



**Regulation, constraints and benefits of colour plasticity in a mimicry system**

Journal:	<i>Biological Journal of the Linnean Society</i>
Manuscript ID	BJLS-4989.R1
Manuscript Type:	Original article
Date Submitted by the Author:	n/a
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Keywords:	colour change, aggressive mimicry, coral reef fish, phenotypic plasticity, bluestriped fangblenny, <i>Plagiotremus rhinorhynchus</i>

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Review

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3 ***Regulation, constraints and benefits of colour plasticity in a mimicry system***  
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**Abstract**

Rapid colour change is used in aggressive interactions, ontogenetic transitions, nuptial displays, and to prevent detection and/or recognition from predators or prey. The underlying mechanisms, constraints and benefits of colour change are often unclear, but examining such factors offer insights into phenotypic plasticity. Here, we investigated the mechanisms behind how an aggressive reef fish mimic (bluestriped fangblenny *Plagiotremus rhinorhynchos*) changes colour rapidly (1-5 min) between mimetic and other colour forms. Black with one neon blue dorsal stripe (mimic), black with two neon blue stripes, brown, olive and orange forms differed in melanophore density. Fish skin biopsies were modulated *in vitro* by hormones, and smaller fangblennies changed colouration more rapidly than larger fish suggesting that the ability to change colour is diminished as fish get larger. Individuals may be limited by differences in pigment cell densities to change colour between extreme colour forms (black and orange), therefore longer morphological changes may also occur or fangblennies may exhibit dimorphic populations. Behavioural observations suggest that small black and orange individuals were equally successful in attacking passing fish to feed on dermal tissue/scales, indicating that deceptive strategies used by each colour form may deliver equal fitness benefits. The present study demonstrates for the first time how fangblennies change colour and highlights that colour plasticity offers important adaptive advantage; however, physiological constraints should also be considered.

**Keywords:** colour change, aggressive mimicry, coral reef fish, phenotypic plasticity, bluestriped fangblenny, *Plagiotremus rhinorhynchos*

## Introduction

Phenotypic plasticity allows animals to alter growth, morphology, physiology, behaviour or life history in response to environmental variation, and contributes to the ecological success of individuals and populations (Pigliucci, 2001). One prominent example of plasticity is the ability to alter body colouration. Many animals are able to adaptively alter their colour in response to changes in seasons, motivational state, light quality, temperature, background and for communication purposes, including nuptial displays or deimatic displays (e.g. Kodric-Brown, 1998; Leclercq, Taylor & Migaud, 2010; Nilsson Sköld et al., 2016; Sköld, Aspengren & Wallin, 2013; Stuart-Fox & Moussalli, 2011). However, there are limitations to colour change in most animals, which reduces the ability to display optimal colour signals in many circumstances (Stuart-Fox & Moussalli, 2011). Understanding the capacity and constraints of colour change will enable us to elucidate the function, benefit and evolution of dynamic visual signals.

In fish, colour change appears to be ubiquitous (Nilsson Sköld et al., 2016): flatfish, groupers and gobies match the visual properties of the background (Stevens, Lown & Denton, 2014; Watson, Siemann & Hanlon, 2014); fish may display nuptial colouration during breeding season, such as the males of Southern pygmy perch, *Nannoperca australis*, which develops red and black colour patches on their fins and bodies prior to breeding (Morrongiello et al., 2010). Colour change also occurs during aggressive interactions: terminal phase male Bluehead wrasse, *Thalassoma bifasciatum*, alter their posterior body coloration from opalescent (pink-gray pearl) to bright bottle green when chasing other males away from individual spawning territories (Dawkins & Guilford, 1993).

Colour change can also be used as a deceptive mechanism to prevent detection or recognition by potential predators or prey (Cheney, Grutter & Marshall, 2008; Cortesi et al., 2015; Cortesi et al., 2016; Côté & Cheney, 2005; Norman, Finn & Tregenza, 2001). The use of colour change in mimicry systems is an interesting phenomenon, and has been studied in a few fish species including the dusky dotyback, *Pseudochromis fuscus*, which alters colouration over a period of two

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3 weeks to match the colour of surrounding schooling fish to prevent detection from  
4 juvenile prey (Cortesi et al., 2015).  
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8 Rapid colour change occurs in the bluestriped fangblenny, *Plagiotremus*  
9 *rhinorhynhos*, which are aggressive mimics of the juvenile bluestreaked cleaner  
10 wrasse, *Labroides dimidiatus* (Côté & Cheney, 2005). Instead of cleaning reef fish by  
11 removing ectoparasites from larger reef fish clients (Côté, 2000; Grutter, 1999),  
12 *Plagiotremus* fangblennies feed by attacking passing reef fish removing scales and  
13 dermal tissue (Kuwamara, 1981). Bluestriped fangblennies exhibit many different  
14 colour forms: as a cleaner wrasse mimic, it exhibits a black body with one neon blue  
15 dorsal stripe; whereas alternative colour forms can have a black body with two blue  
16 stripes, or a brown, olive or orange colour body with two white or light blue stripes  
17 (Fig. 1). Some of these other colour forms are often found associated with shoals of  
18 similarly coloured fish (Cheney et al., 2008; Cheney et al., 2009; Côté & Cheney,  
19 2005). However, the physiological mechanisms behind how colour changes are  
20 achieved and whether there is a significant difference in foraging success between the  
21 distinct strategies of a cleaner wrasse mimic and shoal-related forms remains unclear.  
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33 In a previous study, fangblennies in aquaria that changed colour were  
34 significantly smaller than those that did not change colour, and only small individuals  
35 (< 50mm) changed colour to the black/blue one stripe mimic colour form (Cheney et  
36 al., 2008). Due to a size disparity between adult fangblennies (> 70mm) and juvenile  
37 cleaner wrasse (< 50 mm), it was suggested that fangblennies only mimic juvenile  
38 cleaner wrasse when they are of a similar size, as the adaptive advantage to  
39 resembling them when they were larger was limited. However, the mechanisms that  
40 cause fish to change colour and whether they lose this ability as they grow larger has  
41 not been previously demonstrated. Furthermore, fangblennies have been observed to  
42 switch from black/blue one or two stripes colour forms to brown or olive forms  
43 (which we term here the 'default' colouration as this is the colour fangblennies often  
44 revert to when caught; Fig. 1) and back again (Cheney et al., 2008), and from orange  
45 forms to brown or olive forms (Côté & Cheney, 2005). However, it is unclear whether  
46 individuals have the ability to switch between the extremes of their colour repertoire  
47 (i.e from black/blue to orange), or whether this change is physiologically constrained.  
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3 In this study, we investigated the mechanisms behind colour change in this  
4 species and examined potential underlying physiological and morphological  
5 constraints to colour change. Fish body colour is dependent on different pigment cells  
6 in their skin (chromatophores), which are classed as melanophores (black),  
7 erythrophores (red), xanthophores (yellow), leucophores (white), iridophores  
8 (iridescent) and cyanophores (blue chromatophores in callionymid fish) (Goda &  
9 Fujii, 1995). Some fishes can change their body colouration within minutes using  
10 physiological processes, which cause intracellular mass translocations of pigments  
11 and/or a change in the orientation of reflective crystals inside chromatophores (Goda  
12 & Fujii, 1995). Alternatively, fishes can also change colour over a longer time period  
13 using morphological processes that change the number, type and/or distribution of  
14 chromatophore cells within the skin (Leclercq et al., 2010).  
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24 We first investigated the pigment cells responsible for physiological colour  
25 changes between different fangblenny colour forms from individuals immediately  
26 fixed in the field and from fish kept for a few days in the laboratory. To better  
27 understand the regulation and extent of colour plasticity, trunk biopsies were then  
28 treated with melatonin, prolactin and melanocyte stimulating hormone (MSH),  
29 hormones known to regulate differently coloured chromatophores in other fish species  
30 (Sköld et al., 2013), with the expectation to replicate the colour changes observed in  
31 the wild. We also compared the rate at which skin biopsies changed colour, and  
32 determined whether size imposed morphological constraints on colour change in this  
33 species. Finally, we conducted behavioural observations on small fangblennies  
34 exhibiting black/blue one stripe (mimic) and orange colour forms to investigate  
35 whether there was a significant fitness difference in foraging success for fish that  
36 exhibit different deceptive strategies.  
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## 48 **Materials and Methods**

### 49 *Collection of specimens*

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Bluestriped fangblennies, *Plagiotremus rhinorhynchos*, (n = 24) were located  
and collected from shallow reefs (1-10m) around Lizard Island (14°40'S, 145°27'E),  
Great Barrier Reef, in May 2014. Individuals were collected with hand nets and if  
needed, a small amount of clove oil solution (10% clove oil, 40% ethanol, 50%

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3 seawater) sprayed into the water (this did not cause colour change in individuals).  
4 Immediately after capture, individuals were placed in a hermetically sealed bag and  
5 photographed underwater. We categorised their colouration as either black body with  
6 one neon blue stripe (mimic colour form) (n = 5; range standard length (SL): 35-72  
7 mm), black body with two neon blue stripes (n = 3, range SL: 45-65 mm), brown  
8 body with two white stripes (n = 5, range SL: 43-68 mm), olive body with 2 light  
9 blue/white stripes (n = 7; range SL: 58-68 mm), orange body with 2 light blue/white  
10 stripes (n = 4, range SL: 54-58 mm) (Fig. 1).  
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### 18 ***Chromatophore analysis***

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20 To reveal chromatophore status in the wild, fish (n = 8; black/blue one stripe =  
21 1, black/blue two stripes = 2, olive = 3, orange = 2) were taken back to the boat by a  
22 snorkeler, and decapitated within 2 minutes after capture to preserve colouration. The  
23 trunk was immediately put in 4% neutral buffered formalin. The fixed trunk biopsies  
24 were then brought to the research station for examination under a microscope. A  
25 further set of fish (n=16; black with one stripe = 4, black with 2 stripes = 1, brown =  
26 5, olive = 4, orange = 2) were collected and then kept in individual tanks with plastic  
27 pipe shelters and coral rubble for a few days. While these fish were used for analysing  
28 rate of color change *in vitro* and hormone effects, a piece of the trunk was also fixed  
29 immediately after decapitation in 4% neutral buffered formalin for melanophore  
30 analysis of the main body area between the 2 stripes as a complement to the fish fixed  
31 in the field.  
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42 Melanophore density in the body area was assessed by manually counting the  
43 number of melanophores in a randomly selected 0.5 x 0.5 mm square of a photograph  
44 of each fixed biopsy. The melanophore status was scored manually in the same  
45 photographs using the Melanophore Index (MI; Hogben & Slome, 1931), where MI 1  
46 is totally aggregated pigments and MI 5 totally dispersed pigments. Magnification and  
47 illumination were kept under standardized conditions during photography.  
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### 53 ***In vitro analysis of colour change***

