Hidrobiológica 2012, 22 (2): 181-184 **NOTAS** Agosto 2012

Early Gametogenesis of Kumamoto oyster (*Crassostrea sikamea*)

Gametogénesis temprana en el ostión Kumamoto (*Crasssotrea sikamea*)

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Cáceres-Martínez J., R. Vásquez-Yeomans and Y. Guerrero-Rentería. 2012. Early Gametogenesis of Kumamoto oyster (C*rassostrea sikamea*). *Hidrobiológica* 22(2): 181-184.

ABSTRACT

The Kumamoto oyster, *Crassostrea sikamea,* starts gametogenesis as young as 71 days old from spawning (35 days from post-settlement) with a mean shell height of 3.0 mm. This information constitutes a new record in age-size for gametogenesis in oysters for commercial importance and adds another biological difference comparing this species with the Pacific oyster *Crassostrea gigas*.

Key words: *Crassostrea sikamea*, gametogenesis, gonadal development, Kumamoto oyster, sexual differentiation.

RESUMEN

El ostión Kumamoto, *Crassostrea sikamea*, inicia la gametogénesis a una edad de 71 días después del desove (35 días después del asentamiento) con un promedio de altura de la concha de 3.0 mm. Esta información constituye un nuevo registro en la edad y talla en la cual se inicia la gametogénesis en ostiones de importancia comercial. Adicionalmente, pone en evidencia otra diferencia biológica respecto al ostión Japonés *Crassostrea gigas*.

Palabras clave: *Crassostrea sikamea*, desarrollo gonadal, diferenciación sexual, gametogénesis, ostión Kumamoto.

The Kumamoto oyster, *Crassostrea sikamea* Amemiya, 1928, was introduced for commercial culture into bahía de San Quintín, Baja California, Mexico around 1970 from the West coast of the U.S.A. as a variety of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) (Gobierno del Estado de Baja California Sur, 2010). Nowadays, it is known that the Kumamoto oyster is a separate species from the Pacific oyster based on molecular characterization, microsatellite DNA markers, concordant differences in 16S rDNA and allozymes (Banks *et al*., 1993, 1994; Hedgecock *et al*., 1999; Sekino *et al*., 2003; Reece *et al*., 2008; López-Flores *et al*., 2010). Additionally, there are phenotypic and physiological differences between *C. gigas* and *C. sikamea*, the later has slower growth rate, smaller size, smaller eggs, a more deeply cup-shaped left valve, and a highly wrinkled or ridged shell (Amemiya, 1928; Numachi, 1978). The age and size for gametogenesis could also be different among genetically related species. The smaller size for gametogenesis in *C. gigas* is between 16 to 39 mm of shell height (Buroker, 1983), while for *C. virginica* (Gmelin, 1791) it is smaller than 35 mm of shell height, and as young as 42 days from post-settlement (Stafford, 1913; Thompson *et al*., 1996). The oyster *C. rizophorae* (Guilding, 1828) begins gametogenesis at the smallest sizes; from 5 to 9 mm of shell height and less than 42 days old from post-settlement (Vélez, 1976). Until now, there is no information about the age and size for the beginning of gametogenesis in *C. sikamea*. During a histological survey of Kumamoto oysters of < 4.5 mm of shell height and 35 days old from post-settlement, surprisingly, we observed gametogenesis. Results from this survey are described.

In August 2007, one sample of 120 Kumamoto oysters from a commercial hatchery in Sinaloa, Northwest Mexico, was sent alive to the Laboratory of the Instituto de Sanidad Acuícola, A.C. for histological analysis. The sample was composed by oysters of 71 days old from spawning and 35 days from post-settlement, cultured at 24 °C. A second sample of 80 oysters of similar age and cultured at the same temperature was sent a month later to the same laboratory. The oysters were cleaned using running water

