

## ***In vivo* investigation of oocyte transit and maturation in a broadcast-spawning holothurian**

Jean-François Hamel<sup>1,a</sup> and Annie Mercier<sup>2</sup>

<sup>1</sup> Society for the Exploration and Valuing of the Environment (SEVE), Portugal Cove-St. Philips, Newfoundland, Canada A1M 2B7

<sup>2</sup> Ocean Sciences Centre (OSC), Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

**Abstract.** A sequential *in vivo* approach was used to examine the transformations undergone by oocytes during transit in the gonoduct of the sea cucumber *Holothuria leucospilota*, from ovulation until fertilization competency. Spasms of the ovarian muscle bands, during the pre-spawning locomotor activity of the females, coincided with the extrusion of oocytes from the follicle cells (ovulation). No germinal vesicle breakdown (GVBD) was visible and the oocytes were not fertilizable. As the animal began to display the anterior sweeping movements characteristic of spawning, the oocytes streamed out of the gonad and were stored in the gonad basis. The oocytes, which were still non-fertilizable, were then pressed forward through the first (proximal) section of the gonoduct. GVBD was completed during this rapid transit, but oocytes could not be fertilized unless they had soaked  $\geq 20$  min in seawater. In the second (distal) section of the gonoduct, most oocytes were readily fertilizable; fertilization rates increased noticeably after the formation of a bulge beneath the gonopore, which favored the entry of seawater. Hydration of the jelly coat was apparent (i.e., a 60% increase in oocyte surface area). Gamete release occurred in one powerful spurt  $\sim 85$  min after the onset of ovulation. This oocyte maturation sequence is expected to occur in holothurian species with similar anatomy and spawning behavior.

*Additional key words:* spawning, sea cucumber, *Holothuria leucospilota*, gonoduct

---

In many broadcast-spawning marine invertebrates, the few hours or minutes that precede gamete release are determinant for the final maturation of reproductive cells into fully competent gametes. With the exception of echinoids, gametes released from the gonad via the gonoduct in echinoderms are the culmination of a dependent series of cellular events triggered sequentially by endogenous chemical messengers (Shirai & Walker 1988; Smiley 1990). Numerous investigations at the cellular level on surgically collected and/or laboratory-manipulated oocytes have described the endocrine control of ovulation and oocyte meiosis by chemical or mechanical treatments in holothurians (e.g., Strathmann & Sato 1969; Ikegami et al. 1976; Kishimoto & Kanatani 1980; Maruyama 1980; Smiley 1984; Maruyama 1985; Smiley & Cloney 1985; Maruyama 1986) and other echinoderms (e.g.,

Kanatani 1969; Davidson et al. 1982; Meijer & Guerrier 1984; Davidson 1986; Giese & Kanatani 1987; Shirai & Walker 1988). In these studies, the process of oocyte maturation is examined outside of its natural site of occurrence (i.e., the ovary).

In contrast, the normal sequence of events occurring inside the gonad and along the gonoduct, just before gamete release, has rarely been examined in marine invertebrates. Widowati et al. (1995) conducted a histological investigation of the oocytic pathway in a mollusc (*Pecten maximus* LINNAEUS 1758), Holland (1988) studied the fine structure of oocyte maturation in one female crinoid (*Oxycomanthus japonicus* MÜLLER 1841) by serial biopsy and histology, and Smiley & Cloney (1985) looked at ovulation under a dissecting microscope in the sea cucumber *Stichopus californicus* STIMPSON 1857. The processes of ovulation, maturation, and acquisition of fertilizability of the oocyte, relative to its *in situ* translocation from the gonad to the water column, remain largely unexplored.

---

<sup>a</sup> Author for correspondence.

E-mail: hamel@seve.cjb.net