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BRIEF COMMUNICATION

A method for the automated long-term monitoring of three-spined stickleback *Gasterosteus aculeatus* shoal dynamics

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This paper describes and evaluates a flexible, non-invasive tagging system for the automated identification and long-term monitoring of individual three-spined sticklebacks *Gasterosteus aculeatus*. The system is based on barcoded tags, which can be reliably and robustly detected and decoded to provide information on an individual's identity and location. Because large numbers of fish can be individually tagged, it can be used to monitor individual- and group-level dynamics within fish shoals.

Key words: automatic tracking; computer vision; individual identification.

Studying the dynamics of captive fish groups often requires recognition of individuals over extended time periods. To facilitate this, researchers have developed a range of automated tracking methods (Delcourt *et al.*, 2013). For example, computer vision techniques have been used to reliably track single (Kane *et al.*, 2004; Xiao *et al.*, 2011) and multiple (Zhu & Weng, 2007) individuals, allowing the location (and movement) of fishes to be assessed without using any physical tags or identification markers. They are, however, either limited to the tracking of single fish or only useful over relatively short time periods [<15 min in Zhu & Weng's (2007) system].

Individual tagging using visible external markers is often used to monitor multiple fishes for behavioural experiments (Bégout *et al.*, 2012) and to allow the manual identification of individuals (Beukers *et al.*, 1995; Barber & Ruxton, 2000). Tags are often not reliably recognized from a distance, and fishes can be disturbed if close observations or catching is necessary for identification. Furthermore, the possibility of automatic monitoring using these markers is often not possible. Remote tagging using acoustic, radio or passive inductive transponder (PIT) tags has also been used to monitor the identity and location of individual fishes (Cousin *et al.*, 2012), although these techniques are invariably invasive and a high spatial and temporal resolution is not always guaranteed.

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Therefore, reliably monitoring multiple fishes over an extended period of time is still a great challenge.

This paper describes a flexible, non-invasive tagging system for the automated identification and long-term monitoring of shoaling three-spined sticklebacks *Gasterosteus aculeatus* L. 1758. This species is a widely used model organism for studies in behavioural and evolutionary ecology (Huntingford & Ruiz-Gomez, 2009) as they are relatively easy to capture, house and manipulate in the laboratory. As they are used to study a variety of social behaviours (Ranta & Lindström, 1990; Ward *et al.*, 2002; Frommen *et al.*, 2007; Pike *et al.*, 2008, Wark *et al.*, 2011), it is crucial to be able to differentiate between individuals within a shoal. Various kinds of non-invasive tagging systems have been used for this purpose, including coloured tubing attached to anterior spines (Ward *et al.*, 2002), wire pressed onto pelvic spines (Barber & Ruxton, 2000) and tags bearing unique symbols or colours attached to one of the three dorsal spines (Pike *et al.*, 2008; Webster & Laland, 2009). Existing tagging systems are not suitable for automated monitoring of large shoals, either because tags cannot be reliably detected due to their position on the fish, small size or complexity (Barber & Ruxton, 2000; Ward *et al.*, 2002; Pike *et al.*, 2008), or because there is a limit to the number of tags that can be used simultaneously (Webster & Laland, 2009). The use of coloured tags can also potentially influence the behaviour of the fish (Smith *et al.*, 2004).

The tagging system described here builds directly on the system developed by Webster & Laland (2009). It uses monochromatic, barcoded tags to allow the automatic tracking of large groups of fishes over long periods. When designing the tags, the following constraints were imposed: (1) they must be readable by both humans and consumer-level webcams, allowing simultaneous replicates to be obtained in a cost-effective manner; (2) they must be robust to changes in pose, orientation and size (*e.g.* as fishes move vertically in the water column and feed), and some motion blur (as often occurs when imaging fast-moving fishes such as *G. aculeatus*) and (3) they should allow the locations of individual fish to be monitored over extended periods (months or years), either in real time from videos or from still images, without the need for manual input. This requires a system that can cope well with missing (*e.g.* blurred or occluded) tags.

The tags consist of a white disc divided into three equal-width concentric zones (Fig. 1). The middle zone bears a circular, rotationally unique barcode encoding an *n*-bit binary number as an alternating sequence of *n* black and white elements, each of which subtends an equal angle. Because the fish can be potentially oriented in any direction, making the tags rotationally unique means that barcodes can be read from any starting point without the need for a specific start indicator. This simplifies the design and makes tag identification more robust but reduces the possible number of unique codes available. The number of barcode elements (and hence the number of bits encoded) is determined by the number of fish that need to be monitored simultaneously, and the precision with which they need to be located and identified. For example, for an eight-element (8-bit) barcode, 34 rotationally unique codes are available, while for a 12-element barcode, 350 unique codes can be created, although the relatively smaller angle subtended by each element increases the chance of misidentifying a tag.

