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Obtaining a Biometric Index for Juveniles and Adults of *Holothuria inornata* (Semper, 1868)

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ABSTRACT

The sea cucumber *Holothuria inornata* (*H. inornata*) is a species at risk in Mexico. To help protect this species, it is important to provide information related to biological and fisheries management. The present study aimed to predict the weight of the sea cucumber (*H. inornata*) using biometric measurements such as wet weight and dry weight. A total of 267 *H. inornata* were manually collected from El Borrego Beach in the municipality of San Blas, Nayarit, Mexico. Species identification involved characterizing calcareous ladders. Each specimen was sedated using xylazine 10%, and measurements were taken out of water with a digital scale. Multiple linear regressions were performed, with size serving as the independent variable and wet weight, dry weight, and humidity percentage as dependent variables. A general equation was developed to predict the wet weight of the *H. inornata* at the juvenile stage. For correlations based on dry weight, the following equations were established. In immature adults, including dry weight, the equation was 0.0019 × (length^1.6479); and in mature adults, including dry weight, it was 0.0053 × (length^1.5362). The biometric parameters used for weight estimation exhibited a high degree of correlation with actual weight. Wet weight was the biometric parameter that showed the best fit among the studied parameters. Utilizing these biometric indices facilitates the recording of wet and dry weights for interested parties, thereby supporting population and biomass evaluations within the *H. inornata* fishery and aquaculture sectors.

Keywords: Biometric index, Dry weight, *Holothuria inornata*, Wet weight

INTRODUCTION

Sea cucumbers (Holothurians) have been harvested from Pacific islands for over 170 years (Purcell, 2014). Market prices exhibit considerable variation depending on factors such as species, product quality, size, and location (Perez and Brown, 2012). Among the twenty sea cucumber species sold in Hong Kong, China, *Holothuria inornata* (*H. inornata*) is the most valuable, with prices reaching 294 USD per kilogram (Purcell et al., 2025). Sea cucumbers are classified within the phylum *Echinodermata*, which represents one of the most diverse phyla within the animal kingdom (González-Navarro, 2012). The central Pacific region of Mexico and the Gulf of California demonstrated a significant diversity of echinoderms (Ríos-Jaras et al., 2013). Echinoderms are found in all the world's oceans and are mainly distributed along the seabed (Benthic) from the littoral to abyssal zones, with a particularly high abundance in coral reefs (Calcagno, 2014). Echinoderms play crucial roles in maintaining the structure and function of ecosystems (Alvarado and Chiriboga, 2008). Echinoderms possess a tegument consisting of a delicate cuticle and epidermis; beneath this lies the dermis. The dermis of holothurians is similar to that of other echinoderms in both structure and composition (Liu et al., 2025). The ossicles of the endoskeleton are tiny and embedded in the dermis, and they do not provide support in fish. Because ossicles are so diverse and detailed, they have taxonomic importance. The longitudinal muscle bands located beneath the dermis in the interambulacral zones are responsible for the body's stretching and contraction (Calcagno, 2014). Echinoderms' muscle bands or mutable collagenous tissues can rapidly change their mechanical properties, including elasticity and viscosity, within minutes under nervous system control (Siadat et al., 2021). The muscle tissues contain a large amount of extracellular matrix, mainly composed of collagen fibrils, proteoglycans, and microfibrils. The dermis of the holothurian body wall is a typical connective tissue that can quickly and reversibly change its mechanical properties in response to different stimuli (Tan et al., 2015). Therefore, an indirect method should be used to measure its weight.

