

## Hermaphroditism and preliminary reproductive study of the sea-urchin, *Pseudechinus magellanicus*, in the Beagle Channel (Tierra del Fuego, Argentina)

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**Abstract:** The sea urchin *Pseudechinus magellanicus*, abundant in the Beagle Channel, plays a crucial role in the benthic ecosystem. This study presents the first documented records of hermaphrodites of *P. magellanicus* in the Beagle Channel and provides updated insights into its reproductive dynamics in the studied population. Monthly samplings (30 adult individuals each) were conducted between July and September 2023, assessing sex and reproductive stage through histological analysis. A total of 90 individuals were examined, 45 identified as male, 39 as female, and 6 as hermaphrodites. Excluding hermaphrodites, the sex ratio was 1:1, and the hermaphroditism incidence was 6.67%. The hermaphrodites were found in pre-mature II, mature, or partially spawned stages. Various types of hermaphrodites were identified, including three hermaphrodites with male predominantly characteristics, one with predominantly female characteristics, and two ovotestes. Hermaphrodite gonads were classified based on the presence and distribution of female and male germ cells within the same or different acini. While these findings provide valuable insights into the reproductive biology of *P. magellanicus* in the Beagle Channel, the underlying factors of the observed cases of hermaphroditism remain speculative and require further research to assess potential environmental influences and their implications.

**Key words:** reproductive cycle, ovotestes, echinoderms, gonads, subantarctic

**Resumen:** Hermafroditismo y estudio reproductivo preliminar del erizo de mar, *Pseudechinus magellanicus*, en el Canal Beagle (Tierra del Fuego, Argentina). El erizo de mar *Pseudechinus magellanicus* es una especie abundante en el canal Beagle, donde desempeña un papel crucial en el ecosistema bentónico. Este estudio presenta el primer registro de hermafroditas de *P. magellanicus* en el canal Beagle y proporciona información actualizada sobre su dinámica reproductiva dentro de la población estudiada. Se realizaron muestreos mensuales de 30 individuos adultos entre julio y septiembre de 2023, determinando el sexo y el estadio reproductivo mediante análisis histológico. De un total de 90 individuos examinados, se identificaron 45 como machos, 39 como hembras y 6 como hermafroditas. Excluyendo a los hermafroditas, la proporción de sexos fue de 1:1, mientras que la incidencia de hermafroditismo fue del 6.67%. Los hermafroditas se encontraron en los estadios de pre-madurez II, maduros o parcialmente desovados. Se identificaron varios tipos de hermafroditas, incluidos tres hermafroditas con características principalmente masculinas, un hermafrodita con características principalmente femeninas y dos ovotestes. Las gónadas hermafroditas se clasificaron según la presencia y distribución de células germinales femeninas y masculinas dentro de los mismos o diferentes acinos. Mientras que estos hallazgos destacan aspectos novedosos de la biología reproductiva de *P. magellanicus* en el canal Beagle, las razones detrás de los casos de hermafroditismo observados siguen siendo especulativas y requieren investigaciones adicionales para evaluar posibles influencias ambientales y sus implicancias.

**Palabras clave:** ciclo reproductivo, ovotestes, equinodermos, gónadas, subantártico

## INTRODUCTION

Echinoids play a crucial role both ecologically in the benthic community and commercially due to their ecological and gastronomic value (Calvo *et al.* 1998; Andrew *et al.*, 2002; Sun & Chiang, 2015; Contreras *et al.*, 2019; Suckling *et al.*, 2022). Sea urchins influence the structure of the benthic community by affecting the distribution, abundance, and composition of algae (Heck & Valentine, 1995; Tuya *et al.*, 2004, 2005; Lawrence, 2013) and acting as ecosystem engineers (Hartney & Grorud, 2002). They also play a significant role in converting drift algae into particulate organic matter, providing food that supports the thriving of detritivores and filter feeders in the benthic zone (Moreno *et al.*, 2018). Understanding the reproductive strategies of sea urchins is important for clarifying their population dynamics, life histories, and ecological roles as key benthic species. This information provides insights into how they contribute to the structure and function of benthic ecosystems. (Pearse & Cameron, 1991). The reproductive cycle of echinoids, particularly in mid and high latitudes, typically results in one or two annual spawnings with a high degree of synchronicity between sexes (Marzinelli *et al.*, 2006; Pérez *et al.*, 2010; Brogger *et al.*, 2013; Vafidis *et al.*, 2019; Gil *et al.*, 2020). This periodicity is associated with changes in environmental factors, mainly photoperiod and temperature (Garrido & Barber, 2001; Lester *et al.*, 2007; Pérez *et al.*, 2010; Gil *et al.*, 2020), leading to inter-population variations on both small and large scales (Lester *et al.*, 2007; Lawrence, 2013). In some environments with low seasonality, continuous reproduction throughout the year is possible (Williamson & Steinberg, 2002). Most echinoderms are gonochoric with a 1:1 sex ratio, external fertilization, and development is typically indirect (Tyler *et al.*, 1985; Pearse & Cameron, 1991; Calvo *et al.*, 1998; Lawrence, 2013). However, this developmental pattern is not universal among sea urchins (Barker, 1985; Pearse & Cameron, 1991; Lawrence & Herrera, 2000; McEdward & Miner, 2001; Gil *et al.*, 2009). In sea urchins, five gonads are located between the ambulacral areas in an aboral position. Each gonad is connected by a gonoduct to one of the five gonopores, which open to the exterior around the anus (Lawrence, 2013). The reproductive stage of the gonads can be histologically defined based on their germinal series and accessory cells contained in the acini (Orler, 1992; Pérez, 2009; Lawrence, 2013; Gil *et al.*, 2020).

Although hermaphroditism in echinoids is rare (Meidel & Scheibling, 1998; Hernández *et al.*, 2007) and the frequency of its occurrence is typically low, there are a few documented global cases (Pearse & Cameron, 1991). Echinoids can present different types of hermaphroditism (Hernández *et al.*, 2007), and the number of gonads exhibiting this condition can vary. These types include: a) "ovotestes", where the acini contain both female and male tissues equally; b) hermaphrodites with female predominantly characteristics, in which the gonads are predominantly ovaries; and c) hermaphrodites with male predominantly characteristics where the gonads are predominantly testicles (Pearse & Cameron, 1991).

Although sexual determination in echinoderms is likely genetically driven (Bacci, 1965; Pieplow *et al.*, 2023), the factors causing hermaphroditism still remain unclear (Pearse & Cameron, 1991; Hernández *et al.*, 2007; Pieplow *et al.*, 2023). Some authors suggest that environmental factors may significantly influence gonad development (Moore *et al.*, 1963; Gonor, 1973; Pearse & Cameron, 1991), as they are among the first organs to be affected by stress and energy re-allocation (Dell'Acqua *et al.*, 2019). Deviations from the 1:1 sex ratio and the occurrence of hermaphrodites could help identify the biotic and abiotic factors affecting sexual determination during early development stages (Pearse & Cameron, 1991; Hernández *et al.*, 2007). Pearse and Cameron (1991) dismissed the hypothesis that hermaphroditic echinoderms originate from protandry or sexual reversion due to the lack of evidence.

