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RESEARCH ARTICLE

Comparison of electronic length frequency analysis (ELEFAN) for estimation of growth parameters for lollyfish, *Holothuria* (*Holothuria*) *atra* and sand sea cucumber, *Holothuria* (*Thymiosycia*) *arenicola* (Holothuroidea: Echinodermata) in the north Arabian Sea, Pakistan

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ABSTRACT

To estimate the growth parameters, the non-seasonal von Bertalanffy and Hoenig seasonal von Bertalanffy models were fitted to the length frequency data of *Holothuria atra* and *H. arenicola* obtained from coastal areas of Karachi in the northern Arabian Sea, Pakistan between January and December 2014. The Hoenig seasonal von Bertalanffy growth parameters were estimated as L_∞=36.1 cm total length (TL), K=0.75 year^{-1} for *H. atra* and as L_∞=34.9 cm TL, K=0.70 year^{-1} for *H. arenicola*. *H. atra* individuals reached 73.9% of their maximum total length at the one year old. For *H. arenicola* it was calculated as 72.7%. Monthly mean growth rate of *H. atra* at the same ages was calculated higher than *H. arenicola*. The seasonal oscillation in growth rate for *H. atra* (C=0.50) was larger than it was for *H. arenicola* (C=0.37). The time of the year when the growth is slowest corresponded to the middle of April (WP=0.30) in *H. atra* and at the beginning of August (WP=0.60) in *H. arenicola* may be the result of the extended both reproduction and poor nutrition periods due to monsoonal effects on the marine environment. The relatively high growth rates (K>0.7 year^{-1}) of these two species may have important implications for high survival rate, particularly in environmental condition where cause biological stress and marine confusion but may also increase their potential as a candidate species for aquaculture.

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Introduction

A total of 25 sea cucumber species have been recorded from the coastal waters of Pakistan, so far and seven of them are documented as commercially important species (Tahera and Tirmizi, 1995; Purcell, 2010; Purcell et al., 2012; Ahmed and Ali, 2014, 2020; Ahmed et al., 2016a, 2020b; Moazzam and Moazzam, 2020). Currently, sea cucumber fishing occurs all over the world, but, there is no traditional sea cucumber fisheries in Pakistan (Siddique and Ayub, 2015; Moazzam and Moazzam, 2020). Two of the commercially important sea cucumber species are lollyfish, *Holothuria* (*Holothuria*) *atra* Jaeger, 1833 and sand sea cucumber, *Holothuria* (*Thymiosycia*) *arenicola* Semper, 1868 found in the coastal waters of Pakistan. *H. atra* known also as the black sea cucumber is found in the tropical Indo-Pacific region, its range extends from the Red Sea and East Africa to Australia. It inhabits the inner and outer flats, back reefs, shallow lagoons, sand-mud and rubble, and sea grass beds between 0 and 20 m. In few Pacific Island nations, the body wall, intestines and/or gonads are consumed in traditional diets or in times of hardship (Purcell et al., 2012).

H. arenicola is also found from the Indo-Pacific to the tropical Western Atlantic. The species is reported from tropical Australia, Mozambique, the Red Sea, Ascension Island, Virgin Islands, Antigua, Barbados, Tobago, Belize, Bermuda, Tortugas, Jamaica, Puerto Rico, U.S. (Mosher, 1980; WoRMS, 2020). These two holothurians are found on the seabed, in shallow waters on reefs and under intertidal rocks, sand flats and in seagrass meadows at depths of up to 20-30 m (Mosher, 1980; Pourvali et al., 2014; WoRMS, 2020). *H. atra* stocks in many regions are now being exploited and are often marketed under the name bêche-de-mer or trepang which means “cooked and dried sea cucumbers” (Purcell et al., 2012).

Relatively little information is available on the biology and ecology of these sea cucumbers belong to the class Holothuroidea in the northern Arabian Sea coasts of Pakistan. There are some works about the growth of sea cucumbers; including the weight–length relationships (WLRs) and condition factor (CF) based growth features in holothurian species such as *Ohshimella ehrenbergii* Selenka, 1867, *H. arenicola*, *H. atra*, *H. pardalis* Selenka, 1867 and *H. verrucosa* Selenka, 1867 from this region (Siddique et al., 2015; Ahmed et al., 2018a, 2018b). In addition to these, a detailed study on the population dynamics of sea cucumbers has been carried out on the *H. arenicola* stocks in Manora and Buleji rocky shores in the northern Arabian Sea, Pakistan so far (Siddique and Ayub, 2015). It was reported that an increase of the gonad index (GI) of *H. arenicola* was observed during spring and early summer, followed by a decrease in autumn and winter, which showed the spawning followed by resting phase and also the GI values were reported as a significant negative correlation with salinity and non-significant correlation with temperature (Siddique and Ayub, 2015). Moreover, unimodal distribution, bimodal distribution and three modes were determined for *H. arenicola* by seasons and areas (Siddique and Ayub, 2015). In other geographical regions, previous studies have been conducted for *H. atra* to determine reproduction biology features including sexual and asexual reproduction from Heron Reef (Harriott, 1982, 1985), spawning season, size at sexual maturity and fecundity from Red Sea (Chao et al., 1994; Abdel-Razek, 2005), ecology including distribution, habitat preference and feeding activity from Rongelap Atoll (Bonham and Held, 1963), Bahamas (Mosher, 1980), and Gulf of Mannar (Asha et al., 2015) and also environmental variability, population biology and fishing pressure from South Tarawa Lagoon, Republic of Kiribati (Tamaroa, 2010).

Age determination of holothuroid echinoderms is difficult because of their soft-bodied, polymorphic shape, and the small size of hard structures that could exhibit growth rings (Watanabe et al., 2014; Sun et al., 2019). Analyses of length frequency data (LFDA) can be used for estimating age classes and growth parameters (Pauly and David, 1981), and can also be applied to calculate growth of holothuroid echinoderms (Olaya-Restrepo et al., 2018). An additional challenge for estimating growth of holothuroid echinoderms arises due to the seasonal growth pattern, and a modified von Bertalanffy growth model has been developed to incorporate this (Hoenig and Hanumara, 1982). Seasonal growth patterns have been reported for different *Holothuria* species, such as *H. arguinensis* Koehler and Vaney, 1906 (Olaya-Restrepo et al., 2018), *Isostichopus badionotus* (Selenka, 1867) (Poot-Salazar et al., 2015) and *Australostichopus mollis* (Hutton, 1872) (Morgan, 2012). However, no seasonal growth information exists for the mentioned unexploited *Holothuria* species inhabiting the Arabian Sea. The aim of this study was to investigate seasonal growth rate of *H. atra* and *H. arenicola* in the north Arabian Sea.

