Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Notes and Comments

Protocol for reproduction and ploidy confirmation in *Nodipecten nodosus* (Linnaeus, 1758) by flow cytometry

Y. M. Ferreira[a*], R. R. Silva[a], A. C. Alves[b], L. R. V. Ramos[a], N. F. Nascimento[c], G. S. Yasui[b] and M. P. Santos[a]

[a]Universidade Federal Rural do Rio de Janeiro - UFRRJ, Programa de pós-graduação em Ciência Animal, Seropédica, RJ, Brasil

[b]Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio, Centro Nacional de Pesquisa e Conservação de Peixes Continentais – CEPTA, Laboratório de Biotecnologia de Peixes, Pirassununga, SP, Brasil

[c]Universidade Federal Rural de Pernambuco – UFRPE, Serra Talhada, PE, Brasil

The production of scallops *Nodipecten nodosus* (LINNAEUS, 1758), a marine bivalve species, native and hermaphrodite from Brazil, has great potential in the state of Rio de Janeiro. The species has production protocols in the laboratory defined, ranging from reproduction and larviculture to the production of seeds (post-larvae), which are marketed to mariculturists.

Throughout its cultivation, problems such as high mortality are encountered, yet without a reason (Landuci et al., 2021), and the application of biotechniques such as triploidy may bring improvements and zootechnical increments to production (Maldonado-Amparo et al., 2016). Thus, the present work was carried out with the objective of describing a protocol for species management in the laboratory, describing the methodology for broodstock selection, induced reproduction, gamete collection, ploidy analysis by cytometry, and some discussion possibilities for triploidization in the species.

Faced with the aforementioned problems, there is a constant search for solutions, one of which is the production of triploid scallops. There are already reports of the advantages of triploids over diploids, such as higher growth and higher glycogen and protein content (Maldonado-Amparo et al., 2016).

The procedures were carried out at the Mollusk Reproduction Laboratory of the Instituto de Eco Desenvolvimento da Baia de Ilha Grande (IEDBIG), in Angra dos Reis, Rio de Janeiro. The animals were maintained in the breeding room at 1,000L tanks, in a semi-closed system with filtered salt water with a mechanical filter at 5µm, at a temperature of 20°C, pH 8.0, salinity 37 g/L, and oxygen saturation at 75.6%. The scallops were fed with approximately 20L of microalgae (*Chaetoceros* sp, *Bellorochea* sp.) alternating daily, and with a concentration of $5x10^4$ cell mL^{-1}.

For spawning induction, twelve scallops were selected when they reached maturation stage IV, according to Ramírez-Castillo (2003), and with a shell length of 10 to 12 cm. Then, they were induced to spawn through the triggering of stress mechanisms, through exposure to air and brushing to remove incrustations that adhere to the shells, kept in a 100 L tank with running water, and finally, through thermal shock, with marine water (after filtration in UV filter) added for the temperature gradually heat from 21 to 25°C, with the help of a thermostat.

After the beginning of spawning, the scallops were separated and in a 500mL beaker with filtered and sterilized water. The spawnings were separated to avoid self-fertilization. After spawning, the gametes were filtered through mesh sizes ranging from 45 and 25 µm to remove impurities and then separated for fertilization.

The ratio of 10 sperm to 1 oocyte were used for fertilization, according to the protocol stipulated by Bourne et al. (1989). Every 15 minutes the solution with gametes was homogenized and after 30 minutes a sample of 1 mL from each of the twelve buckets was collected with a 3 mL pipette and observed under a microscope to determine the percentage of fertilization, by counting embryos in a Neubauer chamber. After about 90% of the oocytes were fertilized, the solution was transferred to 6,000-liter larviculture tanks containing filtered seawater, where it proceeded to larval development. The density ranged from 30-50 larvae/ mL.

For ploidy analysis, approximately 20 larvae were collected with a 25 µm mesh sieve at approximately 72 hours after fertilization. Each larva was allocated in microtubes of 1.5 mL with saline solution for further flow cytometry analysis.

For ploidy analysis, samples were sent to the Fish Biotechnology Laboratory of the National Center for Research and Conservation of Continental Fish (CEPTA/ICMBio), in Pirassununga, São Paulo. The samples were placed in a cryogenic tube box and transported for 12 hours in a styrofoam box at low temperature to the laboratory, where an innovative protocol of flow cytometry was standardized for this species, based on a methodology proposed by Xavier et al. (2017).

*e-mail: yannmalini@yahoo.com
Received: December 14, 2022 – Accepted: July 17, 2023

Each larvae separated into microtubules was submitted to a cell lysis solution (9.53 mM MgSO, 4 .7H2O, 47.67 mM KCl, 15 mM Tris, 74 mM sucrose, pH 8.0 and 0.8% of Triton X-100) for 10 minutes to enucleate the cells by removing the membrane.

