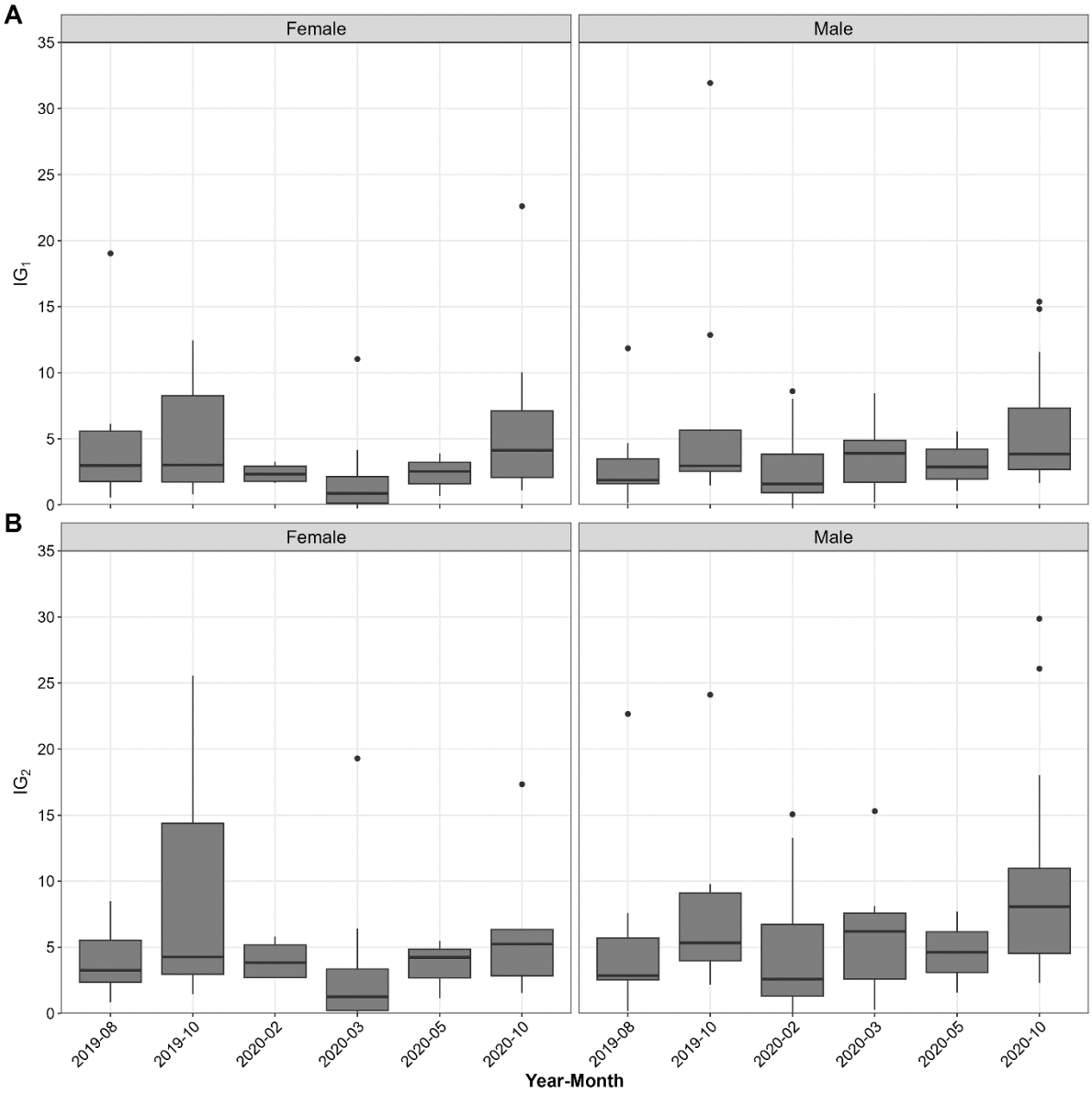
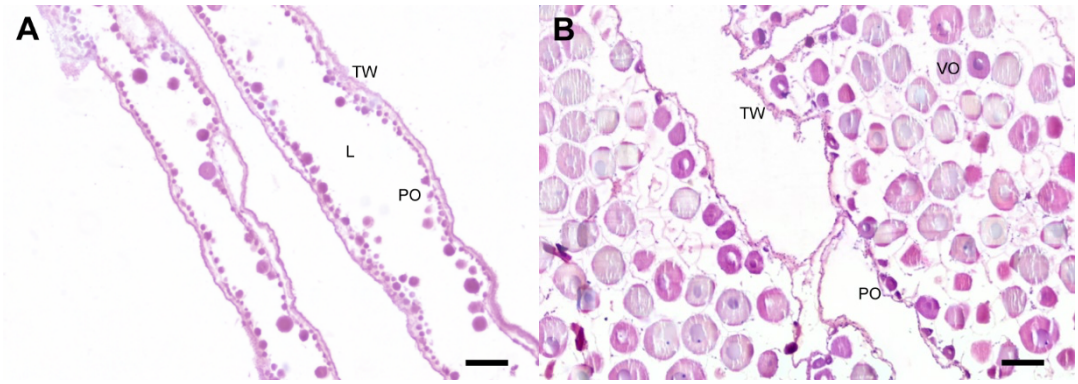
