Characterization of *Hippocampus kuda* (Bleeker, 1852) - yellow seahorse feeding click sound signal in a laboratory environment: An application of probability density function and power spectral density analyses

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Abstract

Do the sounds generated by different-sized fish of different sex differ from each other in temporal, spectral or intensity patterns? As a consequence this study aims at developing a passive acoustic technique to locate seahorses in open water-bodies. Based on the similar perspective, characterization of Hippocampus kuda (Syngnathidae) yellow seahorse feeding click sounds recorded in a controlled laboratory environment has been presented in this work. The characterization involve analysis of the 'probability density function' (PDF), and the 'power spectral density' (PSD) of the seahorse feeding click sound signals from different sizes and sex. The PDF describes general distribution of the magnitude of a random process, and such analysis, employing recorded seahorse clicks point towards the multimodal statistical distribution i.e. the existence of more than one fitting components for majority seahorse click signals over the tank ambient noise. This fact has been appraised towards the involvement of more than one process in the seahorse click signal generation mechanism. However, lack of prior knowledge about the individual PDF components of the data lead towards the mismatch between the data PDFs. The PDF gives no information on the time and frequency content of the processes as provided by the PSD. Under such conditions, curve fitting of the 'power law' expressions to the PSD functions estimates 'slope' and 'intercept' parameters of the seahorse click signal. Striking differences in the feeding click characteristics of various sizes of the H. kuda and the sex were discernable based on the clustering pattern among the estimated 'slope' and 'intercept' parameters. Further studies using the seahorse body sizes and weight (wet) with respect to the peak frequencies estimated from the PSD distribution show equivalent results in comparison with the previous findings.

Keywords: yellow seahorse (*H kuda*); feeding click signal characterization; multimodal process; power law; laboratory condition; seahorse size and sex

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Introduction

Seahorses occupy both temperate and coastal waters covering wide geographical distribution. They may usually be found among corals, macro algae, mangrove roots and sea-grasses, but some live on open sandy or muddy bottoms (Lourie et al. 1999). *Hippocampus kuda* (Bleeker, 1852) is an endangered, carnivorous marine fish and feeds on small invertebrates such as mysids. The seahorse plays an important role in maintaining an ecological balance in water (Vincent et al. 2011). Seahorses generate sound signals similar to snapping of fingers in a variety of situations e.g. during feeding, courtship, copulation, exploring new territory and inter-male competitions (Fish 1953; Anderson 2009). In order to locate the seahorse in world wide open waters, visual techniques were used (Curtis and Vincent 2005), and remote techniques (McGregor 2012) have to be developed. Therefore, laboratory based seahorse click sound signal recording and analysis is an important initiation towards the development of passive acoustics technique (Luczkovich et al. 2008) to locate seahorses in open water-bodies.

Characterization of the sound signals produced by marine animal and fish are ongoing investigations that were initiated long back (Fish and Mowbrey 1970; Greene 1998) and are continuing till date with great interest (Towsey et al. 2012). Moreover, relating such sound signals with respect to the skeletal and muscular details of the animals is an important study subject (Au and Banks 1998). At the time of feeding, seahorses are capable of generating sound signals and capture their prey by simultaneous elevation of the head [stridulatory mechanism: generated due to the rapid depression of the hyoid bones from its rest position during the prey-capture attempt (Colson et al. 1998)], and buccal cavity expansions (sound is caused by cavitations inside the buccal cavity, because many fish species cavitate water during their forceful prey capture attempt). Therefore, there are a few possibilities to distinguish the different methods of generating sound signals (Bergert and Weinwright 1997).

