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Environmental conditions modulate hermaphroditic pathways in *Salpa thompsoni* near the Western Antarctic Peninsula

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Field observations during summer-fall of 2018 in the Antarctic Peninsula region provided the first morphological evidence that under certain environmental conditions, *Salpa thompsoni* blastozooid development can deviate from its traditional sequential protogynous hermaphroditism (SPH). Early male testis development at female blastozooid stages points to a wide-spread overlapping protogynous hermaphroditism (OPH) in regions where warm Antarctic Circumpolar Current waters mix with colder Antarctic Coastal waters. Our findings highlight the importance of the environmental setting in determining the reproductive pathway in *S. thompsoni*. OPH was observed in cooler and less productive waters, while SPH occured in both warmer and cooler productive waters. It appears that food availability may offset the effect of decreasing water temperatures for warm-water *S. thompsoni* reproductive development. It is plausible that OPH may permit the establishment of the *S. thompsoni* populations in the high Antarctic under the warming trend in the Southern Ocean by allowing for more efficient reproduction.

Pelagic thaliaceans, including salps, pyrosomes and doliolids, are a conspicuous and prominent group of gelatinous organisms found throughout the world`s oceans contributing significantly to zooplankton biomass, organic matter cycling and carbon sequestration[1,2]. Their life cycles follow metagenesis, a process through which one generation of animals reproduce asexually before giving birth to a sexually reproducing generation. Despite the inherent complexity of pelagic tunicate life cycles, all their life histories are shaped to maximize reproductive output over a short period. In their review, Alldredge and Madin[3] highlighted several key traits that allow tunicates to achieve a rapid population increase in unpredictable environments. These traits include efficient feeding, rapid growth and high fecundity, short generation times, direct development, viviparity, alteration of generations and hermaphroditism.

Among pelagic thaliaceans, salps have a particularly complex life cycle and proliferate through an obligatory alternation of oozooid asexual and blastozooid sexual generations[3]. Sequential hermaphroditism described in salps suggests that blastozooid females arranged in chains of a few hundred genetically identical individuals possesses a single, fully formed and ready for fertilization egg[4]. The moment blastozooids 'inhale' water first time after being released, they are usually fertilized by a male-stage blastozooid, which releases sperm into the water[5]. The fertilized egg then develops into an embryo, which is a precursor of the oozooid. Upon the embryo release, the blastozooid female develops testis becoming a functional male[6]. There is an indication that recently released blastozooids, while possessing a fully developed egg, also inherit the precursor cells of sperm that are rudiments of the future testis[4,6]. By default, the testis development is only initiated after the embryo is fully developed and released[6]. It is unclear when the development of the testis is initiated, but to avoid self-fertilization it is thought to be delayed till the developed embryo is discharged[6,7]. However, Purcell and Madin[8] mentioned that "*During development of the embryo, the parent aggregate salp becomes a functional male, releasing sperm into the water for the fertilization of other, younger aggregates.*" The latter observations are however lacking.

During summer-fall of 2018 in the western Antarctic Peninsula, we had a unique opportunity to study the *Salpa thompsoni* population with a particular emphasis on its stage of development in various water masses.

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During this expedition, blastozooids that were functional females with embryos at the terminal developmental stage already possessed fully developed male testes[9,10]. We aimed to investigate first, the stage development of *S. thompsoni* in contrasting water masses, examining how environmental variability influences its life cycle. Secondly, we aimed to examine whether embryo and testis development in *S. thompsoni* blastozooids occur in a sequential or parallel manner, thereby providing novel insights into their reproductive flexibility and adaptive capacity in response to changing environmental conditions.

Materials and methods

Salps were collected at 58 stations using mainly the 2.5 m^2 Isaacs-Kidd Midwater Trawl equipped with the 0.5 mm mesh during March-May 2018 onboard RV *Polarstern* (Fig. 1). Samples were collected during the double oblique tows down to ~ 170 m at the speed of 2-2.5 knots. In all catches, if present, salps were counted, sexed, staged, and oral-atrial length (OAL) measured to the nearest mm. Oozooids (solitaries) as well as large blastozooids (aggregates) were analysed from the entire catch. The remaining catch was subsampled (1/4 to 1/40 fraction of the sample) to obtain minimum 150–350 small blastozooids for detailed biological analysis. The environmental data were collected using the CTD Seabird by generally deploying (downward cast) it to a depth of 500 m at every station. In the current study, only surface environmental data, where salps concentrate during nighttime, were used.

