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

Journal:	*Biological Journal of the Linnean Society*
Manuscript ID	BJLS-4989.R1
Manuscript Type:	Original article
Date Submitted by the Author:	n/a
Complete List of Authors:	Cheney, Karen L; The University of Queensland, School of Biological Sciences Cortesi, F; University of Queensland, Queensland Brain Institute Nilsson Sköld, Helen; Goteborgs Universitet, The Sven Lovén Centre for Marine Sciences
Keywords:	colour change, aggressive mimicry, coral reef fish, phenotypic plasticity, bluestriped fangblenny, Plagiotremus rhinorhynchos

SCHOLARONE™
Manuscripts

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

Karen L. Cheney[1,2*], Fabio Cortesi[2], Helen Nilsson Sköld[3, 4]

[1]School of Biological Sciences, The University of Queensland, Brisbane 4072 Australia.

[2]Queensland Brain Institute, The University of Queensland, Brisbane 4072 Australia.

[3]The Sven Lovén Centre for Marine Sciences, Kristineberg, University of Gothenburg, Sweden.

[4] Havets Hus, 453 30 Lysekil, Sweden

[*]Communicating author: k.cheney@uq.edu.au

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 dottyback, *Pseudochromis fuscus*, which alters colouration over a period of two

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

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

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

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 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%

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

Chromatophore analysis

To reveal chromatophore status in the wild, fish (n = 8; black/blue one stripe = 1, black/blue two stripes = 2, olive = 3, orange = 2) were taken back to the boat by a snorkeler, and decapitated within 2 minutes after capture to preserve colouration. The trunk was immediately put in 4% neutral buffered formalin. The fixed trunk biopsies were then brought to the research station for examination under a microscope. A further set of fish (n=16; black with one stripe = 4, black with 2 stripes = 1, brown = 5, olive = 4, orange = 2) were collected and then kept in individual tanks with plastic pipe shelters and coral rubble for a few days. While these fish were used for analysing rate of color change *in vitro* and hormone effects, a piece of the trunk was also fixed immediately after decapitation in 4% neutral buffered formalin for melanophore analysis of the main body area between the 2 stripes as a complement to the fish fixed in the field.

Melanophore density in the body area was assessed by manually counting the number of melanophores in a randomly selected 0.5 x 0.5 mm square of a photograph of each fixed biopsy. The melanophore status was scored manually in the same photographs using the Melanophore Index (MI; Hogben & Slome, 1931), where MI 1 is totally aggregated pigments and MI 5 totally dispersed pigments. Magnification and illumination were kept under standardized conditions during photography.

In vitro *analysis of colour change*

To further understand the regulation of colour plasticity between differently sized fish and between different colour forms, trunk sections of fish (n = 16) were treated with different combinations of hormones. Stock solutions of hormones were

stored at -20ºC and diluted to the experimental concentration in analytical phosphate buffered saline (APBS at pH 7.4; ProSciTech, Thuringova, Qld, Australia) just before use. Melatonin and sheep prolactin (Sigma Aldrich) were stored at -20ºC as 10 mM and 150 IU/ml stock solutions, respectively. Melatonin was used at 10 μM and prolactin at 0.150 IU/ml. MSH (α- melanocyte stimulating hormone; ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA), was stored as 1 mM stock solutions at -20ºC and used at 5 μM. Control trunk sections were incubated in APBS with 0.1% ethanol, the dissolvent of melatonin.

Fish used in the *in vitro* experiments were kept in aquaria for a few days prior to treatments. Each fish was sized, photographed and quickly decapitated using sharp scissors. In many cases, their colouration had altered since capture and so was classified again (black with 2 stripes = 2, brown = 6, olive = 8). The body was thereafter cut into approximately 0.5 cm vertical sections from head to tail and biopsies were rested in saltwater for 5-15 min to allow full chromatophore pigment dispersion before onset of treatments (one section was fixed immediately after decapitation in 4% neutral buffered formalin, see above). For each individual, biopsies were thereafter randomly incubated in hormone and control solutions. Two different *in vitro* experiments were performed. First, the capacity of differently colored individuals to change color was tested by incubating the biopsies for one hour using different combinations of hormones (melatonin, prolactin or MSH, individually or in combinations) or a control solution (APBS), before analyzing pigment movement/colour change under a bright field microscope. Using pilot trials, one hour was found sufficient to trigger the full spectrum of physiological colour change responses. Second, the rate at which colour changes occurred *in vitro* was tested using 10 μM melatonin, which causes movement of pigment granules to the cell centre (aggregated), on differently sized fishes (SL: 49-75 mm). Melanophore pigment was dispersed (MI = 5) for each individual at the beginning of the trial, and scored every 30 s using microscopy and the Melanophore Index for 10 min.

Behavioural observations

To examine whether there was a fitness advantage between extreme colour forms, we conducted 1 or, when possible, 2 behavioural observations for 15 min on black/blue one stripe (n = 15), and orange individuals (n = 9) (n = 39 observations in

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^2, 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^2, MI 2-3) or dense amounts of melanophores (104 melanophores/0.25 mm^2, 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).

In orange forms (n = 2), the body area was orange due to fewer melanophores (49 and 30 melanophores/0.25 mm^2) 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^2, 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^2; 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^2; 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

the melanophores. The skin of smaller fish (< 60mm) on the other hand, went from almost black to considerably pale due to the rapid aggregation of pigment in all observed melanophores.

Behavioural observations

The main fish species that were attacked by fangblennies included: snapper (*Lutjanus fulviflamma*), parrotfish (*Scarus* spp.), damselfish (*Chromis* spp.), squirrelfish (*Myripristis* spp.), and anthias (*Pseudanthias* spp.). For each individual, the number of strikes at fish ranged from between 0 to 12, per 15 min observation; however, strike number did not significantly differ between black/blue one stripe or orange colour forms (Mann Whitney-U = 52, n_1 = 15, n_2 = 9, p = 0.67). The percentage of successful strikes ranged from 20 to 100 %, but again, did not differ between forms (Mann Whitney-U = 44, n_1 = 15, n_2 = 9, p = 0.77) (Fig. 5).

