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**Colour pattern has no influence on the visual recognition of conspecifics by coral reef fish juveniles**

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## **Abstract**

Once hatched, convict surgeon fish larvae (*Acanthurus triostegus*) spend about two months in the ocean before crossing the coral reef to recruit in the lagoon, usually selecting a specific reef habitat already inhabited by conspecifics. To form groups, juveniles must be able to recognise their conspecifics, even when some have a slightly different morphotype. Here, a virtual reality platform was used that collects juveniles' behavioural reactions to fully immersive controlled visual stimuli. We tested which visual parameters influenced recognition when juveniles had to choose between two virtual shoals of adult convict surgeonfish presented on opposite sides of the aquarium, each with specifically manipulated colour patterns. The results showed that the shape of the adult fish was more important than the patterns and colours for the choice made by the juveniles. These results underline the importance of better understanding the mechanisms of visual recognition in coral reef fish.

## **Keywords**

Virtual reality; visual recognition; animal behaviour, *Acanthurus triostegus*

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## **Introduction**

In many animal species, living in a group is beneficial for survival by allowing the many-eyes effect (Caraco et al. 1980), the confusion of the predator (Foster & Treherne 1981) and the dilution effect (Hamilton 1971). It is also useful for reproduction, as it simplifies the search for mates (Ward & Webster 2016). However, gathering in groups primarily involves the capacity to recognise conspecifics, and distinguish them from potentially dangerous other species. On coral reefs, the great density and diversity of fish present will make the species-specific recognition – and thus the shoaling behaviour – more complex (Myrbergg & Fuiman 2002). For shoaling fishes, there are two main situations where

individuals try to join a shoal: an adult might be far from its shoal and try to find it, or a young individual might try to join one (Keenleyside 1955). Only the second case was addressed in the current study. The intraspecific attraction was already demonstrated as an indicator of species recognition, the first step of single-species shoaling. For instance, the Whitetail dascyllus (*Dascyllus aruanus*) is more attracted by conspecifics than heterospecifics, even when images were desaturated (i.e., deprived of their colours) (Katzir 1981). While we limited the current study to the visual domain, species recognition might well involve other factors such as chemical (Kühme 1963) or acoustic (Spanier 1979) cues.

This study aimed to find the characteristics of the visual species recognition in the coral reef group-living fish *Acanthurus triostegus*. No structural modification of visual patterns that could be cues of another species – such as changing the direction of the stripes or reversing the colours – has ever been tested on group-living fishes. Moreover, while showing real shoals in adjacent aquariums allows visual cues with both static patterns and moving cues, it does not allow for the control of all parameters such as the overlapping of individuals in the field of vision or their orientation in relation to the aquarium glass. We used a virtual reality setup with automated video-based tracking of fish responses to simulated 3D models of animated fish shoals (e.g., Vidal et al. 2023). Such a platform allowed precise control over the experimental execution and stimulation, especially the size, spatial arrangement, and orientation of the simulated individuals. Thus, it was possible to create virtual swimming fish with similar morphology to the existing species but with distinct patterns.

In this work, the study model was the convict surgeonfish *A. triostegus*, a group-living fish that typically has a white, yellowish body with black vertical stripes. This diurnal fish possesses a developed visual colour system (Fogg et al. 2022). Although to our knowledge no studies have investigated visual acuity in *A. triostegus*, we can estimate it by comparing it with other convict fish species. In *Microcanthus strigatus* for example, the visual acuity is 13.04 cycles deg<sup>-1</sup>. It is the higher acuity of the 13 species reported by the authors (Parker et al., 2017). After metamorphosis, individuals live in single species groups, in quiet and dark places, along the coast (Besson 2017). This species also shows a high degree of intraspecific variation in terms of colour patterns. For example, some juveniles appear to be black with yellowish stripes (Besson 2017). Convict surgeonfish also get darker as they are stressed, with dark brown squares appearing between their stripes. Recognition within this species is made more challenging by the similarity in appearance to a number of other sympatric species: *Zebrasoma veliferum* has inverted colours; *Naso unicornis* has a similar body colour, without stripes and *Acanthurus lineatus* is similar but with horizontal instead of vertical stripes. Considering the intra-specific pattern variation in *A. triostegus*, to what extent juveniles can recognise adult conspecifics with different colour patterns? Knowing that *A. triostegus* live in single species groups, we can hypothesise that juveniles are more attracted toward “regular” adults than adults with manipulated colour patterns that could resemble other species. In particular, the good colour visual system and the good visual acuity are hypothesised to increase attraction toward “regular” conspecifics relative to inverted or desaturated colours, or horizontal stripes. However, as changes in the environment can stress fish and alter their visual appearance, another hypothesis is that individuals able to recognise variation within the species might have been selected. If this hypothesis is true, we would have no difference in attraction between the various virtual convict surgeonfish shown.

