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Reproduction and embryonic development in two species of squaliform sharks, *Centrophorus granulosus* and *Etmopterus princeps*: Evidence of matrotrophy?

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ABSTRACT

Modes of reproduction and embryonic development vary greatly among the elasmobranchs, and prior studies have suggested that the energetic toll of embryogenesis in lecithotrophic species depletes embryonic organic matter by 20% or more. Matrotrophic species experience a lesser reduction or an increase in organic matter during embryogenesis. To investigate the maternal–embryonic nutritional relationship, we measured changes in organic matter from fertilization to near-parturition in embryos of *Centrophorus granulosus* and *Etmopterus princeps*. Embryos of *C. granulosus* experienced a reduction of 19.5% in organic matter, while *E. princeps* embryos experienced a reduction of 7.7% in organic matter over the course of embryonic development, suggesting some level of matrotrophy occurs, particularly for the latter species. Uterine villi were present in both species and developed concurrently with the embryos, increasing in length and thickness while becoming progressively vascularized. Embryos of *C. granulosus* were dissected to track the partitioning of water, organic matter, and inorganic matter to the liver, external yolk sac, internal yolk sac, digestive tract, and evicerated body throughout development. Mating was aseasonal for both species and spatially-mediated segregation by sex and maturity stage was observed. Ovarian cycles were concurrent for *C. granulosus* and consecutive for *E. princeps*. Size at maturity for *C. granulosus* was determined to be 111 cm TL for males and 143 cm TL for females, with an average fecundity of 5.3 embryos (range=4–7). Size at maturity for *E. princeps* was determined to be 56.5 cm TL for males and 61 cm TL for females north of the Azores and 54 cm TL for males and 69 cm TL for females near the Charlie Gibbs Fracture Zone. Average fecundity was 11.2 embryos (range=7–18) for this species. This is the first reporting of reproductive parameters for these two species, and the information provided will be valuable for informing stock assessment models in areas where these species are fished.

1. Introduction

Modes of reproduction in elasmobranchs are quite varied and include internal (viviparous) and external (oviparous) forms of embryonic development (Wourms, 1977). Viviparous species exhibit one of a number of matrotrophic modes of embryonic development, or the lecithotrophic mode of development known as yolk-sac viviparity (Hamlett et al., 2005; Musick and Ellis, 2005). Yolk-sac viviparity is classified as lecithotrophic because even though embryos develop internally, no maternal nourishment supplements the yolk sac. However, several authors note the lack of available data to properly classify many presumed yolk-sac viviparous species as such (Hamlett et al., 2005; Musick and Ellis, 2005). Matrotrophy occurs in the form of four different processes, with varying levels of maternal input (Table 1): uterine secretions (histotrophy, either lipid or mucoid), embryonic feeding on nutritional ova (oophagy), intra-uterine cannibalism (adelphophagy), placental connection (placentotrophy) (Hamlett et al., 2005).

Mossman (1987) defines histotroph as “any nutritive material, including glandular secretions, cells, and cell debris that is available to the embryo or fetus and is derived directly from maternal tissues other than blood.” Uterine secretion in the form of “lipid histotrophy” is found only in certain batoids and should be differentiated from “limited histotrophy” (also known as mucoid, incipient, or

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Table 1
Modes of reproduction in elasmobranchs, with relation to maternal nutrition supplied during embryogenesis (adapted from Wourms, 1981; Musick and Ellis, 2005).

Reproductive mode	Matrotrophy (M) or lecithotrophy (L)	Relative level of maternal nutritional supplementation*
Oviparous	L	None
Viviparous		
Yolk-sac viviparity	L	None (presumed)
Lipid histotrophy	M	Extensive
Limited, or "mucoid" histotrophy	M	Minimal to moderate
Oophagy	M	Extensive
Adelphophagy	M	Extensive
Placentotrophy	M	Moderate to extensive

* In addition to the yolk-sac.

minimal histotrophy; Hamlett et al., 2005), which has been documented in some triakid and squaliform species (Musick and Ellis, 2005). Lipid histotrophy is fairly easy to identify in pregnant stingrays (Myliobatoidei) due to the presence of a lipid-producing trophonemata and copious amounts of white, milky secretions (histotroph) found in the uterus during embryonic development (Musick and Ellis, 2005). Limited histotrophy is more difficult to identify through field observations, as it involves the secretion of a much more dilute substance (e.g. mucus) than is found in the lipid histotroph and may occur only episodically during gestation (Hamlett et al., 2005). It has been suggested that many shark species exhibit "limited histotrophy", yet few have been documented (Hamlett et al., 2005). Additionally, it has been suggested that the degree of maternal nutritional supplementation follows a continuum across species, with no clear quantifiable threshold to discriminate one reproductive mode from another (Hamlett et al., 2005; Musick and Ellis, 2005). When measuring embryonic organic matter consumption, differences between "yolk-sac viviparous" and "limited histotrophic" species might be indistinct, with varying degrees of yolk dependence found among taxa (Wourms, 1981; Hamlett et al., 2005; Frazer et al., 2012).

Other forms of matrotrophy are more readily identifiable during embryonic development. Ovatrophic species continue to ovulate nutritional ova during embryonic development and these ova can be found free in the oviducts and uterus, as well as in the stomachs of developing embryos (Castro, 2009). Adelphophagy has been documented only in *Carcharias taurus* and is characterized by intra-uterine cannibalism (Gilmore et al., 1983). Evidence of adelphophagy is apparent in the mid to latter stages of embryonic development since the number of live embryos will decrease from beginning to end of gestation as siblings are cannibalized. Also, the surviving embryo in each uterus bears precocious teeth and extremely distended stomachs that contain nutritional ova and the digested bodies of siblings (Gilmore et al., 1983). Placentotrophy is also easily identified by the presence of a yolk stalk and placental connection found in mid- and late-stage embryos.

Previous studies have suggested that the energetic toll of embryogenesis in lecithotrophic species results in a reduction in organic matter of more than 20% from freshly fertilized eggs to full-term embryos (Hamlett et al., 2005). However, few studies have appropriately quantified such changes in organic matter over the course of gestation (Storrie et al., 2009; Frazer et al., 2012). Ranzi's (1932, 1934) seminal studies analyzed changes in water, organic content, and minerals from egg to term embryos for 15 species of oviparous and viviparous elasmobranchs (translated and summarized in Needham, 1942).

The classical method for determination of matrotrophy is simple and requires relatively few samples (Hamlett et al., 2005). Absolute weight gain during embryogenesis occurs in every elasmobranch species, regardless of any maternal input, due to the uptake of water. However, metabolic losses during embryogenesis result in a partial depletion of the organic matter contained in the yolk at the time of fertilization, generally assumed to be a reduction of 20% or greater (Hamlett et al., 2005). Measurements of ash-free dry weight for early- and late-stage embryos can be compared to quantify loss of organic matter over the course of embryogenesis. If final measurement of organic matter is greater than 80% of that contained in a freshly fertilized egg, it is assumed that some form of maternal nourishment is utilized (Hamlett et al., 2005; Musick and Ellis, 2005).

Centrophorus granulosus (Supplementary Fig. 1) is one of the largest species of gulper shark (Family Centrophoridae), with a maximum size of at least 1760 mm TL (Castro, 2011). This species has a patchy global distribution and is typically found along the edges of continental shelves and upper slopes (White et al., 2013). This species is rarely landed in U.S. waters as bycatch in the squid fishery (Capt. Jim Ruhle, F/V *Darana R*, personal communication), in research and monitoring surveys, and other deep-water trawls and longlines (Kiraly et al., 2003).

A great deal of taxonomic uncertainty persists within the genus *Centrophorus* (Compagno et al., 2005). The species examined in the present study has at times been identified as *C. granulosus*, *C. acus*, *C. uyato*, *C. lusitanicus*, *C. machiquensis*, and more recently in the Western Atlantic, *C. niaukang* (White et al., 2013). At the time the present study was conducted the species was identified as *C. niaukang* (Compagno et al., 2005; Cotton, 2010). However, a recent study determined this taxon to be a junior synonym of *C. granulosus* (White et al., 2013).

Etmopterus princeps (Supplementary Fig. 2) is one of the largest species of lanternshark (Family Etmopteridae) and is widely distributed in the North Atlantic Ocean (Compagno et al., 2005) at depths of 400–4500 m (Dyb and Bergstad, 2004; Kyne and Simpfendorfer, 2007). Much of the biology and behavior of this bathydemersal species in unknown (Compagno et al., 2005), yet it is highly abundant in deep waters of the North Atlantic (Fossen et al., 2008). Currently, no conservation measures exist and few landings are reported for this species (Herndon and Burgess, 2006), despite the high bycatch mortality this species likely encounters due to the trauma associated with capture by deep-water trawl, gillnet, or longline (Cotton, personal observation). This species is routinely caught and discarded at sea, with no record of the bycatch, largely due to the fact that the meat has little value and the liver is too small to justify processing (Stale Dyb, captain M/S *Loran*, personal communication). The liver of this species contains proportionally less squalene than other squaliform sharks (Hernández-Pérez et al., 1997), further reducing the impetus to harvest.

Knowledge of the reproductive biology of sharks is important for clarifying their life history strategies. As higher-trophic level predators (Cortés, 1999; Musick and Cotton, this issue), deep-water sharks require a great deal of energy in an energy-poor environment to sustain basic life functions. The process of reproduction places further energetic demands on these species, with mature ova largely composed of energy-dense compounds such as fatty acids, amino acids, and lipids (Remme et al., 2005). Ova of *Centrophorus* spp. can reach enormous sizes, up to 95 mm in diameter and 390 g in weight (Guallart and Vicent, 2001; present study), one of the largest cellular sizes described for any vertebrate. The mode of reproduction and embryonic development employed by a given species therefore offers insight into the energetic requirements of the reproductive cycle.

In this study, we empirically quantify the organic matter depletion from fertilization to parturition in *C. granulosus* and *E. princeps* embryos to test the hypothesis that these species are lecithotrophic. We also characterize for the first time the

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reproductive parameters (i.e. maturity ogives, fecundity, ovarian cycle, and reproductive seasonality) for these two deep-water shark species.

