


**Submission Manuscript**

aan\_fish@yahoo.../Sent

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: jabb@ufersa.edu.br <jabb@ufersa.edu.br>  
Cc: Dony Apdillah <donyapdillah@umrah.ac.id>

Jan 5, 2021 at 7:39 PM

Dear CHIEF EDITOR  
JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY  
An International Journal

Subject: Submission of manuscript for publication in JABB  
Happy greeting from Indonesia!  
Hope, this mail does not disturb your busy activities.

In this moment, we would like submit our research article. Therefore, we are enclosing herewith a manuscript entitled "**Comparison of the target strength of Seahorses based on the presence of a reproductive Organ**" at attachment file.

With the submission of this manuscript we would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

We expect this article is sufficient and feasible and could be published at the journal of animal behaviour and biometeorology.

Thank you very much for your kind attentions and cooperation.

My best regards,

Dr. NOFRIZAL

Riau University

Faculty of Fisheries and Marine Science

Department of Fisheries Resources Utilization

Kampus Bina Widya, KM. 12.5, Simpang Panam, Pekanbaru, Riau, Indonesia

Telp. +62-761-61760, Fax. +62-761-63275

1 File 3.2MB



Manuscript\_TS-Seahorse\_Reproductive-organ\_Nofrizal.docx  
3MB

# Comparison of the Target Strength of Seahorses Based on the Presence of a Reproductive Organ

**Dony Apdillah<sup>a,b</sup> • Indra Jaya<sup>b</sup> • Henry Munandar Manik<sup>b</sup> • Totok Hestirianoto<sup>b</sup> • \*Nofrizal<sup>d</sup>**

<sup>a</sup> Marine Science Department, Marine Science and Fisheries Faculty, Raja Ali Haji Maritime University, Indonesia

<sup>b</sup> Division of Acoustic and Marine Instrumentation, Marine Science and Technology Department, IPB University, Indonesia

<sup>c</sup> Marine and Coastal Resource Research Center, Raja Ali Haji Maritime University, Indonesia

<sup>d</sup> Program Study of Environment Science, Doctoral Program, Riau University, Indonesia

**Nofrizal (\*Corresponding author)**

## Abstract

The aim of this study is to provide reliable information on the comparison of the target strength (TS) values of seahorses based on the reproductive state. This was carried out using the hydro-acoustic technology in a water tank environment. Data was 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±SE) of *Hippocampus kuda* female, male and pregnant male seahorses were  $-56.24 \pm 0.047$ ,  $-57.25 \pm 0.032$  and  $-58.26 \pm 0.06$  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) were highly significant compared to the female (absence of a brood pouch) ( $P < 0.05$ ). The results showed that there is a possibility of finding a pregnant *H. kuda* male in a water column through the use of active acoustic methods in the future.

**Keywords:** seahorse, target strength, hippocampus kuda, reproductive organ

## Introduction

Seahorses are threatened with extinction due to the by-catch impact of the fishing industry (Vincent et al. 2011). There have been an increasing rate of exploitation of these species because they are mostly used for various purposes. This include being a major component of traditional Chinese medicine and as an ornamental fish in marine aquarium (Bertha and Davy, 2000, Vecchione 2013 and Vincent 1996). Seahorses live in habitats that are either stationary or, threatened by anthropogenic activities, such as seagrass, coral, macro algae. Therefore, they are more vulnerable to population decline (Vincent et al. 2011, Zachary et al, 2013;, Foster. et al, 2014, Project Seahorse 2014, and 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 maintain the existence of these species. Furthermore, Correia, et al. (2013) developed a laboratory-scale seahorse cultivation using an artificial environment for habitat restoration. However the provision of natural seahorse habitat was much better than the use of an artificial environment (Vecchione 2013). The identification of the biophysical aspects relating to the life of these specie is also very important when recommending its protection zones.

The hydroacoustic approach has been used as a tool in fisheries and coastal ecosystem research (Frouzova et al., 2005, Greenstreet et al. 2010, DuFour et al. 2018, Manik et al, 2017, and Manik and Apdillah, 2020). Furthermore, the implementation of the use of active

acoustic technology was largely determined through the single target acoustic backscattering information known as Target Strength (TS). This is the main parameter for assessing the density and abundance of fish because their biomass are analyzed using the relationship between the backscattering sound intensity, and variables such as length or weight (Simmonds and MacLennan, 2005 and Manik et al, 2006). The hydroacoustic survey used in providing an estimate of fish abundance was strongly influenced by an understanding of the distribution of the TS value 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 important to have knowledge about TS regarding the presence of brood pouches, and also contribute to the mapping of the spatial distribution of pregnant male seahorses which is used as a guide for information on nursery ground. In addition, an understanding of nurseries and nesting areas is useful for marine conservation zones.

## **Materials And Methods**

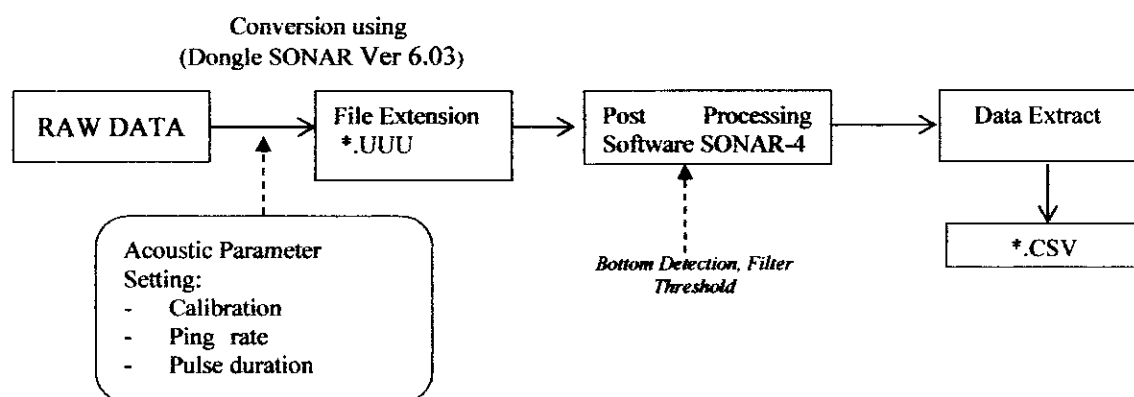
### **Seahorse collection and experimental setup**

The samples were obtained from the waters of Bintan Island, Indonesia, and 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. In addition, both males were identified by the presence of a brood pouch (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 collection of acoustic data 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 in 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 the use of live targets with a tethered technique which allows the target to swim (limitedly) in the experimental container. Furthermore, the seahorses were placed at a distance of 110 cm from the transducer and adjusted for the near field distance (Medwin and Clay 1998). Measurement of the temperature and salinity of the water environment were carried out to obtain the value of the sound speed. Furthermore, acoustic acquisition of the seahorses 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 tungsten carbide ( $TS = -42$  dB) using standard procedures (Simrad, 2012). Before the data acquisition of seahorses, recording of the backscatter from the water tank was carried out without the presence of the sea horse. During the acoustic recording, video shooting was also carried out through an underwater camera that was placed on the side of the water tank wall. 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 the figure below.



**Figure 1.** Flow chart of acoustic data acquisition and processing

**Table 1.** Acoustic parameters and specifications of instruments in 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 <sup>-1</sup> )	10
Minimum threshold (dB)	-68

#### Acoustic data computing

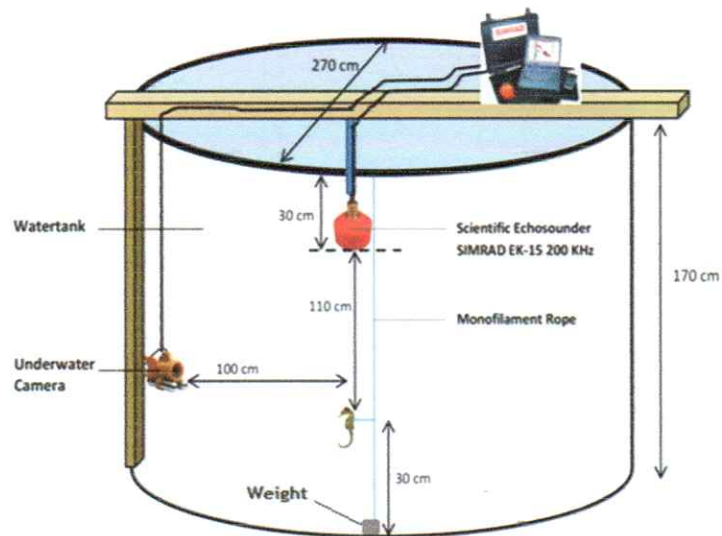
The data analysis for calculating the target strength (TS) of seahorses was carried out using the Sonar-4 software (Balk and Lindem, 2015). Furthermore, the equation for calculating the TS value include:

$$TS = 10 \log (\sigma_{bs})$$

Where  $\sigma_{bs}$  represents the backscattering cross section, on the measurement of the acoustic signal from a single target. Furthermore, the linear equation for measuring the average TS include:

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

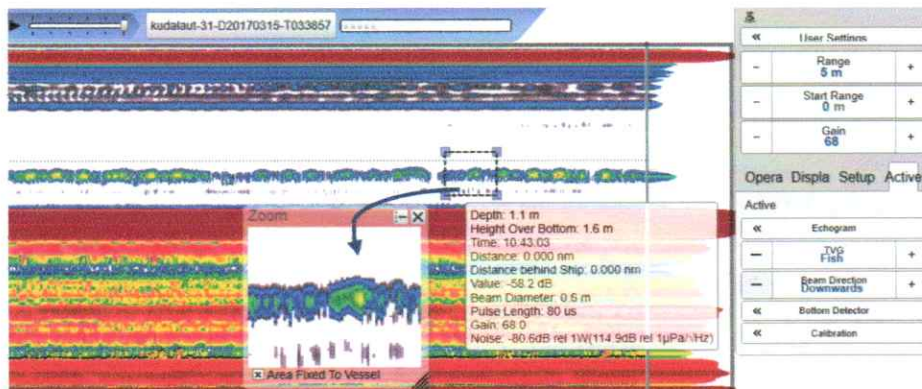
The acoustic data were analyzed statistically using Analysis of variance (ANOVA), to test the TS value response to the development of the reproductive state of the pregnant and non-pregnant male, and female seahorses. Furthermore, the Tukey test was carried out to see the interaction between treatments.



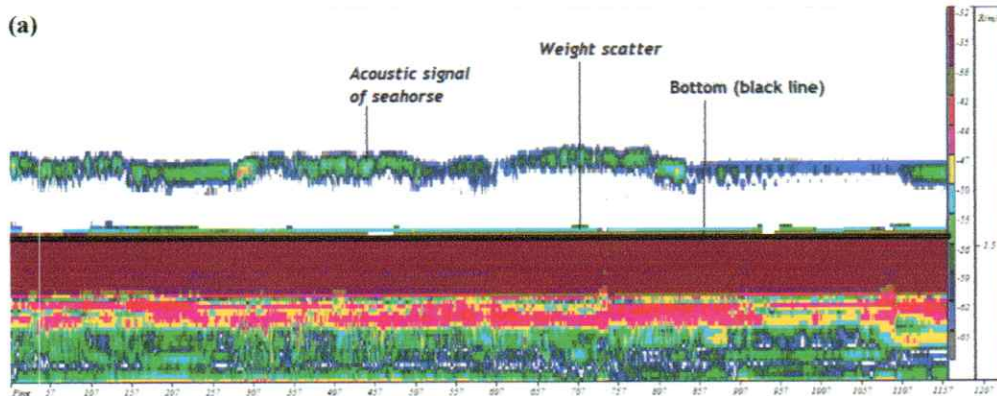
**Figure 2.** The experimental design of TS data recording according to the development of the reproductive organs using the live seahorse approach with the tethered method.

