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A computer-vision system and methodology for the analysis of fish behavior

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ABSTRACT

Behavioral variations of small fish populations are difficult to measure quantitatively. To quantify such measurements, a low-cost computer vision system has been developed to analyze fish behavior in aquaculture tanks. With this system, 9 tanks can be observed simultaneously, enabling the study of one factor, in three triplets for further statistical analysis. The system enables the observation of the tanks at all times, with the use of a web publishing tool, while it allows the remote control of the acquisition to eliminate behavioral variations that might otherwise be caused by human presence. Evaluation of the system was achieved by measuring fish interaction (inspection and biting) on three different net conditions. Measurements were completed in three experimental sets, using stocking density as a stress factor. Results clearly demonstrate that the system successfully recorded fish behavior with minimal frame loss (<21 s in 24 h), while analysis identified every fish interaction with the net. In addition, the measured variations of fish behavior within a single day showed no statistical differences. In conclusion, an inexpensive and efficient computer vision system is presented, assisting in the monitoring and analysis of fish behavior.

1. Introduction

Behavioral variations of fish are very sensitive to stress factors (Masud et al., 2005; Pratt et al., 2005; Miller and Gerlai, 2007; Mancera et al., 2008), such as changes in environmental parameters (Little et al., 1990; Little and Finger, 1990). Furthermore, farmed fish are exposed to a larger variety of dynamic and multifactor stress factors (McFarlane et al., 2004; Huntingford et al., 2006; Johansson et al., 2006). Studies have been completed exploiting fish behavior as an analytical tool for environmental risk assessment and their reaction under specific stimulation like chemical stress analysis (Steinberg et al., 1995; Kane et al., 2004; Masud et al., 2005), or time spent on a specific behavior (Pratt et al., 2005; Miller and Gerlai, 2007). Moreover, it is well known that in aquaculture, high stocking density is one of the major causes of fish stress (Mancera et al., 2008).

To measure and quantify fish behavior, many systems have been developed. Video recording has been used for the remote observation in real time, and also for the offline reproduction of fish activity through video playback (Vogl et al., 1999; Suzuki et al., 2003; Kane et al., 2004; Mueller et al., 2006; Stien et al., 2007; Waggett and Buskey, 2007; Grubich et al., 2008; Salierno et al., 2008). One of the major advantages of video systems is the fact that no human presence is required close to the experimental area to observe fish behavior. Experiments may last many hours, even days, making video systems an invaluable tool in behavioral study of fish. The long duration of the recorded video requires equal hours of observation from a person, making analysis very hard to be performed. Furthermore, the analysis of fish positions in a tank with hand scoring through direct observation results in low spatial and also time resolution (Bjerselius et al., 2001; Teather et al., 2001; Wibe et al., 2001).

Various techniques have been implemented to help automate behavioral studies, each one specially designed to answer specific biological questions, showing that a more general tool is difficult to develop. Examples show that automated image analysis techniques for post processing (Kane et al., 2004; Higham et al., 2005; Park et al., 2005; Stien et al., 2007; Grubich et al., 2008; Duarte et al., 2009) or motion analysis of fish (Brewer et al., 2001; Grubich et al., 2008) are nowadays obligatory [such as the observation of 12 fish individuals in 12 small tanks, and the analysis of their random walk (Kane et al., 2004), the video analysis procedure developed to assess the vertical fish distribution in tanks (Stien et al., 2007), or even more advanced motion analysis techniques like the image processing system for the quantification of fish behavior (Kato et al., 2004)]. Moreover, in the last decade, systems have been developed from hand scoring through direct observation, to more intelligent and complex

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systems that can automatically track individuals (Dusenbery, 1985; Ye and Bell, 1991; Hoy et al., 1996; Kato et al., 1996; Hoy et al., 1997; Delcourt et al., 2009). Although advances in technology allow the design of more sophisticated systems (making hardware more simple to control and software more easily developed by non programmers) no system has been developed yet to monitor specific behavioral traits of fish (such as their willingness to escape).

In this work, we describe a digital recording system and a novel analysis algorithm that allow the remote study of fish behavior and the fast analysis of specific behavioral questions. The system is capable of extracting specific frames from the recorded video for further evaluation (such as when behavioral-related fish responses occur). The description of the technique and the analysis algorithm is explained. The total cost of the system remains very low, allowing widespread implementation. Improvements and modifications of the system are discussed.

