Aquacultural Engineering 77 (2017) 107–111

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aque

# A behavioural sensor for fish stress

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## ARTICLE INFO

*Keywords:*
Fish stress
Sensor
Farmed fish
Early signal

## ABSTRACT

Due to water turbidity, fish stress might be difficult to observe. Evaluation of fish stress by blood sampling requires removing a fish from the water, which is in itself a stressful event. Therefore, we designed and built a sensor to detect fish behaviour that reflects stress. The electronic sensor detected early signs of fish stress by scoring the fish's inactivity. LEDs and detectors are embedded on a steel wand that is held underwater by an operator. In this preliminary (feasibility) study, the new sensor was validated for Tilapia (*Cichlidae*) and Hybrid Striped Bass *(Morone)*. We induced stressful situations in the fish tanks by manipulating oxygen and temperature levels.

*Results:* Lowering the temperature and oxygen levels both significantly increased the average number of signals identified by the sensor, which indicate stress. The effect of reducing water temperature from 24 °C to 15 °C was three times stronger than was the effect of lowering the oxygen saturation level from 85% to 50%. The difference in the number of signals between the good and stressful conditions was statistically significant, amounting to approximately eight sensor signals, 10.57 compared to 2.49 respectively. Lowering the temperature increased the mean number of signals by 5.85 and 6.06 at 85% and 50% oxygen saturation respectively, whereas lowering oxygen levels increased the mean number of signals by 2.02 and 2.23 at 24 °C and 15 °C, respectively. The results indicate that the stress status of cultured fish can be evaluated using the proposed behavioural sensor. The new sensor may provide an earlier indication of a problem in a fish tank or pond than was heretofore possible. This early warning can enable the fish farmer to take action before many fish are harmed.

## 1. Introduction

Many studies exist that support the case that stress affects fish welfare and production (Schreck et al., 2001; Tim et al., 2012). Proper early attention can prevent fish mortality. At present, a common method to identify stressed fish is to analyse their blood cortisol levels (Ellis et al., 2012). However, this means removing a fish from the water, which is by its nature a stressful procedure for the fish (Braithwaite, 2011). The challenge addressed in this study was how to evaluate fish stress conditions without having to remove a fish from the water.

On a fish farm, fish behaviour can be an indicator of fish stress (Rios et al., 2002), or an indicator of polluted water (Galhardo et al., 2011). Studies have therefore focused on behavioural changes in fish due to pollution (Martins et al., 2012) and fishes' sensitivity to their surroundings. (Leal et al., 2011).

Electronic sensors and video cameras have been used to monitor fish behaviour (Greaves and Tuene, 2001) but so far, no specific behavioural pattern associated with fish stress has been quantified (Schreck, 2010).

Very little could be found in the literature about the behaviour of stressed and unstressed fish to support what experienced fish farmers know, i.e. that an unstressed fish can barely be touched. It does its utmost to escape contact with any human being and swim rapidly away from any unknown or moving object. By contrast, a stressed fish cannot swim out of the way. This was implied in the study by Xu et al. (2006), which tested algorithms to quantify the average swimming speed for stressed fish. Consequently, we hypothesized that a sensor would have a hard time touching lively, unstressed fish.

Our **research hypotheses** were that: (1) an unstressed fish can barely be touched; (2) an electronic sensor can detect stressed fish that will not swim away from the sensor. Therefore, **the aim** of this study was to design, build and evaluate a behavioural sensor that would count the number of times that fish do not escape from a moving object. Each signal registered on the sensor represents one fish that was behaving as a stressed fish, and did not swim away from the sensor.

