A new model study species: high accuracy of discrimination between individual freckled hawkfish (*Paracirrhites forsteri*) using natural markings

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Abstract

Variation between distinct natural markings of freckled hawkfish (*Paracirrhites forsteri*) could allow *in-situ* identification of individuals from underwater photography. Receiver Operating Characteristic (ROC) analysis was used to assess the ability of I^3S software to assist in discriminating between images of *P. forsteri* individuals. Our results show a high discriminant ability of I^3S to differentiate between unlike individuals and identify images of the same individual. The ability to use automatic computer-aided assistance in this species will allow future research to explore behaviour and movements of individuals in the wild.

Key words: I^3S, model species, natural marks, *Paracirrhites forsteri*, population dynamics, ROC analysis

Reliable identification of individuals in the wild can provide valuable insight into animal behaviour, spatial distribution and habitat use (Bertulli *et al.,* 2015). The ability to identify individuals over time also allows population demographic structure and dynamics to be inferred (Araujo *et al.,* 2017). Developing an understanding of the behaviour, spatial distribution and population ecology of a species is essential in order to design appropriate management and conservation strategies (Benjamins *et al*., 2018; Couturier *et al.,* 2012).

Identification techniques used to study wild populations often rely on mark and recapture programmes requiring invasive tagging methods such as fin clipping, or external data logger fitting (Walker *et al.,* 2012). As an alternative, non-invasive identification techniques rely on natural markings of an individual. Using differences in appearance, photographic identification has allowed individuals to be identified in several marine species including whale sharks (*Rhincodon typus*), manta rays (*Mobula alfredi*) and white sharks

(*Carcharodon carcharias*) (Marshall *et al.,* 2011; McCoy *et al.,* 2018; Towner *et al.,* 2013).

Automated photographic identification software enables faster analysis of photo databases and allows analysis of databases which would be too large to process manually (Hillman *et al*., 2003). Interactive Individual Identification System (I^3S) is commonly used to differentiate individuals based on pigmentation spot patterns (González-Ramos *et al.,* 2017; Keeping *et al*., 2019; Van Tienhoven *et al*., 2007).

The spot pattern of the freckled hawkfish (*Paracirrhites forsteri)* is a potentially suitable natural marker for identifying individuals of this species. *P. forsteri* (Order: Perciformes Family: Cirrhitidae) is a small mesopredator (22 cm maximum standard length) inhabiting shallow coral reefs in the Indo-West Pacific, Red Sea and East Africa (Lieske and Myers, 2002). This species has been observed to show colour polymorphism throughout its range and has a distinctive “freckle” spot pattern on the sides of the head and the front of the body (Coker *et al*. 2017). *P. forsteri* is a useful model species because it inhabits readily accessible, shallow sunlit waters and has relatively small territory sizes.

The ability to identify individuals of *P. forsteri* would greatly aid *in-situ* studies of growth rates, population dynamics, habitat use and individual behavioural traits of a wild fish species. The territorial behaviour of *P. forsteri* is ideally suited for testing I^3S as a platform to identify individuals and support future research. Here, we aim to assess the efficacy and accuracy of computer aided photo ID to identify individuals of *P. forsteri.*

Our study was observational, using photography to record images of wild fish in-situ. No fish were collected, killed or underwent any surgical or other procedure that could cause severe distress or lasting harm.

A database of 268 images of *P. forsteri* was created from photographs taken over a four-week period at the Abu Sautir Reef (Red Sea, Egypt, 26°12’19.33” N 34°13’11.48” E) (Figure 1a). The site consisted of north and south sloping reef walls enclosing a central sandy inlet allowing shore diving access. Images were captured opportunistically by University of Glasgow students and Open Ocean Dive Centre staff between 5 m and 10 m depth. Divers were recreationally trained and no further specific training was given prior to data collection. Each time a *P. forsteri* individual was encountered, three photographs were taken of its left operculum using Fujifilm, XP130 and Finepix Real 3D W3 digital cameras. Photographs were only discarded if focal fish spot patterns was not fully visible in the frame (i.e. obscured by coral substrate) and the focal fish spot pattern was not perpendicular.

To assess the ability of I^3S classic (V4.02) to correctly match images of *P. forsteri* a digital fingerprint file was built. Three standardised reference points were selected to frame the identification area and 12-30 user-selected markers were placed (Figure 1b). Reference points and markers were selected as described in the I^3S manual (Hartog and Reijns, 2007). No photographic manipulation or editing was conducted, and images were viewed at 100% zoom in the software. An initial database for *P. forsteri* was built from images of fish identified as “new” individuals if they were photographed on opposite sides of a fissure in the reef (> 55 m apart) and by manual identification. *P. forsteri* has been shown to maintain territories of ~290 m^2 (Kadota and Sakai, 2016). Although territories are likely to be non-uniform shapes, *P. forsteri* individuals occupy the same area throughout the day with the distance of any movements likely to be shorter than the 55 m separation distance adopted here.

