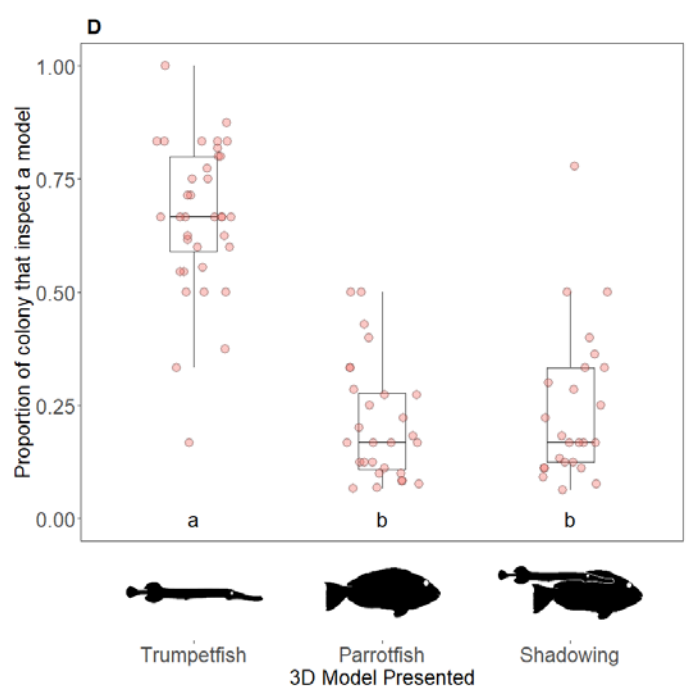
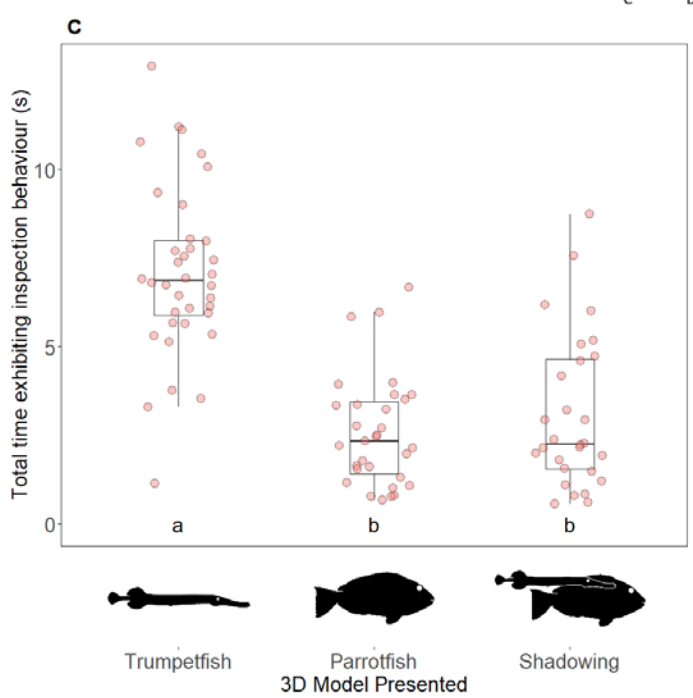
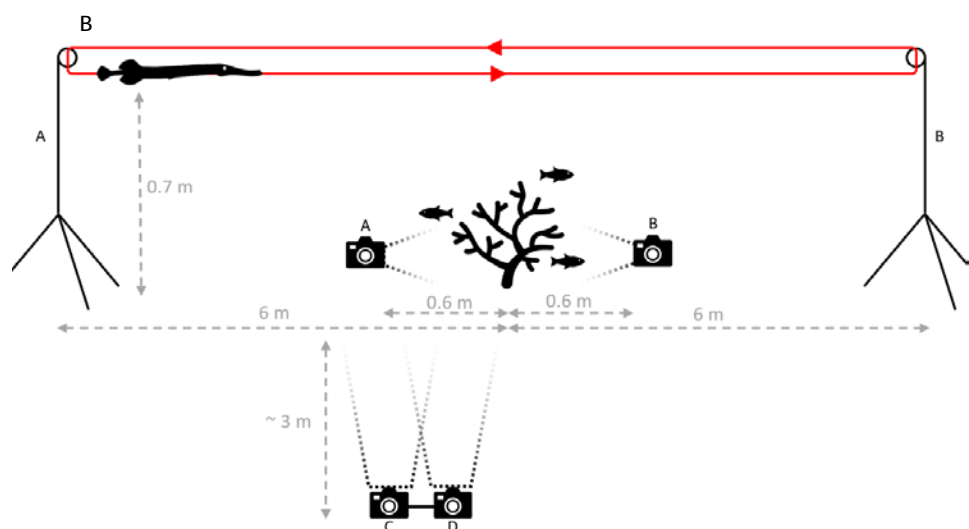
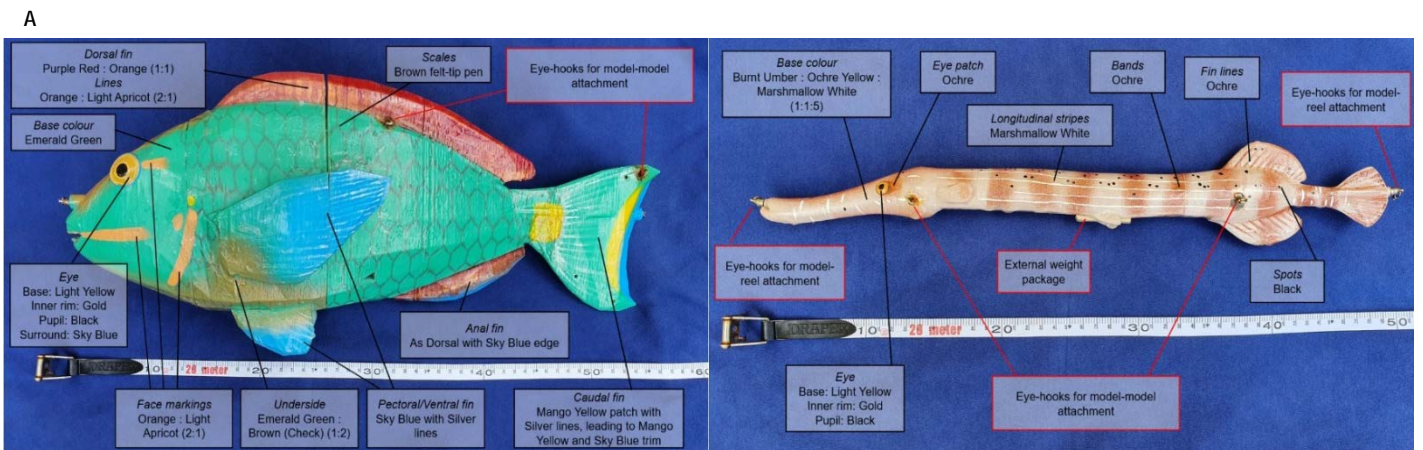


