Title

Allometric relationships to assess ontogenetic adaptive changes in three NE Atlantic commercial sea cucumbers (Echinodermata, Holothuroidea).

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Introduction

Several sea cucumber species are heavily consumed in south-eastern Asia. Their high commercial value has led to an increasing demand resulting in a massive and unregulated exploitation of this resource. The overexploitation of these echinoderms eventually drove most natural stocks to collapse in the Pacific region (Purcell et al., 2013; Purcell, 2014). As a result of local stock depletion and closure of some regional and national fisheries (Purcell et al., 2014), the exploitation of natural stocks expanded to other areas endangering additional species (Purcell et al., 2013; Erikson & Clarke 2015). In the North-eastern Atlantic and the Mediterranean Sea, several sea cucumber species, new to the international trade markets, are now targeted for directed fisheries. The current fishing pressure (some occurring illegally) has already led, in some regions, to a decrease in density, abundance and genetic diversity as well as loss of the largest size classes, increase of the prevalence of some diseases and even local stock depletion (González-Wangüemert et al., 2014; González-Wangüemert et al., 2018).

Allometry is the study of the relation between the size of an organism and aspects of its physiology, morphology, and life-history (Bradshaw & McMahon, 2008; Gould, 1965). The study of allometric relationships allows us to easily identify variations of shape with growth (Hallgrimsson, 2015). These variations lead to an improvement of physiological and ecological efficiency (Gould, 1965). Through length-weight relationships, a tool commonly used in fisheries biology, allometric parameters can be assessed to understand how an increase in length relates to an increase in weight (Froese, 2006) and the underlying ontogenetic adaptations that drive such relationships (Alberts & Harshaw, 2014). However, in sea cucumber's scientific literature, allometric coefficients have mostly been used to understand the change in shape between smaller and bigger animals (Conand, 1993; Herrero-Pérezrul & Reyes-Bonilla, 2008; Kazanidis et al., 2010; Ramón et al., 2010; Poot-Salazar, 2014; Marquet et al., 2017; Ahmed et al., 2018). Also, variation of allometric coefficients between regions and habitats, for the same species, is usually not addressed.

The aim of the present study was to (i) determine the length-weight relationships of *Holothuria (Holothuria) mammata,* Grube, 1840, *Holothuria (Roweothuria) arguinensis* Koehler & Vaney, 1906 and *Holothuria (Panningoturia) forskali*, Delle Chiaje, 1823; (ii) assess the inter-specific ontogenetic differences in response to external factors; and (iii) determine, through the analysis of allometric

relationships, how the variation of the allometric coefficient could provide clues on intra-specific regional ontogenetic adaptation to different protection regulations.

Methods

Study area

The study was focused on a NE-Atlantic coastal area in the southwest of Portugal (38°28'40.7"N 8°58'21.4"W) (Figure 1). Most of this coastline is a marine protected area (MPA) with regulation that bans most fishing activities (none of which susceptible of capturing sea cucumbers) and restricts human presence. It is also physically protected by the surrounding Arrábida mountain range from the dominant swell and prevailing winds (Jacob et al., 2009), providing a sheltered environment (Henriques et al., 1999). Different habitats compose this MPA, such as rocky reefs, kelp forests, sea grass beds and sand banks (Gonçalves et al., 2002). For these reasons, this area allows the study of population parameters in the most pristine-like conditions possible in the Portuguese coastline.

Sampling

Starting in January 2018 and for 16 months, monthly samples of around 30 individuals of each species (*H. forskali*, *H. mammata* and *H. arguinensis*) were collected by scuba diving. In some occasions, due to environmental conditions, the number of sea cucumbers caught varied. Sea cucumbers were individualized in plastic containers, *in situ*, guaranteeing no loss of eviscerated material, and brought back to the laboratory. Length and weight of sea cucumbers are variable and easily influenced by manipulation, due to physiological processes acting as a response to environmental stimuli (e.g. muscle contraction). So, to obtain reasonable comparisons of these biometric parameters, the handling technique was standardized. All individuals were immersed on ice (until there was no apparent mechanical response), measured for total length (TL – to the nearest mm) and total (W) and gutted (Wev) weights (to the nearest 0.01g). Standard error (SE) was used to show uncertainty of the mean. Gut content of all individuals was analysed macroscopically, aiming to determine its main content, either sediment or other.