To assess the effectiveness of I^3S for matching images of *P. forsteri*, a test set of 134 images was presented to the database. I^3S calculates a distance metric (DM) as the sum of the distances between each spot pair divided by the number of spot pairs squared. DM values are calculated between a newly presented image and all other images in the database. More likely matches have a lower DM and all images in the database are presented in ascending DM order. Correctly matched images (true positives) were identified as the same individual if the photographs had been taken consecutively in the series of three taken during each fish encounter. Because I^3S makes no distinction between matching and non-matching images, only providing an DM, incorrect matches are false positives and were identified when the photographs of *P. forsteri* were taken at opposite sides of the reef (as described above). The true and false positives were further confirmed by a thorough visual examination of the images. The flow diagram (Figure 1c) illustrates the image matching protocol.

A receiver operating characteristic (ROC) curve was used to compare the true positive rate with the false positive rate for the test images (Figure 1d). Youden's index was used to identify the point on the ROC curve which maximised the true positive rate while minimising the false positive rate. This point occurs on the ROC curve at the greatest distance from the diagonal line (intercept=0, slope =1) and was used to identify the optimal DM cut off value. We calculated the Area Under Curve (AUC) as an indicator of the effectiveness of I^3S software at identifying matching images and to be able to compare our results with other studies using a similar method (Burns, 2013). High AUC values close to 1 demonstrate better image matching ability of the software. The dot and violin diagram (Figure 1e) graphically represent the range of DM generated through all pairing of individuals known to be the same (1) and known to be different (0), shown on the x-axis. The optimal DM is also displayed as a dashed line on the dot and violin diagram (Figure 1e).

Our results show that the facial spot markings of *P. forsteri* can be used to discriminate accurately between wild individuals. The ROC analysis and AUC of 0.998 showed high discriminant ability using I^3S to assist identifying individual *P. forsteri* with computer-aided photo ID. The optimal DM of 5.065 occurred at a false positive rate of 0% and a true positive rate of 96%. These results indicate that, in this sample group at least, individuals are highly likely to be correctly identified as matching and very unlikely to be confused with another individual.

This study shows that photographic identification is particularly effective at identifying *P. forsteri* individuals. The use of computer-aided photo ID assistance has the potential to speed up studies requiring a database of known individuals. Establishing a threshold DM allows for rapid categorisation of new images as either previously encountered or new individuals. Using a defined threshold DM will allow us to identify *P. forsteri* individuals consistently across studies in multiple locations with a standard protocol for comparable results. The high efficacy of I^3S makes *P. forsteri* an ideal model species for future work requiring the rapid identification of numerous individuals.

Use of I^3S software for photographic identification of *P. forsteri* in long-term or multi-year studies of individuals will be dependent on the temporal stability of pigmentation spot patterns in this species. Successful individual identification may become biased with any changes in natural markings over the course of an individual's lifespan. Previous studies have demonstrated that permanent melanophore spot patterns in Atlantic salmon (*Salmo salar*) enable individual identification over extended periods of their life history (Stien *et al*. 2017). Ontogenetic changes in *P. forsteri* spot patterns have not yet been evaluated. If spot patterns prove to be stable over the life of this species non-invasive studies of population dynamics will be possible.

The results of this study show particularly high precision and accuracy of computer aided photo ID in identifying individuals of *P. forsteri*. The high efficacy of this method coupled with the feeding and territorial behaviour of *P. forsteri* make *in situ* studies of movement patterns, individual behaviours and behavioural traits possible without the use of invasive identification techniques.

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Contributions

M. M. conceived and designed the method, collected and analysed data and prepared the manuscript. N. B. conceived and designed the method, analysed data and edited the manuscript. C. H. conceived and designed the method, analysed the data and edited the manuscript. G. H. designed the method. D. M. edited the manuscript. D. B. conceived and designed the method and edited the manuscript.

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Fig. 1. Study location at 26.205437°N and 34.219930°E on the Red Sea coast of Egypt (a). Three example photographs of *P. forsteri* with spot patterns including the three standard reference markers in blue and user-selected markers in red (b). Flow diagram describing matching protocol used to build the initial *P. forsteri* database of individuals (c). Receiver Operating Characteristic (ROC) curve displaying the high discriminant ability of I^3S to differentiate between individual *P. forsteri* and the optimal discriminant threshold to identify *P. forsteri* individuals (d). Dot-plot showing the range of I^3S distance metric scores (DM) categorised as true negatives and true positives. The dotted line indicates the optimal cut off identified from the ROC analysis (e).

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