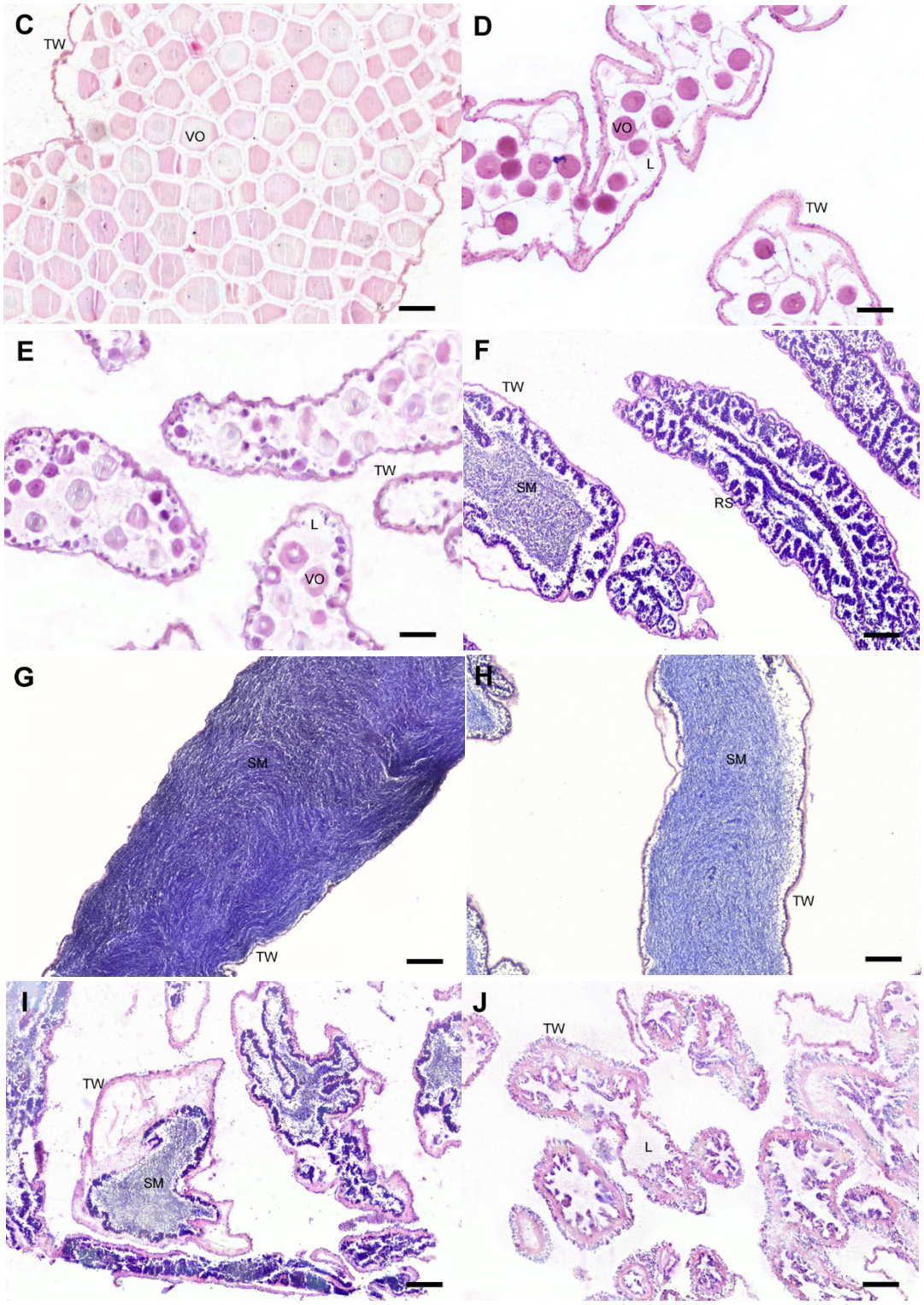
**Supplementary material**