Material and Methods

Study Area and Sampling

Holothuria atra (n=221) and *Holothuria arenicola* (n=258) specimens were collected on monthly basis from Buleji (24°50’20.41” N, 66°49’24.15” E) and Sunehri (24°52’33.49” N, 66°40’40.20” E) (Figure 1) coasts from January to December 2014 except June and July due to rough sea in monsoon season. The sample was collected from intertidal zone by hand-picking through forceps at low tide.

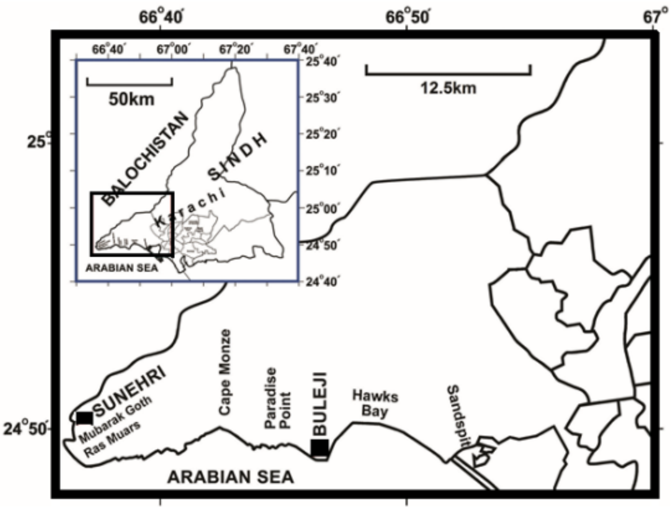

Figure 1. Sampling stations, Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan

Collected specimens were kept alive in water filled containers and then were transported to the laboratory and shifted in well aerated aquaria. For taxonomic studies and identification, morphological features were examined and microscopic studies were conducted. Identification of species of sea cucumbers was based on characteristics body morphology of the sea cucumbers, using the guidebooks from Selenka (1867) and Samyn et al. (2006).

Ossicles were taken from three positions (dorsal and ventral body walls, and tentacles); wet mounts were prepared by placing a small piece of skin tissue on slide and adding few drops of 3.5% bleach, the slides were then rinsed with drops of distilled water. The slides were examined under the Nikon LABOPHOT-2 microscope at 10×10 magnifications. Microphotography was also performed through Fujifilm 16 MP digital camera (see Ahmed et al., 2018a, 2018b for more details). Length (cm) data were collected for each sea cucumber after allowing the sea cucumber to relax in water for 5 min. Total length from mouth to anus was measured with the flexible ruler.

Von Bertalanffy Growth Function Parameter Estimation

Growth in length has been described using the von Bertalanffy (1938) growth function, based on either observed or back calculated length at ages. The length frequency distribution analysis (LFDA) package is also a PC based computer package for estimating growth parameters from length frequency distributions. Version 5.0 of LFDA includes methods for estimating the parameters of both non-seasonal and Hoenig seasonal versions of the von Bertalanffy growth curve (Kirkwood et al., 2003).

The standard or non-seasonal von Bertalanffy (1938) growth function (VBGF) is:

$$L_t = L_\infty\left(1 - e^{-k(t-t_0)}\right) \quad (1)$$

Seasonal growth or five parameters von Bertalanffy growth model (5 Parameters VBGF) was described using the Somers's (1988) version of the VBG equation:

$$L_t = L_\infty\left[1 - e^{\left[-K(t-t_0)-\left(C\frac{K}{2\pi}\right)\sin 2\pi(t-t_S)+\left(C\frac{K}{2\pi}\right)\sin 2\pi(t-t_S)\right]}\right] \quad (2)$$

where, L_t is length at age t, L_∞ is the asymptotic length to which the sea cucumber growth, K is the growth-rate parameter, t_0 is the nominal age at which the length is zero, C is the relative amplitude ($0 \leq C \leq 1$) of the seasonal oscillations, t_S is the phase of the seasonal oscillations ($-0.5 \leq t_S \leq 0.5$) denoting the time of year corresponding to the start of the convex segment of sinusoidal oscillation.

The time of the year when the growth rate is slowest, known as the winter point (WP), was calculated as:

$$WP = t_S + 0.5 \quad (3)$$

Seasonal VBG curves were fitted to the length distributions after first indicating a range of values of L_∞ and K and reducing iteratively the range to maximize the goodness of fit (Rn) of the curves to the data. Rn was calculated as:

$$Rn = \frac{10^{\frac{ESP}{ASP}}}{10} \quad (4)$$

where ASP is the available sum of peaks, computed by adding the best values of the available peaks, and ESP is the explained sum of peaks, computed by summing all the peaks and troughs hit by the VBGF curve.

Analysis of the length data were fitted to length frequency distributions grouped in 2 cm total length size classes using the Electronic LEngth Frequency ANalysis (ELEFAN) procedure in the PC-based computer package Version 5.0 of Length-Frequency Distribution Analysis (Kirkwood et al., 2003). The ELEFAN procedure first restructures length frequencies and then fits a VBGF curve to the restructured data. Both seasonal and non-seasonal VBGF curves were fitted to the seasonal length distribution after providing a range of values for the parameters to be estimated and then iteratively reducing the range until the goodness of fit of the curve to the data is maximized.

