

Individual identification of Eurasian perch *Perca fluviatilis* by means of their stripe patterns

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ABSTRACT

In this study we show that the number, position and shape of stripes were sufficiently unique to demonstrate that juvenile Eurasian perch *Perca fluviatilis* can be individually identified based on their stripe patterns. The stripe patterns in perch and also other species may thus be used in experiments as an alternative to conventional marking techniques that frequently cause stress to the fish.

Keywords:

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In laboratory experiments with fish it is often desirable or even necessary to identify individual fish repeatedly throughout the course of the experiment. For example, in growth studies more information can be gained by tracking individual growth trajectories as compared to averaging growth across groups of fish, in behavioural experiments it can be meaningful to focus on particular individuals, and in common garden experiments where fish that have received a certain pre-treatment are reared together, it is essential to recognize individuals at later stages of the experiment. Common ways of identifying individual fish include marking via externally attached tags, subcutaneous injection of fluorescent dyes, implantation of coded wire tags, transponders or transmitters, or mutilation of fins. The most obvious disadvantage of these techniques is handling stress during the marking procedure. Furthermore, external marks may have adverse effects on swimming ability and growth, and social interactions may be compromised by any change of a fish's appearance or by marking-induced changes of its behaviour. An alternative to these traditional approaches is the visual identification of individual fish by their external characters.

Identification of aquatic animals by external characters is a commonly applied method in aquatic ecology. For example, individuals of whale sharks *Rhincodon typos* could be re-identified through

decades based on their pigmentation patterns (Hueter et al., 2013; Meekan et al., 2006). Recently, Huntingford et al. (2013) have shown that mirror carp *Cyprinus carpio* can be individually identified by their scale pattern.

Eurasian perch *Perca fluviatilis* (henceforth: perch) is a model species in freshwater fish ecology used extensively in field studies and in controlled aquarium and mesocosm experiments. Perch have a conspicuous stripe pattern consisting of vertical darker pigmented stripes on their body flanks from just behind the operculum to the caudal peduncle. The stripe pattern varies in the number of stripes, the presence or absence of y-shaped stripes, stripe width, inter-stripe distance (Pimakhin, 2012), and the intensity to which dorsal vs. ventral ends of the stripes are pigmented; for example, stripes can be broader at the dorsal end and narrower at the ventral end (Figs. 1–3). In this communication we hypothesized that a fish's individual pattern persists on common experimental timescales and that it is unique to the degree that it allows the discrimination of single individuals. These hypotheses were tested by analyzing the stripe-patterns of individual perch in six to seven week feeding experiments in aquaria and a mesocosm during which the fish were anaesthetized and photographed at the beginning and end of each experiment. Importantly, to simulate conditions in the field the fish were not additionally marked individually, hence our analysis rests fully on non-invasive identification.

Fish for the aquaria experiment originated from Lake Karsee, a small eutrophic lake north of Lake Constance (south-west Germany), or from Lake Constance. Two groups of Lake Karsee

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Fig. 1. Eurasian perch *Perca fluviatilis* photographed at the start and the end (left and right photograph, respectively) of a six weeks feeding experiment. Individuals are easily identified by their stripe pattern.

perch and two groups of Lake Constance perch of eight individuals each were stocked into aquaria of 85 L volume at 20 °C with 14 h light per day. During the experiment they were either fed with chironomid larvae (one group from each lake) or they received a dry diet (one group from each lake). Different food types were applied because the fish were used in another study which focused on the influence of food quality on fin pigmentation. Here, we restrict the method description to information relevant for the identification of stripe patterns. The fish's standard length increased from 10.8 ± 0.6 to 12.2 ± 0.6 (mean \pm 1 SD) for Lake Karssee perch and from 13.5 ± 0.5 to 14.8 ± 0.6 cm for Lake Constance perch during the six weeks of the experiment. For the mesocosm experiment 40 wild-caught fish from two littoral sites in Lake Constance were marked with a fin-cut removing approx. 1 mm² tissue from the distal end of the second dorsal or the anal fin, and then stocked together into an outside mesocosm with 10 m \times 1 m \times 1 m base dimensions which was supplied with lake water. The fin-cut was

needed to extract DNA for later kinship analysis which was the primary aim of this experiment. Fish in the mesocosm were fed living chironomids and living zooplankton daily. At the end of the experiment the fish in the outside mesocosm had almost doubled their wet weight from 4.7 ± 1.6 (mean \pm 1 SD) to 9.1 ± 2.5 g.

