#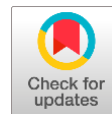 Application of active acoustic technology to assess the target strength of seahorses based on the presence of a reproductive organ

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Abstract This study aims to provide reliable information on comparing the target strength (TS) values of seahorses based on the reproductive state. This study was carried out using hydro-acoustic technology in a water tank environment. Data were obtained using the single-beam scientific echo-sounder SIMRAD EK-15 at a frequency of 200 kHz and analyzed via the Sonar-4 software. The measurement result of the TS (mean±S.E.M.) of *Hippocampus kuda* female, male and pregnant male seahorses were -56.24±0.047, -57.25±0.032, and -58.26±0.06, respectively. There was a significant difference in the mean TS value of *H. kuda* based on the reproductive state ($P < 0.05$). Furthermore, the response to the mean TS value of pregnant *H. kuda* male (the presence of a brood pouch) was lowly significant compared to the female (absence of a brood pouch) ($P < 0.05$). The results showed a possibility of finding a pregnant *H. kuda* male in a water column through active acoustic methods in the future.

Keywords *Hippocampus kuda*, reproductive organ, seahorse, target strength

1. Introduction

Seahorses are threatened with extinction due to the fishing industry's by-catch impact (Vincent et al 2011). There has been an increasing rate of exploitation of these species because they are used for various purposes, besides being a significant component of traditional Chinese medicine and an ornamental fish in a marine aquarium (Bertha and Davy 2000; Vecchione 2013; Vincent 1996). Seahorses live in habitats that are either stationary or threatened by anthropogenic activities, such as seagrass, coral, macroalgae. Therefore, they are more vulnerable to population decline (Vincent et al 2011, Zachary et al 2013; Foster et al 2014; Project Seahorse 2014; Yip et al 2014).

Consequently, a conservation strategy is required to maintain their existence, and also they need to be included in the list of endangered species (IUCN Red List of Threatened Species 2014). Wilson and Vincent (1998) recommended captivity or cultivation as an alternative to maintaining these species' existence. Furthermore, Correia et al (2013) developed laboratory-scale seahorse cultivation using an artificial environment for habitat restoration. However, the provision of natural seahorse habitat was much better than using an artificial environment (Vecchione 2013). Identifying the biophysical aspects of these species' lives is also very important when recommending its protection zones.

The hydro-acoustic approach has been used in fisheries and coastal ecosystem research (Frouzova et al

2005, Greenstreet et al 2010; DuFour et al 2018; Manik et al 2017; Manik and Apdillah 2020). Furthermore, the implementation of active acoustic technology was primarily determined through the single target acoustic back-scattering information known as Target Strength (TS). This is the main parameter for assessing the density and abundance of fish because their biomass is analyzed using the relationship between the back-scattering sound intensity and variables such as length or weight (Simmonds and MacLennan 2005; Manik et al 2006). The hydro-acoustic survey used in providing an estimate of fish abundance was strongly influenced by an understanding of the TS value distribution, which was used as the object of observation.

TS measurement for seahorses was carried out (Apdillah et al 2018) using the live fish approach. Furthermore, the TS value of these species was influenced by the size and changes in orientation (angle) caused by their movement. One of the unique biophysical characteristics of seahorses is that the males have brood pouches (Foster and Vincent 2004; Jones 2004). During pregnancy, the male seahorses raise their chicks in their abdominal pouch until they hatch, while the females only release their eggs into the male's incubation bag (Foster and Vincent 2004; Stölting and Wilson 2007).

The uniqueness of the seahorse reproductive state (Kawaguchi et al 2017) is an area that provides an opportunity for more research—for example, exploring the response of acoustic back-scatter energy that could become

an acoustic signature in determining sex traits through active acoustic methods in the future. Therefore, it is essential to know about TS regarding brood pouches' presence and contribute to mapping the spatial distribution of pregnant male seahorses, used as a guide for information on nursery ground. Besides, an understanding of nurseries and nesting areas is useful for marine conservation zones.

2. Materials and Methods

2.1. Seahorse collection and experimental setup

The samples were obtained from the waters of Bintan Island, Indonesia. They include *H. kuda* female (no presence of a brood pouch), *H. kuda* male (not fertilized/not pregnant), and *H. kuda* pregnant male or Mature males. Besides, both males were identified by a brood pouch's presence (Vincent et al. 1995). An approximate brood pouch volume (ml) was calculated for each male as follows: brood pouch volume = brood pouch length x width x depth x 1.3 (Woods et al 2005). The Brood pouch length was measured in a straight line starting from the pouch opening to the point where it joins the tail, pouch width as the widest lateral distance, and pouch depth as the dorso-ventral distance at the widest point.