Little is yet known regarding the various behavioral patterns of fish. To understand fish behavior and to find the major stress factors that alter their reactions, novel instrumentation and methods need to be developed. In this study, the interaction and behavior of *Sparus aurata* with an aquaculture net is studied, under various conditions, using stocking density as a stress factor. In addition, the variability of their behavior during the day is observed, before and after feeding time. We measure the time that fish spend inspecting the net, and the number of bites that occur on the net surface, as these parameters are strongly associated with the damages reported by fish farmers (Norwegian Fisheries Statistics, 2007; www.preventescape.eu).

2. Methods

2.1. Tank preparation

Nine parallelogram tanks were used (length: 115 cm, width: 34 cm and water depth: 40 cm), filled with 100 L salt water (salinity 38 ppt), while the inside surface was painted white. The fish were observed through longest side of the tank, made out of a 5 mm thick glass. Each tank was separated into two regions, 60:40 by volume, using a mesh made out of a white aquaculture net sized 31 cm × 28 cm with 17 mm hexagonal mesh eyes. The mesh was fixed with a blue plastic frame, which fitted perfectly to all sides of the tank. The tanks were oriented in a vertical-plane 3 × 3 array format, with tanks separated by 50 cm.

2.2. Test species

The system was tested using gilthead sea bream (*S. aurata*), at the Biology Department of University of Crete. During the experiment, fish were kept in the 60% area of the tank (referred as holding area) and were fed once every day at 14:00. The amount of food supplied in each tank was equivalent to 2% of the initial fish-population biomass in the tank. The food was always introduced on one corner of the holding area, to avoid the possible flow of food to the other side of the mesh, due to water currents. The weight of each of the selected fish was 25.35 ± 2.27 g, while the average length was 12.45 ± 0.44 cm.

For this experimental protocol, fish tanks were grouped in three triplicates for further statistical analysis, each triplicate consisting by the vertical axis of the tanks array. In the three triplicates different applied densities were used, classified as low (10 individuals), medium (15 individuals) and high (20 individuals), respectively.

2.3. Vision acquisition system design

Nine color digital CCD cameras with manual iris and focus controls were mounted opposite from the tanks' windows, at a distance of 1 m, each one recording a single tank. The cameras' factory lenses were replaced with lenses that had no infrared-cut coating, allowing the detectors to acquire more red and infrared light. The cameras were connected via firewire (IEEE1394) protocol to a single desktop computer (CPU i7, 6GB RAM, 2TB Hard Disk Drive (HDD)). The required frame rate of the recording was set to 3 frames per second. To achieve this acquisition rate, cameras were connected on 2 different Firewire – PCI cards (Unibrain) (5 cameras to 1 card and 4 cameras to the second card) to exceed the limit of 400 Mbit/s transfer rate. Acquisition was performed with custom made software, written in LabView (National Instruments), specially developed for the experiment in the lab. The cameras were recording 24 h a day for the entire duration of each experiment. It should be noted that the video recordings are currently black for 12 h per day, when the lights are switched off. We plan to use infrared illumination, which will allow night-time observations as well. The recordings of each day (total time 24 h) were automatically stopped at 00:00 time and saved as separate files on the computer's HDD. The software then automatically creates a new file and continues saving of the new video. All frames from the 9 cameras were acquired sequentially. The total acquisition time from all cameras was measured to be lower than 1 ms, while the intermediate time until next acquisition was fixed at 333 ms. Following acquisition, the frames were combined to a single frame for simultaneous observation and so as to have a single video file as an output. MJPEG compression algorithm was used to reduce the huge amount of data recorded daily. Recording during the night minimally affect the amount of data recorded, since the MJPEG algorithm highly compresses the black frames. After completion of each day, video files were remotely transferred, via internet to another computer for data analysis, while still running the experiment. Real-time observations of the fish behavior in all tanks was also achieved with the use of a web-server (LabView, Web Publishing Tool) installed together with the software in order to observe the progress of the experiment without entering the experiment room thus avoiding influencing the behavior of the fish.

2.4. The set of experiments

A total of 405 fish were used in this study, 135 for each experimental set. The experimental design was the same among the three experimental sets, while the only difference involved the condition of the mesh.

