebacks, *Gasterosteus aculeatus*. *Behaviour* **132**: 1223-1240.
- Barlow GW. 1972 The attitude of fish eye-lines in relation to body shape and to stripes and bars. *Copeia* **1**: 4-12. (doi:10.2307/1442777)
- Beldade P, Brakefield PM. 2002. The genetics and evo-devo of butterfly wing patterns. *Nature Reviews Genetics* **3**: 442-452.
- Blest AD. 1957. The function of eyespots in the Lepidoptera. *Behaviour*. **11**: 209-256.
- Boarman M. Askew RR. & Cook LM. 1974. Experiments on resting site selection by nocturnal moths. *J. Zool., Lond.* **172**: 343-355.
- Brakefield PM. Gates J. Keys D. Kesbeke F. Wijngaarden PJ. Monteiro A. French V. Carroll SB. 1996. Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**: 236-242.
- Caro T. 2005. *Anti predator defenses in birds and mammals*. London, UK: The University of Chicago Press Ltd.
- Cooper JM. & Allen JA. 1994. Selection by wild birds on artificial dimorphic prey on varied backgrounds. *Biol. J. Linn. Soc.* **51**: 433-466.
- Coss RG. 1979. Delayed plasticity of an instinct: Recognition and avoidance of two facing eyes by the jewel fish. *Dev. Psychobiol.* **12**: 335-345.
- Cott HB. 1940. *Adaptive coloration in animals*. London, UK: Methuen & Co., Ltd.

- Cox S. Chandler S. Barron C. & Work K. 2009. Benthic fish exhibit more plastic crypsis than non-benthic species in a freshwater spring. *J. Ethol.* **27**: 497–505.
- Cuthill IC. & Székely A. 2009. Coincident disruptive coloration. *Phil. Trans. R. Soc. B.* **364**: 489–496.
- Darwin C. 1859. On the origin of species by means of natural selection. London, UK: Murray.
- Darwin E. 1794. *Zoönomia, or the laws of organic life, vol. 1*. London, UK: Johnson.
- Dimitrova M. & Merilaita S. 2010. Prey concealment: visual background complexity and prey contrast distribution. *Behav. Ecol.* **21**: 176–181. (doi:10.1093/beheco/arp174)
- Dimitrova M. & Merilaita S. 2012. Prey pattern regularity and background complexity affect detectability of background-matching prey. *Behav. Ecol.* **23**: 384–390.
- Dimitrova M. & Merilaita S. 2014. Accuracy of background matching and prey detection: predation by blue tits (*Cyanistes caeruleus*) indicates intense selection for highly matching prey colour pattern. *Funct. Ecol.* (doi:10.1111/1365-2435.12248)
- Feltmate BW. & Williams DD. 1989. A test of crypsis and predation avoidance in the stonefly *Paragnetina media* (Plecoptera: Perlidae). *Anim. Behav.* **37**: 992–999.
- Gagliano M. 2008. On the spot: the absence of predators reveals eyespot plasticity in a marine fish. *Behav. Ecol.* **19**: 733–739. (doi:10.1093/beheco/arn013)
- Garcia TS. & Sih A. 2003. Color change and color dependent behavior in response to predation risk in the salamander sister species *Ambystoma barbouri* and *Ambystoma texanum*. *Oecologia.* **137**: 131–139. (doi:10.1007/s00442-003-1314-4)
- Giles N. 1987. Predation risk and reduced foraging activity in fish: experiments with parasitized and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus*. *L. J. Fish Biol.* **31**: 37–44.