Extensive studies on the dynamic mechanical properties of the sea cucumber (*Actinopyga mauritiana*; Quoy and Gaimard, 1833) dermis have demonstrated that the tissue can exist in at least three different states. These are rigid, standard, and soft states, which can be distinguished based on elastic and viscous properties as well as deformation-dependent behaviors. The mechanical parameters of the standard state are not just intermediate values between the stiff and soft states, suggesting that different mechanisms are responsible for transforming the soft state into the standard state and for converting the standard state into the rigid state (Yamada et al., 2010). The body weight of sea cucumbers

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exhibited significant variability attributable to differences in the water content within the respiratory tree and intestinal contents across different species. Liu et al. (2015) introduced a computational approach to estimate weight in *Apostichopus japonicus*, based on a method using r^2 correlation analysis, but only applicable to this species. Regarding *H. inornata*, the methods used include live biometric measurements such as weight and length, which can produce different results depending on the method applied. The precise method for collecting biometric data remains unclear, especially given the sea cucumber's ability to expel water, escape, or change its form or size. Numerous protocols are available for the biometric sampling of deceased organisms; however, there is no protocol nor a biometric index applicable to living organisms. Therefore, the present study aimed to conduct biometric sampling to establish correlations that facilitate the development of equations for estimating the live weight of juvenile, mature, and immature adult stages of *H. inornata* sea cucumbers based on their size.

MATERIALS AND METHODS

Ethical approval

Wild sea cucumber was collected with prior written permission (SPARN/DGVS/02181/24) from the Mexican Ministry of the Environment and Natural Resources (SEMARNAT). The samples were processed at the Wildlife Conservation Management Unit (UMA), Mexico, with registration number SEMARNAT, DGVS-UMA-IN-2025-NAY/22, which authorized research activities involving *H. inornata*. All relevant international, national, and institutional guidelines for animal care and use were adhered to authors.

Species identification

Initially, the verification of the species *H. inornata* was conducted through the analysis of external characteristics and the types of ossicles present in the tissue. For identification purposes, ten specimens were examined, comprising five juveniles and five adults. A 0.5 cm tissue fragment was carefully extracted from the mid-ventral region of each individual using a scalpel, serving as the basis for ossicle observation and identification. Each tissue sample was placed in a 15 mL test tube, and 5 mL of sodium hypochlorite 6% was added for tissue digestion. After five minutes, the specimens were prepared for microscopic examination using a compound microscope (Optika E-pL, Italy) equipped with a camera (P'Tkam B5, Italy), under which the ossicles were photographed. The identification of *H. inornata* was accomplished utilizing the taxonomic keys developed by the original description by Semper (1868), Solís-Marín et al. (2009), Santos-Beltrán and Salazar-Silva (2011), and Pacheco-Vega et al. (2022).

Biometrics

To find a correlation between biometric variables in *H. inornata*, sampling was conducted on different-sized organisms ranging from 10 to 255 mm, including juveniles, mature, and immature adults. A total of 267 *H. inornata* were collected from February 2023 to March 2024. The collection was performed through autonomous diving in sandy rocky areas at El Borrego beach in the municipality of San Blas, Nayarit, Mexico (Latitude 21.52545, Longitude 105.27849). A total of 267 *H. inornata*, with a minimum sample size of 157, were utilized, and biometrics were conducted. The measurements included length, measured from the mouth to the anus, and fresh weight, with classification into juveniles (Less than 15 cm) and adults (Over 15 cm; Tagliafico et al., 2011). The lengths were measured while the specimens were relaxed, without reacting to external stimuli, using a metric ruler (mm). Each specimen was then sedated with 1 mL of xylazine 10% (Aranda®, Mexico) for juveniles or 2 mL for adults, administered intramuscularly. After 30 minutes to ensure the anesthetic had fully taken effect, the procedure was then executed. This method had been previously standardized for the present study species.

Sea cucumber tissue processing

The sea cucumbers were removed from the tank, and excess water was eliminated using absorbent paper. Their weight and body mass were measured immediately thereafter (Less than one minute) employing a digital scale (Ohaus Scout® Pro, Germany). The analysis did not consider the water loss from the external surface area of the sea cucumbers. The methods employed to accurately determine the stages of maturity (Mature and immature) involved visual (Macroscopic) identification of the gonad maturity stage (Vitale et al., 2006). While the specimens were still sedated, they were sacrificed for gonadal separation according to the rules of the internal committee of the Autonomous University of Nayarit, Mexico, and following guidelines for animal slaughter. Each of the selected parts was placed in a forced convection oven (NOVATECH ®, HS45-AIA, Argentina) at 70°C for 48 hours (Rasul et al., 2022). The water content (%) was determined using the standard gravimetric method with the following formula.

$$\text{Humidity (\%)} = \left(\frac{(\text{Ww} - \text{Ws})}{\text{Wt}}\right) \times 100$$

Ww is the wet weight, Ws is the dry weight, and Wt means the sample weight.

