



# Reproductive cycle, growth and age estimation of *Donax trunculus* in the Méditerranéen sea of Morocco

O. Haroufi<sup>1, 2, 3</sup>, M. Daoudi<sup>2</sup>, M. Maatouk<sup>1</sup>, A. Arias-Pérez<sup>3</sup>

<sup>1</sup>Department of Earth Sciences, Faculty of Sciences and Techniques of Tangier (FSTT), University Abdelmalek Essaadi UAE, Morocco

<sup>2</sup>Fisheries Department, National Institute of Fisheries Research (INRH), Tangier, Morocco

<sup>3</sup>Department of Biomedicine, Biotechnology and Public Health, University of Cádiz (UCA), 11510, Puerto Real, Cádiz, Spain.

e-mail: haroufi.ouassima@alum.uca.es



## INTRODUCTION

Molluscs are among the most important groups of marine invertebrates, representing a very relevant part of marine biodiversity. Within this phylum, bivalves are one of the majority groups. One such group that has been known since the antiquity, being already described by Aristotle 25 centuries ago in his treatise *Historia animalium*. While Xenocrates mainly described his mode of consumption (cited in Voultziadou, 2009).

Fishing for marine invertebrates in Morocco is subject to a system of authorization by species, but there are also closed periods that are published in the official bulletin (BOE 2010). These periods correspond to biological rest periods or to periods in which the levels of biotoxins are high in the organism of the species. The species that do not have been the subject of a request for exploitation authorization before the Ministry of Fisheries Maritime are exploited in an unregulated way as is the case of *Donax trunculus* and other species of marine invertebrates along the entire Moroccan coast.

In order to guarantee a reasonable and sustainable exploitation of the bivalve, the present study on the population of *Donax trunculus* fits in the framework of a follow-up of dynamic ecology of the population *Donax trunculus*, and aims to determine for the first time the reproductive cycle of *D. trunculus* in the Mediterranean sea of Morocco.

## OBJECTIVES

*Donax trunculus* (Linné, 1758) has a wide Atlantic-Mediterranean distribution; It is a bivalve mollusk commonly known as coquina. It belongs to the Donacidae family and is therefore included in the Tellinacea superfamily. Morphologically, it is characterized by having a solid shell, with almost symmetrical, triangular-shaped valves and a smooth outer surface (Figure 1). The coloration of the shell is variable and presents darker concentric and radial bands, the interior of the shell also presents variable coloration (Fischer et al., 1987).



Figure 1. External aspect of *D. trunculus* (Sampling object species)

This work aims first of all a follow-up of dynamic ecology and growth of the population of *Donax trunculus* whose objective to describe and compare among *D. trunculus* sin Mediterranean of Morocco:

- The study of the dynamics of populations of *D. trunculus* by following the distribution of frequencies of size classes and the parameters biometric.
- The histological study of male and female gonads of *Donax trunculus* throughout the year (13 months).

## STUDY AREA

Located in the north of Morocco exactly the Bay of Martil extends over 9 km approximately from the dike of Cabo Negro in the North and of Sidi Abdessalam South (35° 38'30"N; 5° 18'30"O).



Figure 2: location map of the study area

## WORK ON GROUND

Sampling Strategy: The collection of *Donax trunculus* is carried out with the help of a rake to Hand, commonly used for the fishing of molluscs, commonly called "rachtro"; This tool consists of two parts, a metal part composed of two hoops together and a fishback in iron in the form of comb armed with long teeth in metal in the form of comb armed with long teeth in metal; the other part consists of a net of which the mesh size should not be less than 10 mm. The rake is provided of a wrist 1.5 m long, which allows you to adjust the tilt angle of the teeth.



Figure 3: the fishing gear

## LABORATORY WORK

The sampling is done on a monthly basis for a period of one year (May 2017 - May 2018), 60 individuals are caught on a monthly basis and in a random manner at the site level and during the year of study on a depth of 10 cm in the sand and a water column of 0.5 to 1 m depending on the state of the sea.

To carry out the histological studies, samples of male and female gonads will be analyzed for 13 consecutive months.

The samples are then transported to the laboratory in a coolers or trays in plastics containing of the sea water. The biometric study, the dissection and the levy of the mantle are realized after desilting for the study of the reproductive cycle, while the samples intended for the study of the growth are handled the same day.



Figure 4: the samples caught

Histology methods will be applied after dissection of the gonads of individuals mature and will be fixed in the Davidson solution for 72 hours, dehydrated in alcohol, they will be cleaned in xylene, embedded in paraffin and cut (6-8 µm). The tissue cuts stained with Ehrlich's hematoxylin and Eosin and according to the procedures described in (Howard and Smith, 1983). Individuals of *D. trunculus* are measured using a vernier caliper to obtain a precision of 1/10 mm, and classified according to: The length (L) The measurement of the height (h) the thickness (E) The Weight parameter is determined using a precision balance (Sartorius H110, Precision 0,1mg)(Figure 6).