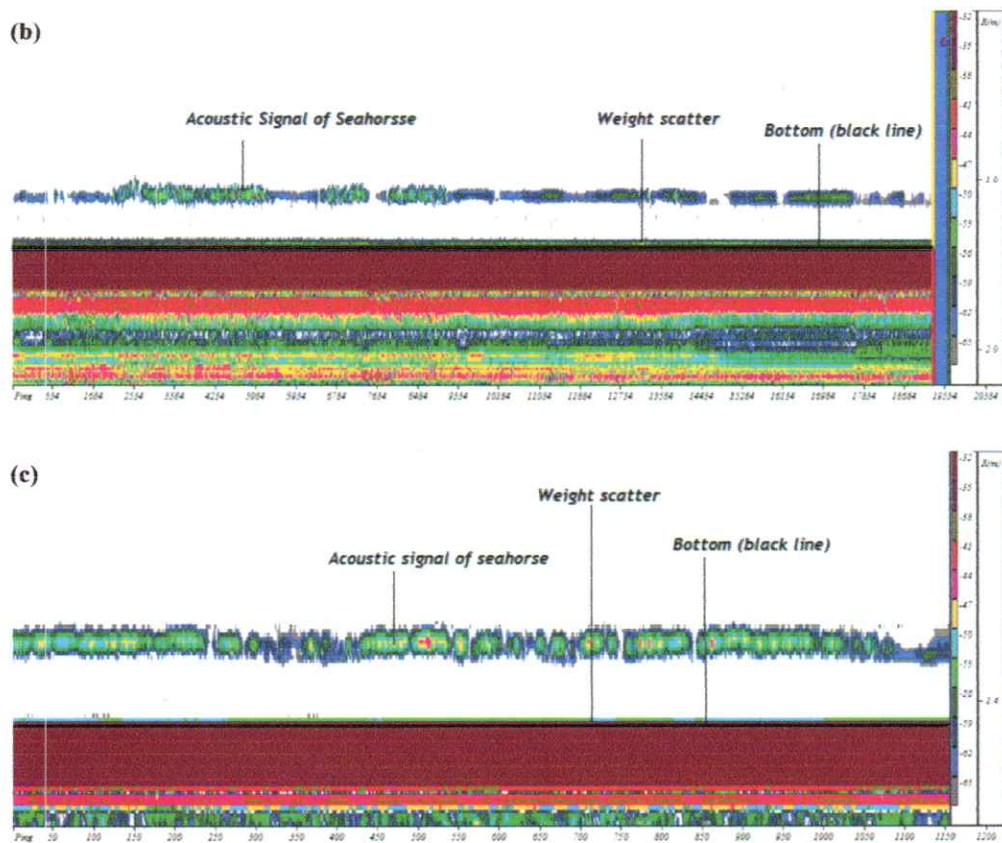
### Result and discussion

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



**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 clearly separated from the acoustic signal of the seahorse *H. Kuda*; (a) Male (no-pregnant), (b) male pregnant, (c) female

The seahorse morphometric measurements were carried out after recording the acoustic data, including length, sex. Furthermore, this method was applied for 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 $\pm$ SE) of female seahorses *H. kuda* was  $-56.24 \pm 0.047$  dB, while for the pregnant and non-pregnant males were  $-58.26 \pm 0.06$  and  $-57.25 \pm 0.032$  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 internal anatomy of the brood pouch organs being able to reduce the seahorse's acoustic signals. In the brood pouch there is a pocket hole that serves 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.

**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	N (Number of Pings)	Mean TS±SE (dB)
<i>H. kuda</i>	19.0	-	Female (no brood pouch)	10.000	-56.24±0.047
<i>H. kuda</i>	18.5	-	Male (brood pouch has not been fertilized)	10.000	-57.25±0.032
<i>H. kuda</i>	18.6	2.52	Male pregnant (presence of a brood pouch)	6.000	-58.26±0.06

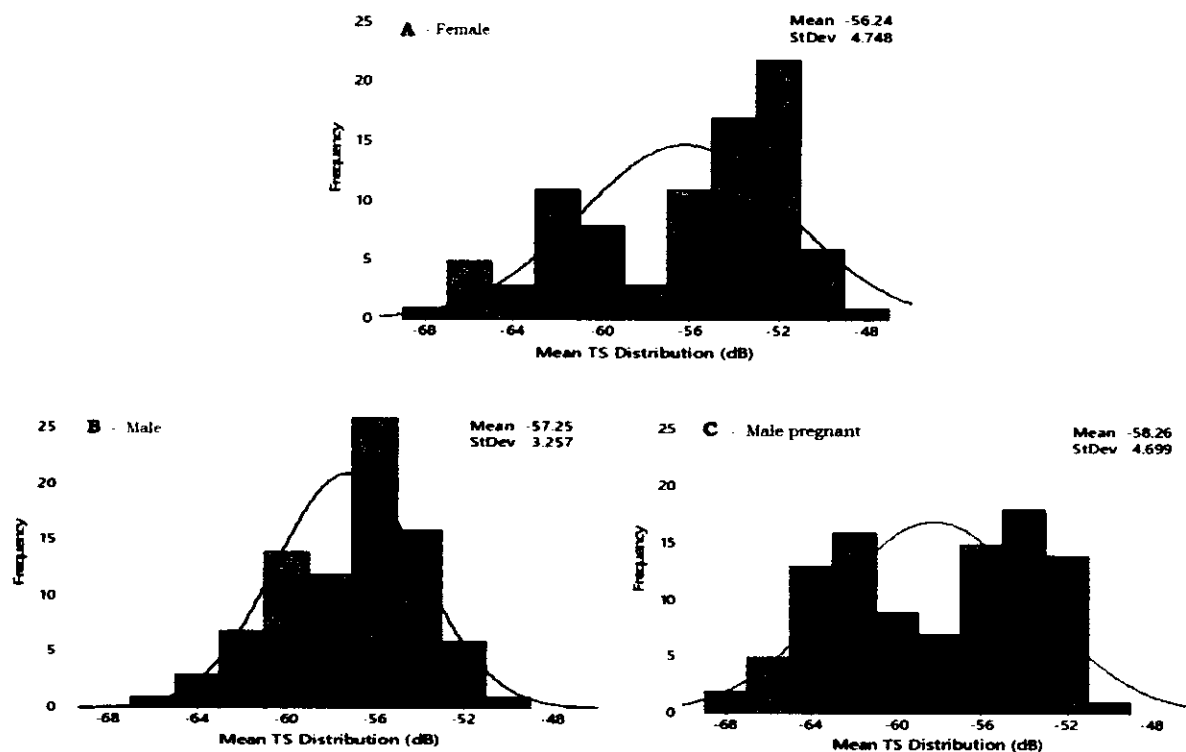
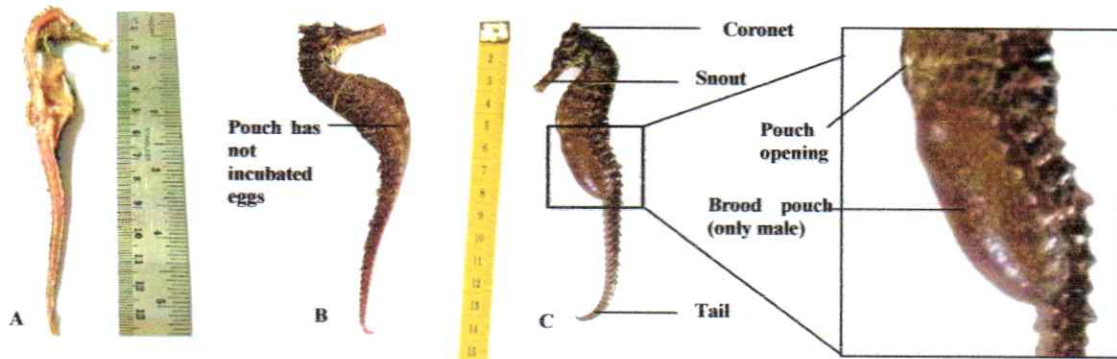
**Figure 5.** Histograms of the normal mean TS distribution of *H. kuda* for A, female, B, male and C, male pregnant

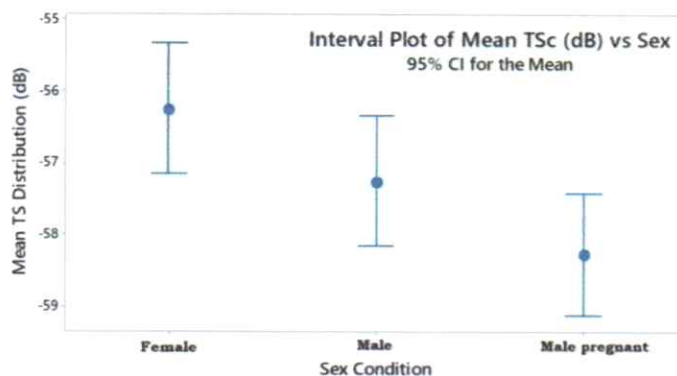
Figure 5 shows that the response to the TS value of female seahorses have the distribution characteristics of TS data that are leaning to the right, with the most frequency being in the TS range of -50 dB to -56 dB, where the maximum TS was -46 dB, and the minimum, -68 dB. Meanwhile, for the non-pregnant 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 TS distribution of the pregnant male seahorses 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.

The ANOVA results showed that the difference in TS responses to the development of the reproductive organs, through 3 levels of conditions, namely, males were 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 the response to the mean TS value in the two conditions of seahorse,

with significance value of ( $P < 0.05$ ). Based on these results there was an interaction between the treatments, and then the 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) were 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. A plot of the ANOVA result interval with the largest to the smallest TS response rates from the 3 phases of reproductive state is presented in Figure 7.



**Gambar 6.** Morfology 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).



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

This research is the first initiative to compare seahorses TS values based on the presence of reproductive organ, using the active acoustic technology. The results of the research indicated that the presence of brood pouch in *H. kuda* has the potential to respond differently to sound reflected. These results are consistent with those reported by Ona (1990), that stated that apart from the volume and shape of the swimbladder, 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, and internal organs are a physiological factor that can affect the sound reflected by a fish (Horne, 2003). The condition of laying eggs and releasing eggs by freshwater giant 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 have an influence on backscattering strength. Carpuccio et al. (2002) reported that morphologically,



the male brood pouch from the *Hippocampus hippocampus* as observed using light and electron microscopy, has a 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 condition 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. Meanwhile, for the Oocytes, they increase in size, then zona radiata appear and cortical alveoli accumulate, until they becoming heterogeneous in size and fill up much of the cytoplasm. The reproductive condition of mature female ovaries contain 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).

The formation and content of the brood pouch of seahorses can affect sound reflection. Kawaguchi et al, (2016) categorized the processes of brood pouch formation during male seahorse development into three stages. The early stage is characterized by the formation of a baggy structure from the primordium, while 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 ventral midline of the tail, 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 characteristics of the altered tissue layers during gestation. They include the normal stage, the embryo-carrying stage, the embryo-release stage and the repair stage. The brood pouch is composed of 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 and 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 has an effect on the intensity of sound reflection, which can reduce the scattering of sound.

### **Conclusion**

The response to TS in female *H. kuda* (without the presence of brood pouch) was significantly different ( $P < 0.05$ ). Furthermore, the TS value of *H. kuda* male pregnant (the presence of a brood pouch) differed significantly compared *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.