An additional visual census sampling was done every month, for the same period of 16 months, to understand the differences of habitat preferences between the three species and relate them to their allometric coefficients. Two habitats were defined: Rocky reef and sandy area. Three transects (replicates) were done in each, parallel to the coast, at the same depth stratum. Every transect was thirty meters long

and three meters wide, covering an area of 90 square meters. Along each transect, sea cucumbers were identified to species level and counted. In rocky reefs there were sand patches and in sandy areas there were isolated rocks so within each habitat, sea cucumbers could be settled on a hard surface or on soft substrate and this information was recorded. Finally, sheltered sea cucumbers and sea cucumbers concealed in rock crevices or other objects, were also registered accordingly. So, for each sea cucumber, three variables were determined:

- Habitat: Rocky reef or sandy area.

- Surface: where they were settled within each habitat: hard surface or soft substrate.

- Shelter: Sheltered or exposed.

Data analysis

To analyse relative changes to sea cucumbers' morphology, allometric relationships were assessed using the allometric equation established by Keys (1928):

$$y = ax^b \qquad (1)$$

where b is the allometric coefficient and a is the intercept, estimated from least-squares fitting method of the $\log_{10}$-transformed variables:

$$\log y = \log a + b \log x \qquad (2)$$

A Pauly's t-test was performed (Pauly, 1984) (eq. 3) to test whether the allometric coefficient estimated in eq. 2, was significantly different from isometric growth (i).

$$t' = \frac{\text{S.D.}x}{\text{S.D.}y} \cdot \frac{|b-i|}{\sqrt{1-r^2}} \cdot \sqrt{n-2} \qquad (3)$$

where: t' is the critical value of t; S.D.x is the standard deviation of TL or W and S.D.y is the standard deviation of W or Wev, depending on the relation; b is the estimated value of the allometric parameter; i is the value that indicates isometric growth; n is the number of sea cucumbers used in the computation. The total weight (W) was used as the proxy for sea cucumber's total volume and Wev for body wall volume. For those reasons, isometric growth between TL and both W and Wev, occurs when $b = 3$ and positive or negative allometric growth when $b > 3$ or $b < 3$, respectively. Between W and Wev, isometric growth occurs when $b = 1$ and positive or negative allometric growth when $b > 1$ or $b < 1$, respectively.

The value of *b* is significantly different from *i*, indicating allometric growth, if t' value is greater than the tabled t values for n-2 degrees of freedom (Pauly 1984).

Confidence and prediction intervals were computed with an error level $\alpha = 0.05$ to the fitted regressions on $\log_{10}$ transformed variables and then fitted to the allometric equations after anti-$\log_{10}$ transformation (Nagy et al., 1999).

Results

Overall, a total of 1414 sea cucumbers were caught, of which 490 belonging to *Holothuria mammata* species, 436 to *Holothuria arguinensis* and 488 to *Holothuria forskali*. Total length (TL), Total weight (W) and Gutted weight (Wev) measurements revealed HM as the longest species and HA the heaviest (Table 1). Analysis of gut content revealed HM and HA ingested exclusively sand, whereas HF showed only organic matter content, the majority of which macroalgae.

Table 1: Summary of the data of the three studied species captured throughout the sampling period – *Holothuria mammata*, *Holothuria forskali* and *Holothuria arguinensis*. N – number of sampled individuals; TL – Average Total length; W – Average Total Weight; Wev – Average Gutted weight.