Reliability of Growth Parameter Estimates

Having estimated a set of growth parameters, one would like to evaluate their reliability. A possible test is the so-called phi-prime test (*Φ'*) known as growth performance index. This test is based on the discovery by Pauly and Munro (1984) that *Φ'* values are very similar within related taxa. So, the growth performance comparisons were made using the growth performance index (*Φ'*) which is preferred rather than using L_∞ and *K* individually (Pauly and Munro, 1984) and is computed as:

$$Q' = \log(K) + 2\log(L_\infty) \quad (5)$$

Results

Population Structure

A total of 221 *Holothuria atra* and 258 *Holothuria arenicola* were sampled between January and December 2014. The total length ranged between 12 and 34.5 cm (mean 19.7±0.38 cm, 95% confidence interval: 19.0-20.5 cm) for the *H. atra* (Figure 2) and between 13 and 33 cm (mean 23.3±0.30 cm, 95% confidence interval 22.7-23.9 cm) for the *H. arenicola* (Figure 3). The length composition data revealed that most individuals in both *H. atra* (76.9%) and (72.5%) *H. arenicola* species ranged from 14 to 24 cm. Larger *H. atra* individuals (>22-24 cm) were not obtained in January, February, March and October. Smaller *H. arenicola* individuals (<16-20 cm) were not determined in August, September, October and December. Size frequency distributions were significantly different (Kolmogorov-Smirnov two-sample test; d=0.395, *P*<0.0001) between two species. The mean total length of *H. arenicola* was significantly (t-test: 4.814E13, *P*<0.0001) greater than the mean total length of *H. atra*.

Seasonal and Non-Seasonal Von Bertalanffy Growth Parameters

The Hoenig seasonal and non-seasonal VBGF curves parameters obtained from the LFDA for each species are summarized in Table 1. The *Rn* value of the non-seasonal growth curve for both *H. atra* and *H. arenicola* improved when the seasonal growth curves were fitted (Table 1), suggesting that, at least for our data, these two sea cucumbers exhibit seasonal growth patterns. This seasonality in growth was also apparent in the results of the relative amplitude values (*C*=0.50 for *H. atra* and *C*=0.37 for *H. arenicola*) of the seasonal oscillations and in Figure 4B and Figure 5B where sinusoidal pattern could be observed in the Hoenig seasonal von Bertalanffy growth curve.

The slowest growth period started at the middle of April for *H. atra* (*WP*=0.30; Figure 6). For *H. arenicola*, however, the start of slow growth period was at the beginning of August (*WP*=0.60; Figure 6).

The calculated growth performance indices (*Φ'*; Table 1) of seasonal growth for *H. atra* (*Φ'*=2.989) was greater than the *H. atra* (*Φ'*=2.930) (Table 1).

Table 1. Seasonal and non-seasonal von Bertalanffy growth parameters estimated from the length frequency distribution analysis and maximum life span for *Holothuria atra* and *Holothuria arenicola*. L_∞, asymptotic total length (cm); *K*, growth coefficient (year^{-1}); t_0, age at zero length; WP, winter point; *C*, amplitude of growth oscillation; *Rn*, goodness of fit index; *Φ'*, growth performance index; A_{95}, the life span to attain 95% of L_∞, calculated from the VBG equation.

Parameters	*Holothuria atra*		*Holothuria arenicola*	
	Hoenig Seasonal	Non-Seasonal	Hoenig Seasonal	Non-Seasonal
L_∞ (cm)	36.09	35.37	34.86	34.86
K (year^{-1})	0.75	0.76	0.70	0.74
t_0 (year)	-0.23	-0.27	-0.19	-0.15
WP	0.30	-	0.60	-
C	0.50	-	0.37	-
Rn	0.296	0.263	0.502	0.423
Φ'	2.99	2.99	2.93	2.95

Age Structure and Growth Rate

Age–length key calculated from the Hoenig seasonal VBGF curves parameters both *H. atra* and *H. arenicola* individuals are showed in Figure 7. Length for the first age class (0 years old) was estimated as 14.2±1.38 cm (between 7.7 and 21.3 cm) for *H. atra* and 13.1±1.41 cm (between 4.8 and 18.6 cm) for *H. arenicola* by LFDA method with ELEFAN model.

The calculated mean total length in the age classes showed that the *H. atra* individuals reached 73.9% of their maximum total length (L_{max}=34.5 cm) at the second age class (one year old; 10.3±0.39 cm). For *H. arenicola* it was also calculated as 72.7%. This fast growth characteristic of small individuals was also apparent in the growth curves in Figure 4 and Figure 5, where it could be observed that the slight slope in the larger