2. Methods

2.1. *Centrophorus granulosus*

Specimens of *C. granulosus* were collected in the Gulf of Mexico and in the Atlantic Ocean off the coasts of Virginia, North Carolina, and Florida (Fig. 1A). These specimens were collected opportunistically during research cruises using demersal longlines or demersal trawl nets during one of the following projects: the Virginia Institute of Marine Science (VIMS) shark survey, collections of voucher specimens for the field guide “The Sharks of North America” (Castro, 2011), the National Marine Fisheries Service (NMFS) Winter Bottom Trawl Survey, and the Deep-C project (www.deep-c.org). One additional specimen was obtained as bycatch from a commercial squid trawler, taken approximately 50 km south of Norfolk Canyon, off the coast of Virginia (Capt. Jim Ruhle, F/V *Darana R*, personal communication). Specimens were measured for precaudal length (PCL), fork length (FL), and total length (TL). Determination of maturity ($n = 138$ specimens) was based on the presence of enlarged

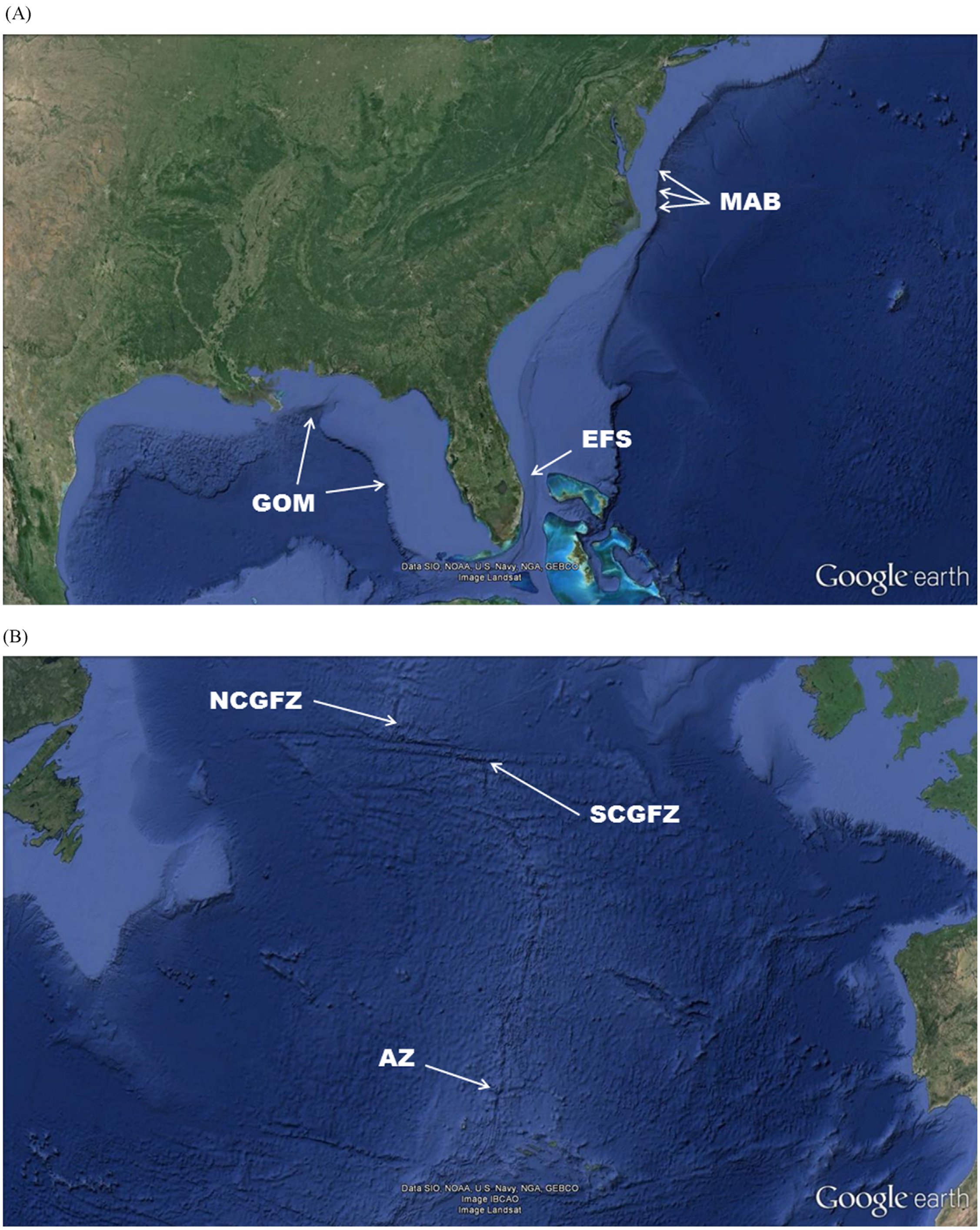

Fig. 1. Locations of (A) *C. granulosus* collections from the Mid-Atlantic Bight (MAB), eastern Florida shelf (EFS), and Gulf of Mexico (GOM). Locations of (B) *E. princeps* collections along the Mid-Atlantic Ridge north of the Azores (AZ), south of the Charlie Gibbs Fracture Zone (SCGFZ) and north of the CGFZ (NCGFZ).

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oocytes (or uterine eggs/embryos), distended uteri, and enlarged oviducal glands (Supplementary Fig. 1) for females and clasper calcification, presence of milt, and degree of vas deferens coiling for males. In assessing female reproductive status, we used the terms ‘oocyte’, ‘ovum, and ‘egg’ to denote the developing gamete, the mature (unfertilized) gamete, and the fertilized gamete, respectively. Eggs and early stage embryos were frozen *in utero* to prevent rupture of the delicate yolk sac (Supplementary Fig. 3). Mid- and late-stage embryos were dissected from the uteri and frozen individually (Supplementary Fig. 4). In some specimens, the maximum oocyte diameter was also recorded. Maturity ogives were constructed with SigmaPlot software using logistic regression with data for each sex binned in 10-cm size-class intervals. The inflection points of the curves (size at 50% maturity) were determined visually from each plot. Plots and regressions of basic reproductive parameters (fecundity by size, egg weights by size, embryo lengths by month, oocyte diameter vs embryo lengths) were generated with Microsoft Excel, and regression analysis was used to determine whether the slope of each regression was significantly different from zero, i.e. no relationship.

Embryos (if visible) were measured for TL to nearest mm, and for embryos larger than 150 mm TL, PCL and FL were also measured. Eggs and embryos smaller than 150 mm TL were weighed whole and intact to the nearest 0.001 g. Embryos larger than 150 mm TL were dissected to remove the external yolk sac (EYS) (Supplementary Figs. 4 and 5A), internal yolk sac (IYS), liver (L), and digestive tract (DT) from the evicerated body (B) (Supplementary Fig. 5B). Each component of those dissected embryos was weighed to the nearest 0.001 g, and the composite weight of all components was recorded as the embryo's total weight. A small loss in weight was unavoidable using this method, but amounted to no more than 1% of the total weight of the embryo, as verified by weighing some embryos before dissection. After weighing, samples were dried in either aluminum trays or ceramic crucibles at 60 °C until the weight stabilized, indicating that all water was removed. Dried samples were transferred to a muffle furnace for incineration. The ashing protocol outlined by Guallart and Vicent (2001) caused excessive bubbling, splattering, and loss of sample, so we formulated a more conservative ashing protocol. Samples were incinerated for 24 h at each successive temperature of 150, 200, 250, 300, and 350 °C, with transitions implemented slowly (1 °C/min) to prevent boil-over and loss of sample. Samples were then incinerated at 550 °C for 72 h, with the remnant ash weighed to the nearest 0.001 g. Water content, organic content, and inorganic content were determined by formulas (1)–(3):

$$\text{Water content} = \text{wet weight} - \text{dry weight} \tag{1}$$

$$\text{Inorganic content} = \text{ash weight} \tag{2}$$

$$\text{Organic content} = \text{dry weight} - \text{ash weight} \tag{3}$$

2.2. *Etmopterus princeps*

Specimens of *E. princeps* were collected during the MAR-ECO expedition of 2004 (www.mar-eco.no; Bergstad and Godø, 2003; Fossen et al., 2008). Two general regions along the northern Mid-Atlantic Ridge (MAR) were sampled: the Charlie Gibbs Fracture Zone (CGFZ), and an area approximately 400 km north of Faial Island, Azores (Fig. 1B). Specimens were captured and processed according to the methods outlined in Fossen et al. (2008). Maturity stage was assessed following Stehmann (2002) for 459 individuals (247 females, 212 males). Whole reproductive tracts were removed from 14 pregnant females, wrapped in cheesecloth, and preserved in 10% neutral-buffered formalin (Supplementary Figs. 6 and 7). Embryos of *E. princeps* were not dissected, but were dried and incinerated intact following the procedure for *C. granulosus* embryos. Accidental mishandling of ashed samples from the largest (near-term) litter of embryos led to sample loss (post-incineration) and underestimation of inorganic content for this batch of samples. A single, frozen term-embryo (TL = 175 mm) was therefore used as a proxy for inorganic content at this final stage of embryonic development. Maturity ogives were constructed as for *C. granulosus*, with data binned in size-class intervals of 3 cm (female) and 2 cm (male) for the Azores data and 5 cm (both sexes) intervals for the CGFZ data, reflecting differences in size distributions among specimens collected in these two areas. Basic reproductive parameters were plotted and analyzed as for *C. granulosus*.

3. Results

3.1. *Centrophorus granulosus*

A total of 138 individuals were collected (118 female, 20 male), with a size range of 690–1725 mm TL. Sexual dimorphism was noted with females reaching a larger size (1725 mm TL) than males (1240 mm TL). Twenty-two of the females collected were pregnant, yielding 88 embryos with sex determined for 35 of these (15 females, 20 males), thus embryonic sex ratio was 0.75 females per male. The EYS was nearly resorbed (0.7–39 g) in the litter containing the largest embryos (374–392 mm TL), suggesting that this size is near parturition (Supplementary Fig. 5A).