In this study, we only focus on development of S. *thompsoni* blastozooids. Their maturity stages have been well described[11–13]. In short, five different blastozooid stages (from 0 to 4) were classified according to the gradual growth of the embryo. At stage 0, the ovarian sac is spherical with no sign of embryo development. By stage 4, the embryo (≥ 4 mm in length) resembles features the early oozooid[11,12]. Stage S or 'spent' was identified by the presence of a placenta scar indicating that embryo was recently released. Blastozooids with visible placenta scar and sperm channels on the salp nucleus were classified as males (stage M). However, during the biological analysis of salps, we paid particular attention to sperm channels on the salp nucleus at all stages of development. If sperm channels were visible, the particular stage was classified with letter "M". For example, if the embryo development was on stage 2 but showed beginning of the sperm channel development, it was classified as blastozooid stage 2 M. Finally, blastozooids with no visible embryos (possibly failed fertilization) or embryos

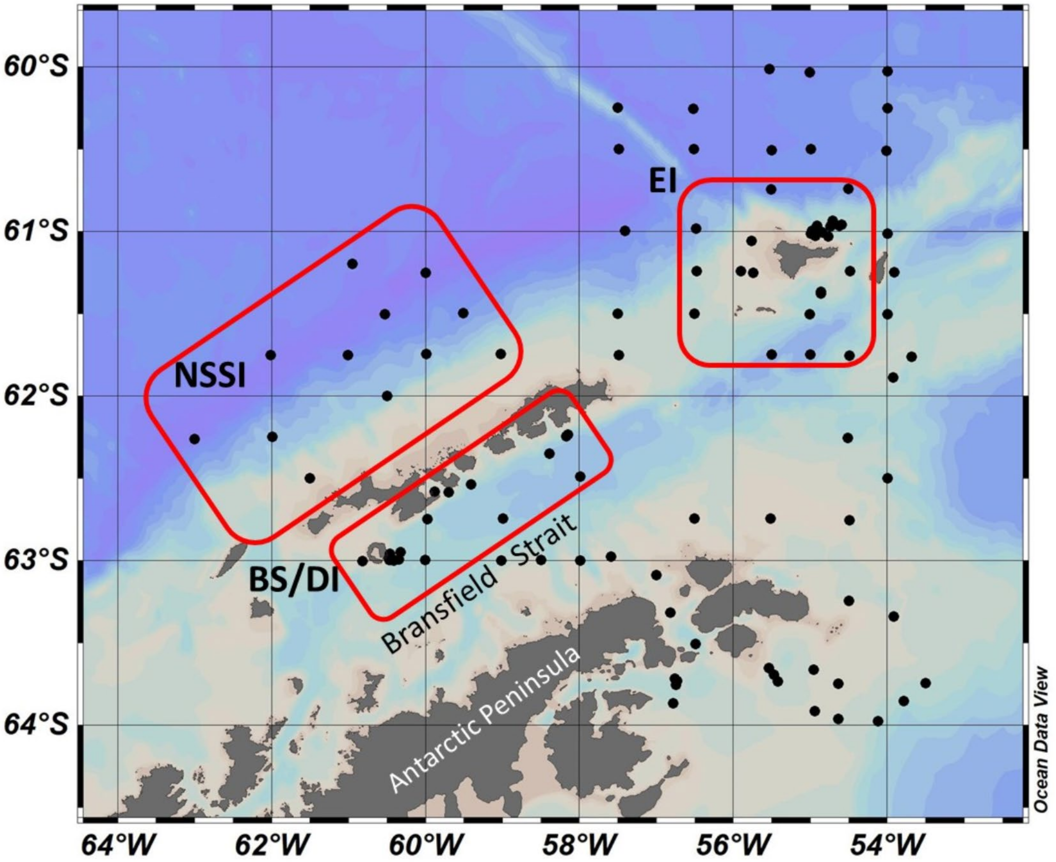

Fig. 1. All stations sampled during March-May 2018 onboard RV *Polarstern*. BS/DI – Bransfield Strait and Deception Island region; NSSI – north of South Shetland Islands region; EI – Elephant Island vicinity region. Red squares include stations used for the salp development analyses and to calculate environmental parameters presented in Table 1. The map was created using the ODV sofware version 5.6.5 (Schlitzer, Reiner, Ocean Data View (https://odv.awi.de), 2023).

that showed a remnant of some degree of development (possibly embryos with a disrupted development), no visible placenta scar, no visible sperm canals were classified following Chiba et al.[14] as stage X.