Statistical techniques such as the 'probability density function' (PDF) as well as the 'power spectral density' (PSD) functions are useful tools to characterize the recorded seahorse click sound signal. PDF describes the relative likelihood occurrence of a random variable at a given point. The PDF of a data showing heavy or long tailed e.g. un-symmetric or skewed distributions supports the fact that the distributions are combinations of non-normal and / or Gaussian (Rachev et al. 2005). Sometimes mixtures of two or more normal / Gaussian distributions show complex distributions indicating quantitative variability in the data i.e. identifying outliers or subpopulations (Pearson et al. 1992). An attempt to employ curve fitting technique assuming multimodal distributions and their resultant to the

data signal PDF provides an idea about the involvement of a number of processes. Though mixture models are extensively used (Roch et al. 2007), lack of prior knowledge about the individual distribution of the mixtures e.g. normal or any other PDF can lead towards the mismatch between the data PDFs. Moreover, the existence of the mixture of many non-normal and / or Gaussian stable distributions is often referred as 'stable paretian distributions' (Newman 2005). This is due to the tails of the non-Gaussian stable distribution (Pareto or power type decay). Moreover, PDF gives no information on the time and frequency content of the processes as provided by the PSD. Power law distribution or function characterizes an important behavior from nature and human endeavor, especially when long / heavy tailed distributions are dominant (Levy and Solomon 1997). Under this condition, PSD functions analyses with respect to the frequencies and curve fitting using power law expressions (Chakraborty et al. 2003) to estimate 'slope' and 'intercept' parameters of the seahorse click sounds are of significance along with the PDF based studies.

In this communication, the click signals of yellow seahorse - *Hippocampus kuda* (Bleeker, 1852) belonging to the *Syngnathid*ae family of fish, were recorded in a laboratory environment. The seahorses were acquired from the Miriya and Shirgam creeks of the western continental margin of India off Ratnagiri district in the state of Maharashtra (Pawar et al. 2011). The primary aim of the present characterization of the yellow seahorse - *H. kuda* feeding click sound signal statistics (PDF and PSD) in a laboratory environment has linkages with the establishment of a passive sensing technique. Moreover, this investigation would be of significant help to locate the seahorse in open water-bodies including their identification in terms of sizes and sex. Besides, feeding seahorse click sound analysis needs many other interrelated study aspects e.g. experimental tank environment (Anderson et al. 2011), and sound generating mechanisms (stridulatory and / or cavitations). Furthermore, correlation studies involving the seahorse body sizes and weight (wet) in relation to the peak frequencies derived from the PSD distribution offer comparative results with respect to the previous study (Colson et al. 1998).

Materials and methods

Study animals

The feeding click sound signals of the oceanic yellow seahorse, *H. kuda (Syngnathidae)* were recorded in the present study. The work was carried out at the seahorse hatchery adjoining the aquaculture laboratory complex of the National Institute of Oceanography, Goa (India), where the techniques for standardization of captive breeding, rearing and culture of Indian seahorse species are being established

(Pawar et al. 2011). New born juveniles (pelagic phase) released from a single Hippocampus kuda spawner belonging to the F2 generation were reared in rectangular light blue background fiberglass reinforced plastic (FRP) tanks (capacity, 100 L) at a juvenile density of 2 fish L⁻¹ until they attained settlement phase. Thereafter, juveniles were reared in large FRP tanks (capacity, 500 L) secured with different types and sizes of holdfasts depending upon the growth of juveniles. Various husbandry practices as described in Pawar et al. (2011) were followed. The seawater used was treated by rapid sand filtration, bio-filtration and then passed through ultraviolet radiation. Adequate aeration was provided to the FRP tanks using air blowers and a photoperiod of 12 h L (0700- 1900h) and 12 h D (1900-0700 h) was maintained using a fluorescent bulb (100WPhilips build) providing a light intensity ~800 lx at the water surface. The tanks were cleaned daily and important water quality parameters were measured twice a week. The measured physico-chemical parameters of seawater in the rearing tanks fell within the optimum levels recommended for the culture of seahorses: temperature (28.5°C), salinity (31 ppt), dissolved oxygen (6.1 mgL⁻¹), pH (7.9) and other parameters as well. Healthy adults measuring (12-18 cm) length and weighing, (06-26) g wet weight were selected for present study. Single adult seahorse (H. kuda) was netted from the rearing tanks and transferred to another glass tank. The tank used for present investigation possesses the length (L_X) , width (L_Y) , and height (L_Z) of 90, 44, and 44 cm respectively, and the thickness of the glass tank was measured around 0.7 cm. The tank was filled with fresh, aerated seawater prior to feeding click sound recordings. During the click sound recording, the tank water temperature, dissolved oxygen and pH values were measured to be 29°C, 6.1mg L⁻¹ and 7.9 respectively. In the present study, we have recorded feeding click sound signals from three male and two female seahorses. A total of twenty-three seahorse feeding click sounds were recorded. These include two, eight and five clicks from three male seahorses of lengths 12cm, 16cm, and 18cm respectively and three and five click signals from two female seahorses of lengths 13cm and 12cm respectively.

