UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

BIOLOGY, ECOLOGY AND FISHERY OF THE BANDED MUREX, *HEXAPLEX (TRUNCULARIOPSIS) TRUNCULUS*, IN THE RIA FORMOSA LAGOON

(Tese para a obtenção do grau de doutor no ramo de
Ciências do Mar, especialidade de Biologia Marinha)

PAULO DA CONCEIÇÃO SILVA VASCONCELOS

Orientador: Doutor Miguel José Baptista Gaspar
Co-orientador: Doutora Maria Margarida Miranda de Castro

Constituição do Júri:
Presidente: Doutora Maria Teresa Dinis
Vogais: Doutor Christopher A. Richardson, Doutor Carlos Miguel Miguez Barroso,
Doutor Carlos Luciano da Costa Monteiro, Doutor Pedro Conte de Barros

FARO
(2007)

UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

BIOLOGY, ECOLOGY AND FISHERY OF THE BANDED MUREX, *HEXAPLEX (TRUNCULARIOPSIS) TRUNCULUS*, IN THE RIA FORMOSA LAGOON

(Tese para a obtenção do grau de doutor no ramo de
Ciências do Mar, especialidade de Biologia Marinha)

PAULO DA CONCEIÇÃO SILVA VASCONCELOS

Orientador: Doutor Miguel José Baptista Gaspar
Co-orientador: Doutora Maria Margarida Miranda de Castro

Constituição do Júri:
Presidente: Doutora Maria Teresa Dinis
Vogais: Doutor Christopher A. Richardson, Doutor Carlos Miguel Miguez Barroso,
Doutor Carlos Luciano da Costa Monteiro, Doutor Pedro Conte de Barros

FARO
(2007)

“For in the end, we will conserve only what we love;
we will love only what we understand;
and we will understand only what we are taught.”

Baba Dioum (Senegalese Conservationist)

To Catarina, João and Beatriz,
and to the memory of Luís Miguel.

ACKNOWLEDGEMENTS

The present work was entirely undertaken in the South Regional Centre for Fisheries Research (Centro Regional de Investigação Pesqueira do Sul - CRIPSul) of the Institute for Fisheries and Sea Research (Instituto de Investigação das Pescas e do Mar - IPIMAR). During the study period, the present candidate benefited from a PhD grant from the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia - FCT: SFRH/BD/5139/2001). In addition, some components of this study were integrated and partially funded by diverse research projects that were in progress in the IPIMAR - CRIPSul, namely the Small-Scale Fisheries Program (IPIMAR), DiverAqua Project (Interreg IIIA), RedAqua Project (Interreg IIIA) and ProMar Project (Interreg IIIA).

The development of a study of this kind obviously depends on the involvement and contribution of many individuals and institutions, to whom I would like to express my sincere acknowledgements:

- To Dr. Carlos Costa Monteiro (President of the IPIMAR) for accepting, encouraging and providing all the adequate conditions necessary for the development of this study in the IPIMAR-CRIPSul;

- To Prof. Margarida Castro (University of the Algarve - FCMA) for accepting the supervision of this PhD project, and especially for the support and statistical advice;

- To Dr. Miguel B. Gaspar (IPIMAR - CRIPSul) for believing in this PhD project since the beginning, and particularly for the constant interest, dedication and support. Also for helping me to stay focused and keep looking forward, but above all for being a good friend…;

- To Dr. Eurico Monteiro (Portuguese Directorate-General for Fisheries and Aquaculture - DGPA) for authorising the experimental fishing surveys with the illegal fishing gear (“wallet-line”) in the Ria Formosa lagoon;

- To the “Associação de Moradores da Ilha da Culatra”, in particular to Sílvia Padinha for her commitment in involving the fishing community of the Culatra Island in this study. Special gratitude is owned to the skippers of the fishing boats (Cristiano Mestre Artífice, Jorge Humberto do Carmo, Rui Alberto do Carmo, José Carlos Pacheco, João Luís Quintino, Celestino Patrício dos Santos and José Patrício dos Santos) for their skill in conducting the fishing surveys. Thanks are also due to Dr. Rogélia Martins and Miguel Carneiro (IPIMAR) for making available the technical drawing of the “wallet-line”;

- To Dr. Pedro Pousão-Ferreira (Olhão Fish Culture Experimental Station, IPIMAR - CRIPSul) for kindly providing an aquaculture earthen pond for undertaking the mark/ recapture experiments and to his collaborators for monitoring and making available the data on the seawater temperature in the Ria Formosa lagoon. Sincere thanks are also due to the colleagues Alexandre Pereira and João Tata-Regala for their help in fencing the aquaculture earthen pond, and to Susana Carvalho, Rita Constantino and João Cúrdia for their assistance during the periodic recapture operations;

- To Domitília Matias and Sandra Joaquim (Tavira Molluscan Aquaculture Experimental Station, IPIMAR - CRIPSul) for their interest in the preliminary assessment of the potential of the banded murex for muriciculture and for providing the facilities for maintaining the banded murex in captivity. Gratitude is also owed to João Maurício Teixeira for installing the tanks circuit and to the colleagues Margarete Ramos, David Fernandes and Rui Gonçalves for the daily work with the maintenance of hatchlings and juvenile gastropods;

- To Dr. Paula F. Pereira, João Cúrdia, Susana Carvalho and Fábio Pereira for their assistance in the identification of epibiotic polychaetes and by-catch species. Special recognition is also due to João Cúrdia for his help in photographing the histological sections of the gonads. To Pedro Lino for being our “informatics consultant”, with his tireless help in solving computer troubles and answering technical questions from “computer dummies”…;

- To the technical staff of IPIMAR - CRIPSul, particularly to José Luís Sofia, Tibério Simões, Rolando Machado, Ana Canas and Lina Oliveira, for their contribution in laboratory sampling procedures;