The nuclei were then stained by adding a dye DAPI - 4.6 Dimidine 2 Phenylidone Di-Hydrochloride (0.01%) in Dulbecco's Phosphate Buffer Saline in a volume of 800 µL (Sigma #D5773, St. Louis, EUA).

Subsequently, the solution was filtered through a 30 µm filter (Celltrics, Partec, GMBh, Germany). After that, the DNA content was measured with a flow cytometer (CyFlow Ploidy Analyzer, Partec, GMBh, Germany). This was the first time that ploidy analysis was performed in the species using flow cytometry, which was confirmed by using diploid larvae as a control (Figure 1). These results are innovative and can be used in future studies of chromosome set manipulation, such as the confirmation of androgenetic, gynogenetic, triploids, and tetraploids.

In conclusion, the protocol described can be used in future studies of chromosome set manipulation in *N. nodosus*, such as the induction of triploidy. This technique can enhance the activity when the triploid animals are sterile and have increased somatic growth, resulting in shorter cultivation time and a lower chance of suffering problems due to weather conditions.

The technique may also provide subsidies for the development of conservation strategies for the species. Triploid induction was also applied in other mollusk species such as *Anomalocardia brasiliana* (Lavander, 2018) or native fish such as *Rhamdia quelen* (Fukushima et al., 2012) and *Colossoma macropomum* (Hashimoto et al., 2020). Notably, these characteristics of triploid individuals are species-specific, and future experiments are necessary.

Acknowledgments: We would like to thank the staff of the Fish Biotechnology Laboratory of the National Center for Research and Conservation of Continental Fish (CEPTA/ICMBio), in Pirassununga, São Paulo, and the Instituto de Eco Desenvolvimento da Baia de Ilha Grande (IEDBIG), in Angra dos Reis, Rio de Janeiro, for technical and laboratory support.

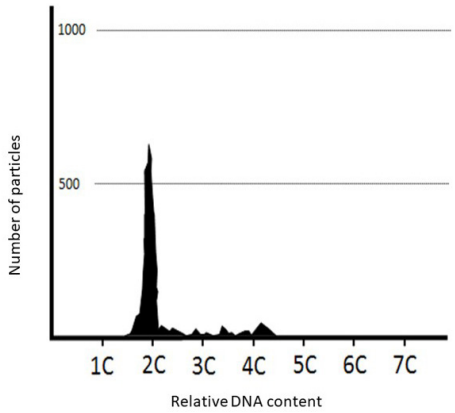

Figure 1. Relative DNA content resulting from a diploid sample of the species *Nodipecten nodosus*.

References

BOURNE, N., HODGSON, C.A. and WHYTE, J.N.C., 1989. *A manual for scallop culture in British Columbia*. Ottawa: Department of Fisheries and Oceans. 215 p. Canadian Technical Report of Fisheries and Aquatic Sciences, no. 1694.

LANDUCI, F.S., BEZ, M.F., RITTER, P.D., COSTA, S., SILVESTRI, F., ZANETTE, G.B., CASTELAR, B. and COSTA, P.M.S., 2021. Mariculture in a densely urbanized portion of the Brazilian coast: current diagnosis and directions for sustainable development. *Ocean and Coastal Management*, vol. 213, p. 105889. http://dx.doi.org/10.1016/j.ocecoaman.2021.105889.

MALDONADO-AMPARO, R., VERDUGO, C.A.R., IBARRA, A.M., RUEDA-PUENTE, E.O., DEL TORO SÁNCHEZ, C.L. and FÉLIX, F.R., 2016. Poliploidía en moluscos de importancia comercial. A Review. *European Scientific Journal*, vol. 12, no. 33, p. 69. http://dx.doi.org/10.19044/esj.2016.v12n33p69.

RAMÍREZ-CASTILLO, E.R., 2003. *Desarrollo reproductivo estacional de la almeja mano de león Nodipecten subnodosus*. Ciudad Obregón: Instituto Tecnológico Agropecuario.

XAVIER, P.L.P., SENHORINI, J.A., PEREIRA-SANTOS, M., FUJIMOTO, T., SHIMODA, E., SILVA, L.A., SANTOS, S.A. and YASUI, G.S., 2017. A flow cytometry protocol to estimate DNA content in the yellowtail tetra Astyanax altiparanae. *Frontiers in Genetics*, vol. 8, p. 131. http://dx.doi.org/10.3389/fgene.2017.00131. PMid:28993791.