Results

Oceanographic setting

The three regions under investigation differed in their oceanographic setting. The north of South Shetland Islands (NSSI) region was influenced exclusively by the Antarctic Circumpolar Current (ACC), with the highest mean sea surface temperature and chlorophyll-a concentrations (Table 1). The Bransfield Strait/Discovery Island (BS/DI) region was also primarily impacted by the southern branch of the ACC, with some mixing with the Antarctic Coastal Current waters. The water mass in this region, while having the lowest mean surface temperature, was similar in chlorophyll-a concentrations to the NSSI region (Table 1). The Elephant Island (EI) region generally represented a mixing zone between the ACC waters (particularly at stations far north of EI) and the mixture of the Antarctic Coastal Current and Weddell Sea waters. The latter water masses dominated the region near the EI and south of it. The waters near the EI had average surface temperatures similar to those of the BS/DI region, but with the lowest chlorophyll-a concentrations (Table 1). Hence, generally three regions under investigation can be characterized as: warm and productive (NSSI), cool and productive (BS/DI) and cool and non-productive (EI) (Table 1).

Salp development dynamics.

The sampling in all three regions lasted less than three weeks and temporal variations in environmental conditions were minimal. Nevertheless, there was a conspicuous difference in the *S. thompsoni* development dynamics between the three regions. Fitting the classical paradigm, there was a remarkable similarity in the *S. thompsoni* development in the BS/DI and NSSI regions (Fig. 2A and B). There was a robust length-related development of salp blastozooids and males (M stage) started appearing in the population at OAL 26–30 mm, reaching 50% of blastozooids by length of ~ 34 mm, and by length of 41–43 mm all blastozooids were males (Figs. 2A, B and 3A, B). It was also clear that M stage only appeared after salp released small oozooids (Fig. 2A, B).

Population dynamics of *S. thompsoni* in the EI region was strikingly different (Figs. 2C and 3C). First, there was a distinguishable overlap in the appearance of male testes in early blastozooids. This occurred as early as at stage 2 and the salp OAL of 14 mm (Fig. 2C). Second, the 50% blastozooids at the OAL of 26 mm had testes at various stages of development (Fig. 3C). Finally, by length of 37 mm all blastozooids could be classified as males (Fig. 3C).

The size and stage development photographic sequence collected in the vicinity of the Elephant Island is shown in Fig. 4. It shows the visible initiation of the testis canal development at the blastozooid stage 2 at a OAL of 15 mm (Fig. 4C). The canals develop fast and by stage 3 (Fig. 4D and E) testes may become functional since mechanical disturbance (needle touching) during the biological analysis often triggered the sperm release in freshly caught blastozooids (E.A Pakhomov, personal observation). Finally, we observed large blastozooids with both embryos and fully developed visible testes (Fig. 4I).

Discussion

It has been documented that the stage development in pelagic tunicates is mainly modulated by environmental temperature, food availability and quality[15–17]. The observed differences in blastozooid development (SPH vs. OPH) in regions influenced by either the ACC or Antarctic Coastal Current/Weddell Sea waters- documented for the first time to our knowledge- are striking and have ecologically important implications for the populations of *S. thompsoni* in the high Antarctic regions. It appears that *S. thompsoni* under certain conditions may deviate from the traditional sequential protogynous hermaphroditism (SPH) by the overlapping development of male testes, which should be called overlapping protogynous hermaphroditism (OPH). This phenomenon was previously suggested for *Cyclosalpa bakeri* by Purcell and Madin[8] and was recently observed during lab-based experiments under controlled conditions for *S. fusiformis*, a species closely related both morphologically and taxonomically to *S. thompsoni*[18]. Our findings highlight the importance of the environmental setting in determining which pathway is triggered. OPH was observed in cooler and less productive waters, while SPH emerged in both warmer and cooler, but more productive, environments. It comes across that the food availability may offset the effect of decreasing water temperatures for warm-water *S. thompsoni*. However, this observation warrants further experimental investigation to fully understand the underlying mechanisms.