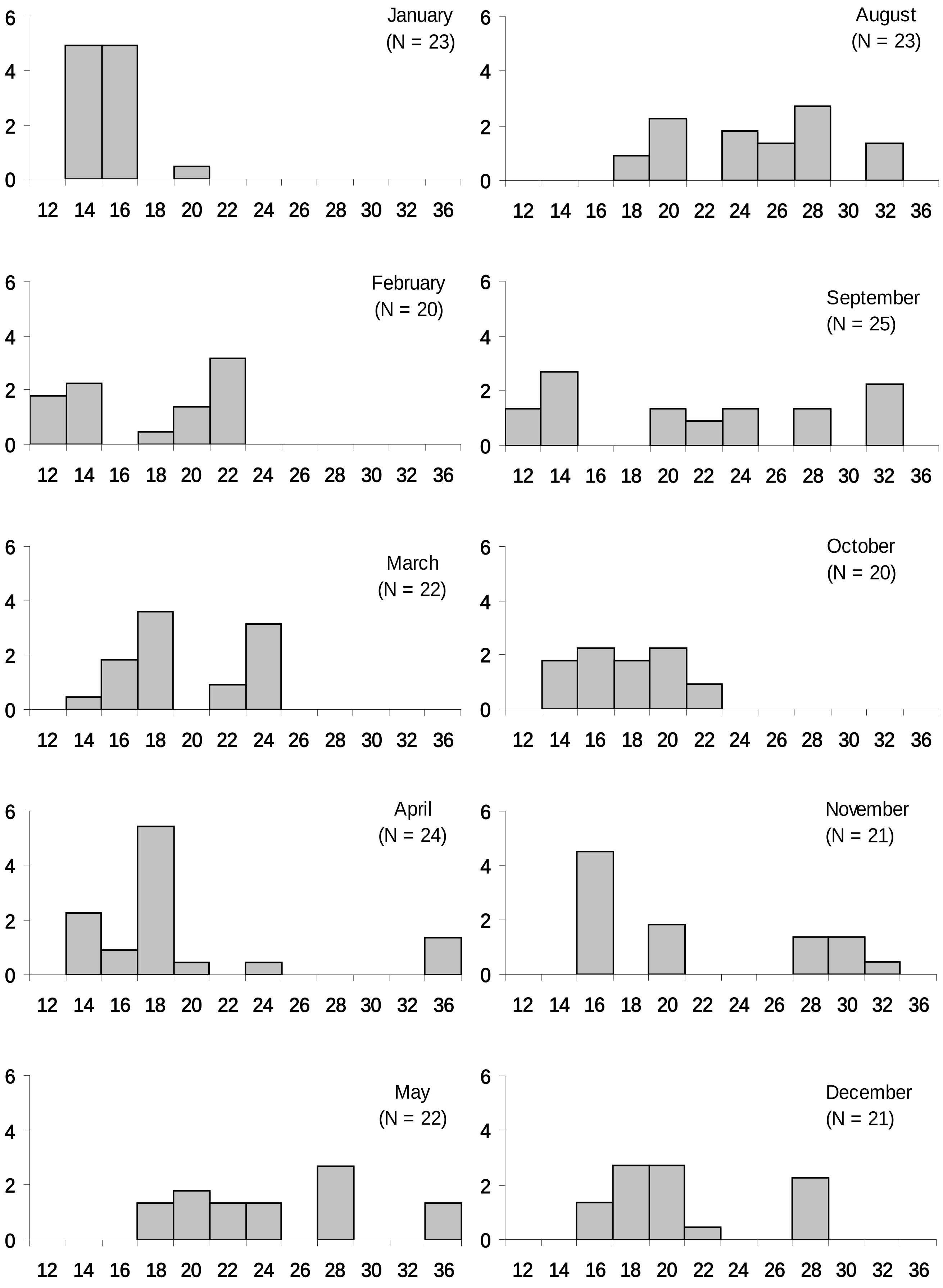

Figure 2. Monthly total length (2 cm size classes) frequency distributions (in percentages) of *Holothuria atra* between January and December 2014

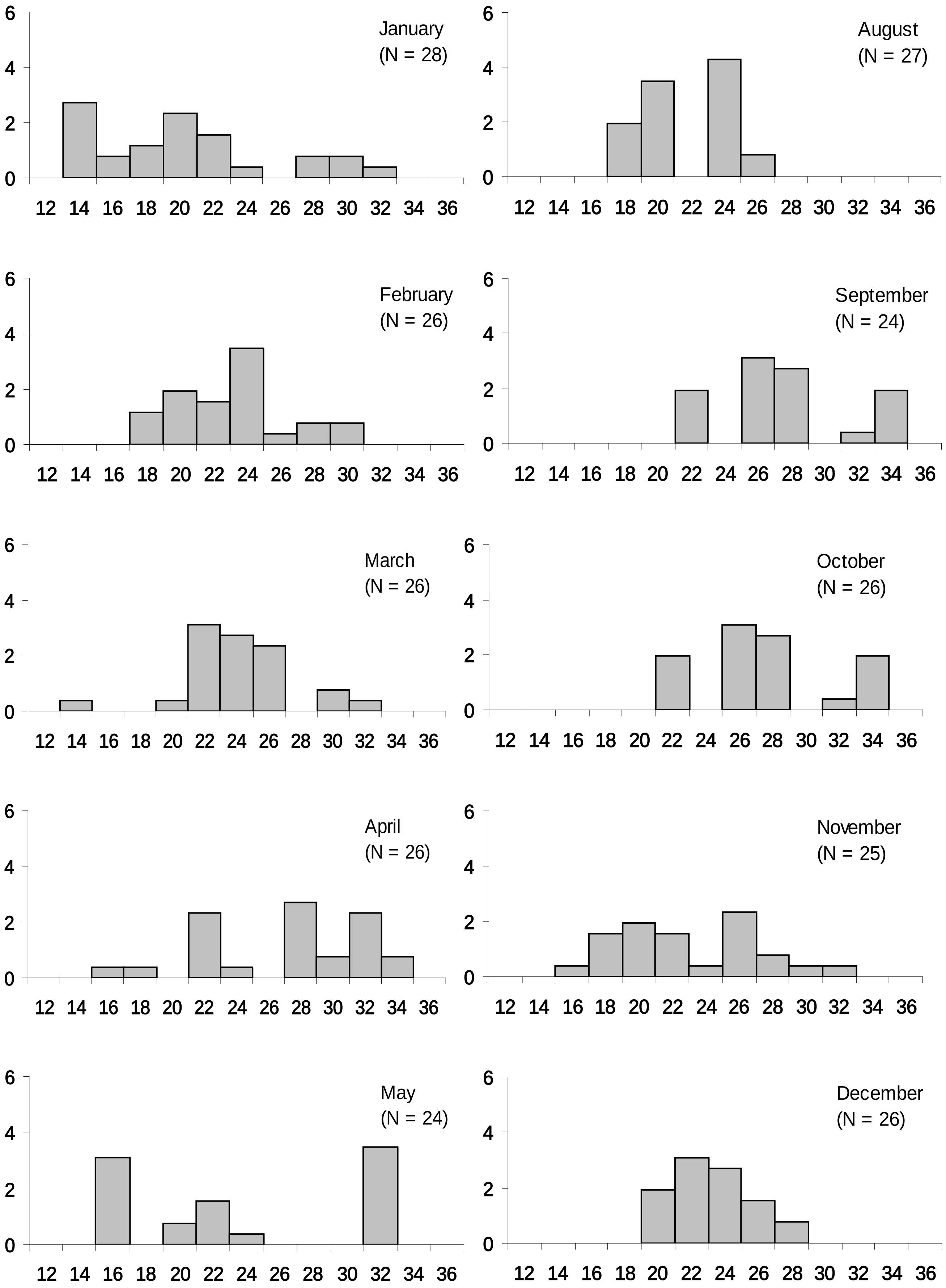

Figure 3. Monthly total length (2 cm size classes) frequency distributions (in percentages) of *Holothuria arenicola* between January and December 2014

individuals compared to smaller sea cucumber leading to small individuals grew faster than large ones.

Monthly mean growth rate of *H. atra* was calculated as 1.18 cm/month for 0-365 days (0 age class), 0.97 cm/month 365-730 days (1 age class), 0.45 cm/month for 730-1095 days (2 age class) by using estimated average total length values in the ages. For *H. arenicola*, however, it was calculated as 1.09 cm/month for 0-365 days (0 age class), 0.91 cm/month 365-730 days (1 age class), 0.46 cm/month for 730-1095 days (2 age class). In older individuals (>1095 days old), it almost tended to steady state for two species.