Sound recording

In this experiment a hydrophone (C55 M/s Cetacean Research Technology, Seattle, having 20 dB preamplifier gain and receiving sensitivity of -185 dB re $1V/\mu$ Pa) was submerged in a glass tank at a depth of 30 cm from the surface. The distance between the animal and hydrophone was maintained around 60 cm. Generally, seahorses were fed thrice a day with different live prey organisms such as copepodites, Artemia nauplii and mysids (Mesopodopsis orientalis). The initial observations were recorded from the water filled tank before feeding the seahorse, i.e. recording of the ambient tank noise without seahorse clicks. The seahorse feed (mysids) was placed in the tank, and the click signals were recorded during the 14:00 hr. To decrease mechanical, electrical and background noise, the tank was placed over 40 mm thick flexible foam and pump and other electrical accessories were switched off. These conditions were maintained throughout the experiment. In order to confirm the presence of seahorse clicks in a tank i.e. in the presence of tank ambient noise and resonance frequency, spectrogram (Figure 1) of the seahorse click signals were utilized. Four distinct set of click signals from 16cm male seahorse were presented along with the corresponding spectrograms. Oscillograms (20s length) of these four clicks have been shown as representative samples (Figure 2).

The hydrophone was connected to the analog to digital (A/D) converter card (Advantech PCI-1712L) installed in a personal computer (PC). The analog data from the hydrophone was digitized through the card i.e. a 12 bit converter with 16 single-ended or 8 differential channels. The digital data was transferred to the PC via a parallel port interface using the MATLAB software at a sampling frequency of 10000 Hz (Saran et al. 2011). In order to perform the experiment, the glass tank was filled with sea water up to a depth of 36 cm. The expression to compute tank resonance frequency using dimensional aspects of the tank is given as (Akamatsu et al. 2002):

$$f = (c/2) \times \sqrt{\left[(l/L_X)^2 + (m/L_Y)^2 + (n/L_Z)^2 \right]}$$
(1)

where *c* is the sound velocity in seawater depending on the water temperature and salinity. The terms *l*, *m*, *n* represents integer numbers and designated as the 'mode number'. The mode numbers were chosen to be 'unity' for the present calculation. The tank resonance frequency (*f*) was computed to be ~ 3000 Hz using equation (1). The tank resonance signal based on the above expression has been indicated in the representative spectrogram (Figure 1b). In this experiment, the tank resonance signal was found to be negligible as compared to the four seahorse click sound signals (Akamatsu et al. 2002). Moreover, the sound generated from a fish can undergo multiple reflections which can create standing waves, and the tank would be in a resonance mode where the presence of several resonance frequencies is possible (Au and Hastings, 2008). However, in this experiment such modes are unlikely due to the chosen separation between the seahorse - hydrophone (60cm), and water depth (36 cm) (except the background noise observed within 0-500 Hz).

Probability Density Functions (PDF) of the click data

In this section, the statistical parameters related to the probability density functions (PDF) of the seahorse feeding click signal amplitudes were studied. The estimation of the central tendency in the

statistical study such as 'mean', 'median' and 'mode' are usually common. Occasionally the PDF's are not always normally distributed and they may be skewed or heavy-tailed (Figure 3c). The existence of such statistical outliers is also observed in the presently recorded seahorse click signals. The Gaussian mixture model is expressed as (Pearson et al. 1992):

$$F(X) = \sum_{n=1}^{n} a_n \times \exp[-\{(X - b_n)/c_n\}^2]$$
(2)