The acoustic data collection was carried out using an experimental water tank at the Marine Science Laboratory of the Faculty of Marine Sciences and Fisheries, Raja Ali Haji Maritime University, Tanjungpinang, from January to March 2018. The experimental water tank was made of concrete with a diameter of 270 cm and a height of 17 cm.

Furthermore, data analysis was carried out at the Acoustics and Marine Instrumentation Laboratory, IPB University.

The seahorses were placed into the experimental water tank using a live fish approach. This approach involves using live targets with a tethered technique that allows the target to swim (limitedly) in the experimental container. Furthermore, the seahorses were placed at 110 cm from the transducer and adjusted for the near field distance (Medwin and Clay 1998). The experimental design is shown in Figure 2. Measurement of the water environment's temperature and salinity was carried out to obtain the sound speed's value. Furthermore, the seahorses' acoustic acquisition was carried out using the single beam SIMRAD EK-15 scientific echosounder, and the transducer was placed in a downward (vertical) position. The instrument specifications are presented in Table 1.

Instrument calibration was carried out using the on-axis acoustic transmission technique with a 38.1 mm diameter sphere ball of tungstens carbide (TS = -42 dB) using standard procedures (Simrad 2012). Before the seahorses' data acquisition, recording the back-scatter from the water tank was carried out without the sea horse's presence. During the acoustic recording, video shooting was also carried out through an underwater camera placed on the water tank wall's side. The results obtained from the acoustic data acquisition in the form of RAW data were analyzed using Sonar-4 post-processing software. The flow chart for acoustic data processing is shown in Figure 1.
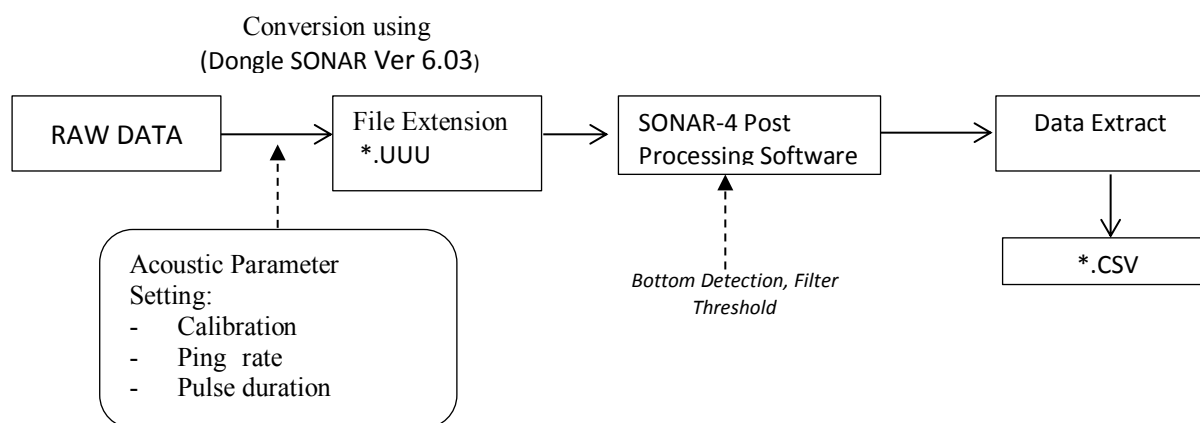

Figure 1 Flow chart of acoustic data acquisition and processing.

2.2. Acoustic data computing

The data analysis for calculating the target strength (TS) of seahorses was carried out using the Sonar-4 software (Balk and Lindem 2015). Furthermore, the equation for calculating the TS value include:

TS = 10 log (σ_{bs})

where σbs represents the back-scattering cross-section on the measurement of the acoustic signal from a single target,

furthermore, the linear equation for measuring the average TS include:

$$Mean\ TS = 10\ log\ \left(\frac{1}{N} \sum_{i=1}^{N} 10^{TSi/10}\right)$$

The acoustic data were analyzed statistically using variance (ANOVA) to test the TS value response to the development of the pregnant and non-pregnant male and female seahorses' reproductive states. Furthermore, the Tukey test was carried out to see the interaction between treatments.

Table 1 Acoustic parameters and specifications of instruments in the recording.