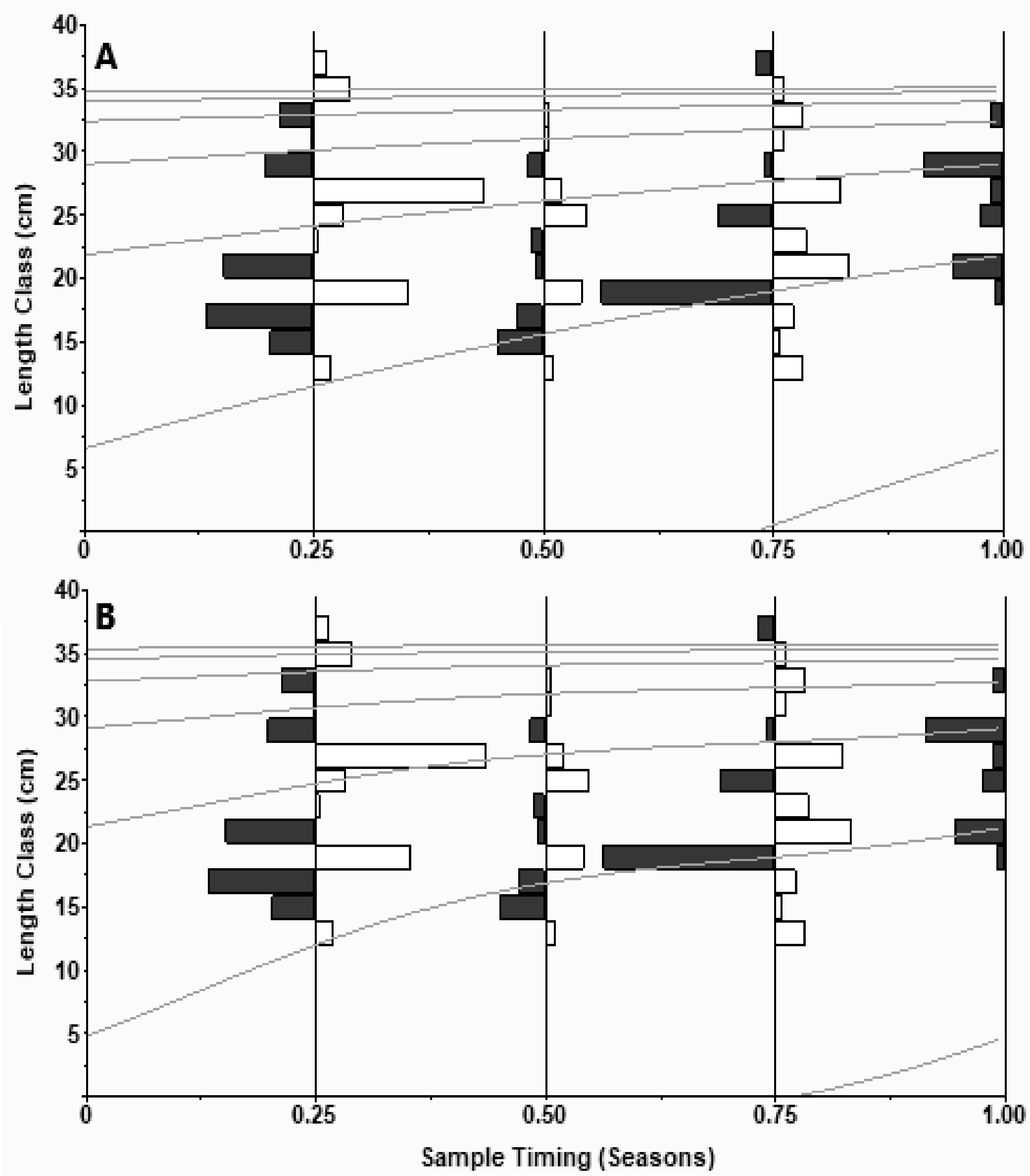

Figure 4. Growth curve (lines) from the von Bertalanffy model with non-seasonal (A) and the Hoeing seasonal (B) by using electronic length-frequency analysis (ELEFAN), to length-frequency data of *Holothuria atra* sampled from Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan between January 2014 and December 2014