**Supplemental Information:**

**Predatory trumpetfish conceal themselves from their prey by swimming alongside other fish**

Samuel R. Matchette, Christian Drerup, Isla Keesje Davison, Stephen D. Simpson, Andrew N. Radford, and James E. Herbert-Read



### Figure S1. The 3D models, experimental set-up, and additional measures of damselfish behaviour

(A) An example of a model parrotfish (above) and a model trumpetfish (below) used in the experiment, each labelled with the paint colours (and ratios, where necessary) that were used to create their appearance. All colour names are those derived from Magicfly (Central, Hong Kong; [www.imagicfly.com](http://www.imagicfly.com)). (B) An infographic detailing the experimental setup. For each presentation, researcher A attached a model to the nylon line, at which point researcher B hand-reeled this model along the nylon line past the focal colony. Cameras A and B were positioned next to the colony to record the behavioural responses of the damselfish, while a stereo camera rig (camera C and D) was positioned further away to extract the distance between the model and the colony *post-hoc*. (C) The total duration of time (s) that at least one individual spent inspecting each treatment for each trial. (D) The maximum proportion of a colony that were observed inspecting each treatment. This proportion was calculated using the maximum number of fish observed exhibiting inspection behaviour during the presentation and the maximum number of fish observed on the colony. For both panels C and D, the box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range. The hollow circles represent the raw data points. Letter labels below the boxes denote the pairwise comparisons between treatments, whereby treatments that have the same letter label did not statistically differ. The pairwise comparison between groups was computed using the *emmeans* and *clt* functions from the *emmeans* package<sup>S1</sup>.

