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# The sensor to estimate the sound pressure level in eggs

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ABSTRACT

Before assessing the effects of sound stimuli on the embryonic development of poultry, the current study asked the following question: what sound pressure level (SPL) would embryos inside eggs be exposed to? The question has motivated the current research, which developed a reduced-size sensor (miniaturized decibel meter) to help measuring SPL inside artificially-incubated eggs (microenvironments). The sensor was developed by using the Arduino® microprocessor - a standard amplifier circuit and electret microphones. Calibrations were performed in a commercial decibel meter to allow confirming the sensor capacity. However, it was necessary using mathematical models to help converting the sound measures to the decibel scale, since the direct conversion of them was not possible. The use of the sensor in studies focused on artificial incubation confirmed the acoustic insulation capacity of eggshells. However, results showed that the internal SPL (air chamber) in eggs externally exposed to 90 dB (A) remains high and probably perceptible to embryos. Such information is highly relevant to studies focused on investigating bioacoustics during incubation.

## 1. Introduction

Bioacoustics and sound analysis emerged as a new research field in poultry production in order to optimize the conditions of the rearing environment (Ben Sassi et al., 2016). For example, sound technologies are used for monitoring feeding behaviors of broiler chickens (Aydin et al., 2015; Aydin and Berckmans, 2016); to determine the adequacy of the thermal environment (Moura et al., 2008) and in the artificial incubation process, which is the focus of this research.

Artificial incubators have sufficient technology such as ventilation, egg turning, humidity and refrigeration systems to assure optimal poultry-embryo development conditions. However, noise is inevitable during such procedures, since engines and fans are constantly working, which results in sound pressure levels exceeding 95 dB (A) (Carvalho et al., 2015).

Accordingly, studies have demonstrated distinct aspects of the effect of sound (rhythmic music, species-specific vocalizations and random noises) on the embryonic development of birds, such as changes in responses associated with the maturation of physiological systems and even with the post-hatching life (Alladi et al., 2005; Kesar, 2013; Sanyal et al., 2013; Tong et al., 2015). However, it is necessary investigating some issues in the case of embryos exposed to external sounds. One of these issues lies on the embryonic functionality of the avian auditory system, which was already proven by Jones et al. (2006). Another issue refers to sound wave absorption and transmission by egg constituents, which was only investigated in preliminary studies, so far. Information about the acoustic parameters inside the incubated eggs is not easily found. For instance, we need to know the sound intensity near the embryo to establish critical exposure values.

Sound is a longitudinal wave that leads to pressure variations in different media such as air, water or solids. The sound pressure level (SPLS) represents the volume auditory perception, whose measuring device is known as decibel meter. According to David et al. (2013), decibel meters are electroacoustic transducers capable of detecting sound and of converting it into an electrical signal, as well as of amplifying and processing it. According to the international standard IEC 61672-1 (2002), such equipment presents many variations, which directly affect its efficiency, accuracy and cost.

Thus, the current study developed a sensor by using the Arduino® platform, which is an open-source microcontroller that presents a range of applications when it is associated with different sensor modules and actuators (Hjort and Holmberg, 2015; Torres et al., 2015). Arduino® was launched in 2005 as an easy-to-apply platform for programming beginners (Haugen and Moore, 2014). According to D'Ausilio (2012), this microcontroller allows using multiple hardware complements and free scripts for different purposes.

After its popularization, Arduino® started being used to develop sensors, fact that made it easily applicable to measure temperature,

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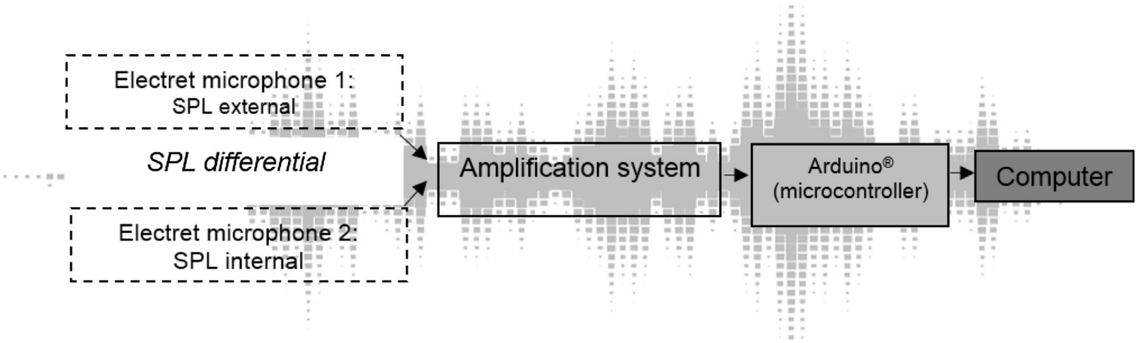