During one behavioural observation at 3m depth on an additional black/blue one stripe individual (SL 35m), the fangblenny was observed to curl its tail around on itself to form a circle and interestingly the overall body colour and both stripes changed to black. The fish then floated to the surface in the adopted circular body posture where it drifted just under the surface with debris, including dark pieces of seaweed against which it was camouflaged. The observer followed the fish for about 200 m and the fish was still alive and floating when left.

Discussion

Here, we report the underlying physiological mechanisms and constraints to colour change in an intriguing mimicry system. Bluestriped fangblennies have the ability to change colour rapidly due to the translocation of pigments in chromatophores. Small fangblennies are also able to cloak a second ventral blue stripe to become an accurate mimic of juvenile cleaner wrasse. *In vitro,* skin biopsies of smaller individuals altered their colouration more rapidly than larger individuals, and this supports what has previously been observed in behavioural and field investigations (Cheney et al., 2008); therefore, translocation of pigment may be limited in larger individuals. Candidate hormones, MSH, prolactin and melatonin are potentially involved in natural rapid colour change regulation. Finally, we report the behavior of small black/blue one stripe (mimic) and orange individuals, and found

there was no significant difference in the number or the success of strikes at passing reef fishes between colour forms. This indicates that strategies including cleaner wrasse mimicry and hiding within shoals of similar coloured fish, may be equally as successful in terms of attacking prey. We also present an anecdotal observation on pelagic floating behavior, which was previously observed in another blenny, *Aspidontus taenatiatus* (Losey, 1972), and is thought to be used for migratory purposes. In this case, the small black/blue one stripe fangblenny turned completely black (without any stripes), which demonstrates colour plasticity may be more broad than originally thought, at least in juveniles.

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

However, from *in vitro* samples, trunk sections of fish with olive body colour were incubated in prolactin or MSH, the body darkened and when another trunk section of the same fish was treated with melatonin in combination with prolactin or MSH, the body became more orange, which suggests that individual fish may be able to switch between colour forms. Noteworthy, the stripes appeared more iridescent and

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).

The fitness benefits of colour change can be limited however, since it may come with a cost. A long-term study on long- and short-term color change in sculpins showed a small but significant cost of color change in terms of reduced survival (Bergstrom, Whiteley & Tallmon, 2012). In a similar study on guppies, repeated skin color change in response to dark and light backgrounds carried an energetic cost in terms of increased food consumption (Rodgers et al., 2013). The fish also preferred habitats and shoals that matched their own coloration.

Environment variation - whether habitat or socially related - is likely to promote the evolution and maintenance of flexible colouration. However, to fully understand the maintenance and benefit of phenotypic plasticity in communication and camouflage systems, we must investigate the mechanisms that allow plasticity to persist, and the underlying physiological constraints and adaptive benefits to the species.

Acknowledgements

References

Agrawal AA. 2001. Ecology - Phenotypic plasticity in the interactions and evolution of species. *Science* **294:** 321-326.

Bergstrom CA, Whiteley AR, Tallmon DA. 2012. The heritable basis and cost of colour plasticity in coastrange sculpins. *Journal of Evolutionary Biology* **25:** 2526-2536.

Cheney KL, Grutter AS, Marshall NJ. 2008. Facultative mimicry: cues for colour change and colour accuracy in a coral reef fish. *Proceedings of the Royal Society B-Biological Sciences* **275:** 117-122.

Cheney KL, Skogh C, Hart NS, Marshall NJ. 2009. Mimicry, colour forms and spectral sensitivity of the bluestriped fangblenny, *Plagiotremus*

rhinorhynchos. *Proceedings of the Royal Society B-Biological Sciences* **276:** 1565-1573.

Cortesi F, Feeney WE, Ferrari MCO, Waldie PA, Phillips GAC, McClure EC, Skold HN, Salzburger W, Marshall NJ, Cheney KL. 2015. Phenotypic plasticity confers multiple fitness benefits to a mimic. *Current Biology* **25:** 949-954.

Cortesi F, Musilova Z, Stieb SM, Hart NS, Siebeck UE, Cheney KL, Salzburger W, Marshall NJ. 2016. From crypsis to mimicry: changes in colour and the configuration of the visual system during ontogenetic habitat transitions in a coral reef fish. *Journal of Experimental Biology* **219:** 2545-2558.

Côté IM. 2000. Evolution and ecology of cleaning symbioses in the sea. *Oceanography and Marine Biology Annual Review* **38:** 311-355.

Côté IM, Cheney KL. 2005. Choosing when to be a cleaner-fish mimic. *Nature* **433:** 211-212.

Dawkins MS, Guilford T. 1993. Color and pattern in relation to sexual and aggressive-behavior in the Bluehead Wrasse *Thalassoma bifasciatum*. *Behavioural Processes* **30:** 245-251.

Goda M, Fujii R. 1995. Blue chromatophores in two species of callionymid fish. *Zoological Science* **12:** 811-813.

Grutter AS. 1999. Cleaner fish really do clean. *Nature* **398:** 672-673.

Hogben L, Slome D. 1931. The pigmentary effector system VI The dual character of endocrine co-ordination in amphibian colour change. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character* **108:** 10-53.

Kodric-Brown A. 1998. Sexual dichromatism and temporary color changes in the reproduction of fishes. *American Zoologist* **38:** 70-81.

Kuwamara T. 1981. Mimicry of the cleaner wrasse *Labroides dimidiatus* by the blennies *Aspidontus taeniatus* and *Plagiotremus rhynorhynchos. Nanki Seibutu* **23:** 61-70.

Leclercq E, Taylor JF, Migaud H. 2010. Morphological skin colour changes in teleosts. *Fish and Fisheries* **11:** 159-193.

Mäthger LM, Land MF, Siebeck UE, & Marshall NJ. 2003. Rapid colour changes in multilayer reflecting stripes in the paradise whiptail, *Pentapodus paradiseus*. *Journal of Experimental Biology*, **206 (20):** 3607-3613.

Morrongiello JR, Bond NR, Crook DA, Wong BBM. 2010. Nuptial coloration varies with ambient light environment in a freshwater fish. *Journal of Evolutionary Biology* **23:** 2718-2725.