where a_n , b_n and c_n , are the estimated scaling amplitude (to scale the height of the curve), mean, and standard deviations of the components of the PDF respectively. These statistical parameters were estimated as fitting parameters to the experimental PDFs of the sampled amplitude related to the seahorse feeding clicks. Curve fitting involves estimating minimum of the sum of the squares due to an error (SSE) i.e. summing up the square of the residuals between the predictive (mixture of the estimated normal distributions) and click signal PDF (Figure 3). Moreover, correlation coefficients of the error between the predictive and seahorse click data PDF was also determined. There were seven click signals, where the correlation coefficients of the error data were much less than the 0.99 along with the higher values of SSE, indicating a poor match between the PDFs (Figure 4). The arrow mark in Figure 4 depict the representative 'click 3' shown in Figure 3c. In Figures 3 a, b and d, the PDFs of the representative clicks (shown in Figure 2 a, b and d) are well fitted, having higher correlation coefficients (~0.99) and lower SSE. Though a maximum four components have been used to fit the PDFs, the mean and variance values associated with three components of the representative PDFs have been presented in Figure 5.

Power law parameter estimation

Power law (Newman 2005) usually describes systems where the larger events are rarer than the smaller, which ensures that the power law is a monotonically decreasing function. The central limit theorem states that the sum of random variables with finite mean and variance will always converge to a Gaussian distribution. The 'mean' and 'variance' are the parameters to indicate how much individuals differ. Interestingly, if relaxation of the constraint i.e. the variance and / or mean be finite (allowing arbitrarily larger steps and / or don't require a characteristic size) then the central limit theorem does not predict Gaussian distributions. Instead, it predicts a variety of sum-stable distributions akin to power law. Gaussian distributions drop off quickly (large events are extremely rare), but power law distributions drop off more slowly. Accordingly the power law is applicable for heavy-tailed

distributions (Levy & Solmom 1997).

The PDF of the seahorse click signals generally show heavy or long-tailed distributions, and curve fitting techniques employed in previous section show success for only sixteen feeding click signals out of twenty-three. Therefore, the PSD of the click signals was computed employing 'power law' fittings. The PSD function describes the average frequency content of a random process. Hence the PSD function was obtained using a smoothed periodogram approach. The input profile was de-trended to remove the best-fit linear trend of the click signal (Chakraborty et al. 2006). Then, the first and last 10% of each profile was tapered with a cos^2 function to minimize the edge effect or leakage. A Fast Fourier Transform (FFT) algorithm was subsequently applied, and the resulting complex spectrum was squared to achieve a sample power spectrum periodogram. The results were plotted into $log_{10}-log_{10}$ plots of power (V²/Hz) versus frequency (Hz) (Figure 6a-d). The power law equation is given by:

$$\log_{10} P_H = (-b)\log_{10} f + \log_{10} a_0 \tag{3}.$$

Comparing equation 3 with a straight-line equation, b (arbitrary units) corresponds to the slope of a straight line fitted to the PSD and a_0 (in Volt) to the intercept of the click signal.

Results

PDF study results

Study results presented this section provide estimated statistical parameters employing fitted PDFs (Figure 3) of the seahorse click signals. The scatter diagram between the correlation coefficients and SSE, and mean and variances associated with the three components of the matching PDFs are presented in Figure 4 and 5 respectively. Excluding the seven sets of click signals, the remaining sixteen clicks show good fit between the predicted and data PDFs. In general, the estimated curve fit parameters of the unimodal distribution show imperfect matching in four clicks out of seven datasets. A representative distribution exhibiting unimodal distribution is presented in Figure 3c. The arrow mark depicted in Figure 5a presents the mean (1.66) and variance (0.04 V) values associated with the distribution. Moreover, relatively low correlation coefficient (0.95) and high SSE (677 V) observed in Figure 4 supports the fact that, the data having unimodal Gaussian distribution show poor curve matching.

The estimated curve fit parameters of individual components provide component wise mean and variances (Figure 5). The mean and variances of the 16 cm male seahorse show the highest values

followed by 18 cm male, 13 and 12 cm male and females. The PDFs related to multiple curve fitting based on the assumption of combinations of normal / Gaussian distributions indicate well fitted multimodal curves generated using MATLAB (Math Works Inc 2005) programs. Out of the twenty three clicks, four clicks (two clicks of 16 and 18cm male, and two clicks of female seahorses having 12 and 13cm) show unimodal distribution. Remaining nineteen clicks show more than one component i.e. two to four components of scaling, mean and standard deviation values. The correlation coefficients used to fit the curves vary within the 0.90 to 0.99 values (for twenty-two clicks), which also include 0.5 for a single click from a 13 cm length female seahorse (Figure 4). Out of twenty three clicks, twelve clicks provide 2-components of the fitting parameters, five clicks have 3 and two clicks have 4-components of fitting parameters (the fourth component is not presented). The multimodal parameters are acceptable as the curve fitted error parameters show 99% correlation for sixteen clicks. The fitted curves of remaining seven observations indicate poor fit, wherein four clicks were unimodal.

