



Scientific Note

A compacted culture system for a marine model polychaete (*Platynereis dumerilii*)

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Abstract. *Platynereis dumerilii* is a marine polychaete, which shows several characteristics that have promoted its use as a model laboratory animal. We describe a compact laboratory culture system appropriate for research activities. We have successfully established a culture of *P. dumerilii* in an extremely reduced space.

Key words: aquaculture, marine model organisms, reproduction, feeding

Resumen. Cultivo compacto del poliqueto modelo *Platynereis dumerilii*. *Platynereis dumerilii* es un poliqueto marino cosmopolita. Es un fósil viviente que presenta varias características que lo han promovido como un organismo modelo. En este trabajo, se describe un exitoso sistema de cultivo compacto de *P. dumerilii* apropiado para apoyar tareas de investigación.

Palabras claves: acuicultura, poliqueto, reproducción, alimentación

There are already several marine invertebrate species that are being cultivated, for example as a food item, for commercial or research purposes. In this context, marine invertebrates have a great potential, since several represent ecologically keystone species in food chains while others represent important stages in the evolution of eumetazoans (Raible and Arendt 2004). Furthermore, there is a present increasing concern among the scientific community to reduce the use of vertebrate species in bioassays (*e.g.* toxicological exposure studies), supporting, therefore, the use of alternative species for such experiments (Robinson 2005). The culture of nereidid polychaete is being developed for several reasons –for example, as a bait for fishing, to play an important role to increase the reproductive fitness of crustacean brood-stock, thus generating a synergism between basic scientific research and the aquaculture of these worms (Olive 1999). Nereidid polychaetes are widely employed in research, environmental monitoring and as key food items in aquaculture (Fischer & Dorresteijn 2004, Gray & Elliott 2009, García-Alonso *et al.* 2008).

Among them, *Platynereis dumerilii* is a new emerging model animal. *Platynereis dumerilii* is a marine polychaete, which shows several characteristics that have promoted its use as a model laboratory animal, for example in reproduction, ecotoxicology and evo-devo studies. Based on phylogenetic trees, classical invertebrate model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* are now branched together as Ecdysozoa, while members of phyla such as Mollusca, Nemertea and Annelida are grouped in the Lophotrochozoa; these latter show less representation in laboratory studies. Over the last decade, several laboratories mainly in Europe have decided to adopt this polychaete species as a model laboratory organism. Several qualities make this species an excellent organism to cultivate (Fischer & Dorresteijn 2004). The worm belongs to the Nereididae a family of worms with a high concentration of essential biomolecules (García-Alonso *et al.* 2008). Robustness, short life cycle, and the huge number of eggs produced have made this species an excellent aquatic animal for culturing.

They reproduce once in a lifetime (semelparous, Fischer 1997), and have been cultivated in the laboratory since the 1950s by Hauenschild (Hauenschild & Fischer 1969). As a result, *P. dumerilii* is employed as a model species in evolutionary studies (Dray *et al.* 2010) as well as in ecotoxicology (Hutchinson *et al.* 1995, García-Alonso *et al.* 2011) and has the potential for use as an enrichment diet in marine culture systems.

Several culture systems of this species exist but most routinely make use of an entire culture room or even larger scale facilities, implying the use of an isolated room with light and temperature control. In this study, we address the question of how *P. dumerilii* can be maintained in a reduced marine culture system for research or as food item (co-culture) purposes. We describe a compact laboratory culture system appropriate for research activities. We have successfully established a culture of *P. dumerilii* in an extremely reduced space, which has proved able to generate a large number of larvae and adult worms. The dedicated, low-cost, compact system requires only easy maintenance involving just a total of 2 hours per week. We consider this system to be a useful tool for researchers choosing to work with an emerging model marine organism for it involves simple handling and, most importantly, can be used in a reduced space.