Statistical analysis

A Shapiro-Wilk test for normality was conducted with a significance level of $p \geq 0.05$, along with a Kruskal-Wallis hypothesis test. The analysis focused only on the length, wet weight, and dry weight of each sampled organism, and the length-weight relationship was evaluated using a potential regression model (Y = axn). All statistical analyses were performed using Python software version 3.4.0 for Windows.

RESULTS

Based on the current observations and following the taxonomic keys, the specimens were identified as *H. inornata*. The collected specimen had a cylindrical body with scattered ambulacral feet on the dorsal surface. The dorsal surface was marked by small reddish papillae (Figure 1). The specimens had a table, a rod, and plate-shaped ossicles (Figure 2).
Based on the present findings from 128 juvenile and 139 adult *H. inornata* specimens, it appeared that the relationship between biometric variables may differ according to developmental and gonadal maturation stages (Table 1). The length-weight relationship helped create an equation that describes how parameters such as length, dry weight, and wet weight in *H. inornata* are related to their developmental stage. The biometric data for *H. inornata* indicated that using the equations is feasible for determining wet or dry weight from the measurement lengths.

The analysis of variance demonstrated statistically significant differences ($p < 0.05$) in at least one group that did not conform to a normal distribution. The current findings indicated significant differences ($p < 0.05$) in wet weight measurements among the groups (Immature, mature, and juvenile). The present data revealed that wet weight values in the mature group were significantly higher ($p < 0.05$) than those in the immature and juvenile groups. Additionally, wet weight values in the immature group were significantly higher ($p < 0.05$) than those in the juvenile group.

Table 1. Comparison of Pearson's and Spearman's coefficients of correlation for selected variables of sea cucumber with data collected from February 2023 to March 2024 in the El Borrego beach of San Blas, Nayarit, México

Base: Wet/dry	Development stage	Weight (g)	Wet weight (%)	Size (mm)	Equation	Correlation coefficients
Wet	Juveniles	$10.7^{c} \pm 9.3$	$86.2^{a} \pm 2.4$	$77.8^{c} \pm 27.5$	$WW = 0.0016 \times (talla^{2.1664})$	$r^2 = 0.84$
	Immature adult	$76.0^{b} \pm 30.8$	$84.0^{a} \pm 14.2$	$141.4^{b} \pm 37.7$	$WW = 0.006 \times (talla^{1.8873})$	$r^2 = 0.89$
	Mature adult	$171^{a} \pm 49.2$	$85.7^{a} \pm 10.3$	$199^{a} \pm 30.1$	$WW = 0.5935 \times (talla^{1.0878})$	$r^2 = 0.90$
Dry	Immature adult	$7.5^{b} \pm 4.2$	$84.0^{a} \pm 14.2$	$141.4^{b} \pm 37.7$	$DW = 0.0019 \times (talla^{1.6479})$	$R^2 = 0.76$
	Mature adult	$18.8^{a} \pm 7.9$	$85.7^{a} \pm 10.3$	$199.0^{a} \pm 30.1$	$DW = 0.0053 \times (talla^{1.5362})$	$r^2 = 0.90$

WW: Wet weight, DW: Dry weight, ^{abc} Different superscript letters within a column indicate statistically significant differences between base wet or dry groups ($p < 0.05$).