3.1.1. *Maturity ogives*

The smallest mature female observed was 1460 mm and the largest immature female was 1630 mm TL. The smallest mature male observed was 1150 mm TL, and the largest immature male was 1090 mm TL. Maturity ogives indicated that 50% of females were mature at approximately 1430 mm TL and 50% of males were mature at approximately 1110 mm TL (Fig. 2). Overall sex ratio was skewed with 5.9 females per male collected. Of the total of 138 specimens collected, 83 (mostly pregnant) were collected near Norfolk Canyon off Virginia and no males have been collected in that area to date (Moore et al., 2003; present study).

3.1.2. *Fecundity and reproductive cycle*

Fecundity varied between 4 and 7 embryos (mean = 5.3) and was uncorrelated ($R^2 = 0.0746$) with maternal size, with a

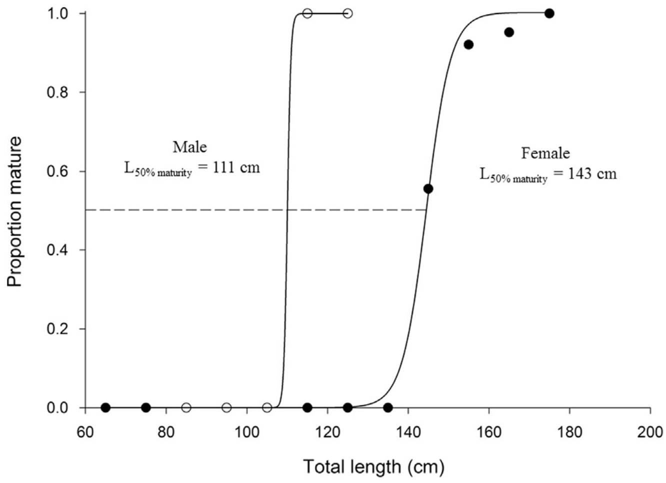

Fig. 2. Length-based maturity ogives for male (open circles) and female (closed circles) *C. granulosus* collected in the northwest Atlantic Ocean and Gulf of Mexico. Size-class intervals of 10 cm were used for both sexes.

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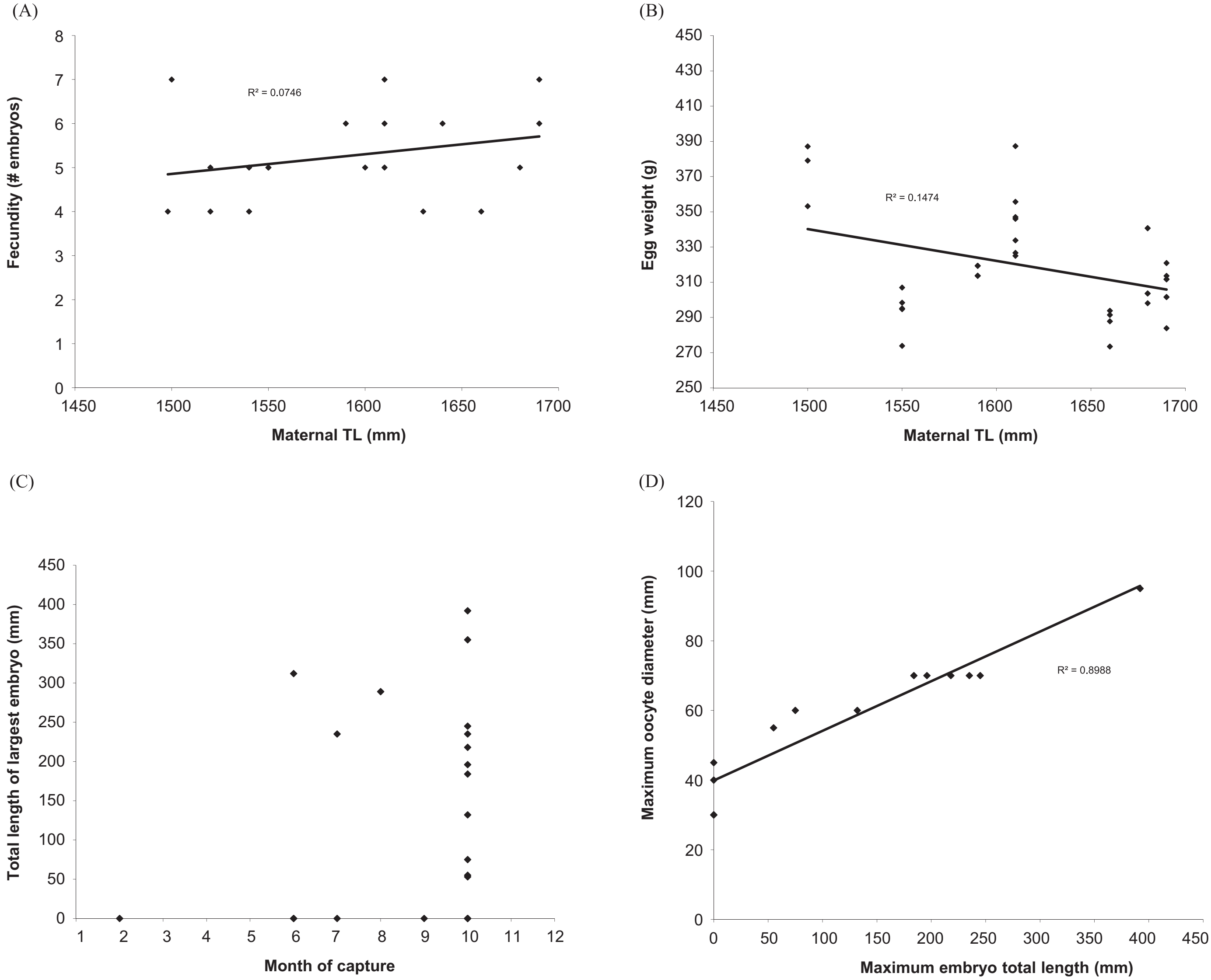

Fig. 3. Reproductive biology of female *C. granulosus*: (A) fecundity related to maternal size (TL), (B) freshly fertilized egg weight ($n=7$ females) related to maternal size (TL), (C) largest embryo size (TL) per litter of pregnant females by month of capture (non-discernable embryos were assigned a size of 0 mm TL), and (D) relationship of maximum oocyte diameter to maximum embryo length for each pregnant female, showing that ovarian cycle is concurrent with gestation.

regression slope not significantly different from zero ($p=0.2579$) (Fig. 3A). Mean egg weight was weakly correlated ($R^2=0.1474$) with maternal size, with a significantly negative regression slope ($p=0.019$), indicating a reduction in mean egg size as female size increased (Fig. 3B).

The distribution of embryo sizes found throughout the year suggests an aseasonal reproductive cycle, due to the presence of 'size-0' embryos (indiscernible in size) found year-round, as well as the wide range of embryo size classes found in the month of October (Fig. 3C). Ovarian development was concurrent with gestation in this species as evidenced by the increase in oocyte size with increasing embryo lengths (Fig. 3D). The maximum oocyte diameter measured was in a female with near-term embryos and was equivalent in size with observed freshly fertilized uterine eggs (~90 mm in diameter).

3.1.3. Length relationships

Length relationships (PCL, FL, and TL) were calculated from measurements of a subset of 25 adults ($n=2$ M ≥ 1150 mm TL; $n=23$ F ≥ 1500 mm TL), 49 juveniles ($n=14$ M, 840–1090 mm TL; $n=35$ F, 690–1630 mm TL), and 27 embryos (172–392 mm TL, sexes combined) (Supplementary Fig. 8). Equations for these length relationships (measured in mm) are given by formulas (4)–(9):

$$\mathrm{TL-PCL\ relationship\ (adult)}: \mathrm{TL}=1.0715(\mathrm{PCL})+192.87 \tag{4}$$

$$\mathrm{FL-PCLrelationship\ (adult)}: \mathrm{FL}=0.9285(\mathrm{PCL})+227.49 \tag{5}$$

$$\mathrm{TL-PCL\ relationship\ (juvenile)}: \mathrm{TL}=1.2084(\mathrm{PCL})+36.67 \tag{6}$$

$$\mathrm{FL-PCL\ relationship\ (juvenile)}: \mathrm{FL}=1.1109(\mathrm{PCL})+1.75 \tag{7}$$

$$\mathrm{TL-PCL\ relationship\ (embryo)}: \mathrm{TL}=1.4066(\mathrm{PCL})-25.95 \tag{8}$$

$$\mathrm{FL-PCL\ relationship\ (embryo)}: \mathrm{FL}=1.2267(\mathrm{PCL})-20.69 \tag{9}$$

3.1.4. Embryonic composition

Mean wet weight, water content, and inorganic content in freshly fertilized eggs were 319.630 ± 4.69 g, 162.555 ± 3.91 g, and 4.043 ± 0.07 g, respectively, and increased to 363.985 ± 9.55 g (+13.9%), 236.727 ± 9.50 g (+45.6%), and 5.412 ± 0.21 g