In the first experiment, an undamaged mesh was placed in each one of the tanks separating them into two areas. In the second, the mesh used was slightly damaged, having one twine of a centrally located mesh eye cut exactly in the middle. Finally, in the third experiment, three adjacent twines were cut forming a tear through which the fish could cross and swim to the other side of the mesh. To form a tear of the appropriate size, the width of the fish was also measured (average width: 4.0 ± 0.1 cm, average length of tear created: 4.0–4.5 cm).

The first phase of the experiment was the acclimatization, which lasted four days and took place before the beginning of each trial. All fish were placed into the tanks and were provided with food in the same quantity and manner as in the rest of the experiments. During this phase, the activity of the fish was not recorded. After acclimation, the next experimental phase followed where the camera system acquired video for a full 24-h period. In the third experimental phase the analysis of the acquired data was involved. The selected day for the analysis was the sixth day after acclimatization, since fish were fully acquainted with the environment. Analysis was performed for five different time periods, each with duration of 15 min, to analyze fish behavioral differences within the day. The start of the selected time periods were: at the beginning of the day (8:30), mid-morning (11:00), before feeding time (13:00), after

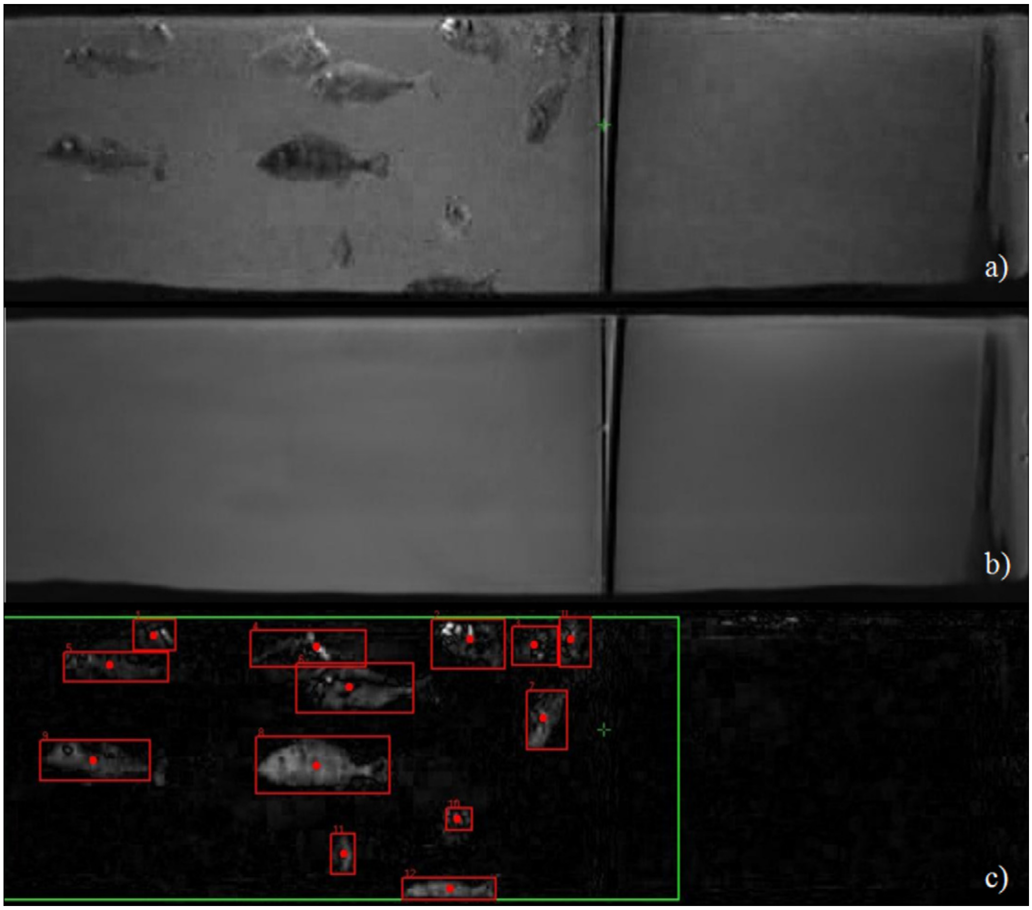

Fig. 1. (a) Selected video frame with no processing; (b) processed average frame; (c) detection of population individuals.

feeding time (15:30), and at the end of the day (19:30). Two behavioral variables were chosen to be measured, activity close to the net (inspection) and net biting. The total inspection time is determined by the total number of frames in which at least one fish approaches the net by less than 2 cm, while also facing the net. Net-biting events are individually counted.