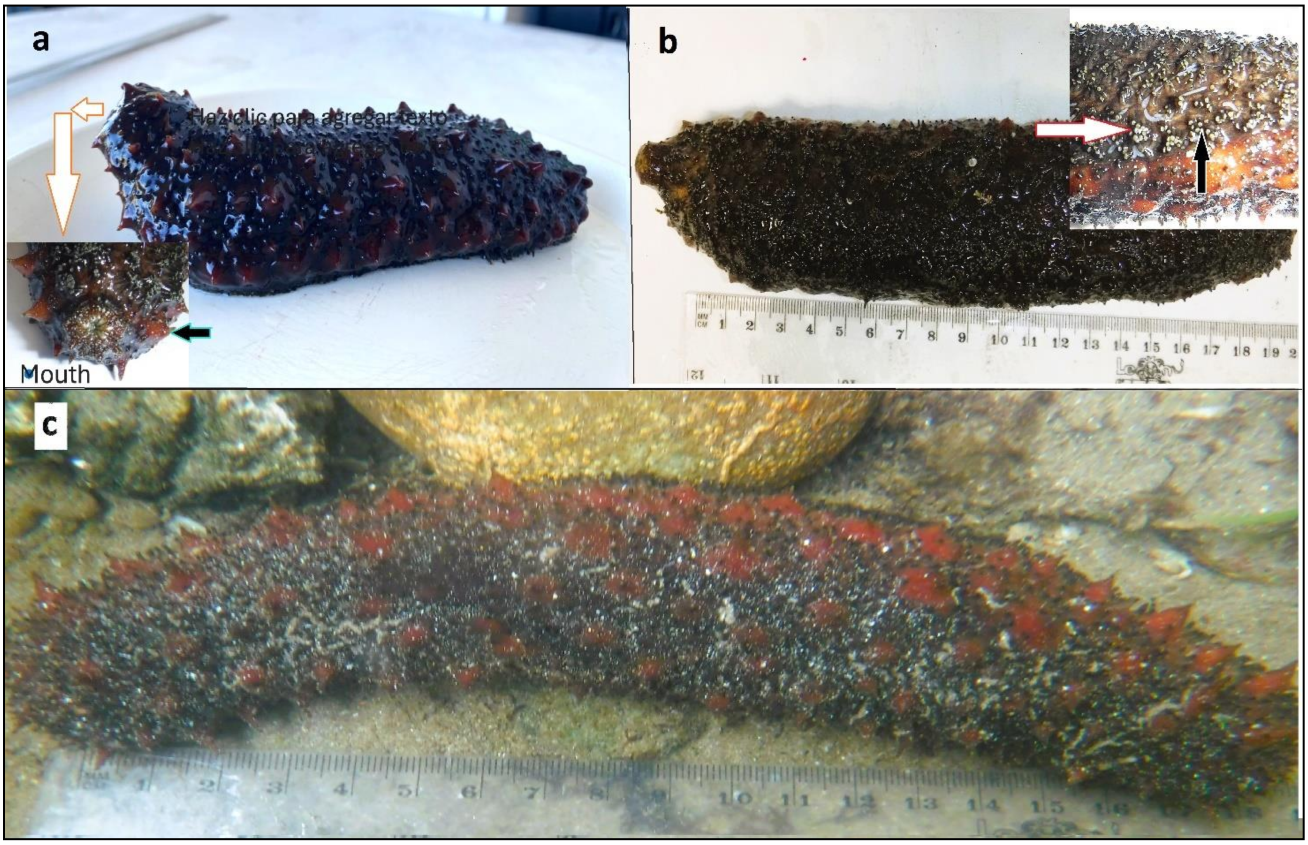

Figure 1. External morphology of sea cucumber *Holothuria inornata* (Semper, 1868). **a**: Dorsal region of the specimen with reddish conical papillae (black arrow) and circle-shaped tentacles externally bordering an area of the buccal membrane in the center of which the circular mouth is located. **b**: Ventral region with ambulacral feet, fairly of 3-5 mm in length. **c**: Adult sea cucumber.