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55 To further understand the regulation of colour plasticity between differently  
56 sized fish and between different colour forms, trunk sections of fish (n = 16) were  
57 treated with different combinations of hormones. Stock solutions of hormones were  
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3 stored at -20°C and diluted to the experimental concentration in analytical phosphate  
4 buffered saline (APBS at pH 7.4; ProSciTech, Thuringova, Qld, Australia) just before  
5 use. Melatonin and sheep prolactin (Sigma Aldrich) were stored at -20°C as 10 mM  
6 and 150 IU/ml stock solutions, respectively. Melatonin was used at 10 µM and  
7 prolactin at 0.150 IU/ml. MSH ( $\alpha$ - melanocyte stimulating hormone; ICN  
8 Pharmaceuticals, Inc., Costa Mesa, CA, USA), was stored as 1 mM stock solutions at  
9 -20°C and used at 5 µM. Control trunk sections were incubated in APBS with 0.1%  
10 ethanol, the dissolvent of melatonin.  
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18 Fish used in the *in vitro* experiments were kept in aquaria for a few days prior  
19 to treatments. Each fish was sized, photographed and quickly decapitated using sharp  
20 scissors. In many cases, their colouration had altered since capture and so was  
21 classified again (black with 2 stripes = 2, brown = 6, olive = 8). The body was  
22 thereafter cut into approximately 0.5 cm vertical sections from head to tail and  
23 biopsies were rested in saltwater for 5-15 min to allow full chromatophore pigment  
24 dispersion before onset of treatments (one section was fixed immediately after  
25 decapitation in 4% neutral buffered formalin, see above). For each individual,  
26 biopsies were thereafter randomly incubated in hormone and control solutions. Two  
27 different *in vitro* experiments were performed. First, the capacity of differently  
28 colored individuals to change color was tested by incubating the biopsies for one hour  
29 using different combinations of hormones (melatonin, prolactin or MSH, individually  
30 or in combinations) or a control solution (APBS), before analyzing pigment  
31 movement/colour change under a bright field microscope. Using pilot trials, one hour  
32 was found sufficient to trigger the full spectrum of physiological colour change  
33 responses. Second, the rate at which colour changes occurred *in vitro* was tested using  
34 10 µM melatonin, which causes movement of pigment granules to the cell centre  
35 (aggregated), on differently sized fishes (SL: 49-75 mm). Melanophore pigment was  
36 dispersed (MI = 5) for each individual at the beginning of the trial, and scored every  
37 30 s using microscopy and the Melanophore Index for 10 min.  
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### 52 ***Behavioural observations***

53 To examine whether there was a fitness advantage between extreme colour  
54 forms, we conducted 1 or, when possible, 2 behavioural observations for 15 min on  
55 black/blue one stripe (n = 15), and orange individuals (n = 9) (n = 39 observations in  
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total) that were haphazardly located on ten coral reefs around Pulau Hoga 05°28'S, 123°45'E, Southeast Sulawesi, Indonesia in July-August 2006. Individuals were located at least 10 m apart using SCUBA at depths of 2–18 m and were all SL < 50 mm based on length estimates in the field. For repeat observations on the same individual (n = 15), small pieces of flagging tape were placed on the reef to relocate individuals. Repeat observations were done on a different day/time and results were averaged. During each observation, we recorded the total number of attacks made by a mimic, defined as a fangblenny darting towards a potential victim; and the number of successful attacks, defined as clear contact made with the victim. All black/blue one stripe (mimic) individuals were found within 1 m of a juvenile cleaner wrasse, and all orange fangblennies were located within a shoal of > 20 orange anthias species (e.g. *Pseudanthias squamipinnis*).

## Results

### *Chromatophore analysis of field samples*

Analysis of chromatophores on trunk biopsies from fish fixed in the field revealed differences between color forms in terms of chromatophore density (Fig. 1, 2). Black/blue forms with one (n = 1) or two stripes (n = 2) showed dense amounts of melanophores in the body area with dispersed black pigment ( $\geq 135$  melanophores/0.25 mm<sup>2</sup>, MI 4-5) but we did not detect xanthophores here. Fins contained chromatophores with yellow and red pigment (xanthophores and erythrophores). The blue ventral stripes showed iridophores (iridescent platelets) overlaying melanophores with aggregated pigment (MI 1-2). In the one fish lacking the ventral blue stripe (n = 1), the pigment was dispersed in melanophores in the ventral stripe region (MI 4-5) (Fig. 2A, B).

Olive forms (n = 3) showed an olive body area due to either few melanophores with partly aggregated pigment (63 and 64 melanophores/0.25 mm<sup>2</sup>, MI 2-3) or dense amounts of melanophores (104 melanophores/0.25 mm<sup>2</sup>, mix of MI 1 and 4). In these individuals, xanthophores with aggregated pigment were also observed. The stripes were almost white and the melanophores had aggregated pigment (MI 1-2) and the fins were red containing erythrophores (Fig. 2C).

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3 In orange forms ( $n = 2$ ), the body area was orange due to fewer melanophores  
4 (49 and 30 melanophores/ $0.25 \text{ mm}^2$ ) despite relatively dispersed melanophore  
5 pigment (MI 4), but with a higher number of erythrophores. As with the olive form,  
6 the blue stripes were white in color and contained melanophores with aggregated  
7 pigment (MI 2). The fins contained only xanthophores, or both xantho- and  
8 erythrophores (Fig. 2D).  
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### 13 14 15 ***Chromatophore analysis of fish kept in laboratory***

16 Of the fish kept in tanks before fixation (black/blue two stripes = 2, brown =  
17 6, olive = 8) results were similar to field fixed fish. The body area in black/blue two  
18 stripes forms were completely covered in melanophores with dispersed pigment  
19 (density  $> 135$  melanophores/ $0.25 \text{ mm}^2$ , MI 4-5) and in brown individuals had fewer  
20 melanophores compared to the black/blue two stripes individuals (range: 56 to 132  
21 melanophores/ $0.25 \text{ mm}^2$ ; mean = 93.9 and melanophore pigment was dispersed (MI  
22 4-5). The body areas in olive coloured individuals had similar melanophore densities  
23 to the body areas in brown forms (range: 42 to 156 melanophores/ $0.25 \text{ mm}^2$ ; mean =  
24 73.4) and also had dispersed melanophore pigment (MI 4-5), while one individual had  
25 relatively aggregated melanophore pigment (MI 2-3).  
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### 35 36 37 ***In vitro analysis of colour change***

38 The body areas of biopsies kept in MSH or prolactin were dark (Fig. 3A), and  
39 similar to treatments kept in the control solution (APBS) only (not shown). However,  
40 the blue stripes appeared more fluorescent in MSH or prolactin compared to the  
41 control. Incubating biopsies in melatonin resulted in paling of the body area and  
42 abdomen due to the aggregation of pigment inside melanophores and xanthophores  
43 (Fig. 3B). Combining melatonin with MSH resulted in more colourful bodies from  
44 more dilated xanthophores and pale abdomen from aggregated melanophore pigment  
45 (Fig 3C).  
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49 With the use of melatonin as a trigger for melanophore pigment aggregation, it  
50 became evident that both the rate and capacity of color change declined with an  
51 increase in size of the fish. Fish length was strongly correlated with MI Index after 90  
52 s (Spearman's rank  $r = 0.76$ ,  $n = 16$ ,  $p < 0.001$ ; Fig. 4). Moreover, in one of the larger  
53 individuals (SL = 70 mm), some of the melanophores did not respond at all within the  
54 10 min time frame. Interestingly, this individual also had melanin deposits outside of  
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3 the melanophores. The skin of smaller fish (< 60mm) on the other hand, went from  
4 almost black to considerably pale due to the rapid aggregation of pigment in all  
5 observed melanophores.  
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### 8 9 10 **Behavioural observations**

11 The main fish species that were attacked by fangblennies included: snapper  
12 (*Lutjanus fulviflamma*), parrotfish (*Scarus* spp.), damselfish (*Chromis* spp.),  
13 squirrelfish (*Myripristis* spp.), and anthias (*Pseudanthias* spp.). For each individual,  
14 the number of strikes at fish ranged from between 0 to 12, per 15 min observation;  
15 however, strike number did not significantly differ between black/blue one stripe or  
16 orange colour forms (Mann Whitney-U = 52,  $n_1 = 15$ ,  $n_2 = 9$ ,  $p = 0.67$ ). The  
17 percentage of successful strikes ranged from 20 to 100 %, but again, did not differ  
18 between forms (Mann Whitney-U = 44,  $n_1 = 15$ ,  $n_2 = 9$ ,  $p = 0.77$ ) (Fig. 5).  
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26 During one behavioural observation at 3m depth on an additional black/blue  
27 one stripe individual (SL 35m), the fangblenny was observed to curl its tail around on  
28 itself to form a circle and interestingly the overall body colour and both stripes  
29 changed to black. The fish then floated to the surface in the adopted circular body  
30 posture where it drifted just under the surface with debris, including dark pieces of  
31 seaweed against which it was camouflaged. The observer followed the fish for about  
32 200 m and the fish was still alive and floating when left.  
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### 40 **Discussion**