### **Acknowledgements**

This research was supported by the Grant Kemenristek Dikti in 2017. The authors are grateful for the facilities given by the Aquaculture Laboratory, Marine Science and Fisheries Faculty of UMRAH, which were used in the data collection process, and to the Laboratory Signal Processing Division Acoustic and Marine Instrumentation IPB Bogor for helping in the data processing.

### **Conflict of Interest**

The author declare no conflict of interest

## References

Apdillah D, Jaya I, Manik HM, T Hestirianoto and Febrianto T (2018) Seahorse Acoustic Reflection on Active and Passive Moving Condition: A Preliminary Observation in Watertank. *Omni-Akuatika*, 14(1).

Balk H, Lindem T (2015) Sonar 4 and Sonar 5-Pro post-processing systems, Operator manual version 6.0.3, 494 p, Lindem Data Acquisition Humleveien 4b. 0870 Oslo, Norway

Bertha M.O & Davy B (2000) Traditional Medicine Chines & Tonic Food in Proceeding of The First International Workshop on The management and Culture of Marine Species Use in Tradional Medicine. Project Seahorse Department of Biology MC. Gill University Montreal; Quebec. Canada.

Carcupino M, Baldacci A, Mazzini M, Franzoi P (2002) Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. *Journal of Fish Biology*, 61(6), 1465-1480.

Correia MJ, Palma, Koldewey HJ, Andrade JP (2013) Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (*Hippocampus guttulatus* Cuvier)? *Journal of Experimental Marine Biology and Ecology*. 448:258-264.

DuFour M.R, Christine MM, Song SQ, Christopher SV, Richard TK, Patrick MK, David MW (2018) Inferred fish behavior its implications for hydroacoustic surveys in nearshore habitats. *Fisheries Research*. Vol 199, 63-75. ISSN 0165-7836.

Frouzova J, Kubecka J, Balk H, Frouz J (2005) Target strength of some European fish species and its dependence on fish body parameters. *Fisheries Research*, 75(1-3), 86-96.

Foster SJ, Wiswedel S, Vincent ACJ (2014) Opportunities and challenges for analysis of wildlife trade using CITES data - seahorses as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Foster SJ, Vincent ACJ (2004) Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* 65: 1-61.

Greenstreet SPR, Holland GJ, Guirey EJ, Armstrong E, Fraser HM, Gibb IM (2010) Combining hydroacoustic seabed survey and grab sampling techniques to assess local sandeel population abundance. *ICES J. Mar. Sci.* 67, 971-984.

Horne JK (2003) The influence of ontogeny, physiology and behaviour on the target strength of walleye pollock (*Theragra chalcogramma*). *Ices Journal of marine science*. 60: 1063-1074.

IUCN Red List of Threatened Species. Version 2014.3. <[www.iucnredlist.org](http://www.iucnredlist.org)>.

Jones AG (2004) Male pregnancy and the formation of seahorse species. *Biologist* 51 (4). Texas A&M University, USA.

Kawaguchi M, Okubo R, Harada A, Miyasaka K, Takada K, Hiroi J, Yasumasu S (2017) Morphology of brood pouch formation in the pot-bellied seahorse *Hippocampus abdominalis*. *Zoological letters*, 3(1), 19.

Kusumaningrum W (2013) Analisis target strength Udang Galah (*Macrobrachium rosenbergii* De Man) menggunakan akustik *split beam* di Delta Mahakam. *Thesis*, Sekolah Pascasarjana IPB, Bogor.

Laksanawimol P, Damrongphol P, Kruatrachue M (2006) Alteration of the brood pouch morphology during gestation of male seahorses, *Hippocampus kuda*. *Marine and freshwater research*, 57(5), 497-502.

Manik HM, Apdillah D (2017) Remote Sensing of Seagrass and Seabed using Acoustic Technology in Bintan Seawater, Indonesia.

Manik. HM, Apdillah D, Dwinovantyo A, Solikin S (2017) Development of Quantitative Single Beam Echosounder for Measuring Fish Backscattering. Chapter 6 from the book *Advances in Underwater Acoustics*. Published by INTECH

Manik HM, Furusawa M, Amakasu K (2006) Quantifying Sea Bottom Surface Backscattering Strength and Identifying Bottom Fish Habitat by Quantitative Echo Sounder. *Jpn. J. of App. Phys.* 45(5B):4865–4867

Medwin H, dan Clay CS (1998) *Applied Ocean Acoustics: Fundamentals of Acoustical Oceanography*. Academic Press, New York. 712 pp.

Ona E (1990) Physiological factors causing natural variations in acoustic target strength of fish. *Journal of the marine science biological association of the UK*. 70: 107-127.

Poortenaar CW, Woods CMC, James PJ, Giambartolomei FM, Lokman PM (2004). Reproductive biology of female big-bellied seahorses. *Journal of fish biology*, 64(3), 717-725.

Project Seahorse (2014) <http://seahorse.fisheries.ubc.ca>.

Simmonds EJ, Maclellan DN (2005) *Fisheries Acoustic Theory and Practice*. Second Edition. Oxford: Blackwell Science Ltd.

Simrad (2012) Installation manual, Simrad EK15 Multi purpose scientific echosounder, Kongsberg Maritime AS, ISBN 978-82-8066-147-0

Stölting KN, Wilson AB (2007) Male pregnancy in seahorses and pipefish: beyond the mammalian model. *Bioessays*, 29: 884-896.

Vecchione A (2013) Prospects challenges in monitoring the seahorse population of South Carolina, USA. *Journal of Marine Animal and Their Ecology* 6 (2), 6-11.

Vincent ACJ, Berglund A, Ahnesjö I (1995) Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* 44:347–61

Vincent ACJ (1996) The International Trade in Seahorses. TRAFFIC International, Cambridge, UK.

Vincent ACJ, Foster SJ, Koldewey HJ (2011) Conservation and management of seahorses and other Syngnathidae. *Journal of fish biology* 78: 1681-1724.

Wilson MJ, Vincent ACJ (1998) Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aq. Sci. Cons.* 2, 179-196.

Woods CMC (2005) Reproductive output of male seahorses, *Hippocampus abdominalis*, from Wellington Harbour, New Zealand: Implications for conservation, *New Zealand Journal of Marine and Freshwater Research*, 39:4, 881-888.

Yip MY, Lim ACO, Chong VC, Lawson JM, Foster SJ (2014) Food and feeding habits of the seahorses *Hippocampus spinosissimus* and *Hippocampus trimaculatus* (Malaysia). *Journal of the Marine Biological Association of the United Kingdom.* :1-8.

Zachary CZP, Ferber NG, Maidment (2013) Summary of Seahorse Population and Distribution Koh Rong Samloem Preah Sihanouk, Cambodia. Report on seahorse demographics and habitats, *Marine Conservation Cambodia 1st Quarter Report 2013 – Jan/Feb/Mar*

**Re: Submission Manuscript**

aan\_fish@yahoo.../Inbox



**Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Jan 6, 2021 at 3:11 AM

Dear Dr. Nofrizal,

Thank you for submitting your manuscript for possible publication in the **Journal of Animal Behaviour and Biometeorology**.

The evaluation of your manuscript begun and you will soon be contacted to find out about the editorial decision.

 Livre de virus. [www.avast.com](http://www.avast.com).

Em qua., 6 de jan. de 2021 às 00:39, nofrizal nofri &lt;aan\_fish@yahoo.com&gt; escreveu:

Dear CHIEF EDITOR  
JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY  
An International Journal

Subject: Submission of manuscript for publication in JABB  
Happy greeting from Indonesia!  
Hope, this mail does not disturb your busy activities.

In this moment, we would like submit our research article. Therefore, we are enclosing herewith a manuscript entitled "**Comparison of the target strength of Seahorses based on the presence of a reproductive Organ**" at attachment file.

With the submission of this manuscript we would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

We expect this article is sufficient and feasible and could be published at the journal of animal behaviour and biometeorology.

Thank you very much for your kind attentions and cooperation.

My best regards,

Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax: +62-761-63275

--

Best regards,

**Dr. João Souza-Junior***Editor-in-chief*[www.jabbnet.com](http://www.jabbnet.com) <https://orcid.org/0000-0002-1912-2185> Livre de virus. [www.avast.com](http://www.avast.com).

**Editorial decision**

aan\_fish@yahoo.../Inbox



**Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Feb 15, 2021 at 12:46 PM

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

Dear Dr. Nofrizal,

Thank you for sending your manuscript to the *Journal of Animal Behaviour and Biometeorology*. I have completed my evaluation of your manuscript. I am pleased to inform you that your above-mentioned article **is accepted for publication** in our journal. **Minor corrections can be requested before final publication.**

*Note that we made a small change to the article title to better fit the scope of our journal.*

Your article is already mentioned on our website: <https://www.jabbnet.com/ed/5f875c610e8825c8093901ae>

Please reply to this email as soon as possible with the **payment method** of your chosen Article Processing Charge (APC). This payment must be made by **February 21, 2021**. See more details at <https://www.jabbnet.com/instructions>

**We await your contact.**

Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

<https://orcid.org/0000-0002-1912-2185>

**Re: Editorial decision**

aan\_fish@yahoo.../Sent



nofrizal nofri &lt;aan\_fish@yahoo.com&gt;

To: Journal of Animal Behaviour and Biometeorology &lt;jabb@ufersa.edu.br&gt;

Feb 15, 2021 at 10:56 PM

Dear Dr. João Souza-Junior  
Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you for our manuscript information.

I am going to immediately make payments before the JABB death line is given, which is on February 21, 2021.

I am contact you as soon as possible, if I have been made a payment and then after I get a receipt from the bank transfer I will sent to you via mail.

Thank you very much for your attention and cooperation.

My best regards

Dr. NOFRIZAL

Riau University

Faculty of Fisheries and Marine Science

Department of Fisheries Resources Utilization

Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia

Telp. +62-761-61760, Fax +62-761-63275

On Monday, February 15, 2021, 12:46:00 PM PST, Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br> wrote:

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

Dear Dr. Nofrizal,

Thank you for sending your manuscript to the *Journal of Animal Behaviour and Biometeorology*. I have completed my evaluation of your manuscript. I am pleased to inform you that your above-mentioned article **is accepted for publication** in our journal. **Minor corrections can be requested before final publication.**

*Note that we made a small change to the article title to better fit the scope of our journal.*

Your article is already mentioned on our website: <https://www.jabbnnet.com/ed/5f875c610e8825c8093901ae>

Please reply to this email as soon as possible with the **payment method** of your chosen Article Processing Charge (APC). This payment must be made by **February 21, 2021**. See more details at <https://www.jabbnnet.com/instructions>


**We await your contact.**

Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnnet.com](http://www.jabbnnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**Bank Account for Publication Payment**

aan\_fish@yahoo.../Sent

Feb 15, 2021 at 11:44 PM

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br>  
Cc: Dony Apdillah <donyapdillah@umrah.ac.id>

Dear Dr. João Souza-Junior  
Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee.  
Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275



Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee.  
Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275

--  
Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>


--  
Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

 Livre de vírus. [www.avast.com](http://www.avast.com).

**Re: Bank Account for Publication Payment**

aan\_fish@yahoo.../Inbox



**Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Feb 16, 2021 at 2:28 AM

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

Dear Dr. Nofrizal,

Thanks for your quick reply.

The APC is \$ 450 USD (American Dollars) + **an additional 10%** is charged when the payment is made by International Bank Transfer. See more details at <http://www.jabbnet.periodikos.com.br/page/apc>

So, you must transfer \$ 495 EUR (American Dollars).

Below are all the bank details for you to make the bank transfer:

Beneficiary: João Batista Freire de Souza Junior / CPF: 05180259428  
Bank of the beneficiary: Caixa Econômica Federal (Code: 104)  
Physical address of the bank: Rua Coronel Gurgel, 406, Centro, Mossoró, RN, Brazil,  
CEP: 59600-200.