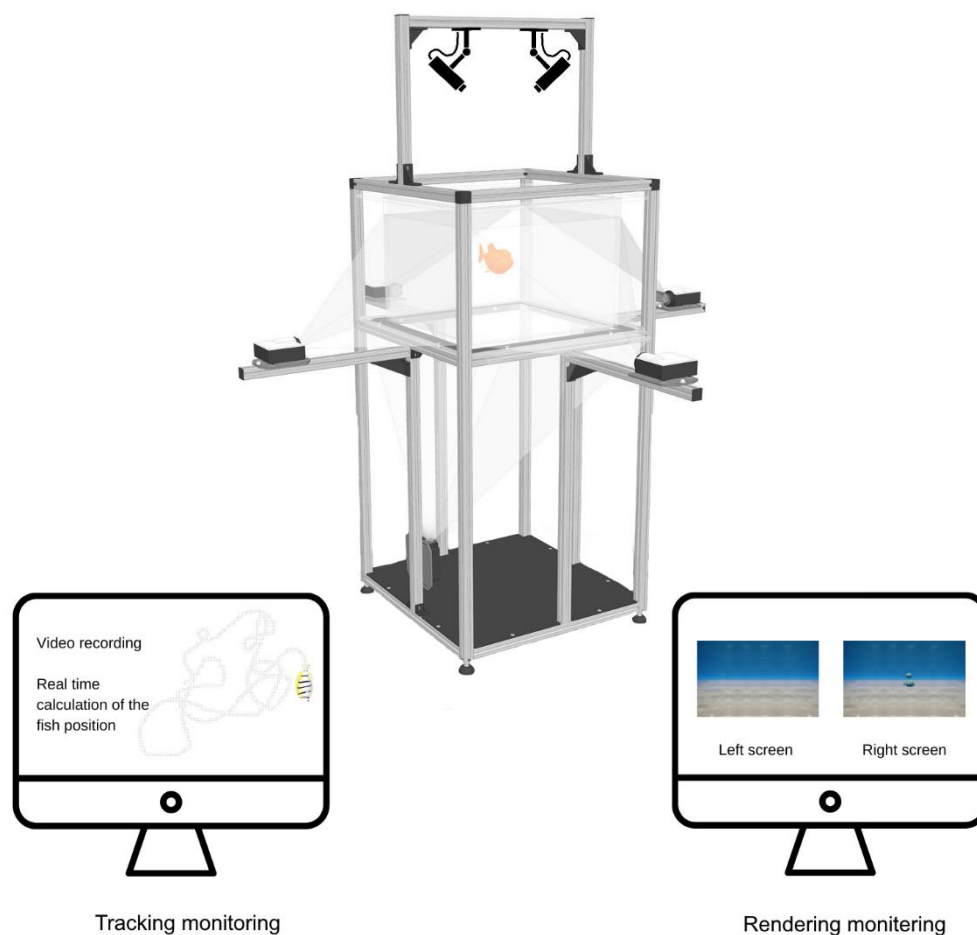
## Methods

### *Juveniles' collection*

A total of 105 juveniles of *A. triostegus* were captured using hand nets, in the nursery area on the fringing reef (northwest coast of Moorea Island, French Polynesia, 17°2'54.3"S 149°5'54.6"W) (as in Fr  d  rich et al. 2012). Individuals were identified using a combination of their size ( $32 \pm 2$  mm) and the colour, number and orientation of their stripes (Holzer et al. 2017). Juveniles were then transferred into acclimatisation aquariums at the CRIOBE research station, filled with UV-sterilized and filtered seawater (10-  m filter), following the holding methods of Besson (2017). Juveniles were tested the day after their capture.