41 Here, we report the underlying physiological mechanisms and constraints to  
42 colour change in an intriguing mimicry system. Bluestriped fangblennies have the  
43 ability to change colour rapidly due to the translocation of pigments in  
44 chromatophores. Small fangblennies are also able to cloak a second ventral blue stripe  
45 to become an accurate mimic of juvenile cleaner wrasse. *In vitro*, skin biopsies of  
46 smaller individuals altered their colouration more rapidly than larger individuals, and  
47 this supports what has previously been observed in behavioural and field  
48 investigations (Cheney et al., 2008); therefore, translocation of pigment may be  
49 limited in larger individuals. Candidate hormones, MSH, prolactin and melatonin are  
50 potentially involved in natural rapid colour change regulation. Finally, we report the  
51 behavior of small black/blue one stripe (mimic) and orange individuals, and found  
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3 there was no significant difference in the number or the success of strikes at passing  
4 reef fishes between colour forms. This indicates that strategies including cleaner  
5 wrasse mimicry and hiding within shoals of similar coloured fish, may be equally as  
6 successful in terms of attacking prey. We also present an anecdotal observation on  
7 pelagic floating behavior, which was previously observed in another blenny,  
8 *Aspidontus taenatiatus* (Losey, 1972), and is thought to be used for migratory  
9 purposes. In this case, the small black/blue one stripe fangblenny turned completely  
10 black (without any stripes), which demonstrates colour plasticity may be more broad  
11 than originally thought, at least in juveniles.  
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20 From our fixed samples and from previous behavioural observations (Cheney  
21 et al., 2008), there appeared to be little evidence that individual fangblennies can  
22 rapidly change from one extreme colour form to another (i.e. from black and blue to  
23 orange, and *vice versa*). Examination of pigment cells in the black and orange trunk  
24 sections from fixed fish revealed that these extreme colour forms may rely on the  
25 density of chromatophores for their primary colouration. In particular, the differences  
26 in density of melanophores may suggest two distinct color forms of the fangblenny.  
27 The black and blue form is likely to be able to tune its body color to olive and/or  
28 brown by means of physiological color change and olive/brown forms with fewer  
29 melanophores and more red/yellow pigment are likely to be able to turn orange.  
30 Unfortunately, we were unable to quantify xanthophore density, and such information  
31 is not achieved easily. While the density of melanophores is possible to measure, this  
32 is difficult for yellow cells as their cell border is less distinct and they appear behind  
33 the melanophores, thus masked from view. If two extreme colour forms do exist,  
34 colour change from orange to black/blue could occur over a longer time frame by  
35 means of changes in the density of differently coloured pigment cells (morphological  
36 colour change), depending on the surrounding habitat and nearby fish species (as per  
37 Cortesi et al., 2015).  
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51 However, from *in vitro* samples, trunk sections of fish with olive body colour  
52 were incubated in prolactin or MSH, the body darkened and when another trunk  
53 section of the same fish was treated with melatonin in combination with prolactin or  
54 MSH, the body became more orange, which suggests that individual fish may be able  
55 to switch between colour forms. Noteworthy, the stripes appeared more iridescent and  
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3 blue in prolactin or MSH compared to in APBS only. Therefore, melatonin, prolactin  
4 and MSH were able to partially restore the colour forms *in vitro* and are quite possibly  
5 the key hormones that modulate the colour forms in fangblennies in the wild;  
6 however, without the use of receptor blockers or other *in vivo* manipulations this  
7 cannot be confirmed. Other candidates for inducing a yellow/orange body may be  
8 noradrenaline or MCH (melanocyte concentrating hormone). These hormones  
9 mediate pigment aggregation in melanophores, erythrophores and xanthophores  
10 similar to melatonin (Sköld et al., 2008). Work on other fish species indicates that  
11 they need, however, to be combined with a hormone that dilates yellow and red  
12 pigments, such as prolactin or MSH, for the overall colour to turn yellow (Sköld et al.,  
13 2008; Sköld et al., 2013). It is therefore likely that at least the orange form is  
14 maintained by a combination of different hormones.  
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24 Also, the iridescent stripes vary in colour: while they are blue in the black/blue  
25 fish, they are white in the orange fish. At this point, we were unable to identify the  
26 mechanisms behind this colour difference in fangblennies. Potential mechanisms may  
27 be color change by means of translocation of iridophore crystals or modulation of the  
28 iridescence by changes in the surrounding coloured pigment cells (Nilsson Sköld et  
29 al., 2016; Mäthger et al 2003).  
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36 Colour change can be used to blend into the background habitat to prevent  
37 detection by potential predators or prey (camouflage), or as in the case of mimicry or  
38 disruptive coloration, as a deceptive mechanism to avoid recognition. Flexible  
39 aggressive mimicry has so far been described for only a limited set of species, i.e. the  
40 bluestriped fangblenny and the dusky dottyback (Cheney et al., 2008; Cortesi et al.,  
41 2015). However, given the likely fitness advantage of such plasticity in the wild, we  
42 believe that more such cases are waiting to be discovered. Animal colouration is a  
43 trait with strong implications for adaptation and specialization. While both genetic  
44 and plastic traits are likely to contribute to adaptation in new environments,  
45 phenotypic plasticity such as colour change allows animals to persist in a wider range  
46 of conditions, provides faster response to environmental changes and facilitates  
47 invasion into novel habitats (Agrawal, 2001; Whiteley et al., 2009).  
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3 The fitness benefits of colour change can be limited however, since it may come  
4 with a cost. A long-term study on long- and short-term color change in sculpins  
5 showed a small but significant cost of color change in terms of reduced survival  
6 (Bergstrom, Whiteley & Tallmon, 2012). In a similar study on guppies, repeated skin  
7 color change in response to dark and light backgrounds carried an energetic cost in  
8 terms of increased food consumption (Rodgers et al., 2013). The fish also preferred  
9 habitats and shoals that matched their own coloration.  
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16 Environment variation - whether habitat or socially related - is likely to  
17 promote the evolution and maintenance of flexible colouration. However, to fully  
18 understand the maintenance and benefit of phenotypic plasticity in communication  
19 and camouflage systems, we must investigate the mechanisms that allow plasticity to  
20 persist, and the underlying physiological constraints and adaptive benefits to the  
21 species.  
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### 28 **Acknowledgements**

29 We thank Martin Luehrmann for help in the field and to the staff at the Lizard  
30 Island Research Station for logistical support. The University of Queensland Animal  
31 Welfare Ethics Committee (QBI/192/13/ARC) approved this work. Animals were  
32 collected under the Great Barrier Reef Marine Parks Permit G12/35688.1 and  
33 Queensland General Fisheries Permit 161624. The project was funded by the  
34 Australian Research Council (DP110105389 to KLC). FC was supported by a Swiss  
35 National Science Foundation Doc.Mobility Fellowship (#155248) and HNS was  
36 supported by a grant from The Lars Hierta Memorial Foundation.  
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## Figure legends

**Figure 1:** Colour variations of the bluestriped fangblenny, *Plagiotremus rhinorhynchus*, with typical skin biopsies depicting black pigmented cells (melanophores) and yellow-pigmented cells (presumable xanthophores). Scale bar = 100  $\mu\text{m}$ . T = temporal, D = dorsal, N = nasal, V = ventral.

**Figure 2:** Trunks of *Plagiotremus rhinorhynchus* preserved in the field revealed differences in chromatophore status and density depending on colour form. **A:** Colour form with black body with a dorsal and no ventral neon blue stripe contains dense amounts of melanophores with dispersed pigment that covers the body area, and dispersed but less dense melanophores in the ventral stripe area (arrowhead). **B:** In a similarly dark color form but with a visible ventral blue stripe, melanophore pigment is aggregated in the ventral stripe area (arrowheads). **C:** Olive form with two light blue stripes show melanophores with dispersed pigments, but that only partly cover the body area (arrowhead) and expose adjacent yellow pigment cells. **D:** Orange form with distinct yellow fins show yellow/orange pigment cells that totally cover the body area and with few overlaying melanophores with partly dispersed pigment (arrowhead). *d* = dorsal stripe. *b* = body. *v* = ventral stripe. Scale bars = 1 mm (left) and 50  $\mu\text{m}$  (right). Arrowheads indicate melanophores.

**Figure 3:** Hormonal regulation of colour change in *Plagiotremus rhinorhynchus*. Trunk biopsies from an olive coloured form incubated in different hormone solutions. **A:** The body area of the biopsy kept in MSH (5  $\mu\text{M}$ ) or prolactin (0.150 IU/ml) were dark (b); however, blue stripes and abdomen appeared more fluorescent compared to control. **B:** Melatonin (Mel) at 10  $\mu\text{M}$  resulted in a pale body and abdomen **C:** Melatonin with MSH resulted in a more orange body resulting from dilated yellow pigment cells. *ds* = dorsal stripe. *b* = body. *vs* = ventral stripe. *a* = abdomen. Scale bars = 1 mm (left) and 50  $\mu\text{m}$  (right).

