



## Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour

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Many coral reef fish possess ultraviolet (UV) colour patterns. The behavioural significance of these patterns is poorly understood and experiments on this issue have not been reported for free-living reef fish in their natural environment. The damselfish *Pomacentrus amboinensis* has UV facial patterns, and spectroradiometric ocular media measurements show that it has the potential for UV vision. To test the potential behavioural significance of the UV patterns, I studied the response of males, in natural territories on the reef and in aquaria, to two conspecific intruders, one presented in a UV-transmitting (UV+) container and the other in a UV-absorbing (UV-) one. Territory owners attacked intruders viewed through UV+ filters significantly more often and for longer than intruders viewed through the UV- filter. In general, the results of the field experiment confirmed those of the laboratory experiment. The results support the hypothesis that *P. amboinensis* males are sensitive to UV light and that reflectance patterns, which appear in high contrast only in UV, modulate the level of aggressive behaviour. A recent survey showed that many predatory fish may not have UV vision and the use of UV colours in select species of reef fish may therefore serve as a 'private communication channel'.

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Many reef fish possess colour patterns that contain ultraviolet (UV) components invisible to the human eye (Marshall 1996, 2000b). A variety of damselfish species, such as *Pomacentrus amboinensis*, display UV patterns on their faces and on their fins. During agonistic behaviour, *P. amboinensis* first rushes towards an intruding fish until it is face to face with it, then fully erects its fins and swims antiparallel (head to tail and tail to head) to the intruder. Both actions are repeated until the intruder retreats. As both face and fins are used extensively in display, this suggests a role in communication (Thresher & Moyer 1983). The question arises whether fish displaying UV patterns are sensitive to UV light and what behavioural significance this may have.

Many invertebrates (e.g. Silberglied 1979) and vertebrates have UV-sensitive photoreceptors (fish: e.g. Losey et al. 1999; amphibians and reptiles: e.g. Govardovskii & Zueva 1974; Arnold & Neumeyer 1987; Fleishman et al. 1993; Loew et al. 1996; Sillman et al. 1997; birds: e.g. Huth 1972; Chen et al. 1984; Palacios & Varela 1992; Bennett et al. 1996; Hausmann et al. 2003; mammals: e.g. Jacobs 1992). The spectral sensitivities of only a few coral reef fish have been investigated so far (Shand 1993; Lythgoe et al.

1994; McFarland & Loew 1994; Losey et al. 2003). However, among these are six close relatives of *P. amboinensis*, which have been shown to possess UV-sensitive photoreceptors (McFarland & Loew 1994; Losey et al. 2003). A prerequisite for UV sensitivity is that the ocular media of the eye (cornea, lens and humours) transmit UV light. A recent ocular media survey showed that 52.3% of the 400 coral reef species investigated have the potential for UV vision based on this criterion (Siebeck & Marshall 2001, unpublished data). *Pomacentrus amboinensis* and 40 other damselfish species have UV-transparent ocular media but the photoreceptor sensitivities of *P. amboinensis* are not yet known.

A comparison of the ocular media survey with a survey of reef fish colours (Marshall 2000a) reveals that not all fish with UV coloration patterns have the potential for UV vision and vice versa (Siebeck & Marshall 2001). It is therefore not possible to predict UV sensitivity based on the presence of UV coloration alone. This is not to say that the UV coloration of fish insensitive to UV light has no behavioural significance. Although invisible to conspecifics, it may well be directed at the visual system of another species.

Few studies have investigated the functional significance of UV sensitivity and UV coloration in terms of an animal's behaviour. However, the following systems have been studied in some detail. UV markings on flowers serve

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as nectar guides for certain insects (reviewed in Silberglied 1979), UV coloration is an important selection criterion for mate choice in some species of butterfly (e.g. *Polyommatus icarus*: Knüttel & Fiedler 2001), bird (e.g. red-billed Leiothrix, *Leiothrix lutea*, and starlings *Sturnus vulgaris*: Maier 1994; Bennett et al. 1997) and freshwater fish (e.g. amarillo fish, *Girardinichthys multiradiatus*, and guppies, *Poecilia reticulata*: Garcia & de Perera 2002; Kodric-Brown & Johnson 2002; Smith et al. 2002), UV is important for foraging in some species of fish (e.g. yellow perch, *Perca flavescens*, rainbow trout, *Oncorhynchus mykiss*, and pumpkinseed sunfish, *Lepomis gibbosus*: Loew et al. 1993; Browman et al. 1994) and bird (e.g. three species of hummingbirds, *Archilochus alexandri*, *Lampornis clemenciae* and *Eugenes fulgens*, kestrels, *Falco tinnunculus*, and blue tits, *Parus caeruleus*: Goldsmith 1980; Viitala et al. 1995; Church et al. 1998) and UV perception is important for orientation in several invertebrates (e.g. honeybees, *Apis mellifera*, amphipods, *Talitrus saltator*, ants, *Polyergus rufescens*, whitefly, *Bemisia argentifolii*: von Frisch 1953; Ugolini et al. 1993; Grasso Donato et al. 1997; Antignus et al. 2001) and fish (e.g. rainbow trout: Hawryshyn et al. 1990). Most of these studies, however, were done in the controlled laboratory environment rather than in the natural habitat of the animal.

Successful reproduction and the avoidance of predation can conflict with one another, as it may require that the animal be conspicuous to potential mates while at the same time not drawing attention to itself to avoid predation. Simultaneous conspicuousness and crypsis has been shown in some fish, such as many labrids and pomacanthids (e.g. *Thalassoma lunare* and *Pygoplites diacanthus*) and the guppy (Endler 1991; Marshall 2000a). Using the UV waveband as a secret communication channel available for those with UV sensitivity (Siebeck & Marshall 2001) and unavailable for those without (like humans) is a way to solve this problem. Short-wavelength UV light is also scattered more strongly in particulate media than light of longer wavelengths (Jerlov 1976). UV signals travelling through the water are therefore attenuated faster than signals comprising longer wavelengths. Therefore, communication in the UV is effective only over short distances.