*Pseudechinus magellanicus* (Fig. 1) is an echinoid with a wide distribution in the southernmost part of South America. Its range extends from Puerto Montt in the Pacific Ocean to the Río de la Plata estuary in the Atlantic Ocean. It is also found in the Beagle Channel and the sub-Antarctic islands of the Southwestern Atlantic (Bernasconi, 1953; Marzinelli *et al.*, 2006; Brogger *et al.*, 2013; Gil, 2015). This is one of the smallest and most abundant sea urchins in Argentina and is associated with strong currents and clear waters (Zaixso & Lizarralde, 2000; Gil *et al.*, 2020). Its bathymetric distribution ranges from the intertidal zone down to depths of 340 m (Bernasconi, 1953; Marzinelli *et al.*, 2006; Gil *et al.*, 2020). *Pseudechinus magellanicus* is the most abundant sea urchin species among the four reported in the Beagle Channel (Vásquez *et al.*, 1984). It has no specific predators in the area,

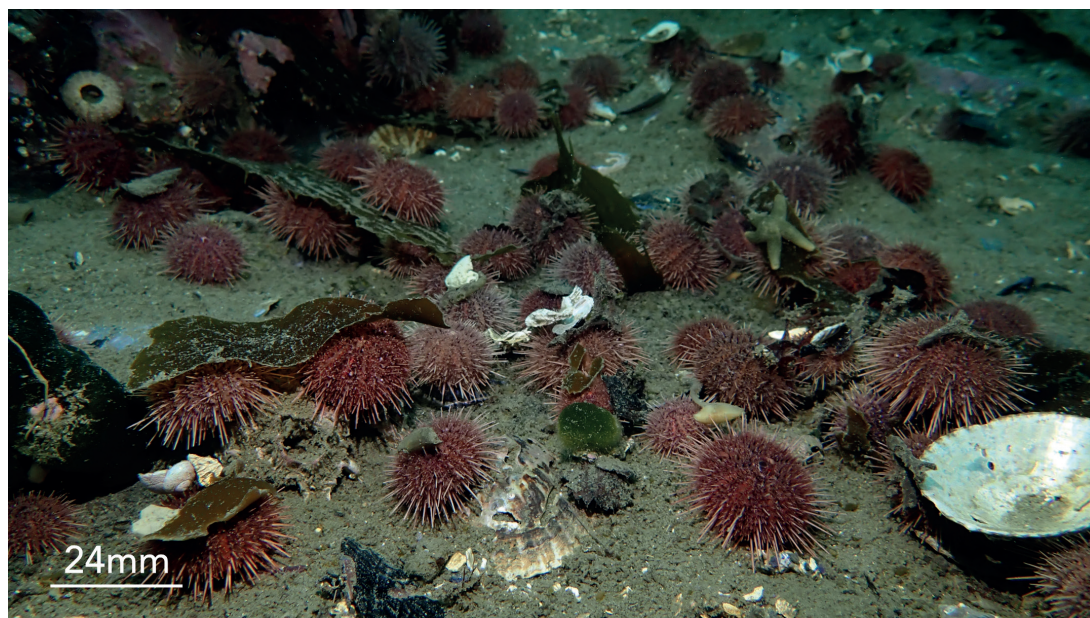


Fig. 1. *Pseudechinus magellanicus* in the study area. Photography by Mariano Rodríguez (Instituto de Ciencias Polares y Antárticas-Universidad Nacional de Tierra del Fuego).

although it has been occasionally preyed upon by the starfish *Cosmasterias lurida* and the snail *Fusitriton magellanicus* (Castilla, 1985). This species feeds on algae and various invertebrates, with its diet varying according to location, depth, and associated community (Brogger *et al.*, 2013; Gil *et al.*, 2021). In the Beagle Channel, it is associated with *Macrocystis pyrifera* forests (Vásquez *et al.*, 1984), although the sea urchin does not overgraze these areas and can also be found outside the macroalgae forests, feeding on drift algae (Castilla & Moreno, 1982).

The reproductive cycle of *P. magellanicus* has been studied on the coasts of Chile (approximately 42°S: Kino, 2010) and in three areas of the Argentine Sea: Golfo Nuevo (approximately 42°S: Bigatti *et al.*, 2004; Marzinelli *et al.*, 2006), Golfo San Jorge (approximately 45°S: Gil, 2015; Gil *et al.*, 2020), and the Beagle Channel (approximately 55°S: Orler, 1992). These studies indicate that *P. magellanicus* is a gonochoric species typically exhibiting a 1:1 sex ratio, with an annual and synchronous reproductive cycle. The variations in the reproductive cycle of this species are strongly related to temperature and photoperiod (Bigatti *et al.*, 2004; Brogger *et al.*, 2013; Gil, 2015). Off Chiloé, the reproductive period occurs from August to October (Kino, 2010); in Golfo San Jorge, from August to September (Gil, 2015); and in Golfo Nuevo, primarily from June

to August, with a second spawning from January to February (Bigatti *et al.*, 2004; Marzinelli *et al.*, 2006). In the Beagle Channel, spawning occurs during winter, from July to August, with the possibility of a second, less intense spawning event during spring (Orler, 1992). Furthermore, males can remain reproductively active for longer periods than females, which exhibit a more pronounced seasonality (Marzinelli *et al.*, 2006). So far, there have been no records of hermaphroditism in this species. This study aims to present the first record of *P. magellanicus* hermaphrodites in the Beagle Channel and update the information regarding their reproduction in the studied population.

## MATERIALS AND METHODS

The study area comprehend the Beagle Channel (54.8764°S, 67.5044°W), approximately 3.75 km from Puerto Almanza, between Upú and Gable islands (Fig. 2). In this particular area, the Beagle Channel narrows and the depth decreases, creating a region with intense currents and shallow waters (Giesecke *et al.* 2021). The substrate of this seabed is primarily fine sediment with gravel and pebbles, and areas with biogenic bottoms are frequent (rhodoliths or shells, personal observation). Although this study focused solely on sampling outside the kelp forests of





Fig. 2. Map showing the studied area. A: Isla Grande de Tierra del Fuego (Argentina) and the Beagle Channel B: Sampling area in Puerto Almanza. The red triangle indicates the collection site.

*Macrocystis pyrifera*, individuals of *P. magellanicus* were abundant both within and outside these forests.

