International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 6, p. 164-173, 2018

INNSPUB

RESEARCH PAPER OPEN ACCESS

Fractal analysis of *Hippocampus* spp. (seahorse) in Danajon Bank, Philippines

Sharon Rose Tabugo[1*], Charity Mae Apale[2], Edwin Dumalagan[2]

[1]*Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines,*

[2]*iSeahorse, ZSL, Philippines*

Key words: Fractal analysis, *Hippocampus*, Seahorse, Box-counting method, Lacunarity.

http://dx.doi.org/10.12692/ijb/12.6.164-173 Article published on June 14, 2018

Abstract

Danajon bank is the home of four out of seven species of seahorses known in the Philippines. Seahorses are considered as highly vulnerable species to overfishing and habitat destruction, especially in Danajon bank thus, there is a need to monitor populations in the area. One method to look into the nature of populations is to use fractal geometry to look into morphometric complexity patterns of different species of seahorses (*Hippocampus* spp.) hence, this study. Irregular non-euclidean objects are better described by fractal geometry and the measurable value is called the fractal dimension. It is hypothesized that fractals are far from the equilibrium state and thus associated with chaos. Hence, high fractal dimensions have implications to vulnerability of species. 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. Based on ANOVA and Tukey's pairwise comparison, results yield significant difference between species in terms of fractal dimension and coefficient of variation (CV) values for lacunarity and could be attributed to difference in morphological complexity and associated microhabitats. However, high mean fractal dimension based on box-counting and CV values was associated with *H. histrix*. Herewith, *H. histrix* yield to be the most highly complex and vulnerable species. Information obtained contributes to understanding the nature of seahorses especially those found in Danajon bank.

* **Corresponding Author:** Sharon Rose Tabugo ✉ sharonrose0297@gmail.com

Introduction

Danajon Bank is a home of over 200 threatened species including species of *Hippocampus* (seahorse) (Pichon, 1977; White and Cruz-Trinidad, 1998; Armada, White, and Christie, 2009). These charismatic creatures, which had been popular in Chinese medicine, are among the many genera whose life histories render them vulnerable to overfishing, overexploitation and habitat destruction. To date, Danajon bank, experienced very high fishing pressure and habitat disturbance. In fact, across Danajon Bank, coral reefs and resources known for their economic and cultural value, are in decline due to anthropogenic activities (such as land-based sources of pollution and sedimentation, overfishing) and climate change (Diaz *et al*., 2012).

In this respect, monitoring seahorses and understanding their nature is crucial hence, the importance of this study. *Hippocampus* spp. (seahorses), are often associated with varied habitats such as sea grass beds, mangroves, sandy silt bottoms, gorgonians corals, sponge gardens, sea fans, sea whips, estuaries and coral reefs, which harbours the highest biodiversity in the marine environment (Foster and Vincent, 2004; Loh *et al.,* 2014).

They are not only excellent for relatively long-term monitoring and ecological studies because of inferred life spans that ranged from 1 to 5 years (Foster and Vincent, 2004) but proved to be fascinating subject for phenotypic studies, owing to complex shapes and forms. 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). Different species are said to be associated with different microhabitats.

This study, highlights morphological complexity. Accordingly, the utilization of shape descriptors can be used as overall indicators of macro states of biological entities. However, many shape descriptions are often subjective and could be highly bias. Hence, a proposed method for Irregular non-euclidean objects is better described by fractal geometry and the measurable value is called the fractal dimension. 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 inadequately studied. Herewith, 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). Hence, high fractal dimensions may have implications to vulnerability which aide in understanding the nature of species.

Accordingly in the past, 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 tree growth, river paths, tumour 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). Hence, pave the way of the importance of fractals in biological forms and analysis.

