Ultrastructure of Oogenesis in the Bluefin Tuna, *Thunnus thynnus*

Francisco J. Abascal and Antonio Medina*

Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Avda. República Saharaui, s/n, E-11510 Puerto Real, Cádiz, Spain

ABSTRACT  Ovarian ultrastructure of the Atlantic bluefin tuna (*Thunnus thynnus*) was investigated during the reproductive season with the aim of improving our understanding of the reproductive biology in this species. The bluefin, like the other tunas, has an asynchronous mode of ovarian development; therefore, all developmental stages of the oocyte can be found in mature ovaries. The process of oocyte development can be divided into five distinct stages (formation of oocytes from oogonia, primary growth, lipid stage, vitellogenesis, and maturation). Although histological and ultrastructural features of most these stages are similar among all studied teleosts, the transitional period between primary growth and vitellogenesis exhibits interspecific morphological differences that depend on the egg physiology. Although the most remarkable feature of this stage in many teleosts is the occurrence of cortical alveoli, in the bluefin tuna, as is common in marine fishes, the predominant cytoplasmic inclusions are lipid droplets. Nests of early meiotic oocytes derive from the germinal epithelium that borders the ovarian lumen. Each oocyte in the nest becomes surrounded by extensions of prefolicle cells derived from somatic epithelial cells and these form the follicle that is located in the stromal tissue. The primary growth stage is characterized by intense RNA synthesis and the differentiation of the vitelline envelope. Secondary growth commences with the accumulation of lipid droplets in the oocyte cytoplasm (lipid stage), which is then followed by massive uptake and processing of proteins into yolk platelets (vitellogenic stage). During the maturation stage the lipid inclusions coalesce into a single oil droplet, and hydrolysis of the yolk platelets leads to the formation of a homogeneous mass of fluid yolk in mature eggs. J. Morphol. 264:149–160, 2005. © 2005 Wiley-Liss, Inc.

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Tunas show asynchronous oocyte development and are considered multiple spawners. Like the other two species of bluefin tunas (*Thunnus maccoyii* and *T. orientalis*), *T. thynnus* exhibits a migratory and spatiotemporally confined reproductive pattern, since spawning is restricted to a short period of time and to specific grounds (Schaefer, 2001). An accurate knowledge of reproductive parameters, such as sexual maturation, fecundity, and spawning, is essential for population dynamics and stock management studies in tunas. Histological examination of ovarian and testicular tissues is used to assess the reproductive cycle of teleost fishes (Grier and Taylor, 1998; Taylor et al., 1998; Brown-Peterson et al., 2002; Lo Nostro et al., 2003), including tunas (Hunter et al., 1986; Schaefer, 1996, 1998, 2001), since it provides the most precise picture of the reproductive state in both females and males. However, despite the economic importance of *T. thynnus*, histological studies on its reproductive biology have been scarce until very recently (Baglin, 1982; Susca et al., 2001; Medina et al., 2002; Sarasquete et al., 2002; Corriero et al., 2003; Abascal et al., 2002, 2004). Most of these studies are based on light microscopy, which has considerable limitations in resolving the entire suite of gametogenic processes, such as folliculogenesis or vitellogenesis, for which only electron microscopy provides adequate resolution and detail (Grier, 2000).

In the developing ovary of fishes, following oogonial proliferation, early meiotic oocytes become surrounded by prefolicle cells and form the ovarian follicles. Subsequently, early oocytes enter primary oocyte growth, which is marked by intense RNA synthesis and formation of the vitelline envelope, and then are arrested in diplotene of prophase I. During vitellogenesis the diplotene oocytes take up vitellogenin, a protein synthesized by the liver which is processed into yolk globules. Eventually, under appropriate hormonal stimuli, the oocytes undergo final maturation, are ovulated, and become fertilizable eggs (deVlaming, 1983; Tyler and Sumpter, 1996). Ultrastructural aspects of oogenesis have been documented in several teleost species (Selman and Wallace, 1982, 1983, 1986; Selman et
RESULTS

The ovaries of *Thunnus thynnus* are paired, elongate organs, attached to the abdominal roof by the dorsal mesentery. Posteriorly, the ovaries extend into short oviducts that open into the cloaca. The ovarian tissue forms longitudinal lamellae that extend into the reduced central lumen. A squamous epithelium, the so-called germinal epithelium (Grier, 2000), lines these lamellae at the luminal surface. The pattern of ovarian development is asynchronous (Baglin, 1982; Susca et al., 2001; Medina et al., 2002; Corriero et al., 2003); therefore, during the breeding season all stages of oocyte development can be found in the gonad (Fig. 1A). The complete process of oogenesis was divided into five stages: formation of early oocytes from oogonia and folliculogenesis, primary oocyte growth, secondary oocyte growth (comprising lipid stage and vitellogenesis), and maturation.