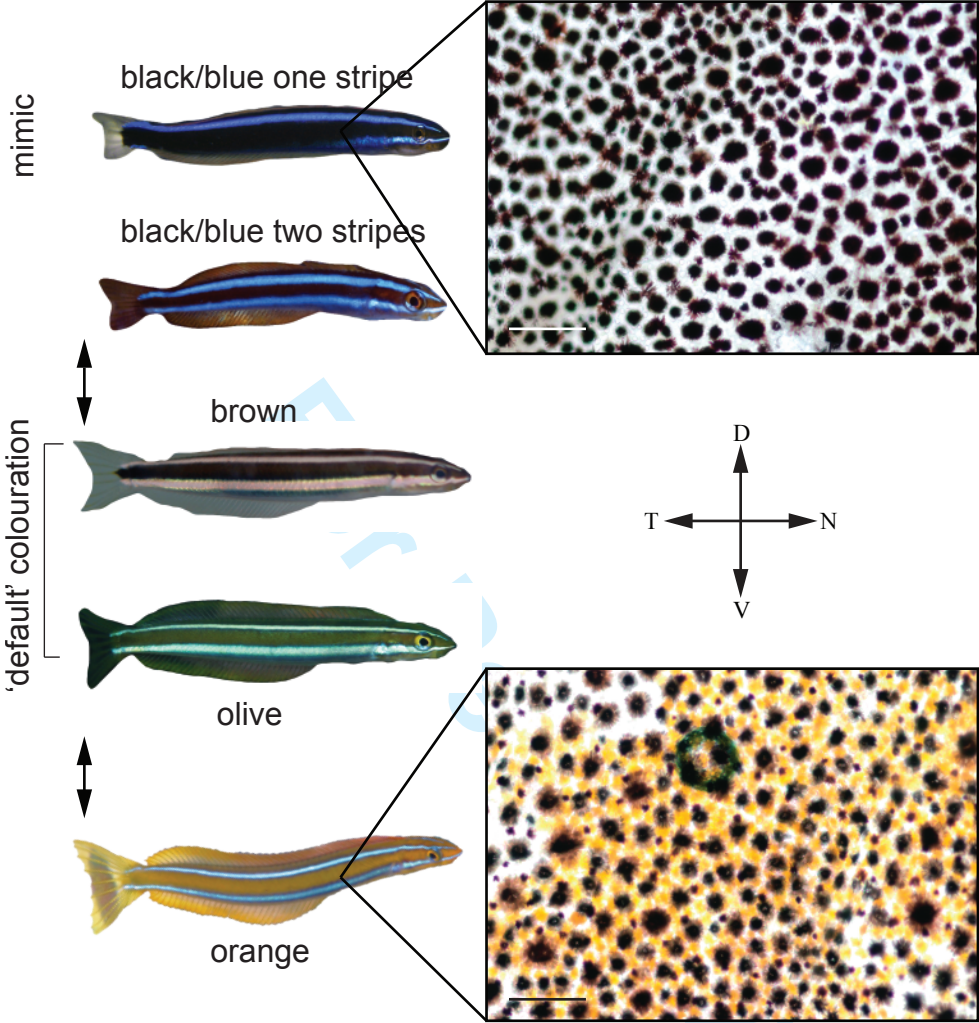
**Figure 4:** Colour change *in vitro* for different sized and coloured forms of *Plagiotremus rhinorhynchus* individuals (n=16). Trunk biopsies with initially dispersed melanophore pigment were incubated in 10  $\mu\text{M}$  melatonin to induce colour change. Colour change was quantified as the Melanophore Index (MI) at every 30 second where MI 1 = totally aggregated pigment and MI 5 = evenly dispersed pigment. Graph shows MI for each fish at

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3 90 seconds in relation to fish length. Black with two stripes, brown and olive describe the  
4 different colour forms when euthanized.  
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8 **Figure 5:** Boxplots of results from behavioural observations of black and orange  
9 fangblennies in the field: i) number of strikes at passing reef fish, and ii) number of  
10 successful attacks. Dark horizontal lines represent the median, with the box representing the  
11 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the whiskers the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Ns, indicates no  
12 significant difference was detected between colour forms.  
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For Peer Review

Figure 1



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Figure 2

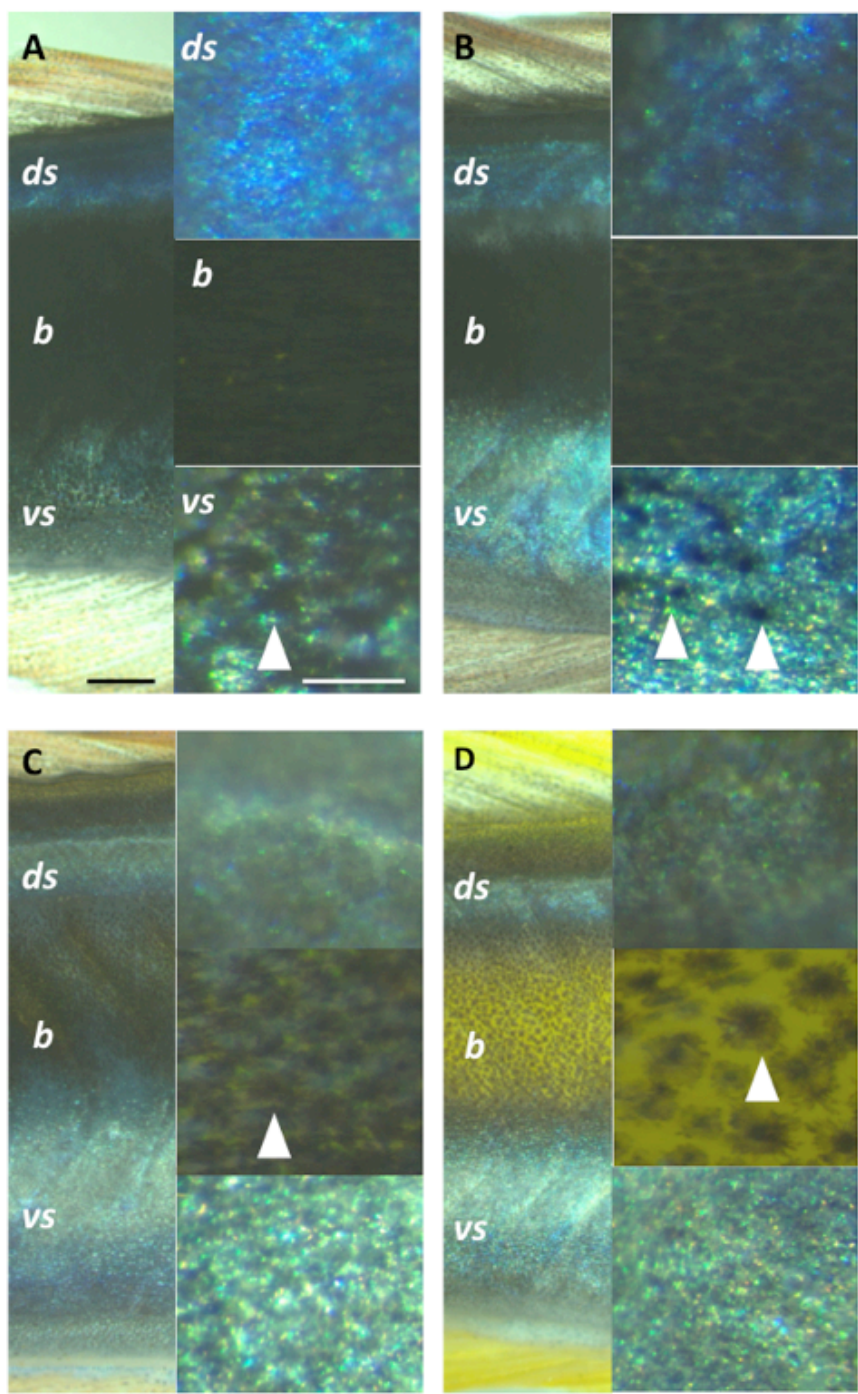
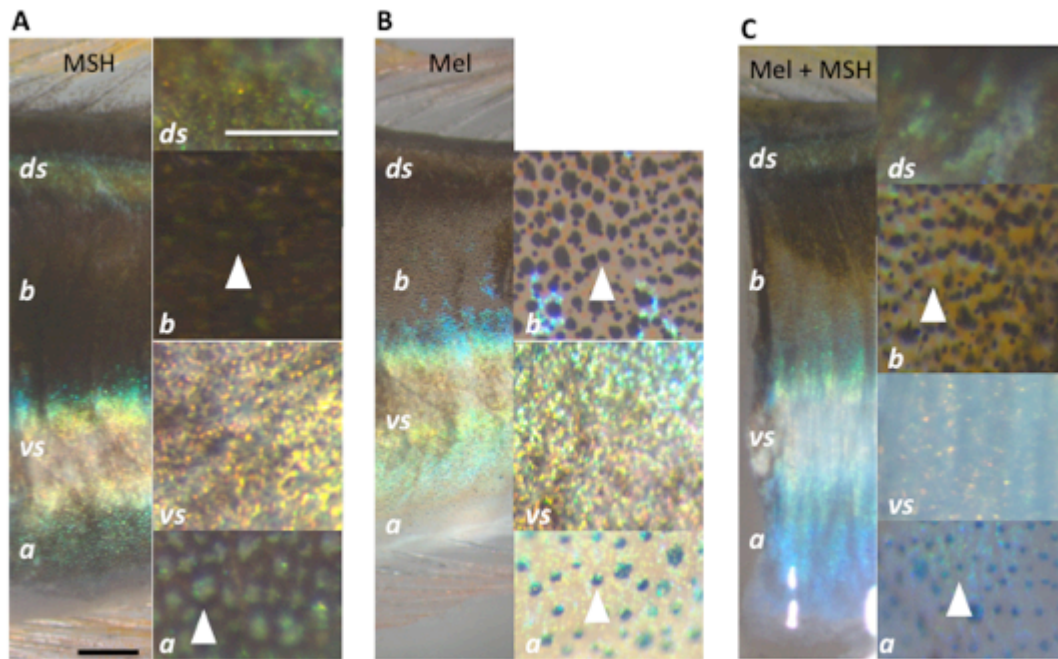