Hence, this serve as an exploratory study to objectively describe morphometric complexity of selected common seahorse *Hippocampus* species: *H. comes*, *H. histrix, H. spinosissimus* and *H. kuda* from Danajon bank through fractal analysis via Box-counting and Lacunarity method respectively for monitoring purposes specially vulnerability. 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. Moreover, considerable research on this area will be beneficial for seahorse conservation and management such that high fractal dimensions may have implications to vulnerability. Information obtained also contributes to understanding the nature of seahorses especially those found in Danajon bank.

Materials and methods

Sampling area, specimen collection and identification

The sampling area was in Danajon bank, a double barrier reef. It is the only well-documented seahorse sanctuary in the Philippines and was considered as an area where marine animals thought to have first evolved (Fig. 1).

Fig.1. Study area: Map of Danajon bank, a double barrier reef.

This reef was considered a rare geologic formation and one of the six double barrier reefs in the world. It spans in the islands of Cebu, Bohol, mainland Leyte and Southern Leyte. It is considered as a home of over 200 threatened species including species of *Hippocampus* (seahorse) (Pichon, 1977; White and Cruz-Trinidad, 1998; Armada, White, and Christie, 2009). Adult seahorse specimens were in courtesy of iseahorse, phils., ZSL. Specimens were bycatch samples of fishermen in the area. 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 iseahorse underwater surveys (through SCUBA diving) undertaken in the area.

Image acquisition, processing, fractal analysis and statistical analysis

Image acquisition of samples was done using Canon DSLR 550D. All images were processed in triplicates.

The full colored images of seahorses were pre-processed in Adobe Photoshop and converted to 24-bitmap type, binary (black & white color) images for noise reduction. Fraclac v.2.5, which is available as a plugin to ImageJ was used to process and analyze images per species. Fraclac is for digital image analysis and apparently suitable for analyzing binary digital images such as contours of biological forms or fractals. It is used to measure difficult to describe morphological features. In 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 Frac Lac 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 $=\partial/\mu$. 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. To compare between species, statistics were calculated using PAST v.2.17, ANOVA and Tukey's pairwise comparison test (post-hoc) were used to see if there is a significant difference per species.

Results and discussion

In the Philippines, Danajon Bank, serves as an important sanctuary for many species.

The sad part, is that seahorses are often targeted by fisher folks supplying traders for medicinal and aquarium use (Lourie *et al.,* 1999; Lourie *et al.,* 2003; Pajaro and Vincent, 2015). Seahorses are often vulnerable to bycatch fishing and habitat degradation.

Albeit, seahorse research had made progress, data is still lacking when it comes to nature of populations and morphological complexity in relation to vulnerability.

Table 1. Summary of fractal dimensions and coefficient of variation values for lacunarity of seahorses found in Danajon Bank.

	Mean D_B	Min D_B	Max D_B	Mean CV	Min CV	Max CV
H. comes (F)	1.5245	1.4859	1.6128	0.3894	0.3394	0.449
H. comes (M)	1.3758	1.3331	1.4221	0.3048	0.2632	0.3677
H. kuda (F)	1.446	1.4119	1.5037	0.3509	0.2533	0.3968
H. histrix (M)	1.5339	1.4856	1.6015	0.4446	0.3459	0.5729
H. spinosissimus (M)	1.4383	1.4039	1.4918	0.3455	0.273	0.383

M-male; F-Female.

Measurement of fractal dimensions via box-counting method and coefficient of variation (CV) values for lacunarity method

In this study, it is hypothesized that fractals are far from the equilibrium state and thus associated with chaos. Hence, 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. comes* (F), predictor range values for D_B is from 1.4859-1.6128; *H. comes* (M) – D_B (1.3331-1.4221); *H. kuda* (F)-D_B (1.4119-1.5037); *H. histrix* (M)-D_B (1.4856-1.6015); *H. spinosissimus* (M)-D_B (1.4039-1.4918) respectively.

