

*Short Communication*

## Comparative analysis of the reproductive strategy of lion's paw scallop *Nodipecten subnodosus* in Baja California Sur, Mexico

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**ABSTRACT.** The reproductive strategy of lion's paw scallop *Nodipecten subnodosus* was evaluated in a culturing system in Bahía Tortugas, Baja California Sur, Mexico during an annual cycle, comparing its response with data previously reported at other localities. High frequencies of ripe gonads throughout the year indicate that reproduction was continuous, with two main ripening/spawning events: July-September and December-March. A continuous breeding is also reported for the species in Bahía Magdalena, Bahía Juncalito, and Bahía de Los Angeles. These eutrophic areas are Biological Active Centers where gametogenesis appears to be regulated by the energy taken from recently ingested food following an opportunistic strategy. However, the digestive gland index decreased and the muscle indices increased during one of the breeding peaks, suggesting that some stored reserves are also used to sustain gametogenesis (conservative strategy) partially. High incidences of atretic oocytes are likely associated with atypical daily variations in water temperature from May through September (12 to 33°C), or with stressful conditions in the culturing system in summer. Despite this, the culturing system set in Bahía Tortugas appears beneficial for a continuous reproduction of *N. subnodosus*.

**Keywords:** culturing system, gametogenesis, lion's paw scallop, lipid index, morpho-physiological indices, reproductive strategy.

## Análisis comparativo de la estrategia reproductiva de la almeja mano de león *Nodipecten subnodosus* en Baja California Sur, México

**RESUMEN.** La estrategia reproductiva de la almeja mano de león *Nodipecten subnodosus* se estudió en un sistema de cultivo en Bahía Tortugas, Baja California Sur, México durante un ciclo anual, comparando su respuesta con datos previamente reportados para otras localidades. La alta frecuencia de gónadas maduras durante todo el año indica que la reproducción es continua, con dos eventos principales de madurez/desove: julio-septiembre y diciembre-marzo. Una gametogénesis continua también se reporta para esta especie en Bahía Magdalena, Bahía Juncalito y Bahía de Los Ángeles. Estas áreas eutróficas son Centros de Actividad Biológica, donde la gametogénesis estaría regulada por la energía del alimento recientemente ingerido siguiendo una estrategia oportunista. Sin embargo, el índice de la glándula digestiva disminuyó y el índice del músculo aumentó durante uno de los máximos de madurez, indicando un uso parcial de reservas energéticas para sustentar la gametogénesis (estrategia conservadora). La alta incidencia de ovocitos atrésicos se atribuye a variaciones diarias atípicas en la temperatura de mayo a septiembre (12 a 38°C) o a condiciones estresantes en el sistema de cultivo en verano. Sin embargo, Bahía Tortugas parece beneficiar una reproducción continua de *N. subnodosus*.

**Palabras clave:** sistema de cultivo, gametogénesis, almeja mano de león, índice lipídico, índices morfo-fisiológicos, estrategia reproductiva.

In temperate bivalves, seasonal changes in environmental factors regulate overall patterns of energy acquisition and allocation, and with this, important

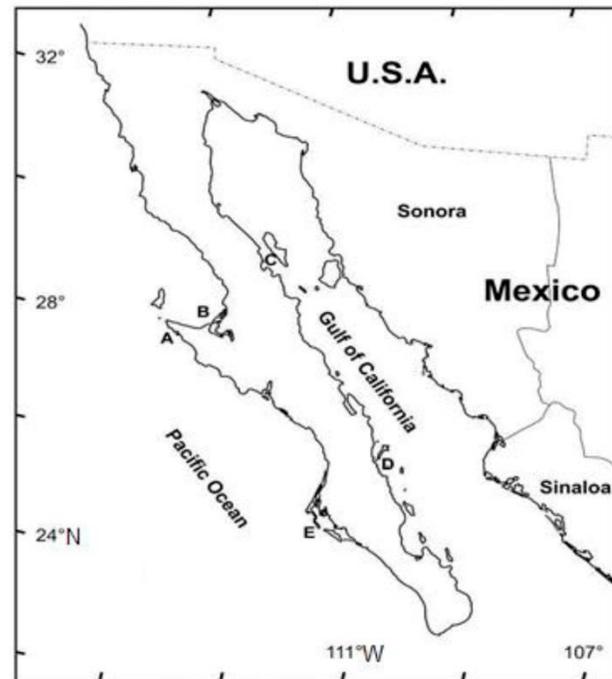
aspects of gametogenesis, such as gamete maturation, growth, release, and reabsorption (Barber & Blake, 2006; Benninger & Le Pennec, 2006). In hermaphrodite

