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A new method for measuring group behaviours of fish shoals from recorded videos taken in near aquaculture conditions

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ABSTRACT

Monitoring fish shoal behaviour is a growing concern for scientists studying fish stress and welfare. This study presents an algorithm developed to calculate, from videos taken from above aquaria, two indexes characterizing fish shoal behaviour. These two indexes quantify the dispersion and the swimming activity of the fish shoal in the aquaria. The reliability of these indexes was tested on fish shoal simulations following the rules of Reynolds' model on flocks, herds and schools. Since coordinates of each simulated fish in the shoal was known, these simulations provided true values of dispersion and swimming speed of each fish in the shoal, which were compared to values calculated using the presented algorithm. Further, the two indexes were tested on videos of rainbow trout in aquaria. Behavioural variations of the shoal were estimated before and after food distribution in one test, and before and after a four hours confinement stress in a second test. Data resulting from simulations indicate that the two indexes are sensitive to the simulated changes in the cohesion or the swimming speed of the group. Thus, indexes faithfully translated true values in simulations, with a minimum of 94% of the total variation in true values explained by indexes. Furthermore, the two indexes were sensitive to shoal behaviour modifications observed in the two case studies. Indeed, as expected, a strong group dispersion decrease associated with an important swimming activity increase could be detected just after food distribution using our method. Similarly, our indexes were sensitive to a group behaviour change observed after the four hours confinement stress. Finally, our method was compared to Israeli's, and was found to be more sensitive and more accurate in our conditions. This method provides therefore a sensitive, non-invasive, simple and widely applicable tool to quantify behavioural changes associated with various challenges in aquacultural conditions.

1. Introduction

Stress is now well documented for its deleterious effect on health and growth of cultured fish (Dror et al., 2006; Iwama et al., 1997; Pankhurst et al., 1997; Welker et al., 2007). Some factors such as water quality, stocking density or physical disturbances can act as powerful environmental stressors, thereby reducing the performance and the welfare of the fish. Behaviour is the first visible change after exposure to a stressor and has proven to be a sensitive indicator of environmental conditions and animal welfare in ecosystems or controlled conditions (Atchison et al., 1987; Hasler et al., 2009; Lee et al., 2003; Scherer, 1992). Monitoring behaviour can therefore help to detect perturbations such as physical or chemical disturbances and might provide researchers and farmers with efficient and accurate information on the magnitude of an environmental or social change.

In fish species, some behaviour such as swimming activity, group distribution or space use is commonly considered as stress indicators since they reflect defense behaviour mechanisms increasing survival probability (Almazán-Rueda et al., 2005; Espmark and Baeverfjord, 2009; Lima and Dill, 1990; Martins et al., 2012). Therefore, a challenging situation usually leads to modifications of swimming and shoaling behaviour (Brodin et al., 2013; Hoare et al., 2004; Koltes, 1985; Kramer, 1987; Weis and Weis, 1974a,b).

To observe animal behaviour, manual methods consisting in writing down observations directly or from recorded videos are time-consuming but remain the only way to detect and number the occurrences of some specific behaviour. During the last decade, important improvements have been made to automate as much as possible measurements on isolated individuals (Huntingford et al., 2011). Video tracking was developed and allowed the measurement of several behaviours such as speed, changing directions, time spent moving or angular velocity on an isolated individual (Kane et al., 2004; Schjolden et al., 2005).

Fish group behaviour still remains very challenging to assess since fish move in a three dimensional environment, difficult to access.

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Recently, methods have been developed to enable the tracking of several fish in the same tank but these methods are either invasive, potentially disturbing or need restricting conditions. Indeed, a common solution consists in marking few fish within a shoal and following their behaviour semi-automatically with Elastomers (Colléter and Brown, 2011; Doupé et al., 2003; Geffroy and Bardonnet, 2012) or automatically with electronic marks (Bégout Anras and Lagardère, 2004; Kristiansen et al., 2004). These methods are reliable and efficient but the number of followed individuals remains low and moreover, a probable behavioural bias exists due to surgery. A second solution relies on algorithms capable of tracking individual fish within a shoal by estimating their trajectory from videos (Barry, 2012; Butail and Paley, 2012; Delcourt et al., 2009; Faucher et al., 2010; Johansson et al., 2009). A good algorithm is able to follow more than hundred fish at the time but these methods need low density conditions to avoid formation of large groups and are therefore not reliable in aquaculture conditions.