## Supplemental Experimental Procedures

### 3D Model Generation

An individual digital model of a stoplight parrotfish (*Sparisoma viride*; product ID: 1099252) and a trumpetfish (*Aulostomus maculatus*; product ID: 1251130) were acquired from TurboSquid (TurboSquid, New Orleans, LA, USA; [www.turbosquid.com](http://www.turbosquid.com)), an online platform for the distribution of graphical models. We then manipulated both digital models using Blender (Blender v.3.1.2, Amsterdam, Netherlands; [www.blender.org](http://www.blender.org)) and FreeCAD (FreeCAD v.0.19; [www.freecadweb.org](http://www.freecadweb.org)) to prepare them for 3D printing using PrusaSlicer (v2.3.3; Prusa Research, Prague, Czech Republic; [www.prusa3d.com](http://www.prusa3d.com)). Due to the size and time limitations of the printing process, the primary manipulation involved splicing each digital model into subsections: the trumpetfish comprised three subsections, whereas the parrotfish comprised five subsections. Printing was completed using an Original Prusa i3 MK3S+ (Prusa Research, Prague, Czech Republic; [www.prusa3d.com](http://www.prusa3d.com)). Each subsection was printed using white 1.75 mm PETG filament (TecBears, Kwun Tong, Hong Kong; [www.tecbears.com](http://www.tecbears.com)). Following printing, we re-joined the subsections using a bespoke 3D-printed dowel system to create a physical 3D model (hereafter, 'model') of each species. The final dimensions of each model fell within the natural size ranges for these species<sup>52</sup>: the parrotfish model had a total length of 53 cm, while the trumpetfish had a total length of 46 cm. To reduce pseudo-replication, we printed three models of each species.

We painted all models by hand using non-toxic acrylic paint (Magicfly, Central, Hong Kong; [www.imagicfly.com](http://www.imagicfly.com); Figure S1A), using fish identification books as a visual guide (see references<sup>52,53</sup>). The parrotfish was painted to resemble a terminal phase stoplight parrotfish, which is a common species shadowed by trumpetfish in the Caribbean<sup>54</sup>, whereas the trumpetfish was painted to resemble the common reddish-brown morph of this species<sup>53</sup>. To waterproof the painted models, we applied two successive coats of non-toxic waterproof epoxy resin (mixed 1:1 with epoxy resin hardener) to each model. To accommodate the connection of a trumpetfish model to a parrotfish model (during the presentation of a shadowing trumpetfish), we screwed four (two mirrored each side) threaded hook eyes (5 mm diameter) into each model (Figure S1A). Two further hook eyes (5 mm diameter) were glued at each end of the models, which facilitated the connection to the nylon reeling line.

### Experimental Set-Up

We presented these models *in situ* to 36 colonies of bicolor damselfish (*Stegastes partitus*) across three locations (12 colonies at each) in Curaçao, Netherland Antilles. A colony was defined as a group containing a minimum of four individuals that inhabited a semi-isolated structure (typically a fire coral, an anemone, or a mound of coral rubble). Three locations were chosen for their shallow reef habitats, the reef accessibility, and the sheltered nature of each of the bays: Playa Cas Abou (N 12.2283, W -69.0922), Playa Kokomo (N 12.1614, W -69.0046) and Playa Porto Mari (N 12.2190, W -69.0863). We visited each location once every three experimental days, with the order visited organised in a randomised block design. We tested two colonies of damselfish each day. The mean ( $\pm$  1SD) depth of damselfish colony was 3.26 m  $\pm$  0.81 m, the mean ( $\pm$  1SD) number of fish within each colony was 9  $\pm$  6, and all colonies at a given location were at least 15 m apart. Presentations were conducted between 9:00 am and 11:00 am by the same two

researchers on SCUBA. All procedures were approved by the University of Cambridge Animal Welfare and Ethical Review Body (OS2021/08). To finalise the experimental procedure prior to the experiment proper, we conducted a small number of pilot trials with damselfish colonies in Piscadera Bay (N 12.1223, W -68.9695). During these trials, we observed that damselfish returned to 'normal behaviour' (for example, low alignment between individuals, swimming steadily above their shelter) within a minute of a passing model, which is reflective of the acclimation times observed for similar reef fish<sup>55</sup>. We did not observe any behavioural response from the damselfish to the use of elasticated 'tank bangers', commonly used as a means of communication between SCUBA divers and, here, to signify the start of a model presentation. In addition, the extended presence of the researchers did not appear to alter the behaviour of the damselfish: the total time spent nearby and the distance maintained from the colony reflect those outlined in a prior study, which highlighted how reef fish habituate to the presence of divers<sup>56</sup>.