Nilsson Sköld H, Aspengren S, Cheney KL, Wallin M. 2016. Fish chromatophores—from molecular motors to animal behavior. *International Review of Cell and Molecular Biology* **321:** 171-219.

Norman MD, Finn J, Tregenza T. 2001. Dynamic mimicry in an Indo-Malayan octopus. *Proceedings of the Royal Society B-Biological Sciences* **268:** 1755-1758.

Pigliucci M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press: Baltimore, MD, United States.

Rodgers GM, Gladman NW, Corless HF, Morrell LJ. 2013. Costs of colour change in fish: food intake and behavioural decisions. *Journal of Experimental Biology* **216:** 2760-2767.

Sköld HN, Amundsen T, Svensson PA, Mayer I, Bjelvenmark J, Forsgren E. 2008. Hormonal regulation of female nuptial coloration in a fish. *Hormones and Behavior* **54:** 549-556.

Sköld HN, Aspengren S, Wallin M. 2013. Rapid color change in fish and amphibians - function, regulation, and emerging applications. *Pigment Cell & Melanoma Research* **26:** 29-38.

Stevens M, Lown AE, Denton AM. 2014. Rockpool gobies change colour for camouflage. *Plos One* **9**.

Stuart-Fox D, Moussalli A. 2011. Camouflage in color changing animals: trade-offs and constraints. In: Stevens M and Merilaita S, eds. *Animal camouflage: mechanisms and function*. Cambridge, UK.: Cambridge University Press. 237-253.

Watson AC, Siemann LA, Hanlon RT. 2014. Dynamic camouflage by Nassau groupers Epinephelus striatus on a Caribbean coral reef. *Journal of Fish Biology* **85:** 1634-1649.

Whiteley AR, Gende SM, Gharrett AJ, Tallmon DA. 2009. Background Matching and Color-Change Plasticity in Colonizing Freshwater Sculpin Populations Following Rapid Deglaciation. *Evolution* **63:** 1519-1529.

Figure legends

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

Figure 2: Trunks of *Plagiotremus rhinorhynchos* 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 μm (right). Arrowheads indicate melanophores.

Figure 3: Hormonal regulation of colour change in *Plagiotremus rhinorhynchos*. Trunk biopsies from an olive coloured form incubated in different hormone solutions. **A:** The body area of the biopsy kept in MSH (5 μM) 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 μM 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 μm (right).
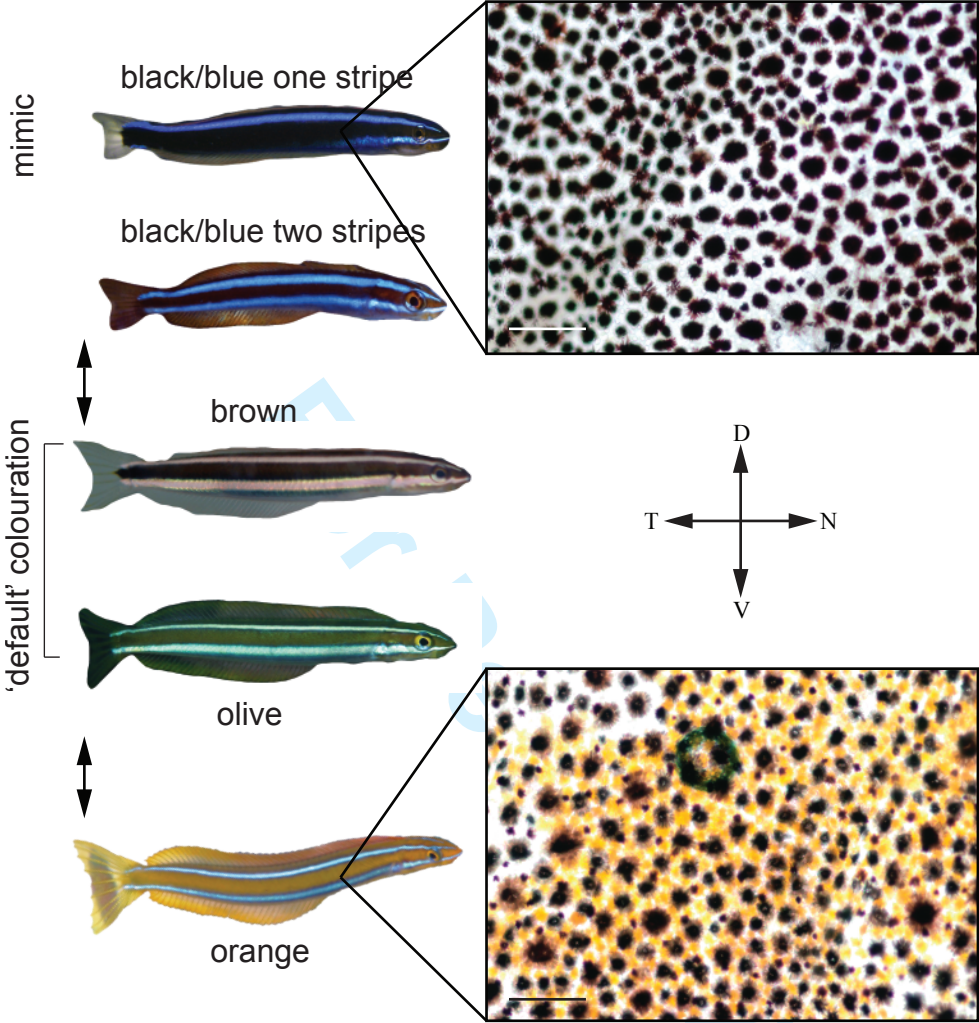
Figure 4: Colour change *in vitro* for different sized and coloured forms of *Plagiotremus rhinorhynchos* individuals (n=16). Trunk biopsies with initially dispersed melanophore pigment were incubated in 10 μM melatonin to induce colour change. Colour change was quantified as the Melanophore Index (MI) at every 30 second where MI 1 = totally aggregated pigment and MI 5 = evenly dispersed pigment. Graph shows MI for each fish at

90 seconds in relation to fish length. Black with two stripes, brown and olive describe the different colour forms when euthanized.