- To Dr. Miguel N. Santos, Dr. Manuela Falcão and Prof. Luís C. Fonseca (IPIMAR - CRIPSul), for their friendly support and constructive suggestions;

- To many friends and colleagues for maintaining a joyful working environment in the IPIMAR-CRIPSul, in particular to many other grant-holders that work (or worked…) there since the beginning of this study, such as Alexandra Garcia, Alexandre Pereira, Ana Moura, Ana Silva, Belisandra Lopes, Carlos Campos, Clara Martins, Cristina Martins, Dalila Serpa, Fábio Pereira, Francisco Leitão, Hugo Saldanha, João Cúrdia, João Tata-Regala, Jorge Ramos, Mafalda Ferreira, Maria Vicente, Marisa Barata, Marta Rufino, Natália Dias, Paula Moura, Paula Pereira, Pedro Lino, Rita Constantino and Susana Carvalho. Thanks a lot for all those good laughs…;

- To my parents, family and friends, for the constant encouragement during my academic career and specially for believing that one day I will finally find a normal job…;

- To Catarina for her endless patience and support, and to João and Beatriz for their infinite and contagious energy, which altogether are the main reasons to keep carrying on. I just expect that one day I will be able to compensate my family for all the time I have not spent with them…;

- To everyone who contributed somehow for this study (with my sincere apologies to the ones that I am sure to have forgotten to include in this extensive list);

- Finally, I have already forgiven all those who were constantly asking the boring question: “So, when will you finish writing the PhD thesis ?...” and I promise never to do the same to those who are still rushing to conclude their theses...

Thank you all...

NOME: Paulo da Conceição Silva Vasconcelos
FACULDADE: Faculdade de Ciências do Mar e do Ambiente (FCMA)
ORIENTADOR: Doutor Miguel José Baptista Gaspar (IPIMAR - CRIPSul)
CO-ORIENTADOR: Doutora Maria Margarida Miranda de Castro (UAlg - FCMA)
DATA: 2007
TÍTULO DA TESE:
Biologia, ecologia e pescaria do búzio, *Hexaplex (Truncolariopsis) trunculus*,
na Ria Formosa

RESUMO

Na Ria Formosa (Algarve - Portugal) existe uma pescaria artesanal de gastrópodes muricídeos dirigida à captura de duas espécies com elevado valor comercial, o búzio [*Hexaplex (Truncolariopsis) trunculus*] e a canilha (*Bolinus brandaris*), a qual é tradicionalmente exercida com uma arte de pesca ilegal localmente designada por “teia-de-carteiras”. Devido à escassez de informação sobre esta pescaria e respectivas espécies alvo, os principais objectivos do presente trabalho foram a caracterização da actividade pesqueira com a “teia-de-carteiras” e o estudo de aspectos relevantes da biologia e ecologia de *T. trunculus*. Relativamente à pescaria, a realização de campanhas de pesca mensais teve por objectivo a descrição das operações de pesca e da arte de pesca, estimação do rendimento de pesca, caracterização da composição das capturas das espécies alvo, identificação de espécies acessórias e rejeições. No respeitante aos estudos sobre a biologia e ecologia do búzio, foi dado particular destaque à estimação da idade e crescimento, estudo do ciclo reprodutivo e avaliação da incidência e nível de imposex em *T. trunculus*. Finalmente, a globalidade da informação obtida no presente estudo permitiu a proposta de diversas medidas de gestão para a implementação de regulamentação específica para esta pescaria artesanal na Ria Formosa.

Palavras-chave: *Hexaplex (Truncolariopsis) trunculus*, Ria Formosa, idade e crescimento, ciclo reprodutivo, imposex, gestão pesqueira.

TITLE OF THE THESIS:

Biology, ecology and fishery of the banded murex, *Hexaplex (Trunculariopsis) trunculus*, in the Ria Formosa lagoon

ABSTRACT

In the Ria Formosa lagoon (Algarve coast - southern Portugal) exists an artisanal fishery for muricid gastropods targeting two commercially valuable species, the banded murex [*Hexaplex (Trunculariopsis) trunculus*] and the purple dye murex (*Bolinus brandaris*), which is traditionally undertaken with an illegal fishing gear locally known as “wallet-line”. Due to the scarcity of information about this fishery and respective target species, the main objectives of the present work were the characterisation of the fishing activity with the “wallet-line” and the study of relevant aspects of *T. trunculus* biology and ecology. Regarding the fishery, monthly fishing surveys aimed the description of the fishing operations and fishing gear, estimation of fishing yield, characterisation of the target species catch composition, identification of by-catch species and discards. Concerning the studies on the biology and ecology of the banded murex, particular emphasis was given to the estimation of age and growth, study of the reproductive cycle and assessment of the incidence and level of imposex in *T. trunculus*. Finally, the overall information gathered in the present study allowed for the proposal of several management measures for the implementation of specific regulations for this artisanal fishery in the Ria Formosa lagoon.

Key-words: *Hexaplex (Trunculariopsis) trunculus*, Ria Formosa lagoon, age and growth, reproductive cycle, imposex, fishery management.

TABLE OF CONTENTS

SECTION 1

GENERAL INTRODUCTION AND OBJECTIVES

SECTION 1

General introduction and objectives

GENERAL INTRODUCTION

Marine molluscs are among the most important invertebrate fisheries in the world and marine gastropods represent approximately 2% of the molluscs fished worldwide (Leiva and Castilla, 2002). Several gastropod species have high commercial value in international markets and play important social roles in small-scale fisheries, namely *Haliotis* spp. (U.S.A., South Africa, Australia and New Zealand), *Strombus* spp. (Caribbean), *Busycon* spp. (U.S.A.), *Concholepas concholepas* (Chile and Peru) and *Turbo truncatus* (Japan and Korea) (Leiva and Castilla, 2002). In Europe, *Buccinum undatum* and *Littorina littorea* are the main species exploited, particularly in France, United Kingdom and Ireland (accounting for over 90% of the European gastropod catches) (Leiva and Castilla, 2002), while in Iberian waters the most important gastropod fishery targets *Bolinus brandaris* along the Catalan coast (northwestern Mediterranean) (Martín *et al.*, 1995). However, besides these well established and developed commercial fisheries, other gastropods populations have been subjected to exploratory and experimental fisheries in order to constitute an alternative to the exploitation of traditional fishing resources (*e.g.*, Duncan *et al.*, 1989; Gunnarsson and Einarsson, 1995; Valentinsson *et al.*, 1999).