From July to September 2023, we performed monthly collections of 30 adult individuals of *P. magellanicus* (test diameter approximately 24 mm) by SCUBA diving at depths ranging from 1.6 to 5 m. The test diameter of each collected individual was recorded using a caliper ( $\pm 1$  mm), followed by a dissection. One of the gonads was preserved in Bouin's fixative and processed histologically by transverse sectioning and hematoxylin-eosin staining, following the methods outlined by Pérez *et al.* (2010). In those cases where macroscopic differences in the gonads were observed, more than one gonad was fixed for comparative analysis of respective sections. To avoid cross-contamination between samples, all dissection tools (petri dishes, scissors, and scalpels) were cleaned with alcohol and absorbent paper after each specimen dissection. Each gonad was preserved in its own Falcon tube and was individualized throughout the histological process. Sections were photographed with a digital camera attached to an Olympus BX40 microscope.

A total of 90 individuals were histologically sexed, and their reproductive stages were de-

termined based on previous studies (modified from Orlor 1992; Gil 2015; and Gil *et al.* 2020). We classified reproductive stages as "recovery", "pre-mature I", "pre-mature II", "mature", "partially spawned", and "spawned", as follows. For both sexes and hermaphrodites, when several stages were observed simultaneously, the overall stage was determined based on the predominant stage across the entire section.

#### Gonads with female characteristics

The female germ cells were classified as: oögonium  $<10\ \mu\text{m}$  (nucleus), previtellogenic oöcytes (9-20  $\mu\text{m}$ ), vitellogenic oöcytes (20-57  $\mu\text{m}$ ), and ova  $\geq 58\ \mu\text{m}$ . The vitellogenic oöcytes exhibited a clear cytoplasm with eccentric basophilic nuclei, whereas the ova did not present a visible nucleolus and were separated from the acinus wall.

- Pre-mature I stage: there were abundant previtellogenic oöcytes at different developmental stages and sizes, with few or no ova. There is a low abundance of nutritive phagocytes.
- Pre-mature II stage: The presence of ova increased compared to the previous stage. The acinar wall contained both previtello-



genic and vitellogenic oocytes. The lumen is observed reduced, with less free space.

- Mature stage: There were abundant ova densely packed in the lumen. The acinus wall was thin and contained a few primary oocytes and oogonia.
- Partially spawned stage: The acinus appeared contracted and nearly empty, with a few mature ova. The germinal epithelium was thin, with few or no accessory cells.
- Spawned stage: The acinus contained no mature ova, but it could have a few residual oocytes. The frequency of nutritive phagocytes increased.

### Gonads with male characteristics

Spermatogonia were observed along the acini walls, while primary spermatocytes extended into the lumen, forming spermatoc columns as the stages progressed towards maturity. These columns projected towards the center of the lumen, where spermatozoa accumulated. We measured the columns in regions where the acini walls were intact.

- Pre-mature I stage: Spermatogenic columns began to form, exhibiting variable thickness. A limited number of spermatozoa were in the lumen.
- Pre-mature II stage: Spermatozoa number increased compared to the previous stage, but they were not compacted, and there was still free space in the lumen. The spermatogenic column was more developed.
- Mature stage: Spermatozoa were densely compacted, acinus appeared swollen and its wall thickened.
- Partially spawned stage: A few spermatozoa remained in the lumen, but they were less dense, with some empty spaces. The acinar walls were thin.
- Spawned stage: Acini appeared empty, without spermatozoa, and with thinner walls than previous stage.
- Recovery stage: The acini were reduced and folded. Occasionally, a few residual spermatozoa were present and to be digested by nutritive phagocytes. The acinar walls appeared disorganized as new spermatogenic columns began to form.

Chi-squared and Fisher's exact tests were performed to analyze the overall and monthly sex ratios, respectively. The monthly variation in reproductive stages was qualitatively assessed,

and the percentage of hermaphrodites was calculated. All analyses were performed using RStudio (version 2024.04.2+764).

## RESULTS

A total of 45 males, 39 females, and six hermaphrodites were identified. The sex ratio did not significantly differ from 1:1 (Chi-squared = 0.42857,  $df = 1$ ,  $p = 0.5127$ ), nor were there significant differences in the sex ratios for July, August, and September when analyzed separately (Fisher's exact test,  $p = 0.2568$ ,  $0.4497$ , and  $0.5637$ , respectively). Individuals were in pre-mature I, pre-mature II, mature, partially spawned, spawned and recovery, while hermaphrodites were in pre-mature II, mature, or partially spawned stages. Hermaphrodites occurred in July ( $n=1$ ), August ( $n=2$ ), and September ( $n=3$ ), comprising 6.67% of the total sample of 90 individuals. The monthly frequency variation of the reproductive stages showed that in July, the pre-mature II stage was predominant among males, females, and hermaphrodites. In August, 50% of females and hermaphrodites were mature, while the remaining 50% were in the pre-mature II stage. In contrast, over 60% of males were in the pre-mature II stage. In September, 50% of females were in the mature stage, followed by the partially spawned stage. Similarly, 50% of males were in the partially spawned stage. In September, hermaphrodites were equally distributed among the pre-mature II, mature, and partially spawned stages (Fig. 3).

Regarding the percentage of female and male tissue in the hermaphrodite gonads, hermaphrodites with predominantly male or female characteristics, and ovotestes were identified. In some instances, individual acini contained both female and male cells simultaneously, termed "mixed acini". While in other cases, the acini were distinctly segregated by sex, a condition referred to as "hetero-acinus hermaphroditism". A histological description of the hermaphrodite gonads is presented here:

- Individual A (Fig. 4): Test diameter: 25 mm. The gonads were macroscopically identified as male. Subsequent histological analysis revealed evidence of hermaphroditism characterized by mixed acini and an ovotestis. The gonad was in a pre-mature II stage in July. Abundant free sperm were accumulated in the lumen (Fig. 4A). Numerous nutritive phagocyte cells were in the periphery

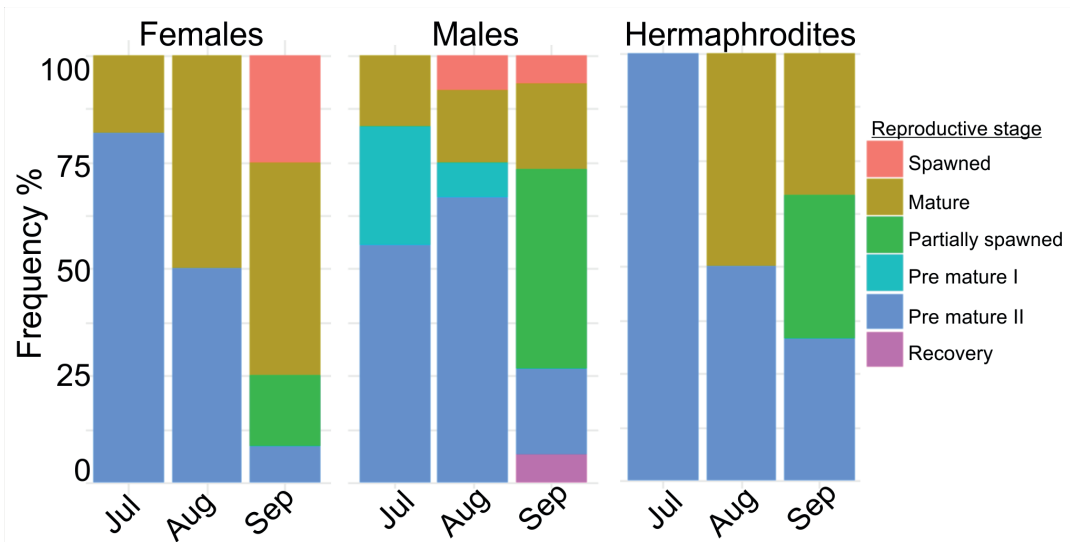


Fig. 3. Percent monthly variation of each reproductive stage for females, males and hermaphrodites.

