Article

Fractal analysis of *Hippocampus* spp. (seahorse) from Surigao City, Philippines

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Abstract
Assessment of species can be promising for ecosystem and environment sustainability. The demand for trading and environmental impact becomes the culprit to decline in seahorse population. Hence, this paved way for the emergence of plausible non-destructive method and tools for monitoring to measure risks and vulnerability. The area near Surigao city, had been popular due to mining practices and also a famous route for seahorse trade in Mindanao. The aim of this study is to use fractal geometry to look into morphometric complexity patterns of different species of seahorses (*Hippocampus* spp.) in Surigao City. Irregular non-Euclidean objects are better described by fractal geometry and the measurable value is called the fractal dimension. Box-counting and lacunarity method were performed with FracLac v.2.5, and available as a plugin to ImageJ. The method was done to test whether significant differences in fractal dimension and lacunarity values can be species-specific and provide evidence of vulnerability. It is hypothesized that fractals are far from the equilibrium state and thus associated with chaos. Thus, high fractal dimensions have implications to vulnerability of species. High fractal dimension based on box-counting and lacunarity method was associated with *H. comes.* The results for fractal dimensions (D_B) were in accordance with the coefficient of variation (CV) values. Information obtained aide in understanding the nature of species in Surigao city.

Keywords fractal analysis; *Hippocampus*; seahorse; Surigao city; box-counting method; lacunarity.

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1 Introduction

In the core of changing environment, it is pertinent that one look into vulnerable and resilient species that can be recommended for monitoring and protection. Assessment of these species can be promising for ecosystem and environment sustainability. The marine environment posed a tremendous challenge as surface waters are contaminated due to anthropogenic activities. The estuaries, coral reefs, mangrove ecosystem and deep seawaters are threatened and species such as seahorses that become flagship species of the marine environment and had gained popularity in Traditional Chinese Medicine (TCM) were greatly affected. The demand for trading and environmental impact becomes the culprit to decline in population. Hence, this paved way for the emergence of plausible non-destructive method and tools to measure risks and vulnerability. In the past, tools in imaging and analysis have been used in search for patterns in nature. A proposed method for Irregular non-Euclidean objects is better described by fractal geometry and the measurable value is called the fractal dimension. Investigators use different types of fractal analysis to study a myriad of intractable phenomena including the complex geometries of many types of biological cells (Kam et al., 2009) and complex patterns such as river paths, tree growth, tumor growth (Cross, 1997), heart rates (Huikiri and Stein, 2012), diabetic retinopathy (Karperien et al., 2008), gene expression (Aldrich et al., 2010), forest fire progression (Turcotte et al., 2002), economic trends, and cellular differentiation in space and time (Waliszewski and Konarski, 2002). Thus, pave the way of the importance of fractals in biological forms and analysis.

Meanwhile, seahorses have attracted attention because of their unique form and shape. It is hypothesized that the seahorse’s S-curved body was a recent evolutionary innovation and that body shape evolve in respond to habitat and function (Wassenbergh et al., 2011). Based on studies, different species are said to be associated with different microhabitats. Accordingly, the utilization of shape descriptors can be used as overall indicators of macro states of biological entities. This study highlights morphological complexity specifically for seahorses.

In this respect, spatial fractals refer to the presence of self-similarity manifested to various enlargements, for instance, the small intestine repeats its form on different scales (Havlin et al*.*, 1995). The forms revealed by repeated enlargement show no loss of details as well as revealing self-similar forms. In like manner, in seahorses the presence of repeated segments of trunk rings and tail rings impose fractal properties. Thus, it is of interest, knowing that seahorse populations in the wild are less studied. In this study, the method test whether significant differences in fractal dimension values based on morphological complexity can be species-specific and provide evidence of vulnerability. It is further hypothesized that fractals are far from the equilibrium state and thus associated with chaos (Klein et al., 2013). Thereby, high fractal dimensions may have implications to vulnerability which aide in understanding the nature of species in Surigao city. The city of Surigao is nested along the northernmost tip of the Province of Surigao del Norte where mountains and hills are prominent indentations throughout the coasts. It is apparently bounded on the north and east by the Hinatuan Passage and on the west by the Surigao Strait and on the south by Tagana-an. The area near Surigao city had been popular due to mining practices and also a famous route for seahorse trade in Mindanao.

