Egg strain in the sea urchin *Pseudechinus magellanicus* (Echinoidea: Temnopleuridae)

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Abstract: Echinoid eggs with sizes greater than the gonopore experience strain resulting from compression during spawning, which can damage them affecting fertilization. The aim of this study was to describe gamete characteristics and analyse aspects related to egg strain during spawning of *Pseudechinus magellanicus* from Golfo Nuevo, Patagonia, Argentina. Mean fresh egg diameter observed was 122 µm with an additional jelly coat of 49 µm. The relationship between gonopore sizes and maximum test diameter showed a positive correlation but egg size did not vary with test diameter, as seen in other sea urchins, indicative of egg strain during spawning. Egg strain calculated from the relationship between egg size and gonopore size was 0.47-0. Eggs of small individuals (<15 mm test diameter) were the most affected by this strain. The egg jelly coat observed in *P. magellanicus* could protect eggs from strain and shear stress during spawning. Rev. Biol. Trop. 56 (Suppl. 3): 335-339. Epub 2009 January 05.

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Echinoids generally spawn large number of eggs that pass from the gonad through a narrow oviduct and a rigid gonopore before reaching the water, where fertilization takes place. Eggs experience flow-induced shear stress forces (Thomas & Bolton 1999). In some species the diameter of the eggs is larger than the diameter of the gonopore. In these cases the egg experience strain resulting from compression of the egg as it passes through the gonopore (Thomas *et al.* 2001).

Echinoid eggs are surrounded by a jelly coat that consists of globular glycoproteins bound to a fibrous fucan superstructure (Bonnell *et al.* 1994). Although jelly coats are typical of echinoid eggs, there are very few studies that report jelly coat size (Podolsky 2004). The jelly coats in echinoids have been hypothesized to have a number of potential roles in the fertilization process. These roles include the reduction of the incidence of polyspermy and cross-fertilization (Hagström 1956); induction of the acrosomal reaction (SeGall & Lennarz 1979); induction of egg-sperm binding (Vaquier & Moy 1977); activation and chemotaxis of sperm (Nomura & Isaka 1985); species-specific egg-sperm recognition (Miller & Ax 1990); and enlargement of the effective size of the egg thereby increasing the likelihood of the sperm-egg collision (Epel 1991, Levitan 1996). Shear stress and strain forces are important in the evolution of extracellular layers surrounding the eggs. Therefore, further estimates of these forces are needed for other species (Bolton & Thomas 2002).

There are very few studies on reproduction of echinoids from Argentina (Brögger *et* *al.* 2003, Bigatti *et al.* 2004, Marzinelli *et al.* 2006). As far as we know, no studies on egg strain of any South American echinoid have been published. *Pseudechinus magellanicus* (Philippi, 1857) is a small, abundant echinoid in Argentinean and south Chilean waters. It is distributed along the South American coast from off Rio de la Plata (35ºS) in the Atlantic Ocean to Puerto Montt (41ºS), Chile, in the Pacific Ocean; it is also found in islands of the Antarctic Sea (Bernasconi 1953). In Golfo Nuevo, Patagonia, they live in habitats of mixed gravel and sandy bottoms and *Macrocystis pyrifera* blades and holdfasts, occurring at depths of five to twenty metres (Penchaszadeh *et al.* 2004). *P. magellanicus* from Golfo Nuevo has an annual reproductive cycle with two spawning events. Major spawning occurs in winter, followed by a second spawning peak in summer (Marzinelli *et al*. 2006).

The present paper describes gamete characteristics and analyses aspects related to egg strain during spawning of *P. magellanicus* (Temnopleuridae).

MATERIALS AND METHODS

Pseudechinus magellanicus (n = 100) were collected by SCUBA divers from depths between 5-10 m off Golfo Nuevo, Puerto Madryn (42º S, 65º W) during winter 2003.

To analyse the relationship between test and gonopore sizes, the test diameter of 100 urchins of different sizes was measured with a calliper. Animals were dissected and the maximum diameter of each gonopore was measured with a Zeiss stereoscopic microscope and photographs were taken with a Philips XL30 Scanning Electron Microscope (SEM). Mean gonopore diameter was calculated for each individual.