Species	N	TL (mm ±SE)	W (g ±SE)	Wev (g ±SE)
Holothuria mammata	490	219.74 ± 1.97	316.91 ± 5.30	134.88 ± 2.01
Holothuria arguinensis	436	188.00 ± 2.49	469.28 ± 9.91	213.59 ± 4.48
Hholothuria forskali	488	164.30 ±1.43	141.35 ± 2.14	81.02 ± 0.95

Through the visual census (Figure 2), 1640 sea cucumbers belonging to *H. mammata*, 2956 to *H. forskali* and 119 to *H. arguinensis*, were recorded. Regarding settlement substrate, hard surfaces had higher percentages of *H. mammata* and *H. forskali* settled (65% and 80%, respectively). *H. arguinensis* was evenly distributed on hard surfaces or soft substrate (50%/50%). Finally, 34% of *H. mammata* and 17% of *H. forskali* individuals were found to be sheltered, whereas only 3% of *H. arguinensis* were concealed in crevices.

The W and Wev reveal negative allometry with TL (b < 3) for the three species. The models present a fair fit for *H. mammata* (W~TL -> $R^2 = 0.52$; Wev~TL -> $R^2 = 0.50$) and a slightly better fit for *H. arguinensis* (W~TL - $R^2 = 0.65$; Wev~TL - $R^2 = 0.61$) (Figure 3). However, for *H. forskali*, this is not the case (W~TL

- $R^2 = 0.36$; Wev~TL - $R^2 = 0.30$). Wev~W allometric relationships show good fits for all species ($0.72 \leq R^2 \leq 0.90$).

The allometric equations ($y = ax^b$) between all measured parameters (TL, W and Wev) and estimates are present in Table 2. All species revealed significant negative allometric growth (W~TL) and significant negative allometric scaling for all relations. *Holothuria arguinensis* revealed the highest allometric coefficient (*b*) for all relations and *H. forskali*, the lowest.

Table 2: Model summaries for the allometric relationships of the three species at the Arrabida Marine Park, including statistical results. Weight (W), gutted weight (Wev) and total length (TL) were the parameters considered for the allometric relationships.

Species	Model	N	Hypothesis	CI 95% for *b*	R^2	Pauly's t-test	*p*
Holothuria mammata	$W = 0.1859*TL^{1.3723}$	490	$b = 3$	0.1161	0.5244	27.51	0.001
	$Wev = 0.2049*TL^{1.1984}$	488	$b = 3$	0.1056	0.5031	31.59	0.001
	$Wev = 1.3813*W^{0.7962}$	488	$b = 1$	0.0457	0.7977	10.61	0.001
Holothuria arguinensis	$W = 0.0356TL^{1.723}$	436	$b = 3$	0.1203	0.6457	21.28	0.001
	$Wev = 0.0184TL^{1.6983}$	436	$b = 3$	0.1277	0.6106	19.84	0.001
	$Wev = 0.5695W^{0.9612}$	437	$b = 1$	0.0394	0.9043	4.36	0.001
Holothuria forskali	$W = 0.6085*TL^{1.061}$	488	$b = 3$	0.1258	0.3603	33.48	0.001
	$Wev = 1.5865*TL^{0.7667}$	487	$b = 3$	0.1027	0.3096	42.88	0.001
	$Wev = 2.8831*W^{0.6749}$	481	$b = 1$	0.0476	0.7215	17.39	0.001

No data were found on allometric relationships for *Holothuria forskali* in other studies (Table 3). The allometric coefficient (*b*) of *Holothuria mammata* for allometric relations in other studies was addressed with gutted weight (Wev) and total length (TL) or gutted length (TLev). In the south of the Iberian Peninsula (Wev~TL), *b* was reported between 0.924 and 1.175, slightly lower than our estimate. However, the *b* estimate in the Aegean Sea (Wev~TLev) was higher ($b = 1.752$). In Sagres, Olhos de Água and Ria Formosa (southern coast of Portugal), the allometric coefficients for the Wev~TL relation, for *Holothuria*

arguinensis species, were 1.038, 1.437 and 0.980, respectively. For the same relation and species, our study revealed a higher allometric coefficient than all of the stated above (b = 1.698).

Table 3. Allometric coefficients (*b*) of *Holothuria mammata* (HM), *Holothuria arguinensis* (HA) and *Holothuria forskali* (HF) populations from other studies in different regions). The parameters considered for the relations on these studies were weight (W), gutted weight (Wev), total length (TL) and gutted length (TLev).