Bank account details:  
IBAN: BR14 0036 0305 0056 0000 1763 382P 1  
Swift Code: CEFXBRSP  
Account: 176338-2  
Operation: 013  
Bank branch: 0560

If you have any questions, please do not hesitate to contact me.

Best regards,

**Dr. João Souza-Junior****Editor-in-chief**[www.jabbnet.com](http://www.jabbnet.com) <https://orcid.org/0000-0002-1912-2185>

Em ter., 16 de fev. de 2021 às 04:44, nofrizal nofri &lt;aan\_fish@yahoo.com&gt; escreveu:

Dear Dr. João Souza-Junior  
Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee.  
Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,

Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12.5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275

--

Best regards,

**Dr. João Souza-Junior****Editor-in-chief**[www.jabbnet.com](http://www.jabbnet.com) <https://orcid.org/0000-0002-1912-2185>


**Editor-in-chief**

[www.jabnet.com](http://www.jabnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**Trs: Re: Bank Account for Publication Payment**

aan\_fish@yahoo.../Sent

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: Dony Apdillah <donyapdillah@umrah.ac.id>

Feb 16, 2021 at 3:18 AM

[Dikirim dari Yahoo Mail di Android](#)

----- Pesan yang Diteruskan -----

**Dari:** "Journal of Animal Behaviour and Biometeorology" <jabb@ufersa.edu.br>

**Kepada:** "nofrizal nofri" <aan\_fish@yahoo.com>

**Cc:**

**Terkirim:** Sel, 16 Feb 2021 pada 17:28

**Judul:** Re: Bank Account for Publication Payment

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

Dear Dr. Nofrizal,

Thanks for your quick reply.

*The APC is \$ 450 USD (American Dollars) + an additional 10% is charged when the payment is made by International Bank Transfer. See more details at <http://www.jabbnet.periodikos.com.br/page/apc>*

*So, you must transfer \$ 495 EUR (American Dollars).*

Below are all the bank details for you to make the bank transfer:

Beneficiary: João Batista Freire de Souza Junior / CPF: 05180259428

Bank of the beneficiary: Caixa Econômica Federal (Code: 104)

Physical address of the bank: Rua Coronel Gurgel, 406, Centro, Mossoró, RN, Brazil, CEP: 59600-200.

Bank account details:

IBAN: BR14 0036 0305 0056 0000 1763 382P 1

Swift Code: CEFXBRSP

Account: 176338-2

Operation: 013

Bank branch: 0560

If you have any questions, please do not hesitate to contact me.

Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

Em ter., 16 de fev. de 2021 às 04:44, nofrizal nofri <aan\_fish@yahoo.com> escreveu:

Dear Dr. João Souza-Junior

Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee. Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,

Dr. NOFRIZAL

Riau University

Faculty of Fisheries and Marine Science

Department of Fisheries Resources Utilization

Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia

Telp. +62-761-61760, Fax. +62-761-63275

--

Best regards,

**Dr. João Souza-Junior**

7/12/2022 4:16 AM

**Re: Bank Account for Publication Payment**

aan\_fish@yahoo.../Sent

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br>  
Cc: Dony Apdillah <donyapdillah@umrah.ac.id>

Feb 17, 2021 at 11:17 PM

Dear Dr. Joao Souza-Junior  
Editor-in-Chief  
Journal of Animal Behaviour and Biometeorology

Happy greeting from ndonesia.

Hope, this mail does not disturb your busy activities.

In this moment, I would like to inform you that I have been transferred the publication fee for our manuscript via Mandiri Syariah Bank. Therefore, I am sending the the bank transfer receipt in attachment file to you. I expect, the bank transfer can reach bank account soon. Please touch me if any problem in bank transfer process. Thank you very much for your kindness and cooperations.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275

On Tuesday, February 16, 2021, 2:28:00 AM PST, Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br> wrote:

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

Dear Dr. Nofrizal,

Thanks for your quick reply.

*The APC is \$ 450 USD (American Dollars) + an additional 10% is charged when the payment is made by International Bank Transfer. See more details at <http://www.jabbnnet.periodikos.com.br/page/apc>*

*So, you must transfer \$ 495 EUR (American Dollars).*

Below are all the bank details for you to make the bank transfer:

Beneficiary: João Batista Freire de Souza Junior / CPF: 05180259428  
Bank of the beneficiary: Caixa Econômica Federal (Code: 104)  
Physical address of the bank: Rua Coronel Gurgel, 406, Centro, Mossoró, RN, Brazil,  
CEP: 59600-200.

Bank account details:  
IBAN: BR14 0036 0305 0056 0000 1763 382P 1  
Swift Code: CEFXBRSP  
Account: 176338-2  
Operation: 013  
Bank branch: 0560


If you have any questions, please do not hesitate to contact me.

Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnnet.com](http://www.jabbnnet.com)

 <https://orcid.org/0000-0002-1912-2185>

Em ter., 16 de fev. de 2021 às 04:44, nofrizal nofri <aan\_fish@yahoo.com> escreveu:

Dear Dr. João Souza-Junior  
Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee.  
Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia

Telp. +62-761-61760, Fax. +62-761-63275

—  
Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**1 File** 208.4kB



Receipt transver (Doni Abdillah).jpg  
208kB

**Re: Bank Account for Publication Payment**

aan\_fish@yahoo.../Inbox



**Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Feb 18, 2021 at 12:47 PM

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

Dear Dr. Nofrizal,

Thank you for confirming your payment.

I will contact you when the money arrives in our bank account here in Brazil.

 Livre de vírus. [www.avast.com](http://www.avast.com).

Em qui., 18 de fev. de 2021 às 04:17, nofrizal nofri &lt;aan\_fish@yahoo.com&gt; escreveu:

Dear Dr. Joao Souza-Junior  
Editor-in-Chief  
Journal of Animal Behaviour and Biometeorology

Happy greeting from Indonesia.

Hope, this mail does not disturb your busy activities.

In this moment, I would like to inform you that I have been transferred the publication fee for our manuscript via Mandiri Syariah Bank.

Therefore, I am sending the bank transfer receipt in attachment file to you.

I expect, the bank transfer can reach bank account soon.

Please touch me if any problem in bank transfer process.

Thank you very much for your kindness and cooperations.

My best regards,

Dr. NOFRIZAL

Riau University

Faculty of Fisheries and Marine Science

Department of Fisheries Resources Utilization

Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia

Telp. +62-761-61760, Fax. +62-761-63275

On Tuesday, February 16, 2021, 2:28:00 AM PST, Journal of Animal Behaviour and Biometeorology &lt;jabb@ufersa.edu.br&gt; wrote:

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

Dear Dr. Nofrizal,

Thanks for your quick reply.

The APC is \$ 450 USD (American Dollars) + an **additional 10%** is charged when the payment is made by International Bank Transfer. See more details at <http://www.jabbnnet.periodikos.com.br/page/apc>

So, you must transfer \$ 495 EUR (American Dollars).

Below are all the bank details for you to make the bank transfer:

Beneficiary: João Batista Freire de Souza Junior / CPF: 05180259428

Bank of the beneficiary: Caixa Econômica Federal (Code: 104)

Physical address of the bank: Rua Coronel Gurgel, 406, Centro, Mossoró, RN, Brazil,

CEP: 59600-200.

Bank account details:

IBAN: BR14 0036 0305 0056 0000 1763 382P 1

Swift Code: CEFXBRSP

Account: 176338-2

Operation: 013

Bank branch: 0560

If you have any questions, please do not hesitate to contact me.

Best regards,

**Dr. João Souza-Junior***Editor-in-chief*[www.jabbnnet.com](http://www.jabbnnet.com) <https://orcid.org/0900-0002-1912-2185>

Em ter., 16 de fev. de 2021 às 04:44, nofrizal nofri &lt;aan\_fish@yahoo.com&gt; escreveu:

Dear Dr. João Souza-Junior

Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

Em ter., 16 de fev. de 2021 às 04:44, nofrizal nofri <[aan\\_fish@yahoo.com](mailto:aan_fish@yahoo.com)> escreveu:

Dear Dr. João Souza-Junior  
Editor-in-chief Journal of Animal Behaviour and Biometeorology (JABB)

Thank you very much for your acceptance information of our manuscript.

By the way, We would like to make a payment for publication fee.  
Therefore, we need your bank account for transfer the publication fee.

Thank you very much for your attention and cooperation.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275

--  
Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

--  
Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>


 Livre de virus. [www.avast.com](http://www.avast.com).

--  
Best regards,

**Dr. João Souza-Junior**

**Editor-in-chief**

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>



**Re: Bank Account for Publication Payment**

aan\_fish@yahoo.../Inbox



**Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Feb 26, 2021 at 9:29 AM

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

Dear Dr. Nofrizal,

Our bank confirmed that your payment was successful. Thanks!

We will contact you shortly to continue the process of publishing your article in our journal.

Em qui., 18 de fev. de 2021 às 17:47, Journal of Animal Behaviour and Biometeorology &lt;jabb@ufersa.edu.br&gt; escreveu:

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

Dear Dr. Nofrizal,

Thank you for confirming your payment.

I will contact you when the money arrives in our bank account here in Brazil.

Livre de vírus. [www.avast.com](http://www.avast.com).

Em qui., 18 de fev. de 2021 às 04:17, nofrizal nofri &lt;aan\_fish@yahoo.com&gt; escreveu:

Dear Dr. Joao Souza-Junior  
Editor-in-Chief  
Journal of Animal Behaviour and Biometeorology

Happy greeting from Indonesia.

Hope, this mail does not disturb your busy activities.

In this moment, I would like to inform you that I have been transferred the publication fee for our manuscript via Mandiri Syariah Bank. Therefore, I am sending the the bank transfer receipt in attachment file to you. I expect, the bank transfer can reach bank account soon. Please touch me if any problem in bank transfer process. Thank you very much for your kindness and cooperations.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax. +62-761-63275

On Tuesday, February 16, 2021, 2:28:00 AM PST, Journal of Animal Behaviour and Biometeorology &lt;jabb@ufersa.edu.br&gt; wrote:

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

Dear Dr. Nofrizal,

Thanks for your quick reply.

The APC is \$ 450 USD (American Dollars) + an **additional 10%** is charged when the payment is made by International Bank Transfer. See more details at <http://www.jabbnnet.periodikos.com.br/page/apc>

So, you must transfer \$ 495 EUR (American Dollars).

Below are all the bank details for you to make the bank transfer:

Beneficiary: João Batista Freire de Souza Junior / CPF: 05180259428  
Bank of the beneficiary: Caixa Econômica Federal (Code: 104)  
Physical address of the bank: Rua Coronel Gurgel, 406, Centro, Mossoró, RN, Brazil,  
CEP: 59600-200.

Bank account details:  
IBAN: BR14 0036 0305 0056 0000 1763 382P 1  
Swift Code: CEFXBRSP  
Account: 176338-2  
Operation: 013  
Bank branch: 0560

If you have any questions, please do not hesitate to contact me.

Best regards,

**Updated Article!**

aan\_fish@yahoo.../Inbox

 **Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Mar 10, 2021 at 4:18 AM



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

Dear Dr. Nofrizal,

Your article has been updated! The DOI of your article recently accepted for publication in our journal was registered with CrossRef: <http://dx.doi.org/10.31893/jabb.21023>

Soon we will contact you to begin the ProofReading phase.

Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**Balas: Updated Article!**

aan\_fish@yahoo.../Sent

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: jabb@ufersa.edu.br  
Cc: Dony Apdillah <donyapdillah@umrah.ac.id>

Mar 11, 2021 at 12:35 AM

Dear Dr. Joao Souza-Junior

Thank you very much for information of the article updating.