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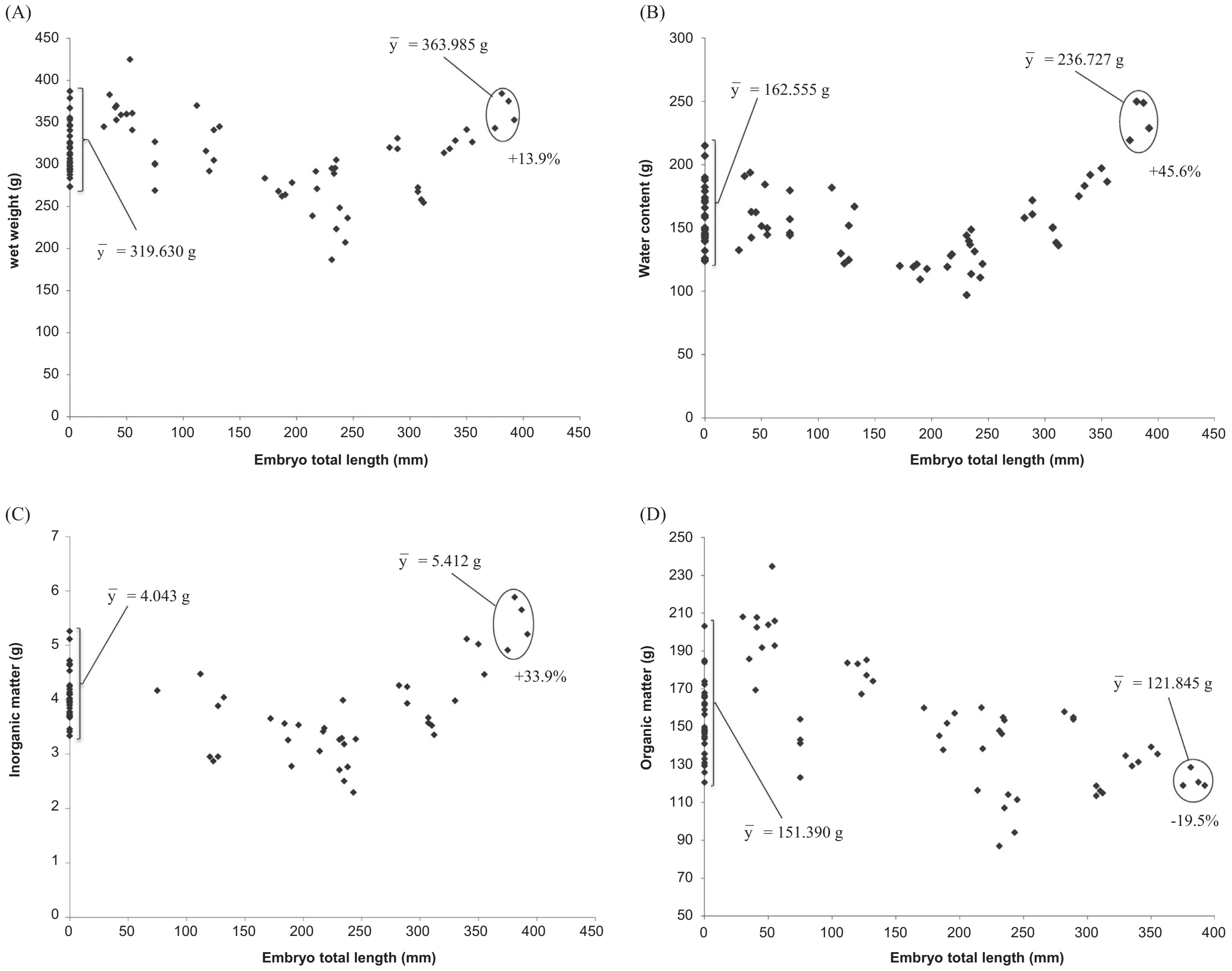

Fig. 4. Relationships between size (TL) of *C. granulosus* embryos and (A) wet weight, (B) water content, (C) inorganic content, and (D) organic content. The mean TL of females with freshly fertilized eggs was 1611 mm (1500–1690 mm), and the TL of the female with term embryos was 1600 mm. *Note*: In panel C, values for embryos in the 30–55 mm TL range are missing due to spillage of samples during incineration.

(+33.9%), respectively, in full-term embryos (Fig. 4A–C). The changes in wet weight, water content, and inorganic matter all followed similar patterns, decreasing slightly during the first half of embryonic development, then increasing after embryos reached approximately 250 mm in length, likely corresponding to the size at which gills become functional. Mean organic matter decreased steadily from 151.390 ± 3.21 g in the egg stage to 121.845 ± 2.51 g in full-term embryos, representing a loss of 19.5% in organic matter over the course of gestation (Fig. 4D).

3.1.5. Embryonic development

Wet weight of the EYS decreased with growth of the embryo, while all other components of the embryo (IYS, L, DT, and B) increased exponentially with increased embryonic length, reflecting the influx of organic matter from the EYS and uptake of uterine fluid, particularly after embryos reached 250 mm TL (Fig. 5). During the latter stages of development, enlargement of the IYS was coincident with the depletion of the EYS, as yolk reserves were relocated from an external to internal position. (Fig. 5A).

The composition of embryonic components varied by type. The EYS is composed of mostly organic material (approximately 60%), except when nearly depleted and most of the lipid-rich yolk has resorbed, leaving a small amount of tissue composing the outer membrane of the EYS (Supplementary Fig. 9). The evicerated body is composed mostly of water (approximately 80%) at all sizes (Supplementary Fig. 9). Growth of the digestive tract was mostly augmented by the uptake of water, whereas growth of the IYS and liver was mostly due to the addition of organic matter to these structures as the embryo developed (Supplementary Fig. 10). The proportion of inorganic matter in the EYS remained fairly constant as yolk was depleted, indicating no further mineral input into this structure (Supplementary Fig. 11). The proportion of inorganic matter in the evicerated body increased with increasing body size, likely as a result of increased uterine fluid uptake as the embryo developed (Supplementary Fig. 11). The proportion of inorganic matter in the IYS and liver decreased with increasing size, as yolk was relocated from the EYS to the IYS and as lipids were added to the developing liver (Supplementary Fig. 12). No obvious pattern of inorganic matter uptake was evident in the growth of the digestive tract (Supplementary Fig. 12).

3.2. *Etmopterus princeps*

A total of 495 individuals were examined for maturity (269 female, 226 male), with a size range of 295–940 mm TL. Sexual

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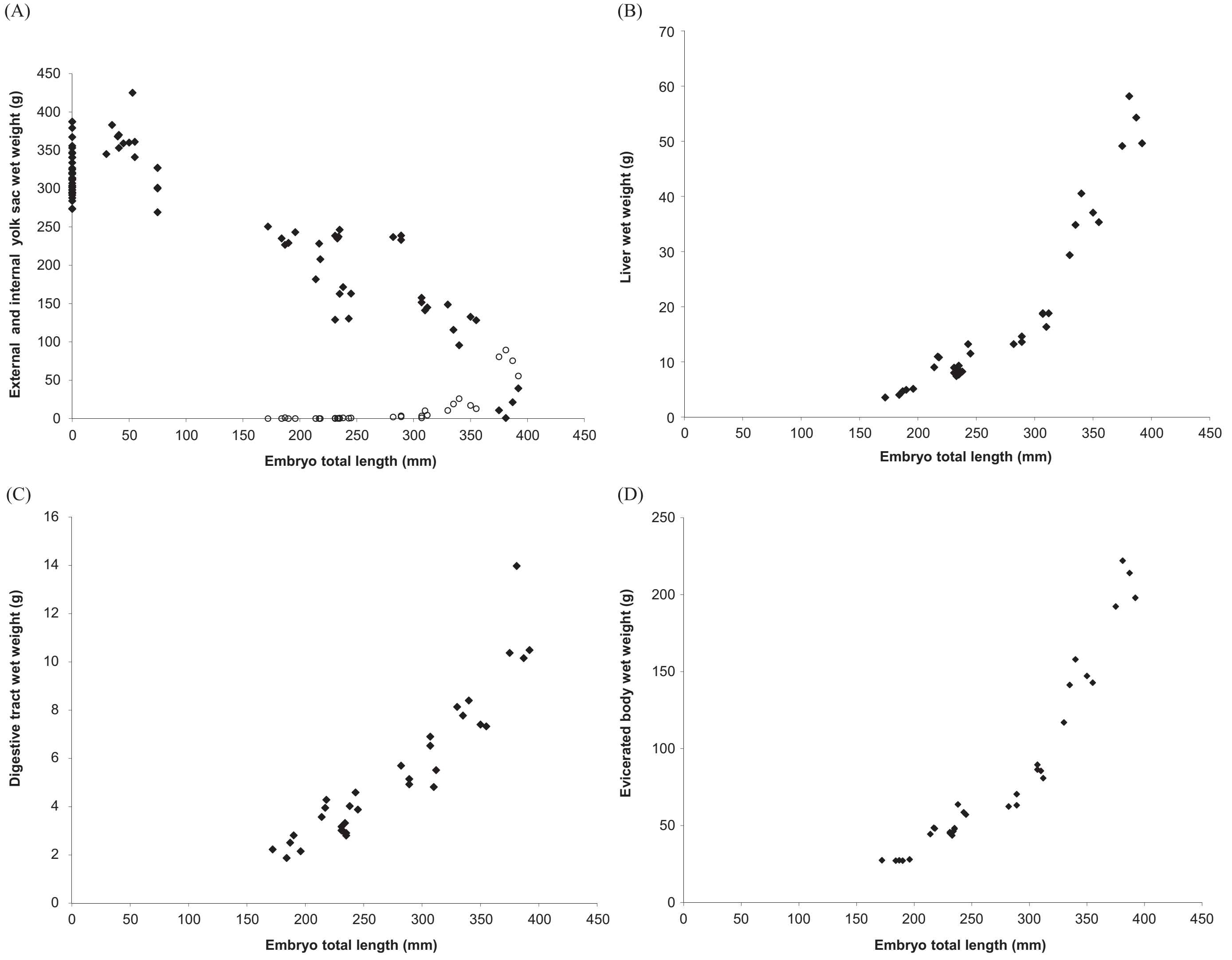

Fig. 5. Relationships between size (TL) of *C. granulosus* embryos and (A) external (closed circles) and internal (open circles) yolk sac wet weights, (B) liver wet weight, (C) digestive tract wet weight, and (D) evicerated body wet weight. Embryos <75 mm TL were not dissected and their weights were considered to be entirely composed of external yolk sac (panel A).