2.5. Analysis software design

All acquired videos were analyzed with the use of custom made software developed in our labs, written in LabView. The software initially locates the turning on and turning off of the fluorescent lights above the fish tanks. Every frame, before processing, is split in its 3 color planes (R, G, and B). The user can select the plane that provides the highest contrast between fish and background. In our setup, the plane with the best contrast was measured to be the green plane, so it was selected to be used for further processing. Subsequently, an automated algorithm averages 300 frames (100 s), to calculate a clear image of the tanks (background image), seemingly with no fish inside. As fish are moving randomly throughout the tank, over 100 s of averaging the fish are blurred uniformly, so that the averaging algorithm evaluates them as noise, largely eliminating their effect on the "background" image. Every frame is analyzed with an image difference algorithm (IMAQ absolute difference, LabView) resulting in a background-subtracted image that contains only the moving objects of the picture such as the fish and the bubbles from the air-stones inside the tanks. The user then selects an area from the image that contains one tank (Fig. 1c, green area). (For interpretation of the references to color in this text, the reader is referred to the web version of this article.) Additionally, a reference point is selected to provide spatial information of individual fish inside the tank. This reference point was set as the center of the net pen that separates the tank in two areas (Fig. 1a–c, green cross) (For interpretation of the references to color in this text, the reader is referred to the web version of this article.), allowing the software to then perform the analysis. Fish are detected within the selected area of interest, with a standard object detection algorithm (LabView, Vision). The user then sets the algorithm variables (max object size, min object size, threshold) so that the fish can be correctly detected as objects. Subsequently, the software detects the objects and determines the positions of the center of mass of each object. The horizontal distance of every object center from the reference point is then calculated. An extra algorithm is used to check if the distance of any object from the reference point is closer than 10 pixels, which occurs for inspection and biting. In this case the algorithm extracts the selected tank from the current frame and stores it to the computers HDD as a BMP image, for later human observation and evaluation. During the analysis, the software detects object the positions and then calculates the distance of every object from the reference point. A common averaging algorithm calculates the position of the population's mass center. Various statistical variables are calculated, such as the total number of fish detected, the horizontal, vertical, and average distance and the average speed of the center of mass of the fish population, as well as the respective standard deviations of averaged variables.

2.6. Statistical analysis

Statistical analysis was performed using SPSS (PASW Statistics 18). To check for statistical differences between replicates and

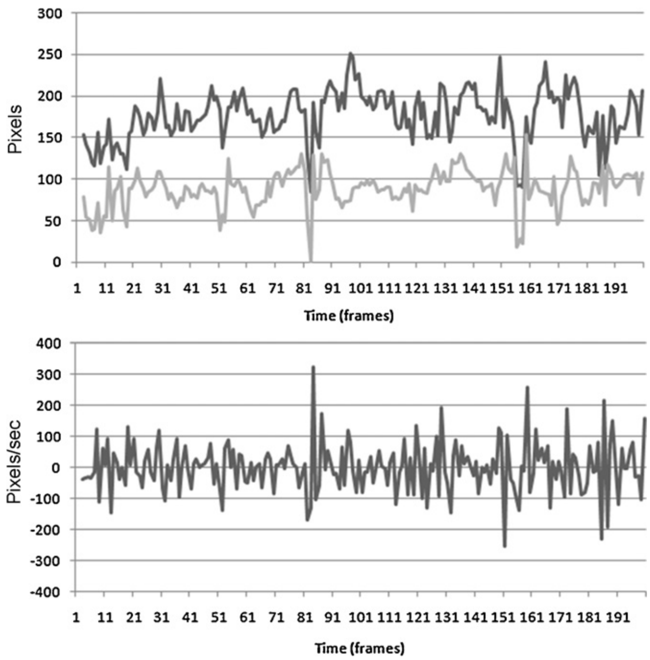

Fig. 2. Top graph represents the center of population mass movement in horizontal axis with the standard error; bottom graph shows the velocity alterations from the center of population mass.

differences between the three population densities, a nested ANOVA model was used, setting the time that fish spent for inspection or the number of bites as the dependent variables, while keeping time periods and the population densities as fixed parameters, and each tank from the triplicate acts as nested factor within the density. A post Hoc Tukey's test was used to detect differences between the groups, keeping the significance level >95% ($P < 0.05$).