Moreover, this serve as an exploratory study to objectively describe morphometric complexity of selected common seahorse *Hippocampus* species: *H. comes*, *H. barbouri* and *H. kuda* from Surigao City through fractal analysis via Box-counting and Lacunarity method respectively. Obtained fractal dimensions can be used as a quantitative marker of morphological shape complexity with respect to changes in biological complexity per species and associated microhabitat. Results of this study contribute to understanding the dynamics and nature of seahorse populations.

2 Materials and Methods

2.1 Sampling area, specimen collection and identification

The sampling area was in Surigao City. The city of Surigao is nested along the northernmost tip of the Province of Surigao del Norte where mountains and hills are prominent indentations throughout the coasts (Fig. 1). It is apparently bounded on the north and east by the Hinatuan Passage and on the west by the Surigao Strait and on the south by Tagana-an. It is worthwhile to say that more than two dozen islands and islets make up two fifths of the city’s total land area. The islands were separated from the mainland by the Hinatuan

Passage, which connects Mindanao Sea to the Pacific Ocean. The largest island of Nonoc recognized by a rusty-red weathering mantle of lateritic nickel ore, which is visible from any vantage point on the mainland coast. The other notable islands were of Hanigad, Sibale, Bayaganan and Awasan. For this study, opportunistic sampling was employed. Adult seahorse specimens were in courtesy of fishermen from Hanigad Island where, specimens were bycatch samples in the area and photos were taken based on standard procedures. Identification of samples was done through illustrated keys, Guide to the identification of Seahorses (Lourie et al., 2004) and consultation of experts. Photographs were taken for all the samples then processed for image analysis. Microhabitats associated with species of seahorses were noted based on underwater surveys.

Fig. 1 Study area: Map of Hanigad, Island, Surigao City.

2.2 Image acquisition, processing, shape analysis and statistical analysis

Images of seahorses from Surigao city were acquired using Canon DSLR 550D. All images were then processed in triplicates to minimize source of error and bias. The full colored images of seahorses were further pre-processed in Adobe Photoshop. The software, FracLac v.2.5, which is available as a plugin to ImageJ was used to process and analyze images per species. Ten (10) images per species were processed in triplicates then further subsampled by the software. FracLac is apparently designed for digital image analysis and suitable for analyzing binary digital images such as contours of biological forms or fractals. It is used to measure difficult to describe morphological features. For this study, Box-counting and lacunarity methods were performed. Herewith, FracLac delivers a measure of complexity, a fractal dimension called the box counting fractal dimension (D_B). It is measured from the ratio of increasing detail with increasing scale (ε). This ratio quantifies the increasing detail with increasing magnification or resolution seen in fractals. The basic technique for calculating the D_B used in FracLac is called box counting. Moreover, lacunarity stands for gappiness or visual texture, it is considered a measure of heterogeneity (inhomogeneity) or translational or rotational variance in an image. This measure practically supplements fractal dimensions in characterizing patterns extracted from digital images. Lacunarity is calculated as the variation in pixel density at different box sizes, using CV for pixel distribution. CV stands for coefficient of variation $=\partial/\mu$ (Zhang and Zhan, 2011; Zhang, 2018). It measures the variation in the dataset and is calculated as the standard deviation over the mean of the data (∂/μ) (Karperien, 2005). FracLac automatically calculates values in a regular scan. For comparison between species, statistics were calculated using PAST v.2.17, software where, ANOVA and Tukey's pairwise comparison test (post-hoc) were used to check if there is a significant difference per species.

3 Results and Discussion

Seahorses are considered as valuable and flagship species because they can occupy diverse habitats in the marine environment (Tabugo-Rico et al., 2017). In the Philippines, specifically in Mindanao, the Islands of Surigao city serve as an important sanctuary for many species. Initial reports show three (3) species of seahorses found in the Island of Hanigad alone and perhaps, there could be more. Herewith, marine habitats like coral reefs, sea grass beds, mangrove forests, sponge gardens and gorgonians serve as a haven for the seahorses. However, the downcast part, is that seahorses are often targeted by fisher folks supplying traders for medicinal and aquarium use (Lourie et al., 1999; Lourie et al., 2004; Pajaro and Vincent, 2015). Moreover, they are often vulnerable also to bycatch fishing and habitat degradation. To date, there has been progress in seahorse research however, there are areas that remain data deficient and information when it comes to nature of populations and morphological complexity in relation to vulnerability and resilience remains lacking. Thus, there is a need to monitor populations in the area for conservation and sustainability.