scallops, reproductive events may operate differently than in protandric bivalves, and differences may occur between species or populations of the same species separated along wide or narrow latitude ranges (Arellano-Martínez *et al.*, 2004, 2011). Thus, the expression of reproductive events in a particular geographic area may be analyzed in relation to the type of strategy (opportunistic or conservative) that a species uses to initiate and sustain gametogenesis from exogenous or endogenous energy reserves.

The lion's paw scallop *Nodipecten subnodosus* (Sowerby I, 1835) is an hermaphrodite bivalve with high potential for commercial cultivation in north-western Mexico, but natural populations are overexploited and exposed to wide variations in temperature and availability of food (Morales-Zárate *et al.*, 2011; Ponce-Díaz *et al.*, 2011). Thus, different reproductive patterns (seasonal or continuous) have been reported for wild (Arellano-Martínez *et al.*, 2004; Yee-Duarte, 2009) and cultivated stocks (Racotta *et al.*, 2003; Villalejo-Fuerte *et al.*, 2004; Arellano-Martínez *et al.*, 2011). This study analyzed the reproductive strategy of *N. subnodosus* in a commercial farm located in a place where reproduction of the species has yet not been studied: Bahía Tortugas, Baja California Sur, Mexico. The results were compared with previously reported data for wild and cultured scallops at other temperate and subtropical localities.

Hatchery-reared *N. subnodosus* juveniles were transferred to the field and seeded in a culturing system in Bahía Tortugas, in the western Pacific coast of the Baja California Peninsula (27°41'30"N, 114°53'45"W) (Fig. 1). Juvenile scallops were grown under suspension conditions at 5 m depth in Japanese lanterns held from 60 m long lines. Overall maintenance to remove fouling and to adjust stocking density to ~50% occupation area of the lantern was conducted every two months, as recommended by Velasco *et al.* (2009).

Thirty scallops (7-8 months old;  $74 \pm 0.69$  mm SD shell height) were collected monthly during the annual cycle April 2009-March 2010. Parallel to samplings, water temperature was registered daily with a digital HOBO thermograph placed at 5 m depth, and concentration of chlorophyll-*a* ( $\mu\text{g L}^{-1}$ ) was obtained for each sampling day using Modis AQUA satellite imagery from the NOAA database (<http://coastwatch.pfeg.noaa.gov>). Collected scallops were taken to the laboratory and dissected to excise male and female gonads, which were fixed in Davidson's solution for 48 h for histological and histochemical analyses using 5- $\mu\text{m}$  slides. Gonads were classified as undifferentiated, early developed, late developed, ripe, partially spawned, spent, and reabsorbed (atretic), as proposed by Arellano-Martínez *et al.* (2004, 2011). A detailed



**Figure 1.** Geographic location of the study area (A: Bahía Tortugas) and other sites of the Baja California Peninsula where reproduction of wild and cultivated lion's paw scallop *Nodipecten subnodosus* has been studied (B: Laguna Ojo de Liebre, C: Bahía de Los Angeles, D: Bahía Juncalito, and E: Bahía Magdalena).

description of each developmental stage can be consulted in Arellano-Martínez *et al.* (2004). Due to stormy weather conditions in August, the collection of scallops for histological analysis was impossible. Additionally, five areas of each ovary were selected and images of 30 oocytes per area were measured to determine oocyte diameter ( $\mu\text{m}$ ). To measure the lipid (triglyceride) content of oocytes, replicated gonad slides were stained with Black Sudan B and processed with Image Pro Plus software to determine a lipid index (LI) (Rodríguez-Jaramillo *et al.*, 2008):

$$I = \left( \frac{AL}{OA} \right) \times 100$$

where *AL* = area covered by lipid droplets in the oocyte (in pixels) and *OA* = oocyte area ( $\mu\text{m}^2$ ).