Clustering of spectral data of the seahorse clicks

Figure 6 depicts the representative Power Spectral Density (PSD) of the four feeding click signals (of 16 cm male seahorse as shown in Figure 2), from which the slope (0.18-1.00) and intercept (-9.94 - -0.76)values were estimated. The interpretation of the slope (b) is somewhat less intuitive (Malamud and Turcotte 1999). When the slope value is equal to zero, the PSD function is independent of the frequency i.e. 'white' noise, and values of the time series data are not correlated with each other. When the slope values are greater than zero (> 0), the PSD functions are dominant at higher frequencies i.e. values of the time series data are positively correlated with each other and are known to be 'persistent'. Conversely, negative values of the slope (< 0) imply dominant PSD at lower frequencies (longer wavelengths) indicating a negative correlation among the time series values, and is referred to as 'anti-persistence'. It has been observed that significant difference in the PSD between the feeding clicks and background noise is observed beyond 1000 Hz and continues up to 4800 Hz (Figure 6). Interestingly, the frequency ranges of measurements made in this work is in corroboration with the findings of previously reported investigations (Fish 1953; Colson et. al., 1998). Therefore, the slopes and intercepts values of feeding signals were estimated by fitting the corresponding PSD values within the frequency range of 1000-4800 Hz, the peak observed around 3000 Hz is due to the 'tank resonance'. Whereas the distribution of PSD function within the frequency ranges of 17.8-1000 Hz is identical in both the cases, representing seahorse feeding signals and background noises respectively (Figure 6). Therefore we denote the peaks within these frequency ranges as the 'ambient noise' present in the tank as it consistently persists in both

the spectrums. The estimated slope and intercept parameters in the presence of twenty-three seahorse clicks and without seahorse clicks give variable straight line error fitting within the range (0.29 to 0.14) and (0.32 to 0.26) respectively.

The clustering patterns among the parameters related to the presence and absence of the seahorse feeding clicks are shown in the scatter plot (Figure 7). In the absence of seahorse clicks within the tank, the slope and intercept parameters are varying within the range (-2.40 to -1.38) and (-4.50 to -1.00) respectively. A clear-cut clustering is observed among the 'higher' slope (0 to 1) and 'lower' intercept (-10 to -7.6) values of 16 cm male seahorses (except for one data point), comprising of seven clicks out of eight signals (including overlapping values). The parameters related to the clicks of eight female seahorses of 12-13 cm lengths show comparatively lower slope and moderate intercept values. The slope and intercept parameters of the remaining five and two clicks of 18 cm and 12 cm length male seahorses also fall within the cluster of female seahorses. Existence of the separate cluster for 16 cm male seahorses.

Relationship between the seahorse body size, weight and sex with the peak frequency

The peak frequencies, derived from the fitted regions of the PSD distributions of the twenty three seahorse feeding click signal (fifteen male and eight female), were utilized to establish a relationship with the seahorse body size, and weight (wet) (Colson et al. 1998) (Figure 8). The peaks generated due to the background noise within the frequency range of 17.8-1000 Hz were excluded from the present analyses (Anderson et al. 2011). As indicated earlier, peak frequencies of the PSD (Figure 6) within the 1000-4800 Hz frequency range signifying the presence of *H. kuda* seahorse clicks were determined. In general, the estimated peaks were found to be varying between the 1000 and 4300 Hz (Figure 8).