In this study, it is hypothesized that fractals are far from the equilibrium state and thus associated with chaos. Thus, high fractal dimensions have implications to vulnerability of species. Table 1 shows the summary of mean fractal dimensions and coefficient of variation with corresponding minimum and maximum values respectively. For *H. barbouri* (F), predictor range values for D_B are from 1.4835-1.5492; *H. comes* (F) – D_B (1.5613-1.6465); *H. kuda* (M)-D_B (1.5183-1.6261) respectively.

Table 1 Summary of fractal dimensions (D_B) and coefficient of variation (CV) values for lacunarity of seahorses found in Surigao City.

	Mean D_B	Min D_B	Max D_B	Mean CV	Min CV	Max CV
H. barbouri (F)	1.5085	1.4835	1.5492	0.2844	0.2422	0.3176
H. comes (F)	1.5941	1.5613	1.6465	0.4108	0.3477	0.4633
H. kuda (M)	1.5634	1.5183	1.6261	0.3411	0.3077	0.3927

An overlapped in the range of fractal dimension values indicates similarity in morphological complexity and linked with common microhabitats. Such as *H. barbouri* species and *H. kuda* were both found around 0.5 to 4m in depth, common in seagrass beds and rocky littoral zones or clinging to hard corals. Whereas, *H. comes* were reportedly landed on shallow waters and water as deep as 20m in coral reefs, sponge gardens and floating sargassum. Among the three, *H. kuda*, was considered to be quite versatile in a way resilient, in terms of occupied microhabitats such that they can be found in estuaries, coastal bays, lagoons, mangroves, harbours, coral reefs, seagrass beds, muddy, macroalgae and sandy bottoms and rocky littoral zones.

The Mean fractal dimension values based on box-counting method were calculated and shown in Fig. 2. The lowest mean fractal dimension value (D_B) of *H. barbouri* (F) is -1.5085 and the highest is *H. comes* (F)-1.5941. Meanwhile, the mean D_B for *H. kuda* (M) is -1.5634. In addition, lacunarity stands for gappiness or visual texture, it is a measure of heterogeneity (inhomogeneity) or translational or rotational variance in an image. Herewith, this measure practically supplements fractal dimensions in characterizing patterns extracted from digital images. It measures the variation in the dataset and is calculated as the standard deviation over the mean of the data (∂/μ) (Karperien, 2005; Zhang and Zhan, 2011; Zhang, 2012, 2018). The mean coefficient of variation (CV) is shown in Fig. 3. The lowest mean coefficient of variation of *H. barbouri* (F) is -0.2844 and the highest *H. comes* (F) is -0.4108, while *H. kuda* (M) has 0.341. Herewith, the results for fractal dimensions (D_B) were in accordance with the coefficient of variation (CV) values.

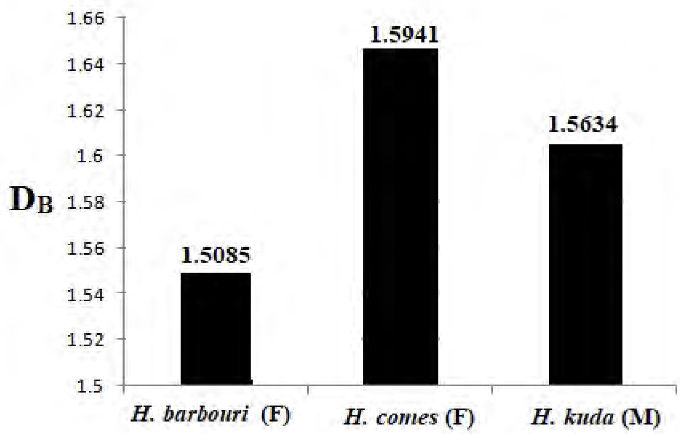


Fig. 2 Mean fractal dimension values (D_B) of seahorse species found in Surigao City, Philippines.