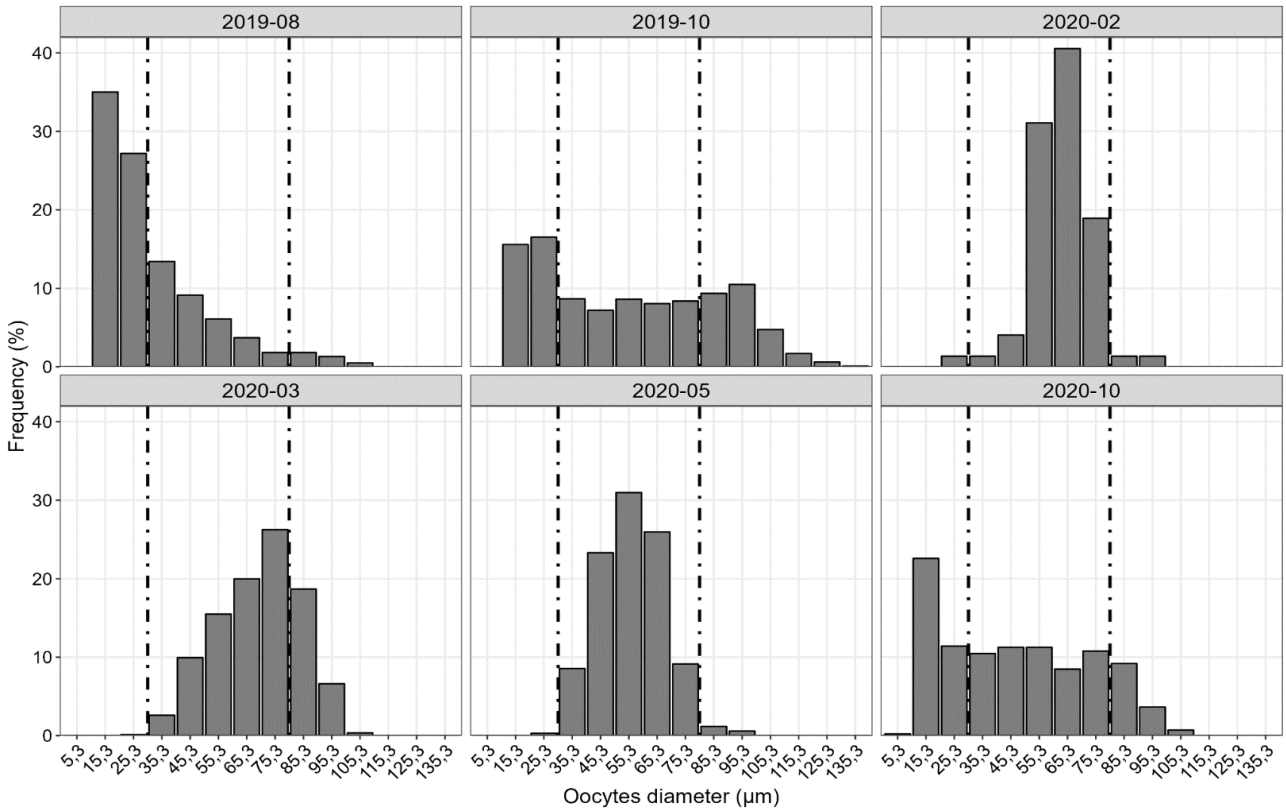



**SMF1.** Gonad indexes of the *H. (H.) grisea* from Armação do Itapocoroy (Santa Catarina, Brazil). **A.** $GI_1$. **B.** $GI_2$.