Species	Location	Relation	*b*	R^2	Study
Holothuria mammata	Turkey, Aegean Sea	Wev~TLev	1.752	0.89	Aydin, 2020
	Murcia, SE Spain, Mediterranean Sea	Wev~TL	0.924	R=0.72	Marquet et al., 2017
	Ria Formosa, S Portugal, NE Atlantic	Wev~TL	1.002	R=0.62	
	Olhos de Água, S Portugal, NE Atlantic	Wev~TL	1.175	R=0.68	
	Arrabida, SW Portugal, NE Atlantic	W~TL	1.3723	0.52	This study
		Wev~TL	1.1984	0.50	
		Wev~W	0.7962	0.80	
Holothuria arguinensis	Sagres, S Portugal, NE Atlantic	Wev~TL	1.038	R=0.53	Marquet et al., 2017
	Olhos de Água, S Portugal, NE Atlantic	Wev~TL	1.437	R=0.75	
	Ria Formosa, S Portugal, NE Atlantic	Wev~TL	0.980	R=0.68	
	Arrabida, SW Portugal, NE Atlantic	W~TL	1.723	0.65	This study
		Wev~TL	1.698	0.61	
		Wev~W	0.961	0.90	
Holothuria forskali	-	-	-	-	No data from other studies
	Arrabida, SW Portugal, NE Atlantic	W~TL	0.7617	0.30	This study
		Wev~TL	0.7667	0.31	
		Wev~W	0.6749	0.72	

Discussion

The soft body of the sea cucumbers is probably responsible for the low R^2 as pointed out by similar results reported for congeneric species (Conand, 1981; Kazanidis et al., 2010; Marquet et al., 2017), revealing the high variability in sea cucumber length measurements (Prescott et al., 2015). However, it is interesting to note that allometric relationships between total wet weight and total length showed either a higher or a similar R^2, when comparing with gutted weight. In other studies, gutted weight (body wall wet weight) is more commonly used to assess length-weight allometric relationships, instead of total wet weight, as the latter typically adds more variability. However, as results indicate, the use of gutted weight for allometric models instead of total wet weight is not the best option for these species. This argument is supported by the results of Kazanidis et al. (2010) where the total wet weight allometric relationship with total length also revealed a higher R^2 and Dereli et al. (2016) where the results for both allometric relationships were similar. If procedures and analyses are to be conducted with proper precautions, the authors propose the use of wet weight and total length in allometric models and estimations of body parameters as a non-invasive sampling technique. Furthermore, it provides a more practical application as it can be used to estimate species biomass in ecological studies (*e.g.* visual census), allowing the collection of demographic data without manipulation or animal sacrifice.

The negative allometric growth, considering W vs. TL, indicates that they become more elongated as they grow, a pattern reported for most sea cucumber species (Ahmed et al., 2018; Aydin, 2020; Jesús-Navarrete et al., 2018; Herrero-Pérezrul & Reyes-Bonilla, 2008; Kazanidis et al., 2010; Marquet et al., 2017; Poot-Salazar, 2014; Ramón et al., 2010). However, the three species show different allometric coefficients, with the highest recorded for *H. arguinensis* and lowest for *H. forskali*. Since we are considering total weight as a proxy of volume, this suggests different life-history strategies, as *H. arguinensis* increases more in volume with growth than the other two species. Because *H. arguinensis* is more commonly found exposed and on soft substrate, a higher increment in weight or volume allows to avoid, more efficiently, being susceptible to environmental stressors (Purcell & Simutoga, 2008).