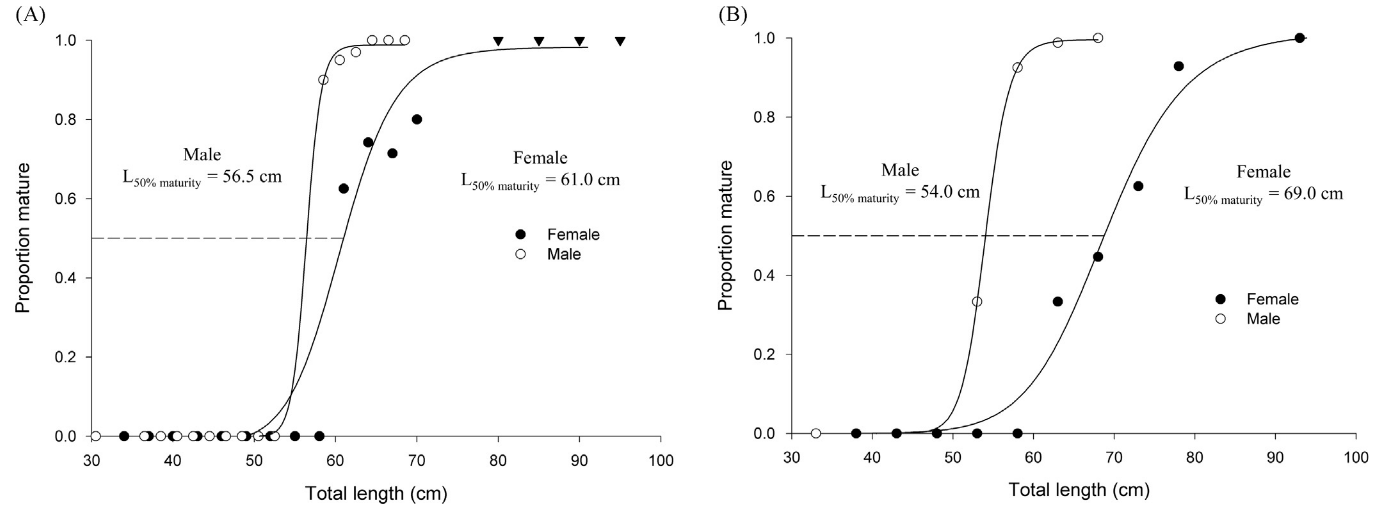

Fig. 6. Length-based maturity ogives for male (open circles) and female (closed circles) *E. princeps* (A) collected north of the Azores ($n=88$ males and 131 females, ratio = 1.00:1.49) and (B) collected near the Charlie Gibbs Fracture Zone (CGFZ) ($n=124$ males and 116 females, ratio = 1.00:0.94). Triangles in panel A represent assumed values, based on data from CGFZ. Size-class intervals of 3 cm (female) and 2 cm (male) were used for Azores data and 5 cm (both sexes) were used for CGFZ data.

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dimorphism was observed in maximum size, with a maximum male TL of 690 mm and maximum female TL of 940 mm. Overall mean size for males examined for maturity was 576 mm TL and for females was 631 mm TL. A total of 117 *E. princeps* embryos were processed from 14 pregnant females and sex was determined for 54 of these (27 males and 27 females), yielding a sex ratio of 1:1. The EYS was almost completely resorbed (0.01–2.08 g) in the litter containing the largest embryos (157–175 mm TL), indicating this was near the size at parturition.

3.2.1. *Maturity ogives*

The smallest mature male observed was 550 mm TL, and the largest immature male was 640 mm TL. The smallest mature female observed was 610 mm and largest immature female was 760 mm TL. Maturity ogives were initially constructed with data from both regions of the MAR combined, indicating that 50% of females were mature at approximately 675 mm TL and 50% of males were mature at approximately 555 mm TL. Further investigation indicated differences in female size at maturity between the CGFZ and Azores sampling sites. Maturity ogives for the Azores region (south) indicated that 50% of males were mature at approximately 565 mm TL and 50% of females were mature at approximately 610 mm TL (Fig. 6A). In the CGFZ region (north), 50% of males were mature at approximately 540 mm TL and 50% of females were mature at approximately 690 mm TL (Fig. 6B). Likewise, sex ratios differed in these two regions, with 1.49 females per male in the Azores area and 0.9 females per male in the CGFZ area.

3.2.2. *Fecundity and reproductive cycle*

Fecundity varied between 7 and 18 embryos (mean = 11.2) and was uncorrelated ($R^2 = 0.0725$) with maternal size, with a regression slope not significantly different from zero ($p = 0.2511$) (Fig. 7A). Mean egg weight was also uncorrelated ($R^2 = 0.0668$) with maternal size, with a regression slope not significantly different from zero ($p = 0.0670$) (Fig. 7B). Sampling of this species was conducted over the span of one month, and therefore a thorough determination of reproductive seasonality was not possible. However, due to the even distribution in size classes of embryos encountered (Fig. 7C; Supplementary Fig. 2), it is likely that the reproductive cycle is aseasonal in this species. Ovarian development is not concurrent with gestation in this species, as evidenced by the lack of developed oocytes in gravid females (Supplementary Figs. 2 and 6).

3.2.3. *Length relationships*

No FL and PCL measurements were obtained during the MAR-ECO cruise, so no length relationships were calculated for adults of *E. princeps*. Embryonic length relationships (PCL, FL and TL) were calculated from measurements of 16 *E. princeps* embryos (Supplementary Fig. 13). Equations for these length relationships are given by formulas (10) and (11):

$$\text{TL}-\text{PCL relationship (embryo)}: \text{TL} = 1.0587(\text{PCL}) + 24.122 \qquad (10)$$

$$\text{FL}-\text{PCL relationship (embryo)}: \text{FL} = 0.9539(\text{PCL}) + 18.855 \qquad (11)$$

3.2.4. *Embryonic composition*

Mean wet weight and water content in freshly fertilized eggs was 22.923 ± 0.29 g and 10.863 ± 0.18 g, respectively, and increased to 31.807 ± 1.01 g (+38.8%) and 20.717 ± 0.74 g (+90.7%), respectively, in full-term embryos (Fig. 8A and B). The changes in wet weight and water content followed a similar pattern over the course of embryonic development, remaining fairly constant during the first half of embryonic development, then increasing some time after the embryos reached approximately 90 mm in length, likely corresponding to the size at which gills become functional. Mean inorganic content in freshly fertilized eggs was 0.207 ± 0.01 g and increased to 0.310 g (+49.6%), using data from a single proxy frozen embryo (Fig. 8C). Samples from a litter of near-term embryos was mishandled after incineration, resulting in underestimation of inorganic content (0.155 ± 0.01 g; −25.1%) so these samples were not used to calculate change in inorganic content. Mean organic matter decreased steadily from 11.852 ± 0.16 g in the egg stage to 10.935 ± 0.32 g in full-term embryos, representing a loss of 7.7% in organic matter over the course of gestation (Fig. 8D).

4. Discussion

4.1. *Maternal-embryo nutritional relationship*

After fertilization and during the very early stages of development *C. granulosus* embryos are contained within a thin egg capsule or “candle”, which dissipates later in embryonic development. Other authors have noted the presence of an egg capsule in early development of some squaloid sharks (e.g. *Squalus acanthias*, Hamlett and Hysell, 1998; *C.* cf. *uyato*, McLaughlin and Morrissey, 2005; *Centroscymnus coelolepis*, Moura et al., 2011). Conversely, the embryos of *E. princeps* are not retained within an egg capsule at any stage of development. Lack of an egg capsule in the embryonic stage has also been reported for other squaloid sharks (e.g. *Centroscymnus owstoni*, Yano and Tanaka, 1988; *Centroscyllium fabricii*, Yano, 1995). Retention of developing embryos within an egg capsule obviously precludes any maternal nourishment during this phase of development. Differences in organic matter depletion observed in the present study might be attributed to the lack of an egg capsule in one of the two species examined.

According to Hamlett et al. (2005), a reduction in organic matter over the course of embryogenesis of greater than 20% should be expected in a lecithotrophic species. Some studies of maternal–embryo nutritional relationships in squaliform sharks have found that reductions in organic matter during embryogenesis were 20% or higher (Guallart and Vicent, 2001; Braccini et al., 2007; Moura et al., 2011) and therefore concluded that these species (*C. uyato* (as *C. granulosus*), *Squalus megalops*, *C. coelolepis*, respectively) were lecithotrophic. However, *E. princeps* exhibited substantially less than a 20% reduction (−7.7%) in organic matter, presumably due to some form of nutritional augmentation by the maternal organism. The notion that lecithotrophic species deplete >20% of the organic matter in a freshly fertilized egg was based on several studies that examined eggs of the oviparous species *Scyliorhinus canicula* (Hamlett et al., 2005), but this species likely has a shorter gestation (Lechenault et al., 1993) than many deep-water elasmobranchs (Kyne and Simpfendorfer, 2007; 2010) and therefore might not be a valid comparison. Other authors have expressed concern about using *S. canicula* as the sole representative for all oviparous species (Huveneers et al., 2011; Frazer et al., 2012). Similar data from a wide range of oviparous species are needed to develop a better understanding of the energetic costs of embryogenesis in these unequivocally lecithotrophic species. Of particular interest would be data from larger oviparous species, e.g. *Bathyraja* spp., with potentially longer gestational periods and higher energetic demands. Indeed a recent study of the Port Jackson shark *Heterodontus portusjacksoni* showed that this oviparous species undergoes a 40% depletion in organic matter during embryogenesis, i.e. twice that of *S. canicula* (Frazer et al., 2012). Likewise, more data is needed from a variety of yolk-sac viviparous species to better estimate the full range of organic matter depletion across species.