Figure 5: Boxplots of results from behavioural observations of black and orange fangblennies in the field: i) number of strikes at passing reef fish, and ii) number of successful attacks. Dark horizontal lines represent the median, with the box representing the 25th and 75th percentiles, and the whiskers the 10th and 90th percentiles. Ns, indicates no significant difference was detected between colour forms.

Figure 1

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
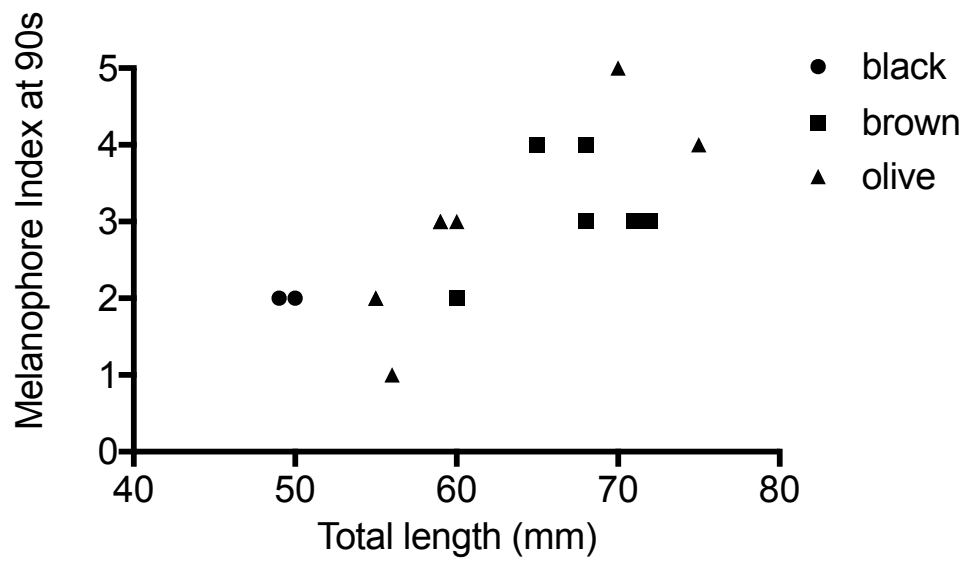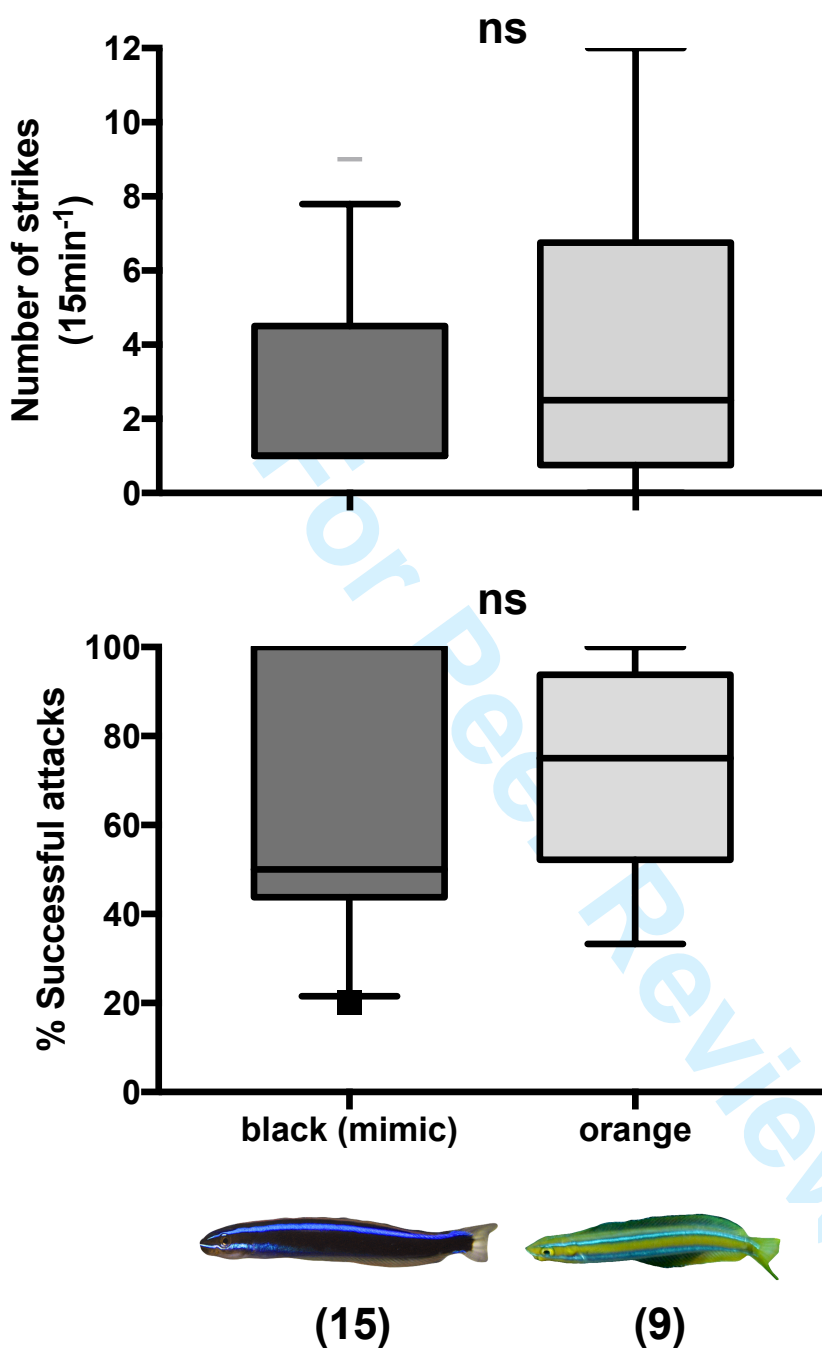46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
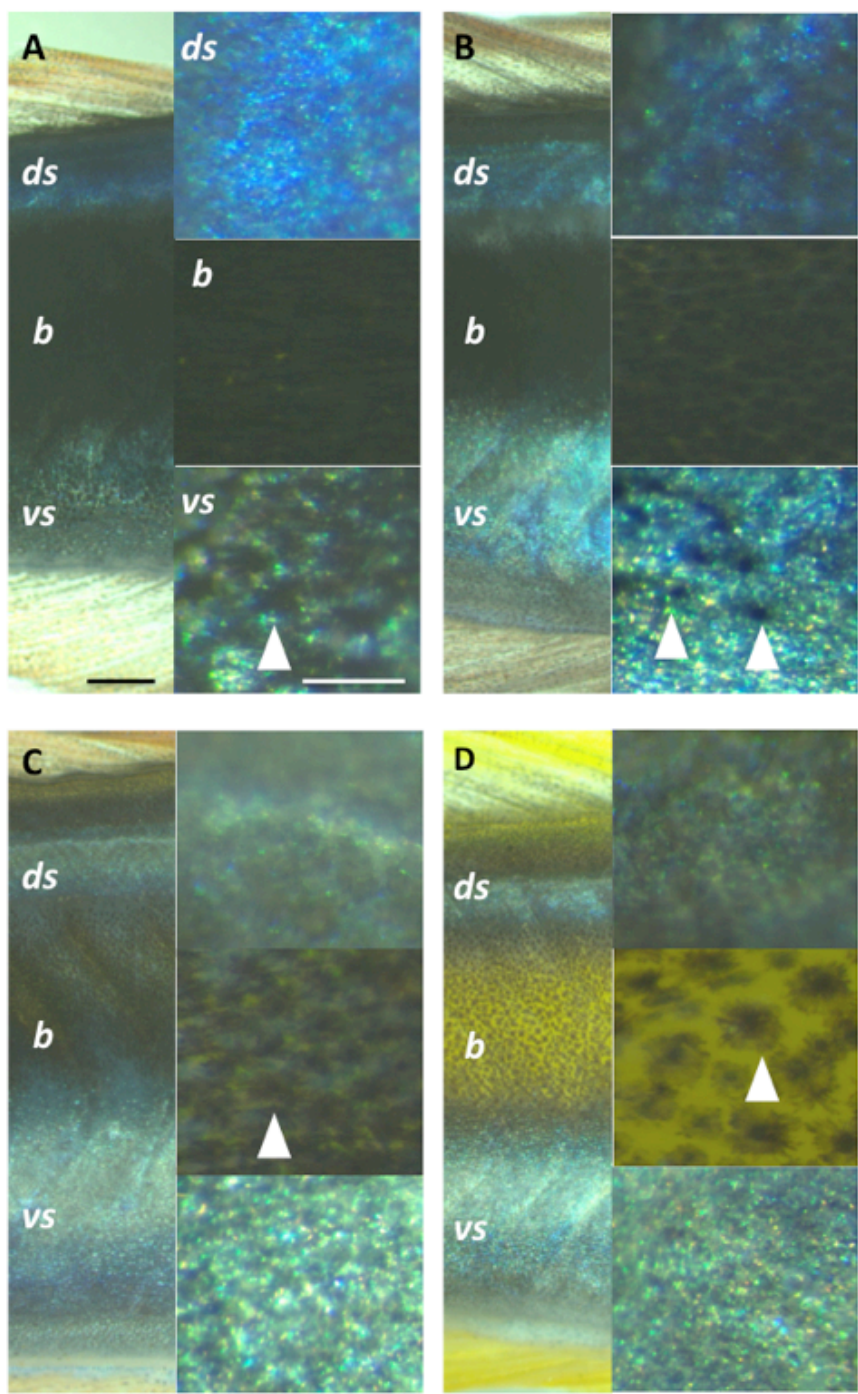
Figure 2
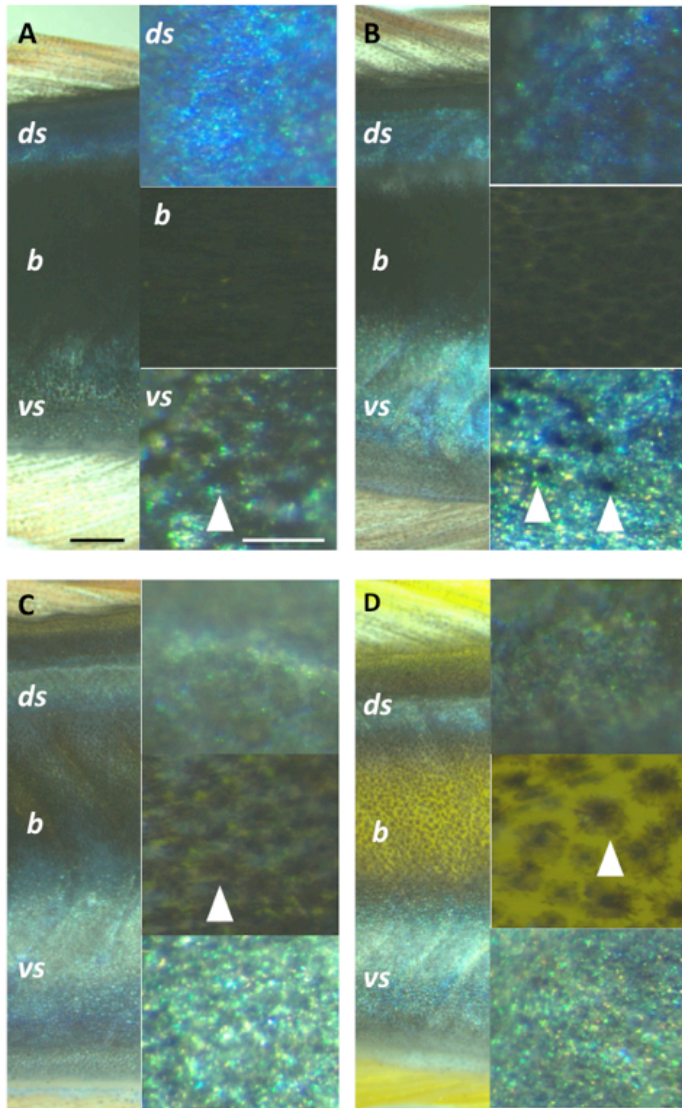
Figure 3
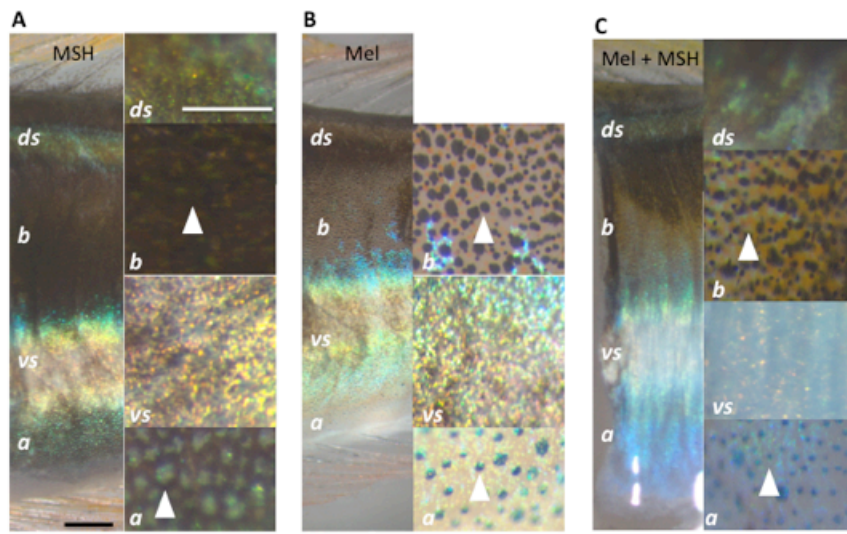
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 4