I am waiting for proof reding phase.

My best regards  
Nofrizal

[Dikirim dari Yahoo Mail di Android](#)

Pada Rab, 10 Mar 2021 pada 19:18, Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br> menulis:



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

Dear Dr. Nofrizal,

Your article has been updated! The DOI of your article recently accepted for publication in our journal was registered with CrossRef: <http://dx.doi.org/10.31893/jabb.21023>

Soon we will contact you to begin the ProofReading phase.

Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**ProofReading [JABB-2021023]**

aan\_fish@yahoo.../Inbox

 **Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Mar 15, 2021 at 10:03 AM



Dear Dr. Nofrizal,

The publication of your article in the *Journal of Animal Behaviour and Biometeorology* is very close. So we're entering the ProofReading phase.

The *Uncorrected Proof* is attached.

**Please note the comments below:**

1. Read the entire article carefully to correct possible typos in all sections.
2. Provide the ORCID ID ([orcid.org](https://orcid.org)) of the authors who own it.
3. Provide, in the text, calls to **Figures 2 and 3**.
4. Declare whether the research received any funding.


Please resend the article by **March 22, 2021**.

Best regards,

**JABB Team**[www.jabbnet.com](http://www.jabbnet.com)**1 File** 1.2MBArticle\_Nofrizal\_Layout.pdf  
1MB

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



Dony Apdillah<sup>a,b</sup> | Indra Jaya<sup>b</sup> | Henry Munandar Manik<sup>b</sup> | Totok Hestirianoto<sup>b</sup> | Nofrizal<sup>c\*</sup> 

<sup>a</sup>Marine Science Department, Marine Science and Fisheries Faculty, Raja Ali Haji Maritime University, Indonesia.

<sup>b</sup>Division of Acoustic and Marine Instrumentation, Marine Science and Technology Department, IPB University, Indonesia.

<sup>c</sup>Program Study of Environment Science, Doctoral Program, Riau University, Indonesia.

\*Corresponding author: [aan\\_fish@yahoo.com](mailto:aan_fish@yahoo.com)

**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  $-56.24 \pm 0.047$ ,  $-57.25 \pm 0.032$ , and  $-58.26 \pm 0.06$ , 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 highly 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, which is 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**

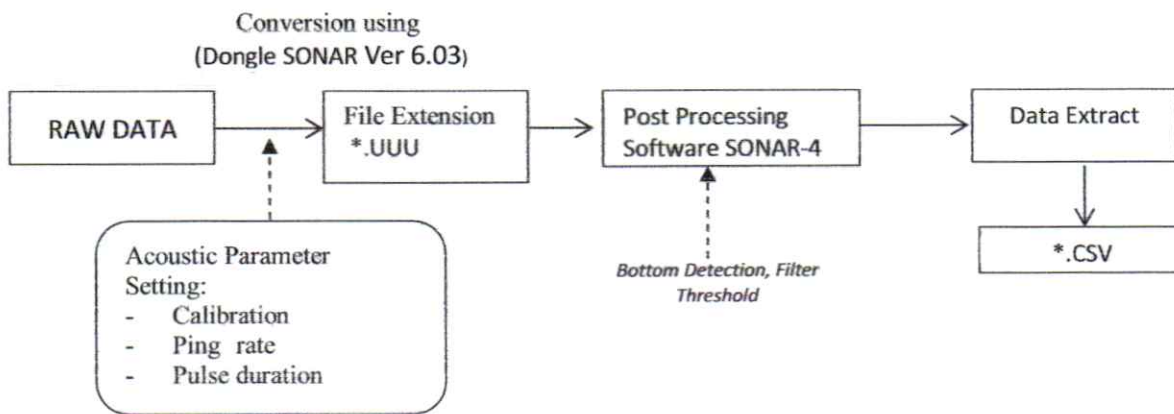
The samples were obtained from the waters of Bintan Island, Indonesia. They include *H. kuda* female (no presence of a brood pouch), *H. kuda* male (not fertilized/not pregnant), and *H. kuda* pregnant male or Mature males. Besides, both males were identified by a brood pouch's presence (Vincent et al. 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). 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 echo-sounder, 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 the figure below.



**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:

$$TS = 10 \log (\sigma_{bs})$$

where  $\sigma_{bs}$  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:

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

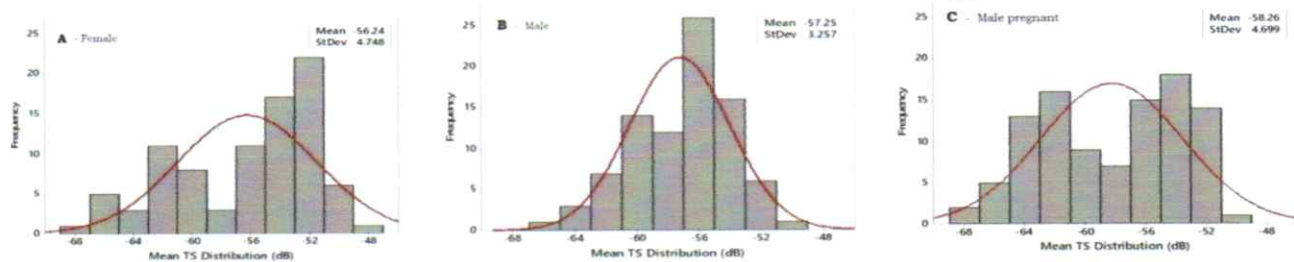
The acoustic data were analyzed statistically using variance (ANOVA) to test the TS value response to the development of the pregnant and non-pregnant male and female seahorses' reproductive states. Furthermore, the Tukey test was carried out to see the interaction between treatments.

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)
<i>H. kuda</i>	19.0	-	Female (no brood pouch)	10.000	-56.24±0.047
<i>H. kuda</i>	18.5	-	Male (brood pouch has not been fertilized)	10.000	-57.25±0.032
<i>H. kuda</i>	18.6	2.52	Male pregnant (presence of a brood pouch)	6.000	-58.26±0.06

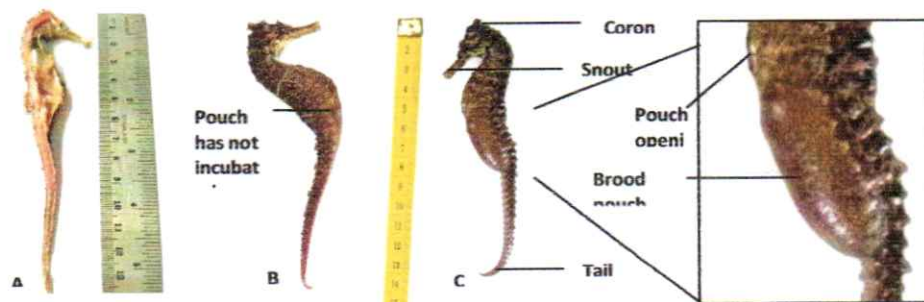


**Figure 5** Histograms of the normal mean TS distribution of *H. kuda* for A, female, B, male and C, male pregnant.

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. Carpuccio 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).

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.

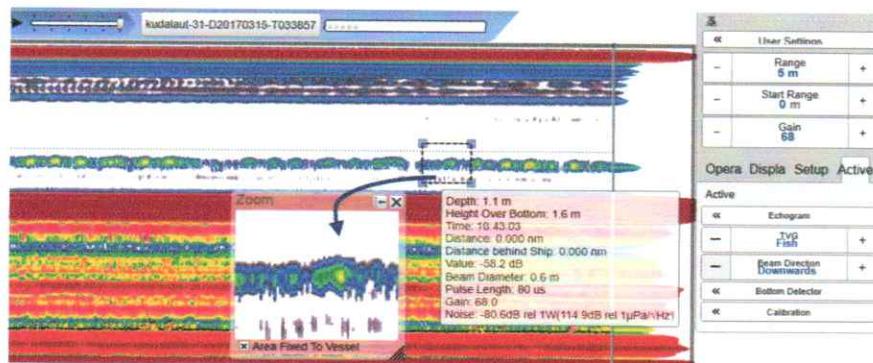


Figure 3 RAW data recorded using the Simrad EK-15 Acquisition software.

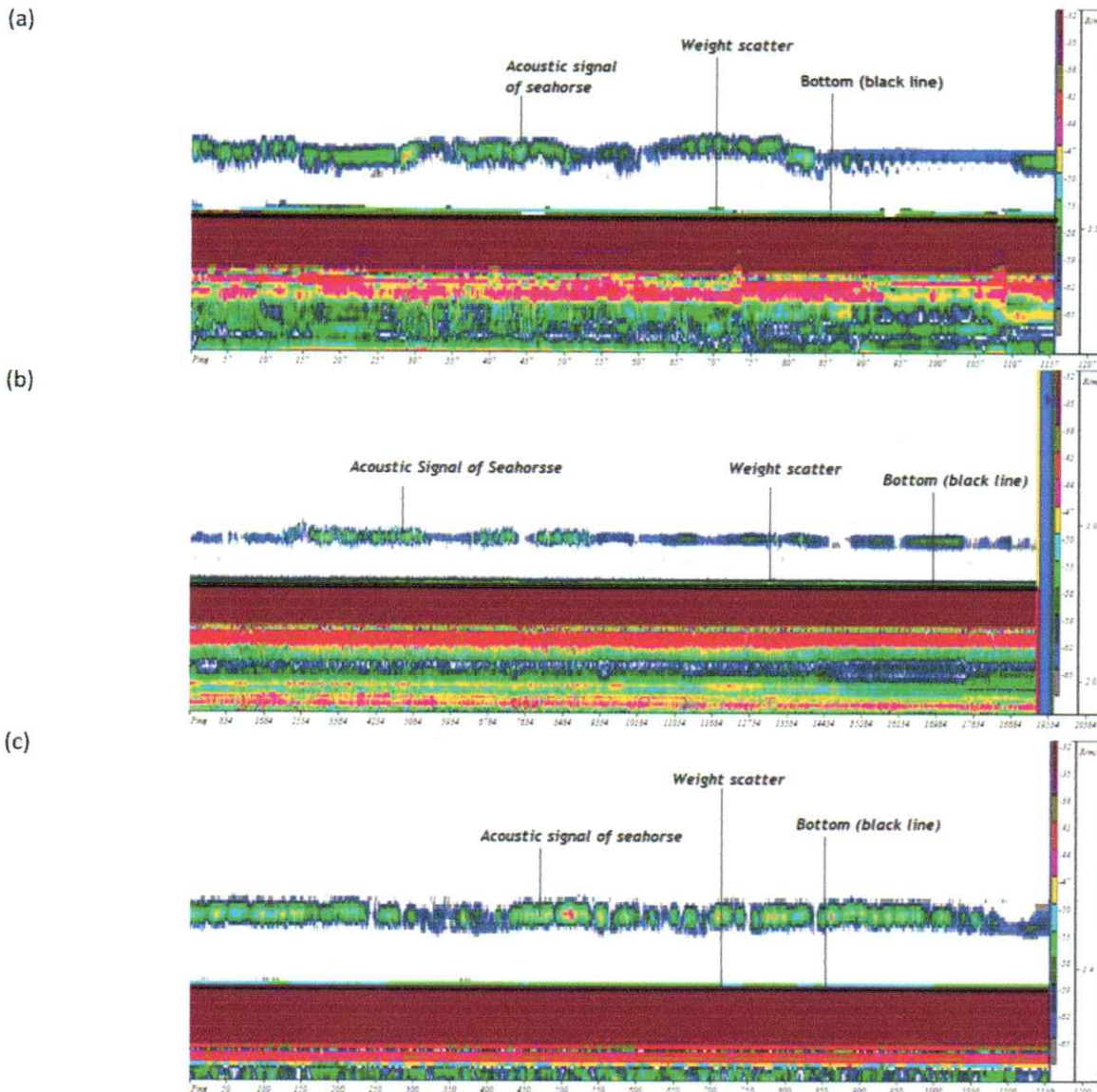
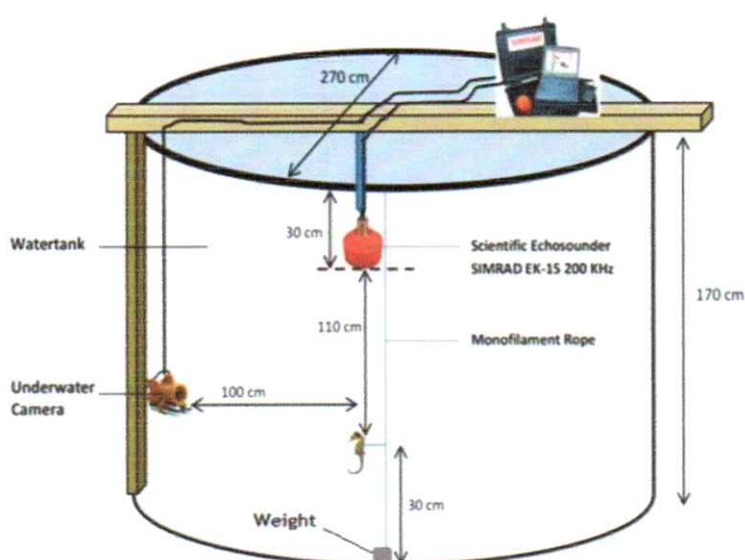