3. Results

3.1. System performance

The system acquired approximately 259,500 frames resulting in a total size of 29GB of compressed data for every 24 h of recording, while only losing 61 frames on average for every 24 h cycle (which refers to 21 s). Every resulting frame required approximately 10 ms for the cameras to transfer their acquired images to the computer, the software algorithm to combine the nine frames to one, the standard MJPEG compression to compress the frame, and finally for the computer to store the data to the HDD.

During the analysis of videos (Fig. 1a), the normalization image, created after averaging, results in a very clear image of the non moving objects of the cameras field of view (Fig. 1b). The detection algorithm was successful in detecting almost every individual (Fig. 1c), except from the cases where fish images were overlapping, in which case the algorithm detected only one individual.

For every 15 min of video analyzed, statistics were performed related to the population center-of-mass positions, as described above. Changes of the movement and velocity in both horizontal and vertical axis were observed. In Fig. 2 the center of population mass horizontal movements are shown together with the standard error representing the populations schooling, and with the horizontal velocity alterations of the center of population mass. The total points in the graph represent the total number of frames analyzed divided by 15, resulting in 180 points (every point is 5 s).

3.2. Experiment

The results from all the experiments involving the behavioral characteristics of fish are presented in Fig. 3. The analysis of the results is described below.

3.3. Undamaged mesh – first experiment

3.3.1. Replicate comparison

The first part of the analysis was to compare the individual replicates in each triplet for significant differences. As stated before, the method used was a randomized block design. Significant differences ($P < 0.05$) on fish inspection were only observed on the replicates of the medium density (15 fish). No significant differences were found on fish biting between all replicates of the same tests.

3.3.2. Time comparison

Behavioral variations between the different time periods were analyzed, under the same analysis method. The same behavioral parameters where analyzed (inspection, biting). There were no significant differences between the different time periods at any treatment, related to the behavioral parameters ($P > 0.05$).

3.3.3. Treatment comparison

Since there were no significant differences between triplets, statistical analysis was performed in relation to the three applied densities. Additionally, using all the data from the experiment, statistical differences were checked between the different hours of the analysis, in relation to the defined behavioral parameters. The test for fish inspection showed that there was a significant difference related to the small and medium population density ($P < 0.01$). The test for fish biting showed that there was a significant difference related to the small and high population density ($P < 0.01$).

3.4. Mesh with a single cut twine – second experiment

3.4.1. Replicate comparison

The same analysis pattern was performed for the second experiment. For fish inspection, the replicates related to the medium population density resulted in a significant difference ($P < 0.01$), while the rest of the replicates showed no statistical differences. Regarding fish biting no significant differences were found between all replicates.

3.4.2. Time comparison

There were no significant differences between the different time periods at any treatment, related to the behavioral parameters ($P > 0.05$).

3.4.3. Treatment comparison

The test for both behavioral parameters showed that there was no significant difference related to the any population density ($P > 0.05$).

3.5. Mesh with a tear – third experiment

3.5.1. Replicate comparison

In the third experiment, the analysis of fish inspection showed significant difference ($P < 0.05$) only among the replicates in the medium density group. Regarding fish biting under the same