Female *P. amboinensis* leave their eggs in the care of the male and thus need to be convinced that their offspring will be protected by the male and have plenty of food. Males establish territories around a central shelter, which they defend vigorously against conspecifics and heterospecifics. After successful courtship, females lay their eggs into the shelter and leave their offspring in the care of the male (McCormick & Nechaev 2002). In their natural habitat, *P. amboinensis* males defend their territories throughout the year and they will also readily adopt and defend new shelters and territories in aquaria in the laboratory. To establish (1) whether *P. amboinensis* is sensitive to UV light and (2) whether the UV patterns of this species have behavioural significance, I investigated the territorial defence behaviour of males confronted with unknown conspecific intruders in the presence and absence of their UV patterns. To manipulate the UV patterns, I placed the intruders inside filter containers

made of UV-transmitting or UV-opaque material. I did this both in the laboratory and in the field. In addition, I conducted three control experiments to test for luminance preference, species specificity of the territorial behaviour and species recognition.

## METHODS

### Study Animals and Maintenance

*Pomacentrus amboinensis* was selected as the study species because pilot experiments showed that it reacts very aggressively towards intruding conspecifics and parts of its body reflect UV light. I used a photographic method to make the UV patterns visible to the human eye. I photographed the fish with a Sony DSC-F707 digital camera through a combination of two filters (Oriol filters 51720 and 51124, Oriol, Stratford, U.S.A.) with a resulting transmission spectrum of 350–400 nm. I photographed the fish through the side of an aquarium made of UV-transparent Plexiglas (GS 2458, Plastral Pty Ltd, Brisbane, Australia).

The experiments were conducted around Lizard Island, Great Barrier Reef, Australia, between September 2001 and February 2002 with permission from the Great Barrier Reef, Marine Park Authority and the University of Queensland Ethics Committee. One of the trips (October) was during the breeding season of *P. amboinensis* and I took care to remove only males that did not guard eggs. This was determined during the initial observation of the fish. I used SCUBA equipment and a hand net to catch fish. I placed each fish into a plastic bag filled with sea water to avoid aggressive interactions between them. I placed the plastic bags side by side into a large bin (1×0.5 m) for the short transport (5 min) to the station. I selected large *P. amboinensis* males (9–10 cm standard length, SL) as the territorial fish (henceforth target fish) and slightly smaller males (7.5–8.5 cm SL) as the conspecific intruder fish (henceforth stimulus fish). In the laboratory 19 target fish were tested with 38 stimulus fish and in field experiments 12 target fish were tested with 24 stimulus fish. All fish were separated into separate holding aquaria to prevent stressful agonistic interactions. In the laboratory, target fish were transferred into the test aquaria 24 h before the laboratory experiments; in the field I selected established territory owners as target fish. Sexes were discriminated by the genital papillae (Thresher 1984) and females and smaller males were released back on to the reefs where they had been caught. Only males were used for the experiments. Each male was used once as a target fish in a laboratory experiment and once as a stimulus fish in a field experiment. For the control experiments, I used 10 *Dascyllus aruanus* (6–7 cm SL) and 12 *Pomacentrus moluccensis* (7.5–8.5 cm SL), which were caught the day before they were used.

At the research station, the fish were housed in aquaria (30×40 cm and 25 cm high) before they were used in experiments. Each aquarium contained a shelter (plastic tube) and was used to house a single fish to prevent stressful interactions with other fish before the experiment. The aquaria were situated on a shaded outside

bench at the station and therefore the light regime was that of the natural light–dark cycle of day and night. All aquaria continuously received fresh sea water supplied by the flow-through system of the station. The temperature was thus that of the sea in front of the station and was equal to the temperature during the field experiments (ca. 27°C). All fish were fed twice daily with flake food containing vitamins, a mixture of shrimp, crab and squid meal and algae (Wardley Corporation, Secaucus, NJ, U.S.A.). They were kept for 3 days and then released at the spot where they had been caught.

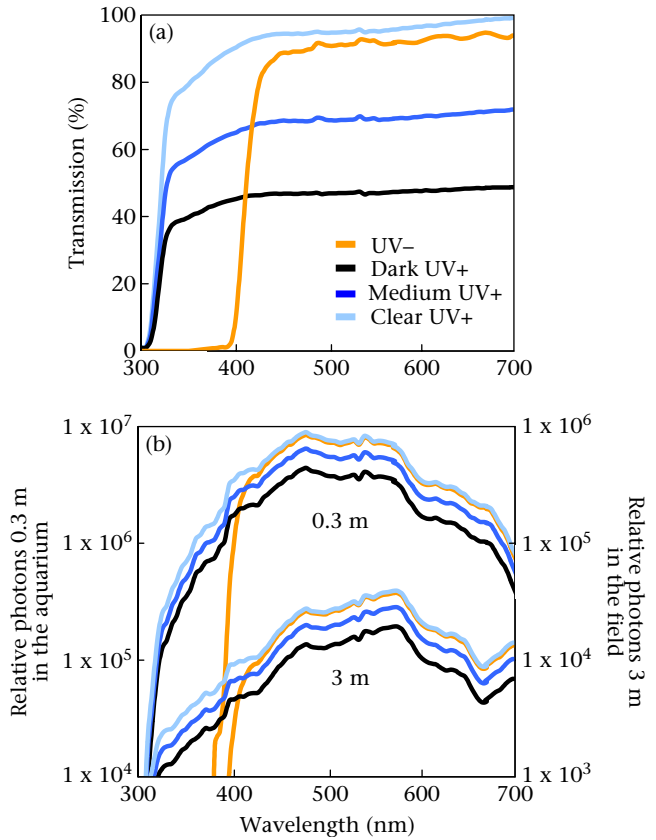
I transferred the fish between aquaria by first catching them with a small hand net and then placing them into small plastic containers that were then taken to the new aquaria. The distance between the holding and experimental aquaria was about 10 m so that the fish were in the plastic containers for less than 1 min.