Figure 4 The results of the signal processing analysis after filtering. The weight scatter was separated from the acoustic signal of the seahorse H.Kuda; (a) male (no-pregnant), (b) male pregnant, (c) female.



**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 <sup>-1</sup> )	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.

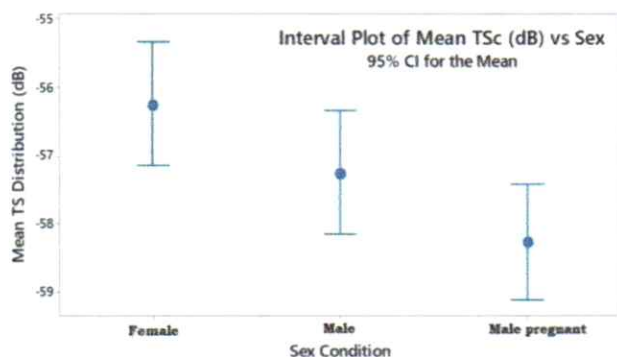
The seahorse morphometric measurements were carried out after recording the acoustic data, including length, sex. Furthermore, this method was applied to each observation object (N = 10,000). The measurement results of the mean TS value of seahorses based on their reproductive state were carried out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

non-pregnant males were  $-58.26 \pm 0.06$  and  $-57.25 \pm 0.032$  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

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 significantly compared *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

Grant Kemenristek Dikti supported this research in 2017. The authors are grateful for the Aquaculture

Laboratory facilities, Marine Science and Fisheries Faculty of UMRAH, used in the data collection process, and the Laboratory Signal Processing Division Acoustic and Marine Instrumentation IPB Bogor for helping in the data processing.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

#### Funding

This research did not receive any financial support.

#### References

- Apdillah D, Jaya I, Manik HM, T Hestirianoto and Febrianto T (2018) Seahorse Acoustic Reflection on Active and Passive Moving Condition: A Preliminary Observation in Watertank. *Omni-Akuatika* 14:1.
- Balk H, Lindem T (2015) Sonar 4 and Sonar 5-Pro post-processing systems, Operator manual version 6.0.3, 494 p, Lindem Data Acquisition Humleveien 4b. 0870 Oslo, Norway.
- Bertha M.O & Davy B (2000) Traditional Medicine Chines & Tonic Food in Proceeding of The First International Workshop on The management and Culture of Marine Species Use in Tradional Medicine. Project Seahorse Department of Biology MC. Gill University Montreal; Quebec. Canada.
- Carpucino M, Baldacci A, Mazzini M, Franzoi P (2002) Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. *Journal of Fish Biology* 61:1465-1480.
- Correia MJ, Palma, Koldewey HJ, Andrade JP (2013) Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (*Hippocampus guttulatus* Cuvier)? *Journal of Experimental Marine Biology and Ecology* 448:258-264.
- DuFour M.R, Christine MM, Song SQ, Christopher SV, Richard TK, Patrick MK, David MW (2018) Inferred fish behavior its implications for hydro-acoustic surveys in nearshore habitats. *Fisheries Research* 199:63-75.
- Frouzova J, Kubecka J, Balk H, Frouz J (2005) Target strength of some European fish species and its dependence on fish body parameters. *Fisheries Research* 75:86-96.
- Foster SJ, Wiswedel S, Vincent ACJ (2014) Opportunities and challenges for analysis of wildlife trade using CITES data - seahorses as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems*.
- Foster SJ, Vincent ACJ (2004) Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* 65:1-61.
- Greenstreet SPR, Holland GJ, Guirey EJ, Armstrong E, Fraser HM, Gibb IM (2010) Combining hydro-acoustic seabed survey and grab sampling techniques to assess local sandeel population abundance. *ICES Journal of Marine Science* 67:971-984.
- Horne JK (2003) The influence of ontogeny, physiology and behaviour on the target strength of walleye pollock (*Theragra chalcogramma*). *ICES Journal of Marine Science* 60:1063-1074.
- IUCN Red List of Threatened Species. Version 2014.3. Available: <[www.iucnredlist.org](http://www.iucnredlist.org)>.
- Jones AG (2004) Male pregnancy and the formation of seahorse species. *Biologist* 51:4.
- Kawaguchi M, Okubo R, Harada A, Miyasaka K, Takada K, Hiroi J, Yasumasu S (2017) Morphology of brood pouch formation in the pot-bellied seahorse *Hippocampus abdominalis*. *Zoological letters* 3:19.
- Kusumaningrum W (2013) Analisis target strength Udang Galah (*Macrobrachium rosenbergii* De Man) menggunakan akustik *split beam* di Delta Mahakam. *Thesis, Sekolah Pascasarjana IPB, Bogor*.
- Laksanawimol P, Damrongphol P, Kruatrachue M (2006) Alteration of the brood pouch morphology during gestation of male seahorses, *Hippocampus kuda*. *Marine and Freshwater Research* 57:497-502.

- Manik HM, Apdillah D (2017) Remote Sensing of Seagrass and Seabed using Acoustic Technology in Bintan Seawater, Indonesia.
- Manik HM, Apdillah D, Dwinovantyo A, Solikin S (2017) Development of Quantitative Single Beam Echo-sounder for Measuring Fish Backscattering. Chapter 6 from the book *Advances in Underwater Acoustics*. Published by INTECH.
- Manik HM, Furusawa M, Amakasu K (2006) Quantifying Sea Bottom Surface Backscattering Strength and Identifying Bottom Fish Habitat by Quantitative Echo Sounder. *Japanese Journal of Applied Physics* 45:4865-4867.
- Medwin H, dan Clay CS (1998) *Applied Ocean Acoustics: Fundamentals of Acoustical Oceanography*. Academic Press, New York. 712 pp.
- Ona E (1990) Physiological factors causing natural variations in acoustic target strength of fish. *Journal of the Marine Science Biological Association of the UK* 70:107-127.
- Poortenaar CW, Woods CMC, James PJ, Giambartolomei FM, Lokman PM (2004) Reproductive biology of female big-bellied seahorses. *Journal of Fish Biology* 64:717-725.
- Project Seahorse (2014) Available: <http://seahorse.fisheries.ubc.ca>.
- Simmonds EJ, MacLennan DN (2005) *Fisheries Acoustic Theory and Practice*. Second Edition. Oxford: Blackwell Science Ltd.
- Simrad (2012) Installation manual, Simrad EK15 Multi purpose scientific echosounder, Kongsberg Maritime AS.
- Stölting KN, Wilson AB (2007) Male pregnancy in seahorses and pipefish: beyond the mammalian model. *Bioessays* 29:884-896.
- Vecchione A (2013) Prospects challenges in monitoring the seahorse population of South Carolina, USA. *Journal of Marine Animal and Their Ecology* 6:6-11.
- Vincent ACJ, Berglund A, Ahnesjö I (1995) Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* 44:347-61
- Vincent ACJ (1996) *The International Trade in Seahorses*. TRAFFIC International, Cambridge, UK.
- Vincent ACJ, Foster SJ, Koldewey HJ (2011) Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology* 78:1681-1724.
- Wilson MJ, Vincent ACJ (1998) Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aquatic Science Conservation* 2:179-196.
- Woods CMC (2005) Reproductive output of male seahorses, *Hippocampus abdominalis*, from Wellington Harbour, New Zealand: Implications for conservation. *New Zealand Journal of Marine and Freshwater Research* 39:881-888.
- Yip MY, Lim ACO, Chong VC, Lawson JM, Foster SJ (2014) Food and feeding habits of the seahorses *Hippocampus spinosissimus* and *Hippocampus trimaculatus* (Malaysia). *Journal of the Marine Biological Association of the United Kingdom* 1-8.
- Zachary CZP, Ferber NG, Maidment (2013) Summary of Seahorse Population and Distribution Koh Rong Samloem Preah Sihanouk, Cambodia. Report on seahorse demographics and habitats, Marine Conservation Cambodia 1st Quarter Report 2013 – Jan/Feb/Mar.

**Balas: Updated Article!**

aan\_fish@yahoo.../Sent

 **nofrizal nofri** <aan\_fish@yahoo.com>  
To: jabb@ufersa.edu.br  
Cc: Dony Apdillah <donyapdillah@umrah.ac.id>

Mar 11, 2021 at 12:35 AM

Dear Dr. Joao Souza-Junior

Thank you very much for information of the article updating.

I am waiting for proof reding phase.

My best regards  
Nofrizal

[Dikirim dari Yahoo Mail di Android](#)

Pada Rab, 10 Mar 2021 pada 19:18, Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br> menulis:



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

Dear Dr. Nofrizal,

Your article has been updated! The DOI of your article recently accepted for publication in our journal was registered with CrossRef: <http://dx.doi.org/10.31893/jabb.21023>


Soon we will contact you to begin the ProofReading phase.

Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>

**ProofReading [JABB-2021023]**

aan\_fish@yahoo.../Inbox

 **Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Mar 15, 2021 at 10:03 AM



Dear Dr. Nofrizal,

The publication of your article in the *Journal of Animal Behaviour and Biometeorology* is very close. So we're entering the ProofReading phase.

The *Uncorrected Proof* is attached.

**Please note the comments below:**

1. Read the entire article carefully to correct possible typos in all sections.
2. Provide the ORCID ID ([orcid.org](https://orcid.org)) of the authors who own it.
3. Provide, in the text, calls to **Figures 2 and 3**.
4. Declare whether the research received any funding.

Please resend the article by [March 22, 2021](#).

Best regards,

**JABB Team**

[www.jabbnet.com](http://www.jabbnet.com)

**1 File** 1.2MB

 Article\_Nofrizal\_Layout.pdf  
1MB

**Re: ProofReading [JABB-2021023]**

aan\_fish@yahoo.../Sent



nofrizal nofri &lt;aan\_fish@yahoo.com&gt;

To: Journal of Animal Behaviour and Biometeorology &lt;jabb@ufersa.edu.br&gt;

Cc: Dony Apdillah &lt;donyapdillah@umrah.ac.id&gt;

Mar 16, 2021 at 11:17 AM

Dear Dr. João Souza-Junior  
Editor-in-chief  
Journal Animal Behaviour and Biometeorology

Thank you very much for sending us the proofreading of manuscript.