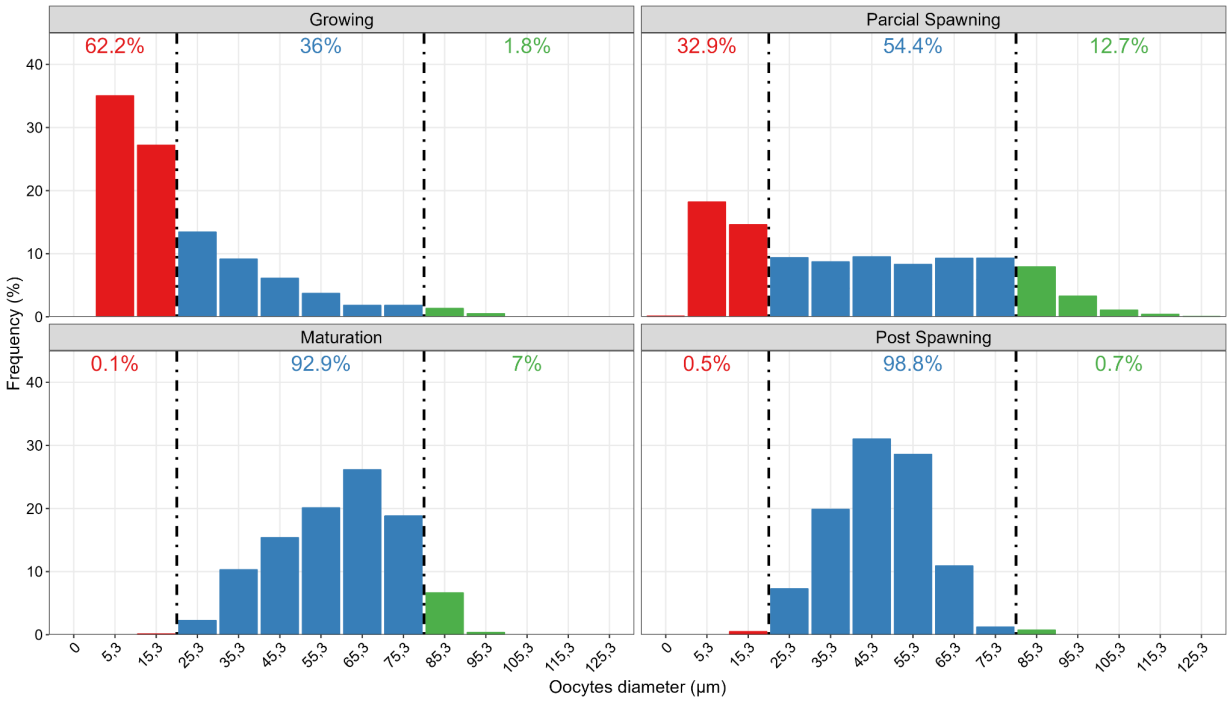




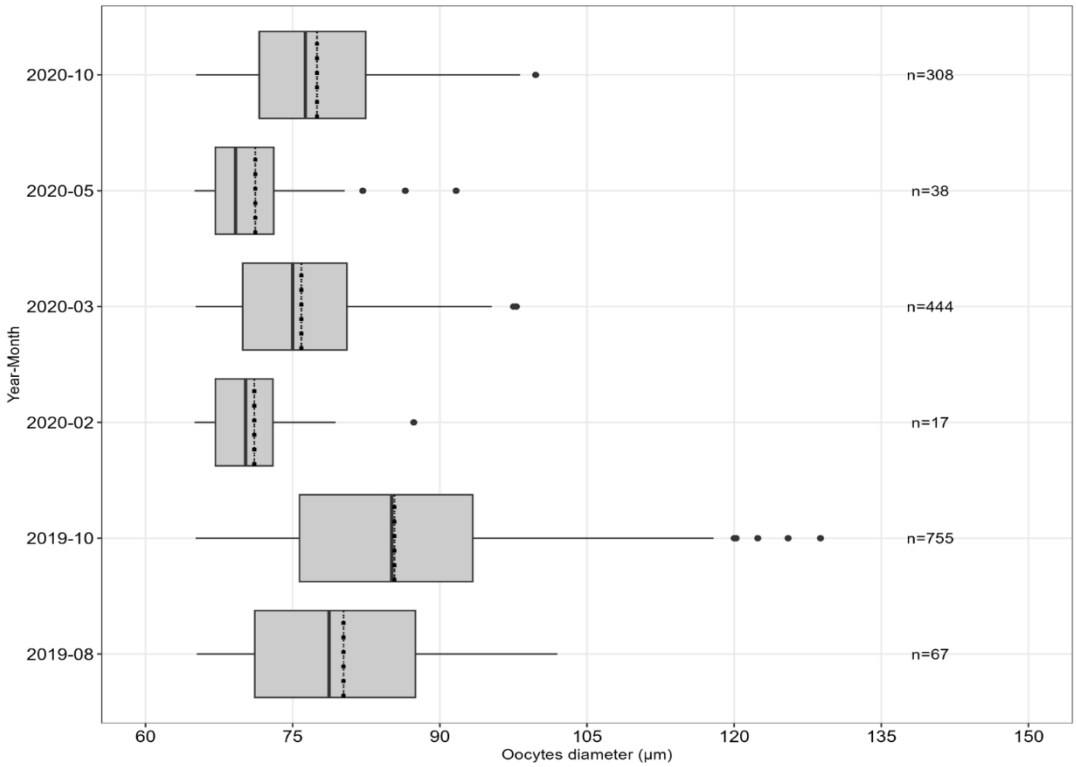

**SMF2.** Tubules development of the *H. (H.) grisea* from Armação do Itapocoroy (Santa Catarina, Brazil). Female: **A.** Growing stage. **B.** Premature stage. **C.** Mature stage. **D.** Spawning stage (total spawning). **E.** Spawning stage (spawning with growing). Male: **F.** Growing and premature stage. **G.** Mature stage. **H.** Beginning of spawning stage. **I.** Spawning stage (spawning with growing). **J.** Post-spawning stage. TW: tubular wall. NP: nutritive phagocyte. L: lumen. PO: previtellogenic oocyte. VO: vitellogenic oocyte. RS: radial series. SM: Spermatozoa mass. Scale bar: 50 µm.