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http://dx.doi.org/10.1016/j.aquaeng.2017.04.001
Received 22 November 2016; Received in revised form 1 April 2017; Accepted 3 April 2017

## 2. Materials and methodology

### 2.1. Design criteria for sensor

A detector to identify stressed fish would have to meet a number of design criteria in order to fulfil the purposes of this study: (a) The detector would have to be reliable, robust, and water-resistant. (b) During the experimentation process, the detected events should be recorded in a file. (c) The sensor should be reliable, with a Mean Time Between Failures [MTBF] of more than five years. (d) A clear and bright readout LCD panel screen that is easy to operate and read in sunlight, and capable of functioning in the high moisture environment prevalent near a fish pond is necessary. (e) The effect of water flow on the sensor should be minimized. (f) The technical maintenance requirements should be low. (g) The labour required to operate the sensor should be minimal. (h) The sensor must be able to function under a wide range of water clarities, including low transparency due to muddy and murky water, and turbidity, the presence of large quantities of algae, etc.[1] (i) The device needs to consume very little electricity, and thus utilize low voltage, e.g. it should require no more electricity than that provided by a 24 v battery. (j) The sensor must be safe for both the operator and the fish, with no stray electrical discharges, especially in the water. (k) The system should be economical to use.

### 2.2. Sensor description

The sensor prototype (Fig. 1) was designed and built at the Institute of Agricultural Engineering, Agricultural Research Organization (ARO) – The Volcani Center, Israel.

The Electronic system is based on four pairs of Panasonic CX-411-P Compact Photoelectric Thrubeam Sensors, consisting of an emitter and a detector of an optical beam. They are highly resistant to water.

The sensors are mechanically connected to the wand, which is 40 cm in length and 3 cm in diameter, and located opposite each other to create an optical connection between them. The sensors signal a processing system that uses a PIC18F877 microprocessor to identify the state of the fish that cross the beam between the pair of sensors. The processor receives information from all four pairs of sensors and detectors. Thus, if only one set of emitter-detector sensor sends a signal, it is counted and displayed on the screen, which is located away from the water.

The sensors are placed along the length of the wand, so as to ensure that fish approaching from any angle will be detected, and to increase the number of slow-moving fish that can be detected simultaneously. As a prototype, the wand utilizes easy-to-assemble parts. Even slow-moving fish never touch a foreign object, so there is no concern that they might be hurt by contact with the wand or the sensors. In fact, the fish were examined carefully after the experiment was completed, and not a single one, including the slow-moving fish, exhibited any wounds. Of course, for future experiments and more widespread use, the design of the wand will be refined. (Fig. 1d). When a fish passes between the light source (Fig. 1b) and the detector (Fig. 1c), it triggers a signal. The signal is transferred to the control unit (Fig. 1a), embedded with microprocessor PIC18F877, recorded and labelled with its time stamp.

### 2.3. Methodology for sensor technical feasibility-test

Two species of fish were used in the experiment: Blue Tilapia (*Cichlidae*) and Hybrid Striped Bass (*Morone*).

Tilapia fingerlings weighing an average of 25 g, which had been raised in a two-hectare open earthen pond on a commercial farm, were taken to the laboratory tanks, located in the central health laboratory on Kibbutz Nir David. One hundred and sixty fish were placed in four containers, i.e. 40 fish in each tank. The transfer was successful and 100% of the fish survived. The fish were not fed the day before the transfer nor during the experiment the following day. The experiment was replicated three times, with an interval of two days between replications. The experiments were conducted in four 100-L tanks. Each tank was filled with 20 L of water, so that the depth of the water was 25 cm. Thus, the wand operator was able to conduct the experiment without putting his hand in the water: he grasps the wand on the 15 cm of the 40 cm-long wand remaining above the water level. Introducing 40 fish weighing 25 g each into 20 L of water yields a density of 1000 g, or 50 gm/L, equal in value to 50 kg/cubic metre of water. This is considered to be a high level of density, and is used in super-intense systems. Consequently, the risks of stress to the fish under such conditions is 20 times greater than it would be in regular ponds or tanks. The oxygen was supplied by compressed air and its level was controlled by a manual valve. The level of oxygen was measured with an OxyGard hand-held meter.