As with other fishing resources, sustainable management of commercially exploited gastropod species and rational decision-making by fisheries biologists require basic and reliable information on the nature of the fisheries and exploitation, as well as on key aspects of the exploited stocks and target species biology (particularly growth and reproduction) (Appeldoorn, 1987; Naylor, 2005). For this reason, several gastropod fisheries have been studied in order to determine the main technical features of the fishing activity and to estimate the fishing parameters required for fisheries management (*e.g.*, Gros and Santarelli, 1986; Sainte-Marie and Hargrave, 1987; Himmelman, 1988; McQuinn *et al.*, 1988; Sainte-Marie, 1991; Lanteigne and Davidson, 1992; Lapointe and Sainte-Marie, 1992). Similarly, there are numerous examples of commercially exploited gastropod species that have been subjected to diverse kinds of studies aiming to obtain relevant biological data for fisheries management (*e.g.*, Brownell and Stevely, 1981; Villiers, 1981; Wood and Olsen, 1983; Santarelli and Gros, 1985; Muñoz *et al.*, 1987; Wicklund and Hepp, 1987; Appeldoorn, 1987, 1988; Davis and Sisson, 1988; Santarelli, 1988; Sisson and Wood, 1988; Walker, 1988; Gendron, 1989, 1991, 1992; Lanteigne and Davidson, 1992; Gunnarsson and Einarsson, 1995; Kenchington and Glass, 1998; Valentinson *et al.*, 1999). Moreover, due to their socio-economic importance in local fishing communities, several gastropod fisheries have already been subjected to specific conservation initiatives and management programmes (*e.g.*, Brownell and Stevely, 1981; Gibson *et al.*, 1983; Hensen, 1983; Wood and Olsen, 1983; Anderson and Eversole, 1984; Santarelli and Gros, 1984; Santarelli *et al.*, 1986; Coulston *et al.*, 1987; Higgs, 1987; Hunt, 1987; Nichols, 1987; Wilkins *et al.*, 1987; Dicosimo, 1988; Kaplan *et al.*, 1988; Kaplan and Boyer, 1992; Fahy *et al.*, 1995, 2000).

However, despite these research and management efforts, due to their high commercial value and excessive capture many marine gastropods show, or have shown, serious problems of overexploitation, which in many cases have led to the decline, collapse or permanent closure of the fishery (Anderson and Eversole, 1988, 1993; Cadée *et al.*, 1995; ten Hallers-Tjabbes *et al.*, 1996; Nicholson and Evans, 1997; Leiva and Castilla, 2002). The social and economic consequences of the collapses of some gastropods fisheries have promoted the development of new management perspectives focused on both biological and economical sustainability (Leiva and Castilla, 2002). In fact, besides classical management measures (minimum landing sizes, total allowable catches, closed seasons and closed areas, etc.), there has been an emergent tendency for the implementation of alternative management strategies (Rowley, 1994; Bell *et al.*, 2005).

In this context, the overexploitation of several marine gastropods populations triggered an increasing interest in improving the knowledge of mariculture of threatened species and integrating stock enhancement and restocking initiatives with other forms of fisheries management as a means to overcome the decline of valuable fishing resources (*e.g.*, Appeldoorn and Ballantine, 1983; Ballantine and Appeldoorn, 1983; Davis and Hesse, 1983; Hensen, 1983; Iversen, 1983; Jory and Iversen, 1983; Laughlin and Weil, 1983; Siddall, 1983; Coulston *et al.*, 1987; Muñoz *et al.*, 1987; Bell *et al.*, 2005; Nasution and Roberts, 2005; Gonzales *et al.*, 2006). Additionally, the implementation of marine protected areas (MPA's) has been recently promoted as a viable complement to classical forms of fishery management, aiming primarily to protect endangered populations and ensure a continuous recruitment of commercially targeted species to fished areas, via protection of a critical minimum spawning stock biomass (Rowley, 1994; Béné and Tewfik, 2003).

RATIONALE AND OBJECTIVES OF THE STUDY

In the Ria Formosa lagoon, located along the Algarve coast (southern Portugal), occurs a fishery for muricid gastropods targeting two commercially valuable species, the banded murex [*Hexaplex (Trunculariopsis) trunculus*] and the purple dye murex (*Bolinus brandaris*), which is traditionally undertaken with an artisanal and illegal fishing gear locally known as "wallet-line". Despite its socio-economic relevance for local fishing communities, at the beginning of the present study the information available on this fishery was very scarce and essentially based on empiric knowledge or generic descriptions (Marques and Oliveira, 1995; Muzavor and Morenito, 1999; Carneiro *et al.*, 2006). Moreover, until then the biology and ecology of the target species (*T. trunculus and B. brandaris*) have not been previously studied in the Ria Formosa lagoon.

Taking into consideration this scarcity (or absence) of information about this fishery and respective target species, the present work aimed to describe the muricid gastropod fishery with the “wallet-line” and study relevant aspects of the biology and ecology of the banded murex (*T. trunculus*), with the ultimate objective of proposing management measures for the implementation of specific regulations for this fishing activity in the Ria Formosa lagoon.