At the beginning of an experiment, the stimulus fish placed in the filter tubes typically inspected the inside of the tube first before settling down somewhere inside it. Occasionally, they would swim around. When under attack the stimulus fish either kept still, or answered the attack by raising the dorsal fin and presenting it perpendicular to the orientation of the attacking target fish. None of the fish showed extreme behaviour or distress as judged by their reactions.

### Filter Construction and Properties

I used filter containers to manipulate the colour signal of the stimulus fish: UV-opaque (UV–) material (LEE filter No. 226, Sydney, Australia) and UV-transparent (UV+) material (overhead transparencies, EXP500 OHP, Corporate Express, Richlands, Australia). The filter containers can be described by two characteristics: wavelength composition of the transmitted light and level of brightness. The human eye cannot distinguish between clear UV+ and UV– filters, because they both transmit almost equal amounts of light in the 400–800 nm range. To a UV-sensitive animal, however, the level of brightness of the two tubes is different, because the UV+ filter transmits relatively more photons than the UV– filter. To determine whether a target fish chose on the basis of the wavelength composition of the stimulus fish's colours rather than the brightness differences, I used three levels of UV+ neutral-density filters. To create different neutral-density filters, I printed various shades of grey on the overhead transparencies with a laser printer. The printouts were used after tests in saltwater showed that the printer toner would not fade or rub off. Sheets of filter material (19×28 cm) were cut and rolled so that tubes were formed (19 cm long, 9 cm in diameter). Small plastic containers with a threaded lid were cut in half and one end of the filter tube was glued (with silicone glue) into the bottom part of the plastic container, while the other half was glued into the threaded top of the container so that the lid could be screwed on to seal the tube.

The spectral transmission of all plastics was quantified individually with a spectrometer ('Sub-Spec', Oriol Instruments, Stratford, U.S.A., details as in Siebeck & Marshall 2001). The three UV-transmitting neutral-density filters



**Figure 1.** (a) Transmission of the ultraviolet-absorbing (UV–) filter and the three neutral-density levels of ultraviolet-transmitting (UV+) filters. (b) Radiance inside all four filters at 3-m depth in the field and 0.3-m depth in the aquarium.

absorbed wavelengths below 300 nm (Fig. 1a). The clear filter transmitted more light than the other two neutral-density filters so that objects behind it always appeared brightest (Fig. 1a). The UV– filter transmitted slightly less light than the clear filter in the wavelength band between 400 and 700 nm and absorbed UV. Objects seen through it would therefore also appear less bright than when seen through the clear filter. The dark filter was chosen on the basis that the fish seen through it was just visible in the aquarium (to human eyes) and an important assumption of the experiments is that objects seen through this will appear darker to the visual system of the fish than those seen through the UV– filter. The medium filter transmitted 20% more light than the dark filter and about 20% less light than the clear filter, and objects seen through it may appear either darker or brighter than those seen through the UV– filter depending on the visual system of the observer.

To measure the spectrum of sunlight available to the fish under experimental conditions in the field and in the laboratory, I used a portable fibre-optic spectrophotometer (S2000, Ocean Optics, Florida, U.S.A.). Measurements were made on a sunny day at the same time as the field and laboratory experiments. In each case the radiance inside the filter tube was measured as that reflected off a spectralon 99% reflection standard placed in the tube. The fibre-optic sensor was inserted into the filter tube

through a small hole in the filter material. Radiance was measured at a 45° angle to the incident light at 0.3-m depth in aquaria and 3–4-m depth in the field (depending on the tide). In each case a 10-m fibre-optic cable with a diameter of 1 mm was attached to the S2000, which was placed in a boat or on a laboratory bench together with the computer running the data acquisition software. In the field, three people were needed, one in the boat recording spectra and two divers positioning the filter tubes, etc. For each filter type in each location 10 measurements were made and their average was used for comparison. The radiance during field and laboratory experiments differed by approximately 2.5 log units (Fig. 1b), resulting in the filters looking darker in the field.