**Fig. 1.** Organogram of the sensor-development project.

luminosity and radiation, relative humidity (Fernandes, 2015; Torres et al., 2015; Jordão et al., 2017; Oates et al., 2017), mechanical vibration (Hjort and Holmberg, 2015; Jaber and Bicker, 2015) and sound pressure levels (Feitosa et al., 2014; Quintana-Suárez et al., 2017). Arduino® is an efficient tool for the measurements, although adaptations are required.

The aim of the current study was to develop, calibrate and test a sensor to help estimating sound pressure levels in microenvironments, such as inside fertile eggs, to gather information about the acoustic insulation capacity of eggshells in researches with bioacoustics in artificial incubation.

## 2. Materials and methods

### 2.1. Sensor development

The sensor herein developed to measure sound pressure levels (SPL) resulted from the need of using a reduced-size equipment to estimate to what extent sounds would be perceived by embryos inside eggs. The project is shown in Fig. 1.

The Arduino® UNO R3 was used as microcontroller. This model is based on the Atmega 328P processor, which presents six input and six output channels that allow connecting several electronic components programmed for and directed to a particular function. Two electret microphones (0.5 cm diameter), Mic 1 and Mic 2, were used to simultaneously measure the sound pressure level in two distinct environments: inside and outside eggs. Overall, microphones respond to sound intensity variations through vibrations in their internal membranes, which transform the sound into an electrical signal. However, it is necessary adopting an amplifier system because this signal is low. Thus, an amplification system with two 100 nf capacitors, four 10 k resistors, two 500 k trinpots and two BC 548 transistors was herein assembled. Details on the final version of it can be found in Fig. 2A.

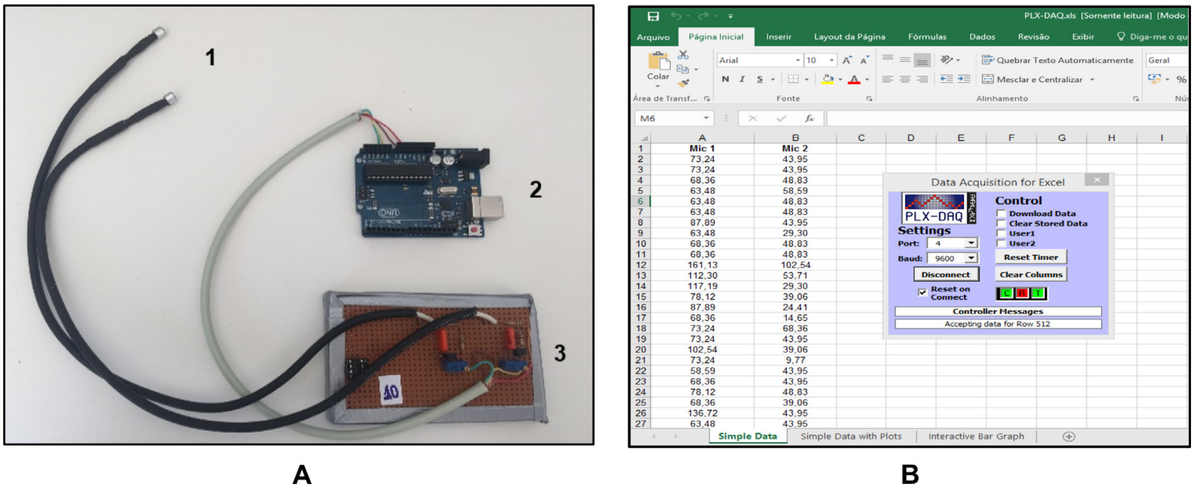
The herein developed sensor was directly connected to the USB port of a computer to enable data acquisition. The programming in C language was done in an open-source processing environment. Parallax Data Acquisition tool (PLX-DAQ®) was used to directly insert data into a Microsoft Excel® spreadsheet, whose output is shown in Fig. 2B (Dworakowski et al., 2016).