This thesis is structured in six major sections, four of them subdivided into chapters containing the compilation of articles published or submitted for publication in the scope of the present study. The specific objectives of these sections or chapters were the following:

- Section 1 is the general introduction and objectives of the study, briefly reviewing the most important gastropod fisheries worldwide, management requirements and initiatives, and introducing the importance of studying the fishery for muricid gastropods in the Ria Formosa lagoon and the main objectives of the present work;

- Section 2 aimed at describing the fishing operations and fishing gear, estimation of fishing yield, characterisation of the target species (*T. trunculus* and *B. brandaris*) catch composition, identification of by-catch species and discards registered in experimental fishing surveys carried out in the Ria Formosa lagoon (Chapter 2.1);

- Section 3 aimed to estimate the growth rate and von Bertalanffy growth parameters of *T. trunculus* through mark/recapture experiments carried out on a fish culture earth pond in the Ria Formosa lagoon (Chapter 3.1). Furthermore, since during routine sampling it was detected that *T. trunculus* shells were frequently colonised by polychaetes (which among other consequences, might affect the gastropod growth by boring into the shell), a complementary study was undertaken aimed at estimating the fouling frequency, degree of epibiosis, preferential areas for colonisation, incidence of shell damages and the main benefits and disadvantages of epibiosis for *T. trunculus* (Chapter 3.2);

- Section 4 aimed at studying *T. trunculus* gametogenic cycle through macro- and microscopic analyses of male and female gonads of specimens from the Ria Formosa lagoon (Chapter 4.1). Since histological techniques are not very practical for the routine assessment of reproductive condition, another study was performed which compared the ability of conventional condition indices (by weighing the whole organism, flesh or shell) and visual quantitative condition indices (by measuring the digestive gland, gonad and capsule gland) for following the reproductive dynamics of *T. trunculus* (Chapter 4.2). Finally, since there was scarcity of information on the relevant aspects of *T. trunculus* reproductive cycle, a further study was performed which aimed at providing additional data on the spawning behaviour and egg-laying pattern, general morphology and dimensions of the spawns, egg capsules, eggs, embryos and early post-metamorphic juveniles, and hatchling and juvenile growth rates (Chapter 4.3);
- Section 5 aimed to assess the incidence and degree of imposex in *T. trunculus* from the Ria Formosa lagoon. This study is essential to detect eventual interferences with normal sexual development and potential occurrence of female sterility caused by the imposex phenomenon in *T. trunculus*, which could ultimately affect the population dynamics of this fishery-exploited species. Simultaneously, this work provides baseline data for future monitoring programmes and for temporal trend surveillance related to TBT pollution in the Ria Formosa lagoon (Chapter 5.1). Furthermore, since *T. trunculus* lacks external sexual dimorphism, its sexual identification implies breaking the shell and sacrificing the organism, which is further complicated by the imposex phenomenon. For this reason, a complementary study was carried out aimed at developing sexual indices based on the dimensions of the male and imposex-affected female penis, which could constitute a non-sacrificial tool for sexing live specimens of *T. trunculus* (Chapter 5.2);

- Section 6 is the general discussion and conclusion of the study, summarising the main results obtained in the previous sections and giving particular emphasis to their significance for supporting the proposal to the fisheries administration of specific management measures for this locally important artisanal fishery. Finally, mention is made of some further developments of this study that could contribute to improve the knowledge on this fishing activity and respective target species, as well as to future research needs in order to assess the potential of these species for molluscan aquaculture and to ascertain the technical and economical feasibility of rearing *T. trunculus* and *B. brandaris*, both for commercial production and for stock enhancement initiatives in the Ria Formosa lagoon.

- Section 7 is the compilation of all the literature cited in the previous sections of the thesis.

REFERENCES

Anderson, W.D.; Eversole, A.G., 1984. Evolution of a *Busycon* fishery in the nearshore South Atlantic Bight. *Abstracts of Technical Papers presented at 1983 Annual Meeting of the National Shellfisheries Association*, Hilton Head Island, South Carolina, 6-9 June 1983. *Journal of Shellfish Research*, 4: 81.

Anderson, W.D.; Eversole, A.G., 1988. Managing a predator: decline of an offshore *Busycon* fishery. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 147.

Anderson, W.D.; Eversole, A.G., 1993. Over exploitation and signs of recovery: Analysis of an offshore whelk fishery. *Abstracts of the technical papers presented at the 85th Annual Meeting of the National Shellfisheries Association*, Portland, Oregon, 31 May - 3 June, 1993. *Journal of Shellfish Research*, 12: 138.

Appeldoorn, R.S., 1987. Practical considerations in the assessment of queen conch fisheries and population dynamics. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 307-324 pp.

Appeldoorn, R.S., 1988. Age determination, growth, mortality and age of first reproduction in adult queen conch, *Strombus gigas* L., off Puerto Rico. *Fisheries Research*, 6: 363-378.

Appeldoorn, R.S.; Ballantine, D.L., 1983. Field release of cultured queen conch in Puerto Rico: implications for stock restoration. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 89-98 pp.

Ballantine, D.L.; Appeldoorn, R.S., 1983. Queen conch culture and future prospects in Puerto Rico. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 57-63 pp.

Bell, J.D.; Rothlisberg, P.C.; Munro, J.L.; Loneragan, N.R.; Nash, W.J.; Ward, R.D.; Andrew, N.L., 2005. Restocking and stock enhancement of marine invertebrate fisheries. *Advances in Marine Biology*, 49: 392 p.

Béné, C.; Tewfik, A., 2003. Biological evaluation of marine protected area: evidence of crowding effect on a protected population of queen conch in the Caribbean. *Marine Ecology*, 24: 45-58.

Brownell, W.N.; Stevely, J.M., 1981. The biology, fisheries, and management of the queen conch, *Strombus gigas*. *Marine Fisheries Review*, 43: 1-12.

Cadée, G.C.; Boon, J.P.; Fischer, C.V.; Mensink, B.P.; ten Hallers-Tjabbes, C.C., 1995. Why the whelk (*Buccinum undatum*) has become extinct in the Dutch Wadden Sea. *Netherlands Journal of Sea Research*, 34: 337-339.