and interspersed among the sperm and the spermatogenic layer (Figs 4B, D). The primary spermatocyte columns projected into the lumen, ranging from approximately 50  $\mu\text{m}$  to 92  $\mu\text{m}$  (Fig. 4D). The vitellogenic oocytes were projected into the lumen amidst the spermatocyte columns. The oocytes were slightly displaced towards the periphery (Fig. 4A). Oocytes were at various developmental stages, including earlier vitellogenic oocytes, which exhibited a pedunculated shape (Fig. 4B) and late vitellogenic oocytes, which tended to be more spherical (Fig. 4C). Both types had an eccentric nucleolus, a feature not observed in the ova. Some ova ( $\sim 66 \mu\text{m}$ ) were accumulated in the lumen (Fig. 4C).

- Individual B (Fig. 5): Test diameter: 20 mm. During dissection, macroscopic observation identified the individual as male. The histological analysis classified the individual as a hermaphrodite with predominantly male characteristics due to the presence of typical male acini along with some ova. This individual was collected in August. It was not possible to determine whether this was a case of hermaphroditism with mixed acini or a hetero-acinus. The external acini were mature, with mature ova ( $\sim 61 \mu\text{m}$ ) located adjacent to the sperm (Fig. 5A). The walls of the acinus were thin ( $\sim 17 \mu\text{m}$ ), while thicker walls ( $\sim 50 \mu\text{m}$ ) were in acini located further from the testicle periphery. In these acini, sperm was concentrated in the lumen,

but not densely packed, indicating a pre-mature II stage (Fig. 5B). No evidence of female germ cells was found in these acini.

- Individual C (Fig. 6): Test diameter: 22 mm. The individual was collected in August. The gonads exhibited a macroscopic hermaphroditic appearance. During dissection, we observed a pale “milky” liquid (sperm) and an orange liquid (indicative of the presence of oocytes). Histologically, the specimen was identified as a hermaphrodite with predominantly male characteristics, characterized by some ova ( $\sim 60 \mu\text{m}$ ) at the periphery and sperm within the lumen, likely originating from the outermost acini of the testicle, which had reached a mature stage. It was not possible to determine whether this represented a hermaphrodite with mixed acini or a hetero-acinus. Acini in a pre-mature II stage were in the central area of the testicle, where spermatocyte columns, ranging from 40  $\mu\text{m}$  to 100  $\mu\text{m}$ , were organized and projected into the lumen. The lumen contained poorly arranged sperm.
- Individual D (Fig. 7): Test diameter: 26 mm. This individual was collected in September. It was macroscopically identified as male based on the gonadal aspect. Histologically, it was classified as a hermaphrodite with predominantly female appearance (Fig. 7A), with fewer male acini present (Fig. 7C). This specimen exhibited hetero-acinus hermaphroditism, where distinct female and male sections were in different acini. The female



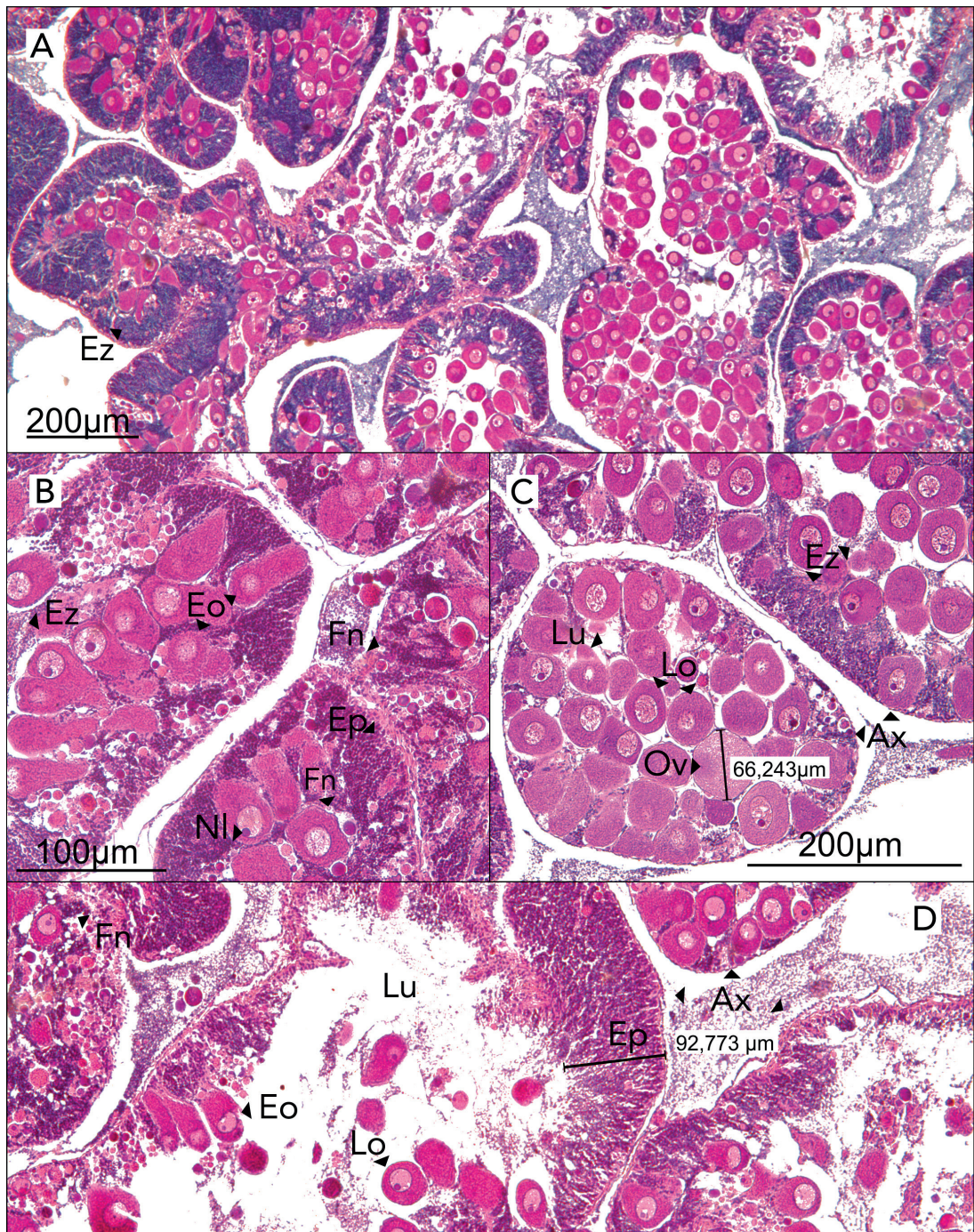


Fig. 4. Gonad of the individual A (Ovotestis with mixed acini in pre-mature II stage observed in July). Ax: Mixed acinus. Lu: Lumen. Ep: Primary spermatocytes. Ez: Spermatozoa. Fn: Nutritive phagocyte. Ov: Ova. Nl: Nuclei. Eo: Early vitellogenic oocyte. Lo: Late vitellogenic oocyte.