Parameter	Scientific Echosounder Simrad EK-15
Transducer shape	Circular
Transmission frequency (kHz)	200
Transmitting power (W)	46
Beamwidth	26°
Pulse length (ms)	0.08
Ping rate (ping s^{-1})	10
Minimum threshold (dB)	−68
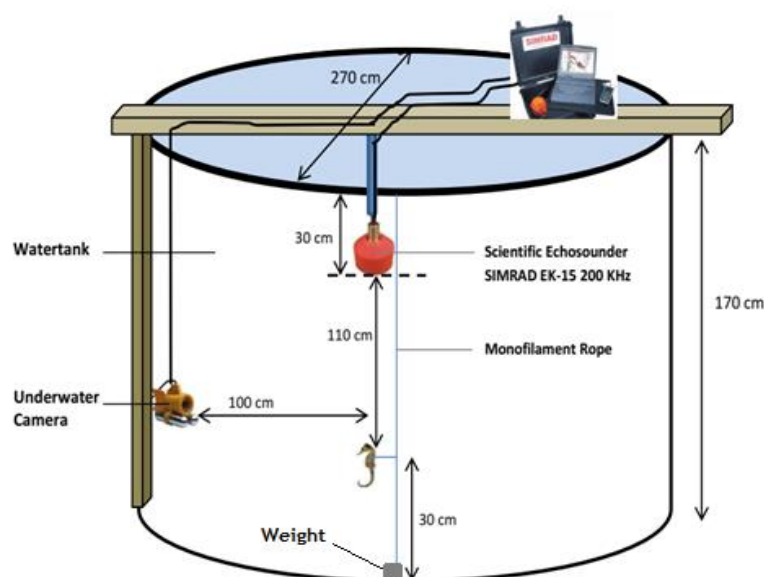
Figure 2 The experimental design of TS data recording according to the reproductive organs' development using the live seahorse approach with the tethered method.

3. Results and Discussion

The seahorse acoustic signals were processed from the raw data obtained using the EK-15 acquisition software and were converted into a typical Sonar-4 file. Furthermore, the TS analysis results were extracted into a CSV file and analyzed statistically. The results of raw visual data and file conversion to Sonar 4 are shown in Figure 3, while the filtering and signal processing analysis results are shown in Figure 4.

The seahorse morphometric measurements were carried out after recording the acoustic data, including length, sex. Furthermore, this method was applied to each observation object (N = 10,000). The measurement results of the mean TS value of seahorses based on their reproductive state were carried out with a live fish approach. Furthermore, this was used to obtain results that were close to the actual condition. The mean TS value (mean±SE) of female seahorses *H. kuda* was -56.24 ± 0.047 dB, while for the pregnant and non-pregnant males were -58.26 ± 0.06 and -57.25 ± 0.032 dB.

The response of the mean TS value of female seahorses (no brood pouch) was greater than that of the pregnant and non-pregnant males (the presence of a brood pouch). This is due to the structure and material forming the brood pouch organs' internal anatomy to reduce the seahorse's acoustic signals. There is a pocket hole in the brood pouch that serves as a medium for receiving eggs from the female seahorses. The pocket hole through which the sound waves exit the transducer causes the sound to be partially absorbed and damped down. Therefore, the back-scattering of the sound which returns to the transducer is smaller. This is thought to have contributed to the lower mean TS value of male seahorses.

Figure 5 shows that the response to the TS value of female seahorses have the distribution characteristics of TS data that are leaning to the right, with the most frequency being in the TS range of -50 dB to -56 dB, where the maximum

TS was -46 dB, and the minimum, -68 dB. Meanwhile, for the non-pregnant male seahorses, the largest data frequency was spread over the TS value of -56 dB, with a maximum of -50 dB and a minimum of -66 dB. Furthermore, the pregnant male seahorses' TS distribution had a maximum value of -50 dB and a minimum of -68 dB. This TS distribution appears to be distributed in two data groups (having two peaks) containing bi-modal.
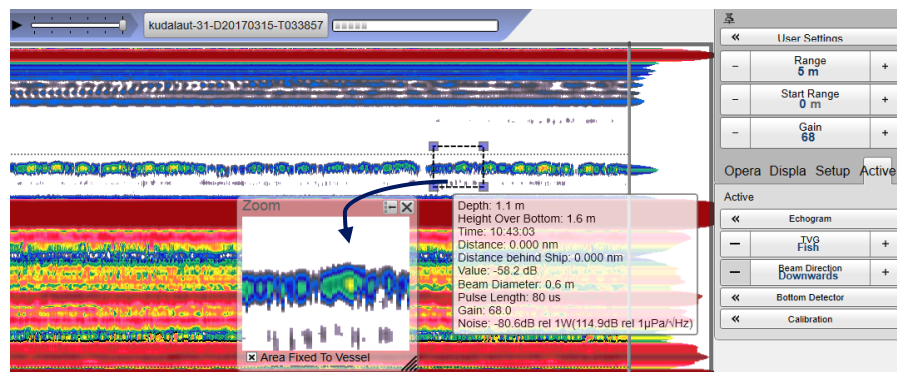