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

	Sum of sqrs	df	Mean square	F	p
Between groups:	0.207666	4	0.0519165	36.34	**7.61E-15**
Within groups:	0.078575	55	0.00142864		
Total:	0.286241	59			

*p<0.05 is significant.

An overlapped in the range of fractal dimension values indicate similarity in morphological complexity and linked with common microhabitats. Such as *H. comes* species are found in sponges so as *H. histrix*. 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) is of *H. comes* (M)-1.3758 and the highest is *H. histrix* (M)-1.5339. Meanwhile, the mean D_B for *H. comes* (F) -1.5245; *H. comes* (M)-1.3758; *H. kuda* (F)-1.446; *H. histrix* (M)-1.5339 and *H. spinosissimus* (M)-1.4383.

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

	H. comes (F)	*H. comes* (M)	*H. kuda* (F)	*H. histrix* (M)
H. comes (F)				
H. comes (M)	0.0001293			
H. kuda (F)	0.0001724	0.0003929		
H. histrix (M)	0.9735	0.0001293	0.0001325	
H. spinosissimus (M)	0.0001414	0.001582	0.9866	0.0001296

*p<0.05 is significant; M-male; F-Female.

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

	Sum of sqrs	df	Mean square	F	p
Between groups:	0.13347	4	0.0333676	18.23	1.38E-09
Within groups:	0.10065	55	0.00183		
Total:	0.234121	59			

*p<0.05 is significant.

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).

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

	H. comes (F)	*H. comes* (M)	*H. kuda* (F)	*H. histrix* (M)
H. comes (F)				
H. comes (M)	0.0002247			
H. kuda (F)	0.193	0.0769		
H. histrix (M)	0.02078	0.0001293	0.0001484	
H. spinosissimus(M)	0.102	0.1508	0.998	0.0001327

*p<0.05 is significant; M-male; F-Female.

The mean coefficient of variation (CV) is shown in Fig. 3. The lowest mean coefficient of variation is of *H. comes* (M)-0.3048 and the highest is *H. histrix* (M)-0.4446. Herewith, results for fractal dimensions were in accordance with the coefficient of variation values. According to Klein *et al.,* 2013 fractals are far from the equilibrium state and thus associated with chaos. With this, high fractal dimension value can be linked with vulnerability. Results show that *H. histrix,* has the highest mean fractal dimension value and coefficient of variation, thus the most morphologically complex which have implications on vulnerability.

