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Accumulation of docosapolyenoic fatty acids in developing oocytes of the winged pearl oyster *Pteria sterna*

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ABSTRACT

Ensuring supplies of pearl oyster spat for commercial grafting operations in Mexico is an ongoing problem. This has refocused research toward improving hatchery propagation protocols. Since gender plays an important role in the physiology of bivalves, we studied the use of fatty acids in the gonad and digestive gland of male and female winged pearl oyster (*Pteria sterna*) over its natural breeding season. Sampling included two peaks of ripening (February and April 2009), a pre-reproductive period (November 2008), and a post-reproductive period (June 2009). We found a significant increase in storage of docosapolyenoic fatty acids during development and ripe stages only in the female gonad, which indicates that these fatty acids could be a limiting factor for successful development of high quality eggs. The content of total monounsaturated fatty acids in male gonads, especially the fatty acid 16:1 n7, was significantly higher than in female gonads at the development and ripe stages. We also found differences between males and females in the use of some fatty acids in the digestive gland, especially at the spawned stage. Our results have future application in developing protocols for rearing of this pearl oyster in hatcheries. Incorporating dietary supplements containing docosapolyenoic fatty acids into diets of pearl oyster broodstock could be a practical way to improve their performance, which is crucial for enhancing the viability of larvae and spat.

1. Introduction

Gender plays a role in the physiology of bivalves. The sex of each bivalve should be determined and taken into account in analyzing and interpreting results when studying, for example, the impact of environmental variables, pathogens, or pollutants (Dang et al., 2012; Duchemin et al., 2007). Additionally, differences in immune responses of bivalves have not been related only to gender, but to the stage in the reproductive cycle (Duchemin et al., 2007; Matozzo and Marin, 2010).

Reproductive metabolism in bivalves demands a high input of energy to maximize the quality of gametes (Barber and Blake, 2006). Lipids are essential to enhance egg quality and increase the number of hatched eggs (Fraser, 1989; Holland, 1978). Hence, lipids are reliable indicators of the quality of gametes (Gallager et al., 1986; Palacios et al., 2007). Of the lipids, poly-unsaturated fatty acids (PUFA) play a key role in maintaining the structural and functional integrity of biological membranes and serve as precursors of eicosanoids (20 C PUFA), that ensure adequate development of the gonads (Hendriks et al., 2003; Howard and Stanley, 1999; Iverson, 2009). However, the effect of gender has rarely been studied in relation to patterns in the use of nutrients and energy.

There are two routes by which fatty acids originate in marine bivalves: (1) transfers of nutrients from the digestive gland (ingested food) to the gonad via the hemolymph during high reproductive activity (Barber and Blake, 1985; Caers et al., 1999; Dridi et al., 2007; Vassallo, 1973) and (2) *de novo* synthesis (lipogenesis) mainly from carbohydrate reserves in the adductor muscle (Gabbott, 1975; Palacios et al., 2007; Racotta et al., 1998). Biosynthesis of long-chain PUFA is greater in phytoplankton than in animals at higher trophic levels; thus, filter-feeding bivalves largely depend on the transfer of PUFA from primary producers to ensure reproductive success (Freites et al., 2010; Napolitano et al., 1997; Soudant et al., 1996).

Improving egg quality at the hatchery is a difficult task and requires measuring reliable indicators of gamete quality, such as fatty acid content, to ensure the production of healthy spat. This is of most relevance in species with high commercial value, such as the winged pearl oyster *Pteria sterna* (Gould, 1851) and abalone *Haliotis* spp. that sustain a small pearl industry in Mexico. These species reflect a current increase in world interest in pearls from genera other than *Pinctada* (traditional pearl oysters) species, including clams, scallops, and gastropods (Strack, 2011; Torres-Martínez et al., 2012). The growth of this activity has been slow, mainly because there is an insufficient supply of

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juveniles, either collected in the wild or produced at a hatchery. This is especially the case for *P. sterna*, despite the high fecundity of females, which produce up to 50 million eggs per spawn (Cáceres-Puig, 2012). There have been improvements in developing reliable hatchery protocols for rearing *P. sterna* (Gómez-Robles et al., 2013; Martínez-Fernández et al., 2004), but commercial hatchery production is unreliable and remains a major bottleneck for the incipient industry.

P. sterna is found in the tropical Eastern Pacific from Mexico to Peru (Keen, 1971). Although tropical in nature, this species seems to prefer temperatures typical of subtropical or warm temperate regions, since it is found in water that is deeper than 15 m. When cultivated, massive die-offs have been reported at temperatures >30 °C (Gómez-Robles et al., 2013; Ruiz-Rubio et al., 2006). In contrast to commercially exploited *Pinctada* species, *P. sterna* spawn mostly during colder months (December to April) at the northern limit of its distribution (Cáceres-Puig et al., 2009; Saucedo and Monteforte, 1997; Vite-García and Saucedo, 2008).

Previous work highlighted important components, such as using saturated fatty acids to describe egg quality in this species (Gómez-Robles et al., 2013). However, information about the effect of gender on internal use of nutrients in the Pteriidae is limited. We aimed to describe how male and female *P. sterna* use fatty acids during the peak of the breeding season and generate relevant information for determining egg quality and improving hatchery propagation of the species.

2. Materials and methods

Pearl oysters were collected in the Bahía de La Paz (24°18′N, 110°20′W) in the State of Baja California Sur, Mexico during the peak of their reproductive season, which lasts from December through April (Saucedo and Monteforte, 1997). We collected 20 adult oysters with a mean shell height of 105 mm every 45 days to include pre-reproductive (November 2008), breeding (February and April 2009), and post-reproductive (June 2009) stages. The collection site is an underwater trestle at a depth of 10 m. During each collection, the gonad and digestive gland were extracted; one portion of the gonad of all oysters was preserved in Davidson's solution for 48 h and then used for histological study of sexual and developmental stages. A second portion of the gonad and a portion of the digestive gland of six oysters from each collection event were preserved at −80 °C and later used to determine the fatty acid composition.