Figure 3



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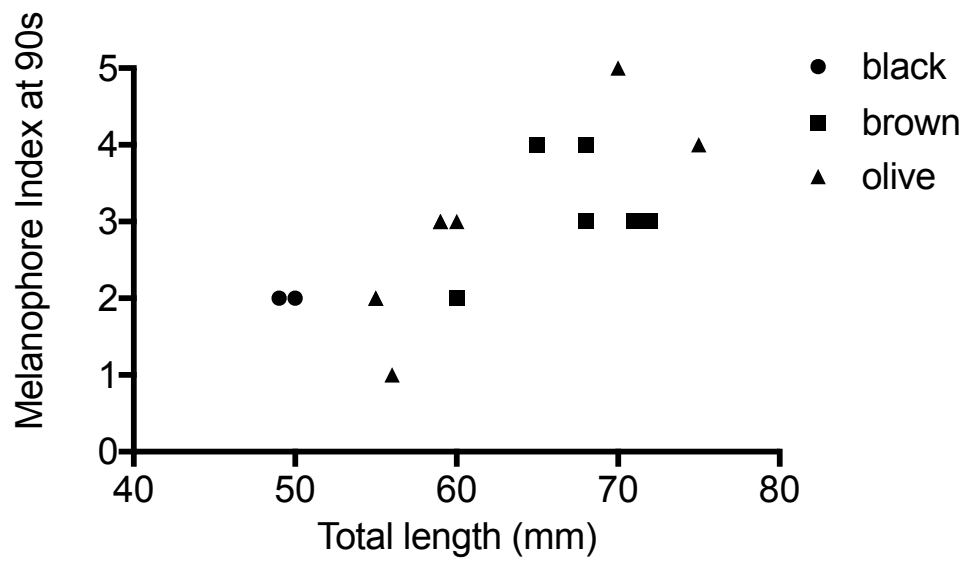
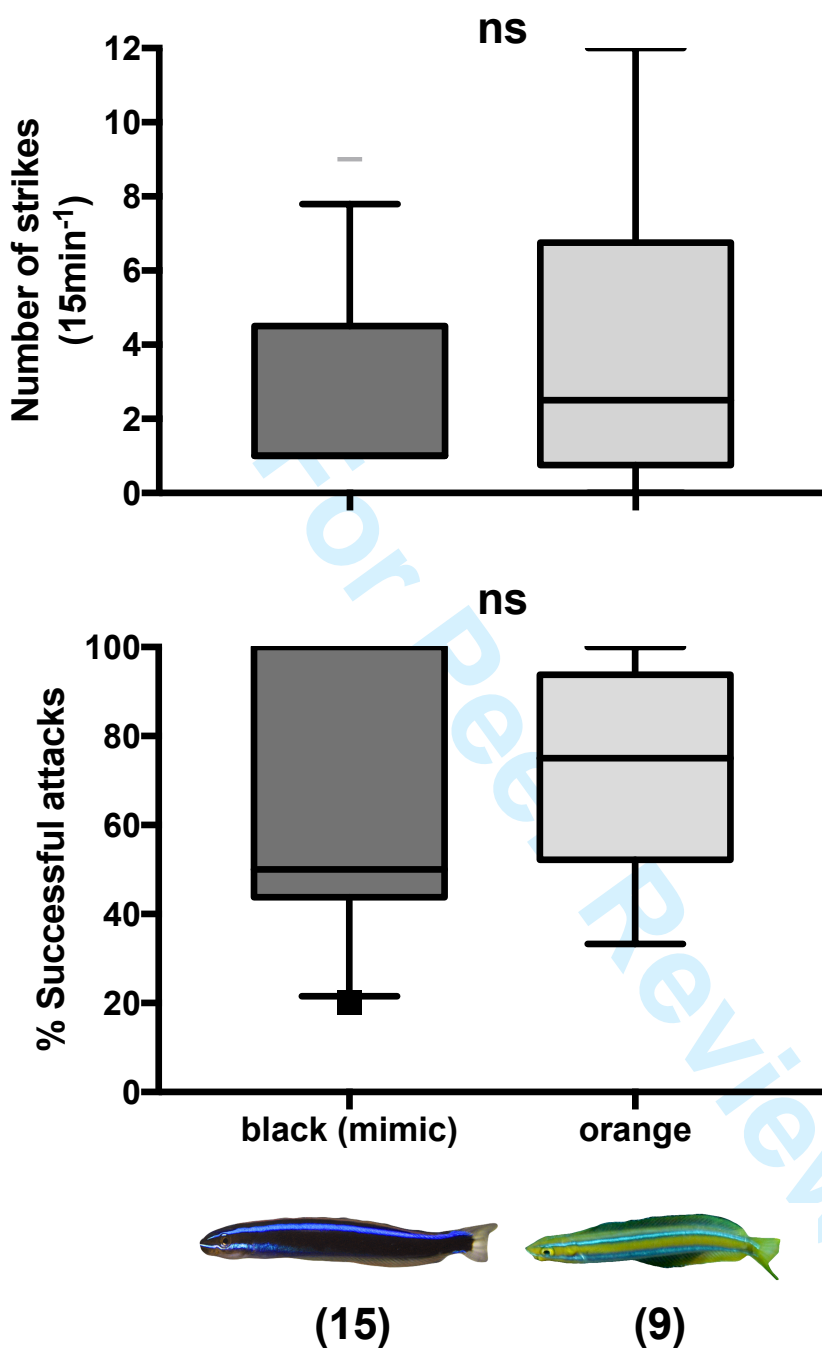


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*Regulation, constraints and benefits of colour plasticity in a mimicry system*

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**Abstract**

Rapid colour change is used in aggressive interactions, ontogenetic transitions, nuptial displays, and to prevent detection and/or recognition from predators or prey. The underlying mechanisms, constraints and benefits of colour change are often unclear, but examining such factors offer insights into phenotypic plasticity. Here, we investigated the mechanisms behind how an aggressive reef fish mimic (bluestriped fangblenny *Plagiotremus rhinorhynchos*) changes colour rapidly (1-5 min) between mimetic and other colour forms. Black with one neon blue dorsal stripe (mimic), black with two neon blue stripes, brown, olive and orange forms differed in melanophore density. Fish skin biopsies were modulated *in vitro* by hormones, and smaller fangblennies changed colouration more rapidly than larger fish suggesting that the ability to change colour is diminished as fish get larger. Individuals may be limited by differences in pigment cell densities to change colour between extreme colour forms (black and orange), therefore longer morphological changes may also occur or fangblennies may exhibit dimorphic populations. Behavioural observations suggest that small black and orange individuals were equally successful in attacking passing fish to feed on dermal tissue/scales, indicating that deceptive strategies used by each colour form may deliver equal fitness benefits. The present study demonstrates for the first time how fangblennies change colour and highlights that colour plasticity offers important adaptive advantage; however, physiological constraints should also be considered.

**Keywords:** colour change, aggressive mimicry, coral reef fish, phenotypic plasticity, bluestriped fangblenny, *Plagiotremus rhinorhynchos*

## Introduction

Phenotypic plasticity allows animals to alter growth, morphology, physiology, behaviour or life history in response to environmental variation, and contributes to the ecological success of individuals and populations (Pigliucci, 2001). One prominent example of plasticity is the ability to alter body colouration. Many animals are able to adaptively alter their colour in response to changes in seasons, motivational state, light quality, temperature, background and for communication purposes, including nuptial displays or deimatic displays (e.g. Kodric-Brown, 1998; Leclercq, Taylor & Migaud, 2010; Nilsson Sköld et al., 2016; Sköld, Aspengren & Wallin, 2013; Stuart-Fox & Moussalli, 2011). However, there are limitations to colour change in most animals, which reduces the ability to display optimal colour signals in many circumstances (Stuart-Fox & Moussalli, 2011). Understanding the capacity and constraints of colour change will enable us to elucidate the function, benefit and evolution of dynamic visual signals.

In fish, colour change appears to be ubiquitous (Nilsson Sköld et al., 2016): flatfish, groupers and gobies match the visual properties of the background (Stevens, Lown & Denton, 2014; Watson, Siemann & Hanlon, 2014); fish may display nuptial colouration during breeding season, such as the males of Southern pygmy perch, *Nannoperca australis*, which develops red and black colour patches on their fins and bodies prior to breeding (Morrongiello et al., 2010). Colour change also occurs during aggressive interactions: terminal phase male Bluehead wrasse, *Thalassoma bifasciatum*, alter their posterior body coloration from opalescent (pink-gray pearl) to bright bottle green when chasing other males away from individual spawning territories (Dawkins & Guilford, 1993).

Colour change can also be used as a deceptive mechanism to prevent detection or recognition by potential predators or prey (Cheney, Grutter & Marshall, 2008; Cortesi et al., 2015; Cortesi et al., 2016; Côté & Cheney, 2005; Norman, Finn & Tregenza, 2001). The use of colour change in mimicry systems is an interesting phenomenon, and has been studied in a few fish species including the dusky dotyback, *Pseudochromis fuscus*, which alters colouration over a period of two

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2 weeks to match the colour of surrounding schooling fish to prevent detection from  
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4 juvenile prey (Cortesi et al., 2015).

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6 Rapid colour change occurs in the bluestriped fangblenny, *Plagiotremus*  
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8 *rhinorhynhos*, which are aggressive mimics of the juvenile bluestreaked cleaner  
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10 wrasse, *Labroides dimidiatus* (Côté & Cheney, 2005). Instead of cleaning reef fish by  
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12 removing ectoparasites from larger reef fish clients (Côté, 2000; Grutter, 1999),  
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14 *Plagiotremus* fangblennies feed by attacking passing reef fish removing scales and  
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16 dermal tissue (Kuwamara, 1981). Bluestriped fangblennies exhibit many different  
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18 colour [forms](#): as a cleaner wrasse mimic, it exhibits a black body with one neon blue  
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20 [dorsal stripe](#); whereas [alternative colour forms](#) can have [a black body with two blue](#)  
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22 [stripes, or a brown, olive or orange](#) colour body with two white or light blue stripes  
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24 (Fig. 1). [Some of these other colour forms are](#) often found associated with shoals of  
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26 similarly coloured fish (Cheney et al., 2008; Cheney et al., 2009; Côté & Cheney,  
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28 2005). However, the physiological mechanisms behind how colour changes [are](#)  
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30 achieved and whether there is a significant difference in [foraging success](#) between the  
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32 [distinct](#) strategies [of a cleaner wrasse mimic and shoal-related forms remains unclear](#).

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34 In a previous study, fangblennies in aquaria that changed colour were  
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36 significantly smaller than those that did not change colour, and only small individuals  
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38 (< 50mm) changed colour to the black/[blue one stripe](#) mimic colour form (Cheney et  
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40 al., 2008). Due to a size disparity between adult fangblennies (> 70mm) and juvenile  
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42 cleaner wrasse (< 50 mm), it was suggested that fangblennies only mimic juvenile  
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44 cleaner wrasse when they are of a similar size, as the adaptive advantage to  
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46 resembling them when they were larger was limited. However, the mechanisms that  
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48 cause fish to change colour and whether they lose this ability as they grow larger has  
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50 not been previously demonstrated. Furthermore, fangblennies have been observed to  
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52 switch from black/[blue one or two stripes](#) colour [forms](#) to brown [or olive forms](#)  
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54 [\(which we term here the 'default' colouration as this is the colour fangblennies often](#)  
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56 [revert to when caught](#); Fig. 1) and back again (Cheney et al., 2008), and from orange  
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58 [forms](#) to brown [or olive forms](#) (Côté & Cheney, 2005). [However](#), it is unclear whether  
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60 individuals have the ability to switch between the extremes of their colour repertoire  
(i.e from black/[blue](#) to orange), or whether [this change is](#) physiologically constrained.