### Early Oocytes and Folliculogenesis (Oocyte Diameter: ~10–20 μm)

Oogonial proliferation was not observed in adult bluefin tuna ovaries, and oogonia (seldom identifiable) were not easily distinguished from early meiotic oocytes. As in another perciform, *Centropomusundecimalis*, oogonia appear to have a more irregular nucleus than the oocytes, whose nuclei are spherical (Grier, 2000). Oogonia and oocytes, prior to initiation of the primary growth stage, are usually found beneath the squamous epithelial layer bordering the ovarian lamellae, and are usually grouped in nests where development seems to be synchronous (Fig. 1B). Both oogonia and early oocytes have a high nucleus–cytoplasm ratio, and the nucleus has a single, central nucleolus. The modest amount of cytoplasm contains many ribosomes, round mitochondria with few cristae, some Golgi complexes, and a poorly developed endoplasmic reticulum (Fig. 1C–F). The nuclear envelope exhibits numerous pores through which an electron-dense, granular material is exported from the nucleus to the cytoplasm and forms the “nuages” (Fig. 1D) that frequently appear associated with mitochondria (Fig. 1C,E). Prefollicle cells, derived from the epithelial somatic cells, surround the germ cell nests and send thin processes between adjacent early meiotic oocytes that separate them from each other (Fig. 1E). This event takes place at the zygote–pachytene stage (Fig. 1E inset). The prefollicle cells surrounding oocyte nests show intricate interdigitations and are joined by desmosomes (Fig. 1F). Eventually, they lose contact with the ovarian lumen, sink under the germinal epithelium, and constitute a follicular epithelium around the oocytes. At this stage desmosomes between adjacent follicle cells are rarely found.

### Primary Oocyte Growth (Oocyte Diameter: ~20–120 μm)

As in other fishes, this stage starts once the oocyte enters into arrested meiotic diplotene (Wallace and Selman, 1990; Grier, 2000). The cytoplasm of primary growth-phase oocytes is highly granular due to an abundance of ribosomes. Nucleoli are first scattered throughout the nucleoplasm and afterwards migrate toward the nuclear envelope (Fig. 2A). The volume of the oocyte increases as a result of proliferation of membranous organelles and accumulation of ribonucleoproteins in the cytoplasm (Fig. 2A). As a consequence, the nucleus–cytoplasm ratio decreases considerably (Fig. 1B). Mitochondria of variable shape, Golgi complexes, and nuage bodies are usually found close to the nucleus (Fig. 2A,B).
Fig. 1. Oogenesis in Thunnus thynnus. A: Section of an ovary with follicles at all developmental stages. LM. B: Section of a nest of early oocytes (ON) located beneath the epithelium bordering the ovarian lumen (L). LM. C–F: Early meiotic oocytes. TEM. Gc, Golgi complex; m, mitochondria; MNO, migratory nucleus oocyte (maturation stage); N, nucleus; n, nuages; nu, nucleolus; p, nuclear pores; PFC, prefollicle cells; PGO, primary growth oocyte; POF, post-ovulatory follicle; sc, synaptonemal complex in pachytene oocyte (see inset in E for further detail); VO, vitellogenic oocyte. Arrows, extensions of prefollicle cells between adjacent pachytene oocytes; arrowheads, desmosomes between prefollicle cells.
Fig. 2. A–F: Primary growth oocytes of *Thunnus thynnus*. TEM. bl, basal lamina; FC, follicle cell; Gc, Golgi complex; ld, lipid droplet; m, mitochondria; mv, microvilli; N, nucleus; n, nuage; nu, nucleolus; O, cytoplasm of oocyte; TC, thecal cell; ve, vitelline envelope.
During this stage, membrane processes begin to extend from the surface of both the oocyte and (to a much lesser extent) the follicle cells. The vitelline envelope (also referred to as zona radiata or chorion in the relevant literature) begins to be deposited as a homogeneous, moderately electron-dense material (Fig. 2C,D). Shortly thereafter, an inner, more electron-dense layer of the vitelline envelope differentiates (Fig. 2E,F). Golgi bodies, some profiles of rough endoplasmic reticulum (RER), and smooth vesicles are present (Fig. 2E). Occasionally, small lipid droplets become apparent in the ooplasm at the end of this stage (Fig. 2F).