		Salpa thompsoni **(ind.1000 m^{-3})**		**Surface temperature (°C)**	**Surface salinity**	**Surface chl-a mg.m^{-3}**
Region	**Sampling dates, 2018**	**mean ± 1SD (range)**	**FO (%)**	**mean ± 1SD (range)**	**mean ± 1SD (range)**	**mean ± 1SD (range)**
Bransfield Strait/Deception Island (BS/DI), *N* = 13	March 22–31	36 ± 49 (0–187)	83.8	0.99 ± 0.21 (0.6–1.25)	34.09 ± 0.06 (34–34.2)	1.84 ± 1.65 (0.3–5.5)
North of South Shetland Islands (NSSI), *N* = 12	April 3–6	1019 ± 808 (39–3012)	100	1.57 ± 0.34 (1.2–2.25)	33.80 ± 0.11 (33.72–34.05)	2.11 ± 1.14 (0.9–4.2)
Near Elephant Island (EI), *N* = 18	April 6–13	984 ± 886 (2–3448)	100	1.03 ± 0.15 (0.8–1.2)	34.03 ± 0.14 (33.7–34.2)	0.41 ± 0.13 (0.25–0.6)

Table 1. Summary of environmental parameters and *Salpa thompsoni* densities during March-April 2018 near the Antarctic Peninsula.