### Experimental Design and Procedure

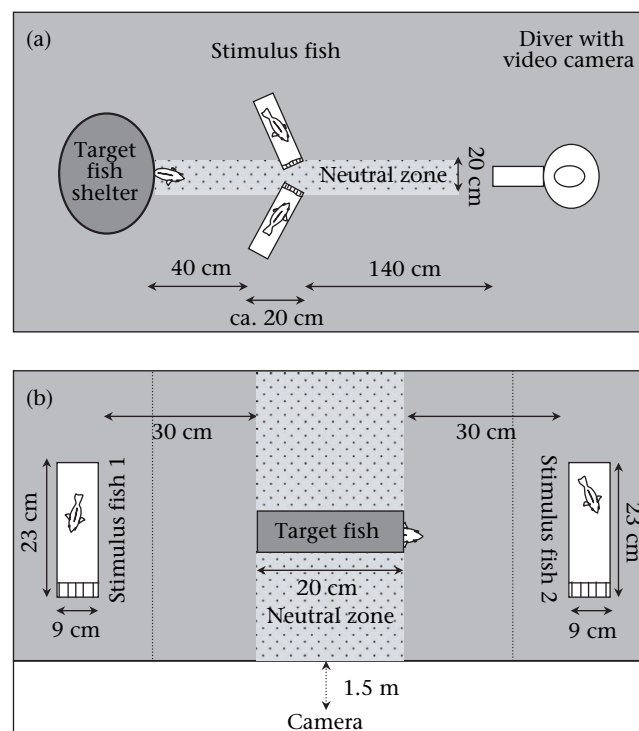
The design of the experiments in the field and laboratory was similar. In each case a single target fish was presented with two conspecific stimulus fish contained in tubes made of filter material with different properties. In each trial, one stimulus fish was presented in a UV-absorbing (UV–) container while the other one was presented in a UV-transmitting (UV+) container. The stimulus fish were of similar size and were similar in size to, or slightly smaller than, the target fish. The behaviour of all fish was filmed with a Sony TR3300 Hi 8 video camera, protected by an underwater housing (Stingray, Light and Motion, Monterey, California, U.S.A.).

I tested each target fish within three blocks of trials, one for each level of neutral-density UV+ filter versus the UV– filter. The order in which the three blocks were used was randomized for different target fish. Within each block there were six trials: two positions of the filter containers (right/left of the shelter of the target fish), two positions of the stimulus fish and two positions of the filter containers when they were empty. Within each block, the first combination of the position of the stimulus fish and filters was picked randomly, for example stimulus fish 1 on the left inside the UV– filter and, simultaneously, stimulus fish 2 on the right inside the UV+ filter. Then the remaining trials in each block were organized in the following way. In the next trial, the positions of the filters with the stimulus fish inside were exchanged. Then the positions of the stimulus fish alone were exchanged and, finally, the positions of the filters containing the stimulus fish were exchanged again. Two trials with empty filter containers were presented either before or after trials with intruders. The trials were organized in this way to minimize the number of times the stimulus fish had to be handled. For each target fish, I selected two stimulus fish that were then used throughout all combinations of filters and positions. I exchanged the positions of the UV+ and UV– filters and the positions of the stimulus fish themselves to eliminate a possible bias towards one side or one stimulus fish. Empty containers (UV+ and UV–) were presented to test the effect of the containers alone.

I conducted field experiments in the morning (0800–1000 hours) and again in the afternoon (1400–1600 hours). Target fish with established territories were

identified and chosen on each dive immediately before each experiment. The criteria for choosing a target fish were that the fish had to be aggressive towards natural intruders and that it had an obvious shelter in which it would withdraw at regular intervals. In most cases the aggressive behaviour of the fish towards intruders (including divers) was so strong that it was easy to spot candidates. An additional criterion was that territories had to be at the edge of the reef, so that the divers could settle in the sandy area away from the reef and face towards the territory and the reef edge. When a fish had been chosen, the divers positioned themselves about 3 m away from its shelter and transferred the previously caught stimulus fish from the holding containers into the selected filter containers (Fig. 2a). Diver 1 then placed the two filter containers with the stimulus fish at about 40 cm from the shelter of the territory, noted the positions of the fish and the filter on a datasheet, and moved to a position behind diver 2. Diver 2 was positioned about 2 m from the shelter with the video camera and was responsible for the filming and timing of the trials. Target fish appeared to ignore the divers and readily attacked stimulus fish in their presence.

A trial started as soon as the target fish approached one of the stimulus fish for the first time and then lasted 2 min. The trials were kept short so that it was possible to film all trials testing a single target fish within one battery life of the camera.



**Figure 2.** Experimental set-up in (a) the field and (b) the laboratory. In the field the stimulus fish were placed side by side about 30–40 cm from the entrance of the target fish's shelter. In the laboratory the shelter of the target fish was in the middle of the territory (aquarium) while the stimulus fish were placed on either side of the shelter. The behaviour of the target fish was analysed only if the target fish was outside the neutral zone (shaded area). Dashed lines show where opaque dividers were inserted between trials.

Whenever the stimulus fish had to be moved from one filter container to another, they were first placed into holding containers and then transferred into the new filter containers. After the experiments, the intruders were released and the behaviour of the target fish was filmed for 5 min. Then the target fish was caught and taken to the laboratory to record its size and confirm its sex. In the next experiment, the former target fish took part as a stimulus fish, together with another fish that had previously been a target fish in the field.

In the laboratory, aquaria (90×37 cm and 37 cm high) were set up on outside tables so that the sun could be used as natural illumination (Fig. 2b). I conducted laboratory experiments between 1100 and 1300 hours so that the sun was directly overhead and evenly illuminated the inside of the aquaria. I created an artificial territory by placing an opaque plastic tube in the centre of the aquarium as shelter and filling the bottom of the aquarium with sand. The 24-h acclimatization period was sufficient for the target fish to accept the plastic tube as shelter and to show the typical aggressive territorial behaviour towards intruders observed in the field. Before an experiment, I inserted two opaque dividers into the aquarium and placed the empty filter containers into the compartments on either side of the shelter. Then the stimulus fish were transferred from their holding aquaria into the filter containers.

The camera was set up on a tripod about 1.5 m from the tank. In each trial the camera was switched on before the dividers were removed. I then left the area so that the fish could not see me during the experiment. This was necessary in laboratory experiments in contrast to field experiments, as the fish would spend most of the time inside their shelter observing me from one of the two entrances if I was visible. After 5 min I returned, switched off the camera, put the dividers in place, exchanged the positions of the stimulus fish or the filter containers or both, switched on the camera, removed the dividers and left the scene. This procedure was repeated for all trials. The shelter had two clearly defined entrances and the tubes could be placed on either side of the shelter so that the target fish could see both stimulus fish from within (Fig. 2b).