A continuous follicular layer (the granulosa) formed by flattened cells encompasses the oocyte (Fig. 2D–F). The follicle cells are elongated, with free ribosomes, mitochondria, small vesicles, and cisternae of the RER. A distinct, thick basal lamina separates this cell layer from the overlying thecal cells (Fig. 2D–F). The inner thecal layer is vascularized and contains small capillaries, whereas the outer thecal cells form a rather thin squamous layer.

**Lipid Stage (Oocyte Diameter: ~120–250 μm)**

The beginning of this stage is marked by the appearance of lipid droplets that are clearly discernible in light microscopy (Fig. 3A). Lipid synthesis continues throughout this period and extends into the vitellogenic stage, leading to an increase in lipid stores that eventually become distributed randomly in the ooplasm (Fig. 3A,B). These lipid droplets are homogeneous, moderately electron-dense, and display a smooth, nonmembrane-bound surface (Fig. 3B,C). The nuclear envelope has numerous pores and the nucleoplasm contains a finely granular chromatin. As in the preceding stage, small amounts of granular material are apposed to the nuclear envelope at the ooplasmic side. Large parts of nucleolar material lie beneath the nuclear envelope in the peripheral nucleoplasm (Fig. 3D).

At this stage the weakly basophilic cytoplasm becomes more vesicular and contains free ribosomes, mitochondria, and some dictyosomes that are more common at the periphery of the oocyte (Fig. 3E). The vitelline envelope has thickened slightly in comparison to the preceding stage, and the follicle cells have become higher (Fig. 3E). In close association with cisternae of the RER, cortical alveolus-like vesicles are occasionally seen. These cytoplasmic inclusions are relatively scarce and have an electron-lucent, finely granular content (Fig. 3F).

**Vitellogenesis (Oocyte Diameter: ~250–500 μm)**

This stage is characterized by substantial growth of the oocyte, which is primarily accounted for by uptake of exogenous material into the ooplasm. During vitellogenesis, the oocytes accumulate membrane-bound yolk platelets that increase in size as oocyte development progresses (Fig. 3A). Vitellogenic oocytes have abundant finger-like projections that are embedded in the layered vitelline envelope (Fig. 4A–E). These well-developed oocyte cytoplasmic microvillar bundles penetrate deeply into the intercellular spaces between follicle cells, they never reach the basal lamina at the opposite side of the follicular epithelium (Fig. 4A,C–E).

The height of the follicular epithelium increases during vitellogenesis; the follicle cells become thicker and contain round mitochondria and a moderately developed RER (Fig. 4A,E). Golgi bodies and cytoplasmic vesicles are relatively scarce. The borders between follicle cells appear clearly marked because of the high electron-density of the extracellular substance (Fig. 4A,C–E). This dense substance also pervades through the bundles of microvilli, thus outlining the microvillar profiles under and between the follicle cells (Fig. 4A,D,E). These observations suggest that the follicular epithelium is rather permeable to substances from the surrounding thecal layers via intercellular spaces. The basal lamina that externally invests the follicle is thick and fibrous, and displays a parallel orientation of the collagen fibers in relation to the follicular surface (Fig. 4A,C,D). Two layers of flattened thecal cells envelope the follicle around the basal lamina. Capillaries are commonly found in the inner thecal layer.

Aside from the presence of microvilli, another evidence of the absorptive capacity of vitellogenic oocytes is the appearance of numerous pits and spherical vesicles (~100 nm in diameter) in the peripheral cytoplasm immediately beneath the plasma membrane. They enclose an electron-dense material that resembles in appearance the intercellular substance found between follicle cells (Fig. 4A,B,D–G). At high magnification these pits and vesicles were found to be clathrin-coated (Fig. 4G), which is indicative of selective transport of specific ligands. Single or multiple coated vesicles are frequently observed to bud from deep tubular membrane invaginations, thus suggesting intensive uptake of exogenous substances (Fig. 4D–G). The cortical region of the ooplasm is rich in free ribosomes but lacks membranous organelles (Fig. 4A,B,F). Under this region the most conspicuous structures are mitochondria with tubular cristae and also electron-lucent vesicles that are somewhat larger than the endocytotic vesicles (Fig. 4A). It is not known whether these vesicles belong to the endoplasmic reticulum or they play a role in the plasma membrane recycling system. Fusion of these vesicles with the plasmalemma is not frequently observed.