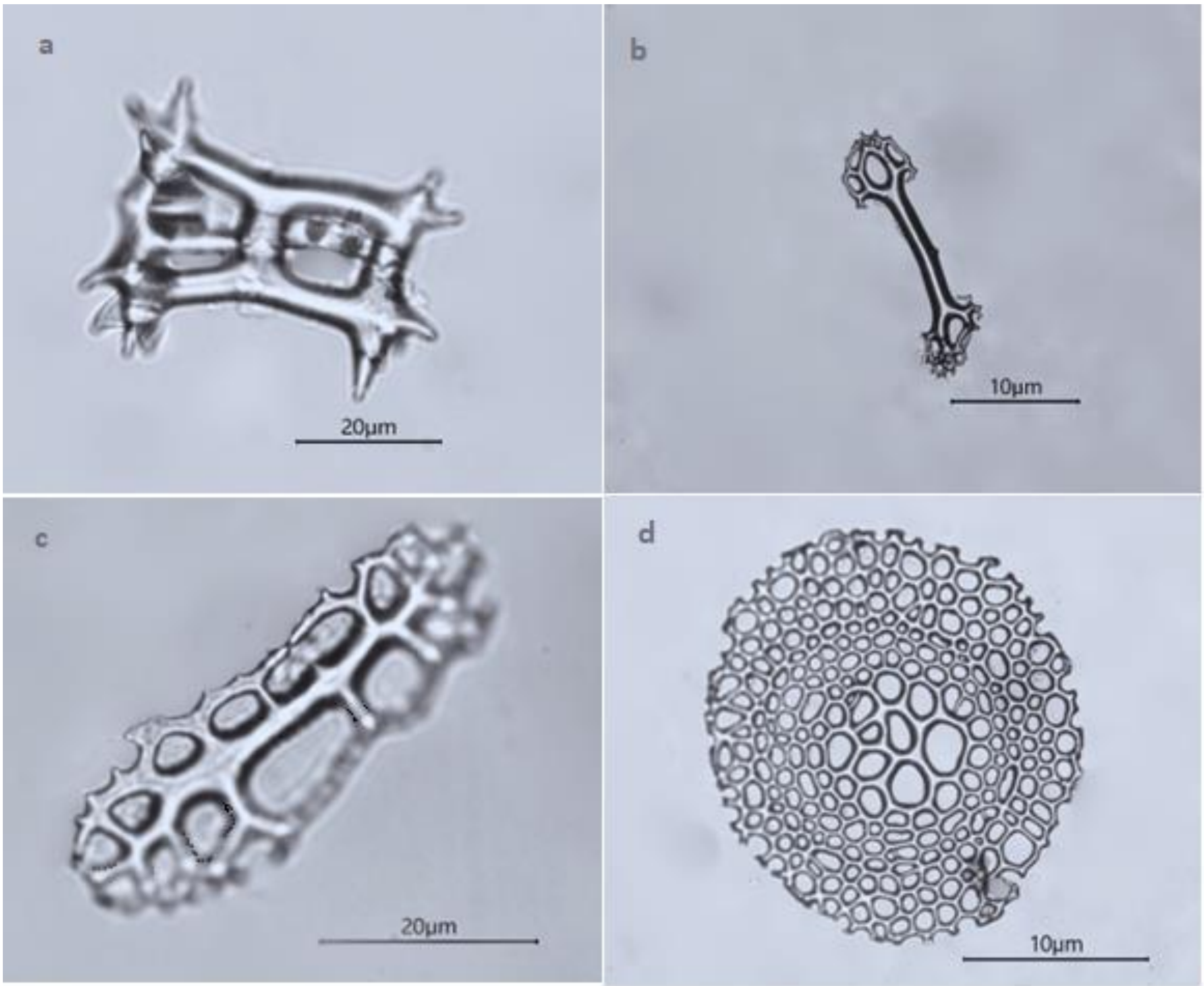

Figure 2. Micrographic images of ossicles observed in the adult stage of the sea cucumber. **a**: Table-shaped ossicles observed from the dorsal region. **b**: Rod-shaped and plate-shaped. **c**: Table-shaped ossicles. **d**: Perforated plate of ambulacral feet. The characteristics of external morphology and ossicles of *Holothuria inornata*.