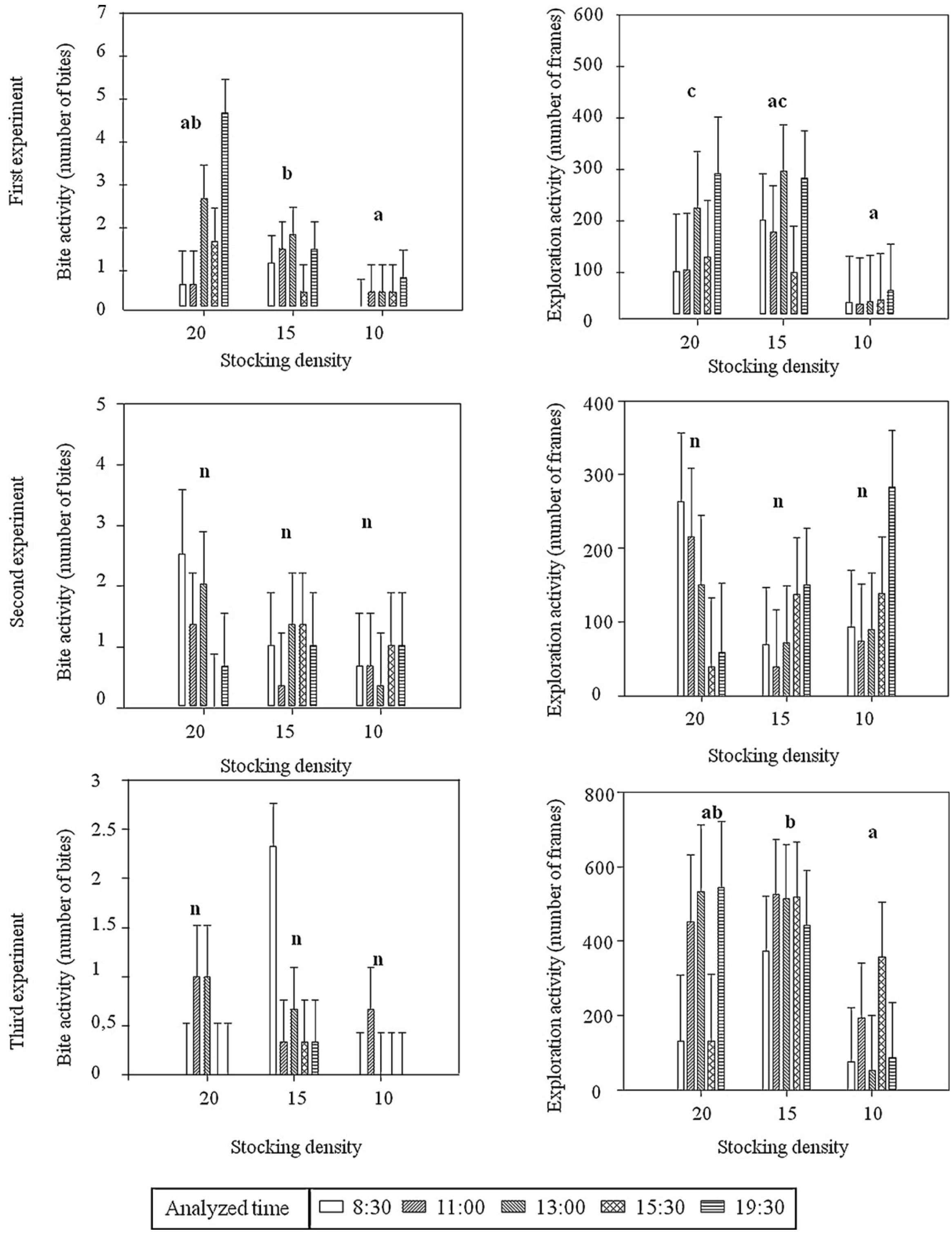

Fig. 3. Replicate analysis. Different grammatical symbols indicate the existence of statistical differences between stocking groups $P < 0.05$. The letter n indicates no statistical difference.

tests no significant differences were found between all replicates ($P > 0.05$).

3.5.2. Time comparison

Similar to the experiments 1 and 2, behavior did not change during the day, as the analysis of the different time periods did not show significant differences for both fish inspection and biting ($P > 0.05$).

3.5.3. Treatment comparison

The results of the nested ANOVA test showed significant differences between the small and medium densities ($P < 0.05$) related to both behavioral parameters, while the rest of replicates showed no statistical differences.

As results indicate, our system is capable of detecting behavioral alterations caused by stress factors. Furthermore there was no significant difference between the time periods within the day, implying that the behavioral variations of fish inspection and biting on the mesh does not vary within a 24-h period.

4. Discussion

Escape events are regarded as a crucial problem for the sustainability of aquaculture industry (Jensen et al., 2010). Especially in the Mediterranean Sea, sea bream is reported to escape from sea cage installations but its extent still remains unknown (Dempster et al., 2007). Furthermore, the increasing demand for maximum productivity requires larger fish populations and therefore higher applied stocking densities. However, considering the biological habits of sea bream, which tends to nibble the aquaculture net and eventually create holes on the net surface [as does the Atlantic cod (Moe et al., 2007)], it can be concluded that high stocking density not only affects the growth performance of sea bream but also alters species' behavior under captivity.