### 2.2. Calibrating and converting the electrical signals of the sensor

Calibration was performed based on Feitosa et al. (2014) and Fernandes (2015). The sensor was compared to commercial equipment in a sequence of tests focused on investigating its responsiveness to variations in the ambient SPL by comparing the herein collected data to those recorded in a duly-calibrated Instrutherm® decibel meter, model DEC - 490. The use of the decibel meter was programmed for "A" weighting, with automatic collection (30–130 dB range) and "slow" mode; records were made every second.

A white noise (100–15000 Hz) was emitted by an amplifier box (Mini Speaker® - BT51) of nominal power 15 W, which was previously calibrated in different volume configurations, according to SPL values set by the commercial decibel meter. The sensor microphones were placed parallel to the microphone of the commercial decibel meter, ten centimeters away from the sound source. The system was initially tested under silent condition. Next, the amplifier box volume was gradually increased (settings: 0, 5, 10, 15, 20, 25 and 30). Five sets of tests were conducted, in total; each set lasted one minute at each volume.

Data collected by the miniaturized decibel meter were electrical signals obtained at rate of ten values per second. Thus, the means of



| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Mic 1 | Mic 2 | | | | | | | |
| 2 | 73,24 | 43,95 | | | | | | | |
| 3 | 73,24 | 43,95 | | | | | | | |
| 4 | 68,36 | 48,83 | | | | | | | |
| 5 | 63,48 | 58,59 | | | | | | | |
| 6 | 63,48 | 48,83 | | | | | | | |
| 7 | 63,48 | 48,83 | | | | | | | |
| 8 | 87,89 | 43,95 | | | | | | | |
| 9 | 63,48 | 29,30 | | | | | | | |
| 10 | 68,36 | 48,83 | | | | | | | |
| 11 | 68,36 | 48,83 | | | | | | | |
| 12 | 161,13 | 102,54 | | | | | | | |
| 13 | 112,30 | 53,71 | | | | | | | |
| 14 | 117,19 | 29,30 | | | | | | | |
| 15 | 78,12 | 39,06 | | | | | | | |
| 16 | 87,89 | 24,41 | | | | | | | |
| 17 | 68,36 | 14,65 | | | | | | | |
| 18 | 73,24 | 68,36 | | | | | | | |
| 19 | 73,24 | 43,95 | | | | | | | |
| 20 | 102,54 | 39,06 | | | | | | | |
| 21 | 73,24 | 9,77 | | | | | | | |
| 22 | 58,59 | 43,95 | | | | | | | |
| 23 | 68,36 | 43,95 | | | | | | | |
| 24 | 78,12 | 48,83 | | | | | | | |
| 25 | 68,36 | 39,06 | | | | | | | |
| 26 | 136,72 | 43,95 | | | | | | | |
| 27 | 63,48 | 43,95 | | | | | | | |

Control
☐ Download Data
☐ Clear Stored Data
☐ User1
☐ User2

Settings
Port: 4
Baud: 9600
☑ Reset on Connect

Controller Messages
Accepting data for Row 512

**Fig. 2.** Sensor developed in the current study: (1) microphones, (2) amplifier circuit, (3) Arduino® board (A); PLX-DAQ® tool used to collect data (B).

these values were used to allow making comparisons to the commercial decibel meter (one signal/second). Descriptive statistics (means, median, standard deviation, minimum and maximum) were used to help visualizing the raw data of the developed sensor and to compare them to the ones recorded in decibel meter.

The sensor did not allow directly recording the sounds in decibels; thus, it was necessary using an equation to relate the measurements of the commercial decibel meter to those of the experimental decibel meter. Modeling was carried out through polynomial regression analysis; the model was adjusted to the data by using the weighted least squares technique in the R statistical software (R Core Team, 2017).