Since the eviscerated sea cucumber can be considered as an empty ball, an allometry (Wev~W) would be the result of the body wall thickness growing either more or less than the length of the animal (Ramón et al., 2010). Gutted weight is the weight of the body wall of the sea cucumber, which functions as a nutrient storage during feeding periods (Hamel & Mercier, 2009). So, gutted weight can be considered as a proxy

for nutrient storage, excluding the variability associated with internal organs, such as the gut contents. Gut contents revealed that *H. mammata* and *H. arguinensis* appear to forage exclusively on sand, as benthic detritivores, and as described for most sea cucumbers (MacDonald et al., 2013), whereas *H. forskali* presents a more selective feeding behaviour, mostly foraging on hard substrates or selectively choosing feeding items, as previously identified in other deposit-feeding holothurians (Navarro et al., 2013). As *H. mammata* and *H. forskali* gutted weight and total wet weight scale with negative allometry, this suggests that both increase less in nutrient storage than in total volume. Both invest resources on defensive behaviours or mechanisms, and this could explain their negative allometry. *H. mammata* is commonly found sheltered in rock crevices during the day while *H. forskali* possesses Cuvierian tubules (Flammang, 1996), a specialized intracoelomic defensive system that occupies a share of the inner volume, whose maintenance comes at an energy cost (Vandenspiegel et al., 2000). This explains the lower allometric coefficient of *H. forskali* and the ontogenetic strategic differences between the two. However, for *H. arguinensis*, despite presenting significant negative allometric scaling, gutted weight and total wet weight scale closer to isometry, indicating the increase in nutritional reserves is higher with the increase in total volume.

This study highlights the highest allometric coefficient reported for *H. arguinensis* of the coast of Portugal, as opposed to a separate study conducted in the south of Portugal in three different locations, in which Ria Formosa presented the lowest (gutted weight – total length relations). Intertidal conditions are potentially more stressful (air exposure, variable temperature, sun light exposure) compared to the more stable conditions in the subtidal. In the subtidal, individuals can spend more energy on growth rather than on physiological changes linked to survival during air exposure (Marquet, 2017).

In this study, both *H. mammata* and *H. arguinensis* presented a higher allometric coefficient than that of different populations in the Southwestern Iberian Peninsula. However, the allometric coefficient of *H. mammata* population of the Aegean Sea was higher than in the present study. This result reinforces the hypothesis of different regional intra-specific growth strategies. The variations in *b* may be attributed to internal and external factors (Le Cren, 1951; Hossain et al., 2014; Muchlisin et al., 2010) such as fishing pressure, that may have short-term and long-term effects on the ontogeny of a population (Reznick, 1993; Rochet, 1998). In Turkey, sea cucumber protection regulations are stricter than in the Southwestern Iberian Peninsula. No-take zones are established for periods of 4 years to allow populations to recover (Aydin, 2020). Meanwhile, in the Southwestern Iberian Peninsula, illegal harvesting of sea cucumbers has been a

reality for several years (González-Wangüemert, et al., 2018). The lower allometric coefficient could be the short-term result of heavier individuals being removed from the system. A long-term result of an ontogenetic adaptation to fishing could also occur, since fishing may create a selective pressure that could shift long-term selective advantage. Either way, stricter protection regulations seem to favour a higher allometric coefficient, reinforcing the fact that regulatory measurements are an effective tool to protect sea cucumber's populations, guaranteeing larger and more fecund animals (Marquet et al., 2017).

References

Alberts, J. R., & Harshaw, C. (2014). Behavioral development and ontogenetic adaptation. In K. Yasukawa & Z. Tang-Martinez (Eds.), Animal behavior: How and why animals do the things they do. 1, 289–324.

Aydın, M. (2020). Length-weight relationships and condition factor of four different sea cucumber species in the Aegean Sea. Journal of Anatolian Environmental and Animal Sciences, 5(1), 80-85.

Cabeçadas, G., Nogueira, M., & Brogueira, M. J. (1999). Nutrient dynamics and productivity in three European estuaries. Marine Pollution Bulletin, 38(12), 1092-1096.

Cameron, J. L., & Fankboner, P. V. (1989). Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson)(Echinodermata: Holothuroidea). II. Observations on the ecology of development, recruitment, and the juvenile life stage. Journal of Experimental Marine Biology and Ecology, 127(1), 43-67.

Conand, C. (1981). Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. Bulletin of Marine Science, 31(3), 523-543.