Collected scallops were measured for shell height ( $\pm 0.1$  mm) and weighed for fresh live biomass ( $\pm 0.1$  g). The weights of the gonad, digestive gland, adductor muscle, and mantle were also registered ( $\pm 0.1$  g) to determine a gonadosomatic index (GSI), digestive gland index (DGI), adductor muscle index (AMI), and mantle tissue index (MI) (Arellano-Martínez *et al.* 2004):

$$DGI, AMI, MI = \left( \frac{TW}{SW} \right) \times 100$$

where  $TW$  = weight of each somatic tissue (g) and  $SW$  = live weight of scallops without shell (g).

One-way ANOVA were used to determine significant differences in oocyte diameter and tissue indices as a function of time, followed by multiple range analysis of media with the Tukey test when needed. The relationship between tissues indices (DGI, AMI, MI), environmental factors, and GSI was analyzed with correlation analyses (Sokal & Rohlf, 1995). Previously, data (%) were *arcsin* transformed. For all analyses, the significance level was preset at  $P < 0.05$ .

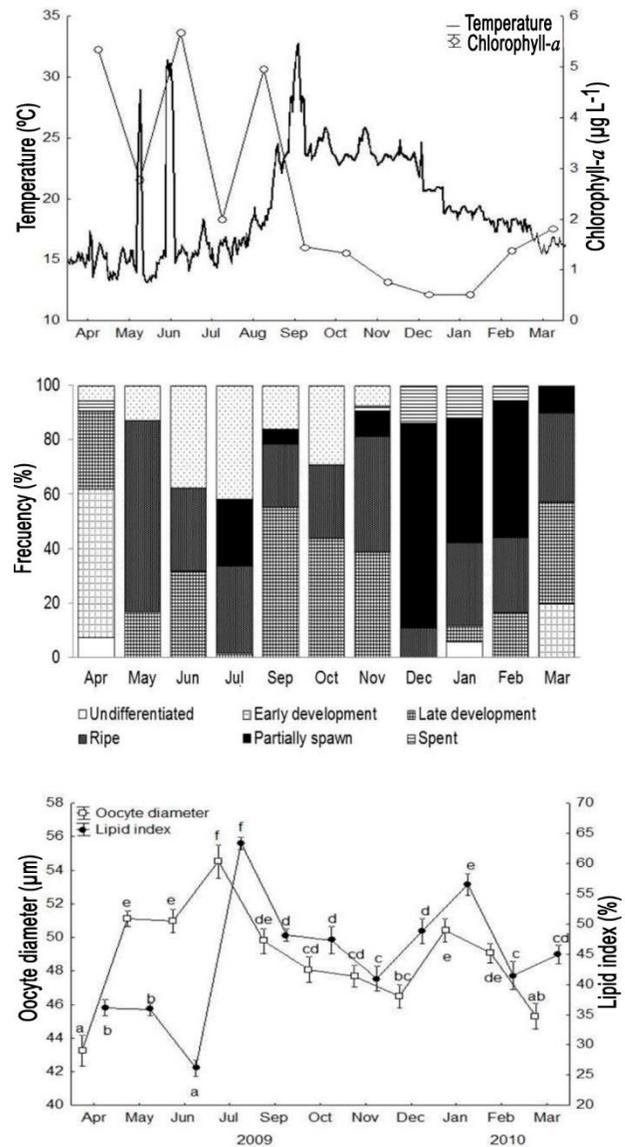
Variations in water temperature and chlorophyll-*a* in Bahía Tortugas followed a typical seasonal pattern (Fig. 2) and maintained an inverse relationship with each other ( $r = 0.87$ ). Lower values occurred in spring (temperature) and summer (chlorophyll-*a*) and higher values in summer-autumn (temperature) and spring-summer (chlorophyll-*a*). There were, however, large temperature oscillations in May-June (13 to 32°C) and September (22.5 to 33°C).