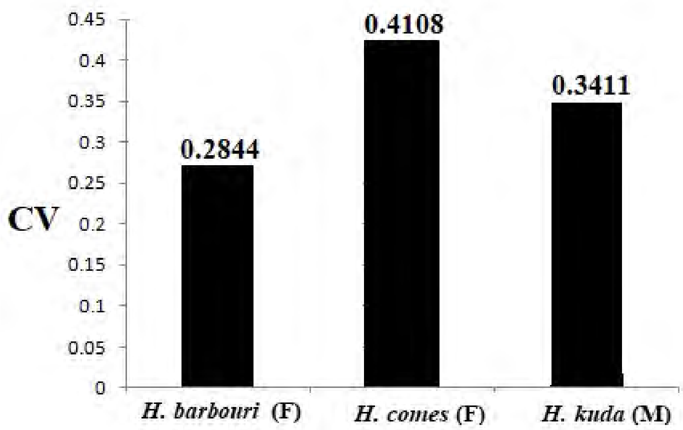


Fig. 3 Mean coefficient of variation (CV) values for lacunarity of seahorse species found in Surigao City, Philippines.

In a study by Klein et al. (2013), it was stated that fractals are far from the equilibrium state and thus associated with chaos. Hence, high fractal dimension value can be linked with vulnerability. The greater the fractal dimension (D_B) value, the more complex the species is thus, the more vulnerable it can be. Results show that *H. comes,* has the highest mean fractal dimension value and coefficient of variation, thus can be morphologically complex and may have implications on its vulnerability. Noteworthy, is that *H. comes,* can be found from shallow waters to water as deep as 20m in coral reefs, sponge gardens and floating sargassum. *H. comes* has average to narrow body; small head relative to the body with rugged spineson body, double cheek spine, prominent nose spine, striped tail and low coronet. Male species highlight the deep ventro-lateral medial portion with conspicuous bellies. *H. comes,* has been listed as Vulnerable (VU A2bd+4bd) based on suspected declines of 30-50% caused by incidental catch, targeted catch and habitat degradation. There were evidences of decline in the availability of this species given its popularity for dried traditional medicine trade and for live aquarium trade. As far as information is concerned, population decline related to this species in other areas outside of the Philippines remain unknown however, the abundance of *H. comes* surveyed in several islands of the Philippines where fishing occurs are low and there is also evidence of decline of availability. Moreover, increase in human population combined with human activities and only minor success in conservation indicates threats in the future for this species (Perante et al. 2002; Morgan and Lourie, 2006).

Moreover, one-way analysis of variance (ANOVA) was performed to check whether there is a significant difference among species in terms of morphological complexity based on fractal dimensions (Table 2). Tukey's pairwise comparison between fractal dimensions (D_B) of species was also performed to identify the difference between species (Table 3). This was visualized through box plot (Fig. 4). Results show significant difference between species and are highly evident between *H. barbouri* (F) and *H. comes* (F); *H. barbouri* (F) and *H. kuda* (M) respectively. Moreover, results were further verified through One-way analysis of variance (ANOVA) for coefficient of variation (CV) for lacunarity (Table 4). Tukey's pairwise comparison between CV values was also performed (Table 5) and results were visualized in box plot (Fig. 5). Based on the results, there is significant difference between species and is highly evident between *H. barbouri* (F) vs *H. comes* (F); *H. barbouri* (F) vs. *H. kuda* (M); *H. comes* (F) vs *H. kuda* (M). Significant difference, could be attributed to difference in morphological complexity and preferred microhabitats. Herewith, *H. comes* yield to be the most complex, variable and vulnerable species. Noteworthy, is that significant difference among fractal dimensions and CV values for lacunarity show great potential as an objective parameter in characterizing morphological complexity in seahorses.

In addition, there is a positive correlation between the fractal dimensions and lacunarity (Karperien et al., 2011). Results show that there is a direct relationship between fractal dimension, coefficient of variation values, morphological complexity and heterogeneity. In this respect, investigating fractals provide indicators of vulnerability. Herewith, information obtained contributed to understanding the nature of seahorses especially those found in Surigao City and can be a basis for conservation efforts.