Carneiro, M.; Martins, R.; Rebordão, F.R., 2006. Contribuição para o conhecimento das artes de pesca utilizadas no Algarve. *Publicações Avulsas do IPIMAR*, 13: 76 p.

Coulston, M.L.; Berey, R.W.; Dempsey, A.C.; Odum, P., 1987. Assessment of the queen conch (*Strombus gigas*) population and predation studies of hatchery reared juveniles in Salt River Canyon, St. Croix, U. S. Virgin Islands. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 294-306 pp.

Davis, M.; Hesse, C., 1983. Third world level conch mariculture in the Turks and Caicos Islands. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 73-82 pp.

Davis, J.P.; Sisson, R.T., 1988. Aspects of the biology relating to the fisheries management of New England populations of the whelks, *Busycotypus canaliculatus* and *Busycon carica*. *Journal of Shellfish Research*, 7: 453-460.

Dicosimo, J., 1988. Commercial fisheries analysis of *Busycon* whelks in Virginia. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 155.

Duncan, S.; Gilling, C.; Hughes, G.; Hawkins, S.J.; Nash, R.D.M., 1989. The viability of establishing a whelk fishery in Manx waters. Mimeo. University of Liverpool.

Fahy, E.; Yalloway, G.; Gleeson, P., 1995. Appraisal of the whelk *Buccinun undatum* fishery of the Southern Irish Sea with proposals for management strategy. *Irish Fisheries Investigations. Series B (Marine)*, 42: 26 p.

Fahy, E.; Masterson, E.; Swords, D.; Forrest, N., 2000. A second assessment of the whelk fishery *Buccinum undatum* in the southwest Irish Sea with particular reference to its history of management by size limit. *Irish Fisheries Investigations*, 6: 67 p.

Gendron, L., 1989. Reproductive biology of the whelk *Buccinum undatum* in the northwest Gulf of St. Lawrence. *Abstracts of Technical Papers presented at the 1990 Annual Meeting of the National Shellfisheries Association*, Williamsburg, Virginia, 1-5 April 1990. *Journal of Shellfish Research,* 8: 483-484.

Gendron, L., 1991. Gestion de l'exploitation du buccin *Buccinum undatum* au Québec: détermination d'une taille minimale de capture. *Rapport Technique Canadien des Sciences Halieutiques et Aquatiques,* 1833: 40 p.

Gendron, L., 1992. Determination of the size at sexual maturity of the waved whelk, *Buccinum undatum* Linnaeus, 1758, in the Gulf of St. Lawrence, as the basis for the establishment of a catchable size. *Journal of Shellfish Research,* 11: 1-7.

Gibson, J.; Strasdine, S.; Gonzales, K., 1983. The status of the conch industry in Belize. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 99-107 pp.

Gonzales, B.J.; Galon, W.M.; Becira, J.G., 2006. Community-based stock enhancement of topshell in Honda Bay, Palawan, Philippines. *Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern* (Primavera, J.H.; Quinitio, E.T.; Eguia, M.R.R., Eds.), Iloilo City, Philippines, 13-15 July 2005, 49-59 pp.

Gros, P.; Santarelli, L., 1986. Méthode d'estimation de la surface de pêche d'un casier à l'aide d'une filière expérimentale. *Oceanologica Acta*, 9: 81-87.

Gunnarsson, K.; Einarsson, S., 1995. Observations on the whelk populations (*Buccinum undatum* L., Mollusca: Gastropoda) in Breidifjördur, western Iceland. *ICES Council Meeting Papers,* ICES-CM-1995/K-20: 13 p.

Hensen, R.R., 1983. Queen conch management and culture in the Netherlands Antilles. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 53-56 pp.

Higgs, C., 1987. Current status of queen conch (*Strombus gigas*) stocks and fisheries regulations in the Bahamas. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute,* Trois-Islets, Martinique, November 1985, 377 pp.

Himmelman, J.H., 1988. Movement of whelks (*Buccinum undatum*) towards a baited trap. *Marine Biology,* 97: 521-531.

Hunt, J.H., 1987. Status of queen conch (*Strombus gigas*) management in the Florida Keys, U.S.A. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute,* Trois-Islets, Martinique, November 1985, 376 pp.

Iversen, E.S., 1983. Feasibility of increasing Bahamian conch production by mariculture. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 83-88 pp.

Jory, D.E.; Iversen, E.S., 1983. Queen conch predators: not a roadblock to mariculture. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 108-111 pp.

Kaplan, I.M.; Boyer, B.C., 1992. Monitoring marine resources: Ecological and policy implications affecting the scientific collecting and commercial value of New England conch (*Busycon*). *Biological Bulletin*, 183: 379-380.

Kaplan, I.M.; Boyer, B.C.; Santos, K.A., 1988. Marine policy implications related to the commercial value and scientific collecting of the whelk *Busycon*. *Biological Bulletin*, 175: 312.

Kenchington, E.; Glass, A., 1998. Local adaptation and sexual dimorphism in the waved whelk (*Buccinum undatum*) in the Atlantic Nova Scotia with applications to fisheries management. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 2237: 43 p.

Lanteigne, M.; Davidson, L.A., 1992. Aperçu des rendements par casier et de la taille à maturité sexuelle de buccins communs du Nord, *Buccinum undatum*, capturés le long de la côte est du Nouveau-Brunswick - 1992. *Rapport Technique Canadien des Sciences Halieutiques et Aquatiques*, 1896: 27 p.

Lapointe, V.; Sainte-Marie, B., 1992. Currents, predators, and the aggregation of the gastropod *Buccinum undatum* around bait. *Marine Ecology Progress Series*, 85: 245-257.

Laughlin, R.A.; Weil, M.E., 1983. Queen conch mariculture and restoration in the Archipiélago de los Roques: preliminary results. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 64-72 pp.

Leiva, G.E.; Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of the catches, management and the Chilean experience. *Reviews in Fish Biology and Fisheries*, 11: 283-300.