Gonads previously preserved in Davidson's solution were dehydrated, placed in embedding media (Paraplast, SPI Supplies, Structure Probe, West Chester, PA), sectioned to 3 μm, and stained with hematoxylin–eosin (Kim et al., 2006). Developmental stages were categorized as inactive, developing, ripe, and spawned (Bayne, 1976) for the fatty acid analyses. For histological description of development of gender, the five-stage scale of Saucedo and Monteforte (1997) was simplified into three stages: developing (inactive and developing), ripe, and spawned (spawning and spent) for easier comparison.

All frozen samples were freeze-dried in a Lyophilizer (VirTis 5 L, SP Scientific, Warminster, PA) and weighed (±0.01 g) with an analytical balance. Lipids of preserved samples of the digestive gland and gonads in the four stages were extracted using a 1:2:0.6 ratio of a solution of chloroform, methanol, and water (Bligh and Dyer, 1959). The extracts were evaporated with gaseous nitrogen (N_2). Lipids were derivatized by transesterification with a solution of hydrochloric acid and methanol (1:19 *v*/*v*) and then heated to 85 °C for 2.5 h (Sato and Murata, 1988). The resulting fatty acid methyl esters (FAME) were analyzed in a gas chromatograph (Varian-CP 3800, Agilent Technologies, Palo Alto, CA) with a mass detector (Varian 1200, Agilent Technologies) with a capillary column (Omegawax, #24136, Supelco/Sigma-Aldrich, St. Louis, MO; 30 m 0.25 m, dr 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The initial temperature was 110 °C and was increased to 165 °C at a rate of 30 °C min^{-1}. This temperature was maintained for 2 min and increased to 210 °C at a rate of 2.2 °C min^{-1}.The final temperature was maintained for 27 min. Detector and transfer line were set at 250 °C with electronic ionization (70 eV) in a dynamic range between 600 and 2000 V. Solvent delay was set at 6 min. Peaks were identified by retention time of standards and interpretation of the mass spectra. Areas were integrated with mass spectrographic software (Wsearch 1.6, Wsearch Software, Melbourne, Australia) and classified as: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids with two or more double bonds (PUFA). Quantification was done by interpolation of peak areas with a calibration curve of 37 fatty acid standards (#47885-U, Supelco, Bellefonte, PA). We expressed the percentage of each fatty acid with respect to total FAME, but only those at >1% are presented.

During the collection of oysters, water temperature (±0.1 °C) was recorded with a portable multi-probe system (#556, YSI, Yellow Springs, OH). Sea surface temperature (SST) anomalies at the collection site during the collection events were downloaded from the NOAA database, using the Coast Watch Searcher (http://coastwatch.pfeg.noaa.gov).

The mean percentages of each fatty acid related to total FAME in males and females were independently analyzed for the developing and ripe stages of the gonad and for the mean content of DPFA between developing and ripe females, using the *t*-test for independent samples and the Levene test for homogeneity of variance in the statistical software (Statistica 6.1, Statsoft, Tulsa, OK). Mean fatty acid content between the gonad in spawned males and females, and the mean content of DPFA between the ripe and spawned stages in females, were analyzed with the *t*-test for a single sample against a constant (fatty acid content of one spawned female). Significance was set at $P < 0.05$, unless noted otherwise.

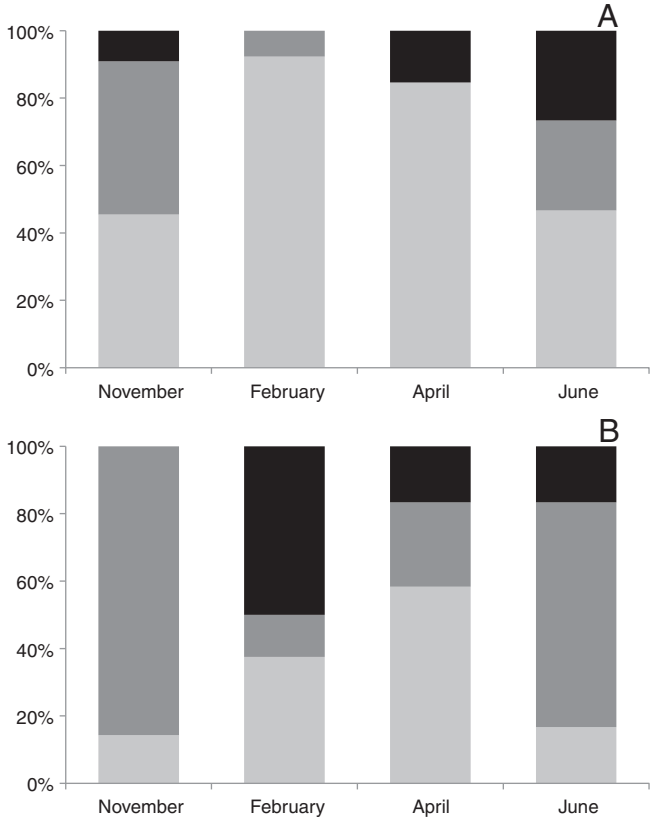

Fig. 1. Proportion of the reproductive stages of the winged pearl oyster *Pteria sterna* during the main breeding season in the Bahía de La Paz, Mexico. Only the three main developmental stages (□) developing; (■) ripe; and (■) spawned, are described for easier comparison between males (A) and females (B).