Table 2 Analysis of variance (ANOVA) for the fractal dimensions (D_B) of species of seahorses found in Surigao City.

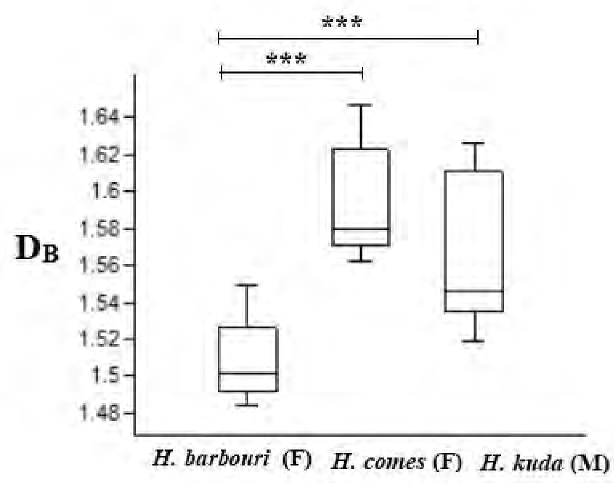
	Sum of sqrs	df	Mean square	F	p
Between groups:	0.045128	2	0.022564	22.47	**6.929E-07**
Within groups:	0.033132	33	0.001004		
Total:	0.078260	35			

*p<0.05 is significant.

Table 3 Tukey's pairwise comparison between fractal dimensions (D_B) of species of seahorses found in Surigao City.

	H. barbouri (F)	*H. comes* (F)	*H. kuda* (M)
H. barbouri (F)	-		
H. comes (F)	**0.0001266**	-	
H. kuda (M)	**0.0005777**	0.06033	-

*p<0.05 is significant.



***highly significant, ns-not significant

Fig. 4 Mean fractal dimension values (D_B) and significant difference between seahorse species found in Surigao City, Philippines.

Table 4 Analysis of variance (ANOVA) for coefficient of variation (CV) values for lacunarity of species of seahorses found in Surigao City.

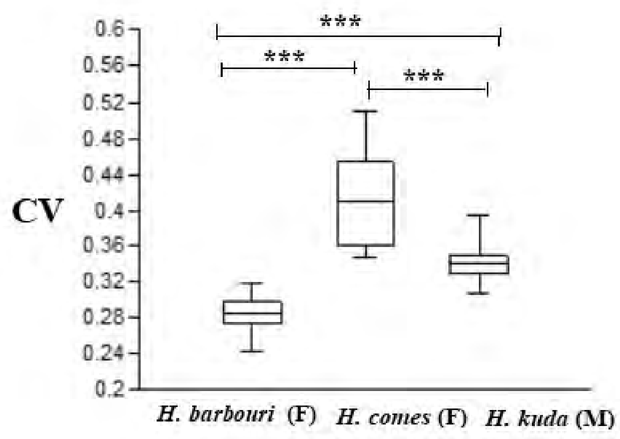
	Sum of sqrs	df	Mean square	F	p
Between groups:	0.0962347	2	0.0481173	40.35	**1.367E-09**
Within groups:	0.039355	33	0.00119258		
Total:	0.13559	35			

*p<0.05 is significant.

Table 5 Tukey's pairwise comparison between coefficient of variation (CV) values for lacunarity of species of seahorses found in Surigao City.

	H. barbouri (F)	*H. comes* (F)	*H. kuda* (M)
H. barbouri (F)	-		
H. comes (F)	**0.0001264**	-	
H. kuda (M)	**0.0009982**	**0.0001806**	-

*p<0.05 is significant.



***highly significant, ns-not significant

Fig. 5 Mean coefficient of variation (CV) for lacunarity and significant difference between seahorse species found in Surigao City, Philippines.