Stressful situations (Trewavas, 1983) were induced in the fish tanks by manipulating the oxygen levels and temperature. Four tanks (Table 1, Table 2) were available. Thus, four combinations of poor and good water temperatures and oxygen levels could be created.

The water temperature, controlled by a central computerized heating system, was lowered to 15 °C in two tanks, and kept at 24 °C, in the other two tanks; the latter temperature is the one at which Tilapia are usually raised.

Each experiment was replicated three times, with each replication constituting one block. Twenty-four underwater sweeps from side to side of the tank were made in each tank. Each sweep lasted 30 s, conducted within a period of three to four hours for each replication of the experiment.

The Hybrid Striped Bass (*Morone*) was the other species of fish used in the experiment. One hundred and sixty fish weighing an average of 20 g were transferred from outdoor fish ponds to four 100-L tanks, located indoors at the Central Health Laboratory on Kibbutz Nir David. Each tank was filled with 20 L of water, so that the water level was no higher than 25 cm. As with the experiment on the Tilapia, this enabled the wand operator to keep his hand out of the water when making the sweeps in the tank. Introducing 40 fish weighing 20 g each into 20 L of water yields a density of 40 gm/litre, equal in value to 40 kg/cubic metre of water. This is considered to be a high level of density, and is used in super-intense systems. Consequently, the risks of stress to the fish under such conditions is 20 times greater than it would be in regular ponds or tanks.

The water temperature was raised to 31 °C in two tanks and kept at 24 °C in the other two tanks (Van den Avyle and Evans, 1990). Combinations of the temperatures and dissolved oxygen are described in Table 2.

The transfer was successful and 100% of the fish survived. The fish were not fed the day before the transfer nor during the experiment the following day. The oxygen was supplied by compressed air and controlled by a manual valve. The level of oxygen was measured with an OxyGard hand-held metre. The experiment was replicated three times, with an interval of two days between replications.

For both species, the operator dipped the electronic sensor wand (Fig. 1, d) into the first tank and activated it. Monitoring was carried out for 30 s while the sensor was moved inside the tank. The operator holds the part of the wand that remains out of the water, and as he puts the rest of the wand under water, he switches on an electronic timer, which automatically stops the sensors from operating after 30 s. He moves the wand from each side of the tank to the other, in width and length, using both straight and curved motions, attempting to get close to the fish. Each replication consisted of 24 underwater sweeps with the wand in each of the four tanks, yielding 96 measurements.

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[1] As a first attempt to test the concept, it was important to the researchers to ensure that the device could work as hypothesised. Therefore, the experiment was conducted under laboratory conditions, with transparent water. Of course, further study entails testing the device in murky water.