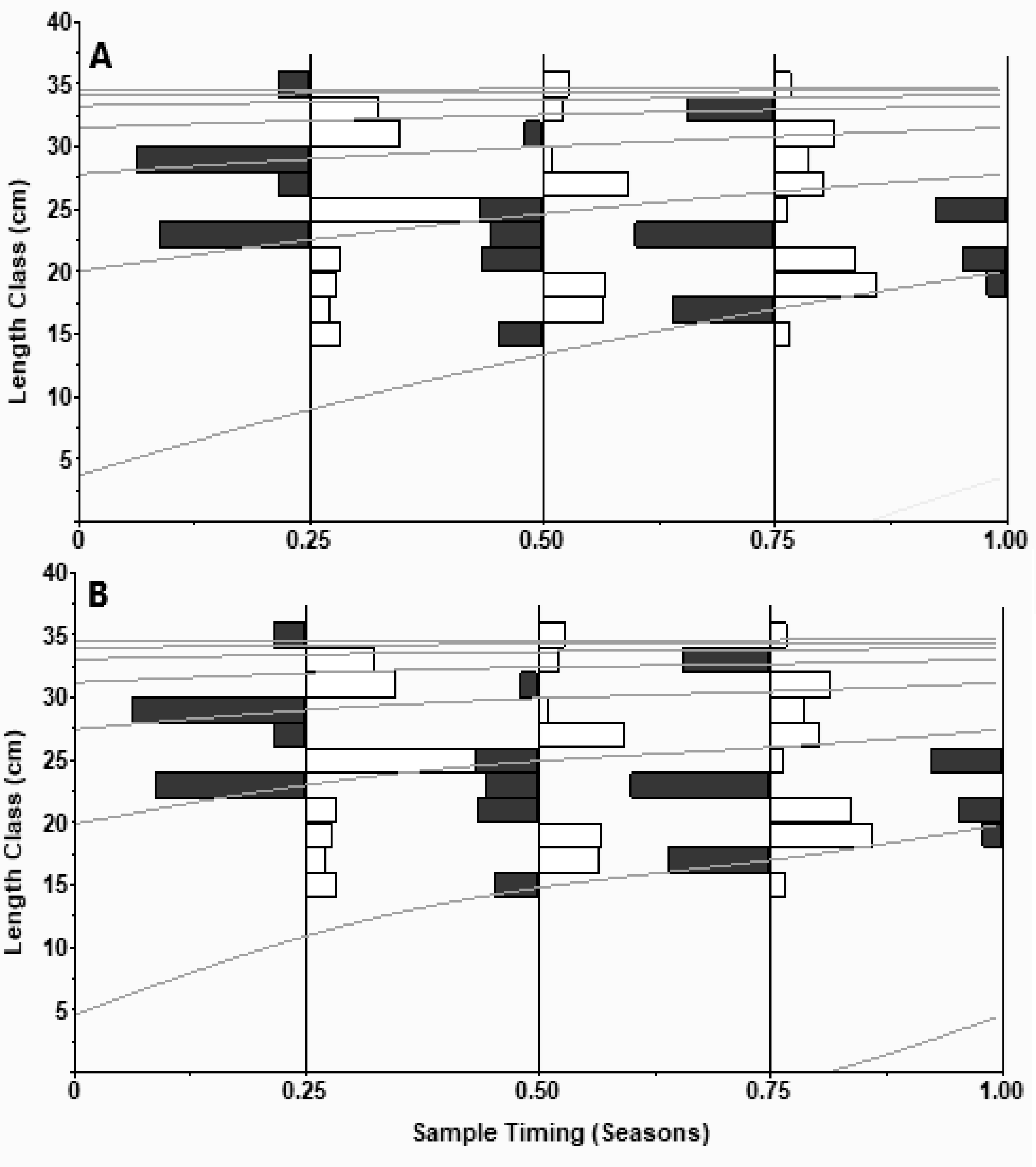

Figure 5. Growth curve (lines) from the von Bertalanffy model with non-seasonal (A) and the Hoeing seasonal (B) by using electronic length-frequency analysis (ELEFAN), to length-frequency data of *Holothuria arenicola* sampled from Buleji and Sunehri in Karachi coast, north Arabian Sea, Pakistan between January and December 2014

Discussion

To the best of our knowledge this is the first study to calculate the Hoenig seasonal growth curve parameters by using ELEFAN, to length-frequency data and age–length key of two holothurians, *H. arenicola* and *H. atra*. Our LFDA results showed that both *H. arenicola* and *H. atra* have similar length-frequency distribution and modes. Different spatial and temporal variation of LFDA and/or modes was reported for *H. atra* from Manora and Buleji in the previous study (Siddique and Ayub, 2005). According to Siddique and Ayub (2005), the small-sized individuals (<12 cm) were equally abundant at both sites, the medium-sized individuals (12-20 cm) were more (37.7% of the total population) at Buleji than at Manora (20.6%) and the larger-sized individuals (>22 cm) were higher in numbers at Manora (21.2%) than at Buleji (2.4%). Moreover, bimodal distribution (consisting of small and medium-sized individuals in summer and autumn), three modes (winter and spring) from Manora and unimodal distribution from Buleji were reported. The mean total length of examined individuals in Buleji (mean length: 11.6±3.5 cm, between 3.8 and 37.6 cm) was also reported as higher than Monara (mean length: 14.1±7.6 cm, between 2.7 and 41.6 cm) during the period from April 2011 to November 2012. In our study, the mean total length (mean: 23.3±0.30, between 13.0 and 33.0 cm) of examined individuals were about 10-11 cm larger than the study of Siddique and Ayub (2005) and also, we have not observed small-sized individuals (<12 cm). In the present study, the sea cucumbers smaller than 10-12 mm could not be obtained in the sampling area both two species, most probably due to the differences in behavioral characteristics (e.g. burrowing cycle) by size. Mercier et al. (2000) reported that juveniles *H. scabra* ≤10 cm burrowed at sunrise and surfaced at sunset, whereas individuals >10 cm burrowed and surfaced a few hours earlier (in the middle of the afternoon). According to these results described above, it can be concluded that the growth and length composition for examined species in the research region differ significantly between years and between regions due to the marine environmental variability such as site health, e.g., water quality (dissolved oxygen and nutrients), hydrodynamics (monsoonal effect, current, water movement). Secondly, because of the different natural mortality and migration or recruitment levels. Migration of *H. atra* from one area to another was reported by different researchers due to rolling with the water movement (Tamaroa, 2010) and also due to lack of dissolved oxygen and nutrients in any environment (Uthicke, 2004).

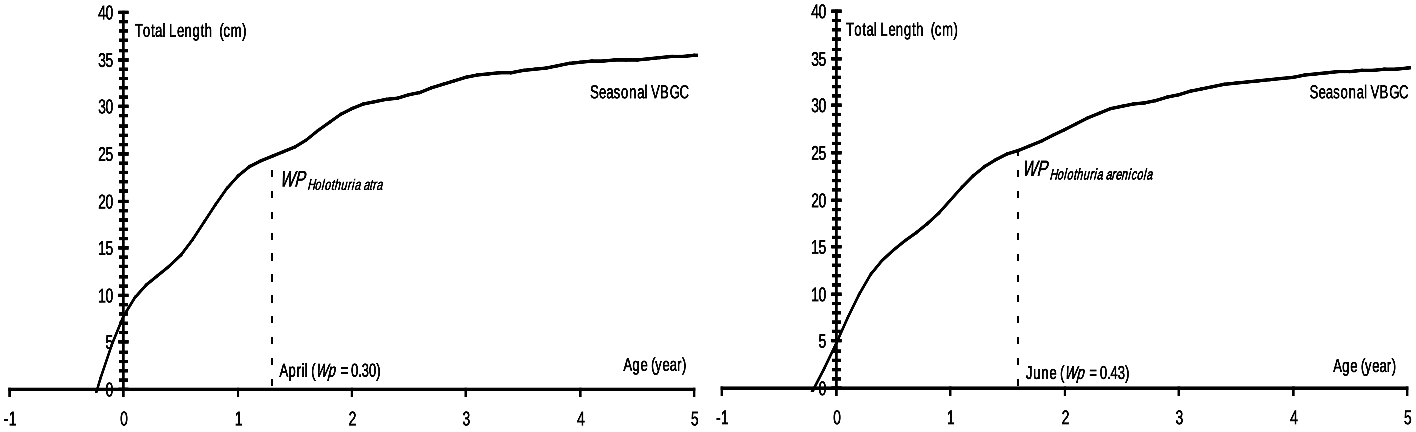

Figure 6. Winter point (*Wp*) of the seasonal oscillations in the seasonal von Bertalanffy growth function curves for *Holothuria atra* and *Holothuria arenicola* in the north Arabian Sea, Pakistan