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In this study, we investigated the mechanisms behind colour change in this species and examined potential underlying physiological and morphological constraints to colour change. Fish body colour is dependent on different pigment cells in their skin (chromatophores), which are classed as melanophores (black), erythrophores (red), xanthophores (yellow), leucophores (white), iridophores (iridescent) and cyanophores (blue chromatophores in callionymid fish) (Goda & Fujii, 1995). Some fishes can change their body colouration within minutes using physiological processes, which cause intracellular mass translocations of pigments and/or a change in the orientation of reflective crystals inside chromatophores (Goda & Fujii, 1995). Alternatively, fishes can also change colour over a longer time period using morphological processes that change the number, type and/or distribution of chromatophore cells within the skin (Leclercq et al., 2010).

We first investigated the pigment cells responsible for physiological colour changes between different fangblenny colour [forms](#) from individuals immediately fixed in the field [and from fish kept for a few days in the laboratory](#). [To better understand the regulation and extent of colour plasticity, trunk biopsies were then treated with melatonin, prolactin and melanocyte stimulating hormone \(MSH\), hormones known to regulate differently coloured chromatophores in other fish species \(Sköld et al., 2013\), with the expectation to replicate the colour changes observed in the wild](#). We also compared the rate at which skin biopsies changed colour, and determined whether size imposed morphological constraints on colour change in this species. Finally, we conducted behavioural observations on small fangblennies exhibiting black/[blue one stripe](#) (mimic) and orange colour [forms](#) to investigate whether there was a significant fitness [difference in foraging success](#) for [fish](#) that exhibit different deceptive strategies.

## Materials and Methods

### *Collection of specimens*

Bluestriped fangblennies, *Plagiotremus rhinorhynchus*, (n = 24) were located and collected from shallow reefs (1-10m) around Lizard Island (14°40'S, 145°27'E), Great Barrier Reef, in May 2014. Individuals were collected with hand nets and if needed, a small amount of clove oil solution (10% clove oil, 40% ethanol, 50%

1  
2 seawater) sprayed into the water (this did not cause colour change in individuals).  
3 Immediately after capture, individuals were placed in a hermetically sealed bag and  
4 photographed underwater. We categorised their colouration as either black body with  
5 one neon blue stripe (mimic colour form) (n = 5; range standard length (SL): 35-72  
6 mm), black body with two neon blue stripes (n = 3, range SL: 45-65 mm), brown  
7 body with two white stripes (n = 5, range SL: 43-68 mm), olive body with 2 light  
8 blue/white stripes (n = 7; range SL: 58-68 mm), orange body with 2 light blue/white  
9 stripes (n = 4, range SL: 54-58 mm) (Fig. 1).  
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### 14 *Chromatophore analysis*

15 To reveal chromatophore status in the wild, fish (n = 8; [black/blue one stripe =](#)  
16 [1, black/blue two stripes = 2, olive = 3, orange = 2](#)) were taken back to the boat by a  
17 snorkeler, [and decapitated within 2 minutes](#) after capture to preserve colouration. The  
18 trunk was immediately put in 4% neutral buffered formalin. The fixed trunk biopsies  
19 were then brought to the research station for examination under [a microscope](#). A  
20 further set of fish (n=16; [black with one stripe = 4, black with 2 stripes = 1, brown =](#)  
21 [5, olive = 4, orange = 2](#)) were collected and then kept in individual tanks with plastic  
22 pipe shelters and coral rubble for a few days. While these fish were used for analysing  
23 rate of color change *in vitro* and hormone effects, a piece of the trunk was also fixed  
24 immediately after decapitation in 4% neutral buffered formalin for melanophore  
25 analysis of the [main body area between the 2 stripes](#) as a complement to the fish fixed  
26 in the field.  
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34 Melanophore density in the body area was assessed by manually counting the  
35 number of melanophores in a randomly selected 0.5 x 0.5 mm square of a photograph  
36 of each fixed biopsy. The melanophore status was scored manually in the same  
37 photographs using the Melanophore Index (MI; Hogben & Slome, 1931), where MI 1  
38 is totally aggregated pigments and MI 5 totally dispersed pigments. [Magnification and](#)  
39 [illumination were kept under standardized conditions during photography](#).  
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### 45 *In vitro analysis of colour change*

46 To further understand the regulation of [colour](#) plasticity between differently  
47 sized fish and between different colour [forms](#), trunk sections of fish (n = 16) were  
48 treated with different combinations of hormones. Stock solutions of hormones were  
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2 stored at -20°C and diluted to the experimental concentration in analytical phosphate  
3 buffered saline (APBS at pH 7.4; ProSciTech, Thuringova, Qld, Australia) just before  
4 use. Melatonin and sheep prolactin (Sigma Aldrich) were stored at -20°C as 10 mM  
5 and 150 IU/ml stock solutions, respectively. Melatonin was used at 10 µM and  
6 prolactin at 0.150 IU/ml. MSH ( $\alpha$ - melanocyte stimulating hormone; ICN  
7 Pharmaceuticals, Inc., Costa Mesa, CA, USA), was stored as 1 mM stock solutions at  
8 -20°C and used at 5 µM. Control trunk sections were incubated in APBS with 0.1%  
9 ethanol, the dissolvent of melatonin.  
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15 Fish used in the *in vitro* experiments were kept in aquaria for a few days prior  
16 to treatments. Each fish was sized, photographed and quickly decapitated using sharp  
17 scissors. In many cases, their colouration had altered since capture and so was  
18 classified again (black with 2 stripes = 2, brown = 6, olive = 8). The body was  
19 thereafter cut into approximately 0.5 cm vertical sections from head to tail and  
20 biopsies were rested in saltwater for 5-15 min to allow full chromatophore pigment  
21 dispersion before onset of treatments (one section was fixed immediately after  
22 decapitation in 4% neutral buffered formalin, see above). For each individual,  
23 biopsies were thereafter randomly incubated in hormone and control solutions. Two  
24 different *in vitro* experiments were performed. First, the capacity of differently  
25 colored individuals to change color was tested by incubating the biopsies for one hour  
26 using different combinations of hormones (melatonin, prolactin or MSH, individually  
27 or in combinations) or a control solution (APBS), before analyzing pigment  
28 movement/colour change under a bright field microscope. Using pilot trials, one hour  
29 was found sufficient to trigger the full spectrum of physiological colour change  
30 responses. Second, the rate at which colour changes occurred *in vitro* was tested using  
31 10 µM melatonin, which causes movement of pigment granules to the cell centre  
32 (aggregated), on differently sized fishes (SL: 49-75 mm). Melanophore pigment was  
33 dispersed (MI = 5) for each individual at the beginning of the trial, and scored every  
34 30 s using microscopy and the Melanophore Index for 10 min.  
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#### 45 ***Behavioural observations***

46 To examine whether there was a fitness advantage between extreme colour  
47 forms, we conducted 1 or, when possible, 2 behavioural observations for 15 min on  
48 black/blue one stripe (n = 15), and orange individuals (n = 9) (n = 39 observations in  
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2 total) that were haphazardly located on ten coral reefs around Pulau Hoga 05°28'S,  
3 123°45'E, Southeast Sulawesi, Indonesia in July-August 2006. Individuals were  
4 located at least 10 m apart using SCUBA at depths of 2–18 m and were all SL < 50  
5 mm based on length estimates in the field. For repeat observations on the same  
6 individual (n = 15), small pieces of flagging tape were placed on the reef to relocate  
7 individuals. Repeat observations were done on a different day/time and results were  
8 averaged. During each observation, we recorded the total number of attacks made by  
9 a mimic, defined as a fangblenny darting towards a potential victim; and the number  
10 of successful attacks, defined as clear contact made with the victim. All black/blue  
11 one stripe (mimic) individuals were found within 1 m of a juvenile cleaner wrasse,  
12 and all orange fangblennies were located within a shoal of > 20 orange anthias species  
13 (e.g. *Pseudanthias squamipinnis*).  
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## 20 Results

### 21 *Chromatophore analysis of field samples*

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23 Analysis of chromatophores on trunk biopsies from fish fixed in the field  
24 revealed differences between color forms in terms of chromatophore density (Fig. 1,  
25 2). Black/blue forms with one (n = 1) or two stripes (n = 2) showed dense amounts of  
26 melanophores in the body area with dispersed black pigment ( $\geq 135$   
27 melanophores/0.25 mm<sup>2</sup>, MI 4-5) but we did not detect xanthophores here. Fins  
28 contained chromatophores with yellow and red pigment (xanthophores and  
29 erythrophores). The blue ventral stripes showed iridophores (iridescent platelets)  
30 overlaying melanophores with aggregated pigment (MI 1-2). In the one fish lacking  
31 the ventral blue stripe (n = 1), the pigment was dispersed in melanophores in the  
32 ventral stripe region (MI 4-5) (Fig. 2A, B).  
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41 Olive forms (n = 3) showed an olive body area due to either few melanophores  
42 with partly aggregated pigment (63 and 64 melanophores/0.25 mm<sup>2</sup>, MI 2-3) or dense  
43 amounts of melanophores (104 melanophores/0.25 mm<sup>2</sup>, mix of MI 1 and 4). In these  
44 individuals, xanthophores with aggregated pigment were also observed. The stripes  
45 were almost white and the melanophores had aggregated pigment (MI 1-2) and the  
46 fins were red containing erythrophores (Fig. 2C).  
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In orange forms (n = 2), the body area was orange due to fewer melanophores (49 and 30 melanophores/0.25 mm<sup>2</sup>) despite relatively dispersed melanophore pigment (MI 4), but with a higher number of erythrophores. As with the olive form, the blue stripes were white in color and contained melanophores with aggregated pigment (MI 2). The fins contained only xanthophores, or both xantho- and erythrophores (Fig. 2D).