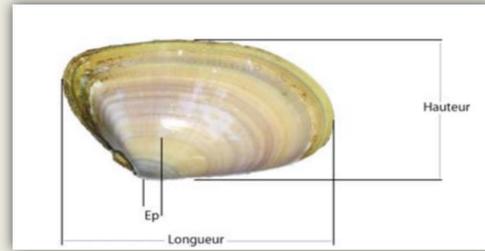


Figure 5: Biometric distances measured in a shell of *D. trunculus*

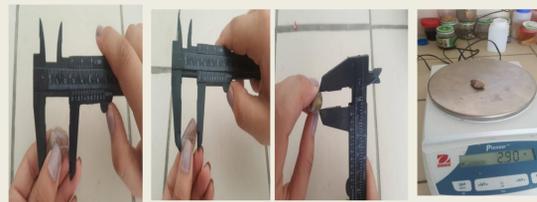


Figure 6: Biometric measurements and measurement of the fresh weight of the shell

For the study of growth and age estimation, about 30 individuals of different sizes (4 to 35 mm) whose antero-posterior lengths will be measured with the help of a measuring Vernier caliper.

Once isolated from their bivalve, the samples will be deposited separately in an oven at 60° C) for 72 hours. The dry weights will be obtained with a precision balance (accuracy 0.1 mg).



Figure 7: Drying use for déshydratation

## HISTOLOGICAL PROTOCOL

Conducted according Martoja and Martoja (1967):

- **Fixing:** the fragment of the gonad is placed in a cassette of histology, and kept in a fixer of histology the liquid alcoholic Davidson during 48 hours.



Figure 8: fixing step of *Donax trunculus*

- **Pre Inclusion:** The samples were then dehydrated in two successive baths of ethanol and three baths of butanol, to finally be impregnated in paraffin, four baths of 24h have been carried out.



Figure 9: dehydration machine

- **Inclusion:** impregnated fabrics of paraffin must then be included in a block of paraffin, that we mold around the tissue on the bench of inclusion. The base of the mold is constituted by the cassette of annotated histology in order to retain the reference of the individual. Once solidified, the block of paraffin is debited in Prism around the fragment of tissue.



Figure 10: the inclusion stage

- **Achievement of the slices:** paraffin blocks containing the tissues are cut using a microtome. They are first rough coated until the cup pass by the totality of the section of visceral mass, then cut to 2 µm thick, the slices are filed on a microscope slide. The slices are then spread to the aid of a heating plate to 30° C and dry in the oven of 60° C for 1 hour.



Figure 11: achievement of the slices

- **Coloring:** In order to obtain a coloring contrasting reproductive tissues, it uses a coloring hematoxylin-eosin Martel (2005). The fabrics are déparaffinés by successive baths Xylene, then hydrated in the alcohol prior to be immersed in the dyes, and rinsed by the baths of alcohol, then xylene.



Figure 12: Coloring/ slide ready for microscopic reading

## EXPECTED RESULTS

In order to ensure a rational management of the species and preserve the resource, this work aims to study the growth and sexual cycle of *D. trunculus* through histological methods (Baved 1990; Neuberger-Cywiak et al. 1990) in order to improve the understanding of its biological cycle which allows to follow:

- The evolution of the general microscopic structure of the ovaries with respect to the gonado-somatic relationship.
- The process of maturation of the ovum.
- The characterization of the phases of sexual maturation of males and females.
- Establish one or two periods of sexual rest according to the seasons. As well as the study of the biology of *Donax trunculus* accompanied of physico-chemical parameters will explain its spatial distribution.

## REFERENCES

- ❖ Amoureux, J.M. 1974. "Etude des peuplements infralittoraux de la côte du Roussillon.III. Variations spatiales et saisonnières." Vie Milieu 24: 321-354.
- ❖ Ansell, A., & Lagardère, F. 1980. "Observations on the Biology of *Donax Trunculus* and *D. Vittatus* at Ile d'Oléron (French Atlantic Coast)." Marine Biology 57: 287-300.
- ❖ Baved, A. 1990. "Reproduction de *Donax Trunculus* Sur La Côte Atlantique Marocaine." Cahiers De Biologie Marine 31:159-69.
- ❖ Guillou, J. & Baved, A. 1991. "Contraintes du milieu sur les populations de *Donax trunculus* L. et *D. venustus* Poli du littoral atlantique marocain." Oceanologica acta 14:291-298.
- ❖ Nantón, A., Freire, R., Arias-Pérez, A., Gaspar, M.B. & Méndez, J. 2015. "Identification of four *Donax* species by PCR-RFLP analysis of cytochrome c oxidase subunit I (COI)." Eur. Food Res. Technol. 240: 1129-1133.