Shortly after the extracellular material is internalized, the endocytotic vesicles lose their clathrin
Fig. 3. Lipid stage oocytes of *Thunnus thynnus*. 

**A:** Ovary showing lipid stage and vitellogenic oocytes. LM. 

**B–F:** Details of lipid stage oocytes. TEM. Arrowheads, traffic of granular material through nuclear pores; bl, basal lamina; cav, cortical alveolus-like vesicle; FC, follicle cell; ld, lipid droplet; LSO, lipid stage oocyte; mv, microvilli; N, nucleus; nu, nucleolus; PGO, primary growth oocyte; rer, rough endoplasmic reticulum; TC, thecal cell; ve, vitelline envelope; VO, vitellogenic oocyte.
Fig. 4. Vitellogenic oocytes of *Thunnus thynnus* (A–G). TEM. bl, basal lamina; e, uncoated endocytotic vesicles (endosomes); FC, follicle cells; is, intercellular space; m, mitochondria; mv, microvilli; N, nucleus; ny, nascent yolk platelets; O, oocyte; T, thecal cell layers; TE, theca externa; TI, theca interna; ve, vitelline envelope; y, yolk platelet. Arrows, gathering of oocyte microvilli into bundles; arrowheads, coated endocytotic pits and vesicles (for further detail, see G, which is an enlargement of the area indicated in E); double arrowheads, multiple budding of endocytotic vesicles from long tubules connecting with the plasma membrane.
coat and appear to coalesce with others to form larger vesicles (endosomes and nascent yolk platelets) (Fig. 4E,F). As a result of this process, the ooplasm of vitellogenic oocytes stores yolk platelets whose size increases centripetally (Figs. 4B, 5A). Large lipid droplets, which continue to accumulate during the vitellogenic stage, alternate with yolk platelets at the central ooplasm (Fig. 5B). The yolk platelets are membrane-bound inclusions that consist of a central, electron-dense core embedded in a more electron-lucent granular matrix (Fig. 5A–C). The peripheral matrix of the yolk platelets is of variable thickness. Sometimes it is lost in areas where the outer membrane is apposed directly to the central core (Fig. 5B). High-magnification micrographs show a crystalline arrangement of the central core material with a periodicity of ~4.5 nm (Fig. 5C, inset). Electron-lucent patches of variable size are seen in some yolk platelets (Fig. 5A).

Maturation (Oocyte Diameter: ~500–1,000 μm)

The primary event of oocyte maturation is the resumption of meiosis. The commencement of this stage is marked by migration of the nucleus toward the animal pole of the oocyte (Fig. 6A), which is followed by breakdown of the nuclear envelope. Concomitant with the nuclear migration, the lipid droplets begin to fuse into larger lipid inclusions.
that occupy the central portion of the oocyte, where they eventually form a very large central lipid droplet (Fig. 6A,B). Throughout this stage the yolk platelets coalesce as they undergo internal disorganization, probably due to hydrolysis of their contents. First, the pale areas in the central core increase in number and size (Fig. 6B), and then the core materials disaggregate into irregular patches that remain embedded in a highly electron-lucent matrix (Fig. 6C,D). The processes of proteolysis and coalescence of yolk globules lead to hydration of the oocyte with the formation of a continuous mass of fluid yolk that occupies the entire volume of the oocyte.

The layered vitelline envelope reaches ~6 μm in thickness and the follicle cells become more vacuolated. Following ovulation the follicular epithelium shrinks and folds, giving rise to postovulatory follicles.