We have read the manuscript carefully, and made revision and rectification on the proof reading manuscript.

Unfortunately, we cannot do any revision and rectification, because the manuscript in PDF file. Therefore, we have converting the manuscript from PDF file to Microsoft word file.

We have a little bit correction in the manuscript proof reading, i.e. :

1. In abstract section line 6, i.e. Furthermore, the response to the mean TS value of pregnant H. kudamale (the presence of a brood pouch) was **highly** significant compared to the female (absence of a brood pouch) ( $P < 0.05$ ). The "highly" (red word) should be deleted, thus becoming "Furthermore, the response to the mean TS value of pregnant H. kudamale (the presence of a brood pouch) was significant compared to the female (absence of a brood pouch) ( $P < 0.05$ ).
2. Corresponding author's address was (cNofrizal) rectified such as there should be added as "cMarine Science Post Graduate Study, Faculty of Fisheries and Marine Science, Riau University, and Program Study of Environment Science, Doctoral Program, Riau University, Indonesia. We also add the author address at the first author (abDony Apdillah).
3. The ORCID id's Dony Apdillah is [0000-0001-7990-1298](https://orcid.org/0000-0001-7990-1298)
4. We were provided, in the text, calls to **Figures 2 and 3**. All rectification has been conducted in red font color at the manuscript.
5. We have declared the research funding in.
- 6.

We expect that the correction we have can make our paper better and perfect.

My best regards,  
Dr. NOFRIZAL  
Riau University  
Faculty of Fisheries and Marine Science  
Department of Fisheries Resources Utilization  
Kampus Bina Widya, KM. 12,5, Simpang Panam, Pekanbaru, Riau, Indonesia  
Telp. +62-761-61760, Fax: +62-761-63275

On Monday, March 15, 2021, 10:03:18 AM PDT, Journal of Animal Behaviour and Biometeorology <jabb@ufersa.edu.br> wrote:



Dear Dr. Nofrizal,

The publication of your article in the *Journal of Animal Behaviour and Biometeorology* is very close. So we're entering the ProofReading phase.

The *Uncorrected Proof* is attached.

Please note the comments below:

1. Read the entire article carefully to correct possible typos in all sections.
2. Provide the ORCID ID ([orcid.org](https://orcid.org)) of the authors who own it.
3. Provide, in the text, calls to **Figures 2 and 3**.
4. Declare whether the research received any funding.

Please resend the article by **March 22, 2021**.

Best regards,

**JABB Team**

[www.jabbnet.com](http://www.jabbnet.com)

1 File 1.7MB



Article\_Nofrizal\_Layout\_Proof Reading-ok.docx  
2MB

**Published Article!**

aan\_fish@yahoo.../Inbox

 **Journal of Animal Behaviour and Biometeorology** <jabb@ufersa.edu.br>  
To: nofrizal nofri <aan\_fish@yahoo.com>

Mar 16, 2021 at 2:11 PM



Dear Dr. Nofrizal,

Thank you for submitting the corrected proof.

Your article is already published on our website: <https://www.jabbnet.com/ed/5ff5a1850e8825ea33fe54a5>

Thank you for choosing JABB to publish your articles.


We hope you will consider our journal to publish your next research!

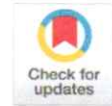
Best regards,

**Dr. João Souza-Junior**

*Editor-in-chief*

[www.jabbnet.com](http://www.jabbnet.com)

 <https://orcid.org/0000-0002-1912-2185>



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

Dony Apdillah<sup>a,b</sup> | Indra Jaya<sup>c</sup> | Henry Munandar Manik<sup>c</sup> | Totok Hestirianoto<sup>c</sup> | Nofrizal<sup>d</sup>

<sup>a</sup>Department of Marine Science, Faculty of Marine Science and Fisheries, Raja Ali Haji Maritime University, Indonesia.

<sup>b</sup>Environmental Science Department, Raja Ali Haji Maritime University, Indonesia.

<sup>c</sup>Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, IPB University, Indonesia.

<sup>d</sup>Marine Science Post Graduate Study, Faculty of Fisheries and Marine Science, Riau University, and Program Study of Environment Science, Doctoral Program, Riau University, Indonesia, Indonesia.

<sup>\*</sup>Corresponding author: [aan\\_fish@yahoo.com](mailto:aan_fish@yahoo.com)

**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±SE) of *Hippocampus kuda* female, male and pregnant male seahorses were  $-56.24 \pm 0.047$ ,  $-57.25 \pm 0.032$ , and  $-58.26 \pm 0.06$ , 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, which is 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**

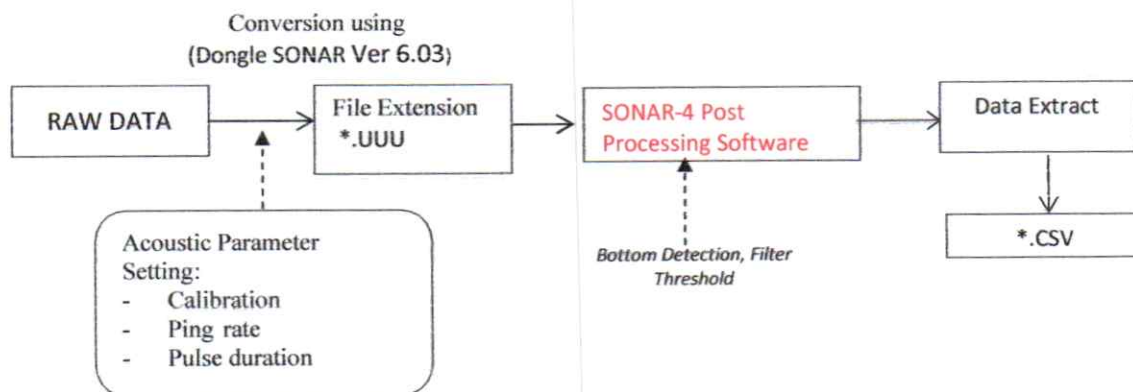
The samples were obtained from the waters of Bintan Island, Indonesia. They include *H. kuda* female (no presence of a brood pouch), *H. kuda* male (not fertilized/not pregnant), and *H. kuda* pregnant male or Mature males. Besides, both males were identified by a brood pouch's presence (Vincent et al. 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 echo-sounder, 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 the 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:

$$TS = 10 \log (\sigma_{bs})$$

where  $\sigma_{bs}$  represents the back-scattering cross-section on the measurement of the acoustic signal from a single target,

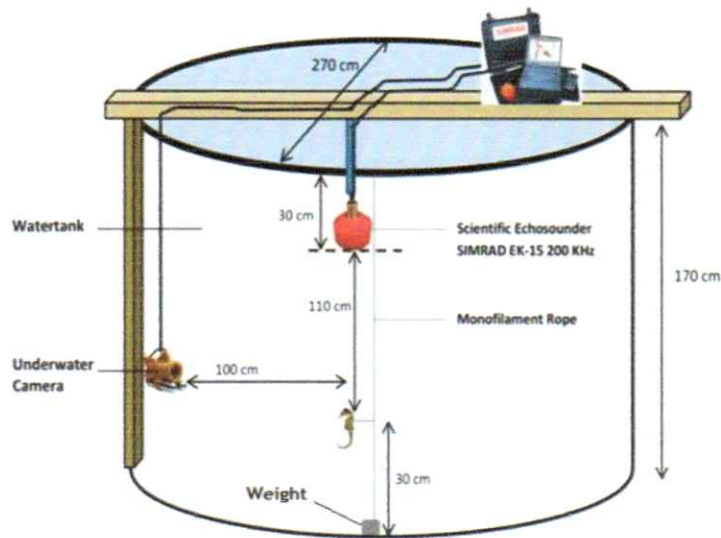
furthermore, the linear equation for measuring the average TS include:

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

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 <sup>-1</sup> )	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  $-56.24 \pm 0.047$  dB, while for the pregnant and

non-pregnant males were  $-58.26 \pm 0.06$  and  $-57.25 \pm 0.032$  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.

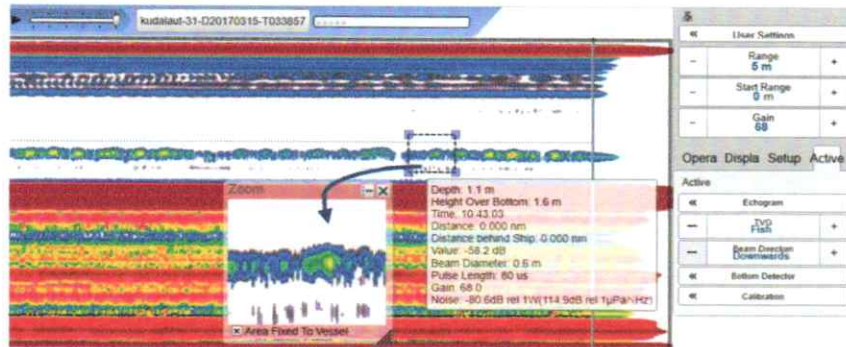


Figure 3 RAW data recorded using the Simrad EK-15 Acquisition software.