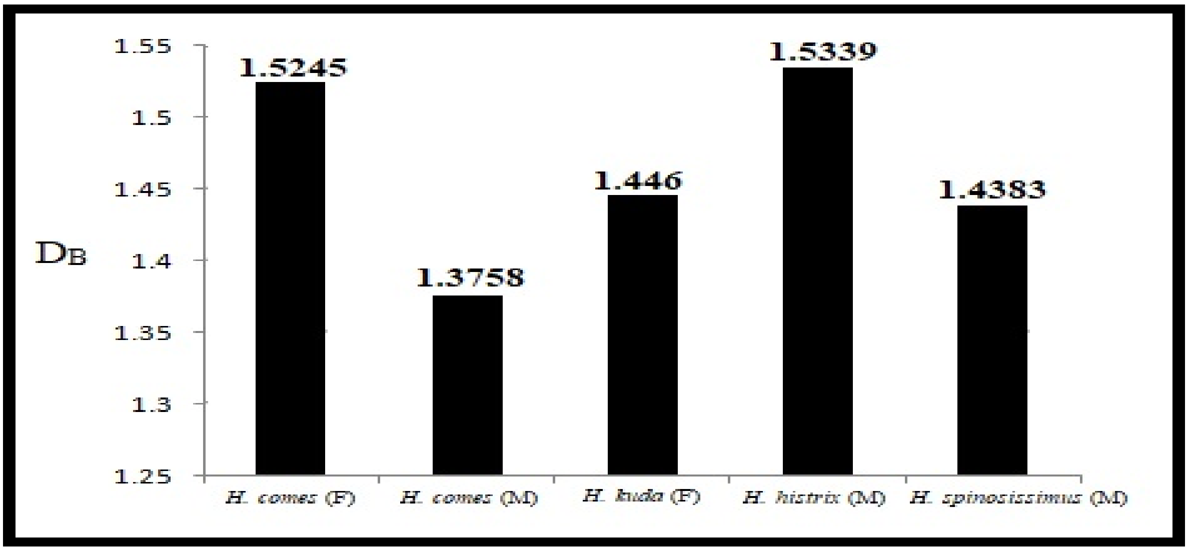


Fig.2. Mean fractal dimension values (D_B) of seahorse species found in Danajon Bank, Philippines.

Noteworthy, is that *H. histrix* are found in depths between 10-40m. Associated micro-habitats were sponges, seagrass beds, weedy rocky reefs, hard & soft corals, sea whips and sea fans. *H. histrix* has long snout and average to narrow body. Male