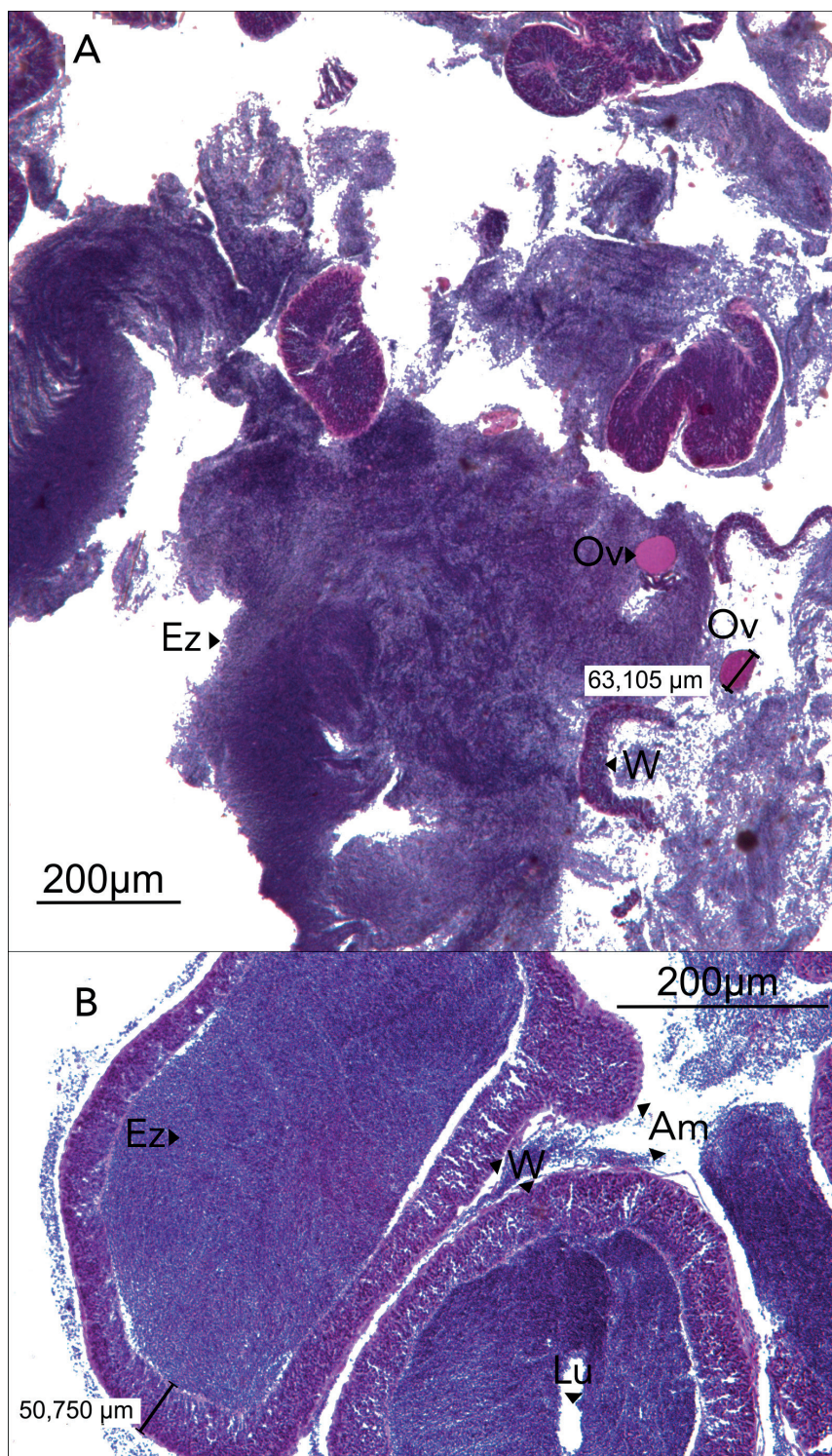


Fig. 5. Cross-section of the gonad of the individual B. (Hermaphrodite with predominantly male gonad in pre-mature II stage observed in August). A: Mature acinus. B: Pre-mature II acinus. Am: Male acinus. Ov: Ova. Ez: Spermatozoa. Lu: Lumen. W: Acinar wall.