Figure 5

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

Karen L. Cheney[1,2*], Fabio Cortesi[2], Helen Nilsson Sköld[3, 4]

[1]School of Biological Sciences, The University of Queensland, Brisbane 4072 Australia.

[2]Queensland Brain Institute, The University of Queensland, Brisbane 4072 Australia.

[3]The Sven Lovén Centre for Marine Sciences, Kristineberg, University of Gothenburg, Sweden.

[4] Havets Hus, 453 30 Lysekil, Sweden

*Communicating author: k.cheney@uq.edu.au

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 dottyback, *Pseudochromis fuscus*, which alters colouration over a period of two

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

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

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

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 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%

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

Chromatophore analysis

To reveal chromatophore status in the wild, fish (n = 8; black/blue one stripe = 1, black/blue two stripes = 2, olive = 3, orange = 2) were taken back to the boat by a snorkeler, and decapitated within 2 minutes after capture to preserve colouration. The trunk was immediately put in 4% neutral buffered formalin. The fixed trunk biopsies were then brought to the research station for examination under a microscope. A further set of fish (n=16; black with one stripe = 4, black with 2 stripes = 1, brown = 5, olive = 4, orange = 2) were collected and then kept in individual tanks with plastic pipe shelters and coral rubble for a few days. While these fish were used for analysing rate of color change *in vitro* and hormone effects, a piece of the trunk was also fixed immediately after decapitation in 4% neutral buffered formalin for melanophore analysis of the main body area between the 2 stripes as a complement to the fish fixed in the field.

Melanophore density in the body area was assessed by manually counting the number of melanophores in a randomly selected 0.5 x 0.5 mm square of a photograph of each fixed biopsy. The melanophore status was scored manually in the same photographs using the Melanophore Index (MI; Hogben & Slome, 1931), where MI 1 is totally aggregated pigments and MI 5 totally dispersed pigments. Magnification and illumination were kept under standardized conditions during photography.

In vitro *analysis of colour change*

To further understand the regulation of colour plasticity between differently sized fish and between different colour forms, trunk sections of fish (n = 16) were treated with different combinations of hormones. Stock solutions of hormones were

stored at -20ºC and diluted to the experimental concentration in analytical phosphate buffered saline (APBS at pH 7.4; ProSciTech, Thuringova, Qld, Australia) just before use. Melatonin and sheep prolactin (Sigma Aldrich) were stored at -20ºC as 10 mM and 150 IU/ml stock solutions, respectively. Melatonin was used at 10 μM and prolactin at 0.150 IU/ml. MSH (α- melanocyte stimulating hormone; ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA), was stored as 1 mM stock solutions at -20ºC and used at 5 μM. Control trunk sections were incubated in APBS with 0.1% ethanol, the dissolvent of melatonin.

Fish used in the *in vitro* experiments were kept in aquaria for a few days prior to treatments. Each fish was sized, photographed and quickly decapitated using sharp scissors. In many cases, their colouration had altered since capture and so was classified again (black with 2 stripes = 2, brown = 6, olive = 8). The body was thereafter cut into approximately 0.5 cm vertical sections from head to tail and biopsies were rested in saltwater for 5-15 min to allow full chromatophore pigment dispersion before onset of treatments (one section was fixed immediately after decapitation in 4% neutral buffered formalin, see above). For each individual, biopsies were thereafter randomly incubated in hormone and control solutions. Two different *in vitro* experiments were performed. First, the capacity of differently colored individuals to change color was tested by incubating the biopsies for one hour using different combinations of hormones (melatonin, prolactin or MSH, individually or in combinations) or a control solution (APBS), before analyzing pigment movement/colour change under a bright field microscope. Using pilot trials, one hour was found sufficient to trigger the full spectrum of physiological colour change responses. Second, the rate at which colour changes occurred *in vitro* was tested using 10 μM melatonin, which causes movement of pigment granules to the cell centre (aggregated), on differently sized fishes (SL: 49-75 mm). Melanophore pigment was dispersed (MI = 5) for each individual at the beginning of the trial, and scored every 30 s using microscopy and the Melanophore Index for 10 min.

Behavioural observations

To examine whether there was a fitness advantage between extreme colour forms, we conducted 1 or, when possible, 2 behavioural observations for 15 min on black/blue one stripe (n = 15), and orange individuals (n = 9) (n = 39 observations in

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^2, 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^2, MI 2-3) or dense amounts of melanophores (104 melanophores/0.25 mm^2, 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).

In orange forms (n = 2), the body area was orange due to fewer melanophores (49 and 30 melanophores/0.25 mm^2) 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^2, 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^2; 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^2; 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

the melanophores. The skin of smaller fish (< 60mm) on the other hand, went from almost black to considerably pale due to the rapid aggregation of pigment in all observed melanophores.