4 Conclusion

This study provided a baseline data on morphological complexity of species of seahorses based on fractals which can be a basis for conservation efforts. It is hypothesized that fractals are far from the equilibrium state and thus associated with chaos. Hence, high fractal dimensions have implications to vulnerability. Results show that *H. comes*, has the highest mean fractal dimension value and coefficient of variation, thus the most morphologically complex and vulnerable species found in Surigao City. Statistically, comparing fractal dimensions (D_B) and coefficient of variation (CV) values reveal significant difference between species and could be attributed to difference in morphological complexity and preferred microhabitats. Herewith, *H. comes* yield to be the most complex, variable and highly vulnerable species. Moreover, significant difference among fractal dimensions and CV values for lacunarity show great potential as an objective parameter in characterizing morphological complexity in seahorses hence can be employed for monitoring purposes. The information obtained contributed in understanding the nature of seahorses especially those found in Surigao City, Philippines.

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References

Aldrich PR, Horsley RK, Ahmed YA, Williamson JJ, Turcic SM. 2010. Fractal topology of gene promoter networks at phase transitions. Gene Regulation and Systems Biology, 4: 5389

Cross SS. 1997. Fractals in pathology. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 182:1-8

Havlin S, Buldyrev SV, Goldberger AL, Mantegna RN, Ossadnik SM, Peng CK, Simons M, Stanley HE. 1995. Fractals in biology and medicine. Chaos, Solitons and Fractals, 6:171-201

Huikuri HV, Stein PK. 2012. Clinical application of heart rate variability after acute myocardial infarction. Frontiers in Physiology, 3: 41

Kam Y, Karperien A, Weidow B, Estrada L, Anderson AR, Quaranta V. 2009. Nest expansion assay: a cancer systems biology approach to in vitro invasion measurements. BMC Research Notes, 2: 130

Karperien A, Jelinek HF, Milosevic NT, Cracow P. 2011. Reviewing lacunarity analysis and classification of microglia in neuroscience. In: Proceedings of the 8th European Conference on Mathematical and Theoretical Biology. Cracow, Poland

Karperien A, Jelinek HF, Leandro JJ, Soares JV, Cesar Jr RM, Luckie A. 2008. Automated detection of proliferative retinopathy in clinical practice. Clinical Ophthalmology, 2: 109

Karperien A. 2005. FracLac V.2. Charles Stuart University, NSW, Australia

Klein K, Maier T, Hirschfeld-Warneken VC, Spatz JP. 2013. Marker-free phenotyping of tumor cells by fractal analysis of reflection interference contrast microscopy images. Nano Letters, 13: 5474-5479

Lourie SA, Foster SJ, Cooper EW, Vincent ACJ. 2004. A guide to the identification of seahorses. Project Seahorse and TRAFFIC North America. University of British Columbia and World Wildlife Fund, Washington DC, USA

Lourie SA, Vincent ACJ, Hall HJ. 1999. Seahorses: An Identification Guide to The World's Species and Their Conservation. Project Seahorse, London, UK

Morgan SK, Lourie SA. 2006. Threatened fishes of the world: Hippocampus comes Cantor 1850 (Syngnathidae). Environmental Biology of Fishes, 75(3): 311-313

Pajaro MG, Vincent ACJ. 2015. The catch and export of the seahorse trade in the Philippines, pre-CITES. Fisheries Centre Working Report, 1: 2

Perante NC, Pajaro MG, Meeuwig JJ, Vincent ACJ. 2002. Biology of a seahorse species, Hippocampus comes in the central Philippines. Journal of Fish Biology, 60(4): 821-837

Tabugo-Rico SRM, Tumanda LA, Cabrido CK, Apale CM, Dumalagan E. 2017. Elliptic Fourier Analysis of body shape variation of *Hippocampus* spp. (seahorse) in Danajon Bank, Philippines. Computational Ecology and Software, 7(4): 140-151

Turcotte DL, Malamud BD, Guzzetti F, Reichenbach P. 2002. Self-Organization, the cascade model, and natural hazards. Proceedings of the National Academy of Sciences of USA, 99: 2530-2537

Waliszewski P, Konarski J. 2002. Neuronal differentiation and synapse formation occur in space and time with fractal dimension. Synapse, 43: 252-258

Wassenbergh SV, Roos G, Ferry L. 2011. An adaptive explanation for the horse-like shape of seahorses. Nature Communications, 2(1): 164

Zhang WJ. 2018. Fundamentals of Network Biology. World Scientific Europe, London, UK

Zhang WJ, Zhan CY. 2011. An algorithm for calculation of degree distribution and detection of network type: with application in food webs. Network Biology, 1(3-4): 159-170