Prior to stocking into the experimental aquaria or the mesocosm all fish were anaesthetized in MS 222 (0.1 g L⁻¹) and photographed (Panasonic® Lumix DMC-FS35, ISO: 400; 12 Megapixels, no flashlight, automatic white adjustment) in air in lateral aspect (left body side) including a ruler in each photo. Photos were taken under a soft box of white canvas that was illuminated with two video floodlights. In this way surface reflections from the fish body were completely eliminated and hard shadows almost absent. The four groups of eight fish were again photographed at the end of the six weeks experiment. Fish in the outside mesocosm were also photographed at the end of the seven weeks experiment. To further test how recognizable stripe-patterns would be under water, we used a

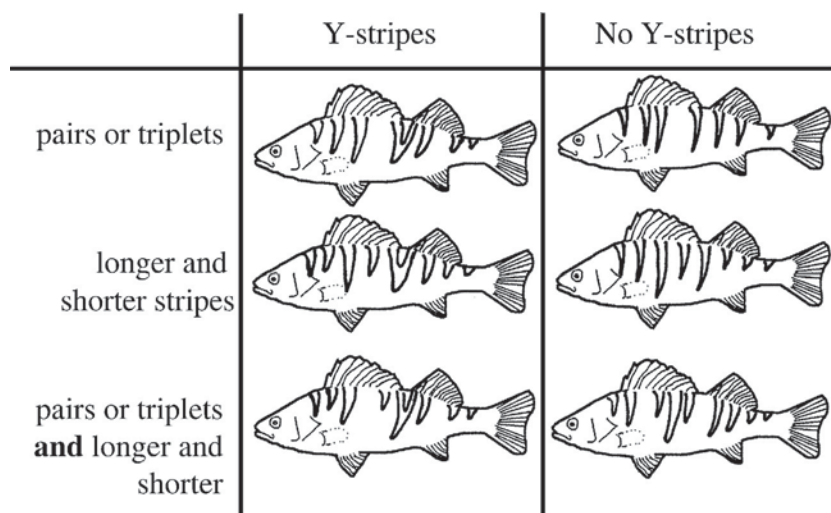


Fig. 2. Stripe pattern categories and examples of pattern combinations used to group Eurasian perch *Perca fluviatilis* for easier matching of pre- and post-experiment photos. Y-stripes may appear at other positions than shown here and number of stripes ranges from five to nine, allowing for a high number of pattern combinations.