Figure 3 RAW data recorded using the Simrad EK-15 Acquisition software.
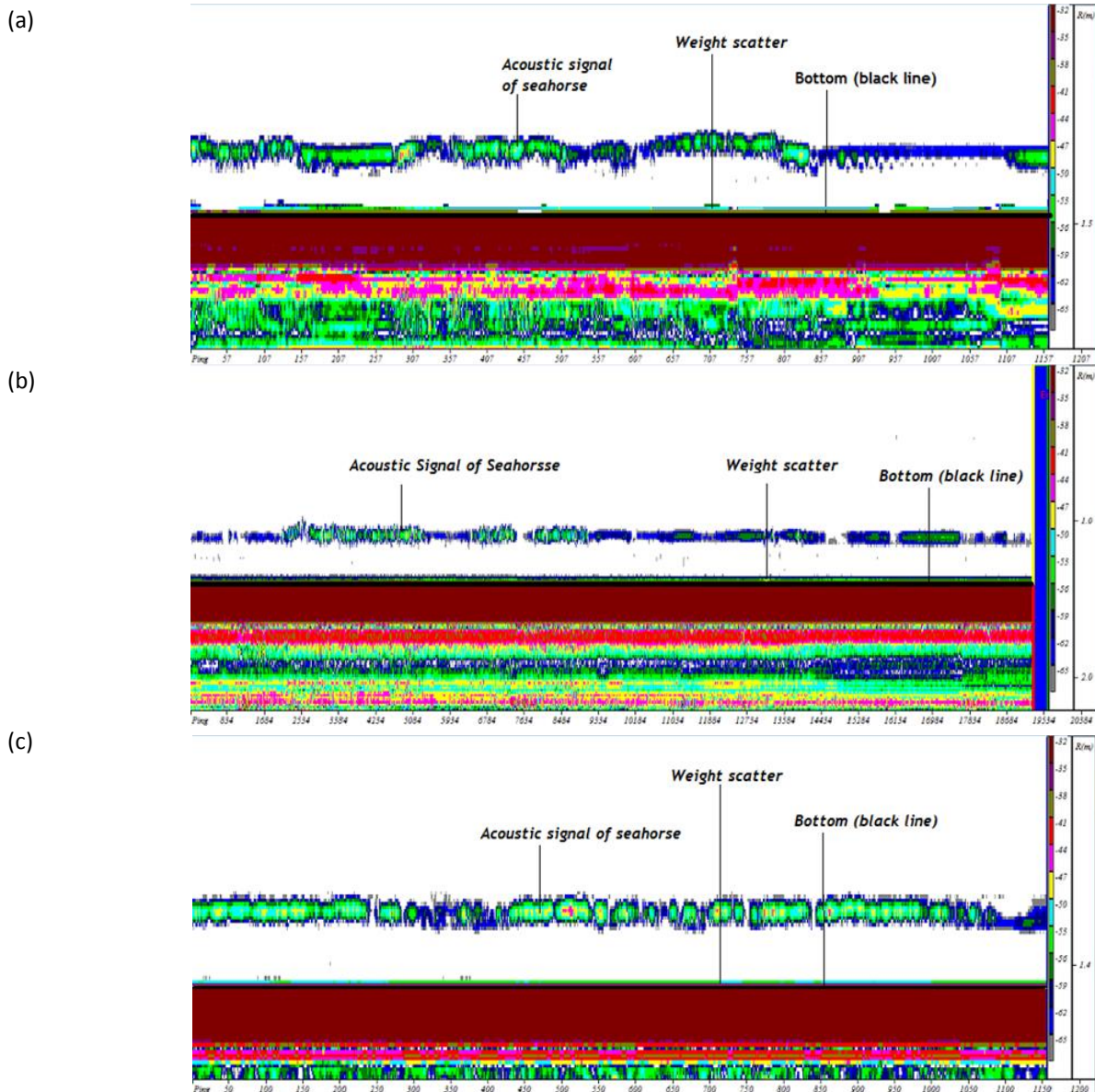

Figure 4 The results of the signal processing analysis after filtering. The weight scatter was separated from the acoustic signal of the seahorse *H. Kuda*; (a) male (no-pregnant), (b) male pregnant, (c) female.