The initial tests allowed noticing that the electrical signals of the microphones (Mic 1 and Mic 2) in a single sensor were not identical; thus, it was necessary investigating whether they could be used to compare sound pressure levels in different environments, as it was initially proposed in the current study. The non-parametric Wilcoxon *t*-test was applied to paired data (measured at the same times), whereas the Kendall rank correlation coefficient was used to set the correlation between the electrical signals of the microphones, both at 5% significance level, in the R statistical software (R Core Team, 2017).

### 2.3. Applying the developed sensor

The application of the herein developed sensor took into consideration two conditions to measure SPL inside eggs: (1) empty eggs (shell only) and (2) intact eggs (with normal content). The eggs analyzed in both conditions had the same origin (white eggs weighting ≈ 50 g). Each tested condition used 15 egg units, which were considered the experimental repetitions.

The eggs were opened (approximately one centimeter) at the larger pole (air chamber region) and their inner contents (yolk/albumen) were removed to allow studying eggshells (alone) as insulators. The eggshells were washed with running water and dried in an oven at 60 °C, for 24 h (Jones et al., 2010). The opening made in the eggshells to remove internal content allowed inserting the sensor microphone for the tests. The microphones were placed in the region of the air chamber, about one centimeter towards the yolk. In the empty eggs, a marking was used on the microphone wire so that the position of the sensor was the same for intact eggs. The remaining spaces between the microphone wire and the opening of the eggs were filled with synthetic mass.

Similar to what was done in the calibration tests, a white noise (100–15000 Hz) emitted by an amplifier box was used in the sensor test. Two external sound pressure levels associated with studies about sound stimuli during artificial incubation were herein tested: 70 and 90 dB (A). These levels were previously calibrated. The tests were performed in an isolated and quiet room to prevent external noise. A test box was used to hold the microphones at the same height, parts of the commercial decibel meter, as well as of the herein developed sensor, were placed on the outer side of the box; the herein developed sensor was connected to a computer for data collection purposes, as shown in Fig. 3A.

The eggs subjected to both conditions were individually tested: type “1” eggs at 70 and 90 dB (A) and type “2” eggs at 70 and 90 dB (A), alternately. The eggs were vertically positioned in a holder placed 20 cm away from the amplifier box. In both cases, one of the microphones of the developed sensor was carefully inserted in the air chamber (Fig. 3B) to avoid touching the liquid content of the intact eggs. The second microphone was positioned at the same height as the first one and as the microphone of the commercial decibel meter; all three microphones were placed at the same distance from the sound source.

At the end of the test, each egg repetition in the two evaluated conditions generated 1000 electric signals of which 100 means/second were recorded and converted into dB. As the external SPLs were set and compared to the commercial decibel meter, we made the option of presenting only values recorded by the decibel meter. Finally, external and internal sound pressure levels were compared at the two herein evaluated levels [70 and 90 dB (A)], through the Kruskal-Wallis test, at 5% significance level, in the R statistical software (R Core Team, 2017).

## 3. Results and discussion

### 3.1. Calibrating and converting the electrical signals of the sensor

The comparison between pre-conversion data of the herein developed sensor and data of the commercial decibel meter in dB (A) is shown in Fig. 4. It is possible seeing that they presented similar behavior towards each volume variation in the amplifier box.

According to the commercial decibel meter, SPL varied from 31.53 ± 0.25 dB (A) to 95.22 ± 0.32 dB (A), from the silence to the maximum volume. On the other hand, the herein developed sensor responded to the range 100.39 ± 5.32 to 2324.84 ± 163.12 (mean of the microphones).

The signals of microphones subjected to increased external-sound volume were more dispersed when they were compared to each other. Such result may be explained by basic acoustic principles: increased volume results in higher sound wave pressure in the microphone membranes - a phenomenon called sound intensity - which is consistent with the mean energy flow per area unit ($W/m^2$). Numerical sound-intensity values vary exponentially; thus, the magnitudes of the sensor's electrical signals ranged from 100 to 2300 units, which, consequently, led to the great dispersion of these signals. Commercial decibel meters conventionally adopt the decibel unit, i.e., they use sound intensity values in a base-10 logarithmic scale (Halliday et al., 2012; David et al., 2013).