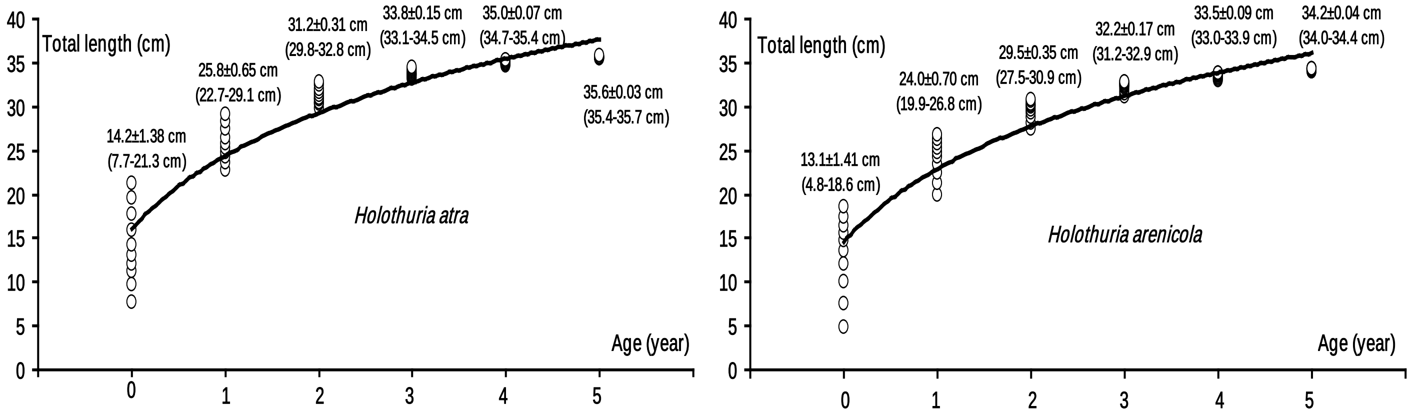

Figure 7. Length–age key (estimated length for each year class) from the seasonal von Bertalanffy growth function curves parameters both *Holothuria atra* and *Holothuria arenicola* in the north Arabian Sea, Pakistan. Values as mean ± std. err. (minimum–maximum)

The observed growth rates (*K*>0.70) indicate that examined two species achieve asymptotic size quickly, even faster some of the other holothurians, such as *I. badionotus* (*K*=0.2), *I. fuscus* (*K*=0.18), *H. pulla* (*K*=0.24). On the other hand, similar growth parameters were also reported for many other holothurian species such as *H. arguinensis* (*K*=0.88), *I. badionotus* (*K*=0.7) (Table 2). The general assumptions for different length-based growth models for the growth determination of holothurians reported as (Olaya-Restrepo et al., 2018): *i*) species reproduce and recruit once per year; *ii*) species reach a specific size; and *iii*) species grow isometrically (*b*=3). Most commercial sea cucumber species fulfill the first two assumptions, but usually they do not exhibit isometric growth (Olaya-Restrepo et al., 2018). For *H. atra* and *H. arenicola*, negative allometric growth has been reported (Siddique et al., 2015; Ahmed et al., 2018b). However, much larger species such as *I. badionotus* are long lived and usually have relatively low values for *K*. In such cases it could be expected that life span is extended considerably. For *I. badionotus* in Sisal, at asymptotic population size was reported ten years while *K* was as low as 0.20 year^{-1} (Poot-Salazar et al., 2015). Faster growth rates may have important and positive effects on the mortality of holothurians such as *Australostichopus mollis* (Hutton, 1872), primarily under high stress conditions and in disturbed environments (Morgan, 2012) and so, the fast growth could be considered as an adaptive advantage. Namely, larger sea cucumbers would be capable of moving and stabilizing themselves in more mobile sediments, such as sandy substrates, and they would also be more likely to escape predation (Mercier et al., 2000; Olaya-Restrepo et al., 2018). In our study, calculated *K* (0.75 year^{-1}) and L_∞ (36.1 cm) values for *H. atra* were found to be larger than the *K* (0.11 year^{-1}) and L_∞ (32.4 cm) values reported in the previous study, conducted between 1975 and 1976 on the seaward reef bench of Ananij Island, Enewetak Atoll, Marshall Islands (Elbert, 1978) (see: Table 2). This difference between our study and Elbert's study, which was carried out about 42 years ago, may have resulted from the difference between biotic and abiotic factors such as prey availability, predators, genetic variation, salinity, habitat structure, etc. and also may be due to the difference of the method used to calculate of the growth. When there is a seasonal growth pattern for holothurian species belonging to the same family in a geographical region, the estimations of L_∞ and *K* may differ significantly between the seasonal and non-seasonal models. In the present study, the non-seasonal VBG model provided realistic results. However, when seasonality was included (with the Hoenig model), more reliable values were obtained, which confirmed the seasonality in the growth of *H. arenicola* and *H. atra*.