The ANOVA results showed that the difference in TS responses to the development of the reproductive organs through 3 levels of conditions, namely, males was not pregnant (brood pouch had not been incubated with eggs), males were pregnant (brood pouch that had been incubated with eggs) and females as controls (morphology see Figure 6). There was a significant difference in response to the mean TS value in the seahorse's two conditions, with a significance value ($P < 0.05$). Based on these results, there was an interaction between the treatments, and Tukey's test was carried out. The mean TS value of the *H. kuda* male pregnant (the presence of a brood pouch) versus female (no presence of a brood pouch) was different significantly ($P < 0.002$), meanwhile for the seahorse *H. kuda* of male no-pregnant versus female and *H. kuda* male no-pregnant versus male pregnant, the TS response values were not significantly different. The ANOVA result interval plot with the largest to the lowest TS response rates from the 3 phases of the reproductive state is presented in Figure 7.

Table 2 Recapitulation of length measurement results and mean TS value of seahorse.

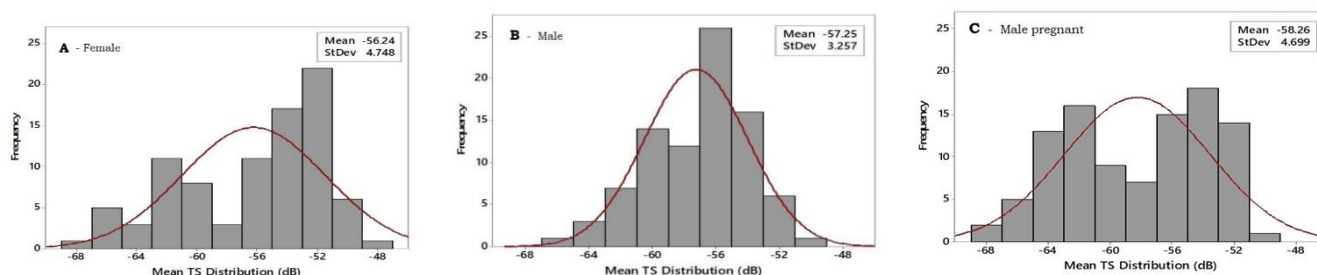| Species	Total length (cm)	Volume of Brood pouch (ml)	Condition of reproductive state	Number of Pings	MeanTS±SE (dB)
H. kuda	19.0	-	Female (no brood pouch)	10.000	-56.24±0.047
H. kuda	18.5	-	Male (brood pouch has not been fertilized)	10.000	-57.25±0.032
H. kuda	18.6	2.52	Male pregnant (presence of a brood pouch)	6.000	-58.26±0.06

Figure 5 Histograms of the normal mean TS distribution of *H. kuda* for A, female, B, male and C, male pregnant.

This research is the first to compare seahorses' TS values based on reproductive organ presence using active acoustic technology. The research results indicated that the brood pouch in *H. kuda* could respond differently to sound reflected. These results are consistent with those reported by Ona (1990), which stated that apart from the swimbladder's volume and shape, another significant factor affecting the TS of Atlantic cod (*Gadus morhua*) is the presence of the gonads. Furthermore, the feeding cycle in walleye pollock (*Theragra chalcogramma*) and gonad production area potentially introduces variability in the swimbladder surface areas. Internal organs are physiological factors that can affect the sound reflected by a fish (Horne, 2003). The condition of laying eggs and releasing eggs by giant freshwater prawns is a physiological factor that can influence the target strength (Kusumaningrum et al 2013).