In plan-view images, the tags appear approximately circular and have predictable radii that fall within a narrow range (mediated mostly by their vertical position in the water column; Fig. 1). Given a suitably contrasting background, they can therefore be

FIG. 1. A group of *Gasterosteus aculeatus* tagged with uniquely identifiable barcoded tags. For scale, each tag has a diameter of 5 mm. Note: the quality of this image is substantially higher than those used to collect the pilot data.

robustly detected using the generalized Hough transform (Duda & Hart, 1972), a technique for identifying the positions of arbitrary shapes, such as circles (Davies, 2012), allowing their centres and radii to be extracted. True tags can be readily differentiated from other falsely-detected circles using the magnitudes of the accumulator array peaks (Davies, 2012), and only retaining circles whose magnitude exceeds a given empirically-determined threshold. This threshold can be either fixed or determined dynamically using the distribution of magnitude values (*e.g.* by *k*-means clustering).

The decoding algorithm analyses a single scanline extracted from the barcode area of the tag, which can be readily located given the tag's radius and the co-ordinates of its centre. The scanline takes the form of a normalized intensity profile indicating the greyscale value of the constituent pixels, read anti-clockwise over all angles from an arbitrary starting point. This scanline is compared to template profiles predicted for each code that are known to be present in the image (see Fig. 2). The process of tag identification is made easier by the fact that the tags present in any image or frame should be known *a priori*. The match between a given template and the observed scanline is calculated from the discrepancy between the intensity profile of the scanline and the template, following Gallo & Manduchi (2009). A template $T(k)$ is defined for code k as a continuous piecewise constant function that alternates between 0 (for a black element) and 1 (for a white element). A template is therefore an archetypal representation of one possible code for a given scanline. A cost function, C, is then defined for each template as

$$C(n) = \begin{cases} \max\left(I(n) - \mu_b, 0\right)^2, & T(k,n) = 0 \\ \min(I(n) - \mu_w, 0)^2, & T(k,n) = 1 \end{cases} \quad (1)$$

where $I(n)$ is the scanline intensity profile over all N points in the scanline ($n = 1, 2, 3, \ldots, N$), and the quantities μ_w and μ_b represent the mean of the largest 50% and smallest 50% values of scanline intensities, respectively. This function penalizes values of $I(n)$ that are small when $T(k,n) = 1$ or large when $T(k,n) = 0$. The most likely code is the one that minimises the sum of C.

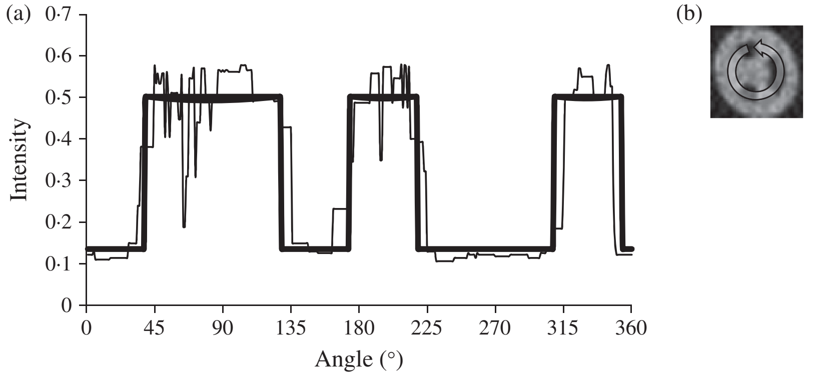

FIG. 2. (a) Intensity profile from a representative scanline (——) extracted from the tag shown in (b), with the best-matched binary template (——) superimposed. (b) Enlarged image of an extracted tag. ◌ indicates the start position, read direction and location of the scanline.

When conducting the pilot testing of the system, it was found that misidentification often occurred when the cost for the lowest-scoring and next lowest-scoring tags was very similar. The likelihood of misidentification was substantially reduced by only considering instances in which the ratio of cost scores between the lowest- and second lowest-scoring tags (referred to as the confidence score) is less than a predefined threshold value (as occurs, for example, when tags are detected but unreadable due to noise or motion blur, as all templates will generate similar cost scores). There was a trade-off between identification accuracy and the number of tags read overall because potentially misidentified tags were ignored. Matlab (Mathworks; www. mathworks.com/) code for performing the detection and decoding routines described here is available from http://eprints.lincoln.ac.uk.