Although the numerical values of the signal picked up by the microphones of the herein developed sensor were not exactly equal, mainly in higher volumes, they did not differ in distribution, according to the Wilcoxon test, at 5% significance level. Thus, the equality between such values ($r = 0.9159$) allowed simultaneously using them, as idealized at the beginning of the current project (Fig. 5).

The main limitation of the herein developed sensor lies on the non-direct conversion of the electric signal into the decibel scale. A survey conducted to gather information about the use of the Arduino® platform to measure SPL came across the methodology by Feitosa et al. (2014), who compared data recorded by the developed sensor to those recorded by a commercial decibel meter, which was followed by mathematical adjustments focused on modeling such relation, in order to overcome such limitation.

Therefore, a polynomial regression based on the least squares technique was adopted as alternative to convert the sensor's electrical signals into the decibel scale. Due to the great dispersion of data recorded by the herein developed sensor, these data were considered the response variable at the time to adjust the model, whereas the measurements of the commercial decibel meter characterized the explanatory variable. As the signals of the microphones did not differ from each other, the conversion process took into consideration the mean signals of the microphones in each volume variation. The adjusted function was a 5th degree model (1), which is shown in Fig. 6 and expressed by:

$$y = 305.57 - 0.98x^2 + 4.09 \times 10^{-2}x^3 - 6.28 \times 10^{-4}x^4 + 3.48 \times 10^{-6}x^5 \qquad (1)$$

The coefficient of determination was set at 0.984; thus, 98.4% of the variation in the signal of the developed sensor could be explained by measurements of the commercial decibel meter, fact that makes its use as sound pressure meter feasible. Although the fourth and fifth order terms presented low coefficients, they were significantly different from zero (test t, $\alpha = 0.05$), so they were maintained (Table 1).

Dias Neto et al. (2016) emphasized that each sensor is unique, as well as that adjustments should be made according to the specificity of each sensor; i.e., individual adjustments should be made to sensors