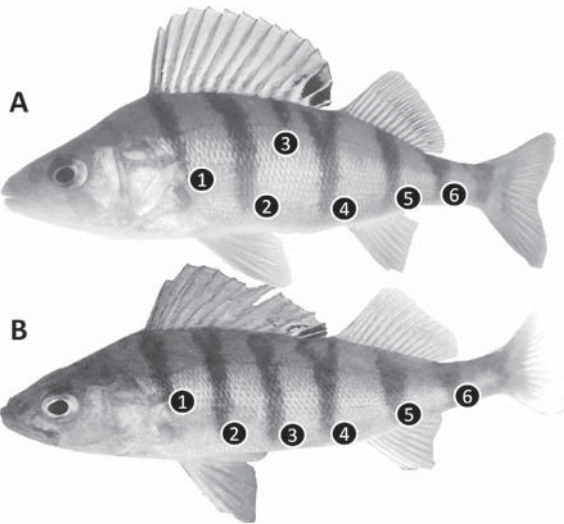


Fig. 3. Field-example of perch stripe patterns. A and B show two perch from one shoal photographed in the littoral zone under water. The series of observations (see Fig. 2 and text) applied to these perch clearly reveals differences despite the stripe-patterns appear similar: both perch contain 6 stripes and both feature y-stripes (stripe number 4 in A, B). However, the individual in A contains a pair of stripes and one stripe (number 3) is shorter (cf. category ‘Y-stripes’ and ‘pairs and triplets and longer and shorter stripes’ in Fig. 2.). The perch in panel B is missing a shorter stripe and all stripes are equidistant to one another.

water-proof digital camera (Sony Cybershot® THX-10, underwater settings to auto) to take pictures of perch swimming in a shoal close to the littoral zone at approx. 2 m depth.

Stripe pattern-matching in the aquaria- and mesocosm-held fish followed the same procedure. For the fish in the outside mesocosm, photographs were first grouped according to their fin-cut into two groups and then classified into categories (exemplified in Fig. 2). The categorization followed a series of observations that the observer went through, thus narrowing down the number of photos to match to each other. Eventually, a match of a pair of photos could easily be assigned by eyesight. The observations were based on the following categories: number of stripes, Y-stripes present or absent, stripes all +/- equidistant or stripes in pairs or triplets that are closer or further to one another than the remaining stripes, stripes all of similar length (with stripes being longest in the middle and decreasing evenly towards the caudal and distal end) or some interspersed stripes shorter or longer than others, breaking the even length distribution. The sequential application of these categories to each photo narrowed down the number of pre- and post-experiment pictures in the mesocosm-held fish to approx. 5–10 photographs for each of the major categories. For each group of eight fish from the aquarium experiment, the start photo of one individual was compared with all eight photos taken after six weeks until the best match was achieved. Next, the start photo of the second individual was compared with the remaining seven photos and so on, until all 16 photos of one group were explicitly assigned to eight individuals. When the classification based on stripe patterns was not 100% correct in the first round, in the second round other external non-classifiable features were incorporated on an individual basis. Those included particular scale patterns such as missing or peculiarly pigmented scales, or a conspicuous curvature of the lateral line.

For three of the four groups of eight fish each, the photos taken at the beginning and at the end of the six weeks aquarium experiment could be matched without difficulty (Fig. 1). In the fourth group the first assignment proved to be wrong in four out of eight fish because the remaining photos could not be matched satisfactorily. The classification was restarted with the start photo of the first

individual in the group, including other external features (cf. above) and now the assignments were successful in all cases.

From 80 fish stocked into the mesocosm, four died and 74 of the remaining photographs taken at the end of the experiment could be unambiguously paired with the photographs taken prior to the stocking into the mesocosm. Two photographs remained unassigned because the fish on the photograph prior to stocking were so weakly pigmented that the stripes could not be classified.

This communication was conceived as a proof of concept for the individual identification of perch based on unique stripe patterns. The results of the identification procedure supported the hypotheses that stripe-patterns in perch remain unchanged through individual growth and are unique enough to allow for a tracking of individuals throughout several weeks long experiments. This agrees with Stolbunov and Pavlov (2006) citing studies by Shaikin (1990) and Zelenetskii and Izyumov (1994) that “the transverse-striped pigmentation does not change throughout life and is inherited”. The temporal stability and uniqueness of stripe patterns also in wild-caught fish kept in an outside mesocosm hints at the applicability of this method even under semi-natural conditions. The fact that perch stripe patterns could be unambiguously identified on pictures taken by a consumer-grade digital camera under water (Fig. 3) further demonstrates the potential of this method even for field studies. It should be noted that, in this study, to minimize handling stress, the fish were not additionally individually marked with other markers (e.g. PIT-tags). The limited number of individuals and the uniqueness of patterns made an additional marking of our fish unnecessary for a successful identification. Given the growing number of software tools for picture-based animal identification, one might ask: why did we not test and apply computer-based matching procedures as e.g. Wild-ID (Bendik et al., 2013)? There is no reason to assume that any computer-based technique would outperform the human eye in identifying perch based on their, rather simple, stripe patterns. Computer-based methods can facilitate identification only when recognition is based on extremely complex pigmentation patterns (e.g. used to identify animals with complex web-like pigmentation patterns such as salamanders (Bendik et al., 2013)). However, such software requires a considerable amount of time to prepare the pictures and, in the most commonly applied software solutions, the final matching of picture-pairs is, again, left to the human eye. Therefore, because the human eye is superior to computers, when it comes to pair-matching of contrast-rich and comparatively simple patterns (e.g. consisting of a maximum of 9 stripes), and because there is no digital preparation of the photographs needed, a simple human-eye-based approach is the most cost- and time-efficient method which is also applicable to field conditions.