- Gillis JE. 1982. Substrate matching cues in the cryptic grasshopper *Circotettix rabula rabula* (Rehn & Hebard) *Anim. Behav.* **30**: 113-116.
- Godin JGJ. & Sproul CD. 1988. Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. *Can. J. Zool.* **66**: 2360-2367.
- Hart PJB. & Gill AB. 1994. Evolution of foraging behaviour in the threespine stickleback. In *The evolutionary biology of the threespine stickleback* (eds MA Bell, SA Foster), pp. 207-239. Oxford, UK: Oxford University Press.
- Hossie TJ & Sherratt TN. 2012. Eyespots interact with body colour to protect caterpillar-like prey from avian predators. *Anim. Behav.* **84**:167-173.
- Hossie TJ & Sherratt TN. 2013. Defensive posture and eyespots deter avian predators from attacking caterpillar models. *Anim. Behav.* (doi:10.1016/j.anbehav.2013.1005.1029)
- Janzen DH. Hallwachs. W. & Burns JM. 2010. A tropical horde of counterfeit predator eyes. *Proc. Nat. Acad. Sci. USA* **107**: 11659-11665.
- Johnsson IJ. & Källman-Eriksson K. 2008. Cryptic prey colouration increases search time in brown trout (*Salmo trutta*): effects of learning and body size. *Behav. Ecol. Sociobiol.* **62**: 1613-1620. (doi:10.1007/s00265-008-0590-8)
- Kelley JL. Fitzpatrick JL. & Merilaita S. 2013. Spots and stripes: ecology and colour pattern evolution in butterflyfishes. *Proc. R. Soc. B* **280**: 20122730.
- Kettlewell HBD. & Conn DLT. 1977. Further background-choice experiments on cryptic Lepidoptera. *J. Zool. Lond.* **181**: 371-376.
- Kettlewell HBD. 1955. Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. *Nature.* **175**: 943-944.
- Kjernsmo K. & Merilaita S. 2013. Eyespots divert attacks by fish. *Proc. R. Soc. B* **280**: 20131458.

- Kodandaramaiah U. Vallin A. Wiklund C. 2009. Fixed eyespot display in a butterfly thwarts attacking birds. *Anim. Behav.* **77**: 1415–1419.
- Lees DR. 1975. Resting site selection in the geometrid moth *Phigalia pilosaria* (Lepidoptera: Geometridae). *J. Zool. Lond.* **176**: 341–352.
- Litvak MK. Leggett WC. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. *Mar. Ecol. Prog. Ser.* **81**: 13–24.
- Lyytinen A. Brakefield PM. & Mappes J. 2003. Significance of butterfly eyespots as an anti-predator device in ground-based and aerial attacks. *Oikos*. **100**: 373–379.
- Lyytinen, A. Brakefield, P. M., Lindström, L. & Mappes, J. 2004. Does predation maintain eyespot plasticity in *Bicyclus anynana*? *Proc. R. Soc. Lond. B.* **271**: 279–283. (doi:10.1098/rspb.2003.2571)
- Meadows DW. 1993. Morphological variation in eyespots of the four-eye butterflyfish (*Chaetodon capistratus*): implications for eyespot function. *Copeia*. **1**: 235–240.
- Merilaita S. & Jormalainen V. 1997. Evolution of sex differences in microhabitat choice and colour polymorphism in *Idotea baltica*. *Anim. Behav.* **54**: 769–778.
- Merilaita S. & Jormalainen V. 2000. Different roles of feeding and protection in diel microhabitat choice of sexes in *Idotea baltica*. *Oecologia*. **122**: 445–451.
- Merilaita S. 2001. Habitat heterogeneity, predation and geneflow: color polymorphism in the isopod *Idotea baltica*. *Evol. Ecol.* **15**: 103–116.
- Merilaita S. 2003. Visual background complexity facilitates the evolution of camouflage. *Evolution* **57**: 1248–1254.
- Merilaita S. Vallin A. Kodandaramaiah U. Dimitrova M. Ruuskanen S. & Laaksonen T. 2011. Number of eyespots and

- their intimidating effect on naïve predators in the peacock butterfly. *Behav. Ecol.* **22**: 1326-1331.
- Monteiro A. Prijs J. Bax M. Hakkaart T. & Brakefield PM. 2003. Mutants highlight the modular control of butterfly eyespot patterns. *Evol Dev.* **5**:180–187.
- Neudecker S. 1989. Eye camouflage and false eyespots: chaetodontid responses to predators. *Env. Biol. Fish.* **25**: 143–157.
- Nijhout HF. 1991. *The development and evolution of butterfly wing patterns*. Washington and London: Smithsonian Institution Press.
- Nonacs P. & Blumstein D. 2010. Predation risk and behavioural life history. In *Evolutionary behavioural ecology*. (eds DF Westneat, CW Fox), pp - 207. New York, USA: Oxford University Press.
- Ohguchi O. 1978. Experiments on selection against color oddity of water fleas by 3-spined sticklebacks. *Z. Tierpsychol.* **47**: 254–267.
- Ohno Y. & Otaki JM. 2012. Eyespot colour pattern determination by serial induction in fish: Mechanistic convergence with butterfly eyespots. *Scientific reports* **2**: 290. (doi:10.1038/srep00290)
- Popham EJ. 1943. Ecological Studies of the Commoner Species of British Corixidae. *Jour. Anim. Ecol.* **12**: 124-136.
- Poulton EB. 1890. *The colours of animals: their meaning and use especially considered in the case of insects*. London, UK: Kegan Paul, Trench, Trubner and Co. Ltd.
- Rowe MP. Baube CL. Loew ER. Phillips JB. 2004. Optimal mechanism for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. *J. Comp. Physiol. A.* **190**: 241–256.
- Ruxton GD. Sherrat TN. Speed MP. 2004. *Avoiding attack*. Oxford, UK: Oxford University Press.

