

REAL TIME MOTION ANALYSIS AS A USEFUL TOOL TO MONITOR BEHAVIOURAL RHYTHMS AND ACTIVITY STATUSES IN FISHES

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1. Introduction

There is a growing interest in developing remote and non invasive techniques for the automated and long-lasting monitoring of behaviour in aquatic organisms of ecological and commercial importance [1]. Quantifiable alterations in the behaviour of ecological indicator species (i.e. with narrow preferences for certain habitat conditions) are the object of ecotoxicological studies, when their presence can be correlated to different categories of pollutants [2].

In this study, we monitored with software for image acquisition and movement processing, the Real Time Motion Analysis (RTMA), the activity rhythm and the activity status of three freshwater fish species: *Alburnus alburnus*, *Pseudorasbora parva* and *Gambusia holbrooki*. We choose these species since: a) they compete for the same resources (diet, habitat and time) but are often found together, suggesting that this coexistence occurs for behavioural differences based on the activity rhythm phase [3]; b) for their small size proposing interesting technological challenges to the automation of the analysis.

2. Results and Discussion

The behaviour of 3 individuals was continuously monitored for 5 days in constant darkness (DD). Animals were held in transparent tanks (15x30 cm). An IR web-cam was placed above the tank, perpendicularly at 1 m height. Two 50W IR illuminators were posed below the aquarium in order to allow video filming in DD. The video camera was connected with a computer loaded with the RTMA software. This program was developed a custom application. It is comprised of a videograbber module and of an image analysis module. The first one was set to acquire a b/w 800x600 image per second, while the second one spotted the fish position by subtracting each next image from the previous one, and then by applying an average filter in a 5x5 window (useful for noise reduction) and thresholding the resulting image. The centroid of the target which was the closest to the tank centre was assumed as the centroid of the fish (this check was needed because sometimes a ghost image, reflected on the tank wall was also present), and its coordinates were recorded.

With the output data we studied:

a) The behavioural rhythm (Fig. 1): centroid coordinates were transformed into distances (cm), giving information on the rate of displacement per sec, as an index of behavioural activity. Resulting time series were binned per 60 min and sectioned into 24-h segments.

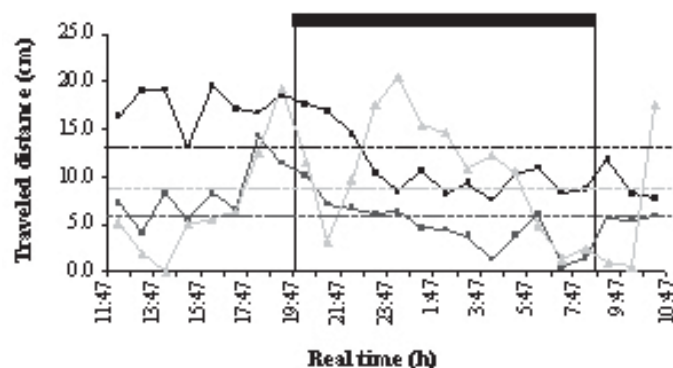


Figure 1. Swimming activity pattern (cm per 60 min) showed by a specimen of *Gambusia holbrooki* in DD during 3 days (1st day black, 2nd day dark grey; 3rd day clear grey) as an example. The black bar represents the night duration; the dashed lines are the MESOR (24-h mean of activity): 12.9 in the 1st day; 6.0 in the 2nd day; 8.0 in the 3rd day.

The individual in Fig. 1 showed a broad diurnal swimming activity pattern during the first and the second day of test. The phase of that rhythm was lost in the third day. This data suggest that *G. holbrooki* is a diurnal species. The rhythm amplitude (as indicated by MESOR) varied through days. Such pattern of noisy swimming activity was typical of all tested individuals and was attributed to the stress during the period of acclimation to laboratory conditions. The same analysis for *A. alburnus* and *P. parva* suggest that the first species is active at crepuscular (down) hours, while the second has a nocturnal swimming pattern.

The analysis of time series obtained with the real motion analysis allows also the determination of the following behavioural parameters (not shown): 1) the exact periodicity of the rhythm by periodogram analysis; 2) the phase determination of the rhythm (by waveform analysis); the quantification of amplitude (intensity) of the rhythm (max-min).

b) The activity status (Fig. 2): (a) images were subdivided into 12x16 squares computing the preferential presence into different areas; (b) trajectories of displacement were computed (90 min duration in the example).