To test the system, tags (5 mm diameter, 4.3 mg; Webster & Laland, 2009) bearing an 8-bit barcode were printed onto white waterproof paper (Memory-Map Toughprint; www.memory-map.co.uk/). This paper is rigid enough to minimize distortion and retains the barcode pattern for at least 3 months. It also reflects infrared light so that tags can be identified at night, using an infrared-sensitive camera and appropriate illumination. By piercing a small hole in the middle of the tag, they can be placed over the first or second of the three dorsal spines, where the serration of the spine is sufficient to hold the tag in place without additional fixation (Webster & Laland, 2009). No anaesthetization is necessary to fit the tags. During the procedure, each individual is held out of water for up to 20 s, and on return to their holding tanks show no adverse effects to the tagging procedure. In the longer term, similar tags cause no harm, are retained for extended periods and cause no known behavioural (Webster & Laland, 2009) or physiological (T. Pike, unpubl. data) changes.

Uniquely tagged *G. aculeatus* ($n = 3$, mean ± S.D. standard length, L_S: 33.0 ± 0.1 mm) were placed in a black circular tank (30 cm diameter), containing 8 cm of water held at a constant 10° C. The tank was illuminated by fluorescent ceiling lights, although care was taken to minimize reflections. An overhead webcam (Microsoft LifeCam Show; www.microsoft.com/) linked to a computer allowed still images to be taken at regular intervals (approximately every 22 s for 3 h). Images were saved as greyscale uncompressed TIFFs, with a resolution of 1280 × 960 pixels. In the resulting images, tags had

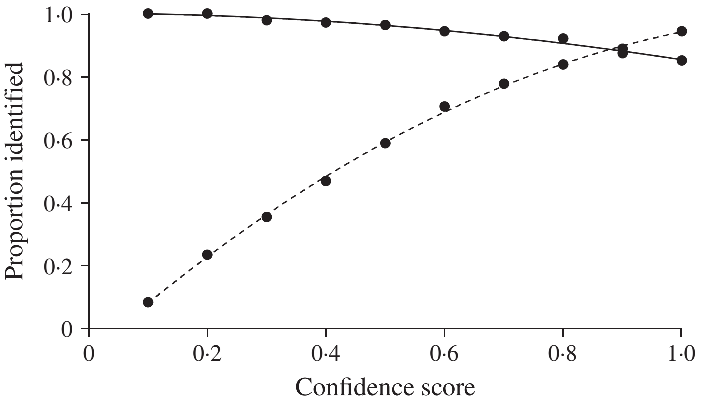

FIG. 3. Representative data showing the proportion of correctly identified tags (——) and the proportion of tags identified, whether correctly or not (-----) as a function of confidence score. These data are based on repeated analysis of 490 images, each containing three *Gasterosteus aculeatus*. Quadratic least-square fits are shown. Overall, 72% of tags were detected (*i.e.* were not prohibitively blurred, occluded or obliquely tilted).

a radius of *c.* six pixels, which is approaching the lower limit for accurate detection and identification.

The algorithm described here can locate and decode tags with a high success rate, although there is inevitably a trade-off between the number of tags detected and the proportion of tags that are successfully decoded; when strict acceptance criteria are imposed (*i.e.* a low confidence score), the number of false-positive identifications can drop to *c.* 0% at the expense of the overall proportion of tags detected (<40% in the example shown in Fig. 3). These results are indicative only, and performance will vary depending on factors such as the resolution and quality of the camera, the size of the tags and the number of bits encoded by each tag. The probability of misidentifying a tag can also be reduced by ensuring that the tags assigned to a particular group differ by the maximum possible number of elements (*i.e.* have the maximum possible Hamming distance), as tags that differ by only a single element are susceptible to decoding errors. The precise setup will depend on the nature of the study and the level of acceptable error.

The tagging system described here is simple and robust and fulfilled the set criteria of being detected and decoded using consumer-level webcams, accommodating changes brought about by an individual's movement and behaviour and, because it does not rely on real-time tracking, can be used to track individuals over extended periods. The tags could potentially encode far more information than just identity and location of individuals. For example, it may be possible to use the apparent size of the tag in an image to measure the height of a fish in the water column, the orientation of the tag to detect direction and the angle of tilt to infer behaviour (*e.g.* feeding or courtship; Wootton, 1976). When combined with data on the relative positions of other fish in the group, it may also be possible to detect directed (*e.g.* aggression) or cooperative (*e.g.* simultaneous feeding) behaviours within shoals. Finally, the flexible design of the tags would allow them to be used on species other than *G. aculeatus* with minimal modification, whenever there is a suitable means of attachment.