For each colony, we placed two tripods (Emart; [www.emartus.com](http://www.emartus.com)) 12 m apart, with the colony situated halfway between the two (Figure S1B). Each tripod was weighed down with 10 lb of weight cable-tied to its base. A carabiner was attached at the top of each tripod, 0.7 m from the seabed. The reeling line—a 24 m thread of clear 1 mm nylon with snap clips attached at each end—was then looped between each tripod, passing through both carabiners in turn, and reattached via the snap clips. We positioned two GoPros (A and B; Hero 10; 3840 x 2160 px, 30 fps, wide angle; GoPro, San Mateo, CA, USA; [www.gopro.com/en/us/](http://www.gopro.com/en/us/)) ~0.6 m on either side of the colony, each with a view of the colony and the opposite tripod. These cameras were positioned to capture the behavioural responses of the damselfish. We attached two further GoPros (C and D; Hero 10; 3840 x 2160 px, 30 fps, linear angle) to a stereo camera rig (left and right; Neewer; Shenzhen, Guangdong, China; [www.neewer.com](http://www.neewer.com)), which, with the addition of short PVC pipe elbow, was free-standing. We positioned this stereo camera rig ~3 m from the colony, such that it could capture the approach of the model. Recording was started from each camera as it was positioned in turn. A minimum of 5 minutes separated the completion of experimental set-up and the initiation of treatment presentations.

Each presentation followed the same process. Researcher A was positioned at one tripod, together with the unrepresented models, whereas researcher B was positioned at the opposite tripod. Researcher A then temporally disconnected the snap clips of the reeling line so that they could be attached to the anterior and posterior hooks of the first model. A five-minute acclimation period then started. After acclimation, researcher B used a tank banger to generate two short sharp tones. Although mainly to signify the start of the presentation to the other researcher, these tones also simultaneously enabled the synchronisation of all four cameras. Following the tones, researcher B slowly pulled the model (using the connected reeling line) from its starting position with researcher A towards researcher B, passing over the top of the colony enroute. Models were presented to each colony at a height of ~0.7 m. Researcher B aimed to pull each model at a steady and consistent speed for the total presentation duration. Once the model reached researcher B, the presentation was deemed to be over, and the model was detached from the reeling line. The next model was then attached to the reeling line by researcher A, before starting a new five-minute acclimation period. This process was repeated until all three models had been presented. On completion of the third presentation, we presented a 7 x 9-square checkerboard with 25 mm squares to the stereo cameras; this was used to calibrate these cameras during video processing. The model replicate (i.e., 1, 2 or 3: there were three available models for each species), the order in which treatments were presented, and the side in which the trumpetfish appeared on the

parrotfish in the shadowing treatment (i.e., left-hand-side or right-hand-side) were organised in a randomised block design.

### *Video Processing*

For each colony, we left all four cameras (A-D; two focal, two stereo) to film continuously until all three model presentations had been completed. It was therefore necessary to synchronise the four videos and identify the start of each presentation. This was achieved using a bespoke script in MATLAB (The Mathworks Inc; Natick, MA, U.S.A.; [www.uk.mathworks.com/products/matlab](http://www.uk.mathworks.com/products/matlab)), which located the synchronisation tones in the audio profile from each raw video. Once synchronised, we then extracted the video segments from each camera that related to each presentation, saving each of these as a one-minute-long clip. This was completed for all three presentations for all 36 colonies. An identical method was used to generate clips that contained the calibration board. All subsequent clips were uploaded to Loopy (Loopbio, Vienna, Austria; [www.loopbio.com/loopy/](http://www.loopbio.com/loopy/)).

In Loopy, we collated the clips generated from the focal cameras for behavioural scoring. By combining scores from both viewing angles (A and B), we ensured that all behavioural responses were captured for each presentation. We quantified two types of behavioural response from the damselfish: inspection and avoidance behaviour. Inspection behaviour by a colony was defined as at least one individual aligning with and swimming towards the model (as observed in guppies<sup>57</sup>), whereas an avoidance response involved at least one individual fleeing rapidly towards the colony shelter. Specifically, for each presentation clip, we recorded (a) the duration of time that a colony spends inspecting with the corresponding video frames when this behaviour started, (b) the maximum number of fish observed exhibiting inspection behaviour at a given time, (c) the number of every avoidance response exhibited by fish and the corresponding video frames, and (d) the maximum number of fish observed on the colony overall.