### *Virtual reality setup*

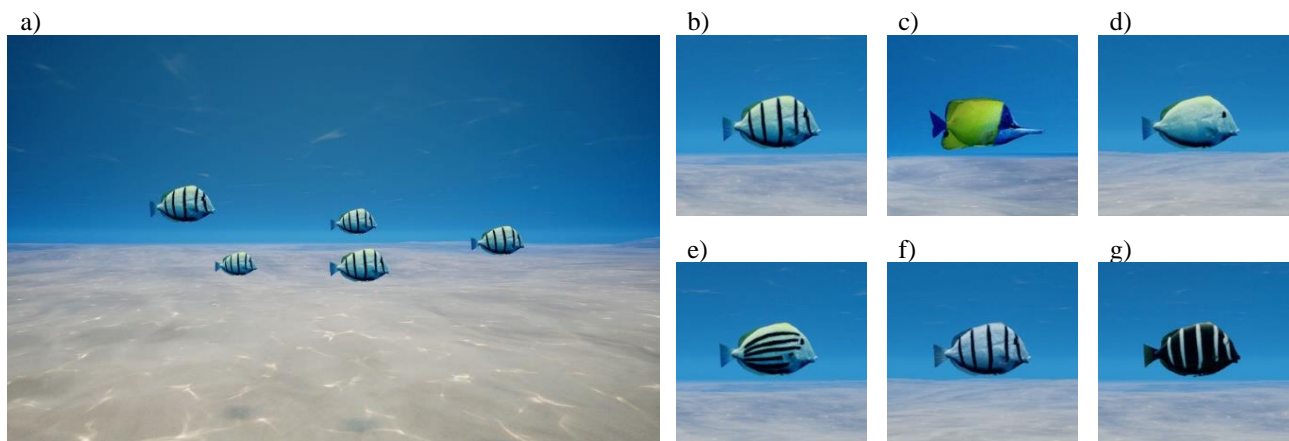
The experimental setup was composed of three connected modules: the focal smaller aquarium freely suspended inside the main aquarium, the rendering module which projected the interactive 3D virtual environment depicting a subaquatic natural scene with fish shoals, and the tracking module which recorded juveniles' behaviour in real-time. Juveniles were swimming in the focal aquarium (Fig. 1; more details in supplementary materials).



**Fig. 1** Experimental setup with the main aquarium, the rendering system (computer, five video projectors and screens), the tracking system (computer, and two cameras). The focal aquarium suspended in the main aquarium is not shown here.

### Experimental protocol

The protocol was designed to identify the role of colours and stripe orientations in the recognition of adult convict surgeonfish by juveniles. Groups of juveniles were transported from the acclimatisation aquarium to the experimental room, where they were kept in the dark for 30 minutes in order to reduce their stress. Each individual was then tested separately in the setup. Individuals were given four minutes to acclimate, the last 60 seconds of which were recorded. Juveniles were tested on six successive treatments, each consisting of a pair of virtual fish shoals presented on opposite sides of the main aquarium. The reference stimulus was a virtual shoal of conspecifics (convict surgeonfish with regular shapes and colour patterns, Fig. 2b), which was displayed on one side with the appearance of being 94 to 164 cm from the centre of the aquarium. As fish usually keep a distance of about 0.5 to 1 body length to other shoal members (Pitcher & Parrish 1993), virtual models were simulated far enough from the focal fish for it to feel apart from the virtual shoal in any location of the aquarium. We controlled for side bias by alternating the side that the reference shoal was presented on. The test stimulus, displayed on the opposite side at the same distance, was either nothing (no shoal control) or one of the five following virtual shoals (Fig. 2c-g): conspecific-sized control fish (butterflyfish, *Forcipiger longirostris*), convict surgeonfish without black stripe, convict surgeonfish with horizontal stripes, convict surgeonfish with desaturated colour pattern and convict surgeonfish with inverted colours (yellowish stripes over a black body). All presented shoals consisted of five virtual fish swimming in place, with the same texture manipulation and with identical spatial distributions (Fig. 2a). Stimuli were presented in an infinite sandy environment with animated caustics. The no shoal control corresponded to this background presented without a shoal. The order of treatments for each juvenile was determined by a Latin square (Grant 1948) to cancel presentation order biases. Treatments lasted 60 seconds and were interleaved with 60 seconds re-acclimatisation periods with only sand. Each time, the fish shoals emerged by fading in the environment in a couple of seconds. The position of each tested juvenile was recorded and analysed as a proxy of attraction behaviour by computing the proportion of time spent close to each of the presented stimuli. The tracking system performance was assessed. On average, detection was not successful in only 2.2 % of the video frames (Fig. A1 in the Appendix).