#### *Chromatophore analysis of fish kept in laboratory*

Of the fish kept in tanks before fixation (black/blue two stripes = 2, brown = 6, olive = 8) results were similar to field fixed fish. The body area in black/blue two stripes forms were completely covered in melanophores with dispersed pigment (density > 135 melanophores/0.25 mm<sup>2</sup>, MI 4-5) and in brown individuals had fewer melanophores compared to the black/blue two stripes individuals (range: 56 to 132 melanophores/0.25 mm<sup>2</sup>; mean = 93.9 and melanophore pigment was dispersed (MI 4-5). The body areas in olive coloured individuals had similar melanophore densities to the body areas in brown forms (range: 42 to 156 melanophores/0.25 mm<sup>2</sup>; mean = 73.4) and also had dispersed melanophore pigment (MI 4-5), while one individual had relatively aggregated melanophore pigment (MI 2-3).

#### *In vitro analysis of colour change*

The body areas of biopsies kept in MSH or prolactin were dark (Fig. 3A), and similar to treatments kept in the control solution (APBS) only (not shown). However, the blue stripes appeared more fluorescent in MSH or prolactin compared to the control. Incubating biopsies in melatonin resulted in paling of the body area and abdomen due to the aggregation of pigment inside melanophores and xanthophores (Fig. 3B). Combining melatonin with MSH resulted in more colourful bodies from more dilated xanthophores and pale abdomen from aggregated melanophore pigment (Fig 3C).

With the use of melatonin as a trigger for melanophore pigment aggregation, it became evident that both the rate and capacity of color change declined with an increase in size of the fish. Fish length was strongly correlated with MI Index after 90 s (Spearman's rank  $r = 0.76$ ,  $n = 16$ ,  $p < 0.001$ ; Fig. 4). Moreover, in one of the larger individuals (SL = 70 mm), some of the melanophores did not respond at all within the 10 min time frame. Interestingly, this individual also had melanin deposits outside of



1  
2 the melanophores. The skin of smaller fish (< 60mm) on the other hand, went from  
3 almost black to considerably pale due to the rapid aggregation of pigment in all  
4 observed melanophores.  
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### 7 **Behavioural observations**

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9 The main fish species that were attacked by fangblennies included: snapper  
10 (*Lutjanus fulviflamma*), parrotfish (*Scarus* spp.), damselfish (*Chromis* spp.),  
11 squirrelfish (*Myripristis* spp.), and anthias (*Pseudanthias* spp.). For each individual,  
12 the number of strikes at fish ranged from between 0 to 12, per 15 min observation;  
13 however, strike number did not significantly differ between black/blue one stripe or  
14 orange colour forms (Mann Whitney-U = 52,  $n_1 = 15$ ,  $n_2 = 9$ ,  $p = 0.67$ ). The  
15 percentage of successful strikes ranged from 20 to 100 %, but again, did not differ  
16 between forms (Mann Whitney-U = 44,  $n_1 = 15$ ,  $n_2 = 9$ ,  $p = 0.77$ ) (Fig. 5).  
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22 During one behavioural observation at 3m depth on an additional black/blue  
23 one stripe individual (SL 35m), the fangblenny was observed to curl its tail around on  
24 itself to form a circle and interestingly the overall body colour and both stripes  
25 changed to black. The fish then floated to the surface in the adopted circular body  
26 posture where it drifted just under the surface with debris, including dark pieces of  
27 seaweed against which it was camouflaged. The observer followed the fish for about  
28 200 m and the fish was still alive and floating when left.  
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### 33 **Discussion**

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35 Here, we report the underlying physiological mechanisms and constraints to  
36 colour change in an intriguing mimicry system. Bluestriped fangblennies have the  
37 ability to change colour rapidly due to the translocation of pigments in  
38 chromatophores. Small fangblennies are also able to cloak a second ventral blue stripe  
39 to become an accurate mimic of juvenile cleaner wrasse. *In vitro*, skin biopsies of  
40 smaller individuals altered their colouration more rapidly than larger individuals, and  
41 this supports what has previously been observed in behavioural and field  
42 investigations (Cheney et al., 2008); therefore, translocation of pigment may be  
43 limited in larger individuals. Candidate hormones, MSH, prolactin and melatonin are  
44 potentially involved in natural rapid colour change regulation. Finally, we report the  
45 behavior of small black/blue one stripe (mimic) and orange individuals, and found  
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2 there was no significant difference in the number or the success of strikes at passing  
3 reef fishes between colour forms. This indicates that strategies including cleaner  
4 wrasse mimicry and hiding within shoals of similar coloured fish, may be equally as  
5 successful in terms of attacking prey. We also present an anecdotal observation on  
6 pelagic floating behavior, which was previously observed in another blenny,  
7 *Aspidontus taenatiatus* (Losey, 1972), and is thought to be used for migratory  
8 purposes. In this case, the small black/blue one stripe fangblenny turned completely  
9 black (without any stripes), which demonstrates colour plasticity may be more broad  
10 than originally thought, at least in juveniles.

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16 From our fixed samples and from previous behavioural observations (Cheney  
17 et al., 2008), there appeared to be little evidence that individual fangblennies can  
18 rapidly change from one extreme colour form to another (i.e. from black and blue to  
19 orange, and *vice versa*). Examination of pigment cells in the black and orange trunk  
20 sections from fixed fish revealed that these extreme colour forms may rely on the  
21 density of chromatophores for their primary colouration. In particular, the differences  
22 in density of melanophores may suggest two distinct color forms of the fangblenny.  
23 The black and blue form is likely to be able to tune its body color to olive and/or  
24 brown by means of physiological color change and olive/brown forms with fewer  
25 melanophores and more red/yellow pigment are likely to be able to turn orange.  
26 Unfortunately, we were unable to quantify xanthophore density, and such information  
27 is not achieved easily. While the density of melanophores is possible to measure, this  
28 is difficult for yellow cells as their cell border is less distinct and they appear behind  
29 the melanophores, thus masked from view. If two extreme colour forms do exist,  
30 colour change from orange to black/blue could occur over a longer time frame by  
31 means of changes in the density of differently coloured pigment cells (morphological  
32 colour change), depending on the surrounding habitat and nearby fish species (as per  
33 Cortesi et al., 2015).

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43 However, from *in vitro* samples, trunk sections of fish with olive body colour  
44 were incubated in prolactin or MSH, the body darkened and when another trunk  
45 section of the same fish was treated with melatonin in combination with prolactin or  
46 MSH, the body became more orange, which suggests that individual fish may be able  
47 to switch between colour forms. Noteworthy, the stripes appeared more iridescent and  
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blue in prolactin or MSH compared to in APBS only. [Therefore, melatonin, prolactin and MSH were able to partially restore the colour forms \*in vitro\* and are quite possibly the key hormones that modulate the colour forms in fangblennies in the wild; however, without the use of receptor blockers or other \*in vivo\* manipulations this cannot be confirmed. Other candidates for inducing a yellow/orange body may be noradrenaline or MCH \(melanocyte concentrating hormone\). These hormones mediate pigment aggregation in melanophores, erythrophores and xanthophores similar to melatonin \(Sköld et al., 2008\). Work on other fish species indicates that they need, however, to be combined with a hormone that dilates yellow and red pigments, such as prolactin or MSH, for the overall colour to turn yellow \(Sköld et al., 2008; Sköld et al., 2013\). It is therefore likely that at least the orange form is maintained by a combination of different hormones.](#)

Also, the iridescent stripes vary in colour: while they are blue in the [black/blue](#) fish, they are white in the orange fish. At this point, we were unable to identify the mechanisms behind this colour difference in fangblennies. Potential mechanisms may be color change by means of translocation of iridophore crystals or modulation of the iridescence by changes in the surrounding coloured pigment cells (Nilsson Sköld et al., 2016; Mäthger et al 2003).

Colour change can be used to blend into the background habitat to prevent detection by potential predators or prey (camouflage), or as in the case of mimicry or disruptive coloration, as a deceptive mechanism to avoid recognition. Flexible aggressive mimicry has so far been described for only a limited set of species, i.e. the bluestriped fangblenny and the dusky dottyback (Cheney et al., 2008; Cortesi et al., 2015). However, given the likely fitness advantage of such plasticity in the wild, we believe that more such cases are waiting to be discovered. Animal colouration is a trait with strong implications for adaptation and specialization. While both genetic and plastic traits are likely to contribute to adaptation in new environments, phenotypic plasticity such as colour change allows animals to persist in a wider range of conditions, provides faster response to environmental changes and facilitates invasion into novel habitats (Agrawal, 2001; Whiteley et al., 2009).

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2 The fitness benefits of colour change can be limited however, since it may come  
3 with a cost. A long-term study on long- and short-term color change in sculpins  
4 showed a small but significant cost of color change in terms of reduced survival  
5 (Bergstrom, Whiteley & Tallmon, 2012). In a similar study on guppies, repeated skin  
6 color change in response to dark and light backgrounds carried an energetic cost in  
7 terms of increased food consumption (Rodgers et al., 2013). The fish also preferred  
8 habitats and shoals that matched their own coloration.  
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13 Environment variation - whether habitat or socially related - is likely to  
14 promote the evolution and maintenance of flexible coloration. However, to fully  
15 understand the maintenance and benefit of phenotypic plasticity in communication  
16 and camouflage systems, we must investigate the mechanisms that allow plasticity to  
17 persist, and the underlying physiological constraints and adaptive benefits to the  
18 species.  
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### 23 Acknowledgements

24 We thank Martin Luehrmann for help in the field and to the staff at the Lizard  
25 Island Research Station for logistical support. [The University of Queensland Animal](#)  
26 [Welfare Ethics Committee \(QBI/192/13/ARC\) approved this work. Animals were](#)  
27 [collected under the Great Barrier Reef Marine Parks Permit G12/35688.1 and](#)  
28 [Queensland General Fisheries Permit 161624. The project was funded by the](#)  
29 [Australian Research Council \(DP110105389 to KLC\). FC was supported by a Swiss](#)  
30 [National Science Foundation Doc.Mobility Fellowship \(#155248\) and HNS was](#)  
31 [supported by a grant from The Lars Hierta Memorial Foundation.](#)  
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### Figure legends

**Figure 1:** Colour variations of the bluestriped fangblenny, *Plagiotremus rhinorhynchus*, with typical skin biopsies depicting black pigmented cells (melanophores) and yellow-pigmented cells (presumable xanthophores). Scale bar = 100  $\mu$ m. T = temporal, D = dorsal, N = nasal, V =ventral.