Spawning was induced by injecting each individual with 0.5 ml of 0.5 M KCl. Fresh eggs (n = 30) of 20 individuals were measured under optical microscope to determine egg strain. For size measurements, eggs were viewed in a suspension of Sumi ink. Strain experienced by the gametes as they pass through the gonopore was estimated as:

$$ES = (\phi\ o - \phi\ g) / \phi\ o$$

Where ϕ o = egg diameter (without jelly coat), and ϕ g = gonopore diameter (Thomas *et al.* 2001).

Spearman's correlation analysis was done to test the hypothesis that there is a positive relationship between gonopore diameter and test diameter. All statistical analysis was carried out using the STATISTICA 6 statistical package.

RESULTS

A scanning electron microscopic photograph of a gonopore of *P. magellanicus* is shown in Fig. 1. The mean gonopore diameter of *P. magellanicus* ranged from 80 µm to 500 µm and is positively correlated with maximum test diameter (Spearman, $p<0.0001$, $r = 0.87$). A microphotograph of an egg of *P. magellanicus* is shown in Fig. 2. The mean egg diameter was 122 ± 5 µm with a jelly coat of 49 ± 6 µm and regardless of test diameter. The total mean egg plus jelly coat diameter was of 220 ± 15 µm (Fig. 2). Egg diameter (without jelly coat) exceed mean gonopore diameter in small individuals (<15 mm). As a result, strain range from 0.47 to no strain at all (Fig. 3).

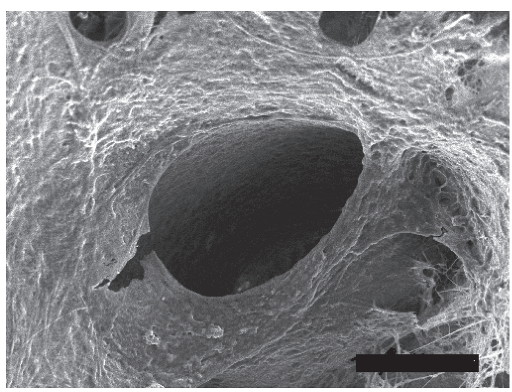

Fig. 1. Scanning Electron Microscopy photograph of a gonopore of *P. magellanicus* (bar = 50 µm).

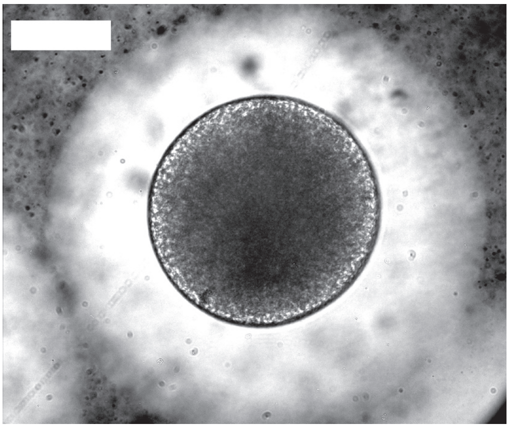

Fig. 2. Microphotography of an egg of *P. magellanicus* in suspension of Sumi ink (bar = 50 μm).

DISCUSSION

In echinoids, gonopore diameter increases linearly as they grow (Emlet 1989). However, there is no apparent relationship between body size and egg size (Thomas *et al.* 2001), as found here for *P. magellanicus*. Because *P. magellanicus* becomes reproductive at a small size (12 mm test diameter) (Orler 1992, Guisado 1995, Marzinelli *et al.* 2006) the eggs of small individuals experience strain when spawned (strain range from 0.47 to 0). In larger individuals (>15 mm), eggs with a diameter smaller than the gonopore experience other forces as a result of flow-induced shear stress. Both forces may damage the eggs, thereby reducing their fertilizability (Thomas & Bolton 1999). Egg damage in small (with strain) and large (without strain) individuals was not, however, analysed in this study. It is, on the other hand, unlikely that strain with total egg diameter (i.e. egg + jelly coat) would affect the egg if the egg itself is smaller than the gonopore in larger individuals because the jelly layer preferentially compresses, reducing the transmission of force to the egg (Thomas *et al*. 2001). At the same time, there could be an effect if more than one egg passes through the rigid gonopore at the same time. Future manipulative experiments are needed to study this model for *P. magellanicus*.