**Fig. 2** a) Spatial arrangement of the presented virtual individuals (apparent size differences are due to the projection perspective), and the six colour pattern and model manipulations tested: b) regular adult surgeonfish, c) butterflyfish, d) convict surgeonfish without stripes, e) convict surgeonfish with horizontal stripes, f) convict surgeonfish with desaturated colours, and g) convict surgeonfish with inverted colours. The background represented a sandy subaquatic environment with animated ripples on the surface and their corresponding caustics on the ground (as in Vidal et al. 2023). For the estimated perceived spatial resolution of the juveniles, see Fig. A3 in the Appendix.

### Ethics note

This study was carried out in accordance with the guidelines of the French Polynesia committee for animal ethics, and the experiments were approved by the CRIOBE-IRCP animal ethics committee. This study did not involve endangered or protected species.

### Data analysis

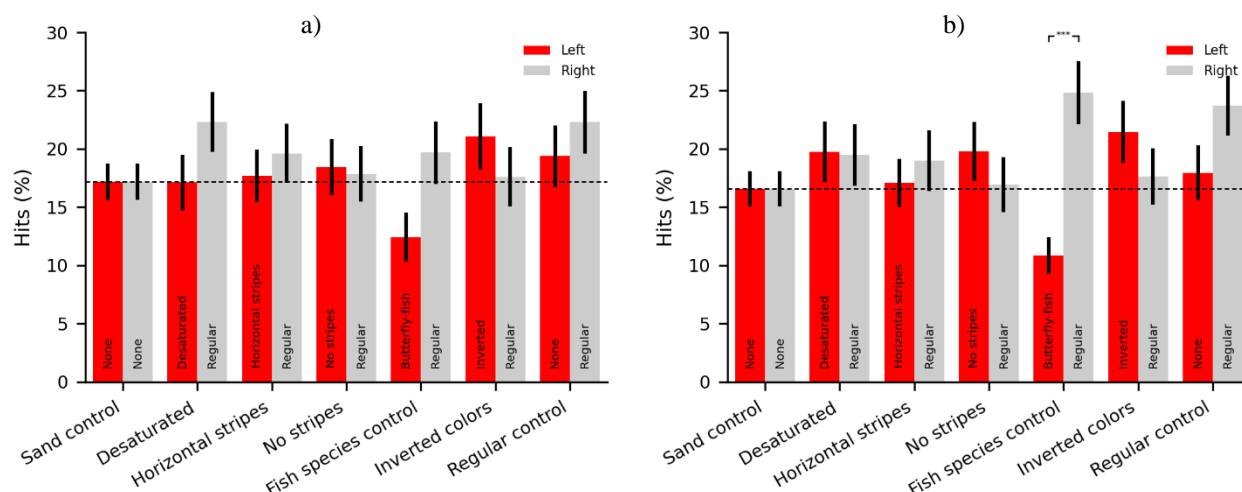
A dedicated Python code performed the pre-processing, statistical analyses, and plots (see Vidal et al. 2023 for details). Individuals who stayed in the same corner of the aquarium for at least three conditions were considered as non-respondent, as they did not see both stimuli, and were excluded from the analysis (see an example in Fig. A2 in the Appendix), leaving a total of  $N = 98$  fish for analyses. Each treatment was split into two periods, the first regarded as the initial assessment and identification of the presented virtual fish, and the second as the behavioural response relative to the preferred stimulus. For each treatment and tested fish, hit proportion was defined as the proportion of time spent in the 2.5 cm area near the aquarium sides where each stimulus was displayed (reference and test). Lower values indicate that juveniles spent little time in the considered area and higher values that they spent more time. A paired Wilcoxon test was then performed to compare hit ratios between the two sides.

### Results & Discussion

The left and right sides in the initial sand control period were not different (t-test,  $t = 0.66$ ,  $p = 0.510$ , Fig. A4 in the Appendix) preventing any preference for either side. During the assessment phase, juveniles spent the same amount of time on either side of the aquarium (Fig. 3a). In this first part of the test, juveniles explored their environment and did not show any preference between the different surgeonfish models presented (Table 1). During the last 30 seconds, juveniles spent significantly more time close to conspecific shoal than to the butterflyfish shoal (Wilcoxon's test,  $t = 996$ ,  $p < 0.001$ , Fig. 3b). For the other experimental conditions, juveniles spent the same amount of time close to the normal convict surgeonfish shoal than to the ones with manipulated colour patterns (Table 1). Finally, juveniles tend to spend more time close to the adult convict fish side than to the control side with no shoal ( $t = 1683$ ,  $p = 0.104$ ).