**Figure 2:** Trunks of *Plagiotremus rhinorhynchus* preserved in the field revealed differences in chromatophore status and density depending on colour form. **A:** Colour form with black body with a dorsal and no ventral neon blue stripe contains dense amounts of melanophores with dispersed pigment that covers the body area, and dispersed but less dense melanophores in the ventral stripe area (arrowhead). **B:** In a similarly dark color form but with a visible ventral blue stripe, melanophore pigment is aggregated in the ventral stripe area (arrowheads). **C:** Olive form with two light blue stripes show melanophores with dispersed pigments, but that only partly cover the body area (arrowhead) and expose adjacent yellow pigment cells. **D:** Orange form with distinct yellow fins show yellow/orange pigment cells that totally cover the body area and with few overlaying melanophores with partly dispersed pigment (arrowhead). *d* = dorsal stripe. *b* = body. *v* = ventral stripe. Scale bars = 1 mm (left) and 50  $\mu$ m (right). Arrowheads indicate melanophores.

**Figure 3:** Hormonal regulation of colour change in *Plagiotremus rhinorhynchus*. Trunk biopsies from an olive coloured form incubated in different hormone solutions. **A:** The body area of the biopsy kept in MSH (5  $\mu$ M) or prolactin (0.150 IU/ml) were dark (b); however, blue stripes and abdomen appeared more fluorescent compared to control. **B:** Melatonin (Mel) at 10  $\mu$ M resulted in a pale body and abdomen **C:** Melatonin with MSH resulted in a more orange body resulting from dilated yellow pigment cells. *ds* = dorsal stripe. *b* = body. *vs* = ventral stripe. *a* = abdomen. Scale bars = 1 mm (left) and 50  $\mu$ m (right).

**Figure 4:** Colour change *in vitro* for different sized and coloured forms of *Plagiotremus rhinorhynchus* individuals (n=16). Trunk biopsies with initially dispersed melanophore pigment were incubated in 10  $\mu$ M melatonin to induce colour change. Colour change was quantified as the Melanophore Index (MI) at every 30

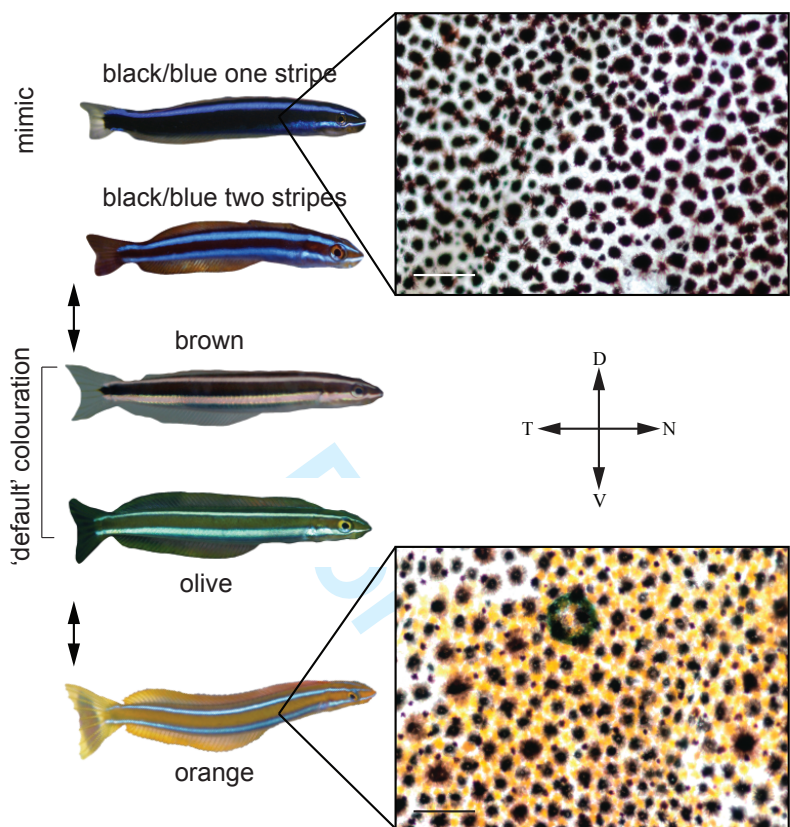
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2 second where MI 1 = totally aggregated pigment and MI 5 = evenly dispersed  
3 pigment. Graph shows MI for each fish at 90 seconds in relation to fish length. Black  
4 with two stripes, brown and olive describe the different colour forms when  
5 euthanized.  
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9 **Figure 5:** Boxplots of results from behavioural observations of black and orange  
10 fangblennies in the field: i) number of strikes at passing reef fish, and ii) number of  
11 successful attacks. Dark horizontal lines represent the median, with the box  
12 representing the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the whiskers the 10<sup>th</sup> and 90<sup>th</sup>  
13 percentiles. Ns, indicates no significant difference was detected between colour  
14 forms.  
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For Peer Review



Figure 1



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Figure 2

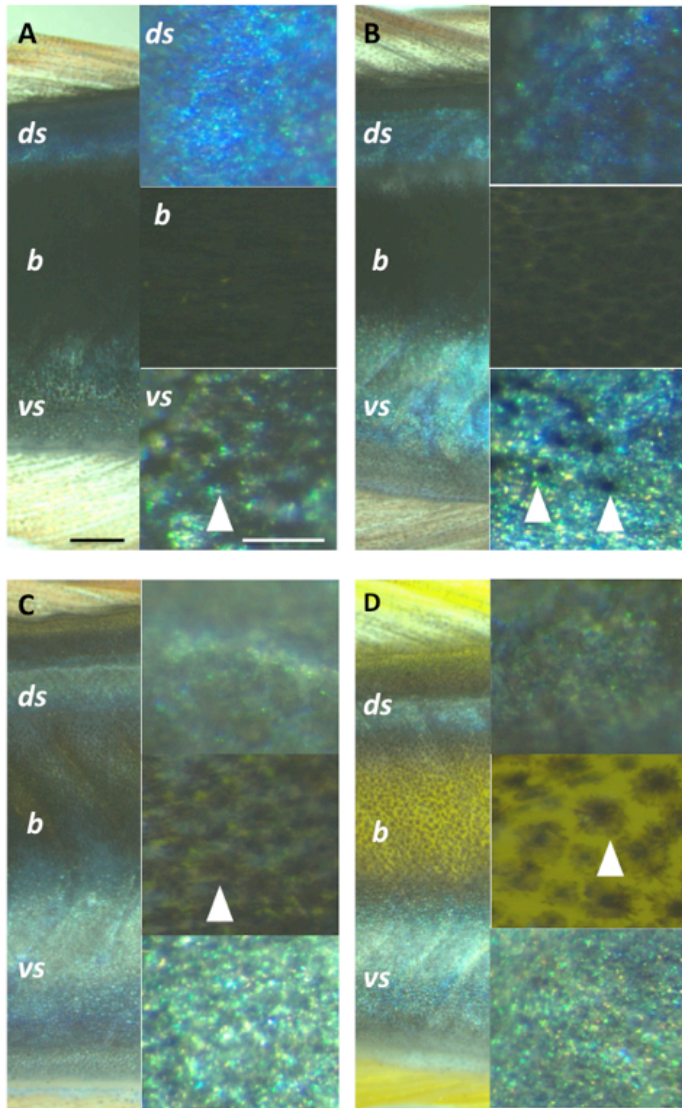
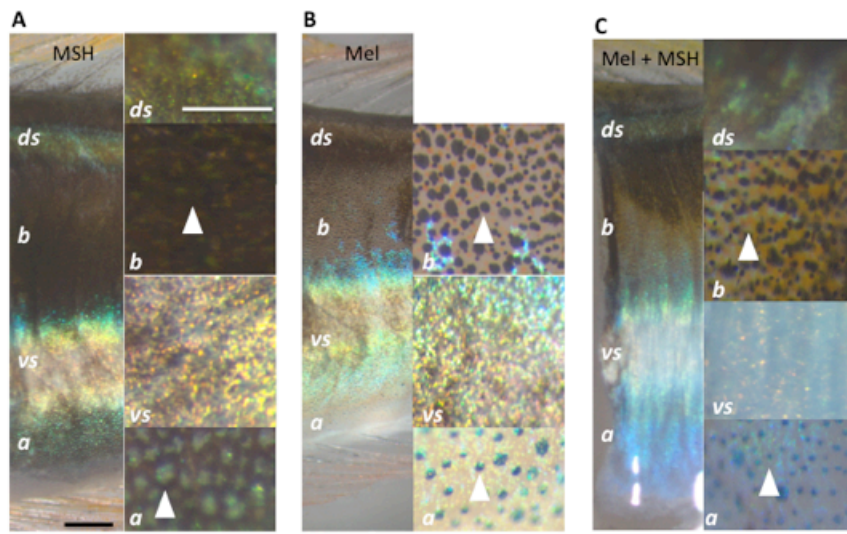


Figure 3



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Figure 4

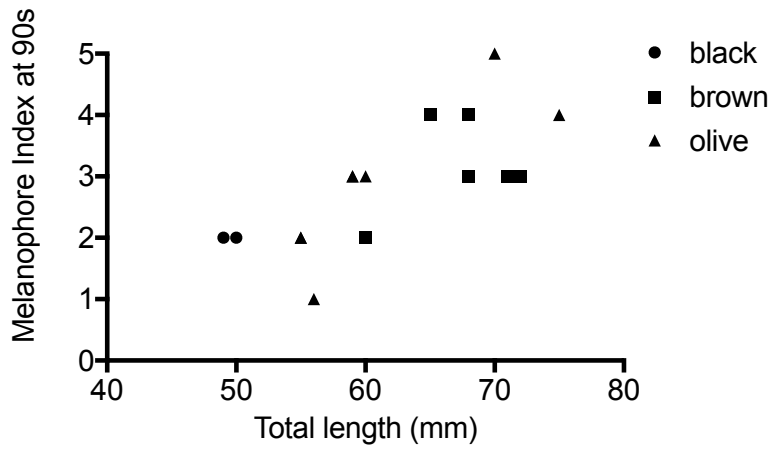


Figure 5