Basically, the system consists of an adapted temperature-controlled drinks container (Bosch, 165 cm x 40 cm x 50 cm, 0.33 m³) refrigerated to 18 °C with a thermostat. Daylight is effected by a Natur 9000K light tube (Solar, JBL, 450 mm) controlled by a timer set for a photoperiod 16:8, Light:Dark. Moonlight is provided by an Arcadia Marine Blue 420 (450 mm) tube. A lunar cycle is fundamental to induce spawning in these worms. The cycle is initiated by keeping this light off for 21 days and on for 7 days. The 'moonlight' is switched on 3 days before the date of the natural full moon and is switched off 3 days after this date, encompassing the natural moon cycle. Animals are kept in plastic Rotilabo®-airtight storage boxes (20 cm x 20 cm x 6 cm) (Roth) with 750 cm³ artificial seawater (ASW, e.g. Tropic Marin®, 33 salinity). Alternatively, natural seawater could be employed, but this would require sterilisation either by filtration or pasteurisation (3 hours in a water-bath at 80 °C). All containers are aerated with an air pump (50 Hz, 45W). For feeding a mixture of live phytoplankton (unicellular *Tetraselmis suecica*), spinach and fish food flakes (Tetra Marine) is enough for good growth of the worms and enables them to reach epitoky and reproduce. Fish food flakes and thawed frozen spinach are macerated and mixed with *T. suecica* which is co-cultured inside the culture system.

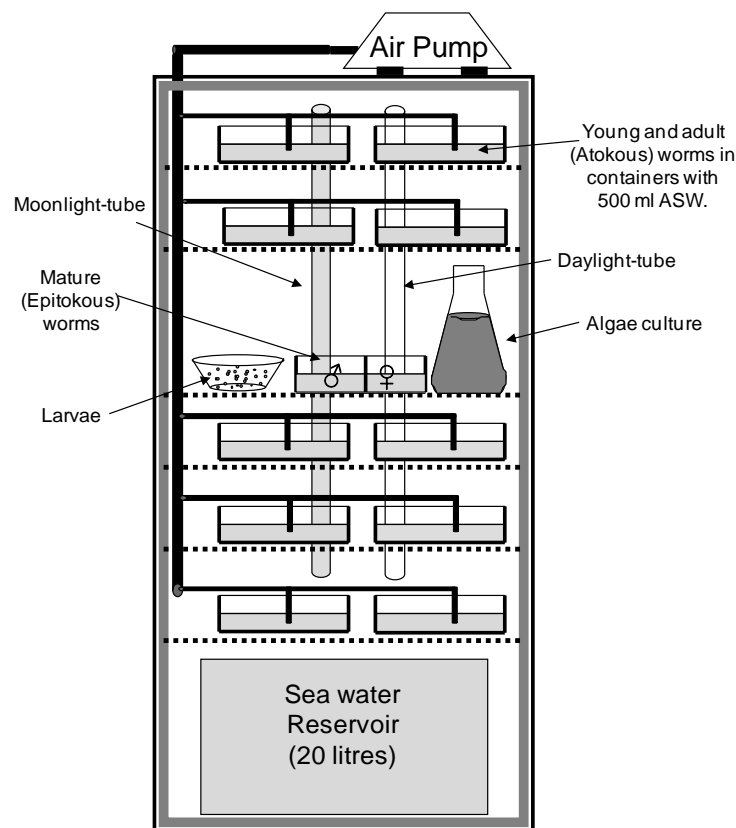


Figure 1. Scheme showing the compacted culture system for *Platynereis dumerilii*.