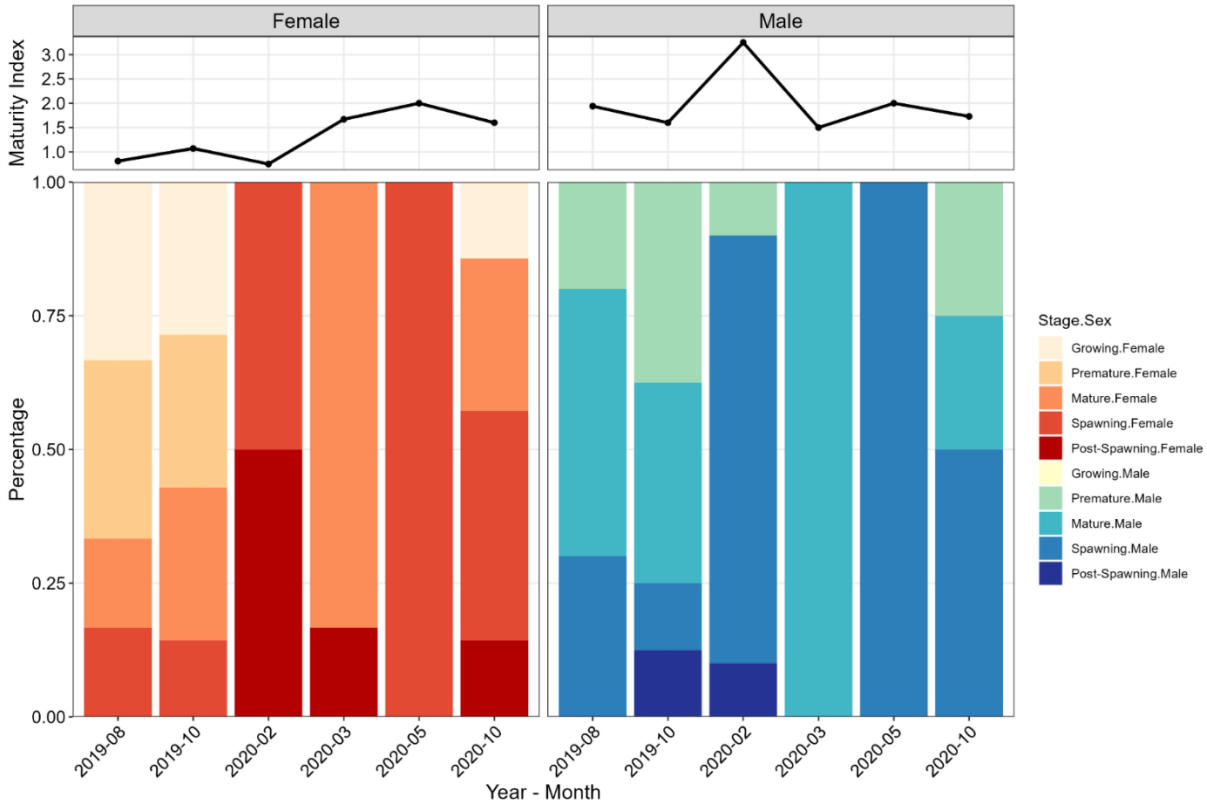

**SMF3.** Monthly distribution of *H.* (*H.*) *grisea* frequency of oocytes diameter average from Armação do Itapocoroy (Santa Catarina, Brazil). The dotted lines represent the cohorts based on Bhattacharya's method.




**SMF4.** Female GDS based on oocyte diameter intervals of the *H. (H.) grisea* from Armação do Itapocoroy (Santa Catarina, Brazil). The dotted lines represent the cohorts based on Bhattacharya's method.




**SMF5.** Mean and standard error of oocyte diameter (µm) of *H.* (*H.*) *grisea* from Armação do Itapocoroy (Santa Catarina, Brazil). n = number of oocytes.




**SMF6.** Frequency of tubule development stages (GDS) and Maturity Index (MI) of *H. (H.) grisea* from Armação do Itapocoroy (Santa Catarina, Brazil).