species highlight the deep ventro-lateral medial portion with conspicuous bellies. *H. histrix* has been reported in international trade since the mid 1990s. To date, interviews with fishers and traders reported declines in the availability of this species (McPherson and Vincent, 2004; Perry *et al.,* 2010).

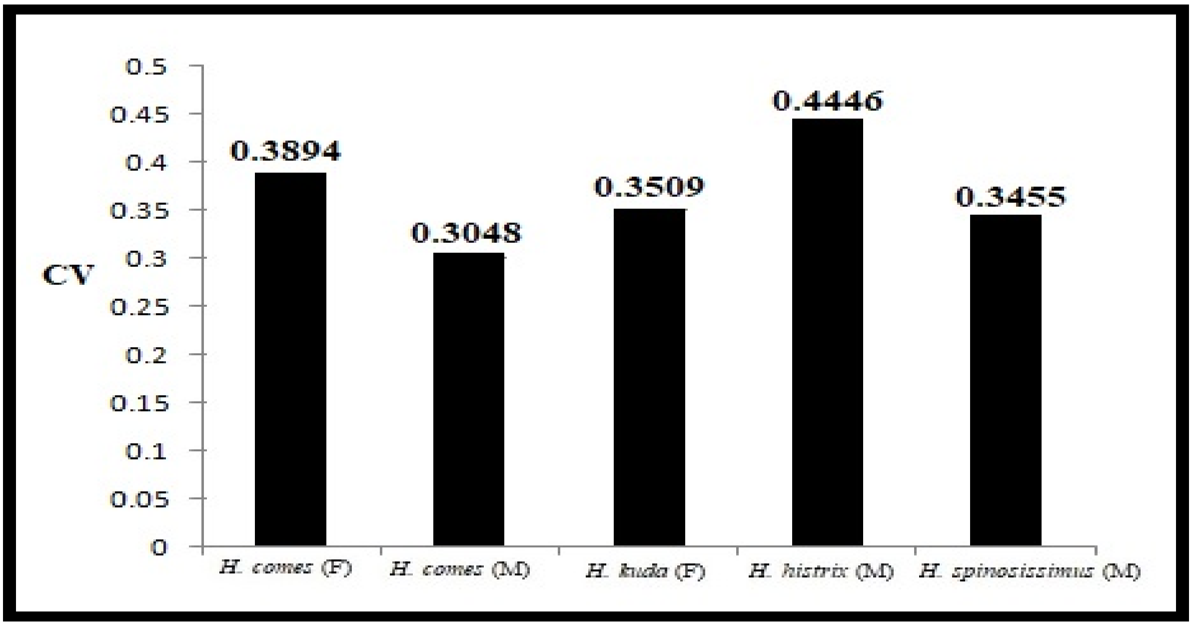
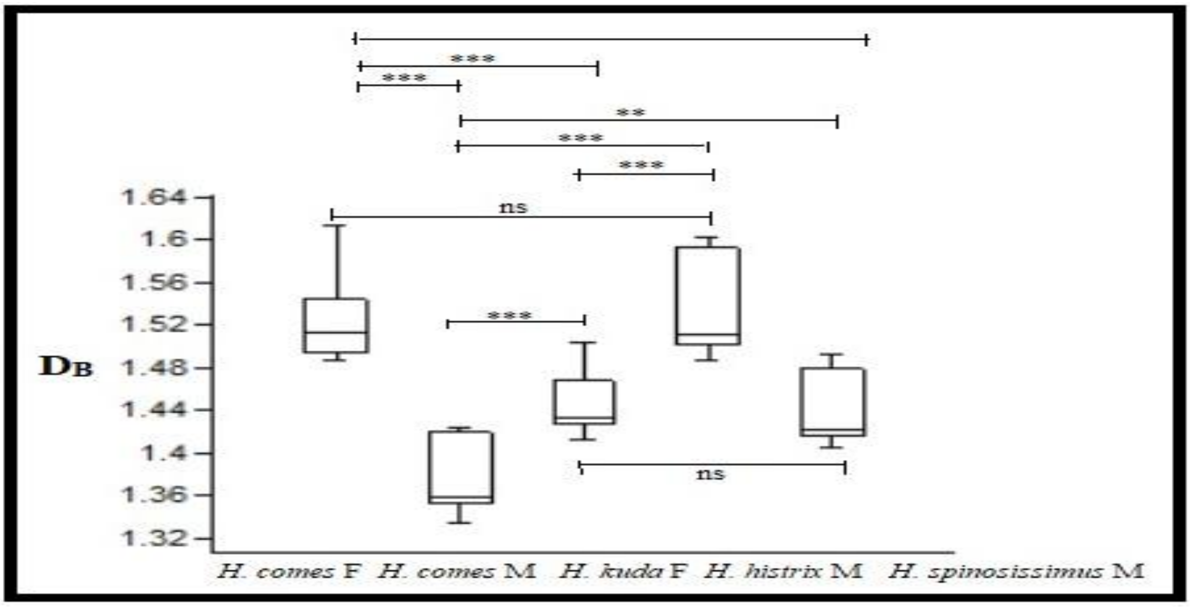