Growth and the attainment of epitoky (the possession of a modified body for sexual reproduction) are the best assessments to confirm that the culture is working properly. When worms are in correct and healthy condition, they show a normal feeding activity. Around 50 adult worms per box were fed twice a week with a mixture of food to deliver the essential parts of the *P. dumerilii* diet. As soon as the nectochaeta larva develops the mouth, it starts to feed on phytoplankton. Later, during the atokous worm stage, the worms graze on the microorganisms that grow in the vicinity, together with small pieces of fish food flakes and spinach. Maintenance of the phytoplankton cultures was achieved in autoclaved Erlenmeyer flasks in filtered or sterilized seawater with added culture media (e.g. Bold Basal Medium with 3-fold nitrogen and vitamins; modified (3N-BBM + V, CCAP, UK)). The phytoplankton culture flask is placed in the system as close to the 'daylight' source as possible. All worm culture boxes were cleaned periodically with 0.5% NaOH and washed with distilled water. Worms that were sexually mature (showing evidence of epitoky) were isolated into labelled boxes (Fig. 2E and F). When they show active swimming, they are collected and put together (ideally several males and couple of females) in a

small glass bowl (100 mL). This bowl is kept inside the culture cabinet near the 'daylight' source (Fig. 1), where later the larvae will be maintained. If the water looks quite cloudy as a result of a large amount of released sperm, it is diluted as soon as possible by adding more water, avoiding polyspermy, which produces unviable fertilized eggs. After 10 minutes of fertilization, the egg jelly release processes should be complete, generating a translucent surrounding area, which does not allow the eggs to touch each other. If the eggs have been fertilized, they become clearly distinguishable and can be differentiated from non-fertilized eggs. After fertilization the larvae remain in the glass bowl for 5 days. Twenty-four hours after fertilization, the egg jelly is removed, water carefully replaced and the flask is marked with the date and time of fertilization. At this stage, larvae are called trochophores (Fig. 2A) with a typical ciliated ring at the equator of the larva. Around 2 thousand larvae can be obtained per fertilization, although after the following metamorphosis processes, the number of polychaetes is gradually reduced. After 48 h, metatrochophore larvae develop and any jelly remaining in this "jelly" flask must be removed and discarded to avoid the growth of fungi and bacteria.



Figure 2. Different life stages of *Platynereis dumerilii*. (A) Trochophore larvae, at 24 hours post fertilization, with a ciliated equatorial ring. (B) Nectochaeta larvae, at 72 hours post fertilization, with elongated shape and developing head and segments. (C) and (D) juvenile and non-mature adult (atokous) worms grazing on food surrounding their burrows. (E) Mature (epitokous) female worm. (F) Mature (epitokous) male.

From 72 hours post fertilization, the nectochaete larval stage emerges (Fig. 2B), showing an elongated body and parapodia with occasionally independent movements. The larvae are removed by pouring them (with the assistance of a fine paint brush) into plastic box containers with 1cm depth of seawater. One week later, more seawater and an air supply are introduced. A small amount of phytoplankton (an aliquot containing around 1×10^5 cells) is introduced and after a further 2 weeks the first water change is made. Any earlier change of water will result in the loss of the tiny developing worms. For a comprehensive description of the developmental process of this species see Fischer *et al.* (2010). Juvenile worms (Fig. 2C), construct mucopolysaccharide burrows in which they live (Daly 1973). Non-mature (atokous) adult worms graze on the food that appears surrounding the burrows (Fig. 2D). Gametogenesis takes place, as well as vitellogenesis in females. If the worm density is too high, large quantities of faeces will remain in the container and could negatively affect the physiology of the worms. When *P. dumerilii* start the epitoky metamorphosis process, they remain inside the burrows without eating, and a change in the colour pattern is observable (Fig. 2E and F).