The jelly coat, which has been hypothesized to have a number of potential roles in the fertilization process (Nomura & Isaka 1985, Epel 1991, Levitan 1996), could also protect the egg during spawning (Thomas *et al.* 2001). For example, 100% of the eggs of *Arbacia punctulata* with a jelly coat exposed to a shear stress up to 2 Pa survived, whereas 29% of

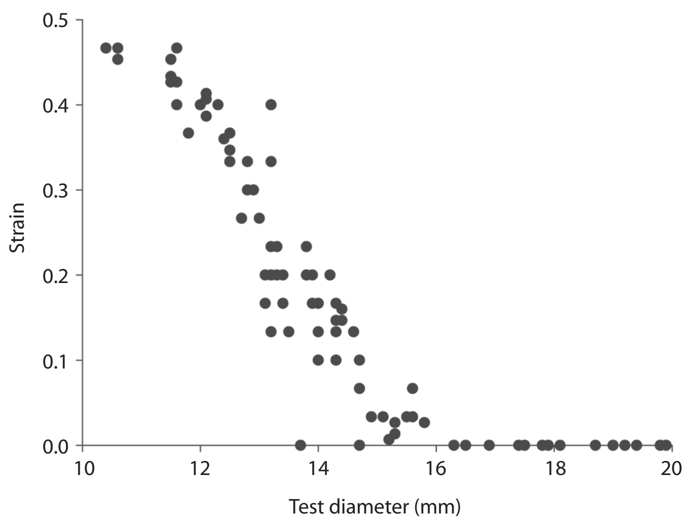

Fig. 3. Relationship between strain and test diameter in *P. magellanicus* (n = 100).

eggs without a jelly coat exposed to the same shear stress were destroyed (Thomas & Bolton 1999). The jelly coat on sea urchin eggs can also reduce the strain imposed on them under a compressive force (Davidson *et al.* 1999). According to Tyler (1986) egg size is indicative of the kind of larval development. Small eggs (mean diameter 100 µm) correspond to a planktotrophic development. In *P. magellanicus* mean fresh oocyte diameter is 122 □m, indicating a planktotrophic development. Another Temnopleuridae species, *Mespilia globules,* with the same development pattern, shows a similar strain range (0-0.5 *vs* 0-0.47 in *P. magellanicus*). On the other hand, the Temnopleuridae species *Holopneustes inflatus*, with lecitotrophic development, shows a greater strain range (0-0.92) (Thomas *et al.* 2001). In all cases, high strain values were seen in the smaller individuals. The jelly coat observed in *P. magellanicus* could protect the eggs from the forces experienced during spawning and until it is fertilized, allowing reproductive success in this temperate environment. Once in the water column, the jelly coat could increase the sperm-egg encounter probability reducing the sperm limitation selection pressure (Levitan 1998, Podolsky 2001, Marshall *et al.* 2002). However, appropriate manipulative experiments need to be done to test hypotheses of these models.

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RESUMEN

Los huevos de equinoideos con un tamaño mayor que el gonoporo experimentan tensión debido a la compresión durante el desove, lo que puede dañarlos y afectar la fertilización. El propósito de este estudio fue describir las características de los gametos y analizar los aspectos relacionados con la tensión en los huevos durante el desove de *Pseudechinus magellanicus* del Golfo Nuevo, Patagonia, Argentina. La media observada en el diámetro de huevos frescos fue de 122 µm con una capa adicional de gelatina de 49 µm. La relación entre los tamaños del gonoporo y el diámetro máximo de la testa mostró una correlación positiva pero el tamaño del huevo no varió con el diámetro de la testa, como se observa en otros erizos de mar, lo cual es indicativo de la tensión que sufre el huevo durante el desove. La tensión en el huevo calculada de la relación entre el tamaño del huevo y el tamaño del gonoporo fue de 0.47-0. Los huevos de individuos pequeños (<15 mm de diámetro de la testa) fueron los más afectados por esta tensión. La capa de gelatina en el huevo observada en *P. magellanicus* podría proteger los huevos de tensión y de estrés por rompimiento durante el desove.

Palabras clave: erizo de mar, *Pseudechinus magellanicus,* desove**,** tensión en el huevo, capa de gelatina.

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