Behavioural observations

The main fish species that were attacked by fangblennies included: snapper (*Lutjanus fulviflamma*), parrotfish (*Scarus* spp.), damselfish (*Chromis* spp.), squirrelfish (*Myripristis* spp.), and anthias (*Pseudanthias* spp.). For each individual, the number of strikes at fish ranged from between 0 to 12, per 15 min observation; however, strike number did not significantly differ between black/blue one stripe or orange colour forms (Mann Whitney-U = 52, n_1 = 15, n_2 = 9, p = 0.67). The percentage of successful strikes ranged from 20 to 100 %, but again, did not differ between forms (Mann Whitney-U = 44, n_1 = 15, n_2 = 9, p = 0.77) (Fig. 5).

During one behavioural observation at 3m depth on an additional black/blue one stripe individual (SL 35m), the fangblenny was observed to curl its tail around on itself to form a circle and interestingly the overall body colour and both stripes changed to black. The fish then floated to the surface in the adopted circular body posture where it drifted just under the surface with debris, including dark pieces of seaweed against which it was camouflaged. The observer followed the fish for about 200 m and the fish was still alive and floating when left.

Discussion

Here, we report the underlying physiological mechanisms and constraints to colour change in an intriguing mimicry system. Bluestriped fangblennies have the ability to change colour rapidly due to the translocation of pigments in chromatophores. Small fangblennies are also able to cloak a second ventral blue stripe to become an accurate mimic of juvenile cleaner wrasse. *In vitro,* skin biopsies of smaller individuals altered their colouration more rapidly than larger individuals, and this supports what has previously been observed in behavioural and field investigations (Cheney et al., 2008); therefore, translocation of pigment may be limited in larger individuals. Candidate hormones, MSH, prolactin and melatonin are potentially involved in natural rapid colour change regulation. Finally, we report the behavior of small black/blue one stripe (mimic) and orange individuals, and found

there was no significant difference in the number or the success of strikes at passing reef fishes between colour forms. This indicates that strategies including cleaner wrasse mimicry and hiding within shoals of similar coloured fish, may be equally as successful in terms of attacking prey. We also present an anecdotal observation on pelagic floating behavior, which was previously observed in another blenny, *Aspidontus taenatiatus* (Losey, 1972), and is thought to be used for migratory purposes. In this case, the small black/blue one stripe fangblenny turned completely black (without any stripes), which demonstrates colour plasticity may be more broad than originally thought, at least in juveniles.

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

However, from *in vitro* samples, trunk sections of fish with olive body colour were incubated in prolactin or MSH, the body darkened and when another trunk section of the same fish was treated with melatonin in combination with prolactin or MSH, the body became more orange, which suggests that individual fish may be able to switch between colour forms. Noteworthy, the stripes appeared more iridescent and

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).

The fitness benefits of colour change can be limited however, since it may come with a cost. A long-term study on long- and short-term color change in sculpins showed a small but significant cost of color change in terms of reduced survival (Bergstrom, Whiteley & Tallmon, 2012). In a similar study on guppies, repeated skin color change in response to dark and light backgrounds carried an energetic cost in terms of increased food consumption (Rodgers et al., 2013). The fish also preferred habitats and shoals that matched their own coloration.

Environment variation - whether habitat or socially related - is likely to promote the evolution and maintenance of flexible colouration. However, to fully understand the maintenance and benefit of phenotypic plasticity in communication and camouflage systems, we must investigate the mechanisms that allow plasticity to persist, and the underlying physiological constraints and adaptive benefits to the species.

References

Agrawal AA. 2001. Ecology - Phenotypic plasticity in the interactions and evolution of species. *Science* **294:** 321-326.

Bergstrom CA, Whiteley AR, Tallmon DA. 2012. The heritable basis and cost of colour plasticity in coastrange sculpins. *Journal of Evolutionary Biology* **25:** 2526-2536.

Cheney KL, Grutter AS, Marshall NJ. 2008. Facultative mimicry: cues for colour change and colour accuracy in a coral reef fish. *Proceedings of the Royal Society B-Biological Sciences* **275:** 117-122.

Cheney KL, Skogh C, Hart NS, Marshall NJ. 2009. Mimicry, colour forms and spectral sensitivity of the bluestriped fangblenny, *Plagiotremus*

rhinorhynchos. *Proceedings of the Royal Society B-Biological Sciences* **276:** 1565-1573.

Cortesi F, Feeney WE, Ferrari MCO, Waldie PA, Phillips GAC, McClure EC, Skold HN, Salzburger W, Marshall NJ, Cheney KL. 2015. Phenotypic plasticity confers multiple fitness benefits to a mimic. *Current Biology* **25:** 949-954.

Cortesi F, Musilova Z, Stieb SM, Hart NS, Siebeck UE, Cheney KL, Salzburger W, Marshall NJ. 2016. From crypsis to mimicry: changes in colour and the configuration of the visual system during ontogenetic habitat transitions in a coral reef fish. *Journal of Experimental Biology* **219:** 2545-2558.

Côté IM. 2000. Evolution and ecology of cleaning symbioses in the sea. *Oceanography and Marine Biology Annual Review* **38:** 311-355.

Côté IM, Cheney KL. 2005. Choosing when to be a cleaner-fish mimic. *Nature* **433:** 211-212.

Dawkins MS, Guilford T. 1993. Color and pattern in relation to sexual and aggressive-behavior in the Bluehead Wrasse *Thalassoma bifasciatum*. *Behavioural Processes* **30:** 245-251.

Goda M, Fujii R. 1995. Blue chromatophores in two species of callionymid fish. *Zoological Science* **12:** 811-813.

Grutter AS. 1999. Cleaner fish really do clean. *Nature* **398:** 672-673.

Hogben L, Slome D. 1931. The pigmentary effector system VI The dual character of endocrine co-ordination in amphibian colour change. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character* **108:** 10-53.

Kodric-Brown A. 1998. Sexual dichromatism and temporary color changes in the reproduction of fishes. *American Zoologist* **38:** 70-81.