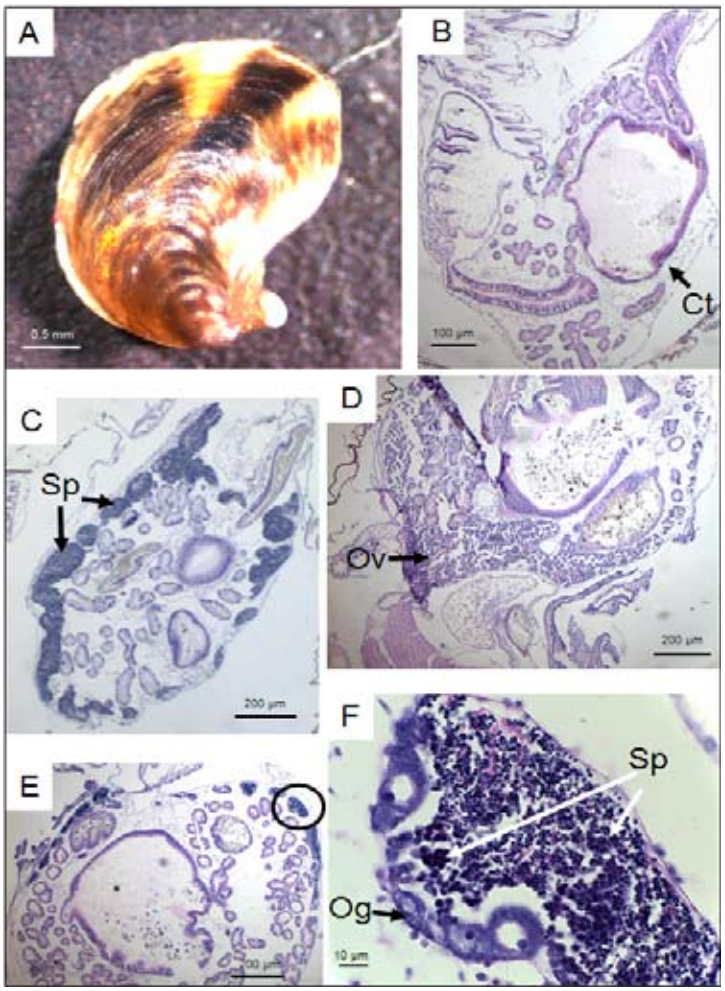

Figure 1A-F. A. Live juvenile Kumamoto oyster. B. Undifferentiated oyster of 2.5 mm of shell height. There is no follicular development in the connective tissue (Ct) surrounding the digestive gland. C. Male oyster of 2.7 mm of shell height with sperm (S) surrounding the digestive gland. D. Female oyster of 3.5 mm of shell height with ovocytes (Ov). E. Hermaphrodite oyster of 3.00 mm of shell height. F. Detail of hermaphrodite (encircled), showing ovogonies (Og) attached to the germinal wall of the follicle and spermatids and spermatozoids (Sp) in the central area of the follicle.

to eliminate debris. From each sample, 54 and 58 organisms were chosen respectively due to best appearance and entire shells. These organisms were considered as subsamples and were measured using a micrometer placed in a stereo microscope, considering the shell height (distance from the umbo to the distal posterior border of the shell; Helm *et al.*, 2006). The whole oysters were fixed by 24 h in Davidson's fixative solution (Shaw & Battle, 1957) and washed in running water. Later, oysters were decalcified by immersion in a solution of 10% EDTA for approximately 12 h (Howard *et al.*, 2004) and washed in running water to eliminating excess of EDTA. Thereafter, oysters were placed in histocassetes for histological process according to Shaw and Battle (1957) and they were checked for tissue alterations and general condition. The size of oysters from different subsamples and reproductive conditions was compared for possible significant differences using the *t*-test (Zar, 1974).

The shell height of the oysters from the first subsample ranged from 2 to 4.1 mm, the mean value was 2.99 mm (± 0.51 SD). In Figure 1A is showing a live oyster of about 3 mm of shell height. In 64% of individuals from this subsample, we do not detect gonad development and this sub-group was considered as undifferentiated (Fig. 1B); however, 36% of these oysters showed development of reproductive follicles with sperm (Fig. 1C). The shell height of this sub-group varied from 2 to 4 mm with a mean value of 2.95 mm (± 0.52 SD). There was no significant difference between the shell heights of both subgroups (*t*-test $p > 0.05$). The shell height of oysters from the second subsample varied from 2 to 4.4 mm, the mean value was 3.19 mm (± 0.58 SD), 62% of the oysters showed gonad development, from which 80.55% were males, 13.89% females (Fig. 1D) and 5.56% hermaphrodites (Figs. 1E, 1F).

There was no significant difference between the shell height of both subsamples (*t*-test, $p > 0.05$); however, there was a trend of larger oysters in the second subsample.

Results indicate that gametogenesis of cultured Kumamoto oyster starts in very young and small size. This finding adds another biological difference between *C. gigas* and *C. sikamea*, the later starts gamete production at about 3 mm of shell height versus 16 mm in *C. gigas*. It is important to carry out studies on natural populations of *C. sikamea* for determining if this early development of gametes takes place in nature or if it was influenced by the hatchery operation, possible due by use of chemicals during culture (Cooper & Wintermyer, 2009). Presence of gametes in these oysters do not means that they are physiologically viable. Moreover, there was not observed empty follicles or the presence of hemocytes which could indicate spawning or reabsorption process. Specific studies are needed for determining if these small oysters may reproduce successfully and if so, how this early reproduction accounts for oyster population dynamics in nature, and how this information may impact management practices in hatchery oyster production.

The fact that the percentage of oysters showing gametogenesis was greater in oysters from the second sample could be related to their slightly larger size; in this sense, Quayle (1988) noted that in *C. gigas* sexual maturity appears to be a function of size rather than age. This information constitutes a new record in the size of gametogenesis in oysters of aquaculture importance, placing the Kumamoto oyster (*Crassostrea sikamea*) as the most precocious species among cultivable oysters in the world. Precocity in bivalve mollusks has been documented in *Mytilus galloprovincialis* Lamarck, 1819 (Mikhailov *et al*., 1995; Paz *et al*., 2001).

ACKNOWLEDGEMENTS

The authors would like to thank Ing. Daniel Carreño Montreal from Sea Farmers, S. A. de C. V. for providing us with the oysters for the study and allowing us to publish the data obtained.

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Recibido: 24 de febrero de 2012.

Aceptado: 03 de mayo de 2012.