## Control Experiments

I conducted three control experiments in the laboratory. In all cases, details of the design of the experiments followed those of the main laboratory experiment described above.

(1) Test for luminance preference alone. The response of the target fish ( $N = 8$ ) was recorded towards a choice of two conspecifics of equal size presented in two different UV+ neutral-density filters (clear and dark UV+). The positions of the stimulus fish and the filters were exchanged once to control for a possible influence of the side or the stimulus fish. Therefore, each target fish was tested in four trials that lasted 5 min each.

(2) Test for species specificity of the observed behaviour. The response of the target fish ( $N = 5$ ) was recorded when given a choice of two heterospecific stimulus fish presented in UV- versus UV+ filters. *Dascyllus aruanus*

was chosen as it occurs in the same habitat and has a completely different coloration (black-and-white stripes) lacking the UV facial patterns. Each target fish was tested in four trials that lasted 5 min each.

(3) Test for species recognition. The response of the target fish ( $N = 12$ ) was recorded when given a choice of similar-sized heterospecific (*P. moluccensis*) versus conspecific stimulus fish presented in clear UV+ filters. The positions of the two stimulus fish and the filters were exchanged once so that each fish was tested in four trials that lasted 5 min each. *Pomacentrus moluccensis* was chosen as it lives in the same habitat as *P. amboinensis*, also appears yellow to the human eye with slight pink tinges, and has UV facial patterns that differ from those of *P. amboinensis* (Siebeck 2002).

## Analysis

### Video analysis

The experiment was analysed with BEAST (event-recorder, G. Losey, University of Hawaii, Honolulu, U.S.A.). Individual keys of the computer keyboard were assigned to different components of the behaviour of the fish. While the video was replayed, I analysed the behaviour by pressing the corresponding keys. BEAST records how often each key is pressed and for how long. In the field the filming started when the first attack was observed and in the laboratory the camera was switched on just before I left the scene. Typically, the target fish would then take a little while before its first appearance, so that the actual testing time was variable and dependent on the behaviour of the target fish. The following responses were observed and analysed in terms of their duration and frequency.

(1) The position of the target fish was recorded, that is whether it was in the right or left half of the aquarium in the laboratory, or closer to the right or left stimulus fish in the field. In the laboratory, the target fish was considered close to a stimulus fish if it was positioned between one entrance of its shelter and the aquarium wall on the same side as the entrance. The zone between the two entrances of the shelter was considered neutral and no measurements were made while the fish was within this zone (Fig. 2b). In the field, the neutral zone was defined by the position of the two stimulus fish containers (Fig. 2a).

(2) Behaviour was counted as aggressive if the target fish left its shelter, attacked one of the stimulus fish and returned into its home. A typical aggressive response usually started with the target fish rushing towards one stimulus fish with all fins erected. This was then followed by trying to bite the stimulus fish, more fin presentations and rushing back into the shelter.

(3) Nonaggressive responses during patrol swims such as eating and swimming without erected fins were also recorded.

For the analysis of the results, I determined the number and duration of aggressive and nonaggressive responses towards the right and left sides and then, after the video analysis of the behaviour of the target fish, I determined the positions (right/left) of the UV+ and UV- filters by cross-referencing the results with the position of the filters as noted on the datasheets. I was blind to the treatment

where the UV- filter was tested with the clear and medium UV+ neutral-density filters. However, this was not possible where the dark UV+ filter was used in combination with the UV- filter because of the large brightness difference between the two.

Because the trial durations in the field and laboratory experiments were variable, I used BEAST to measure the time between the first and the last aggressive response of the target fish. I then determined the number of attacks and their duration as well as the number and duration of the nonaggressive behaviours during that time and for the analysis I used the average response of the fish per min.

### Statistical analysis

To analyse the behaviour of the target fish towards the stimulus fish presented in a UV- filter versus three levels of UV+ filters, I used a repeated measures analysis of variance (ANOVA). The trials controlling for an effect of the position of the stimulus fish and of the filters were treated as replicates. Two measures of the observed aggressive and nonaggressive behaviours were analysed: the frequency of the response per min and the average duration of the response. To test for significance between different neutral-density conditions, I made pairwise comparisons using one-way ANOVAs.

## RESULTS

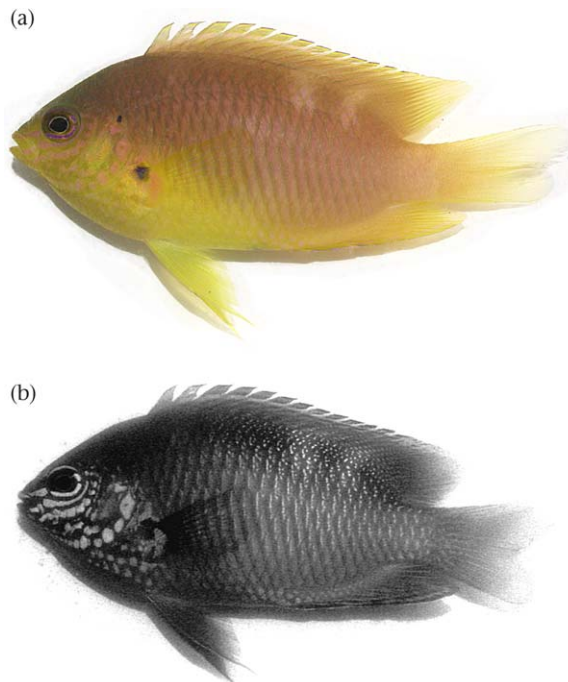
### Ultraviolet Patterns

To the human visual system, the *P. amboinensis* that I photographed appeared mostly yellow with a slight pink tinge (Fig. 3). Their ultraviolet patterns became visible when photographed in the 350–400-nm waveband and displayed as a black-and-white photograph, where white indicates UV-reflective and black UV-absorbing areas (Fig. 3). Fine regular dots lined each scale on their bodies, whereas the facial pattern was less regular and appeared to differ between individuals.