The peaks of the male *H. kuda* with a length of 16 cm and 18 cm male seahorses are covering a broader frequency range than that of female seahorses of smaller body sizes (12-13 cm) (Figure 8a). The frequency ranges covered by female seahorses are generally low (except for a single click of female seahorse having 12cm length showing highest frequency at 4300 Hz). Similarly, the smaller size (12cm) male seahorses show peak towards the low frequency end than the larger sizes. The body weights (wet) of male seahorses were found to be greater than the female, and as the size increases the wet weight also increases (Figure 8). The correlation coefficients between the peak frequencies with respect to the body sizes and weights (wet) of the seahorses were found to be 0.172 and 0.062 respectively. The

observations made in the present study show similarity with results from the work carried out for stridulation sounds of many other fish (Mann & Jarvis 2004).

Discussion

The present study objectives intend towards the identification of the seahorses prevalent in the open seas using click sound signals. Therefore, statistical techniques (PDF and PSD) were employed to characterize the recorded seahorse click sound signal. The presented PDF analyses of the feeding click sound signals reveal multimodal PDFs. Existence of more than one component indicate involvement of the multiple processes during the seahorse feeding strike kinematics prey capture. Colson et al. (1988) had made extensive study using video and audio techniques to understand the mechanism. However, PDF study does not provide click signals relationship with the seahorse size, sex or click sound signal generation mechanisms. Here, the estimated parameters have limited reliability using least square error fit or correlation estimates between the data and predicted PDFs for seven clicks out of twenty-three clicks. The multimodal parameters are acceptable for the cases where curve fitted error parameters show 99% correlation and low SSE. Therefore, PSD was computed for click signals to employ 'power law' towards the estimation of the 'slope' and 'intercept' parameter of the fitted straight lines, and it explains the average frequency content of the random process. The cluster diagram presented between the estimated slope and intercept values were found to be a useful tool to characterize the seahorse feeding click signal. The low slopes (<< 0) and high intercepts values indicate negative correlation in the recorded time series data i.e. anti-persistent condition prevalent to the situation within the tank in the absence of seahorse clicks. The spectral based study (PSD) also establishes that, the body organs which control the biomechanical aspect of the sound signal generation, show higher activities (higher slope and lower intercept) for 16 cm male seahorses than the remaining organisms.

The peak frequencies (within 1000 - 4300 Hz), derived from the fitted regions of the PSD distributions of the seahorse feeding click signal, were utilized to establish a relationship with the seahorse body size, weight (wet) and sex. The correlation coefficients of the peak frequency versus body size and weight (wet) of the seahorses were 0.172 and 0.062 respectively, which are in agreement with the findings of Colson et al. (1998) where the values observed were 0.245 and 0.006 respectively. The smaller size (12cm) male seahorses show peak towards the low frequency end than the larger sizes. Even though the peak frequency of male (or seahorse of longer lengths) ranges from low to high, the present study reveals that, the longer the body length the lower the peak frequency. This study also

emphasizes the importance of the spectral technique employed to the seahorse click signal, and establishes the importance of the spectral parameters i.e. slope-intercept estimation based analyses to identify the size and sex of the *H. kuda* in the field condition. The likelihood of identification would be enhanced significantly based on the present characterizations using spectral techniques than the commonly used parameters e.g. duration of the temporal signal and intensity patterns.

Recent auditory sensitivity studies of H. erectus (Perry) seahorses in terms of sound pressure and particle acceleration establish that their hearing abilities were within 1000 Hz (Anderson and Mann, 2011). However, absence of similar information in the present study of H. kuda seahorses constrains appropriate assessment in this regard. The frequency range (1000- 4800 Hz) utilized in this study is comparable to the observations reported by Colson et al. (1980), wherein the H. zosterae (2.65 - 3.43) kHz) and H. erectus (1.96 - 2.35 kHz) seahorse click sounds were characterized. Similarly, Fish (1953) had also reported broadband click signals ranging from (0 - 4.8 kHz) for *H. hudsonius*. Anderson (2009) had analyzed the hearing ability and clicks sound frequency ranges of seahorses in detail, articulating further investigations. Moreover, seahorses producing louder clicks may generate standing waves within the tank that may introduce resonance peaks other than that of the original click frequencies. However, hearing generalist fish (e.g. H. erectus) can hear conspecific sound signal out of the various resonance components generated due to the distortion of the original click signal. In the present study, the tank related (function of the tank dimensions) resonance peaks (Akamatsu et al. 2002) were computed and found to be ~ 3000 Hz (Figure 1b). Hence, hearing ability test of the H. kuda seahorse is an important aspect to be investigated along with further recordings of the seahorse click signal under different conditions, particularly in tanks of different dimensions.