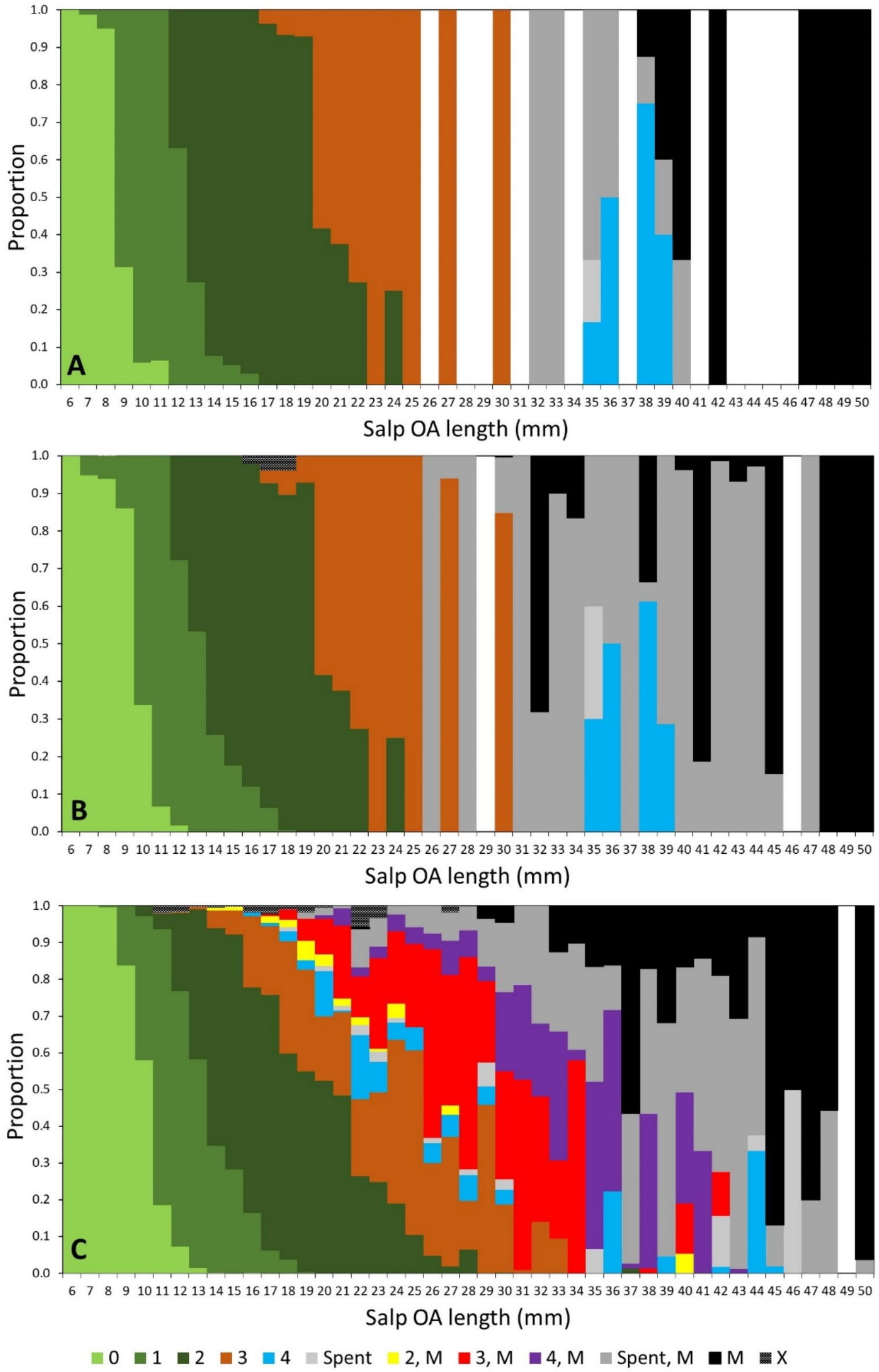

Fig. 2. Size-dependent developmental stage composition of *Salpa thompsoni* during March-April 2018. (**A**) Bransfield Strait and Deception Island (BS/DI) region; (**B**) north of South Shetland Islands (NSSI) region; (**C**) Elephant Island region (EI).

At this point, we can only hypothesize that cool temperatures and low food availability triggered the parallel development of embryo and testis. It is important to note that our observations were based solely on the visible morphological expressions of testis development. However, recent transcriptomic analysis of *S. thompsoni* sexual reproductive stages identified genes linked to the male sex differentiation and spermatogenesis as soon as the egg is fertilized and definitely before the embryo is released[19]. Our morphological evidence is thus consistent