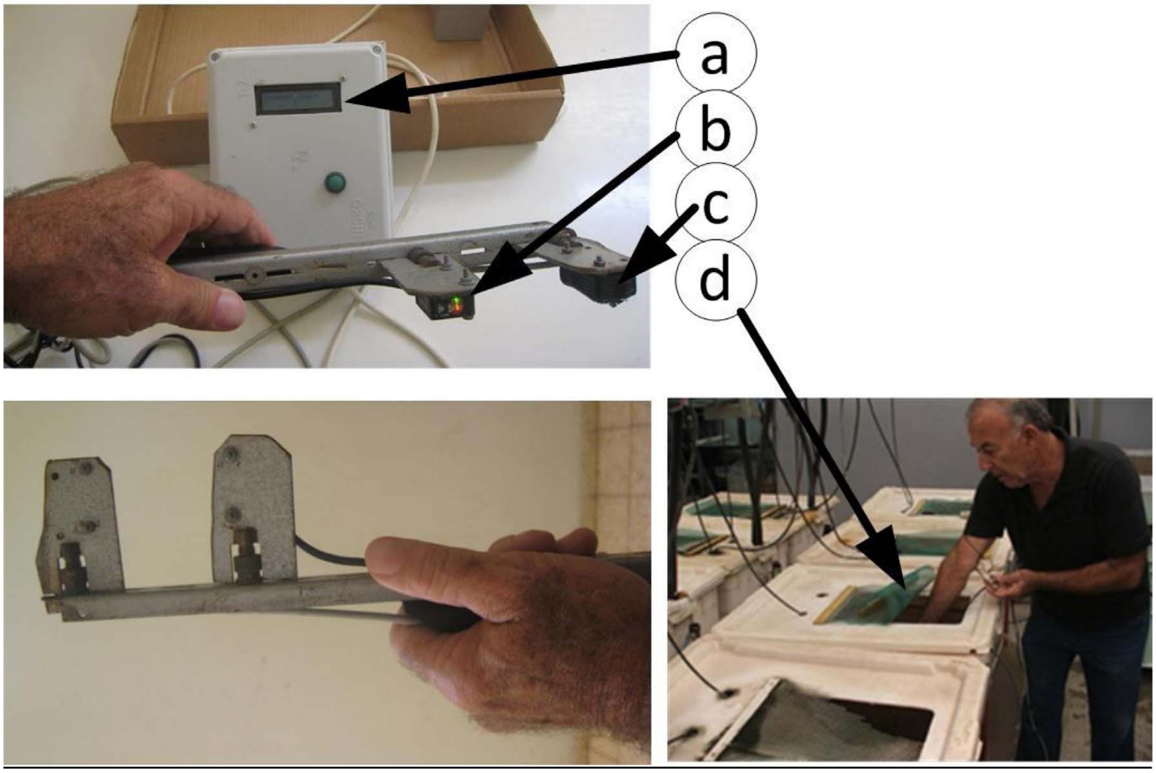

**Fig. 1.** The sensor includes an electronic counter and LCD display: (a). Sensor components include LED emitters (b) and detectors (c). When a fish crosses the light beam, a signal is triggered. An operator holds the sensor underwater (d), monitoring is carried out for 30 s while the sensor is moved inside the tank.

**Table 1**
Tilapia tanks treatments.

| Tank No. | Temperature (°C) | Oxygen level (%) | Condition | Number of replications | Number of samplings |
|---|---|---|---|---|---|
| 1 | 24 | 85 | Good welfare | 3 | 24 |
| 2 | 15 | 85 | Suboptimal 1 (lowered temperature) | 3 | 24 |
| 3 | 24 | 50 | Suboptimal 2 (lowered oxygen level) | 3 | 24 |
| 4 | 15 | 50 | Poor welfare (lowered temperature and oxygen level) | 3 | 24 |

**Table 2**
Hybrid Striped Bass tanks treatments.

| Tank No. | Temperature (°C) | Oxygen Level (%) | Condition | Number of replications | Number of samplings |
|---|---|---|---|---|---|
| 1 | 24 | 85 | Good welfare | 3 | 24 |
| 3 | 31 | 85 | Suboptimal 1 (higher temperature) | 3 | 24 |
| 2 | 24 | 50 | Suboptimal 2 (lowered oxygen level) | 3 | 24 |
| 4 | 31 | 50 | Poor welfare (lowered temperature and oxygen level) | 3 | 24 |

The tanks were 70 cm × 30 cm, and were filled to a depth of only 25 cm. Each 30 s sweep was able to cover the distance from one side of the tank to the other, both length-wise and width-wise, as well as diagonally. Thus, the entire volume of the tank was covered. Twenty-four separate underwater sweeps were conducted (Fig. 1d).

The direction of the wand relative to the movement of the wand was in all the volume of water in the tank. Wherever the operator moves the wand on which a slow fish are caught by the sensor, it was marked on the electronic display. The exact same process was then repeated with each of the three other tanks. Unstressed fish swim rapidly away, usually together; slower ones are registered by the sensors. To ensure the absence of an "operator effect", the operators did not know which tank was assigned to each set of conditions and the order in which the four tanks were tested was randomized in each cycle, i.e. a double-blind randomized crossover experimental design was used. Each replication consisted of 24 underwater sweeps with the wand in each of the four tanks, yielding 96 measurements.