Conclusion

In this paper the recordings of the feeding click sound produced by the yellow seahorse - *H. kuda* (Bleeker, 1852) were carried out in a controlled laboratory environment. Presentation of the spectrograms of the representative click signal helps in identifying seahorse click timing and frequency bandwidth. The statistical analyses employing recorded seahorse clicks i.e. PDFs, indicate multimodal statistical parameters. The existence of more than one component of the fitted normal / Gaussian PDFs for 16 click signals (out of 23) over tank ambient noise (without click signal) denote involvement of more than one process in the click signal generation mechanism. The feeding clicks were further analyzed employing power law fitted PSD (frequency: 1000-4800 Hz) curves, from which the slope and

intercept parameters for twenty-three seahorse clicks were estimated. The scatter diagram of the estimated slope and intercept data reveal three major clustering patterns designating male, female and signals recorded without the seahorse clicks. The cluster of 16 cm male seahorses was found to be distinctly separated from the cluster of 18, and 12 cm males and 12 and 13 cm female seahorses. The separate clustering of 16 cm male seahorses signifies the existence of high frequency fluctuations in their clicks. Hence, the bio-mechanic involvement of the body organs in sound signal generation indicates greater activities of 16 cm male seahorses as compared to the remaining ones. Though, the power law fit to the PSD curves supports the multimodal related aspects covered in the PDF analyses, however, the clear-cut indication of the background noise interference in the multimodal PDF needs detailed study. The seahorse click signals parameters (slope and intercept) were observed to be dominant within the 1000-4800 Hz, whereas part of the PSD having frequencies less than 1000 Hz indicates presence of ambient noise related to the tank. In general two separate processes corresponding to the tank and the seahorses were present in the PSD curves of the 23 seahorse clicks. The estimated correlation coefficients between frequencies (at peak level of the PSD curves) versus seahorse size and weights were well corroborated with the earlier reported results (Colson et al. 1998), which indirectly validates the present study. The PSD related study results would potentially lead to locate and identify the seahorses prevalent in the open sea. In order to relate these parameters with biomechanical aspect of a feeding seahorse further studies are necessary.

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Figure Captions:

Figure 1: Feeding click signals of 16 cm male seahorse are shown as a generic example: (a) oscillogram of four clicks with background noise (b) spectrogram of the above signal showing four clicks (1-4), tank resonance signal (3000 Hz) and background noise (< 500 Hz).

Figure 2: Panels a-d represents enlarged oscillograms of the four representative click signals (shown in Figure 1 as 1-4) produced by 16 cm male seahorse.

Figure 3: Panels a-d represents Probability Density Functions (PDFs) and its fitted multi-modal curves of the four representative click signals produced by 16 cm male seahorse. Note that two components are associated with panel (a) and (d), whereas panel (b) and (c) shows three and one components respectively. The total (with fat-tail) and Gaussian multi-modal curves are fitted well to the distribution of PDFs in all cases except for panel (c) wherein the distribution is uni-modal.

Figure 4: Scatter plot showing the values of error (SSE) and corresponding correlation coefficients associated with the data and fitted multi-modal PDFs curves of twenty-three click signals. The arrow mark depicts the click shown in Figure 3c.

Figure 5: Panels a-c represents component wise values of estimated mean (V) and corresponding variance (V) associated with the data and fitted multi-modal PDFs curves of twenty-three click signals. The arrow mark in panel (a) depicts the click shown in Figure 3c.

Figure 6: Power Spectral Density (PSD) of the four feeding click signals and background noise (shown in Figure 1). The slopes and intercepts of feeding signals and background noises were estimated by fitting the corresponding PSD values within the frequency range of 1000-4800 Hz.

Figure 7: Result showing prominent clustering patterns among the slope and intercepts (estimated from PSD curves as shown in Figure 6) of twenty-three click signals.

Figure 8: Panels (a) and (b) represents variation of peak frequency (determined from the PSD curves within the frequency range of 1000-4800 Hz) with regard to the seahorse body size and weight respectively.



Figure 1





Figure 4



Figure 5



Figure 6



Figure 7



Figure 8