**SMT1**

Gametogenic events for tubules in females and males of the *H.* (*H.*) *grisea* from Armação do Itapocoroy (Santa Catarina, Brazil)

| Event | Tubule wall | Lumen | Germinative cells | Accessory cells |
|---|---|---|---|---|
| Growing | Female tubules are thick with | Rather empty | Small oocytes (Da < 25 µm) arranged in a single layer in a thin the germinal epithelium (grow zone). | Rare or non-existent |
| | Male tubules with a large amount of connective tissue; extremely convoluted | | Ridges of connective tissue develop towards the center of the lumen where primary cells (spermatogonia and spermatocytes) are present. Few spermatozoa mass later started to invade the tubule lumen. | |
| Premature | Increase of wall distension | Empty space still visible | As vitellogenesis progressed (25-65 µm) fully-grown oocytes began to take up a central position in the lumen. Accessory (follicle) cells surround the growing oocytes. | Rare and close to germinal epithelium |
| | | | Spermatozoa mass increase to the center of the tubule. Spermatocyte areas can be observed. Ridges of connective tissue remain at the tubule periphery. | |
| Mature | Maximum distension | Filled with mature gametes | Few numbers of previtellogenic oocytes close to the ovarian tubule wall. Large vitellogenic oocytes (up to 75 µm) fill almost completely the tubule lumen. Mature oocytes have a polyhedral outline and the presence of cortical granules on the cell surface indicates the maximum degree of maturation). | |
| | | | Spermatocytes can still form a well distinct peripheral layer. Spermatozoa mass in their maximum development. | |
| Spawning | Tubules very flaccid and wrinkled | Rather empty due to the release of gametes | Remarkable decrease in the abundance of rounded oocytes number. Atresia signs (visible reduction in size and shape) and progressive disappearance of gametes (resorption process) by phagocytic cells (total spawning). Grow zone (small gametes along the germinal | Few number |

| | | | | |
|---|---|---|---|---|
| | | | epithelium) can be observed in the germinal epithelium (spawning with growing). | |
| | | | Detachment of the radial series from the germinal epithelium and decreased of spermatozoa mass. In some males grow zone can still observe (spawning with growing). | |
| Post-spawning | Maximum thickness with a large amount of connective tissue | Empty but in restricted areas with traces of follicular epithelium and nutritive phagocytes | End of resorption process. A little grow zone can be observed. | Rare or non-existent |

**SMT2**

Macro-microscopic diagnosis (sex, GDS, tubules colors) for females and males of the *H. (H.) grisea* from Armação do Itapocoroy

| Tubule development stages | Males | Females |
|---|---|---|
| Growing | - | transparent<br>transparent-pink |
| Premature | white<br>creamy-white | Pink |
| Mature | transparent-pink<br>white | pink |
| Spawning | creamy-white<br>transparent-white | transparent-pink<br>pink<br>transparent-pink |
| Depletion | - | transparent<br>creamy |

**SMT3**

Literature review on the biological and reproductive characteristics of some tropical species (exploited or not) in *Holothuria* (special remarks to maturity and release of gametes periods in grey frames with details for *H. H. grisea*)