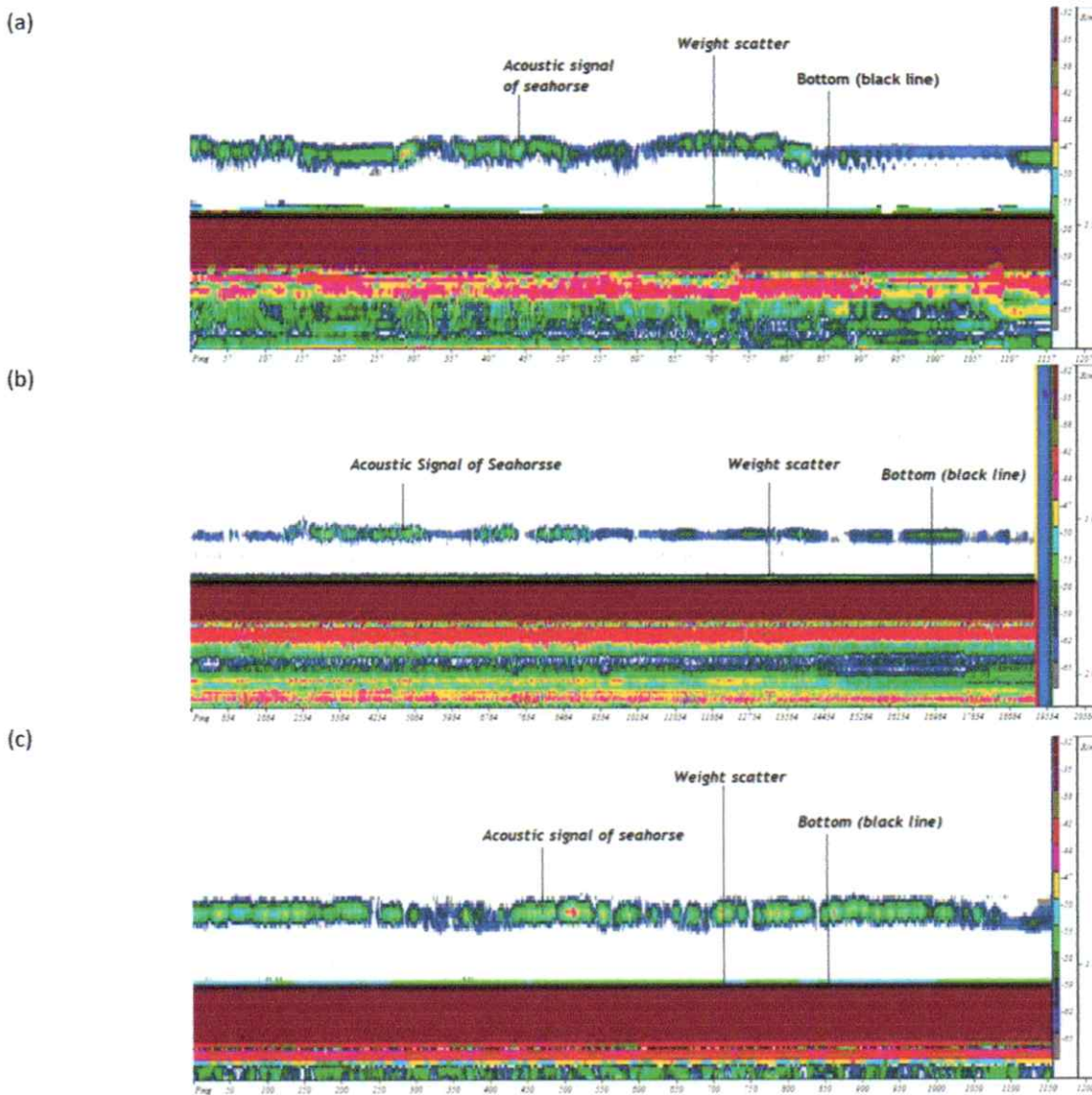


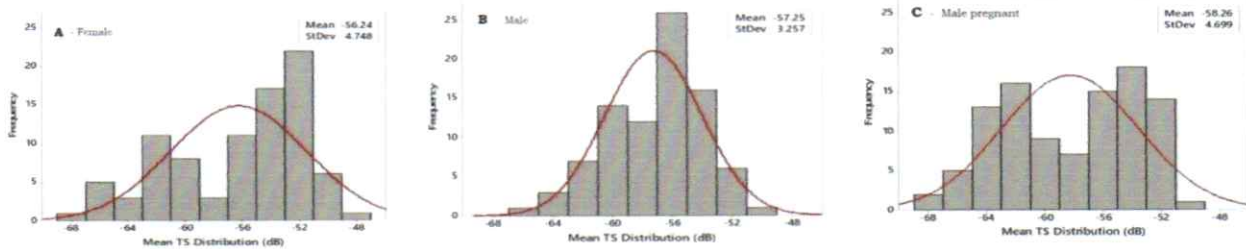
Figure 4 The results of the signal processing analysis after filtering. The weight scatter was separated from the acoustic signal of the seahorse H.Kuda; (a) male (no-pregnant), (b) male pregnant, (c) female.

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)
<i>H. kuda</i>	19.0	-	Female (no brood pouch)	10.000	-56.24±0.047
<i>H. kuda</i>	18.5	-	Male (brood pouch has not been fertilized)	10.000	-57.25±0.032
<i>H. kuda</i>	18.6	2.52	Male pregnant (presence of a brood pouch)	6.000	-58.26±0.06

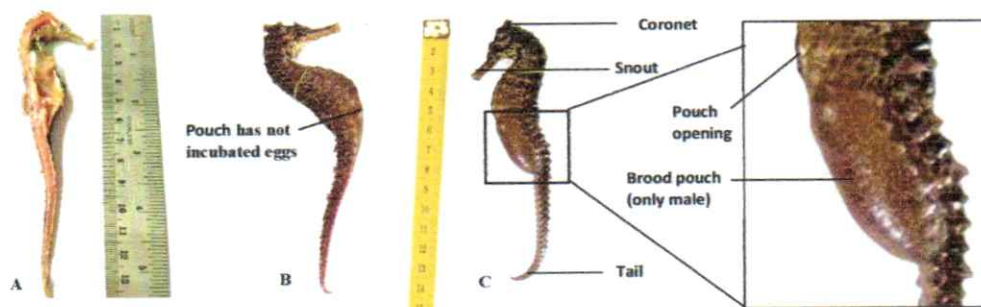


**Figure 5** Histograms of the normal mean TS distribution of *H. kuda* for A, female, B, male and C, male pregnant.

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).

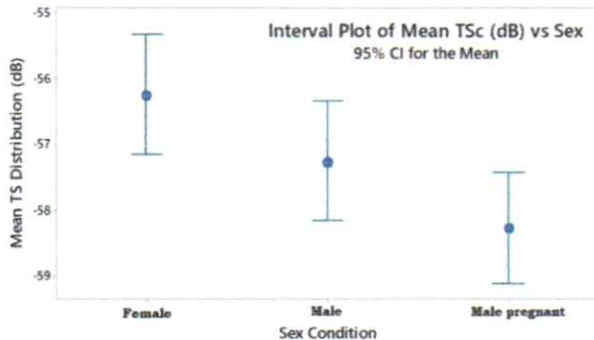
It is suspected that the brood pouch morphological factors from seahorses influence back-scattering strength. Carpuccio et al

(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 oögonia and oocytes in the first growth phase. The Oögonia 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 significantly compared *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

The authors are grateful for the Aquaculture Laboratory facilities, Marine Science and Fisheries Faculty of Raja Ali Haji Maritime University, and the Laboratory Signal

Processing Division Acoustic and Marine Instrumentation IPB Bogor for helping in the data collecting and processing.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

#### Funding

This study was funded by KEMRISTEKDIKTI research grant, No. Contract: 101 / UNS3.01.1 / KONTRAK-LP / I/ 2017. Raja Ali Haji Maritime University.

#### References

- Apdillah D, Jaya I, Manik HM, T Hestirianoto and Febrianto T (2018) Seahorse Acoustic Reflection on Active and Passive Moving Condition: A Preliminary Observation in Watertank. *Omni-Akuatika* 14:1.
- Balk H, Lindem T (2015) Sonar 4 and Sonar 5-Pro post-processing systems, Operator manual version 6.0.3, 494 p, Lindem Data Acquisition Humleveien 4b. 0870 Oslo, Norway.
- Bertha M.O & Davy B (2000) Traditional Medicine Chines & Tonic Food in Proceeding of The First International Workshop on The management and Culture of Marine Species Use in Tradional Medicine. Project Seahorse Department of Biology MC. Gill University Montreal; Quebec. Canada.
- Carpucino M, Baldacci A, Mazzini M, Franzoi P (2002) Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. *Journal of Fish Biology* 61:1465-1480.
- Correia MJ, Palma, Koldewey HJ, Andrade JP (2013) Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (*Hippocampus guttulatus* Cuvier)? *Journal of Experimental Marine Biology and Ecology* 448:258-264.
- DuFour M.R, Christine MM, Song SQ, Christopher SV, Richard TK, Patrick MK, David MW (2018) Inferred fish behavior its implications for hydro-acoustic surveys in nearshore habitats. *Fisheries Research* 199:63-75.
- Frouzova J, Kubecka J, Balk H, Frouz J (2005) Target strength of some European fish species and its dependence on fish body parameters. *Fisheries Research* 75:86-96.
- Foster SJ, Wiswedel S, Vincent ACJ (2014) Opportunities and challenges for analysis of wildlife trade using CITES data - seahorses as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems*.
- Foster SJ, Vincent ACJ (2004) Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* 65:1-61.
- Greenstreet SPR, Holland GJ, Guirey EJ, Armstrong E, Fraser HM, Gibb IM (2010) Combining hydro-acoustic seabed survey and grab sampling techniques to assess local sandeel population abundance. *ICES Journal of Marine Science* 67:971-984.
- Horne JK (2003) The influence of ontogeny, physiology and behaviour on the target strength of walleye pollock (*Theragra chalcogramma*). *ICES Journal of Marine Science* 60:1063-1074.
- IUCN Red List of Threatened Species. Version 2014.3. Available: <[www.iucnredlist.org](http://www.iucnredlist.org)>.
- Jones AG (2004) Male pregnancy and the formation of seahorse species. *Biologist* 51:4.
- Kawaguchi M, Okubo R, Harada A, Miyasaka K, Takada K, Hiroi J, Yasumasu S (2017) Morphology of brood pouch formation in the pot-bellied seahorse *Hippocampus abdominalis*. *Zoological letters* 3:19.
- Kusumaningrum W (2013) Analisis target strength Udang Galah (*Macrobrachium rosenbergii* De Man) menggunakan akustik *split beam* di Delta Mahakam. *Thesis*, Sekolah Pascasarjana IPB, Bogor.
- Laksanawimol P, Damrongphol P, Kruatrachue M (2006) Alteration of the brood pouch morphology during gestation of male seahorses, *Hippocampus kuda*. *Marine and Freshwater Research* 57:497-502.

- Manik HM, Apdillah D (2017) Remote Sensing of Seagrass and Seabed using Acoustic Technology in Bintan Seawater, Indonesia.
- Manik HM, Apdillah D, Dwinovantyo A, Solikin S (2017) Development of Quantitative Single Beam Echo-sounder for Measuring Fish Backscattering. Chapter 6 from the book *Advances in Underwater Acoustics*. Published by INTECH.
- Manik HM, Furusawa M, Amakasu K (2006) Quantifying Sea Bottom Surface Backscattering Strength and Identifying Bottom Fish Habitat by Quantitative Echo Sounder. *Japanese Journal of Applied Physics* 45:4865–4867.
- Medwin H, dan Clay CS (1998) *Applied Ocean Acoustics: Fundamentals of Acoustical Oceanography*. Academic Press, New York. 712 pp.
- Ona E (1990) Physiological factors causing natural variations in acoustic target strength of fish. *Journal of the Marine Science Biological Association of the UK* 70:107-127.
- Poortenaar CW, Woods CMC, James PJ, Giambartolomei FM, Lokman PM (2004) Reproductive biology of female big-bellied seahorses. *Journal of Fish Biology* 64:717-725.
- Project Seahorse (2014) Available: <http://seahorse.fisheries.ubc.ca>.
- Simmonds EJ, MacLennan DN (2005) *Fisheries Acoustic Theory and Practice*. Second Edition. Oxford: Blackwell Science Ltd.
- Simrad (2012) Installation manual, Simrad EK15 Multi purpose scientific echo-sounder, Kongsberg Maritime AS.
- Stölting KN, Wilson AB (2007) Male pregnancy in seahorses and pipefish: beyond the mammalian model. *Bioessays* 29:884-896.
- Vecchione A (2013) Prospects challenges in monitoring the seahorse population of South Carolina, USA. *Journal of Marine Animal and Their Ecology* 6:6-11.
- Vincent ACJ, Berglund A, Ahnesjö I (1995) Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* 44:347–61
- Vincent ACJ (1996) *The International Trade in Seahorses*. TRAFFIC International, Cambridge, UK.
- Vincent ACJ, Foster SJ, Koldewey HJ (2011) Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology* 78:1681-1724.
- Wilson MJ, Vincent ACJ (1998) Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aquatic Science Conservation* 2:179-196.
- Woods CMC (2005) Reproductive output of male seahorses, *Hippocampus abdominalis*, from Wellington Harbour, New Zealand: Implications for conservation, *New Zealand Journal of Marine and Freshwater Research* 39:881-888.
- Yip MY, Lim ACO, Chong VC, Lawson JM, Foster SJ (2014) Food and feeding habits of the seahorses *Hippocampus spinosissimus* and *Hippocampus trimaculatus* (Malaysia). *Journal of the Marine Biological Association of the United Kingdom* 1-8.
- Zachary CZP, Ferber NG, Maudment (2013) Summary of Seahorse Population and Distribution Koh Rong Samloem Preah Sihanouk, Cambodia. Report on seahorse demographics and habitats, *Marine Conservation Cambodia 1st Quarter Report 2013 – Jan/Feb/Mar*.