Results from the present study should be cautiously compared with those of Ranzi (1934) and Guallart and Vicent (2001), as those studies involved a congeneric species from the Mediterranean Sea. Though both studies listed the focal species as *C. granulosus*, recent

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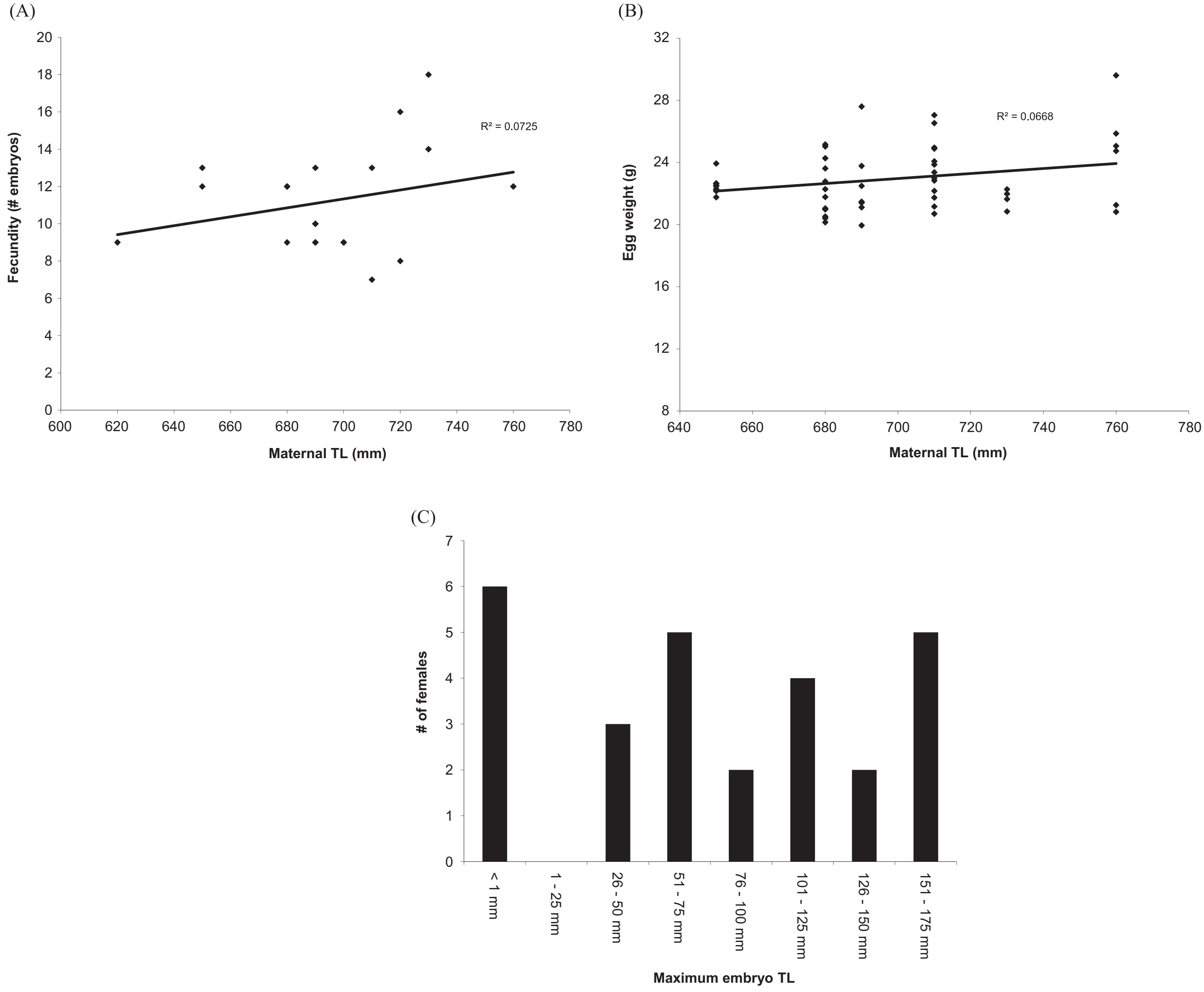

Fig. 7. Reproductive biology of female *E. princeps*: (A) fecundity related to maternal size (TL), (B) freshly fertilized egg weight ($n=6$ females) related to maternal size (TL), (C) distribution of maximum embryo lengths (TL) per litter of *E. princeps*, collected in July, 2004 along the northern Mid-Atlantic Ridge.

taxonomic revisions have shown that *C. granulosus* does not occur in the Mediterranean Sea (Veríssimo et al., 2014) and that the only species of gulper shark reported from the Mediterranean Sea is currently under taxonomic redescription and will bear the name *C. uyato* upon completion of that study (White et al., 2013; Veríssimo et al., 2014).

Limited histotrophy among sharks is likely more widespread than is currently known and the degree of maternal nourishment may occur along a gradient among species (Wourms, 1981). Hamlett et al. (2005) further assert that it is likely that all viviparous species exhibit some degree of histotrophy, which questions the validity of the classification of “yolk-sac viviparity” as distinct from “limited histotrophy”. The results of the present study support this notion, as the apparent degree of maternal nourishment was quite different in the two squaloid sharks examined.

4.1.1. *Potential mode of matrotrophy*

We were unable to definitively identify a source of supplemental maternal nourishment for either species, but we easily excluded most forms of matrotrophy (oophagy, adelphophagy, placental viviparity, lipid histotrophy) by macroscopic examination of the uteri and embryos from each species. The only other form of matrotrophy known among elasmobranchs is limited histotrophy so it was assumed that at least one of the species examined in the present study utilizes this reproductive mode, as has been reported for some squaliform species (Musick and Ellis, 2005). Macroscopic detection of limited histotrophy is not possible as the uterine secretions are very dilute and low in organic content (Hamlett et al., 2005). Biochemical analyses of uterine fluids may also prove difficult in detecting mucoid histotrophy as the secretions may be episodic. Paiva et al. (2012) showed that *Deania calcea* is a matrotrophic species, based on the presence of secretory cells in the uterine wall, the changes in organic matter over embryonic development, and the tracking of fatty acids and mercury from pregnant females to the developing embryos. Tanaka et al. (1990) reported maternal nourishment in *Chlamydoselachus anguineus*, based on dry weights, but the authors offered no evidence of the mechanism. Likewise, Girard and Du Buit (1999) reported maternal nourishment in *C. coelolepis*, but this was based solely on wet weights of eggs and term embryos, so the claim is inconclusive as the observed weight gain could have been attributable to water uptake. Moura et al. (2011) examined this same species and concluded it to be lecithotrophic. Further investigation (e.g. histological examination of

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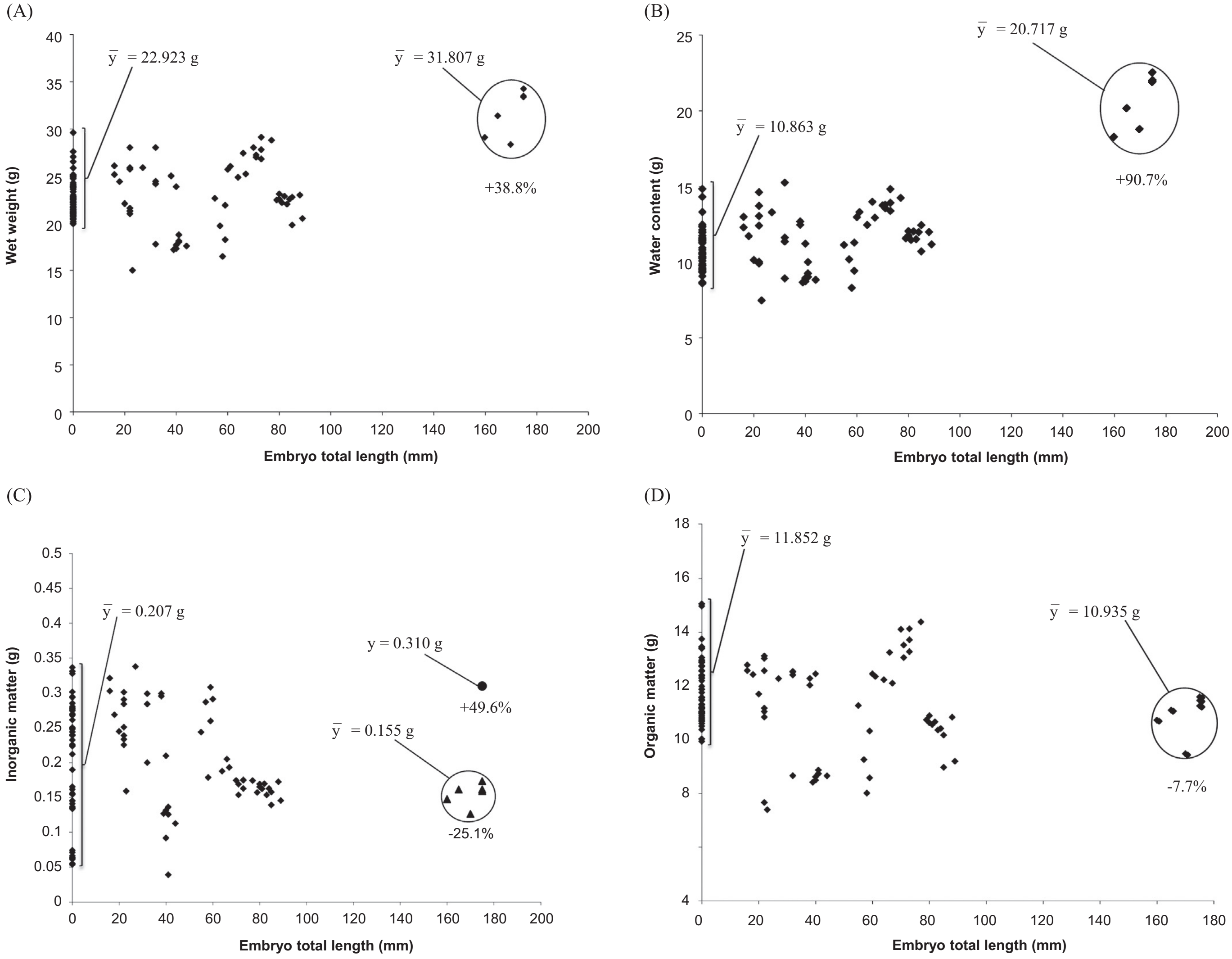

Fig. 8. Relationships between size (TL) of *E. princeps* embryos and (A) wet weight, (B) water content, (C) inorganic content, and (D) organic content. The mean TL of females with freshly fertilized eggs was 703 mm (650–760 mm), and the TL of the female with near-term embryos was 700 mm. *Note*: In panel C, triangles denote a litter of near-term embryos that was mishandled after incineration, resulting in ash weight underestimation, so these samples were not used to calculate change in inorganic content. The closed circle denotes the inorganic content of a single term embryo that was used as a proxy for inorganic content at this final stage of development.

uterine lining) is needed to verify the mechanism for limited histotrophy in the present study.