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References

Barber, I. & Ruxton, G. D. (2000). The importance of stable schooling: do familiar sticklebacks stick together? *Proceedings of the Royal Society B* **267,** 151–155.

Beukers, J. S., Jones, G. P. & Buckley, R. M. (1995). Use of implant microtags for studies on populations of small reef fish. *Marine Ecology Progress Series* **125,** 61–66.

Bégout, M.-L., Kadri, S., Huntingford, F. & Damsgård, B. (2012). Tools for studying the behaviour of farmed fish. In *Aquaculture and Behavior* (Huntingford, F., Jobling, M. & Kadri, S., eds), pp. 65–86. Oxford: Blackwell Publishing Ltd.

Cousin, X., Daouk, T., Péan, S., Lyphout, L., Schwartz, M.-E. & Bégout, M.-L. (2012). Electronic individual identification of zebrafish using radio frequency identification (RFID) microtags. *Journal of Experimental Biology* **215,** 2729–2734.

Davies, E. R. (2012). *Machine Vision: Theory, Algorithms, Practicalities*, 4th edn. Oxford: Academic Press.

Delcourt, J., Denoël, M., Ylieff, M. & Poncin, P. (2013). Video multitracking of fish behaviour: a synthesis and future perspectives. *Fish and Fisheries* **14,** 186–204.

Duda, R. O. & Hart, P. E. (1972). Use of the Hough transformation to detect lines and curves in pictures. *Communications of the ACM* **15,** 11–15.

Frommen, J. G., Luz, C. & Bakker, T. C. M. (2007). Kin discrimination in sticklebacks is mediated by social learning rather than innate recognition. *Ethology* **113,** 276–282.

Gallo, O. & Manduchi, R. (2009). Reading challenging barcodes with cameras. *Technical Report UCSC-SOE-09-26*. Santa Cruz, CA: University of California.

Huntingford, F. A. & Ruiz-Gomez, M. L. (2009). Three-spined sticklebacks *Gasterosteus aculeatus* as a model for exploring behavioural biology. *Journal of Fish Biology* **75,** 1943–1976.

Kane, A. S., Salierno, J. D., Gipson, G. T., Molteno, T. C. A. & Hunter, C. (2004). A video-based movement analysis system to quantify behavioral stress responses of fish. *Water Research* **38,** 3993–4001.

Pike, T. W., Samanta, M., Lindström, J. & Royle, N. J. (2008). Behavioural phenotype affects social interactions in an animal network. *Proceedings of the Royal Society B* **275,** 2515–2520.

Ranta, E. & Lindström, K. (1990). Assortative schooling in three-spined sticklebacks? *Annales Zoologici Fennici* **27,** 67–75.

Smith, C., Barber, I., Wootton, R. J. & Chittka, L. (2004). A receiver bias in the origin of three-spined stickleback mate choice. *Proceedings of the Royal Society B* **271,** 949–955.

Ward, A. J. W., Botham, M. S., Hoare, D. J., James, R., Broom, M., Godin, J. J. & Krause, J. (2002). Association patterns and shoal fidelity in the three-spined stickleback. *Proceedings of the Royal Society B* **269,** 2451–2455.

Wark, A. R., Greenwood, A. K., Taylor, E. M., Yoshida, K. & Peichel, C. L. (2011). Heritable differences in schooling behavior among three-spined stickleback populations revealed by a novel assay. *PLoS ONE* **6,** e18316.

Webster, M. M. & Laland, K. N. (2009). Evaluation of a non-invasive tagging system for laboratory studies using three-spined sticklebacks *Gasterosteus aculeatus*. *Journal of Fish Biology* **75,** 1868–1873.

Wootton, R. J. (1976). *The Biology of the Sticklebacks*. London: Academic Press.

Zhu, L. & Weng, W. (2007). Catadioptric stereo-vision system for the real-time monitoring of 3D behavior in aquatic animals. *Physiology and Behavior* **91,** 106–119.

Electronic Reference

Xiao, G., Zhang, W., Zhang, Y., Chen, J., Huang, S. & Zhu, L. (2011). Online Monitoring System of Fish Behavior. *11th International Conference on Control, Automation and Systems in KINTEX, Gyeonggi-do, Korea*, pp. 26–29. Available at http://ieeexplore.ieee.org/xpls/abs_all.jsp?arnumber=6106127