Marques, M.R.; Oliveira, J.S., 1995. A pesca das "carteiras" na Ria Formosa. *Actas das Comunicações do 8º Congresso do Algarve*, Vilamoura, Portugal, 7-9 Abril 1995, 905-909 pp.

Martín, P.; Sánchez, P.; Ramón, M., 1995. Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research*, 23: 319-331.

McQuinn, I.H.; Gendron, L.; Himmelman, J.H., 1988. Area of attraction and effective area fished by a whelk (*Buccinum undatum*) trap under variable conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, 45: 2054-2060.

Muñoz, L.; Alcolado, P.; Fraga, I.; Llorente, P., 1987. Status of populations and fisheries of *Strombus gigas* in Cuba, with some results of juvenile rearing in pens. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 353-361 pp.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Nasution, S.; Roberts, D., 2005. Laboratory trials on the effects of different diets on growth and survival of the common whelk, *Buccinum undatum* L. 1758, as a candidate species for aquaculture. *Aquaculture International*, 12: 509-521.

Naylor, E., 2005. Chronobiology: implications for marine resource exploitation and management. *Scientia Marina*, 69: 157-167.

Nichols, K.E., 1987. Conch management program - St. Lucia. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 379 pp.

Nicholson, G.J.; Evans, S.M., 1997. Anthropogenic impacts on the stocks of the common whelk *Buccinum undatum* (L.). *Marine Environmental Research*, 44: 305-314.

Rowley, R.J., 1994. Marine reserves in fisheries management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 4: 233-254.

Sainte-Marie, B., 1991. Whelk (*Buccinum undatum*) movement and its implications for the use of tag-recapture methods for the determination of baited trap fishing parameters. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 751-756.

Sainte-Marie, B.; Hargrave, B.T., 1987. Estimation of scavenger abundance and distance of attraction to bait. *Marine Biology*, 94: 431-443.

Santarelli, L., 1988. Estimación de la densidad poblacional de *Buccinum undatum* L. (Gasteropoda: Prosobranchia) por medio del modelo de extracción sucesiva de DeLury. *Anales del Instituto de Ciencias del Mar y Limnología*, 15: 65-72.

Santarelli, L.; Gros, P., 1984. Modélisation bioéconomique de la pêcherie de buccin (*Buccinum undatum* L.: Gastropoda) du port de Granville (Manche Ouest). Eléments de gestion de la ressource. *Revue des Travaux de l'Institut des Pêches Maritimes*, 48: 23-32.

Santarelli, L.; Gros, P., 1985. Détermination de l'âge et de la croissance de *Buccinum undatum* L. (Gasteropoda: Prosobranchia) à l'aide des isotopes stables de la coquille et de l'ornamentation operculaire. *Oceanologica Acta*, 8: 221-229.

Santarelli, L.; Véron, G.; Huet, J., 1986. Exploitation du buccin dans le golfe Normano-Breton. *La Pêche Maritime*, 1294: 48-52.

Siddall, S.E., 1983. Biological and economic outlook for hatchery production of juvenile queen conch. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 46-52 pp.

Sisson, R.T.; Wood, R.S., 1988. Observation on some life history aspects of a commercially exploited population of *Busycon canaliculatum* (Linne) in Narragansett Bay, Rhode Island. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 176.

ten Hallers-Tjabbes, C.C.; Everaarts, J.M.; Mensink, B.P.; Boon, J.P., 1996. The decline of the North Sea whelk (*Buccinum undatum* L.) between 1970 and 1990: A natural or a human-induced event? *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 17: 333-343.

Valentinsson, D.; Sjödin, F.; Jonsson, P.E.; Nilsson, P.; Wheatley, C., 1999. Appraisal of the potential for a future fishery on whelks (*Buccinum undatum*) in Swedish waters: CPUE and biological aspects. *Fisheries Research*, 42: 215-227.

Villiers, L., 1981. Croissance, dynamique du “stock” et de la biomasse de *Turbo setosus* (Gastropode - Turbinidae). Atoll de Hao (Tuamotu, Polynésie Française). *Vie et Milieu*, 31: 3-13.

Walker, R.L., 1988. Intertidal populations of four species of whelks (*Busycon*) in Wassaw Sound, Georgia. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 180.

Wicklund, R.I.; Hepp, L., 1987. Ongoing population studies of juvenile queen conch, *Strombus gigas*, in the Exuma Cays, Bahamas. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 378 pp.

Wilkins, R.M.; Goodwin, M.H.; Reid, D.M., 1987. Research applied to conch resource management in St. Kitts/Nevis. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 370-375 pp.

Wood, R.S.; Olsen, D.A., 1983. Application of biological knowledge to the management of the Virgin Islands conch fishery. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 112-121 pp.

SECTION 2

THE FISHERY FOR MURICID GASTROPODS IN THE RIA FORMOSA LAGOON

CHAPTER 2.1

The artisanal fishery for muricid gastropods (banded murex and purple dye murex) in the Ria Formosa lagoon (Algarve coast - southern Portugal)

Vasconcelos, P.; Carvalho, S.; Castro, M.; Gaspar, M.B. The artisanal fishery for muricid gastropods (banded murex and purple dye murex) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Scientia Marina* (submitted).

ABSTRACT

The artisanal fishery for muricid gastropods in the Ria Formosa lagoon (Algarve coast - southern Portugal) constitutes a locally important fishing activity because the banded murex [*Hexaplex (Trunculariopsis) trunculus*] and the purple dye murex (*Bolinus brandaris*) are greatly appreciated seafood with high commercial value in the Portuguese seafood market. An integrated study was implemented to monitor the muricid gastropod fishery with the artisanal fishing gear (“wallet-line”) through monthly experimental fishing operations carried out during one year in the Ria Formosa. The whole aimed to describe the fishing operations and fishing gear, estimate the fishing yield, characterise the target species catch composition, and identify the by-catch species and discards. The “wallet-line” is neither species-specific nor size-selective fishing gear, because the catches comprised a variety of by-catch species and a high proportion of commercially under-sized target species. The vast majority of the by-catch is discarded on-board and therefore mortality is presumably negligible. The CPUE of both target species and by-catch species decreased during consecutive fishing days mainly due to declining bait attraction. Considering the overall information gathered on this fishing activity, some management measures are proposed for this artisanal fishery, which might ultimately contribute to the long-term sustainable exploitation of the fishing resource.