### Behaviour

The target fish showed the same aggressive behaviour towards stimulus fish in the field and laboratory. Between aggressive attacks, the target fish spent some time swimming around their territories, occasionally picking at something in the sand. During such patrol swims in the field, the target fish often encountered other intruders, which they then also attacked. In most cases, the intruders immediately left the territory before the target fish could bite them. In the field and laboratory, the target fish regularly returned to their central shelter from which they observed their territory for a while before going on another patrol swim or attack.

The main difference in territorial behaviour between natural and experimental conditions was that the intruders in the experiments could not flee. An attack of an intruder in the wild usually consisted only of a single rush towards the intruder and display of dorsal fins, resulting in the retreat of the intruder. During an experiment, the



**Figure 3.** Coloration of *Pomacentrus amboinensis*. (a) Coloration as seen by the human visual system via a Sony digital still camera and (b) UV reflectance patterns captured with the same camera with UV-pass filter attached. White areas reflect UV (350–400 nm) light and dark areas absorb UV light.

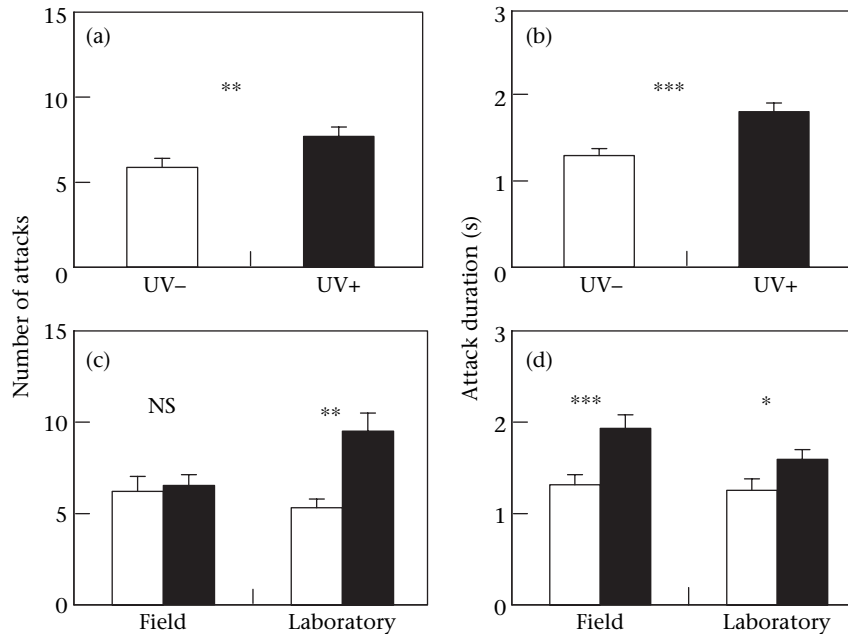
aggression of the target fish persisted and intensified and they often reduced or even stopped their patrol swims through the rest of the territory to concentrate on the intruders in the containers.

### Influence of UV

Overall, significantly more attacks were directed against the intruders in the UV+ filter (ANOVA:  $F_{1,29} = 7.46$ ,  $P = 0.011$ ; Fig. 4a) than against the intruder in the UV- container, regardless of the neutral-density level. Aggressive responses to the intruder in the UV+ filter were also significantly longer (ANOVA:  $F_{1,29} = 27.5$ ,  $P = 0.0001$ ; Fig. 4b). There was no difference in the nonaggressive responses observed in each half of the aquarium containing the UV+ and UV- stimulus fish, showing that the change in behaviour was specific to the change in the appearance of the stimulus fish rather than to the change in the side of the aquarium containing the filters (ANOVA: frequency:  $F_{1,11} = 0.76$ ,  $P = 0.4$ ; duration:  $F_{1,11} = 0.67$ ,  $P = 0.43$ ). This could be measured only in the laboratory, as the target fish often left the area covered by the visual field of the camera in the field.

### Influence of the Environment

In the field, the difference between the number of attacks directed at the UV+ and UV- intruders was not significant (ANOVA:  $F_{1,18} = 0.2$ ,  $P = 0.66$ ; Fig. 4c). However, attacks directed at UV+ intruders were significantly



**Figure 4.** (a) Number and (b) duration of attacks recorded in the combined field and laboratory data. (c) Number and (d) duration of attacks from the field and laboratory experiments. Means are given + SE. Target fish viewed the stimulus fish through UV-absorbing ( $\square$ , UV $-$ ) or UV-transmitting ( $\blacksquare$ , UV $+$ ) filters. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; repeated measures ANOVA.

longer than those directed towards UV $-$  intruders ( $F_{1,18} = 23.2$ ,  $P = 0.0001$ ; Fig. 4d). In the laboratory, the number of attacks and their average duration were both significantly greater for UV $+$  intruders (frequency:  $F_{1,11} = 10.4$ ,  $P = 0.008$ ; duration:  $F_{1,11} = 5.3$ ,  $P = 0.04$ ; Fig. 4c, d). Overall, there were no significant differences between the field and laboratory experiments ( $F_{1,29} = 0.55$ ,  $P = 0.46$ ).

