APPLICATION OF IMAGE ANALYSIS FOR THE QUANTIFICATION OF BEHAVIOUR PATTERNS IN SOLE (*SOLEA* SPP.)

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Introduction

Study of animal behaviour is often subjective and rather difficult depending on the species, for example those which are benthic or with nocturnal habits like sole (*Solea* spp.; Kruuk, 1963). Image analysis is a useful tool which has been applied to many research and industrial fields in order to enhance the results obtained through direct observation.

The aim of this work was to evaluate the reliability of combining the quantification of behaviour using image analysis software, with the qualification of fish attitudes through direct observation, in order to obtain a comprehensive approach to sole behaviour in different conditions.

Materials and methods

A total of 64 *S. senegalensis* were distributed in two groups: a low density group (LD) consisting in 32 fish divided in two experimental units (standard length 16.00±2.03 cm; and 15.00±3.53) and stocked at a 60% of tank bottom surface coverage, and a high density group (HD), composed by 32 individuals stocked in two experimental units (standard length 15.30±1.86 cm; and 13.70± 3.46) at 180 % of bottom surface coverage. Behavioural studies were carried out at night, with water temperature of 13±0.5 °C. Continuous feeding system was scheduled during the period from 1800h to 0600h. Two red spotlights (wavelength, 620-770 nm) were required for good fish visualization in the monitor.

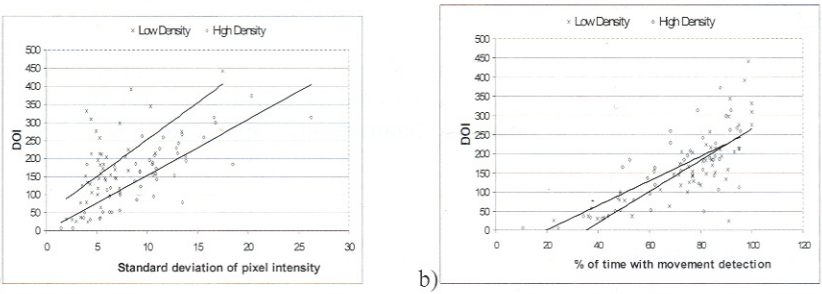
Image acquisition and analysis required a specific software (Image Pro Plus 5.0), a frame grabber PCVision Plus, an a CCD camera Jai A11. Recordings of eight intervals of 40 minutes at 1 frame $2s^{-1}$ were made up for the posterior analysis of the image. The intervals were taken from sunset to dawn (18h, 20h, 22h, 00h, 02h, 04h, 06h, 08h) during 3 nights in each unit. Subtraction of pixel intensity from two consecutive frames allowed to obtain a new "difference frame" showing only the differences between the two frames due to the fish movement or activity. Two different parameters were determined as potential fish activity indicators from image analysis: a) standard deviation of pixel intensity in each "difference frame", b) percentage of "difference frames" with a standard deviation of pixel intensity greater than 3, which will evidence the movement of at least one fish.

In order to calibrate the above mentioned parameters, typical attitudes of sole behaviour (take off, surface swimming, burying, and caudal-lift; Kruuk, 1963, Dalla Via *et* al., 1998) were registered by direct observation during the same intervals. Direct observation index (DOI) was calculated by weighting up each kind off attitude according to its average duration (3, 10, 1 and 1 sec respectively).

Relationship between image analysis parameters and DOI was obtained by lineal regression.

Results

Coefficient of correlation for DOI *vs.* standard deviation of pixel intensity was R^2=0.6741 for HD (n=48) and R^2=0.4042 for LD (n=48) (Fig.1a), and for DOI *vs.* % of time with movement detection was R^2=0.5632 for HD (n=48) and R^2=0.5808 for LD (n=48) (Fig.1b). Better coefficient of correlation was found for DOI *vs.* standard deviation of pixel intensity of R^2=0.8523 for HD (n=24).



a) b)

Fig. 1. Relationship between image analysis parameter and direct observation index (DOI), a) by standard deviation of pixel intensity, and b) by % of time with movement detection.

Discussion

Digital image analysis could be a powerful and reliable tool to assess sole activity, both quantitative and qualitatively, in an easy and time-sparing way. Further work must be done to get a better characterization of different fish attitudes, which could allow the development of new image analysis processes focused to obtain a more accurate description of fish behaviour patterns.

References

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