### 2.4. Statistical analysis

The average of the 24 measurements taken in each of the four tanks was calculated for each of the three replications of the experiment, yielding 12 values. These data were subjected to a three-factor analysis of variance, which examined the main effects of the two environmental factors (oxygen and temperature) and the effect of the interaction between them, as well as the differences between the blocks (the three replications). In this study, the value for each replication was the average of 24 measurements taken over a period of four hours, making these values reliable. The null hypothesis for both main effects was rejected at *P*-value = 0.05 (probability of type I error).

**Table 3**
The effect of lowering water temperature and lowering oxygen level on Tilapia, as measured by the average number of signals registered by the sensor.

| Temperature Oxygen | 24 °C | 15 °C | The effect of lowering water temperature |
|---|---|---|---|
| 85% | $2.49^{a}$ | $8.34^{b}$ | 5.85 |
| 50% | $4.51^{c}$ | $10.57^{d}$ | 6.06 |
| The effect of lowering oxygen content | 2.02 | 2.23 | **5.95**<br>**2.12** |

The error variances (among replicates) were tested and found to be similar in all 4 Temperature by Oxygen combinations, allowing ANOVA (Table 4) and calculation of the same standard error to all 4 means (SEM = 0.411).
Means with no common superscript differ significantly at $P < 0.05$.

## 3. Results

### 3.1. Tilapia

The average number of signals recorded under the four experimental conditions is presented in Table 3. Stressful conditions, i.e. lowered temperature and lowered oxygen level, significantly increased the average number of signals. When the temperature and oxygen levels were comfortable for the fish, an average of 2.49 signals were registered. When the temperature was lowered and the oxygen level was kept at an acceptable level, the average number of signals jumped to 8.34. The effect of lowered oxygen level was less obvious, with the number of signals increasing from 2.49 to 4.51. When both the temperature and the oxygen level were lowered, the number of signals jumped to 10.57. Lowering the temperature increased the mean number of signals by 5.85 and 6.06 at 85% and 50% oxygen saturation respectively, whereas lowering oxygen saturation increased the mean number of signals by 2.02 and 2.23 at 24 °C and 15 °C respectively. The difference between the good and stressed conditions was approximately 8 sensor signals (10.57–2.49), which equals the sum of the average effects of the two factors (approximately 6 and 2 signals for temperature and oxygen, respectively (Table 2)). Compared to the average number of signals (2.49) under standard welfare conditions (85% oxygen and 24 °C), lowering the water temperature and the oxygen increased the mean number of signals by 5.95 and 2.12 respectively, and under the poorest welfare conditions (50% and 15 °C), the average signal number was 10.57 (Table 3). The effect of reducing water temperature from 24 °C to 15 °C was three times stronger than was the effect of lowering oxygen content from 85% to 50%. When the two factors were combined, the number of signals was highest, indicating the poorest welfare conditions (Table 3).

Analysis of variance revealed significant effects of changes in both temperature and oxygen level, in the replications (blocks) as well, but there was no significant interaction between them, $R^2 = 0.979$ (Table 4). The difference in the number of signals between the two temperatures was larger than that between the two oxygen levels, and no significant interaction between the two factors was observed. The effects of lowering both the temperature ($p < 0.0001$) and oxygen ($p < 0.0021$) were highly significant (Table 4). There was no significant interaction between the temperature and oxygen effects ($p = 0.8209$, Table 4).