Conand C. (1993) Reproductive biology of the holothurians from the major communities of the New Caledonian Lagoon. Marine Biology 116, 439–450.

Conand, C. (2008). Population status, fisheries and trade of sea cucumbers in Africa and the Indian Ocean. Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper, 516, 143-193.

Cravo, A., Relvas, P., Cardeira, S., Rita, F., Madureira, M., & Sánchez, R. (2010). An upwelling filament off southwest Iberia: Effect on the chlorophyll a and nutrient export. Continental Shelf Research, 30(15), 1601–1613.

Despalatovic M., Grubelic I., Simunovic A., Antolic B. and Zuljevic A. (2004) Reproductive biology of the holothurian *Holothuria tubulosa* (Echinodermata) in the Adriatic Sea. Journal of the Marine Biological Association of the United Kingdom 84, 409–414.

Engstrom, N. A. (1980). Development, Natural History and Interstitial Habits of the Apodous Holothurian *Chiridota rotifera* (Pourtales, 1851) (Echinodermata: Holothuroidea). Brenesia. San Jose, 17, 85-96.

Flammang, P. (1996). Adhesion in echinoderms. Pp. 1-60 in Echinoderm Studies, Vol. 5, M. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Froese, R. (2006). Cube law, condition factor and weight–length relationships: history, meta- analysis and recommendations. Journal of Applied Ichthyology, 22(4), 241-253.

Gonçalves E.J., Henriques M. & Almada V.C. (2002) Use of a temperate reef-fish community to identify priorities in the establishment of a marine protected area. In Beumer J.P., Grant A., Smith D.C. (eds) Aquatic Protected Areas: What Works Best and How Do We Know? Proceedings of the World Congress Aquatic Protected Areas, Cairns, Australia: Australian Society for Fish Biology: 261-272.

González-Wangüemert, M., Aydin, M., & Conand, C. (2014). Assessment of sea cucumber populations from the Aegean Sea (Turkey): First insights to sustainable management of new fisheries. Ocean & Coastal Management, 92, 87-94.

González-Wangüemert, M., Dominguez-Godino, J. A., & Canovas, F. (2018). The fast development of sea cucumber fisheries in the Mediterranean and NE Atlantic waters: from a new marine resource to its over-exploitation. Ocean & coastal management, 151, 165-177.

Hamel, J. F., & Mercier, A. (1996). Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences, 53(2), 253-271.

Hamel, J. F., & Mercier, A. (2009). Endogenous and exogenous control of gametogenesis and spawning in echinoderms. Academic Press.

Henriques M., Gonçalves E.J. & Almada V.C. (1999) The conservation of littoral fish communities: a case study at Arrábida coast (Portugal). In Almada V.C., Oliveira R.F. and Gonçalves E.J. (eds) Behaviour and Conservation of Littoral Fishes Lisboa: Instituto Superior de Psicologia Aplicada, pp. 473-519

Herrero-Pérezrul, M. D., & Reyes-Bonilla, H. (2008). Weight-Length relationship and relative condition of the holothurian *Isostichopus fuscus* at Espíritu Santo Island, Gulf of California, México. Revista de Biología Tropical, 56(3), 273-280.

Ignatiades, L. (2005). Scaling the trophic status of the Aegean Sea, eastern Mediterranean. Journal of Sea Research, 54(1), 51-57.

Jacob, J., Gama, C., Salgado, R., Liu, J. T., & Silva, A. (2009). Shadowing effects on beach morphodynamics during storm events on Tróia-Sines embayed coast, southwest Portugal. Journal of Coastal Research, 73-77.

Jesús-Navarrete, A. D., Poot, M. N. M., & Medina-Quej, A. (2018). Density and population parameters of sea cucumber *Isostichopus badionotus* (Echinodermata: Stichopodidae) at Sisal, Yucatan. Latin american journal of aquatic research, 46(2), 416-423.

Keys, A. B. (1928). The weight-length relationship in fishes. Proceedings of the National Academy of Science, Vol. XIV, no. 12, Washing-ton, DC, 922–925.

Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J. F., & Mercier, A. (2004). Advances in sea cucumber aquaculture and management. Can J Fish Aquat Sci, 61, 519-528.

MacDonald, C. L. E., Stead, S. M., & Slater, M. J. (2013). Consumption and remediation of European Seabass (Dicentrarchus labrax) waste by the sea cucumber *Holothuria forskali*. Aquaculture International, 21, 1279–1290.

Macedo, M. F., Duarte, P., & Ferreira, J. G. (2002). The influence of incubation periods on photosynthesis–irradiance curves. Journal of Experimental Marine Biology and Ecology, 274(2), 101–120.

Marquet, N., Conand, C., Power, D. M., Canário, A. V., & González-Wangüemert, M. (2017). Sea cucumbers, *Holothuria arguinensis* and *H. mammata*, from the southern Iberian Peninsula: variation in reproductive activity between populations from different habitats. Fisheries Research, 191, 120-130.

Mercier, A., Battaglene, S. C., & Hamel, J. F. (1999). Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology, 239(1), 125-156.

Moutin, T., & Raimbault, P. (2002). Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise). Journal of Marine Systems, 33, 273-288.

Nagy, K. A., Girard I. A., and Brown T. K. (1999). Energetics of free-ranging mammals, reptiles, and birds. Annual Review of Nutrition 19:247–277

Navarro, P. G., García-Sanz, S., Barrio, J. M., & Tuya, F. (2013). Feeding and movement patterns of the sea cucumber Holothuria sanctori. Marine Biology, 160(11), 2957-2966.

Olaya-Restrepo, J., Erzini, K., & Gonzalez-Wanguemert, M. (2018). Estimation of growth parameters for the exploited sea cucumber *Holothuria arguinensis* from South Portugal. Fishery Bulletin, 116(1), 1-8.

Pauly, D. 1984. Fish population dynamics in tropical water: a manual for use with programmable calculators. ICLARM Studies and Reviews 8.

Poot-Salazar, A., Hernández-Flores, A., & Ardisson, P. L. (2014). Use of the SLW index to calculate growth function in the sea cucumber *Isostichopus badionotus*. Scientific Reports, 4, 5151.

Prescott, J., Zhou, S., & Prasetyo, A. P. (2015). Soft bodies make estimation hard: correlations among body dimensions and weights of multiple species of sea cucumbers. Marine and Freshwater Research, 66(10), 857-865.

Purcell, S. W. (2014). Value, market preferences and trade of beche-de-mer from Pacific Island sea cucumbers. PloS One, 9(4), e95075.

Purcell, S. and Simutoga, M. (2008). Spatio- temporal and size- dependent variation in the success of releasing cultured sea cucumbers in the wild. Reviews in Fisheries Science16, 204–214.

Purcell, S. W., Lovatelli, A., & Pakoa, K. (2014). Constraints and solutions for managing Pacific Island sea cucumber fisheries with an ecosystem approach. Marine Policy, 45, 240-250.

Purcell, S. W., Mercier, A., Conand, C., Hamel, J. F., Toral- Granda, M. V., Lovatelli, A., & Uthicke, S. (2013). Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries, 14(1), 34-59.

Quratulan, A., Poot-Salazar, A., Ali, Q. M., & Bat, L. (2018). Seasonal variation in the length-weight relationships and Condition Factor of Four Commercially Important Sea Cucumbers Species from Karachi Coast-Northern Arabian Sea. Natural and Engineering Sciences, 3(3), 265-281.

Quratulan, A., Qadeer, M. A., & Sabri, B. Weight–length relationship of Sclerodactylidae sea cucumber, Ohshimella ehrenbergii (Selenka, 1868) (Echinodermata: Holothuroidea), from Karachi coast, Pakistan (2018). SPC Beche-de-mer Information Bulletin #38

Reznick, D. N. (1993). Norms of reaction in fishes. In" The exploitation of evolving resources"(TK Stokes, JM McGlade and R. Law, eds).

Rochet, M. J. (1998). Short-term effects of fishing on life history traits of fishes. ICES Journal of Marine Science, 55(3), 371-391.