The aim of this study is the development of a novel system, based on computer vision techniques, to quantify behavioral variations of fish under various stress factors. The system was evaluated by using stocking density as a stress factor. As results indicate, the system is capable of detecting behavioral variations in very short time responses (333 ms), while only losing very small amount of time on each 24 h cycle (<21 s). The total number of frames lost are due to the time required from the computer IO (Input/Output) operations, such as closing one previous file and creating a new one at 00:00 h. Other commercial systems can achieve faster acquisition times, however, a significant amount of time is lost. In those systems, frames from each camera are stored to a separate file, thus requiring a fast computing system capable of handling a large number of concurrent IO operations. The addition of more cameras should be considered with caution, as it may increase frame loss. Our system combines frames from all cameras into a single frame, which is then written to the storage medium, thus resulting in minimum frame loss.

The significant capabilities of our system make it a suitable tool for testing behavioral variations under various stress factors, like chemicals and biological contaminants. The system is easily adaptable so that variations in many different behavioral factors can be tested for dependence on environmental factors. Based on future technological advances, the system will continue to upgrade so that more complex behavioral factors will be detected. Software has been developed in a high-level graphical programming interface (LabView, National Instruments) enabling fast modifications as well as later advances with more complex behavioral parameters. Its detection capabilities and the way that data are exported cannot be achieved with existing commercial behavioral quantification systems (Ethovision, Behavioquant and Expert Vision). The analysis of the data, yields detailed information (such as individual and averaged positions and speeds) which can provide further insight into fish behavior. For small population sizes, individuals can be tracked continuously. In contrast, for large population sizes, only statistical averages of population parameters can be determined, which can provide further information related to specific behavioral traits of fish like schooling behavior. In addition the system is remotely controllable and can display video data in real time through a web-based interface for observations around the globe. During analysis of each 15-min-time period, the time required to check the two behavioral parameters (inspection and biting) was around 5 min on average. In addition, no incident was found to be lost, as it is possible to happen on video observations caused by human mistake. That reduced the total time for analysis, to less than 30 percent of the total time that video observations would require.

Sea bream feeding habits are well documented, focussing on both biological and ecological aspects. *S. aurata* is a mobile benthic fish that in nature feeds on decapods (*Carcinus* sp.), gastropods, bivalves, amphipods, isopods, fishes, and errant and sedentary polychaetes (Tancioni et al., 2003; Mariani et al., 2002; Pita et al., 2002; Tancioni et al., 1998). These feeding habits indicate that the type of food preferred by sea bream individuals is located at the bottom of the sea in solid substrates and not at the water column. We saw that when sea bream individuals are reared in tanks, the change in their living conditions does not alter their feeding behavior entirely. As a result, we show that the cultured fish express their feeding behavior on the mesh by inspection and biting. In the case of sea-cage aquaculture, natural sea substrate is replaced by the cage netting. Our results indicate that there is a connection between fish behavior and stocking density and/or net condition. In captivity, fish feeding is influenced by stocking density (e.g. in trout and seabass), and indirectly by social interactions in species in which dominant individuals monopolize the available food (as in salmonids).

Many stress factors affect the biochemical and physiological conditions of fish, in nature as well as in aquaculture (Uglem et al., 2009). Stocking density is a major stress factor that affects fish growth and energetic expenditure (Li and Brocksen, 1977; Morgan et al., 1999; Lefrancois et al., 2001; McFarlane et al., 2004; Salvanes and Braithwaite, 2006). For the aquaculture industry it is very important to know why and how the fish bite the nets, to develop better materials and avoid the escape of fish from the cages that cause economic and ecological damage.

Compared to previously designed systems, this system can be applied to quantify behavioral variations associated with the exposure to any type of chemical stress factors (such as toxins and any industrial or agricultural waste). In addition, the analysis of behavioral responses can provide information related to the neural and mechanical disruption, that result from biochemical and physiological alterations (Brewer et al., 2001). Utilizing this methodology, researchers can develop an inexpensive way to analyze many behavioral variations under a large variety of stress factors, remotely, while eliminating any behavioral interference.

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