INTRODUCTION

Gastropods represent approximately 2% of the marine molluscs fished worldwide, but due to their importance in local consumption and high commercial value in international markets, some species play significant social roles in small-scale artisanal fisheries (Leiva and Castilla, 2002). In Europe, the countries with the most important gastropod fisheries are France, United Kingdom and Ireland (accounting for over 90% of the European gastropod catches), being the common periwinkle (*Littorina littorea*) and the whelk (*Buccinum undatum*) the main target species (Leiva and Castilla, 2002).

The banded murex, *Hexaplex (Truncilariopsis) trunculus* (Linnaeus, 1758), is mainly distributed in the Mediterranean Sea (Poppe and Goto, 1991), but also occurs in the adjacent Atlantic Ocean, from the Portuguese coast (as far west as Lagos) southward to Morocco and to the Madeira and Canary Archipelagos (Poppe and Goto, 1991; Houart, 2001). The purple dye murex, *Bolinus brandaris* (Linnaeus, 1758), is very common throughout the Mediterranean Sea (Poppe and Goto, 1991; Martín *et al.*, 1995; Houart, 2001), whereas its distributional range in the Atlantic Sea is restricted to Portugal (as far north as Cascais) and Morocco (as far west as Tangier) (Poppe and Goto, 1991; Houart, 2001). In terms of depth distribution, *T. trunculus* generally occurs in the shallow sub-littoral and *B. brandaris* in the deeper sub-littoral (Dalla Via and Tappeiner, 1981).

Presently, both *T. trunculus* and *B. brandaris* are commercially valuable species in several Mediterranean countries (Poppe and Goto, 1991; Houart, 2001), traditionally fished for human consumption by using diverse types of artisanal fishing gears (*e.g.*, pots, basket traps, dredges, scoop nets, trawl nets, gill nets, trammel nets) (Gaillard, 1987; Martín *et al.*, 1995). The banded murex is regularly or occasionally fished/harvested in Italy, Cyprus, Turkey (Gaillard, 1987), Croatia (Peharda and Morton, 2006) and Tunisia (Gharsallah *et al.*, 2004), while the purple dye murex is fished for seafood in France (Bartolome, 1985), Italy (Ramón and Amor, 2001) and occasionally in Tunisia and Turkey (Ramón and Flos, 2001). In Iberian waters, *T. trunculus* and *B. brandaris* are commercially exploited by artisanal fisheries both in Spain, essentially along the Mediterranean and Atlantic coasts of Andalucía (Martín *et al.*, 1995; Anon., 2001; Ramón and Amor, 2001; Tirado *et al.*, 2002), and in Portugal, along the Algarve coast, principally in the Ria Formosa lagoon (Marques and Oliveira, 1995; Muzavor and Morenito, 1999; Carneiro *et al.*, 2006).

In the Ria Formosa, the artisanal fishery targeting these muricid gastropods constitutes a locally important fishing activity, because both the banded murex and the purple dye murex are greatly appreciated seafood with high commercial value in the seafood market (reaching values around 10-15 €/kg and 20 €/kg for first sale, respectively). Besides the hand harvesting in inter-tidal areas, this activity is traditionally undertaken with an artisanal fishing gear (also baited with cockles), locally known as "wallet-line", an illegal fishing gear according to the regulations for the fishery in the Ria Formosa lagoon (D.R., 1990). Additionally, due to their easy capture and high fishing yield, the massive agglomerations of *T. trunculus* and/or *B. brandaris* females during collective spawning (that can reach hundreds of individuals in larger aggregations) are subjected to hand harvesting by recreational users and professional fishermen during low tide (Vasconcelos *et al.*, 2004a), hand harvesting by scuba divers operating illegally inside the lagoon and also by professional fishermen using prohibited entangling nets (Muzavor and Morenito, 1999), altogether catching considerable quantities of these species.

Due to their high economical value and excessive capture, several marine gastropods show (or have shown) serious problems of over-exploitation, which in many cases has led to the collapse or permanent closure of the fisheries (Leiva and Castilla, 2002). Similarly, probably due to over-fishing, detrimental harvesting practices and a disregard to the minimum landing size (MLS) stipulated for these species, the abundances of banded murex and purple dye murex in the Ria Formosa lagoon have been decreasing for more than a decade, possibly indicating over-exploitation of the fishing resource (Marques and Oliveira, 1995). Unfortunately, no reliable official statistics are available on the catches of *T. trunculus* and *B. brandaris* in the Ria Formosa and their appraisal is almost impossible because much of the trade is conducted through a “black economy”, in which sales are not declared for VAT purposes.

Despite the declining abundances of *T. trunculus* and *B. brandaris* in the Ria Formosa lagoon, in the last five years there has been some pressure from local fishermen to legalise the fishery with the “wallet-line”. Since, the information available on this fishing activity is essentially based on empiric knowledge (Marques and Oliveira, 1995; Muzavor and Morenito, 1999) or generic descriptions (Carneiro *et al.*, 2006), an integrated study was implemented to monitor the fishery for muricid gastropods using the “wallet-line” (Vasconcelos *et al.*, 2002), including monthly experimental fishing operations carried out during one year in the Ria Formosa lagoon. This study aimed primarily to describe the fishing operations and fishing gear, to estimate the fishing yield, characterise the target species (*T. trunculus* and *B. brandaris*) catch composition, and identify the by-catch species and discards. In the hypothetical legalisation of this fishing activity in the near future, the overall information gathered in this study (complemented by studies of relevant aspects of the target species biology, namely growth and reproduction), would allow for the proposal to the fisheries administration of adequate management measures for this artisanal fishery, which could ultimately contribute to the long-term sustainable exploitation of this locally important fishing resource.