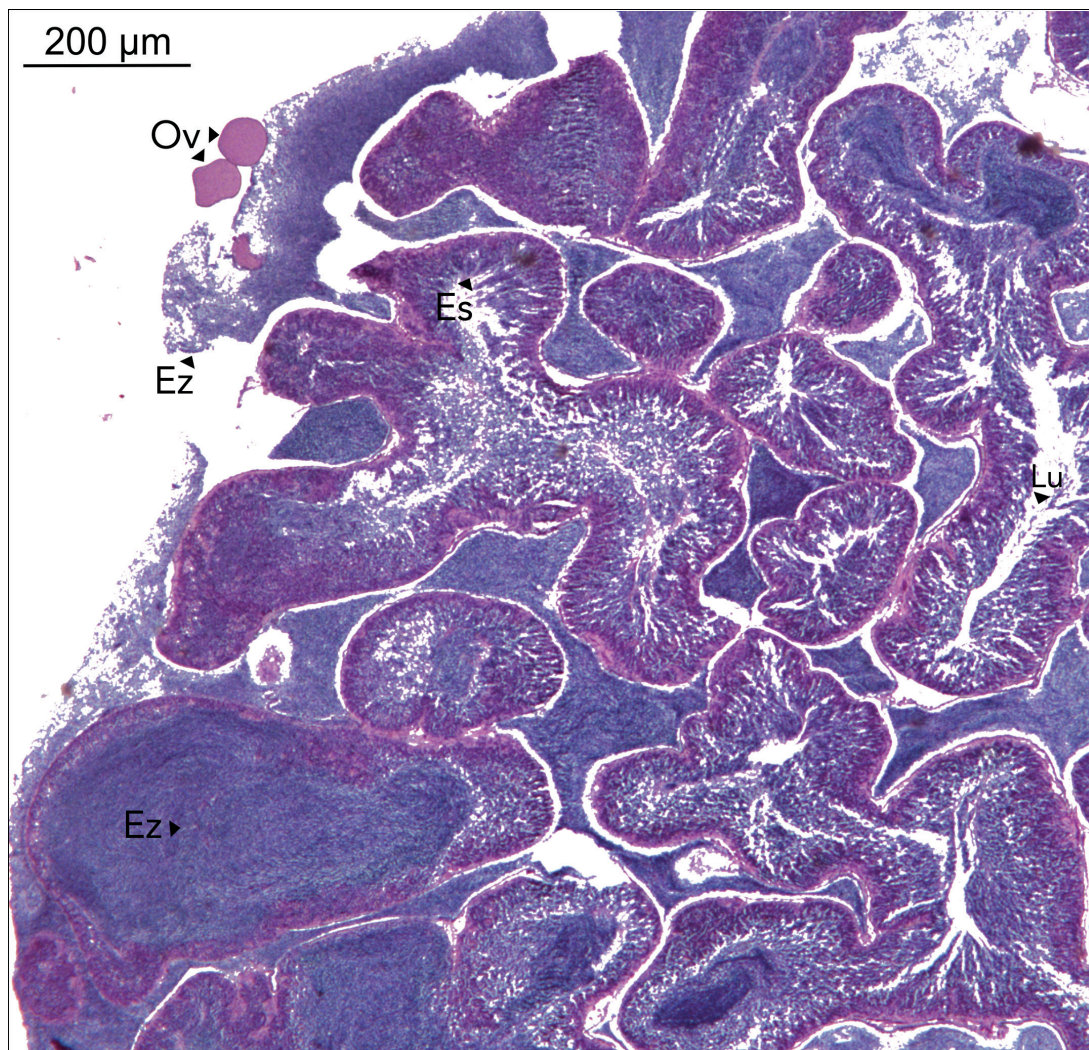


Fig. 6. Cross-section of the gonad of the individual C (Hermaphrodite with predominantly male gonad in mature stage observed in August). Ov: Ova. Ez: Spermatozoa. Es: Spermatocytes.

section showed partial spawning with a few ova ( $\sim 60 \mu\text{m}$ ) in the lumen. These acini had thin walls and contained oogonia ( $\sim 10 \mu\text{m}$ ) and nutritive phagocytes. Towards the periphery of the section, a large quantity of densely packed sperm was outside the acini. The amount and density of sperm indicated that the male fraction was mature (Fig. 7B), suggesting simultaneous spawning and evacuation in both fractions. In other regions of the gonad, male acini exhibited organized spermatocyte columns projecting into the lumen (with a thickness of  $\sim 29 \mu\text{m}$ ), where sperm in a pre-mature II stage was also observed (Fig. 7C).

- Individual E (Fig. 8): Test diameter: 24 mm. The individual was collected in September. It was initially identified as a mature male based on the macroscopic examination of the gonads; however, histological analysis revealed evidence of hermaphroditism with predominantly male characteristics. It was not possible to definitively determine whether this was due to mixed acini or a hetero-acinus. The specimen was in a mature stage, characterized by the nearly complete disappearance of spermatocyte columns, with the acinar lumen completely occupied by sperm. Additionally, some mature ova ( $\sim 49 \mu\text{m}$ – $54 \mu\text{m}$ ) were



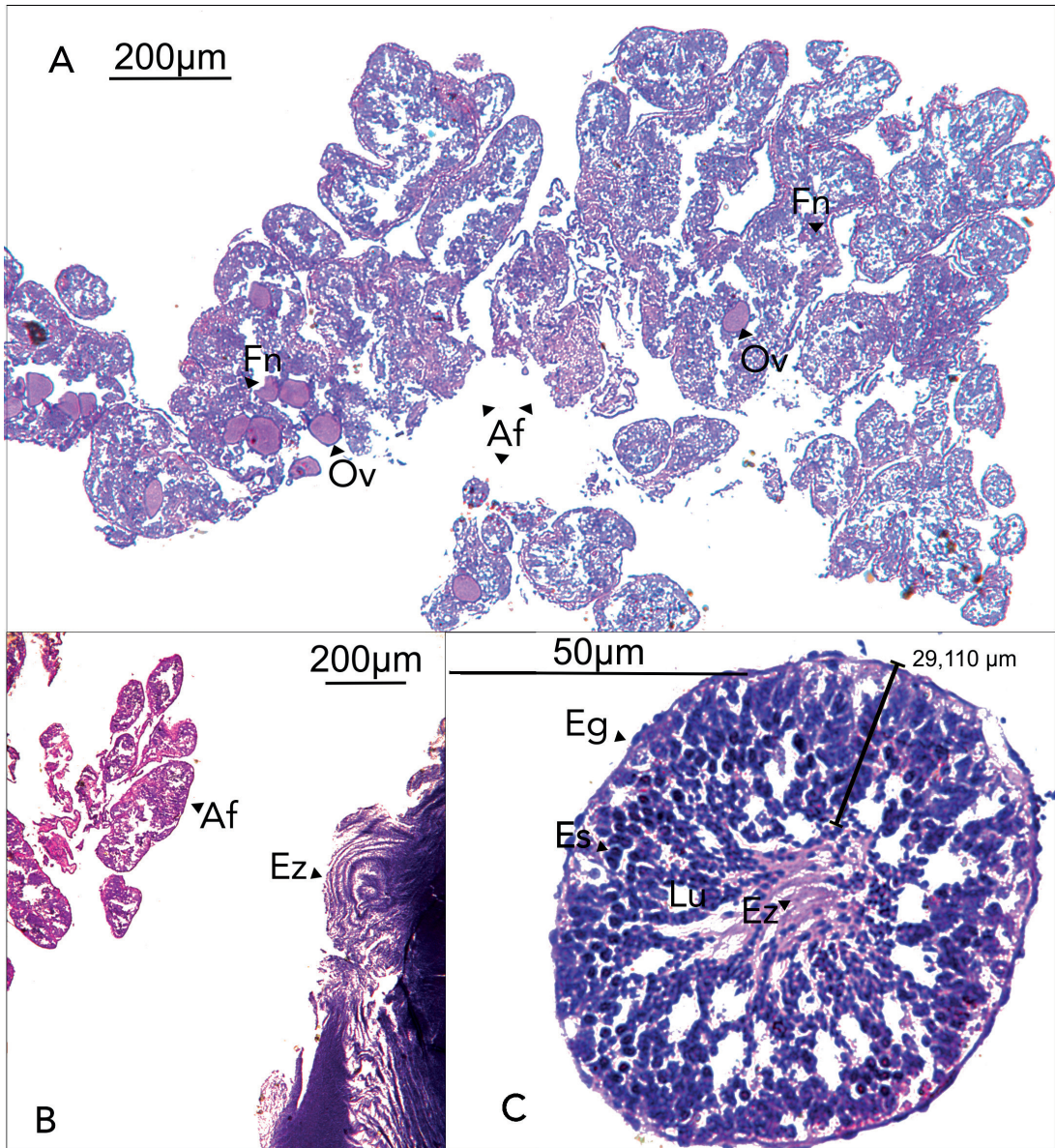


Fig. 7. Gonad of the individual D (Hermaphrodite with predominantly female gonad with hetero-acinus in partially spawned stage observed in September). A: Female partially spawned acini. B: Female acini and spermatozoa. C: Male acinus. Es: Spermatocytes. Ez: Spermatozoa. Af: Female acinus. Ov: Ova. Fn: Nutritive phagocyte. Lu: Lumen. Eg: Spermatogonia.