**Table 1** Paired Wilcoxon tests results for both phases: the assessment phase from 0 to 30 seconds and the decision phase from 30 to 60 seconds. Significant values are shown in bold.

Condition	Phase	$t$	$p$
Desaturated	[0; 30]	1701	0.163
	[30; 60]	1799	0.757
Horizontal stripes	[0; 30]	1773	0.673
	[30; 60]	1796	0.890
No stripes	[0; 30]	1772	0.806
	[30; 60]	1677	0.510
Fish species control	[0; 30]	1229	0.061
	[30; 60]	996	<b>&lt; 0.001</b>
Inverted colours	[0; 30]	1565	0.418
	[30; 60]	1649	0.340
Regular	[0; 30]	1961	0.389
	[30; 60]	1683	0.104



**Fig. 3** Hits ratio representing the proportions of time spent by *A. triostegus* juveniles ( $N = 98$ ) in the 2.5 cm area near the test stimulation (red bars) or reference stimulation (grey bars), during a) the first 30 seconds, and b) the last 30 seconds of the treatment. Three stars indicate a significant difference ( $p < 0.001$ ). Dashed lines indicate the average time spent by juveniles close to either side. Error bars indicate the standard errors.

In coral reefs, only seven studies have explored the importance of visual cues during the habitat selection of coral reef fish larvae (for a review, see Barth et al. 2015). Selecting a habitat implies recognising the right species to shoal with. Our results tend to emphasize that juveniles are more likely to recognise adults by their shapes than their pattern and colour. Indeed, when faced with a choice between the butterflyfish and the convict surgeonfish, juveniles preferred conspecifics whereas when faced with two different patterns of surgeonfish, they did not elicit any significant preference. However, as the butterflyfish model happens to be the brighter and *A. triostegus* juveniles have a developed visual system (Fogg et al. 2022), we cannot exclude the possibility this same-size species is more aversive for the juveniles than the conspecifics are attractive. Nevertheless, *A. triostegus* juveniles might have evolved to recognise all adult conspecifics, even those with altered colour patterns, such as an inversion of colours following stressful events (Besson 2017). Alternatively, while juveniles have good colour discrimination hence discriminating the bright yellow of butterflyfish from the non-yellow of conspecifics, they may not have a good enough visual resolution to discriminate the level of pattern variation we show them. Even if *A. triostegus* have a good visual acuity similar to *Microcanthus strigatus*, we do not know at which age they acquire it. Juveniles at this developmental stage might not have the capacity to recognise conspecifics, and only discriminate similar-sized harmless species to shoal with to get group protection. Juveniles might need to shoal with their conspecifics especially for mating. To discriminate between these two hypotheses, we could do this experiment with older surgeonfish, even though it would require a bigger set up. As our results did not emphasize any discrimination with inversed colour patterns, juveniles either recognise these virtual fish as conspecifics or at least as belonging to a harmless species. In the future, we could try improving how juveniles' response is measured. We could force juveniles to choose between the two groups presented by adding a repulsive stimulus such as a predator, a bluefin jackfish (*Caranx melampygus*) for example, and make the shoal swimming away. Vidal et al. (2023) found that virtual shoals swimming along a linear path elicit a bigger response from convict surgeonfish juveniles. Thus, we could see which shoal is followed by the juveniles. Finally, to settle if juveniles indeed recognise adults by their shape, more experiments need to be carried out in which the regular colour pattern of an adult convict surgeon fish is used on 3D models of different fish species such as the copperband butterflyfish (*Chelmon rostratus*), the short-nosed unicornfish (*Naso brevirostris*), or the clown anemonefish (*Amphiprion ocellaris*). For

instance, to reject the hypothesis that butterflyfish recognition relies mostly on brightness or amount of yellow colour, a control experiment could use virtual 3D models with the shape of butterflyfish but with the colour pattern of convict surgeon fish.

## Statements and Declarations

### *Conflict of interest*

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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