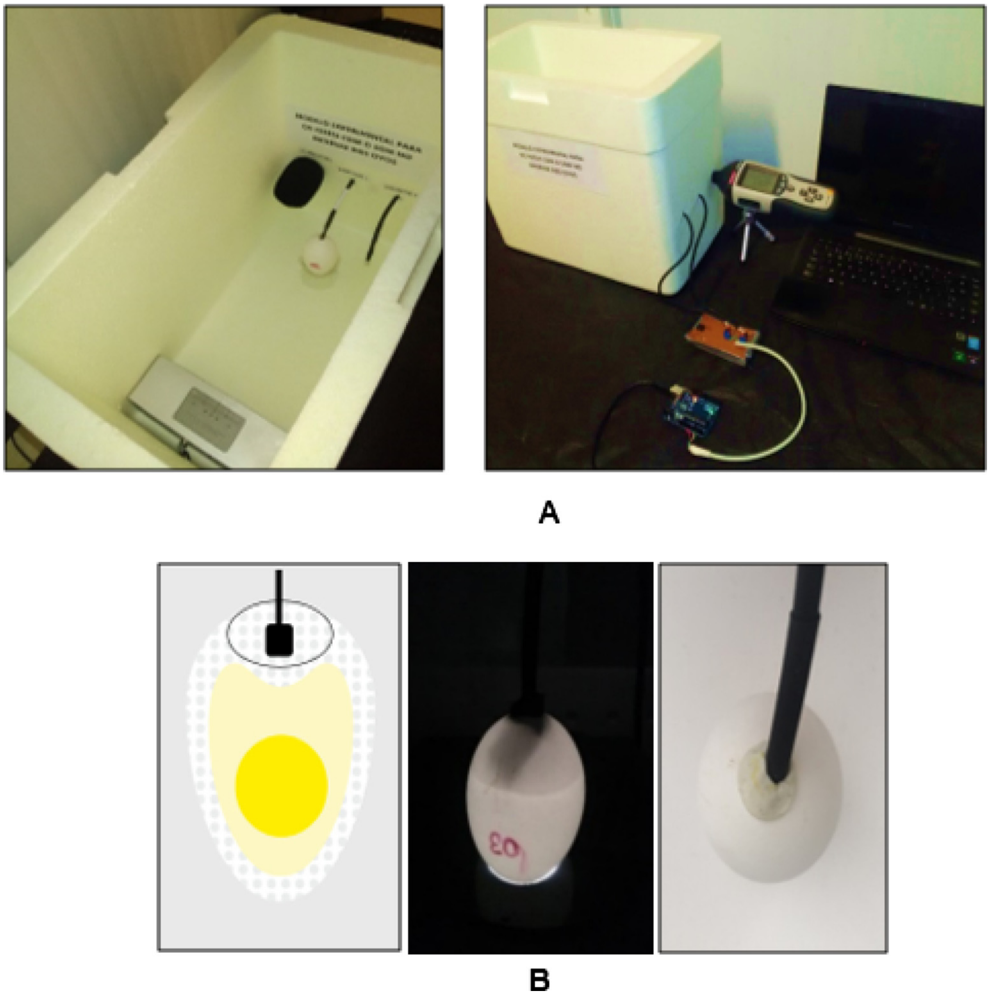


**Fig. 3.** Sensor application test focused on measuring the sound pressure level inside the eggs (A); details of an egg with a microphone (B).

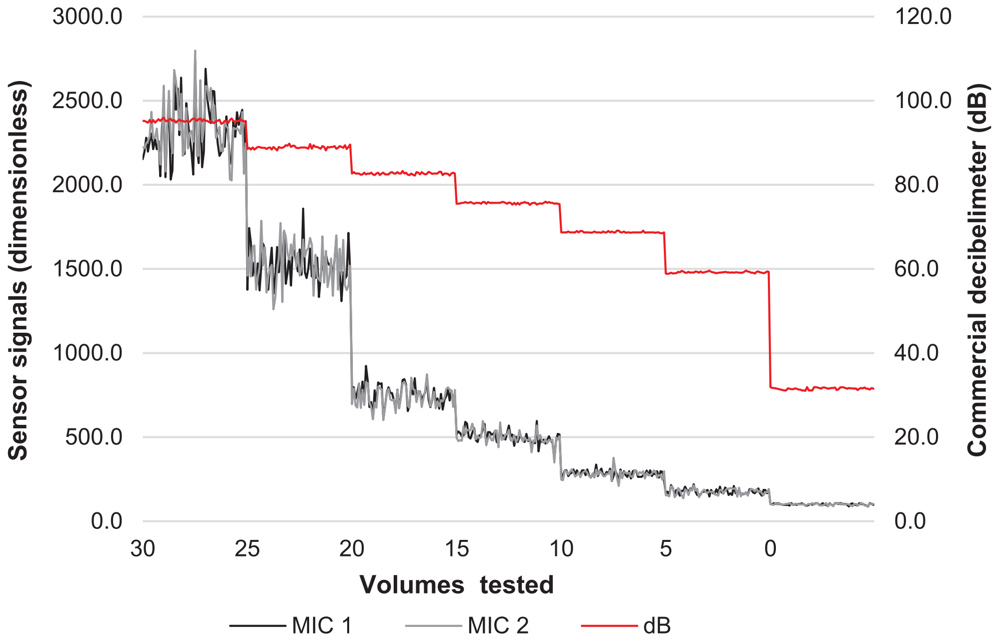


**Fig. 4.** Signals of the developed sensor (left axis) in comparison to commercial decibelimeter signals (right axis) due to the reduction of the sound volume tested.