Kuwamara T. 1981. Mimicry of the cleaner wrasse *Labroides dimidiatus* by the blennies *Aspidontus taeniatus* and *Plagiotremus rhynorhynchos*. *Nanki Seibutu* **23:** 61-70.

Leclercq E, Taylor JF, Migaud H. 2010. Morphological skin colour changes in teleosts. *Fish and Fisheries* **11:** 159-193.

Mäthger LM, Land MF, Siebeck UE, & Marshall NJ. 2003. Rapid colour changes in multilayer reflecting stripes in the paradise whiptail, *Pentapodus paradiseus*. *Journal of Experimental Biology*, **206 (20):** 3607-3613.

Morrongiello JR, Bond NR, Crook DA, Wong BBM. 2010. Nuptial coloration varies with ambient light environment in a freshwater fish. *Journal of Evolutionary Biology* **23:** 2718-2725.

Nilsson Sköld H, Aspengren S, Cheney KL, Wallin M. 2016. Fish chromatophores—from molecular motors to animal behavior. *International Review of Cell and Molecular Biology* **321:** 171-219.

Norman MD, Finn J, Tregenza T. 2001. Dynamic mimicry in an Indo-Malayan octopus. *Proceedings of the Royal Society B-Biological Sciences* **268:** 1755-1758.

Pigliucci M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press: Baltimore, MD, United States.

Rodgers GM, Gladman NW, Corless HF, Morrell LJ. 2013. Costs of colour change in fish: food intake and behavioural decisions. *Journal of Experimental Biology* **216:** 2760-2767.

Sköld HN, Amundsen T, Svensson PA, Mayer I, Bjelvenmark J, Forsgren E. 2008. Hormonal regulation of female nuptial coloration in a fish. *Hormones and Behavior* **54:** 549-556.

Sköld HN, Aspengren S, Wallin M. 2013. Rapid color change in fish and amphibians - function, regulation, and emerging applications. *Pigment Cell & Melanoma Research* **26:** 29-38.

Stevens M, Lown AE, Denton AM. 2014. Rockpool gobies change colour for camouflage. *Plos One* **9**.

Stuart-Fox D, Moussalli A. 2011. Camouflage in color changing animals: trade-offs and constraints. In: Stevens M and Merilaita S, eds. *Animal camouflage: mechanisms and function*. Cambridge, UK.: Cambridge University Press. 237-253.

Watson AC, Siemann LA, Hanlon RT. 2014. Dynamic camouflage by Nassau groupers Epinephelus striatus on a Caribbean coral reef. *Journal of Fish Biology* **85:** 1634-1649.

Whiteley AR, Gende SM, Gharrett AJ, Tallmon DA. 2009. Background Matching and Color-Change Plasticity in Colonizing Freshwater Sculpin Populations Following Rapid Deglaciation. *Evolution* **63:** 1519-1529.

Figure legends

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

Figure 2: Trunks of *Plagiotremus rhinorhynchos* 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 μm (right). Arrowheads indicate melanophores.

Figure 3: Hormonal regulation of colour change in *Plagiotremus rhinorhynchos*. Trunk biopsies from an olive coloured form incubated in different hormone solutions. **A:** The body area of the biopsy kept in MSH (5 μM) 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 μM 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 μm (right).

Figure 4: Colour change *in vitro* for different sized and coloured forms of *Plagiotremus rhinorhynchos* individuals (n=16). Trunk biopsies with initially dispersed melanophore pigment were incubated in 10 μM melatonin to induce colour change. Colour change was quantified as the Melanophore Index (MI) at every 30
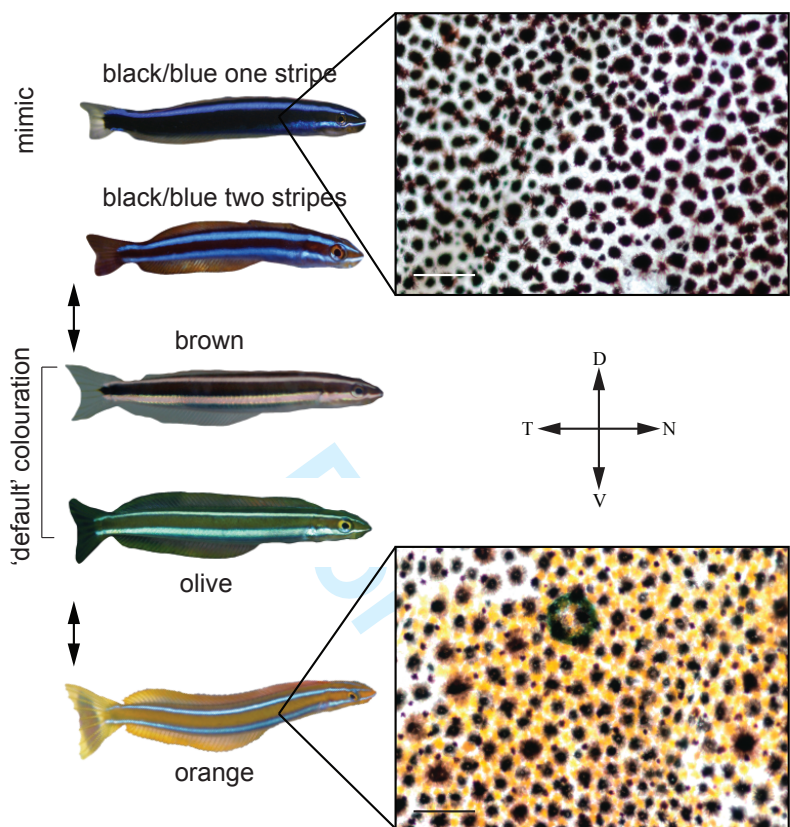
second where MI 1 = totally aggregated pigment and MI 5 = evenly dispersed pigment. Graph shows MI for each fish at 90 seconds in relation to fish length. Black with two stripes, brown and olive describe the different colour forms when euthanized.

Figure 5: Boxplots of results from behavioural observations of black and orange fangblennies in the field: i) number of strikes at passing reef fish, and ii) number of successful attacks. Dark horizontal lines represent the median, with the box representing the 25th and 75th percentiles, and the whiskers the 10th and 90th percentiles. Ns, indicates no significant difference was detected between colour forms.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5