Fig. 3. Mean coefficient of variation (CV) values for lacunarity of seahorse species found in Danajon Bank, Philippines.

It is suspected that the species had undergone a decline and may be accelerating plus primary threats have not ceased. *H. histrix*is further assessed as vulnerable under criterion A2cd+4cd of Red list category. Among the threats are brought about by overfishing, pollution, habitat destruction, bycatch trawlers, fishing practices and coastal development (Lourie, 2016; Pollom, 2017). However, the major threat to *Hippocampus histrix,* is over-exploitation because this species is often caught in both targeted fisheries and as bycatch in other non-selective fisheries, particularly shrimp trawls (McPherson and Vincent 2004; Meeuwig *et al.,* 2006; Perry *et al.,* 2010). As per the record, albeit daily catch rates on individual vessels are quite low, the cumulative impact of the Indo-Pacific trawl fleet leaves little doubt that bycatch is affecting populations of this species (Vincent *et al.* 2011; Lawson *et al.*, 2017).



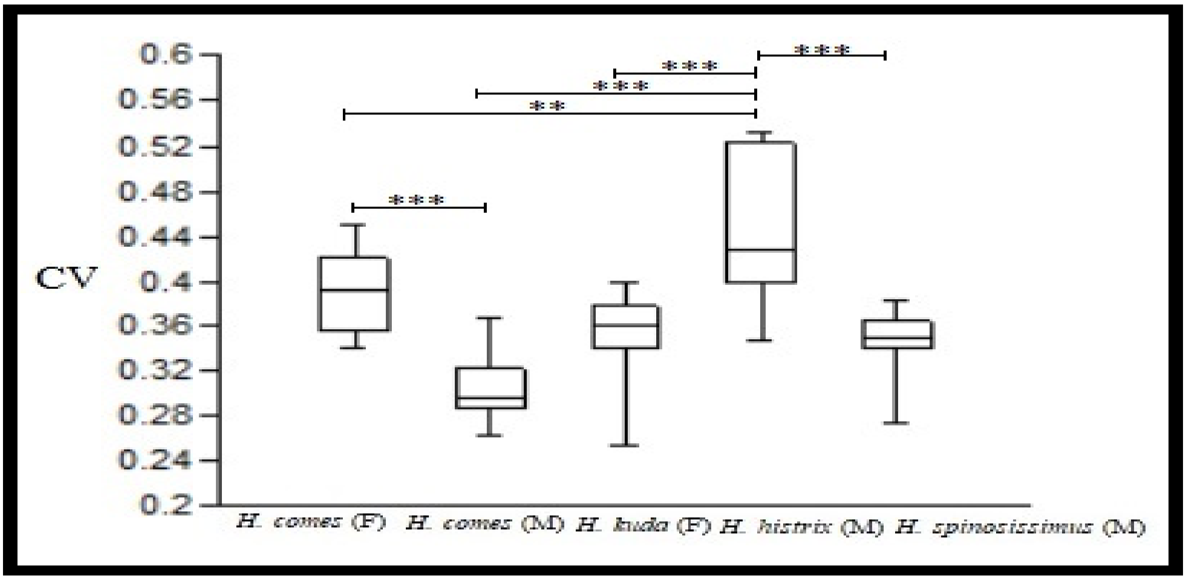
***highly significant, **significant; ns-not significant;M-male; F-Female.

Fig. 4. Mean fractal dimension values (DB) and significant difference between seahorse species found in Danajon Bank, Philippines.

One-way analysis of variance (ANOVA) and Tukey's pairwise comparison test

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 is highly evident between *H. comes* male and female; *H. kuda* and *H. comes*; *H. comes* and *H. spinosissimus*; H. histrix (M) and H. comes (M); *H. histrix*and *H. kuda*; *H. histrix* and *H. spinosissimus*. 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 result there is significant difference between species and is highly evident between *H. histrix*vs. male and female *H. comes*; *H. kuda* and *H. spinosissimus*. Significant difference could be attributed to difference in morphological complexity and associated microhabitats. Herewith, *H. histrix* yield to be the most complex, variable and vulnerable species. Significant difference among fractal dimensions and CV values for lacunarity show great potential as an objective parameter in characterizing morphological complexity.

Furthermore, 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 contributes to understanding the nature of seahorses especially those found in Danajon bank.



***highly significant, **significant; ns-not significant; M-male; F-Female

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

Conclusion

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. histrix,* has the highest mean fractal dimension value and coefficient of variation, thus the most morphologically complex and vulnerable species. 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 associated microhabitats. Herewith, *H. histrix* 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. Herewith, information obtained contributes to understanding the nature of seahorses especially those found in Danajon bank.

Acknowledgment

The researchers would like to express their heartfelt gratitude to ZSL and to their families and friends who become a considerable source of inspiration and determination.

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. https://doi.org/10.4137/GRSB.S53.89

Armada N, White AT, Christie P. 2009. Managing Fisheries Resources in Danajon Bank, Bohol, Philippines: An Ecosystem-Based Approach. Coastal Management **37**, 308-330. http://dx.doi.org/10.1080/089207509028516.09

Cross SS. 1997. Fractals in pathology. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland **182**, 1-8. https://doi.org/10.1002/(SICI)10969896(199705)1.82:1

Diaz R, Apistar D, Porpetcho W, Delizo D, Gatus R, Martinez R, Amolo R. 2012. Danajon Bank Double Barrier Reef. Coastal Conservation and Education Foundation, Inc. (CCEF),1-5.