It should be noted that an individual identification will inevitably become harder with increasing numbers of fish. We are confident however, that the uniqueness of stripe patterns is sufficient to allow individual identification of even more than the 40 fish we tested. But we acknowledge that the individual identification could become more time-consuming as more photographs have to be compared. It could also be relevant to assess the total range variation in stripes patterns in perch destined for an experiment. In the case where fish can be selected from a larger group our method would also allow to pre-screen individuals with especially conspicuous stripe patterns. These selected individuals could then be easier to re-identify because they were pre-selected to be different upon commencing the experiment.

Developing methods for individual tracking of experimental animals without handling will grow in relevance because researchers increasingly prioritizes animal welfare in experiments. Our approach still requires to anaesthetize the fish which clearly also comes with some stress. But apart from the anaesthesia needed

to take a picture, our method does not require any other handling of the fish. Other marking techniques as for example, markings with dye on the fish's caudal fin, are quick to apply and can minimize stress compared to internal tags (Magnhagen and Borcharding, 2008). But in a growing number of countries animal welfare regulations prohibit such markings without anaesthetizing the fish. In those cases such marking techniques will eventually be considered an additional handling stress on top of the anaesthesia. Albeit not completely stress-free, we therefore consider anaesthesia for taking a photograph a minimal level of stress.

Fish can respond to handling stress by changing their pigmentation rapidly (within minutes). This physiological response differs from more long-term (within days or months) morphological changes of pigmentation (Sugimoto, 2002). We have noticed that perch can indeed respond rapidly to handling stress by changes in pigmentation. However, we never observed that the physiological response changed the appearance or location of stripes. This fits well with the assumption that physiological changes allow for an overall change from brighter to darker or vice versa but that patterns of pigmentation remain (Gusen, 2010; Sugimoto, 2002).

Inclusion of individual identification based on stripe patterns can help to further improve computer-based identification methods that are being developed for the identification of species of fish underwater (Joo et al., 2013). Indeed, using filed video recordings from other experiments we were able to identify perch-stripes on juvenile perch filmed swimming in aquaria. This data could not be further analyzed but future studies should investigate the possibility of identifying perch individually based on video recordings. At any case, an established method for the identification of individuals based on unique stripe patterns holds promise for further exploration of the role of such patterns in e.g. mate choice, individual recognition in shoals, and camouflage. This study used Eurasian perch, but considering the close similarity in morphology, physiology, behaviour and ecology between *P. fluviatilis* and yellow perch *P. flavescens* (Thorpe, 1977), we are confident that also yellow perch may be individually identified by their stripe patterns. Future studies might test the applicability of this method for other fish species including percids like pikeperch *Sander lucioperca* or walleye *Sander vitreus* and centrarchids and cichlids that also frequently feature stripe patterns. Finally, we would like to emphasize that this study was conceived as first presentation of a method to stimulate more research into this promising topic. Future studies

will have to demonstrate the applicability of this method in other geographic and experimental contexts and species.

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