To test the objectivity of scoring inspection and avoidance responses in damselfish, we compared our scores from a subset of presentations to those of an independent observer. The presentation clips used in this task (N = 36) were limited to those from focal camera A (i.e., that did *not* capture the model's approach; Figure S1B) and were trimmed prior to the model treatment arriving in shot: at no point during the clip could the observer see the model treatment being presented. The resultant clips were renamed (with an arbitrary number), and their order shuffled. The principal investigator rescored these clips – to ensure scores related only to behaviours that could be viewed from this camera angle and during this timeframe – before being scored by the independent observer. Scores pertained to whether an inspection event or avoidance response occurred (1 or 0) and, if so, the time that this response was observed in the video (frame number). There was no significant difference between scorers for the number of inspection events (Kruskal-Wallis:  $H_1 = 0.01$ ,  $p = 0.918$ ) or avoidance responses observed ( $H_1 < 0.01$ ,  $p = 0.983$ ), nor was there a significant difference in time between scorers for the events observed (inspection events:  $H_1 < 0.01$ ,  $p = 1.000$ ; avoidance responses:  $H_1 = 0.06$ ,  $p = 0.806$ ). The scoring of the behavioural responses by damselfish were therefore deemed to be objective.

The clips that were generated from the stereo camera were used to extract the distance of the model from the colony at which point the first inspection event and avoidance response were exhibited. We first generated a calibration

profile for the colony-specific calibration clips. We then manually annotated the position of the model and the colony within the video frames that corresponded to the first avoidance response and the first inspection event. Annotations were completed for the same frame in both the left and the right stereo clips. Loopy then used the corresponding calibration profile to extract the 3D position of each annotation in space, generated as a real-world XYZ coordinate. We then used a bespoke MATLAB script to calculate the distance between the model XYZ and the XYZ coordinates corresponding to the closest edge of the colony (to the model's approach). If the first inspection ( $n = 12$ ) or avoidance response ( $n = 1$ ) occurred when the model was not fully in shot for either the left or right camera (i.e., when responses were particularly early or late in the presentation), the distance between the model and the colony was calculated using annotations within the first (when early) or last (when late) frame that the model was fully visible in both cameras and a 3D position was obtainable. We used a similar method to assess the mean speed and the variation in speed that the model was reeled past the colony. In this case, we annotated a total of eight frames that spanned the model's journey towards and beyond the colony and calculated the speed (m/s) between successive annotations. The mean and variation (i.e., standard deviation) of these model speeds were then stored for each presentation for later analysis.

### *Statistical Analyses*

All statistical analyses were performed in R v. 3.3.2 (R Foundation for Statistical Computing, [www.R-project.org](http://www.R-project.org)), where we used a combination of linear and generalised linear mixed models (functions *lmer* and *glmer* in the *lme4* package<sup>58</sup>). To check the assumptions of each model, the DHARMA package<sup>59</sup> was used to interpret the dispersion and distribution of the residuals. Independent linear mixed models (Gaussian error structure) were used to assess the effect of presentation type (nominal fixed effect) and presentation order (ordinal fixed effect) on the total time that a colony spent inspecting (square-root transformation), the model distance for the first inspection event, the number of avoidance responses (square-root transformation), and the model distance for the first avoidance response. Independent generalised linear mixed models were used to assess the effect of presentation type (nominal fixed effect) and presentation order (ordinal fixed effect) on the maximum proportion of the colony that were observed inspecting (binomial error structure). For all models, colony ID was provided as a nested random effect within location, denoted in *lme4* syntax as (1 | location / colony ID). In each case, the change in deviance between a model with and without the fixed effect was tested against a chi-square distribution with degrees of freedom equal to the difference in degrees of freedom between the models. In cases where treatment had a significant effect on a response measure, we used the *emmeans* function (with "Tukey" contrasts) from the *emmeans* package<sup>51</sup> to compute the pairwise differences between treatments. Based on these pairwise comparisons, the *clt* function<sup>51</sup> could then be used to group each treatment depending on their relative statistical effect (denoted by a letter label in the figures).

To avoid overfitting the mixed models, we used an unpaired Kruskal-Wallis test to assess how each response measure may have been affected by the position of the trumpetfish when presented as the shadowing model (i.e., on the left side or right side of the parrotfish). We found that there was no significant effect of trumpetfish position on any of the response measures outlined above (Data S1B). Although this was a control measure in the current study, we believe that this aspect warrants further investigation: trumpetfish frequently shift their position on the shadowed species

during shadowing events (Video S1), which may be indicative of an individual optimising their occlusion from their targeted prey.