### Luminance versus Colour Cues

In the field, there were significantly more aggressive responses towards the clear UV $+$  intruder (ANOVA:  $F_{1,18} = 5.88$ ,  $P = 0.026$ ) and towards the medium UV $+$  intruder ( $F_{1,18} = 11.86$ ,  $P = 0.003$ ) than towards the UV $-$  intruder (Fig. 5a). However, the difference between the dark UV $+$  and the UV $-$  intruder was not significant ( $F_{1,18} = 3.52$ ,  $P = 0.07$ ; Fig. 5a). In contrast, aggressive attacks directed at UV $+$  intruders were significantly longer for the medium and dark, but not for the clear, neutral-density level (medium:  $F_{1,18} = 14.75$ ,  $P = 0.001$ ; dark:  $F_{1,18} = 16.41$ ,  $P = 0.001$ ; clear:  $F_{1,18} = 3.25$ ,  $P = 0.088$ ; Fig. 5b).

In the laboratory, the UV $+$  intruders were always attacked significantly more often (ANOVA: clear:  $F_{1,11} = 6.48$ ,  $P = 0.004$ ; medium:  $F_{1,11} = 13.0$ ,  $P = 0.004$ ; dark:  $F_{1,11} = 4.99$ ,  $P = 0.047$ ; Fig. 5c), but only in the dark neutral-density level condition were attacks also significantly longer for UV $+$  intruders (clear:  $F_{1,11} = 0.27$ ,  $P = 0.61$ ; medium:  $F_{1,11} = 0.32$ ,  $P = 0.59$ ; dark:  $F_{1,11} = 11.9$ ,  $P = 0.005$ ; Fig. 5d).

### Controls

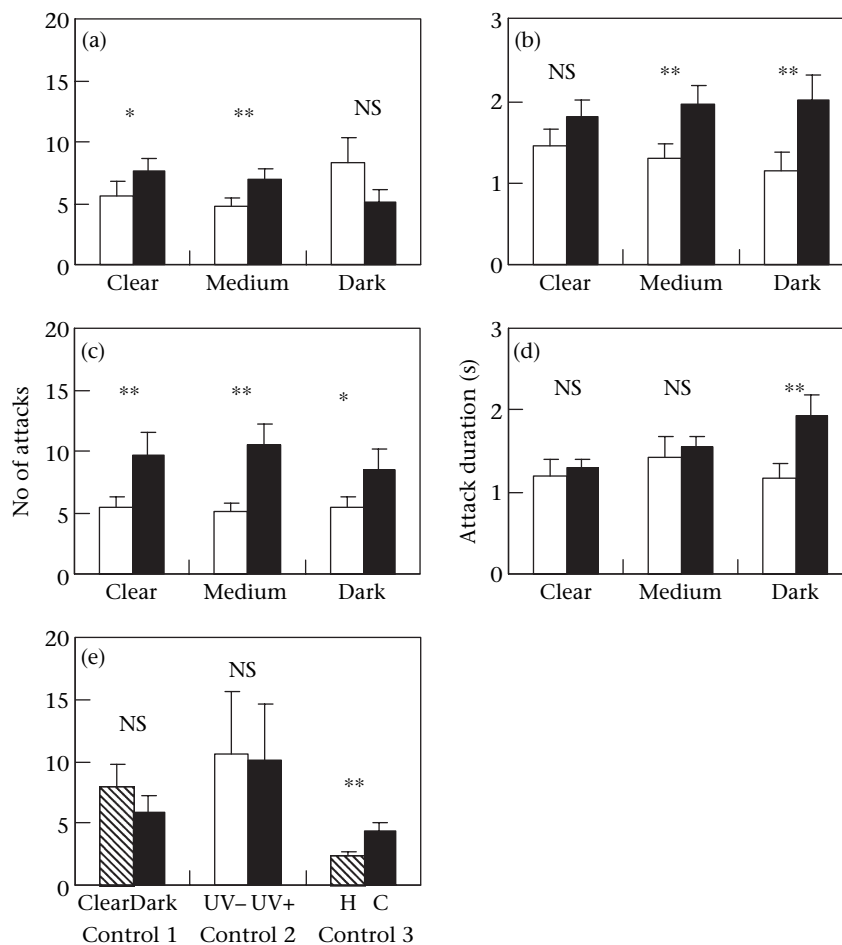
The empty tubes did not elicit an aggressive response by themselves and were treated no differently from the rest of

the territory. In the first control, there was no preference for the intruder contained in the clear or dark UV $+$  filter (ANOVA: frequency:  $F_{1,7} = 1.61$ ,  $P = 0.25$ ; duration:  $F_{1,7} = 0.99$ ,  $P = 0.35$ ; Fig. 5e). In the second control, the two heterospecific intruders contained in UV $+$  or UV $-$  filter tubes were attacked similarly (frequency:  $F_{1,4} = 0.029$ ,  $P = 0.87$ ; duration:  $F_{1,4} = 0.4$ ,  $P = 0.56$ ; Fig. 5e). In control 3, the territory owner attacked the conspecific intruder significantly more often than the heterospecific ( $F_{1,11} = 11.3$ ,  $P = 0.006$ ; Fig. 5e).

## DISCUSSION

*Pomacentrus amboinensis* males generally attacked conspecific stimulus fish lacking their UV patterns significantly less often and for shorter periods than conspecific stimulus fish displaying their UV patterns. They could visually distinguish between heterospecific and conspecific stimulus fish and the change in UV coloration modulated the behaviour only towards the latter. I therefore conclude that *P. amboinensis* males are able to see UV light and that UV patterns play a role in conspecific male–male communication during territorial interactions. Further experiments are needed to investigate the possible role of the UV facial patterns and why the behaviour of male *P. amboinensis* towards a conspecific intruder changed when the patterns were obscured. This is the first study to demonstrate that UV vision is important for free-living fish in their natural habitat.