- Sandoval CP. 1994. Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae: Timemidae) and its consequences for host range. *Biol. J. Linn. Soc.* **52**: 341-356.
- Sargent TD. & Keiper RR. 1969. Behavioural adaptations of cryptic moths. I. Preliminary studies on bark-like species. *Journal of The Lepidopterists' Society.* **23**: 1-9.
- Shine R. 1986. Sexual differences in morphology and niche utilization in an aquatic snake, *Acrochordus arafurae*. *Oecologia.* **69**: 260-267.
- Stevens M. & Merilaita S. 2009a. Introduction. Animal camouflage: current issues and new perspectives. *Phil. Trans. R. Soc. B.* **364**: 423-427. (doi:10.1098/rstb.2008.0217)
- Stevens M. & Merilaita S. 2009b. Defining disruptive coloration and distinguishing its functions. *Phil. Trans. R. Soc. B.* **364**: 481–488.
- Stevens M. & Merilaita S. 2011. Animal camouflage function and mechanisms. In *Animal camouflage: mechanisms and function* (eds M. Stevens & S. Merilaita), pp. 1–16. Cambridge, UK: Cambridge University Press.
- Stevens M. 2005. The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biol Rev.* **80**: 573–588.
- Stevens M. Cantor A. Graham J. & Winney IS. 2009. The function of animal 'eyespot': Conspicuousness but not eye mimicry is key. *Current Zoology* **55**:319-326.
- Stevens M. Hardman CJ. & Stubbins CL. 2008. Conspicuousness, not eye mimicry, makes “eyespot” effective antipredator signals *Behav. Ecol.* **19**: 525-531.
- Stevens M. Hopkins E. Hinde W. Adcock A. Connelly Y. *et al.*, 2007. Field experiments on the effectiveness of 'eyespot' as predator deterrents. *Anim. Behav.* **74**: 1215-1227.

- Thayer AH. 1909. *Concealing-coloration in the animal kingdom*. New York: The Macmillan CO.
- Vallin A. Jakobsson S. & Wiklund C. 2007. An eye for an eye''—on the generality of the intimidating quality of eyespots in a butterfly and a hawkmoth. *Behav. Ecol. Sociobiol.* **61**: 1419–1424.
- Vallin A. Jakobsson S. Lind J. & Wiklund C. 2006. Crypsis versus intimidation—anti-predation defence in three closely related butterflies. *Behav. Ecol. Sociobiol.* **59**: 455-459.
- Vallin A. Jakobsson S. Lind J. & Wiklund C. 2005. Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. *Proc. R. Soc. .B* **272**: 1203–1207.
- Vallin A. Dimitrova M. Kodandaramaiah U. & Merilaita S. 2011. Deflective effect and the effect of prey detectability on anti-predator function of eyespots. *Behav. Ecol. Sociobiol.* **65**: 1629–1636. (doi:10.1007/s00265-011-1173-7)
- Vlieger L. & Brakefield PM. 2007. The deflection hypothesis: eyespots on the margins of butterfly wings does not influence predation by lizards. *Biol. J. Linn. Soc.* **92**: 661–667.
- Winemiller KO. 1990. Caudal eyespots as deterrents against fin predation in the neotropical cichlid *Astronotus ocellatus*. *Copeia*. **3**: 665-657.
- Wootton RJ. 1976. *The biology of the sticklebacks*. London, UK: Academic Press.

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Anti-predator Adaptations in Aquatic Environments

This thesis investigates how protective coloration can be used, either as such or together with behavioural adaptations, to manipulate predator behaviour and decrease predation risk. The results demonstrate that protective coloration provides numerous distinctive ways for aquatic prey to escape predation. Thus, visual perception and behaviour of fish are important factors shaping the appearance and behaviours of aquatic prey.