The histological analysis showed that late developed and ripe gonads occurred all year round (Fig. 2), with two spawning events in July through September and December through March. Regressing gonads occurred from April through November, with a peak in July (41.5%) that coincided with atypical increases in water temperature.

Significant changes in the oocyte diameter ( $P < 0.05$ ) occurred over time, with two periods of size increase in July ( $54.5 \pm 3.08 \mu\text{m SE}$ ) and January ( $50.4 \pm 2.64 \mu\text{m}$ ). These changes were significantly correlated with changes in the LI ( $r = 0.82$ ), which peaked again in July ( $63.3 \pm 3.41$ ) and January ( $56.1 \pm 4.6 \mu\text{m}$ ) (Fig. 2).

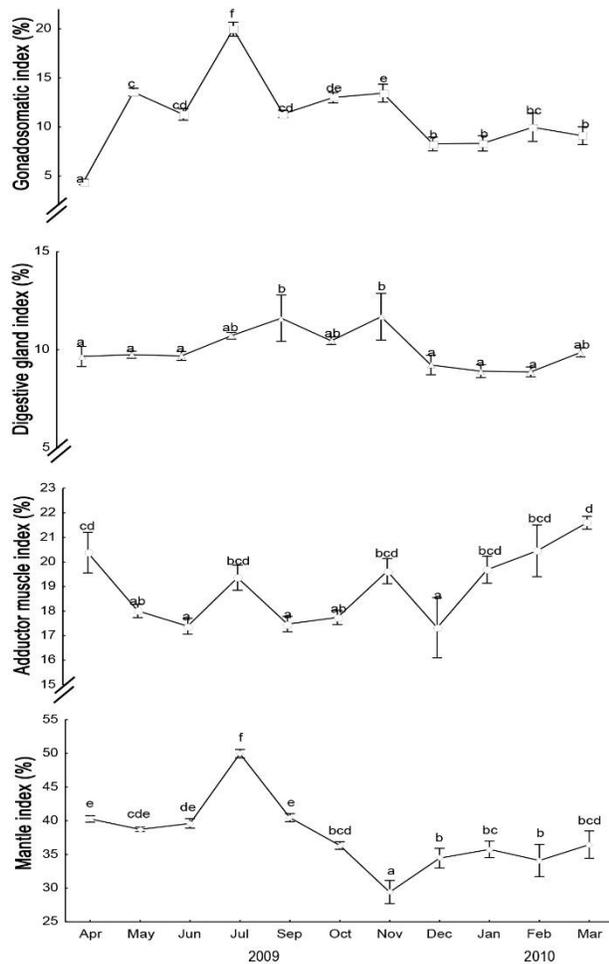
All condition indices varied significantly over time ( $P < 0.05$ ), with peak values occurring in July (GSI), September–November (DGI), March (AMI), and July (MI). Changes in the GSI (4.4% to 20%) were not significantly correlated with changes in temperature ( $P > 0.05$ ). Mean DGI (9.6% to 10.7%) and MI (49.9 to 28.7%) values were not significantly correlated with the GSI ( $P > 0.05$ ), but it was significant ( $P < 0.05$ ) between the AMI (17.3 to 21.8%) and the GSI.

The high proportion of ripe gonads (with large oocytes) in most sampled months indicate that gametogenesis of *N. subnodosus* in Bahía Tortugas was continuous under the conditions prevailing in the culturing system. A continuous reproduction is also reported for the species at other subtropical and temperate localities of the Baja California Peninsula (Fig. 1), including scallops cultivated in Bahía Magdalena (Racotta *et al.*, 2003) and Bahía Juncalito



**Figure 2.** Mean variations in water temperature at 5 m depth (daily) and concentration of chlorophyll-*a* (monthly); frequencies of gonad developmental stages, and diameter and lipid index of oocytes of lion’s paw scallop *Nodipecten subnodosus* in a suspended culturing system in Bahía Tortugas, Mexico during the annual cycle April 2009-March 2010. Means sharing the same superscript denote lack of significantly differences ( $P < 0.05$ ). Bars show the mean  $\pm$  standard errors.