Two additional linear mixed models were used to compare the mean model speeds (log transformation) and the variation in model speeds (i.e., standard deviation; log transformation) for each presentation type (nominal fixed effect). Colony ID was provided as a random effect. Overall, speeds ranged from 0.08 to 0.59 m/s across all treatments. Specifically, the mean ( $\pm 1$  SD) model speeds of the treatments were: non-shadowing trumpetfish = 0.24 m/s  $\pm$  0.11; parrotfish = 0.22 m/s  $\pm$  0.11; shadowing trumpetfish = 0.22 m/s  $\pm$  0.14. The mean model speeds for our 3D parrotfish model fell within the range of known swimming speeds for parrotfish species<sup>S10, S11</sup>. We found that neither the model speed (LMM: LRT = 4.80, df = 2,  $p$  = 0.091) nor the variation in model speed (LMM: LRT = 0.12, df = 2,  $p$  = 0.941) differed significantly between presentation type.

### Data Availability

Data can be found on the data depository, figshare (<https://doi.org/10.6084/m9.figshare.22764866>).

### Author Contributions

Conceptualisation, S.R.M., C.D., I.K.D., S.D.S., A.N.R., and J.E.H-R.; Methodology, S.R.M., C.D., I.K.D., S.D.S., A.N.R., and J.E.H-R.; Investigation, S.R.M. and C.D.; Formal analysis, S.R.M., A.N.R. and J.E.H-R.; Writing (original draft), S.R.M.; Writing (review and editing), S.R.M., C.D., I.K.D., S.D.S., A.N.R., and J.E.H-R.; Funding acquisition, S.R.M. and J.E.H-R.

### Supplemental References

- S1. Lenth, R., Buerkner, P., Herve, M., Love, J., Riebl, H., and Singmann, H. (2020). Estimated Marginal Means, aka Least-Squares Means. Comprehensive R Archive Network (CRAN). Retrieved from <https://cran.r-project.org/package=emmeans>.
- S2. Humann, P., and DeLoach, N. (2008). Reef Fish Identification: Florida, Caribbean, Bahamas Third. (Jacksonville, FL: New World Publications).
- S3. DeLoach, N., DeLoach, A., and Humann, P. (2019). Reef Fish Behaviour: Florida, Caribbean, Bahamas Second. K. Marks, ed. (Jacksonville, FL: New World Publications).
- S4. Aronson, R. (1983). Foraging behavior of the west Atlantic trumpetfish, *Aulostomus maculatus*: use of large, herbivorous reef fishes as camouflage. Bull. Mar. Sci. 33, 166–171.
- S5. Nanninga, G.B., Côté, I.M., Beldade, R., and Mills, S.C. (2017). Behavioural acclimation to cameras and observers in coral reef fishes. Ethology 123, 705–711. 10.1111/eth.12642.



- S6. Nedelec, S.L., Mills, S.C., Radford, A.N., Beldade, R., Simpson, S.D., Nedelec, B., and Côté, I.M. (2017). Motorboat noise disrupts co-operative interspecific interactions. *Sci. Rep.* 7, 1–8. 10.1038/s41598-017-06515-2.
- S7. Godin, J.G.J., and Davis, S.A. (1995). Who dares, benefits: predator approach behaviour in the guppy (*Poecilia reticulata*) deters predator pursuit. *Proc. R. Soc. London. Ser. B Biol. Sci.* 259, 193–200. 10.1098/RSPB.1995.0028.
- S8. Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 1–48. doi:10.18637/jss.v067.i01
- S9. Hartig, F. (2020). Package DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models. Comprehensive R Archive Network (CRAN). Retrieved from <https://cran.r-project.org/package=DHARMA>.
- S10. Korsmeyer, K.E., Steffensen, J.F., and Herskin, J. (2002). Energetics of median and paired fin swimming, body and caudal fin swimming, and gait transition in parrotfish (*Scarus schlegeli*) and triggerfish (*Rhinecanthus aculeatus*). *J. Exp. Biol.* 205, 1253–1263.
- S11. Fulton, C.J. (2007). Swimming speed performance in coral reef fishes: Field validations reveal distinct functional groups. *Coral Reefs* 26, 217–228. 10.1007/S00338-007-0195-0/TABLES/3.