4.1.2. Uterine fluid

Needham (1942) noted the presence of "few" mucus-secreting cells in uteri of *C. granulosus*, *Dalatias licha*, *S. acanthias*, and *Squalus blainville*, and reported the percentage of organic matter contained in uterine fluid of these species at 1.8–2.8%, by weight. According to Guallart and Vicent (2001), prior studies have also shown secretory activity in the uterine walls of *C. uyato* (as *C. granulosus*), and dilute ($<2.5\%$) organic compounds in the uterine fluids, but these were assumed to serve a "lubricant" function, rather than a nutritive function (Bouchet et al., 1982). No study has since confirmed or refuted this assumption. Despite this evidence of organic material in the uterine fluids and identification of secretory cells in the uterine epithelium of gulper sharks, many authors maintain that *C. uyato* and other squaliform sharks are lecithotrophic species (Ranzi, 1934; Guallart and Vicent, 2001; Braccini et al., 2007; Moura et al., 2011). Though the present study suggests that maternal nourishment occurs in *E. princeps* and possibly *C. granulosus*, the mechanism for nutritional transport was not determined. Future studies may rely on other techniques, e.g. histological examination of uterine lining, chemical tracers, or biomarkers, to detect active uptake of uterine fluid by the developing embryo (Huveneers et al., 2011).

4.1.3. Uterine villi

It is likely that the uterine villi secrete nutrients as development of these structures progresses concurrently with embryogenesis. This growth was particularly evident in *C. granulosus*. In uteri with freshly fertilized eggs, villi were approximately 3–5 mm in length, pale in color, thin, and arranged in longitudinal rows (Supplementary Fig. 3). Uteri with early- and mid-stage embryos (35–55 mm TL; 170–190 mm TL), had much thicker, denser, and highly vascularized villi of approximately 5 mm in length (Supplementary Fig. 14). Uteri with slightly larger embryos (230 mm TL) had longer villi (~7 mm) with the same general appearance. Uteri with near-term embryos (375–390 mm TL) had longer villi (~10–12 mm) but the color had changed to dark red, with brownish or dark purple patches (Supplementary Fig. 15). Uterine villi of *E. princeps* greatly increased in length over the course of gestation (from approximately 3–5 mm to 15–18 mm), but overall did not become as dense or vascularized as those of *C. granulosus* (Supplementary Figs. 7 and 16). Although the function of these uterine villi is not fully understood, they have been

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noted in other studies of squaloid sharks (Needham, 1942; Yano, 1995; Girard and Du Buit, 1999; Veríssimo et al., 2003; Moura et al., 2011), with most authors noting that length and density of villi increases concurrently with embryonic growth. Hamlett and Hysell (1998) suggest uterine villi in *S. acanthias* are used strictly for respiration and the removal of waste products, though direct evidence for this is lacking. A recent study by Ellis and Otway (2011) demonstrated that pregnant *Orectolobus ornatus* periodically flush the uteri by opening and closing the cloaca, presumably for the purpose of disposing of metabolic waste from the embryo. If other elasmobranch species are also capable of uterine flushing, there would be no need for the more energy-intensive processes of respiration, active transport of embryonic waste products, and development of a dense "carpet" of uterine villi for the sole purpose of respiration and/or removal of waste products, as proposed by Hamlett and Hysell (1998).

4.2. Embryonic development

4.2.1. Size at parturition

Sizes and weights of *C. granulosus* near-term embryos (374–392 mm TL, 363–405 g) in the present study were in close agreement with full-term pups collected previously from the Gulf of Mexico (400–415 mm TL, 320–390 g; J. Castro, unpublished data). Term embryos (350–400 mm TL) with fully resorbed yolk sacs have also been reported from pregnant females collected near Norfolk Canyon (Kiraly et al., 2003).

Lengths of *E. princeps* near-term embryos in the present study (160–175 mm TL) were larger than some free-swimming juveniles from Iceland (120–180 mm TL; Jakobsdóttir, 2001), suggesting a wide range in size at parturition. Given that the specimens from Iceland were collected by trawl, it is possible that the smaller size range included aborted embryos.

4.2.2. Embryonic composition

Ranzi (1934) reported a reduction in organic matter of 54% for *C. uyato* (as *C. granulosus*) embryos during embryogenesis. This value was later adjusted (18–25% reduction) in a subsequent study with a larger sample size and a full series of developmental stages of embryos (Guallart and Vicent, 2001). Though taxonomic uncertainty persists within this genus (White et al., 2013), these two studies likely examined the same species since both studies collected specimens from the Mediterranean Sea, which harbors only one species of *Centrophorus* (Guallart and Vicent, 2001; Veríssimo et al., 2014). The value reported by Ranzi (1934) was derived from only one egg and one term embryo, and therefore did not take into account individual variation. The results of Guallart and Vicent (2001) were more representative since their data were derived from multiple eggs and term embryos. As such, their reported organic matter reduction for *C. uyato* (as *C. granulosus*) embryos was quite similar to that of the present study.

The reduction in organic matter recorded for *E. princeps* embryos (−7.7%) was very close to that observed in *S. blainville* (−7.4%; Frazer et al., 2012). This value is much lower than that observed for *C. granulosus* (−19.5%) in the present study, as well as some other studies of squaliform sharks (Guallart and Vicent, 2001; Braccini et al., 2007; Moura et al., 2011). Paiva et al. (2012) showed a 3.8% increase in organic matter for embryos of *D. calcea*, suggesting a wide range in magnitude of uterine nourishment for histotrophic squaliform species. Similarly, Frazer et al. (2012) suggested a wide range in organic matter depletion (reduction of ~20% to ~40%) is found among lecithotrophic species.

Problems encountered with estimation of inorganic content of near-term *E. princeps* embryos were relatively inconsequential to the overall estimation of organic content since these values differed by two orders of magnitude (Fig. 8). The decision to use a frozen specimen as a surrogate value for term-embryo inorganic content was justifiable because the inorganic content (minerals) in a frozen sample does not degrade or change under adverse condition (i.e. desiccation or thawing). This single specimen was not used to calculate wet weight, % water or % organic matter since desiccation likely occurred while in frozen storage prior to the study.

4.2.3. Embryonic growth

By dissecting embryos of *C. granulosus*, we were able to track the growth of individual components of the embryo. The rapid growth of the IYS as the EYS was depleted has been reported in several other studies (Guallart and Vicent, 2001; Veríssimo et al., 2003; Braccini et al., 2007). Repositioning yolk from the external yolk sac to an internal position before parturition affords the newborn shark a hydrodynamic advantage by reducing drag. These substantial yolk reserves in the IYS sustain the pup until first feeding (Yano, 1995; Hamlett et al., 2005). The size-related increase of inorganic material found in the evicerated body (Supplementary Fig. 11) is driven by an increased mineral demand by the formation of teeth, skeletal elements, finspines, and denticles as the embryo grows.

Embryonic gills of *C. granulosus* appear to become functional at approximately 250 mm TL (Fig. 4A–C) as wet weight, water content, and inorganic content steadily decline until the embryo reaches this size and then markedly increase afterward. This increase in water and inorganic material is presumably tied to the uptake of uterine fluids through the gills. A similar pattern has been reported for *C. uyato* (as *C. granulosus*; Guallart and Vicent, 2001) and *C. anguineus* (Tanaka et al., 1990), with embryos exhibiting a sharp uptake of water about midway through development.

Tracking the changes in wet weight, organic content, and inorganic content of different components of the developing embryo reveals how water, nutrients, and minerals are partitioned throughout the body during embryogenesis (Supplementary Figs. 9–12). Water and minerals are largely diverted to the evicerated body and digestive tract while the liver and yolk sacs (external and internal) are largely composed of organic matter. The tracking of individual somatic components was not possible for *E. princeps*, since the embryos were not dissected. As observed in *C. granulosus*, embryonic gill function presumably begins about midway through development, as evidenced by the sharp increase in water and mineral uptake at about 90 mm TL (Fig. 8B and C).

4.3. Reproductive biology of *Centrophorus granulosus*

4.3.1. Maturity ogives

Size at maturity (female = 1430 mm TL; male = 1110 mm TL) was in close agreement with that proposed by Kiraly et al. (2003) (female = 1460 mm TL; male = 1100 mm TL; as *C. niaukang*). The smallest mature female observed in the present study was 1460 mm TL, and largest immature female was 1630 mm TL. This large immature female had large oocytes (70 mm) and a small uterus (30 mm width), which was not distended or vascularized (indicative of recent gestation). It is not likely that this individual was in a postpartum "resting" state, since there is no resting stage for species with a concurrent ovarian cycle as indicated for this species (Fig. 3D).

4.3.2. Segregation by sex and maturity stage

Among samples collected from the Gulf of Mexico, mostly immature specimens were found in the northern area and mostly pregnant females were found in the eastern area (off Tampa, FL)

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(Fig. 1). In 1986, during a "shakedown" cruise (about 225 km east of Melbourne, FL), three mature male *C. granulosus* were collected, ranging from 1170 mm to 1210 mm TL (J. Musick, unpublished data; American Museum of Natural History catalog #'s 78262, 78266, 78265).

Kiraly et al. (2003) reported aggregated captures (e.g. $n=40$) of female *C. granulosus* in trawls near Norfolk Canyon (off Virginia) and hypothesized that the animals were moving into shallower waters at night to feed, since daytime trawls did not capture this species. The VIMS longline survey found similar diel differences in catch records with 25 stations fished at Norfolk Canyon, mostly during daylight hours between 1990 and 1991 yielding only three *C. granulosus*. All three of these specimens were captured during one nighttime set (J. Musick, unpublished data). In contrast, 17 stations fished in the same area overnight between 2006 and 2008 yielded 26 *C. granulosus*. On one of these sets 8 pregnant *C. granulosus* were captured on 15 hooks. Conversely, the only daylight sets ($n=2$) during this 2006–2008 period yielded no *C. granulosus*. These results suggest this species undergoes diel migrations into shallower water at night, however a telemetry study is needed to confirm this.