In this paper, we have described an easy and time-efficient *P. dumerilii* culture system at a reduced size scale. Mature adult worms were obtained around 4 months after the start of the culture, encompassing a moon cycle, which is fundamental for the completion of the life cycle in this organism (Hauenschild 1956). Several dozen successful fertilizations per year is the minimum expected number. As an emerging marine model species, the potential of installing small culture systems of *P. dumerilii* in laboratories for experimental purposes is enormous. In parallel, due to the rich food properties of these worms this reduced system could be employed as a co-culture to improve marine live-stock. It is fundamental to keep the system free of chemicals, search regularly for mature animals and periodically check the temperature and salinity of the system. In comparison with the standard culture of the species, it has the great advantage that can be developed in a laboratory, which can be used for several purposes. There is a natural limitation of a compact system such as the total number of adults achievable, although the number of animals produced is sufficient for several research studies. In order to avoid inbreeding, it is recommended to introduce into the system new animals from the field and/or from other laboratories as well, at least every 2 or 3 years.

In conclusion, we describe here a compact system and a procedure to cultivate the polychaete *P. dumerilii* that is attracting increasing international scientific attention due to several biological characteristics, which make this polychaete a marine model organism for several fields of marine and estuarine science.

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References

- Daly, J. M. 1973. Behavioural and secretory activity during tube construction by *Platynereis dumerilii* Aud & M. Edw. [Polychaeta: Nereidae]. **Journal of the Marine Biological Association of the UK** 53: 521-529.
- Dray, N., Tessmar-Raible, K., Le Gouar, M., Vibert, L., Christodoulou, F., Schipany, K., Guillou, A., Zantke, J., Snyman, H., Behague, J., Vervoort, M., Arendt, D. & Balavoine, G. 2010 Hedgehog signaling regulates segment formation in the annelid *Platynereis*. **Science**; 329(5989): 339-342.
- Fischer, A. 1997. Reproducing once in a lifetime: A different mode of organizing gametogenesis. **Journal of Reproductive and Development** 43: 41-42.
- Fischer, A. & Dorresteijn, A. 2004. The polychaete *Platynereis dumerilii* (Annelida): A laboratory animal with spiralian cleavage, lifelong segment proliferation and a mixed benthic/pelagic life cycle. **BioEssays** 26: 314-325.
- Fischer, A. H., Henrich, T. & Arendt, D. 2010. The normal development of *Platynereis dumerilii* (Nereididae, Annelida). **Frontiers in Zoology**. 30: 7-31.
- Gray, J.S., Elliott, M., 2009. Ecology of Marine Sediments: From Science to Management, second edition. Oxford University Press, Oxford.
- García-Alonso, J., Müller, C. T. & Hardege, J. D. 2008. Seasonal changes and influence of food regimes on fatty acid composition of the ragworm *Nereis diversicolor* from Cardiff Bay. **Aquatic Biology** 4: 7-13.
- García-Alonso J., Ayoola J.A.O., Crompton J., Rebscher N. & Hardege J. 2011. Development and maturation in the nereidid polychaetes *Platynereis dumerilii* and *Nereis succinea*

- exposed to xenoestrogens. **Comparative Biochemistry and Physiology Part C**: 154: 196-203.
- Hauenschild C. 1956. Neue experimentelle Untersuchungen zum Problem der Lunarperiodizität. **Naturwissenschaften**, 16: 361-363.
- Hauenschild C. & Fischer A. 1969. *Platynereis dumerilii*. Mikroskopische Anatomie, Fortpflanzung, Entwicklung. **Großes Zoologisches Praktikum Heft 10b**: 1-55.
- Hutchinson T.H., Jha A. N. & Dixon D. R. 1995. The polychaete *Platynereis dumerilii* (Adouin and Milne-Edwards): A new species for assessing the hazardous potential of chemicals in the marine environment. **Ecotoxicology and Environmental Safety** 31: 271-281.
- Olive, P. J. W. 1999. Polychaete aquaculture and polychaete science: a mutual synergism. **Hydrobiologia** 402: 175-183.
- Raible, F., Arendt, D. 2004. Metazoan evolution: some animals are more equal than others. **Current Biology** 14(3): 106-108.
- Robinson, V. 2005. Finding alternatives: an overview of the 3Rs and the use of animals in research. **School Science Review**, 87: 319-322.

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