It is suspected that the brood pouch morphological factors from seahorses influence back-scattering strength. Carpucino et al (2002) reported that morphologically, the male brood pouch from the *Hippocampus hippocampus*, as observed using light and electron microscopy, has skin to cover the eggs. Furthermore, the pouch serves to protect the eggs, which are pear-shaped from the external environment, and it consists of a thin, more folded epidermis. The morphology of the epidermal cells of H. hippocampus shows superficial cells with cytological features similar to flame cone cells. Flame cone cells are specialized cells that contain hydrolytic enzymes, high reductase, and oxidoreductase, which are involved in metabolic pathways. Poortenaar et al (2004) reported that the reproductive and histological conditions of *H. abdominalis* contain oogonia and oocytes in the first growth phase. The Oogonia is concentrated within the luminal epithelium at the germinal ridge and is characterized by a high nucleus to cytoplasm ratio.
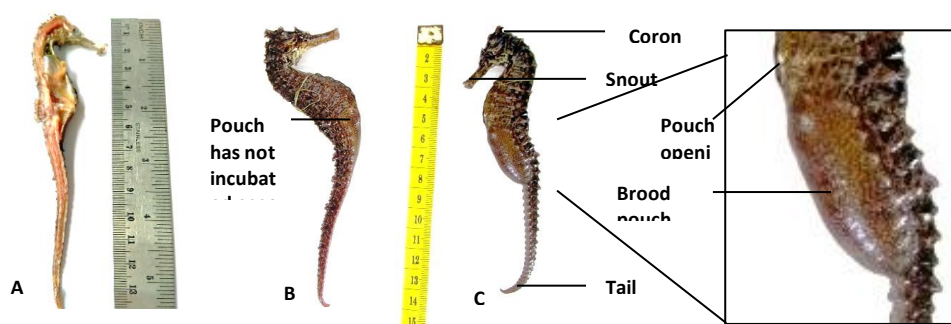

Figure 6 Morphology of *H. kuda* for A; female (no presence of a brood pouch), B; male (brood pouch has not been fertilized), C; male pregnant (the presence of a brood pouch).

Meanwhile, for the oocytes they increase in size, then zona radiata appear, and cortical alveoli accumulate until they become heterogeneous in size and fill up much of the cytoplasm. The reproductive condition of mature female ovaries contains maturing oocytes, large central fluid yolk mass, and a thin peripheral rim of cortical alveoli and lipid. Oocytes are pear-shaped and have a similar shape to the eggs of a male H.hippocampus (Carpucino et al 2002).
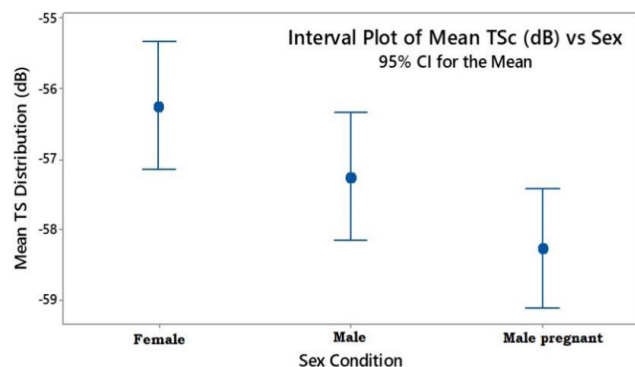

Figure 7 Interval plot of the ANOVA results of the mean TS distribution.

The formation and content of the brood pouch of seahorses can affect sound reflection. Kawaguchi et al (2016) categorized brood pouch formation processes during male seahorse development into three stages. The early stage is characterized by the formation of a baggy structure from the primordium. In contrast, the middle stage is characterized by the differentiation and establishment of brood pouch-specific tissues. Finally, the third and last stage is characterized by a fully formed pouch with developing blood vessels and a pouch fold ultimately capable of carrying and incubating embryos. The brood pouch is formed along the tail's ventral midline, and the lumen of the brood pouch is surrounded by loose connective tissue, called pseudoplacenta and dermis.

Furthermore, this pouch can be divided into four sequential stages based on the altered tissue layers' characteristics during gestation. They include the normal stage, the embryo-carrying stage, the embryo-release stage, and the repair stage. The brood pouch comprises a folded inner pseudostratified columnar epithelium and a smooth outer stratified cuboidal epithelium. There are three tissue layers between the inner and the outer epithelia. They are an inner loose connective tissue layer, a middle smooth muscle layer, and an outer dense irregular connective tissue layer (Laksanawimol et al 2006). The complexity of the tissue layers affects the intensity of sound reflection, reducing the scattering of sound.

4. Conclusions

The response to TS in female *H. kuda* (without brood pouch) was significantly different. Furthermore, the TS value of *H. kuda* male pregnant (the presence of a brood pouch) differed significant compared to *H. kuda* female (no the presence of a brood pouch); meanwhile, in the condition, *H. kuda* male no-pregnant versus female and male pregnant were not significantly different.

Acknowledgments

Conflict of Interest

The authors declare that they have no conflict of interest.

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