**DISCUSSION**

The terminology and features used to differentiate successive stages of oocyte formation and matura-
tion vary according to authors and species. In this study the follicle development in *Thunnus thynnus* was divided into five distinct stages on the basis of histological and ultrastructural observations. This staging reflects morphological as well as physiological and biochemical events, and is compatible with other classifications commonly used in teleosts (de-Vlaming, 1983; Nagahama, 1983; Selman and Wallace, 1986; Wallace and Selman, 1990; Selman et al., 1993; Tyler and Sumpter, 1996). We herein use the term “lipid stage” to refer to the transitional stage between primary growth and vitellogenesis. However, we avoid the term “cortical alveolus stage” commonly used in teleosts because, as in other marine species, cortical alveoli are scarce in, if not absent from, bluefin tuna oocytes. Lipid stage oocytes are characterized by the presence of cytoplasmic lipid droplets that can be distinctly identified on the light microscope (Susca et al., 2001; Medina et al., 2002; Corriero et al., 2003). Although in most teleosts studied thus far cortical alveoli have been reported to become apparent before lipid droplets and yolk granules, there are exceptions (West, 1990). Within the perciforms, Mayer et al. (1988) found cortical alveoli to appear after both lipid droplets and yolk granules in the sea bass, while in the red sea bream (Matsuyama et al., 1991) and the amberjack (Grau et al., 1996) they were observed shortly after the commencement of lipid droplet formation.

The present ultrastructural observations on bluefin tuna oogenesis do not differ significantly from those reported previously for other teleost fishes (Selman and Wallace, 1986; Wallace and Selman, 1990; Selman et al., 1993; Grier, 2000). It is generally accepted that teleost oocytes arise within the germinal epithelium bordering the ovarian lumen (Wallace and Selman, 1990; West, 1990; Grier, 2000). The presence of early meiotic oocytes within the ovarian luminal epithelium suggests the germinal role of this layer in bluefin tuna, although, as reported in previous studies (Selman and Wallace, 1986; Wallace and Selman, 1990; Selman et al., 1993), oogonial proliferation was not observed. Oogonial mitotic divisions are difficult to detect in adult teleost ovaries during the breeding season, since the overwhelming majority of germ cells are more advanced in development. Early meiotic oocytes appeared grouped in nests and, as in other teleost species (Selman et al., 1993; Grier, 2000), their development seems to be synchronous even though the germ cells within the same cluster soon become isolated from each other by prefollicle cells. In the common snook, *Centropomus undecimalis*, desmosomes and tight junctions are lost once the epithelial cells associate with leptotene oocytes to become prefollicle cells (Grier, 2000). Conspicuous desmosomes, however, keep the prefollicle cells tightly joined during zygotene and pachytene in the bluefin tuna, but as the oocyte becomes surrounded by follicle cells and sinks into the ovarian stroma these junctions are no longer observed. A characteristic of early oocytes is the presence of electron-dense nuages close to the nuclear envelope. Nuages continue to be present throughout primary growth until the lipid stage, in contrast to the condition in the common snook (Grier, 2000), where the nuages disappear prior to the completion of folliculogenensis.

According to Nagahama (1983), Wallace and Selman (1990), West (1990), Tyler and Sumpter (1996), Grier (2000), and Guimarães and Quagio-Grassiotto (2001) the primary growth stage is characterized by an intense transcriptional activity. The occurrence of multiple nucleoli at the periphery of the nucleus, as well as numerous perinuclear nuages, appears to indicate an intense transport of ribonucleoproteins from the nucleus to the cytoplasm. Aggregations of organelles, mainly mitochondria in association with nuages, are seen close to the nucleus. At the end of this stage the vitelline envelope begins to form, penetrated by short microvilli.

During the following secondary growth phase the oocyte accumulates nutrients for the development of the future embryo. The first inclusions to become visible in the ooplasm of bluefin tuna oocytes are small lipid droplets that gradually increase in number and size throughout the lipid stage and also during most of the remaining secondary growth. As commonly observed in teleosts, particularly the marine species (deVlaming, 1983), the lipid droplets begin to coalesce centripetally into larger lipid inclusions at the beginning of the maturation stage, eventually forming a single central oil droplet. The lipid content of the bluefin tuna ovary was found to increase significantly throughout secondary growth, neutral lipids (primarily triacylglycerol and steryl/wax ester classes) predominating over polar lipids (Mourente et al., 2002). These data appear to indicate that the lipids accumulated into the egg oil droplet during secondary growth mostly consist of neutral lipids.

In many teleosts, the precursors of the cortical alveoli appear when the follicle diameter is ~200 μm (deVlaming, 1983; Selman et al., 1986, 1988; Tyler and Sumpter, 1996; Guimarães and Quagio-Grassiotto, 2002). Membrane-bound vesicles resembling the cortical alveoli of other teleosts appeared to originate in association with RER cisternae in bluefin tuna oocytes. The extremely low frequency of these structures makes it unlikely that they play a significant role during the cortical reaction at fertilization, as occurs in other species (deVlaming, 1983; Selman et al., 1986, 1988; Tyler and Sumpter, 1996).