(Villalejo-Fuerte *et al.*, 2004), as well as wild scallops from Bahía de Los Angeles (Yee-Duarte, 2009). The three sites, together with Bahía Tortugas, are eutrophic areas reported as Biologically Active Centers or BAC, which offer high primary productivity all year round and optimum conditions for filter feeders to maintain a prolonged breeding (Lluch-Belda *et al.*, 2000; Morales-Zárata *et al.*, 2011). Under these conditions, gameto-



**Figure 3.** Mean monthly variations in the gonadosomatic index, digestive gland index, adductor muscle index, and mantle tissue index of lion's paw scallop *Nodipecten subnodosus* in a suspended culturing system in Bahía Tortugas, Mexico during the annual cycle April 2009-March 2010. Means sharing the same superscript denote lack of significant differences ( $P < 0.05$ ). Bars show the mean  $\pm$  standard error.

genesis appears to be sustained by the energy taken from recently ingested food, following an opportunistic strategy. However, the DGI significantly decreased and the AMI significantly increased during one of the two breeding peaks (January through March), suggesting that the species also uses some of its stored reserves to partially sustain gametogenesis (conservative strategy). Based on this evidence, we believe that *N. subnodosus* can adopt either an opportunistic or a conservative strategy to maximize reproductive output depending on prevailing environmental conditions in Bahía Tortugas.

In Laguna Ojo de Liebre, a temperate site close to the study area (Fig. 1), reproduction of wild *N. subnodosus* is seasonal; here, a short ripening and spawning period from September through November is

followed by a large quiescent period from December onwards (Arellano-Martínez *et al.*, 2004, 2011). This seasonal pattern of reproduction in Laguna Ojo de Liebre is likely the result of low primary productivity limiting nutrient availability to filter feeders during most of the year (Lluch-Belda *et al.*, 2000). The scenario points towards the usage of a conservative strategy to manage energy reserves during narrow breeding peaks, which contrasts with the dynamic pattern of Bahía Magdalena (Racotta *et al.*, 2003), Bahía de Los Angeles (Yee-Duarte, 2009), and Bahía Tortugas (this study) as BAC areas (Morales-Zárate *et al.*, 2011).

The marked thermic oscillations registered in Bahía Tortugas during May-September 2009 appear to be anomalous and not representative of the area for a normal year not influenced by ENSO events. In this period, a large percentage of ripe gonads experienced a spawning interruption and a resorption process (atresia). Atresia has been well described in scallops (Benninger & Le Pennec, 2006), and usually indicates nutrient recycling as a result of stress originated from unsuitable conditions at the wild (Arellano-Martínez *et al.*, 2004, 2011) or within the culturing system because of overcrowding, presence of predators, lack of maintenance, etc. (Velasco *et al.*, 2009). In our study, it is likely that stress would originate only when atypical thermal oscillations occurred, since our overall evidence suggests that the culturing system set in Bahía Tortugas offers suitable conditions for a continuous reproduction of *N. subnodosus*. Based on this evidence, this scallop species may become a viable candidate for profitable cultivation in Bahía Tortugas and other BAC areas of the eastern or western coasts of the Baja California Peninsula. To achieve this, and optimize a protocol for successful cultivation of the species in these areas, we recommend analyzing certain aspects of its physiological response at the wild and under cultivation conditions, such as immune, antioxidant, and genetic. The plasticity of the species to use conservative or opportunistic strategy also needs to be validated as for the quality of gametes produced and overall performance of larvae and spat reared in hatcheries.

#### ACKNOWLEDGMENTS

We thank the following staff at CIBNOR: Horacio Bervera, Juan J. Ramírez, Enrique Calvillo, and Mario Cota for assistance during field collections; Carmen Rodríguez-Jaramillo and Eulalia Meza for support in processing of tissue samples; and Ira Fogel for editorial improvements. This research was funded through SIP 20101187 of Instituto Politécnico Nacional, and

CONACyT 2009-2010 and 81249 grants. MAAD is a fellow student of PIFI (IPN) and CONACyT, and the results presented here are part of his MSc Thesis.

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Received: 27 July 2014; Accepted: 24 March 2015