There are several possible functions of the UV patterns for *P. amboinensis*. The UV component could be essential for the recognition of conspecifics. The fourth control experiment showed that *P. amboinensis* males could distinguish between conspecific and heterospecific stimulus fish



**Figure 5.** (a) Number and (b) duration of attacks by target males in the field experiment. (c) Number and (d) duration of attacks by target males in the laboratory experiment. Target fish viewed the stimulus fish through clear, medium or dark UV-absorbing (□, UV<sup>-</sup>) or UV-transmitting (■, UV<sup>+</sup>) filters. (e) Number of attacks by target males in the controls. In control 1 the filters were dark UV<sup>+</sup> and clear UV<sup>+</sup>; in control 2 the stimulus fish were two humbugs, *Dascyllus aruanus*, behind UV<sup>-</sup> and UV<sup>+</sup> filters; in control 3 the stimulus fish were a conspecific (C) and a heterospecific (H). Means are given + SE. \* $P < 0.05$ ; \*\* $P < 0.01$ ; repeated measures ANOVA.

with visual cues alone. Similar behaviour has been demonstrated for the threespot damselfish, *Eupomacentrus planifrons*, which also attacks conspecifics more strongly than heterospecifics (Thresher 1979). The recognition of conspecifics is thought to be based on coloration (Fricke 1973), whereas the recognition of heterospecific food competitors is thought to be based on shape (Myrberg & Thresher 1974). The difference in attack rates found here could therefore be explained by the target fish not recognizing the UV<sup>-</sup> stimulus fish as a conspecific fish because of the absence of the UV component of the colour pattern. The target fish would still be expected to attack the conspecific lacking UV coloration, as its shape signals that it is a heterospecific food competitor. Alternatively, the UV patterns could signal the territorial status of a target fish and its willingness and ability to fight, in a manner similar to the nuptial coloration of the three-spined stickleback, *Gasterosteus aculeatus* (Baube 1997). Assuming that the fish can modulate the UV component of its coloration, strong UV reflectance could signal a high level of aggression, whereas weak or no UV reflectance could signal submissiveness and therefore help to reduce confrontations.

A combination of both functions is also possible, where the UV reflectance is never switched off completely and therefore aids species recognition, and the level of reflectivity is used to signal intention to fight. Further experiments are needed to investigate the ability of the fish to change the UV reflectance of their facial patterns.

Some differences were observed between the field and laboratory experiments. In the laboratory, the stimulus fish contained in UV<sup>+</sup> filters were attacked significantly more, regardless of the neutral-density level, whereas in the field, this was true for the clear and medium, but not for the dark, neutral-density level. At the same time (in laboratory and field), each attack directed at stimulus fish seen through the dark UV<sup>+</sup> container lasted significantly longer than that directed at stimulus fish seen through the UV<sup>-</sup> container.

Before I consider possible explanations, an important difference between the laboratory and the field needs to be considered. The experiments took place at different depths, that is, 0.3 m in the aquarium and 3–4 m in the field, but the same filter containers were used in both cases, which means that the fish in the field had relatively

fewer photons available to them than the fish in the laboratory, because of the filtering properties of the water. Also, the laboratory experiment was conducted at midday (sun directly overhead) to avoid shading effects of the aquarium walls, whereas the field experiment took place in the early morning or in the afternoon, before and after the laboratory experiment. Both factors led to brightness differences between the experimental conditions in the laboratory and those in the field. A stimulus fish seen through the same neutral-density filter therefore appeared darker in the field than in the laboratory.

There are two possible explanations for the observed behavioural differences. (1) The target fish has a preference for the stimulus fish displaying UV colours, and colour cues are responsible for the change in behaviour. The stimulus fish inside the darkest UV+ filter in the field might have been below the detection threshold of the target fish but, once detected, the target fish attacked it for longer than the stimulus fish inside the UV- container. (2) The target fish has a preference for the brighter stimulus fish and therefore luminance cues are responsible for the observed change in behaviour. Thus, a stimulus fish seen through the darkest filter in the laboratory still appeared relatively brighter to the target fish than a stimulus fish inside the UV- filter.

Several findings support the first but not the second explanation. None of the males preferred the brighter stimulus fish when presented with a choice of stimulus fish seen through the dark or clear UV+ filter. Also, because of the reduced radiance in the field, the stimulus fish inside the darkest UV+ filter was barely visible to the experimenter and it is likely that the target fish could not detect this stimulus fish from its shelter. Instead, the target fish had to approach the tube closely to realize that there was a stimulus fish inside. Most of the time, however, the target fish decided which intruder to attack from a distance (often from the entrance of their shelter) and then quickly swam in a straight line from their home to that stimulus fish. The dark plastic viewed from a distance underwater has a mirror-like appearance, which makes the detection of the stimulus fish inside it even more difficult. Consequently, a reduced attack rate would be expected. The important finding supporting the hypothesis that the target fish's behaviour depends on UV coloration rather than brightness is that once the target fish detected the stimulus fish in the dark filter it attacked it for significantly longer than the stimulus fish in the UV- filter.

There are several advantages of using UV light for communication under water. As mentioned earlier, UV colours are visible only over short distances (because of scattering) and are inconspicuous to predators foraging a long way away. An additional benefit of using UV signals for communication is that many predators on coral reefs possess UV-absorbing ocular media (Siebeck & Marshall 2001) so that, even when close to their prey, UV signalling is unlikely to attract their attention. The use of this 'secret communication channel' may therefore enable reef fish such as *P. amboinensis* to communicate effectively with bright UV colours without the associated danger of attracting the attention of predators.

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