A massive incorporation of exogenous substances appears to be mainly responsible for the enormous enlargement that the oocyte undergoes during secondary growth. The presence of a well-developed micr villar border, and the formation of numerous pits and vesicles at the oocyte surface, provide ample evidence of the great capacity of bluefin tuna vitel-
logenic oocytes to incorporate extracellular substances. The pathway of yolk incorporation into the ooplasm has been revealed in *Cyprinodon variegatus* (Selman and Wallace, 1982) and *Fundulus heteroclitus* (Selman and Wallace, 1983) using electron-dense tracers. Circulating macromolecules were shown to pass between the endothelial cells of the thecal capillaries, then between the follicle cells, and finally through the pore canals of the vitelline envelope (Selman and Wallace, 1982, 1983, 1986). In *Thunnus thynnus* the intercellular spaces of the follicular epithelium are filled with an electron-dense substance that is very similar in appearance to that found in the endocytotic pits and vesicles of the oocyte. Bundles of microvilli from the oocyte accumulate in the subfollicular space, and some of them penetrate deeply into the intercellular spaces between follicle cells, a phenomenon that also has been observed in *C. variegatus* (Selman and Wallace, 1982) and in *F. heteroclitus* (Selman and Wallace, 1983, 1986). The dense extracellular substance diffuses between the microvilli located in the interfollicular and subfollicular spaces; hence, the long microvillar processes convey the exogenous substances to the oocyte surface by capillarity. Once this material has reached the oocyte surface, it appears to enter the ooplasm by endocytosis. Since clathrin-coated pits are major ports of entry of macromolecules into the cell (Smythe, 2003), the abundance of coated pits and vesicles in the cortical cytoplasm of teleost oocytes (Selman and Wallace, 1982, 1983, 1986; Wallace and Selman, 1990; Selman et al., 1993) points to a selective uptake of the yolk precursor, vitellogenin, which would then be translocated to the nascent yolk platelets. The sequence of events leading to formation of the mature yolk platelets can be inferred from ultrastructural observations of the cortical ooplasm. Endosomes apparently resulting from coalescence of endocytotic vesicles fuse with one another or with preexistent yolk vesicles in the cortical ooplasm (Selman and Wallace, 1982, 1983), thus contributing to the formation of larger yolk platelets that reside in the central region of the oocyte. The whole process is quite fast, since in *C. variegatus* circulating horseradish peroxidase is incorporated into peripheral yolk platelets within 20 min after intraperitoneal injection of the tracer (Selman and Wallace, 1982). In calf chromaffin cells, Artalejo et al. (2002) calculated that the duration of clathrin-coated vesicle-based endocytosis is ~10 min. Thus, although the volume of the clathrin-coated vesicles of bluefin tuna oocytes is rather small (~5 × 10^{-4} \text{µm}^3), their great number and the supposedly fast completion of the endocytotic process suggest a high rate of vitellogenin uptake so as to be capable to maintain the production of a batch of mature oocytes every 1.2 days, which is the average interspawning interval estimated for *T. thynnus* during the breeding season (Medina et al., 2002).

Incorporation of exogenous materials by pinocytosis continues to occur until the early maturation stage in oocytes of *Fundulus heteroclitus*, but it stops abruptly at the time of germinal vesicle breakdown (Selman and Wallace, 1983). Likewise, morphologic evidence of endocytosis was not observed in maturational follicles of *Thunnus thynnus*.

As in other teleost species (Kjesbu and Kryvi, 1989; Selman et al., 1993), mature yolk globules of *Thunnus thynnus* have a main proteic body embedded in a superficial matrix. As oocyte development proceeds through maturation, the yolk platelets lose their regular inner structure. Apart from nuclear transformations (germinal vesicle migration and breakdown), the most striking event that occurs during the maturation stage in many marine teleosts is proteolysis and fusion of yolk platelets, which culminate in the formation of a continuous mass of fluid yolk. Hydration during final maturation causes a considerable increase in volume and appears to be important in the production of pelagic (buoyant) eggs (deVlaming, 1983; Tyler and Sumpter, 1996).

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**LITERATURE CITED**