Shiell G.R. and Uthicke S. (2006) Reproduction of the commercial sea cucumber *Holothuria whitmaei* [Holothuroidea: Aspidochirotida] in the Indian and Pacific Ocean regions of Australia. Marine Biology 148, 973–986.

Sicuro, B., & Levine, J. (2011). Sea cucumber in the Mediterranean: a potential species for aquaculture in the Mediterranean. Reviews in Fisheries Science, 19(3), 299-304.

Tuwo, A., & Conand, C. (1992). Reproductive biology of the holothurian *Holothuria forskali* (Echinodermata). Journal of the Marine Biological Association of the United Kingdom, 72(4), 745-758.

Wiedemeyer, W. L. (1992). Feeding behaviour of two tropical holothurians, *Holothuria (Metriatyla) scabra* (Jager 1833) and *H. (Halodeima) atra* (Jager 1833), from Okinawa, Japan. In Proceedings of the Seventh International Coral Reef Symposium (Vol. 2, pp. 863-870).

Wiedemeyer, W. L. (1994). Biology of small juveniles of the tropical holothurian *Actinopyga echinites*: growth, mortality, and habitat preferences. Marine Biology, 120(1), 81-93.

Legends for Figures

Figure 1: Map of the study region, with indication of the sampled area (in red).

Figure 2: *Holothuria mammata* (1640 individuals), *Holothuria arguinensis* (119 individuals) and *Holothuria forskali* (2956 individuals) distribution on: a) different habitats; b) settlement surfaces. Ratios of sheltered vs exposed sea cucumbers are listed on c).

Figure 3: Allometric relationships for *Holothuria mammata* (HM), *Holothuria arguinensis* (HA) and *Holothuria forskali* (HF) - Weight (W), Wev (gutted weight) and TL (total lenght). **I** – Prediction intervals 95%. **I** – Confidence intervals 95%.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Title

Allometric relationships to assess ontogenetic adaptative changes in three NE Atlantic commercial sea cucumbers (Echinodermata, Holothuroidea).

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Abstract

Holothuria arguinensis, *Holothuria mammata* and *Holothuria forskali* are three common sea cucumber species found in the NE Atlantic, traded in international markets and susceptible to capture. Allometric relationships reveal if the scaling relationships between biometric characters are proportional with growth, being a useful tool to understand species growth strategies. Allometric relationships of the three species were estimated and compared between them and with populations from different regions. These allometric relationships revealed that the three species have negative allometric growth. However, they have different growth strategies between them and reveal different regional intra-specific growth strategies when compared to other populations, suggesting ontogenetic adaptation as a consequence of external factors.

Keywords

fishing pressure - *Holothuria forskali* - Holothuria mammata - Holothuria *arguinensis* - length-weight relationships.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

The authors would like to thank the constructive comments of the reviewers and address their comments in a point-by-point reply, below.

Reviewer #1

The aim of the paper should be described at the end of the introduction session

R: The aim of the study was clarified and structured.

I understand that it is a complex issue that deserves a separate paper but I suggest reporting at least the average densities or biomasses of the species encountered in individual or kg /ha.

R: Density information is indeed relevant and a complex issue, as the reviewer indicates, because sea cucumbers have a patchy distribution, which depends on environmental conditions. Hence, there are three main reasons why the authors would prefer not to include this information in this manuscript. First, because it needs dedicated analysis and that is why different articles are being prepared/submitted to tackle this particular issue for these populations; secondly because we believe it would add no relevant information to the discussion; and finally because it ends up falling out of the scope of this paper.

Reviewer #2

The Latin name of sea cucumbers should be in italics, where ever in main text or figures.

R: The names of the species were corrected as suggested.

Section of result: "Wev~W allometric relationships show good fits for all species (0.72 ≤R2 ≥ 0.90)." should be "(0.72 ≤R2 ≤ 0.90).

R: The correction was made accordingly.

Figure 1

10 km

N

Figure 2

Figure 3   Click here to access/download;Figure;Fig3.jpg

Figure 3