- found interspersed among the sperm.
- Individual F (Fig. 9): Test diameter: 24 mm. It was collected in September. Macroscopic examination of the gonads initially suggested the possibility of a male specimen. However, the histological analysis revealed the presence of both female and male acini (ovotestis), distinctly separated (hetero-acinus hermaphroditism). The female acini

appeared as typical acini in a pre-mature II stage. Previtellogenic oocytes were on the acinus wall in small quantities, along with vitellogenic oocytes in various stages of development projecting into the lumen, where a few mature egg cells ( $\sim 60 \mu\text{m}$ ) were present (Fig. 9A). The male acini were also observed in a typical pre-mature II stage, characterized by areas where the walls were



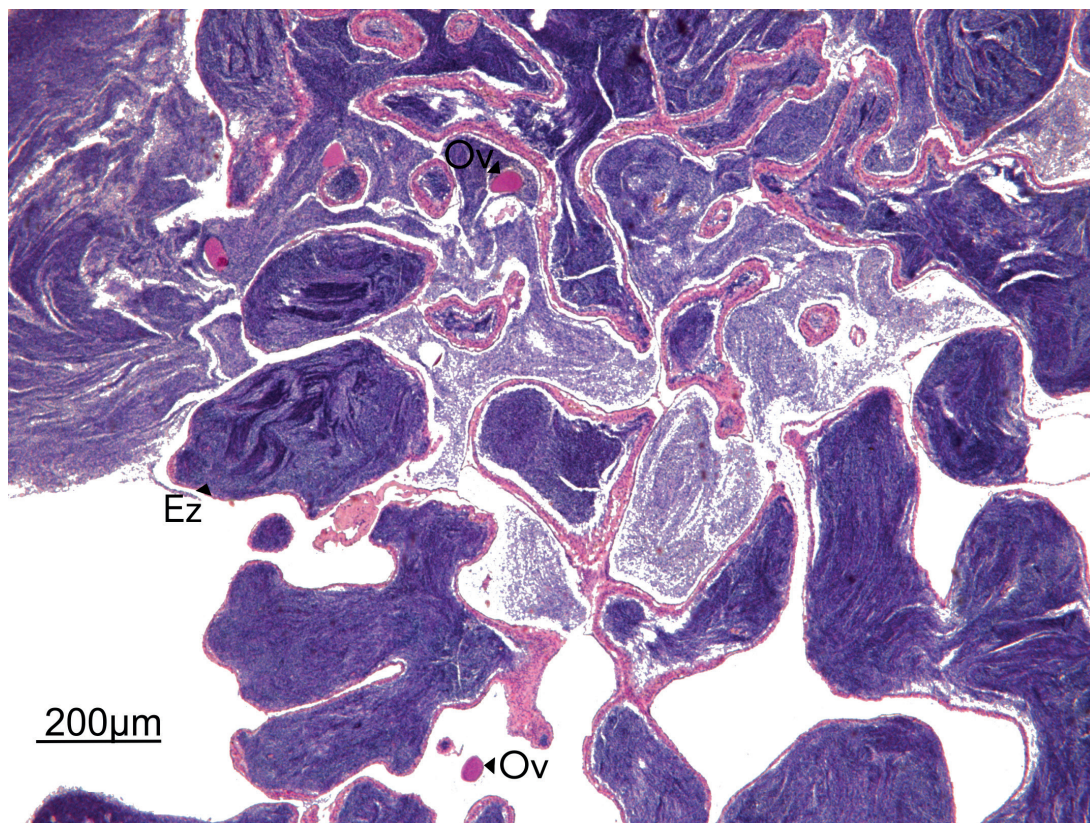


Fig. 8. Gonad of the individual E (Hermaphrodite with predominantly male gonad in mature stage observed in September). Ov: Ova. Ez: Spermatozoa.

slimming. Numerous nutritive phagocytes were observed where the columns remained thick ( $\sim 40 \mu\text{m}$ ) (Fig. 9B).

## DISCUSSION

This study represents the first documented case of hermaphroditism in *Pseudechinus magellanicus* in the Beagle Channel. Previous literature indicates that hermaphrodite sea urchins can exhibit a predominance of either female or male tissue, or an equal proportion (Pearse & Cameron, 1991). In our study, hermaphrodites with predominantly male characteristics were the most common, followed by hermaphrodites with ovotestes. Interestingly, hermaphroditism manifested in some cases within the same acinus, termed “mixed acini”, while in others, male and female tissues were segregated into separated acini, here described as “hetero-acinus” hermaphrodites. Although determining the precise type of acini was not always feasible, our find-

ings suggest a prevalence of hetero-acinus hermaphrodites in this population. In hetero-acinus hermaphrodites, the female and male fractions of the gonad are in distinct acini. Similar cases of hetero-acinus hermaphroditism have been reported in other echinoids (King *et al.*, 1994; Hernández *et al.*, 2007), alongside instances of mixed acini hermaphrodites (Olivares & Avila-Poveda, 2018).

Deviations from the expected 1:1 ratio of male to female sea urchins may indicate underlying environmental influences on hermaphroditism (Pearse & Cameron, 1991; Hernández *et al.*, 2007). However, factors affecting sex ratios, such as differential mortality rates and environmental variability, may not directly impact hermaphroditism but can contribute to observed variations across populations (Pearse & Cameron, 1991). Our study did not identify significant differences in sex ratios, yet we observed a notable presence of hermaphrodites. Previous hypotheses suggesting a link between hermaphroditism and popu-