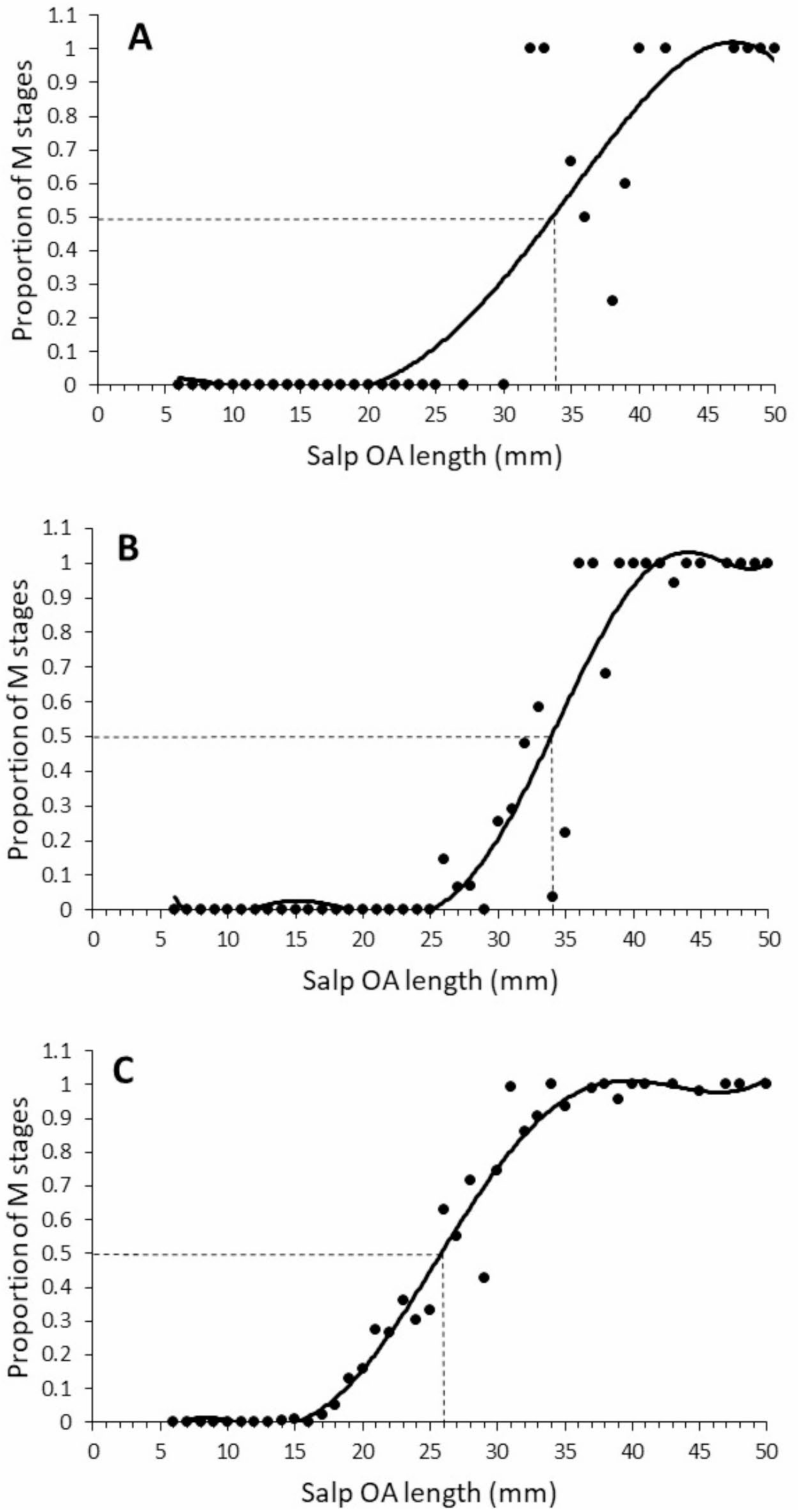

Fig. 3. Size-dependent proportion of M (male) stages of *Salpa thompsoni* during March-April 2018. (**A**) Bransfield Strait and Deception Island (BS/DI) region; (**B**) north of South Shetland Islands (NSSI) region; (**C**) Elephant Island region (EI).

with genetic findings, further challenging the conventional understanding of the reproductive processes in *S. thompsoni*. It is plausible that, to avoid self-fertilization, an internal biological clock triggers testis development after a specific time to ensure egg fertilization by older blastozooids. In the typical salp habitat (such as ACC-influenced region), which is warmer and moderately productive, the egg development occurs faster, thus showing a pathway that closely resembles SPH. Also, SPH would persist even in colder but productive waters

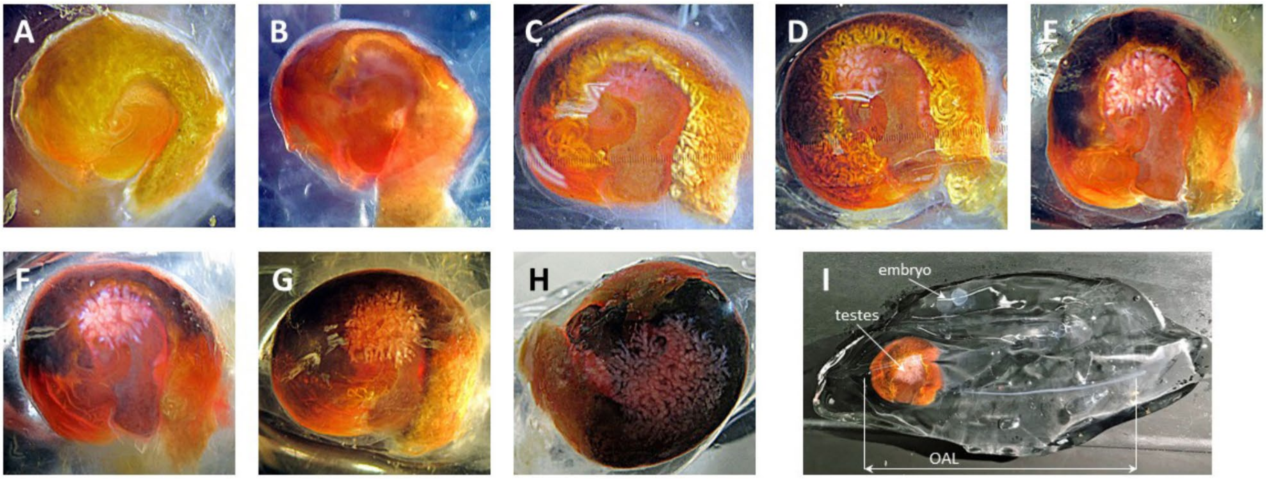

Fig. 4. A sequence of *Salpa thompsoni* blastozooid nuclei in the proximity of the Elephant Island during summer-fall of 2018. (**A**) No testes, stage 1 blastozooid Oral Atrial Length (OAL) 10 mm; (**B**) no testes, stage 2 blastozooid, OAL 13 mm; (**C**) early testes, stage 2 M blastozooid, OAL 15 mm; (**D**) developing testes, stage 3 M blastozooid, OAL 20 mm; (**E**) well developed testes, stage 3 M blastozooid, OAL 28 mm; (**F**) developed testes, stage 4 M blastozooid, OAL 31 mm; (**G**) developed testes, stage Spent M blastozooid, OAL 38 mm; (**H**) developed testes, functional male blastozooid, OAL 40 mm; (**I**) functional male blastozooid with the embryo still inside, OAL 45 mm.