Table 2. Growth parameters of different sea cucumber species belong to different family. L_∞, asymptotic total length (cm); *K*, growth coefficient (year^{-1}); *Φ*', growth performance index

Family	Species	L_∞ (cm)	*K* (yr^{-1})	*Φ*'	Area	Ref.
Stichopodidae	*Isostichopus badionotus*	31.9	0.60	2.786	Celestun, Mexico	1
	Isostichopus badionotus	38.0	0.20	2.461	Sisal, Mexico	1
	Isostichopus badionotus	23.5	0.70	2.587	Progreso, Mexico	1
	Isostichopus fuscus	36.1	0.18	2.370	Gulf of California, Mexico	2
Stichopodidae	*Stichopus vastus*	31.6	0.55	2.739	Karimunjawa, Indonesia	3
	Stichopus quadrifasciatus	38.7	0.34	2.707	Karimunjawa, Indonesia	4
Holothuriidae	*Holothuria arguinensis*	66.9	0.88	3.595	South Portugal	5
	Holothuria atra	32.4	0.11	2.062	Ananij, Marshall Islands	6
	Holothuria scabra	29.0	0.52	2.641	Bolinao, Philippines	7
	Holothuria pulla	30.7	0.24	2.354	Bolinao, Philippines	7
	Holothuria pardalis	18.0	1.00	2.511	Karachi, Pakistan	8
	Holothuria verrucosa	18.0	0.86	2.445	Karachi, Pakistan	8
	Holothuria atra	36.1	0.75	2.990	Karachi, Pakistan	9
	Holothuria arenicola	34.9	0.70	2.931	Karachi, Pakistan	9

Note: 1) Poot-Salazar et al., 2015; 2) Herrero- Pérezrul et al., 1999; 3) Sulardiono et al., 2012; 4) Sulardiono and Muskananfola, 2019; 5) Olaya-Restrepo et al., 2018; 6) Elbert, 1978; 7) Pauly et al., 1993; 8) Ahmed et al., 2020a; 9) Present study.

The *Rn* and *C* values with visual growth curves both *H. arenicola* (Figure 4B) and *H. atra* (Figure 5B) evidenced that these two species exhibited marked seasonality in growth. Seasonal growth pattern was also reported from different regions for different holothurians such as *H. arguinensis* from South Portugal (Olaya-Restrepo et al., 2018) and *I. badionotus* off the northwest coast of Yucatan state, Mexico (Poot-Salazar et al., 2015), *I. fuscus* at Espiritu Santo Island, Gulf of California, Mexico (Herrero-Perezrul et al., 1999) and also for *H. pardalis* and *H. verrucosa* population along Karachi coast, northern Arabian, Sea Pakistan (Ahmed et al., 2020a). Since there is no information in the literature reporting the parameters showing seasonality in growth for *H. arenicola* and *H. atra*, we were unable to compare our findings with others. However, the major factors that cause seasonality in the growth of marine organisms belong to different taxa such as marine invertebrate including shrimps and sea cucumbers were reported to be *i*) photoperiod, *ii*) variation in water temperature and salinity fluctuating over the year, *iii*) seasonal change in nutrient quality/availability, *iv*) energy input into reproduction during the breeding season (Bilgin et al., 2009a, 2009b; Poot-Salazar et al., 2014; Olaya-Restrepo et al., 2018). Unfortunately, detailed studies neither of productivity along the northern Arabian Sea coasts nor of the sea cucumber species reproduction biology in these regions yet exists (except for the spawning time of *H. arenicola*). For *H. arenicola*, it has been reported that the gonad activity began to increase regularly in March, reached the highest value at the beginning of the summer (in June), and decreased the lowest value at the end of the summer (in August) (Siddique and Ayub, 2015). However, *H. atra*, reproduction features based on gonad development reported in the Red Sea coast of Egypt (Abdel-Razek et al., 2005), in southern Taiwan (Chao et al., 1994), New Caledonian lagoon (Conand, 1993), and at Heron Reff, Greak Barrier Reff (Harriott, 1985). Temperature, salinity, food abundance and photoperiod have all been known as factors regulating holothurian reproductive cycle. The coast of Pakistan is for most of the year influenced by high-salinity surface water (36-38‰) and the sea surface temperature during summer is 28-30°C while during winter, it is 21-24°C (Siddique and Ayub, 2015). Furthermore, there is monsoonal variations in nutrient concentration due to monsoon winds and fluctuations of rainfall (Hussaina et al., 2010). Such variations may also be related to productivity and avai1ability of food and to the reproductive cycle and growth of *H. arenicola* and *H. atra* and also other sea cucumber species in the north Arabian Sea, Pakistan (Ahmed et al., 2020a). All these could be considered as the factors forcing seasonality in growth both *H. arenicola* (Figure 4B) and *H. atra* (Figure 5B) in the study area.

The variations in the period of the slowest growth time (*WP*) generally related to environmental condition which cause biological stress, physiological conditions of the marine animal, fullness of stomach and gonads stages (Bilgin et al., 2009a, 2009b; Ahmed et. al., 2016b, 2020a). Temperature also plays an important role in the reproductive events and the abundance of food, and therefore especially the water temperature indirectly effective on the *WP* of the marine organisms. The seasonal and/or monsoonal variations in the mean condition factors of *H. arenicola*, *H. atra*, *H. pardalis* and *H. verrucosa* was reported from the coasts of Karachi, Pakistan (Siddique et al., 2014; Ahmed et al., 2018a, 2018b) and also the gonad index (*GI*) based reproduction time for *H. arenicola* was reported during spring and early summer from these regions (Siddique and Ayub, 2015). The period of slowest growth for *H. arenicola* corresponded to June when the highest *GI* value occurs (see Siddique and Ayub, 2015). The slowest growth period for *H. atra*, however, was estimated to be April, which is the period with a relatively low water temperature and with the most probably when reproductive cells (gonad development) begin to mature or develop (Harriott, 1985; Conand, 1993; Chao et al., 1994; Abdel-Razek et al., 2005). Since the growth rate of holotorians depends on the food availability and quality of the habitat and/or organic matter content of sediment, dissolved oxygen concentration in the water column and in sediment (Tamaroa, 2010) and also reproduction events, the slow growth of *H. atra* in April may be the result of the extended both poor nutrition and reproduction periods growth but this was not tested in this study. Therefore, the effects of these factors on growth should be examined in the future studies.

Conclusion

Together with this study seasonal growth was first pronounced for both *H. arenicola* and *H. atra*. Although the causes of this seasonality are not tested in this study, the effect of fluctuations in the amount of nutrient and maturity levels are probably important for this situation. The effects of the climatic events as monsoon rain and reproduction activities on growth should be studied in detail in order to provide data for holotorian fisheries management in the north Arabian Sea, Pakistan.

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Compliance with Ethical Standards

Authors' Contributions

The data of this study was provided by the first author. The statistical analysis and other stages with writing of the manuscript were all carried out by corresponding author.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was conducted in accordance with ethics committee procedures of animal experiments.

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