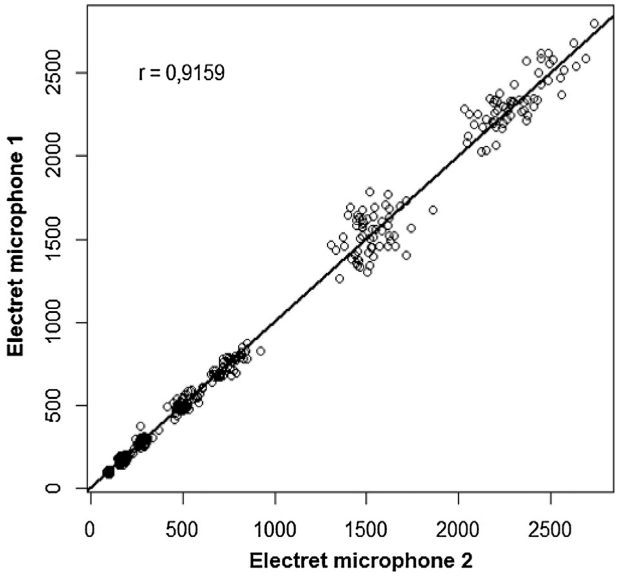


**Fig. 5.** Scatterplot of the signals of microphones 1 and 2 based on Kendall rank correlation coefficient (r).

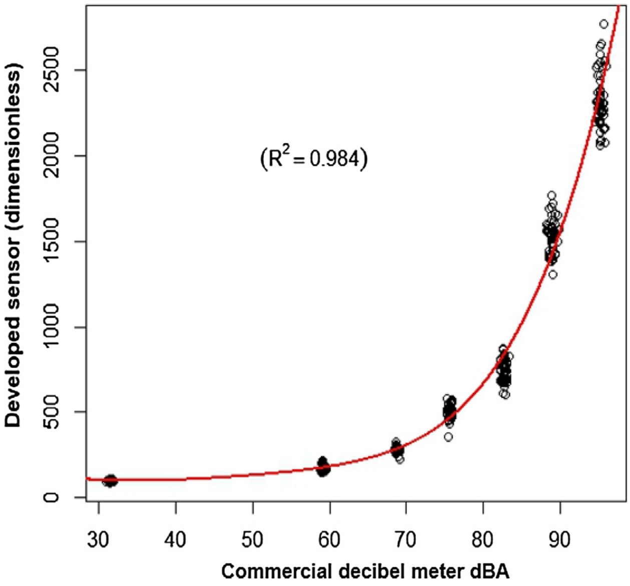


**Fig. 6.** Adjustment of the 5th degree polynomial model used to relate the signal of the herein developed sensor to the commercial decibel meter; wherein y is the mean signal of the two microphones (dimensionless) and x is the measurement of the commercial decibel meter in dB (A).

**Table 1**
Estimates, standard- and test-errors of the coefficients of the fifth-degree polynomial adjusted to data recorded by the herein developed sensor and by the commercial decibel meter.

| Coefficient | Estimate | Standard error | t value | p value |
|---|---|---|---|---|
| Intercept | 305.57 | 9.00 | 3.39 | < 0.001 |
| $X^2$ | 0.98 | 0.31 | −3.17 | 0.002 |
| $X^3$ | $4.09 \times 10^{-2}$ | 0.01 | 3.94 | < 0.001 |
| $X^4$ | $-6.28 \times 10^{-4}$ | $1.25 \times 10^{-4}$ | −5.01 | < 0.001 |
| $X^5$ | $3.48 \times 10^{-6}$ | $5.20 \times 10^{-7}$ | 6.70 | < 0.001 |

similar to the herein developed one. In addition, the converted values are expressed in dB, without weighting filter, as it is done in commercial decibel meters, which can be programmed for filter “A”. This filter is most used because it takes into consideration the frequency bands between 20 Hz and 20 kHz (Malchaire, 2001).

The Arduino® can be programmed for distinct purposes, besides receiving/sending information to be processed and interpreted, just as a commercial decibel meter does. The sensor developed to help estimating the sound pressure level in the current study presented satisfactory operation and met the expectations about its use. Thus, the herein developed and tested sensor was named “*miniaturized decibel meter*”.

### 3.2. Applying the miniaturized decibel meter in field (egg tests)

The small size of the electret microphones was essential to enable using the miniaturized decibel meter to measure the sound pressure level inside eggs. Despite the size advantage, there was a limitation in the use of the sensor in tests applied to eggs: microphones cannot get wet, otherwise there is signal loss and, consequently, the sensor operation is compromised. It could happen if the microphones touch the egg albumen and yolk. Thus, microphone-use results are limited to the upper part of the eggs, which is known as air chamber: an empty and dry space between the eggshell inner and outer membranes (Decuypere and Bruggeman, 2007).