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

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

Huikuri HV, Stein PK. 2012. Clinical application of heart rate variability after acute myocardial infarction. Frontiers in physiology **3**, 41. http://dx.doi.org/10.3389/fphys.2012.00041.eCollection2012

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. https://doi.org/10.1186/1756-0500-2-1.30

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

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, 38.

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. http://dx.doi.org/10.1021/nl4030402

Lawson JM, Foster SJ, Vincent AC. 2017. Low bycatch rates add up to big numbers for a genus of small fishes. Fisheries**42**, 19-33. https://doi.org/10.1080/03632415.2017.12599.44

Loh TL, Knapp C, Foster SJ. 2014. Iseahorse Saving seahorses together Trends Tool Kit. Ver. 1.1.Vancouver, Canada: The University of British Columbia, 6.

Lourie SA. 2016. Seahorses: A Life-Sized Guide to Every Species. The University of Chicago Press, Ivy Press Limited, Chicago, USA.

Lourie SA, Foster SJ, Cooper EW, Vincent AC. 2004. A guide to the identification of seahorses. Washington DC,USA: Project Seahorse and TRAFFIC North America, 114.

Lourie SA, Randall JE. 2003. A new pygmy seahorse, Hippocampus denise (Teleostei: Syngnathidae), from the Indo-Pacific. Zoological Studies **42**, 284-291.

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

McPherson JM, Vincent AC. 2004. Assessing East African trade in seahorse species as a basis for conservation under international controls. Aquatic Conservation: Marine and Freshwater Ecosystems **14**, 521-538. https://doi.org/10.1002/aqc.6.29

Meeuwig JJ, Ky TS, Job SD, Vincent AC. 2006. Quantifying non-target seahorse fisheries in central Vietnam. Fisheries Research **81**, 149-157. https://doi.org/10.1016/j.fishres.2006.07.00.8

Pajaro MG, Vincent ACJ. 2015. The catch and export of the seahorse trade in the Philippines, pre-CITES. Fisheries Centre Working Paper**102**, 27.

Perry AL, Lunn KE, Vincent AC. 2010. Fisheries, large-scale trade, and conservation of seahorses in Malaysia and Thailand. Aquatic Conservation: Marine and Freshwater Ecosystems**20,** 464-475. https://doi.org/10.1002/aqc.11.12

Pichon M. 1977. Physiography, morphology and ecology of the double barrier reef of north Bohol (Philippines).In Proceedings of the Third International Coral Reef Symposium, Miami, USA, 261–267.

Pollom R. 2017. Hippocampus histrix. The IUCN Red List of Threatened Species 2017. e. T10070 A54905206. http://dx.doi.org/10.2305/IUCN.UK.20173.RLTS.T10070A54905206.en

Turcotte DL, Malamud BD, Guzzetti F, Reichenbach P. 2002. Self-Organization, the Cascade Model, and Natural Hazards.Proceedings of the National Academy of Sciences **99**, 2530–37.

Vincent AC, Foster SJ, Koldewey HJ. 2011. Conservation and management of seahorses and other Syngnathidae. Journal of Fish Biology **78**, 1681-1724. https://doi.org/10.1111/j.1095-8649.2011.030.03.x

Waliszewski P, Konarski J. 2002. Neuronal differentiation and synapse formation occur in space and time with fractal dimension. Synapse **43**, 252-258. https://doi.org/10.1002/syn.100.42

Wassenbergh SV, Roos G, Ferry L. 2011. An adaptive explanation for the horse-like shape of seahorses.Nature Communications **2**,164. http://dx.doi.org/10.1038/ncomms116.8

White AT, Cruz-Trinidad A. 1998. The Values of Philippine Coastal Resources: Why Protection and Management are Critical. Coastal Resource Management Project of the Department of Environment and Natural Resources. Cebu City, Phils: United States Agency for International Development, 96.