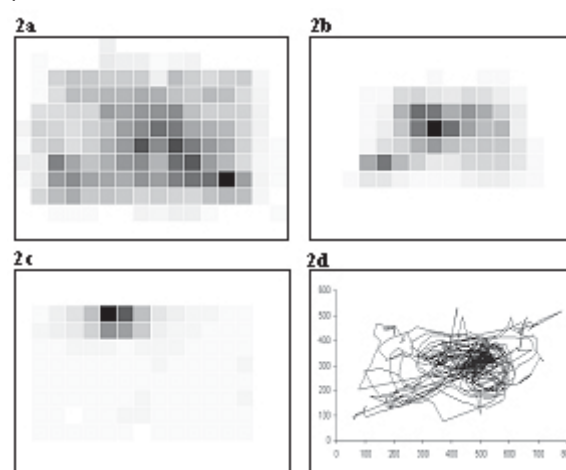


Figure 2. The analysed temporal permanence of *Alburnus alburnus* (a), *Pseudorasbora parva* (b) and *Gambusia holbrooki* (c) in different portions of the experimental aquarium. The trajectory (d) displayed by the swimming activity of a specimen of *A. alburnus* during 60 min is also shown as an example of other behavioural parameters that can be computed.

A. alburnus preferentially swim away from the aquarium walls. Differently, *G. holbrooki* goes closer. *P. parva* mainly swim in the centre. There is a correspondence with the natural behaviour because *G. holbrooki* typically swims near the edges of streams and lakes [4] for feeding on small terrestrial insects larvae usually in the drift and amongst aquatic plants, actively selecting very small preys [5]. *P. parva* moves in the water column to the surface at dusk to feed on microcrustaceans, midge larvae, and algae; descends to deeper waters at daybreak [6]. *A. alburnus* occurs in shoals near the surface feeding mainly on plankton, but can also feed insects and mosquito larvae near the edges of rivers and lakes [3].



3. Conclusions

The species presented different activity pattern and status in agreement with published data (e.g. [4]). The differential rhythmic activity and behavioural status during the swimming performance is at the base of their coexistence. The advantages of the real time motion analysis are: 1) its working capability with any video source and image size (pixel format); 2) its long lasting autonomy in data recording and processing; 3) its independency from memory size for the automated elimination of old frames after digitization; 4) its non-invasive character, allowing studies on a wide variety of aquatic organisms, including protected species.

4. References

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METHODOLOGY FOR THE IDENTIFICATION OF BUSINESS OPPORTUNITIES: THE CHILEAN AQUACULTURE CASE

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The hispano-chilean consulting firm IALE coordinated a project entitled "Identification of Global Business Opportunities based on Technologies for Aquaculture and Related Clusters" pursued within the Bicentenary Program of Science and Technology of the Government of Chile.

The aim of this study was to help initiate an increase in business opportunities based on market and technology tendencies so as to reinforce and act as catalyst for a significant development within the aquaculture cluster and its related industries, which play an important role in the country's economy.

These targets were achieved through the development of three components (products) which were labeled by the Program as a) Evaluation of world technologies and markets; b) Technology Maps, and c) Consortium Development Model.

IALE, with the constant help and support of Adolfo Alvial (director of the Salmon Technology Institute INTESAL) developed the Technology Maps. They focus on the technical aspects of aquaculture by combining results from Technology Foresight, Technology Watch and Competitive Intelligence. The challenges facing the different aquatic species were examined through methodologies such as Delphi or such as the cienciometric analysis of scientific publications and patents. In this work, we describe the methodology which we implemented for the Technology Map product as well as some of the results that we obtained.

The scientific publication database which was selected for this study was ASFA (Aquaculture Science and Fisheries Abstract), which is considered to be the leading reference in science and technology and management of aquatic environments and organisms, for both salt and fresh waters.

As for patents, we consulted both databases of the Patent Offices from the USA and the European Union (USPTO and EPO), for the period between 2000 and 2005.

This study of Technology Maps allows us to identify the main scientific lines of interest and tendencies of recent years as well as the different technology areas. We also developed technology maps of thematic clusters in order to identify emerging signals. Thus, we were able to detect important challenges from different technology areas based on different groups of species (cold water fish and mollusks) in relation to Chile's aquaculture.

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