One of the aims of the current research was to evaluate the acoustic insulation of eggshells by taking into consideration that they host developing embryos that supposedly have access to external sounds, which could be measured through the sound pressure level (SPL). Therefore, the present study measured the difference between the SPL in the external environment and that recorded inside empty eggs in order to assess the eggshell insulation effect. Next, the difference between the external environment and the air chamber of intact eggs was assessed.

External SPLs were set at 70 and 90 dB (A). These levels were selected based on previous studies on sound stimulus during artificial incubation (Kesar, 2013; Sanyal et al., 2013; Tong et al., 2015). In fact, the SPL was lower inside the eggs, fact that was proved in the Kruskal-Wallis test, which indicated differences in data distribution between the two environments, proving the shell's ability to isolate the external sound. The statistical results of these tests were: $x^2 = 35.885$ and $p < 0.0001$ at 70 dB (A); and $x^2 = 32.657$ and $p < 0.0001$ at 90 dB (A).

Empty eggshells presented insulation values 32.85% and 11.11%, at 70 and 90 dB (A), respectively, whereas intact eggs recorded insulation value 17.14% at 70 dB (A) and 8.88% at 90 dB (A). Sound waves are partially reflected, absorbed and transmitted when they touch any surface and the exact quantification of these fractions requires sophisticated acoustics studies. In addition, the current study was not focused on investigating differences between empty eggs and intact eggs; however, it is suggested that the insulation rates may be associated with the reverberation of the fraction of the absorbed sound wave, which is probably different between empty and intact (filled with liquid) eggs. It is worth highlighting that albumin and yolk differ from each other in viscosity and mass, fact that may change the resonance frequency of sound waves (Akashi and Kushibiki, 1997; Attar and Fathi, 2014).

Results of the miniaturized decibel meter application proved that eggshells are sound barriers to avian embryos. However, despite their insulation capacity when they were externally exposed to 90 dB (A), their internal SPL remained high (≈80 dB). In theory, it could impair the embryonic development (Roy et al., 2014). Thus, it is possible explaining the results of studies conducted by Sanyal et al. (2013) and Kesar (2013), who exposed embryos to 110 dB (A) and recorded physiological changes in chicks, such as increased size and number of hearing-related neurons, as well as changes hormone levels and behavior post hatching.

On the other hand, Tong et al. (2015) concluded that embryo exposure to 72 dB SPL (A) did not affect the embryonic growth, hatchability/mortality rates, hormone levels, among others. It is suggested that the 72 dB (A) level test had little relevance to embryos because of the sound insulation of the eggshell and the liquid part of the eggs (not verified in this research). According to tests with intact eggs, the sound intensity would be around 58 dB in the air chamber, which is not averse to biological organisms.

There are no references about exposure limits applied to developing avian embryos and this was one of the points that motivated this research. According to Brouček (2014), several animal species do not present physiological and behavioral changes when they are exposed to adverse sounds lower than 80 dB (A). This last information and the acoustic isolation of the eggshell that were verified in this present research justifies the results recorded by Kesar et al. (2013), Sanyal et al. (2013) and Tong et al. (2015), as well as by other researchers who investigated bioacoustics in artificial incubation processes. It is important to emphasize that the SPL registers inside the eggs are restricted to the air chamber, suggesting the improvement of the sensor so that it can come into contact with the liquid part of the eggs (albumen and yolk).

## 4. Conclusions

This work is one of the first attempts to measure the sound pressure levels in microenvironments (inside incubated eggs) and the results obtained are important for bioacoustics studies in artificial incubation. The sensor developed met the expectations of the current research and had its applicability tested in eggs in different conditions. As expected, the eggshells are acoustic insulations. However, results showed that the internal SPL (measured in the air chamber) in eggs externally exposed to 90 dB (A) remains high and probably is perceptible to embryos. It is suggested to improve the sensor by placing it further into the eggs (liquid part) thus precisely estimating the sound intensity close to the embryos of birds.

## Funding

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