**Table 4**
Results of analysis of variance with Tilapia.

| **Source** | ***F*-Ratio** | **Prob[1] > *F*** |
|---|---|---|
| **Replication (block)** | **20.9886** | **0.0020** |
| **Temperature** | **210.0725** | **< 0.0001** |
| **Oxygen** | **26.7201** | **0.0021** |
| **Temp. × Oxygen interaction** | 0.0559 | 0.8209 |
| **The entire model: $R^2 = 0.979$** | | |

**Table 5**
The effect of lowering water temperature and lowering oxygen content on Hybrid Striped Bass, as measured by the average number of signals registered by the sensor.

| Temperature Oxygen | 24 °C | 31 °C | The effect of increasing water temperature |
|---|---|---|---|
| 85% | 9.4 std | 11.1 std | 2.01 |
| 50% | 10.4 std | 21.0 std | 1.18 |
| The effect of increasing water temperature | 1.00 | 9.9 | 6.15<br>5.45 |

### 3.2. Hybrid striped bass

The results are similar with the Hybrid Striped Bass. Table 5 indicates that stressors (increasing temperature and lowering the oxygen level both) significantly increased the average number of signals (Table 5: from 9.4 to 11.1 [temperature] and from 10.4 to 21.0 [temperature]; from 9.4 to 10.4 [oxygen]; from 11.1 to 21.0 [oxygen]). The effect of increasing water temperature increased the mean number of signals by 2.01 at 85% oxygen saturation and by 1.18 at 50% oxygen saturation.

The electronic sensor performed robustly throughout the replications, on two different fish species of two different sizes. Electronic, software or mechanical adaptations were unnecessary.

## 4. Discussion

The working hypothesis was that fish under good conditions will successfully avoid the sensor, and as conditions worsen, the sensor will count the fish that do not avoid the moving sensor. However, poor conditions are difficult to create. We manipulated oxygen levels and water temperature; low levels of oxygen and low temperatures are well known factors that negatively affect tropical fish. Tilapia are tropical fish; see: http://www.fishbase.org. The Bass are temperate-zone fish (Hodson, 1989), which are raised in Israel at temperatures above 22 °C. Further research should test the performance of this sensor by using it to test the reaction of cold-water fish species to other stressors, other temperature levels, other oxygen levels, water ammonia poisoning, etc.

Based on the working hypothesis, Tables 3–5 indicate that the sensor identified behaviour related to fish stress, which was expressed as an increase in the number of signals that the device registered. The new sensor is not designed to identify a specific stressor. Nevertheless, under field conditions, stress might also result in a higher number of signals from the sensor, and may yield results similar to those exhibited by fish undergoing the induced stress tested in this study. Further research should examine the effects of stress issues. Nonetheless, the device has practical applications: it can be used to draw the attention of the fish farmer to a specific pond or water tank wherein sub-optimal conditions are suspected, especially if he is managing many fish ponds, or if his employees are untrained. Once sub-optimal conditions are found, whether caused by stress, water quality, parasites or illness, the fish farmer could physically go to the suspected pond and examine the problem with his own eyes and determine what caused the problem. Once having made the specific diagnostic, the farmer – not the device – will take the appropriate action.

## 5. Conclusion

Early detection of stress conditions in commercially cultured fish is of the utmost importance. The new sensor can provide early warning of problems in the tank. After further experiments, it is hoped that the

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[1] Significant effects are highlighted in bold face.

sensor will be useful in fish ponds as well. If avoidance of the moving sensor is low, and the sensor registers a high number of signals, then the fish can be considered to be stressed. Thus, this early warning enables the fish farmer to take action before many fish are harmed. Our preliminary results, ($R^2 = 0.979$ for the entire model and $p < 0.0001$ for lowering the temperature for tropical fish) warrant further testing of the proposed sensor with other fish species and other stressors. The latter can include physically handling the fish, high levels of nitrogen, high density/overcrowding, etc. Assuming that the device is found to be efficacious, the device can ultimately be effectively used by many fish farmers.

We also suggest that the device be tested in a wider variety of fish farms. While the device is promising, further testing and validation is obviously necessary.

## Acknowledgments

Many thanks go to the editor, Marsha Brown. The authors acknowledge the time and effort made by Dr. Yoav Gal from the ARO's Precision Livestock Farming [PLF] Lab. This paper is contribution number 007-2016 of the Institute of Agriculture Engineering, ARO, The Volcani Centre.

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