as food availability may buffer slower development caused by colder water temperatures. However, in cold and unproductive waters, salp egg development takes longer, overlapping with the onset of testis development, leading to the OPH pathway. It is also possible that during the female stage, blastozooids may expedite to becoming males, perhaps at the expense of lowering their growth rates. These potential scenarios warrant further investigation using field and transcriptomic studies.

Besides *C. bakeri* and *S. thompsoni*, OPH has also been observed in *S. fusiformis* blastozooids. During a laboratory experiment, *S. fusiformis* kept under controlled conditions in kreisel tanks developed testes while the embryo was still developing[18]. Interestingly, this included individuals that had not been previously fertilized, indicating further the presence of new developmental pathways that may be in contrast to the classic protogynous hermaphroditism and somewhat influenced by time-regulated physiological mechanisms[18]. Gene expression patterns in a follow-up study demonstrated that the salp life cycle may physiologically prepare blastozooids for reproduction during the potential reproductive period, regardless of the fertilization status (Müller, personal communication). This may be a mechanism facilitating a rapid response to favorable environmental conditions. However, since these studies were conducted under controlled conditions that only mirror in situ environments, potential triggers such as temperature and food availability may still modulate different developmental pathways in natural environments as it was observed in the present study.

Previous studies suggested that *S. thompsoni* may complete at least two full life cycles (from egg-to-egg development) during the austral summer in regions affected by the ACC waters[16,20,21]. The recent southward expansion of *S. thompsoni* may thus revert to one cycle, as exposure to cold (<0 °C) and unproductive waters may significantly slow down their development, preventing the completion of the egg-to-egg cycle in the high Antarctic[16]. As a result, the southward expansion remains challenging, with no established *S. thompsoni* populations currently found in the high Antarctic. Their occasional appearance in these regions is likely due to lateral advection, where seeding salp specimens are transported southward beyond the Southern Boundary Front[13,22].

Salps may already be set for reproductive success at birth, increasing the potential for bloom formation as ocean temperatures rise[6,19]. However, it appears that in the real world, the first crop of blastozooid chains from a single oozooid may end up with unfertilized eggs unless there are other oozooids present that release blastozooids early enough, allowing them to develop into males. In case of a single oozooid, it is not clear whether blastozooid chains with unfertilized eggs will develop testes instead, or simply grow, and become males that produce gametes and thus can fertilize the second crop of blastozooid chains. This, however, has limitations because genetically identical specimens will cross-fertilize, curtailing genetic radiation. In fact, the protogynous hermaphroditism has been hypothesized to prevent self-fertilization[6]. Such reproductive strategy would explain the challenges of salp population establishment in new habitats. When only a limited number of salp oozooids are advected southward, the low population density significantly reduces the likelihood of meeting each other, hindering successful reproduction. Hence, for the salp population establishment in the high Antarctic, the advection of numerous oozooids at various stages of development is important. In addition, the short productive period in the high Antarctic further complicates salp proliferation. However, these environmental constraints may shift under the ongoing warming trend in the Southern Ocean. The shift to alternative hermaphroditic pathways, and particularly to OPH, could therefore facilitate the establishment and persistence of the *S. thompsoni* populations in the high Antarctic.

Data availability

"The biological datasets used and/or analysed the current study available form the corresponding author on reasonable request. Raw CTD data are published in PANGAEA: Meyer, Bettina; Rohardt, Gerd (2018): CTD raw data files from POLARSTERN cruise PS112, link to tar-archive [dataset]. Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, PANGAEA, https://doi.org/10.1594/PANGAEA.895969."

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Author contributions

"E.A.P. conceived the study, collected samples, and performed the analysis with the support of S.J.M. and B.M. E.A.P. wrote the manuscript. All authors contributed to the interpretation of results, revised the manuscript, and approved the submitted version."

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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