DISCUSSION

The importance of biometric relationships lies in their ability to determine whether there is a correlated growth of the organism (Hammond and Purcell, 2024). Live weight across different sea cucumber species can be predicted using biometric data measurements. Hammond and Purcell (2024) highlighted the importance of biometric indices in fisheries and the findings related to *Pearsonothuria graeffei* (Semper, 1868), as these enabled the prediction of the *Pearsonothuria graeffei* length or weight based on morphometric variables. Additionally, Aydin (2019) indicated a low correlation of length and weight in *H. mammata* Grube, 1840, while Aydın (2020) reported negative allometric growth in *H. tubulosa Gmelin*, 1791, *Holothuria* (*Roweothuria*) *poli Delle Chiaje*, 1824, *H. mammata*, and *Holothuria* (*Platyperona*) *sanctori Delle Chiaje*, 1823. In previous studies, biometric data were not distinguished by development stage or gonadal maturation, which is important in species where fishing occurs, as it helps in establishing management plans that include closed seasons. According to the current results, the developmental stage (Juveniles and adults) and maturation stage exhibited differences in weight-length morphometric correlations.

In *H. inornata*, the variation in the correlation between weight and length may be linked to ontogenetic changes occurring from larval to adult stages. Additionally, biometric relationships in the sea cucumber *Cucumaria frondosa* are influenced by factors such as species, water temperature, salinity, food availability, light intensity, and substrate (Gianasi et al., 2018). Although *H. inornata* is both commercially valuable and endangered, no assessments have been conducted. This data insufficiency hinders the creation of more effective management strategies for the sea cucumber fishery in the Mexican Pacific (Herrero-Pérezrul and Chávez, 2024).

Generally, species within the Holothuroidea class demonstrated notable phenotypic plasticity, resulting in considerable variation in weight and length (Trenholm et al., 2024). This plasticity makes them sensitive to changes in the traits related to growth or gonadal maturity. The conventional method of conducting biometric assessments involves physically handling the specimen to determine its weight. Nonetheless, collecting this data from organisms such as *H. inornata* is challenging, as manipulation induces stress, prompting the organism to alter its size by depleting water and releasing intestinal contents (Jobson et al., 2021). In addition, they species are considered to have negative allometric growth (Berrú-Paz and Ríos-Peña, 2020; Aydın, 2020). Therefore, a different equation was created for juveniles and adults. Liu et al. (2015) proposed utilizing photographic images to estimate body weight in *Apostichopus japonicus*, establishing a correlation coefficient of $r^2 = 0.96$ between length and weight, with no significant differences observed among organisms exhibiting varying degrees of gonadic development maturation. However, in the present study, the equation that described the correlation between length and weight in *H. inornata* changed due to the effect of their maturation stage. The impacts of environmental factors, including seasons, water temperature, and food availability, influence sexual maturation and the reproductive period in sea cucumbers (Hammond and Purcell, 2024), as well as the correlation between length and weight (Ramili et al., 2024). Consequently, an alternative equation should be employed depending on the respective reproductive periods.

CONCLUSION

Based on the current findings, it can be concluded that distinct length-weight indices should be employed for juveniles and adults. Additionally, the gonadic maturation stage in *H. inornata* affects biometric parameters; thus, a specific index corresponding to its reproductive stage should be used. Using these indices and their associated equations that define the length-weight relationship, accurate weight data can be derived from length for *H. inornata* juveniles, immature adults, and mature adults. These biometric indices will assist in recording wet and dry weights, supporting population and biomass assessments in the *H. inornata* fishery and its aquaculture. Based on the current insights, it is recommended to implement similar procedures for other sea cucumber species.

DECLARATIONS

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Authors' contributions

Mirian Guadalupe Zumaya-Higuera and Alexa Omont collected specimens and processed the data. Juan Manuel Pacheco-Vega was the principal investigator of the study, wrote the manuscript, and posed the original idea. José Trinidad Nieto-Navarro critically reviewed the study. Erika Torres-Ochoa and Julian Gamboa-Delgado contributed to the literature review and interpretation of results. All authors contributed to the analysis of data, read and confirmed the final edition of the manuscript.

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Competing interests

The authors have no conflict of interest to declare.

Ethical considerations

This paper was originally written by the authors and has not been published.

Availability of data and material

The data supporting the findings of the present study are available upon reasonable request from the corresponding authors.

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