| Species | Ecorregion | Latitude | Authors | $L_{t(cm)}$ | $W_{w(g)}$ | GI | $O_{m(\mu)}$ | M:F | DJF | MAM | JJA | SON |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Holothuria tubulosa* | Temperate N Atlantic | 39-41°N & 8°-10ºE | Pasquini et al. (2022) | 9-37 | 52-989 | $7^{M}$-$12^{F}$ | 110 | 1:1 | | | | |
| | | 36°0'N & 03°0'W | Bahida et al. (2022) | 19 | 260 | $11^{Max}$ | - | 1:1 | | | | |
| | | 35°46'N & 0°49'W | Tahri et al. (2019) | - | - | 11-$23^{Max}$ | - | 0.8:1.2 | | | | |
| *Holothuria forskali* | | 39°21'N & 09°22'W | Santos et al. (2015) | - | 40-120 | $13^{F}$-$15^{M}$ | 112-130 | 1.6:1 | | | | |
| *Holothuria forskali* | | 36°0'N & 03°00'W | Bahida et al. (2022) | 16 | 213 | $15^{Max}$ | | 1:2 | | | | |
| *Holothuria mammata* | | 38°26'N & 09°01'W | Venâncio et al. (2022) | 23 | 345 | $29^{M}$-$43^{F}$ | 125 | 1.2:1 | | | | |
| *Holothuria arguinensis* | | 37°24'N & 01°37'W | Marquet et al. (2017) | 15-40 | - | $60^{Max}$ | 136* | 1:1 | | | | |
| | | 37°00'-37°05'N & 07°59'-08°55'W | | 10-50 | - | 74-$88^{Max}$ | | 1:1 | | | | |
| *Holothuria sanctori* | | 27°51'-28°9'N & 15°23'-15°41'W | Navarro et al. (2012) | 11-30 | 34-175 | $4^{M}$-$8^{F}$ | 140-160 | 1:1 | | | | |
| | | 36°0'N & 03°0'W | Bahida et al. (2022) | 19 | 261 | $23^{Max}$ | - | 1:1 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Holothuria polii* | W Indo-Pacific | 38°21'N & 26°46'E | Tolon & Engin (2019) | 14 | 120 | $16^{F}$-$19^{M}$ | - | 1:1 | | | | |
| *Holothuria scabra* | | 04°39'S & 39°13'E | Muthiga et al. (2009) | - | 12-598 | $5^{Av}$ | - | 1:1 | | | | |
| | | 23°20'-23°30'S & 43°40'-43°45'E | Rasolofonirina et al. (2005) | - | - | $5^{Max}$ | 140 | 1:1 | | | | |
| *Holothuria atra* | | 08°04'-08°22'N & 80°43'-80°47'E | Dissanayake & Stefansson (2010) | - | 419-1125 | $14^{M}$-$16^{F}$ | - | 1:1 | | | | |
| *Holothuria leucospilota* | Central Indo-Pacific | 22°35'N & 114°31'W | Huang et al. (2018) | >20 | >400 | $39^{M}$-$79^{F}$ | 141 | 1:1 | | | | |
| | | 21°07'S & 55°32'E | Gaudron et al. (2008) | - | 84-861 | 42 $^{Max}$ | >200 | 1:11 | | | | |
| *Holothuria scabra* | | 4°39'-6°49'N & 116°51'-118°15'E | Arsad et al. (2017) | 5-27 | 48-529 | 1,7 $^{Max}$ | - | 1.14:1 | | | | |
| *Holothuria fuscocinerea* | Tropical E Pacific | 15°44'N & 96°07'W | Benítez-Villalobos et al. (2013) | - | - | $11^{M}$-$20^{F}$ | $121^{Md}$ | 1:1 | | | | |
| *Holothuria mexicana* | Tropical NW Atlantic | 16°47'-16°05'N & 88°42'-88°04'W | Rogers et al. (2018) | 24 | - | $10^{M}$-$19^{F}$ | $163^{*}$ | 1:1 | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Holothuria (Halodeima) grisea* | Tropical SW Atlantic | 02°47'S & 41°14'W | Leite-Castro et al. (2016) | | 20 | 90 | 42[M] - 45[F] | 90-116 | 1:1 | | | | |
| | | 22°45'S & 41°54'W | Martins & Rezende-Ventura (2013) | | - | - | 17 [Av] | - | 1,2:1 | | | | |
| | Temperate SW Atlantic | 25°53'S & 48°33'W | de Lima-Bueno et al. (2015) | | - | 115 | 7[F]-12[M] | 75 [Av] | 1:1 | | | | |
| | | 26°47'S & 48°36'W | Present study | ♀ | 9-20[r] | 30-200[r] | 6-12 Max | 78-129 | 1:1 | mat/spa | mat/spa | gro/pre | pre/mat/spa |
| | | | | ♂ | | | 7-16 Max | - | - | mat/spa | spa | mat/spa | pre/mat/spa |

Lt: total length average. Ww: total wet weight average. Om: mature average oocyte diameter. F:M: female and male sex ratio. GI: gonad indexes ($GI_2$ in bold). gro: growing. pre: premature. mat: maturation. spa: spawning. Av: average. *Approximate value.