Our limited data, along with other catch records for this species indicate that Norfolk Canyon and the adjacent continental shelf break may provide exclusive habitat for gravid females of this species. The VIMS catch in this region was entirely composed of females ($n=28$), 82% of which were gravid and the remainder were near the size at maturity. Moore et al. (2003) reported that 100% of the *C. granulosus* (as *Centrophorus* sp.) caught in NOAA trawl surveys off the eastern coast of the U.S. ($n\sim100$) were composed of gravid females, some with near-term embryos (Kiraly et al., 2003). It seems clear that this outer-shelf habitat is not strictly a "pupping ground" (e.g. *Carcharhinus plumbeus* in Chesapeake Bay; Grubbs and Musick, 2007) because pregnant females with embryos of all stages of development were found in this area. Likewise, this does not appear to be a case of mere sexual segregation, because large immature females were mostly absent from Norfolk Canyon. The segregation observed in the present study appeared to be limited to gravid females, as has been observed in other squaloid sharks. Girard and Du Buit (1999) and Clarke et al. (2001) found only immature and postpartum female *Centrophorus squamosus*, along with mostly mature, reproductively active males on the shelf break west of Scotland and Ireland, but no gravid females were found among their samples. Also, during the MAR-ECO cruise, the *C. squamosus* catch was composed of 82 mature males and one immature female on the MAR north of the Azores (Dyb and Bergstad, 2004). McLaughlin and Morrissey (2005) reported sexual segregation in *C.* cf. *uyato* around Jamaica and hypothesized that males move into the area during the winter months. In addition to geographical segregation, some squaloids exhibit depth-mediated segregation by maturity stage (Yano and Tanaka, 1988; Clarke et al., 2001).

4.3.3. Fecundity and reproductive cycle

A paradoxical reduction in mean egg size with increased maternal length was observed in *C. granulosus* (Fig. 3B). This might have been explained as a correlation with increased fecundity (i.e., lipid reserves divided among an increased number of oocytes), however the regression of fecundity and maternal length (Fig. 3A) showed no relationship. However, both of these regressions, particularly fecundity, may have been limited by the small samples sizes of each. Two female specimens in the present study had an observed fecundity of one; however, these were omitted from Fig. 3A due to the likelihood of aborted embryos. In both cases, each female had a single mid-term embryo, yet both uteri were highly vascularized and distended, with well-developed villi, indicating recent activity in each uterus. Aborted embryos were occasionally observed on the deck of the boat during sample collection. This presents an unfortunate uncertainty in determining fecundity, since abortions may also occur prior to hauling specimens on deck.

Ovarian development is concurrent in this species (Fig. 3D) such that vitellogenesis occurs during gestation with a subsequent batch of oocytes ovulated shortly after parturition. This pattern has also been reported for *C.* cf. *uyato* (McLaughlin and Morrissey, 2005) and *Squalus* cf. *mitsukurii* (Cotton et al., 2011). A closer examination of the ovaries of *C. granulosus* indicated that there are two distinctly different vitellogenic batches (size classes) of oocytes found in the ovaries during gestation (Supplementary Figs. 1 and 4), presumably developing for two subsequent ovulations.

The aseasonal reproductive cycle observed for this species (Fig. 3C) is common among deep-water sharks (Kyne and Simpfendorfer, 2010), probably owing to the relative stability in environmental conditions of the deep ocean, and has been reported for many deep-water squaloids, e.g. *S.* cf. *mitsukurii* (Cotton et al., 2011), *Etmopterus granulosus* (Wetherbee, 1996), *C. fabricii* (Yano, 1995), *C. coelolepis* (Yano and Tanaka, 1988; Girard and Du Buit, 1999; Veríssimo et al., 2003), and *C. owstoni* (Yano and Tanaka, 1988).

4.4. Reproductive biology of *Etmopterus princeps*

4.4.1. Maturity ogives

Size at maturity was approximately the same in both regions (Fig. 1B) for males but females seem to mature at a larger size in the northern region (CGFZ). This discrepancy in female size at maturity might reflect delayed maturity or differences in growth rates if the sharks occupying these two regions compose distinct populations. These differences in size at maturity might also be due to data limitations as the samples from the southern area (AZ) excluded large, mature individuals such that size at maturity for the largest sizes classes were assumed rather than measured (Fig. 6A).

Jakobsdóttir (2001) reported the length at which 50% of *E. princeps* reached maturity around Iceland as 573 mm TL for males and 622 mm TL for females. In the northern stations of the MAR-ECO cruise, we found the size at 50% maturity was 540 mm TL for males and at 690 mm TL for females (Fig. 6B). However, Jakobsdóttir (2001) used different criteria to assess maturity, classifying stage "B" (or "maturing") individuals as mature, whereas we classified those individuals as immature. Recalculation of female maturity ogives using Jakobsdóttir's method yielded a similar size at 50% maturity for females, 627 mm TL.

4.4.2. Fecundity and reproductive cycle

Observed fecundity in *E. princeps* ranged from 7 to 18 embryos and the relationship between female size and fecundity was uncorrelated ($R^2=0.0725$), however this may be an artifact of the small sample size (Fig. 7A), aborted embryos, or the physiological burden of parasitism, observed in some gravid females. Though the lowest observed fecundity was 7, this particular female (710 mm TL) was covered in 21 ectoparasites (unidentified monogenean flatworms and parasitic goose barnacles, *Anelasma squalicola*) and thus may have been compromised. Similarly, the largest pregnant female (760 mm TL) examined had a relatively low fecundity ($n=12$), but this female was also covered in 35 ectoparasites. Previous studies reported reduced fecundity in female etmopterids parasitized by *Anelasma squalicola* (Hickling, 1963; Yano and Musick, 2000). The ovarian cycle is consecutive in this species, as reported for *C. fabricii* (Yano, 1995), *C. owstoni*, and

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C. coelolepis (Yano and Tanaka, 1988). The differing ovarian cycles between *E. princeps* and *C. granulosus* may reflect varying magnitudes of matrotrophy or perhaps the differences in energetic demands of a matrotrophic and a lecithotrophic species, respectively. If *E. princeps* females allocate lipid stores and energy to the production of mucoid histotroph throughout gestation, they may be incapable of concurrent vitellogenesis.

4.4.3. Segregation by sex and maturity stage

Pregnant *E. princeps* were "rarely found" around Iceland and the Reykjanes Ridge (Jakobsdóttir, 2001) and the author suggested that segregation by maturity stage occurs in this species. A high abundance of postpartum and "resting stage" females was also reported among the catch in that study. In the present study, only 1 of 247 females examined was postpartum and this individual was found in the northernmost extent of the area sampled. Wetherbee (1996) also reported segregation by reproductive stage and found low numbers of pregnant *E. granulosus* around New Zealand, suggesting that gravid females segregate from the rest of the population.

Male maturity data collected in the present study also support the hypothesis of segregation by maturity stage. South of the CGFZ, only about 40% of the male *E. princeps* collected were mature, whereas 95% of the males north of the CGFZ were mature and mostly milting (reproductively active). As Yano (1995) reported for *C. fabricii* around Greenland, male *E. princeps* likely remain reproductively active after reaching maturity since there is apparently no mating season for this species (Jakobsdóttir, 2001; present study).

Jakobsdóttir (2001) showed a highly MAR-focused distribution of *E. princeps* around Iceland, including many postpartum females, very few reproductively active males, and some small neonates (≥ 120 mm TL). The Icelandic shelf break and the Reykjanes Ridge may therefore serve as a pupping ground for this species. Jakobsdóttir's (2001) observations, combined with the results of the present study, suggest that females aggregate along the MAR near Iceland for pupping. After the subsequent resting period, mating possibly occurs in the high concentration of reproductively active males near the CGFZ and these mated females may then travel further south along the MAR (north of the Azores) to gestate. This, however, would require females to undergo extensive migrations along the MAR, yet this small-bodied species may be incapable of migrating such great distances.

5. Summary

In this study, we show that organic matter is depleted by 19.5% for embryos of *C. granulosus* and 7.7% for embryos of *E. princeps* between the time of fertilization and parturition. Though these species are presumed lecithotrophic yolk-sac viviparous, our results suggest that *E. princeps* is matrotrophic, likely utilizing the "limited histotrophy" mode of reproduction. Alternate interpretation implies that the range of organic matter depletion from fertilization to parturition may vary greatly among species, depending on taxon-specific conversion efficiency of yolk, and that a depletion of 20% in embryonic organic matter does not necessarily delimit matrotrophic and lecithotrophic species (Frazer et al., 2012). Likewise, as has been suggested by prior authors (e.g. Hamlett et al., 2005), it is possible that all yolk-sac viviparous species exhibit some level of histotrophy, with the degree of maternal nutritional supplementation following a continuum across species.

Mating was determined to be aseasonal in both species examined and segregation by maturity stage was observed. Ovarian cycles were concurrent for *C. granulosus* and consecutive for *E. princeps*. The capacity for sperm storage was not investigated for either species but this mechanism could influence reproductive cycles, particularly for *E. princeps* which has a resting period after parturition. Size at maturity for *C. granulosus* was determined to be 111 cm TL for males and 143 cm TL for females, with an average fecundity of 5.3 embryos (range = 4–7). Size at maturity for *E. princeps* was determined to be 56.5 cm TL for males and 61 cm TL for females north of the Azores and 54 cm TL for males and 69 cm TL for females near the CGFZ. Average fecundity was 11.2 embryos (range = 7–18) for this species.

This is the first reporting of the reproductive biology of *C. granulosus* and *E. princeps* and our analyses of embryonic development challenge the notion that these are lecithotrophic species. Future studies like this are needed to clarify the degree to which different viviparous species deplete yolk (organic matter) during gestation (Frazer et al., 2012). Likewise, the hypothesis of matrotrophy (limited histotrophy) needs to be tested for these species, by histological examination of uterine lining and/or the use of tracer molecules to track the movement of uterine nutrients into the developing embryo. Our characterization of reproductive parameters such as size at maturity and fecundity will be crucial for informing stock assessment models in areas where these species are fished.

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Appendix A. Supporting information

Supplementary data and figures associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2014.10.009.

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