All the above mentioned methods are based upon tracking single individuals in a group. An alternative to these tracking devices is a method based upon group behaviour monitoring without any recognition of individual fish, giving a global value of the shoal behaviour. Some studies inspired from acoustic techniques investigating fish abundances and behaviours in the oceans proved the feasibility of monitoring fish shoal in aquaculture cages (Juell et al., 2003; Oppedal et al., 2007) or tanks (Conti et al., 2006; Rosny and Roux, 2001) using acoustics. However, a number of limitations for tank use were discussed by the authors, indicating the difficulty to apply the technique to aquaculture conditions. Another approach consists in recording videos from the tank and analysing fish shoal behaviour using an automated algorithm. This approach is commonly used to assess preferential areas in the tank (Bratland et al., 2010; Folkedal et al., 2012a,b; Stien et al., 2007). However to our knowledge, only three studies have been based on this approach to study global swimming behaviour like swimming activity or dispersion of the shoal (Duarte et al., 2011; Israeli and Kimmel, 1996; Xu et al., 2006). Xu et al. and Duarte et al. focused on fish activity by subtracting two consecutives frames. Israeli and Kimmel created a group dispersion measure of a shoal without recognition of individual fish (Israeli and Kimmel, 1996). All these studies were however tested either on a low density fish shoal or on flat fish limiting fish overlapping. In this context, our work, inspired by Israeli and Kimmel's and Xu's, focused on the development of an automatic, simple, non-invasive and economic method to monitor shoaling and swimming behaviour of free swimming fish in aquaculture conditions without any practical upper limit of density or number. The objective of the present study was to develop a computerized method extracting an index of dispersion of a rainbow trout shoal and an index of its swimming activity. Reliability of the method was verified on computer simulations where fish group behaviours were changed in order to increase swimming activity or group formation. The method was also tested on two real sets of observations using two radically different environmental conditions: (i) food distribution and (ii) confinement. Finally, our results were compared to Israeli's method and clearly showed an improvement in reliability and accuracy with our method.

2. Materials and methods

2.1. Image analysis procedure

An algorithm was developed with the free software ImageJ 1.47b (http://rsbweb.nih.gov/ij/) and automated with a macro, precisely described below. This algorithm was designed to automatically treat and analyse several images extracted from videos taken from above the aquaria or tanks, each step is detailed below. The only condition of use is to have images where fish colour is different from the colour of the bottom of the tank. First, the macro will ask the user to identify the position in the images of every aquarium to analyse. It will then ask him to set the threshold from which fish are different from the background, and finally, the user will be asked to give the minimum size of a fish. The algorithm will then use these settings to treat images in order to get fish in black pixels on a white background and then calculate two indexes of group behaviour.

The different steps of treatment and analysis of the macro are more precisely described below.

First, images are transformed into 8-bit images (grey colours) and are smoothed using a median followed by a Gaussian filter. If fish are lighter than background, images are inverted. A background picture is created by selecting for each pixel its maximum value out of 30 images taken in the whole sequence. All images are then divided by the background image and pixel values are multiplied by 255 (maximum value in an 8-bit picture). A threshold is then applied to each image and images are binarized so that background becomes white and fish black. The macro keeps then only groups of black pixels having an area bigger than a fish size. Finally, out of these transformed images, four fish shoal behaviour indexes, detailed below, are calculated. The effect of these steps is shown in Fig. 1. The macro creates a text file containing all the results. This text file is analysed using the free software R 2.14.0 (http://cran.r-project.org/).

- Fish group dispersion index This first index was designed to give an indication on the dispersion of the group in the tank or the aquarium.

  The index was calculated by summing the perimeters of each group of individuals (Fig. 1A). A group is a single black area created by the outlines of overlapping fish. A group may consist of one fish only. Using this technique, we expected that index would be high when shoal is spread and low when it is aggregated.

  $$P_{tot} = \sum_{i=1}^{n} P_i.$$

  Where n is the total number of groups in the picture and P_i is the perimeter of a given group of individuals for image t.
- Fish group activity index For each image, an index of swimming activity (Fig. 1C) is estimated by subtracting from an image any dark areas that overlap with dark areas in the previous image in a chronological order. The remaining area is proportional to the fish movement in a given time interval and gives therefore a good estimation of the swimming activity of the shoal. When swimming activity is important, we expected therefore a high index.

  $$A_{diff} = \left(\left(A_{t+dt} + A_t\right)/A_{tot}\right)/dt.$$

  Where A_t corresponds to the projected area of all fish for a given image t. To avoid bias linked to the size of the fish in the tank, fish group activity index was normalized by the total dark area in an image where fish are not overlapping. Results are then divided by the time interval (dt) between the two images. This final index noted A_{diff} translates therefore a percentage of movement of the shoal per time.
- Israeli's method Indexes of fish group behaviour were developed by Israeli and Kimmel (Israeli and Kimmel, 1996) with the coordinates of centre of gravity of the fish groups on the X and Y axes. The standard deviation of the different centres of gravity on both axes is calculated and can be interpreted as the dispersion of the shoal for image t.

  $$\text{On the X axis:} \quad SDX_t = \sqrt{\frac{\sum_{i=1}^{n} A_i (X_i - CX_i)}{\sum_{i=1}^{n} A_i}}.$$