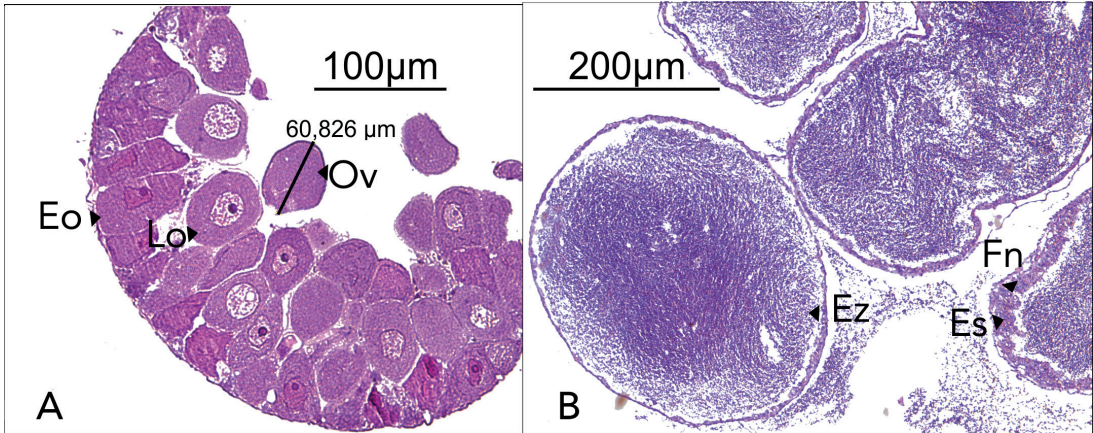


Fig. 9. Gonad of the individual F (Hermaphrodite with ovotestis hetero-acinus in pre-mature II stage observed in September). Eo: Early vitellogenic oocyte. Lo: Late vitellogenic oocyte. Ov: Ova. Ez: Spermatozoa. Es: Spermatocytes. Fn: Nutritive phagocyte.

lation density whether low (Bak *et al.*, 1984) or high (Hernández *et al.*, 2007) were difficult to substantiate due to the limited number of hermaphrodite observations ( $n=1$ ) in these studies. Several factors have been considered to influence hermaphroditism in echinoderms. For instance, in previous studies the substrate type (King *et al.*, 1994; Hernández *et al.*, 2007) does not appear to be a determining factor as the sea-urchins were recorded across various seabed types. A variation in pH has been shown to have significant effects on gonadal alterations (Dell'Acqua *et al.*, 2019), however, we did not record this parameter. Studying the effect of varying pH is crucial in the context of climate change, particularly for species with calcareous structures. Another factor that has been suggested is the temperature, with speculation regarding its potential role in influencing hermaphroditism, which merits further investigation as suggested by Moore *et al.* (1963) and Orlor (1992). In Orlor's (1992) study on the reproductive cycle of *P. magellanicus* in the Beagle Channel, no hermaphrodites were found. However, Orlor reported a case of hermaphroditism in *Loxechinus albus* from a nearby area. Orlor (1992) hypothesized that the low temperatures recorded during the winter of 1988 might explain the occurrence of hermaphroditism. This hypothesis, however, remains speculative due to the lack of periodic temperature records for verification. Similarly, Pérez also documented hermaphrodites in *L. albus*, albeit at an extremely low frequency (0.01%) during the period of 2004-2005 (Pérez, unpublished data).

Noteworthy, the potential underestimation

of hermaphrodites in the population due to incomplete gonad analysis (Moore *et al.*, 1963) may influence the results of reproductive studies. This omission may lead to inaccuracies in identifying and quantifying hermaphroditic individuals. In the case of the genus *Pseudechinus*, previous reports on *P. novaezealandiae* highlight the occurrence of hermaphroditism, where one out of the five gonads analyzed exhibited this condition (McClary & Barker, 1998). Our study acknowledges a similar limitation, as we histologically analyzed only one gonad per individual. Moving forward, it is imperative to prioritize comprehensive conservation practices and methodological rigor in future studies by analyzing all five gonads of each individual. This approach will help mitigate potential biases and provide a more accurate understanding of hermaphroditism within the population. Another consideration is the potential contamination of samples with individuals of different sexes. Despite meticulous dissection and histological procedures, contamination between samples cannot be entirely ruled out, particularly given the high degree of fragmented acini observed in some cases. Although it was not feasible to repeat the histological process using other gonads from the same individual, we repeated several cuts of the same gonad section to ensure no overlap occurred between the sections from different individuals. In the specific case of the individual C, hermaphroditism was suspected early on due to the macroscopic appearance of its gonads and two gonads were analyzed.

Based on our data analyzed from July to



September 2023, *P. magellanicus* likely presents a synchronous reproductive cycle in males and females at the Beagle Channel. However, this inference is limited to the studied period and should be further validated with data covering a complete annual cycle. Moreover, males exhibited more reproductive stages than females during the analyzed period, particularly in August. Additionally, several stages were observed within the same gonad, regardless of sex, suggesting that gonads do not mature simultaneously. Synchronization suggests adaptation to stable environments, where reproductive efforts focus on a single reproductive event, while in fluctuating or unpredictable conditions, reproductive effort is distributed across multiple opportunities (Calvo *et al.*, 1998). The reproductive characteristics of both sexes here reported from July to September appear slightly delayed compared to those reported by Orler (1992) in a nearby area. In that study, a higher proportion of mature and post-spawning individuals was recorded in July, with no broader range of reproductive stages in males than in females during the same period. The occurrence of a secondary, less intense spawning event (Orler, 1992; Gil *et al.* 2020) implies that the environment may not be entirely predictable for this species, particularly if encounters with a different sex are constrained, such as by limited mobility. In this context, hermaphroditism has been proposed as a solution for populations facing a low probability of sexual encounters (Ghiselin, 1969). The occasional presence of simultaneous hermaphroditism in a gonochoric population may either be an error in sex determination without significant consequences at the population level or a mechanism enabling isolated individuals to reproduce via self-fertilization (Calvo *et al.* 1998). Although gametes were normal and potentially capable of self-fertilization in most analyzed echinoid hermaphrodites, this development may not yield normal larvae (Bak *et al.*, 1984; Pearse & Cameron, 1991). Furthermore, mechanisms inhibiting self-fertilization exist in some cases (Pieplow *et al.* 2023). Currently, it remains unclear whether hermaphroditism represents a beneficial reproductive strategy or is influenced by environmental variables.

Given that hermaphroditism is typically associated with isolated or low-density populations, it is unlikely that the high abundance of *Pseudechinus magellanicus* in the Beagle Channel drives this reproductive strategy (Vásquez *et al.*, 1984; Pfoh unpublished data).

However, further studies on population abundances across different substrates and depths are required to evaluate whether hermaphroditism could serve as an adaptive strategy in this context.

Finally, since this study was conducted during the winter months, it is essential to expand sampling across all seasons to determine whether the incidence of hermaphrodites remains elevated throughout the reproductive cycle, whether the sex ratio remains stable, and whether populational synchrony can be confirmed. Encompassing the entire annual reproductive cycle, including juvenile and smaller adult individuals, will allow a better understanding of the dynamics of hermaphroditism. Such data are essential to clarify whether the observed high proportion of hermaphrodites is linked to a specific environmental stressor affecting gametogenesis during the study period or if it represents an adaptive reproductive strategy consistent across size classes in the population.

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