MATERIAL AND METHODS

Before starting the study, a confidential inquiry was made to professional fishermen from the Culatra Island involved in the fishery for muricid gastropods in the Ria Formosa, who voluntarily accepted to participate in these experimental fishing surveys. These interviews aimed basically to pre-characterise the fishing activity (location and type of bottom, frequency and duration of the fishing operations, number and size of fishing gears, etc.) and set up an experimental design that would realistically reflect the fishing operations usually carried out by the local fishing community. Additionally, five fishing gears that had been previously apprehended by the fishery authorities were measured in order to identify their main characteristics and specifications (materials and dimensions) and prepare a technical plan of a "wallet-line" containing the most relevant features of the fishing gear (adapted from Carneiro *et al.*, 2006).

Following the information gathered during the enquiries made to local fishermen, each monthly fishing survey comprised three consecutive fishing days (day 0 - setting the fishing gears, days 1 and 2 - retrieving the catches, day 3 - hauling the fishing gear and retrieving the catches). Generally, the "wallet-lines" were set early in the morning and hauled in the next morning, after a soaking time of approximately 24 hours. The time required for setting the fishing gear (day 0), retrieving the catches (days 1 and 2), hauling the fishing gear and retrieving the catches (day 3) was registered in each fishing operation. In every fishing survey, six "wallet-lines" (similar to the ones used by local fishermen) each one comprising 100 "wallets" baited with live cockles (*Cerastoderma edule*) were used.

At the end of each fishing day, all individuals caught were removed from the "wallet-lines" and studied in the laboratory. Specimens of the target species (*T. trunculus* and *B. brandaris*) were counted, measured for shell length (SL - mm) with a digital calliper (precision of 0.01 mm), weighed for total weight (TW - g) on a top-loading digital balance (precision of 0.01 g) and the catches per unit effort (CPUE - number or total weight) were standardised for 100 “wallets”/24 hours. All specimens of the by-catch species were also counted and identified to the lowest possible taxonomic level. Finally, at the end of each fishing survey, when hauling the fishing gear the bait was removed from 20 randomly selected “wallets” and the live cockles and empty shells were counted to estimate the bait mortality rate at the end of three fishing days. During the study period, data on the surface seawater temperature registered by the Faro Oceanographic buoy (offshore the Ria Formosa lagoon) was downloaded from the freely available web page of the Portuguese Hydrographical Institute (I.H., 2005-2006), to assess potential relationships between this environmental parameter, the fishing yield and the bait mortality rate.

The experimental fishing operations were carried out using the fishing boats of the professional fishermen from Culatra Island involved in this study (each month, a fisherman was randomly selected to perform the fishing surveys, accompanied by the scientific staff of the IPIMAR-CRIPSul). The experimental fisheries were undertaken in locations chosen by the fishermen, usually on muddy-bottoms with seagrasses (namely *Zostera* spp.) in the vicinities of Culatra Island (the geographical coordinates of the fishing grounds were recorded using a portable GPS) (Figure 1). During a one-year study period, a total of 12 monthly fishing surveys were carried out between July 2005 and June 2006. Overall, 36 fishing days (12 months x 3 fishing-days) and 216 fishing operations (36 fishing-days x 6 fishing-gears) were performed. In the three consecutive fishing days, the average soaking time of the gear was 23:35 ± 01:10 hours (ranging from 20:24 to 26:36 hours of daily immersion of the fishing gear, data used for the calculation of standardised CPUE).

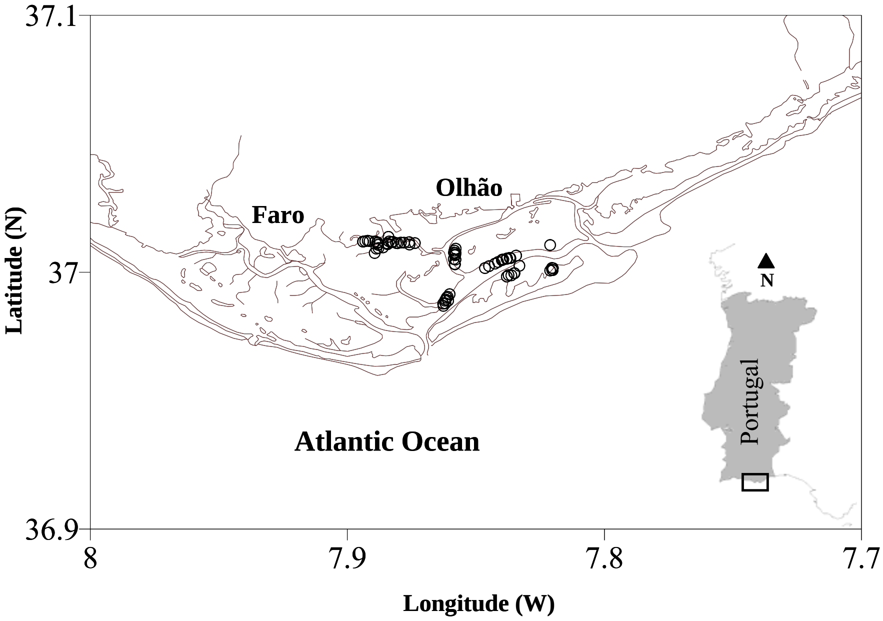


Figure 1. Geographical location of the experimental fishing surveys with the "wallet-lines" (circles) carried out in the Ria Formosa lagoon (Algarve coast - southern Portugal).

RESULTS

Fishing gear and fishing operations

The artisanal fishing gear locally designated by "wallet-line" is basically composed of a long main line with numerous gangions, where small square bags made of rigid plastic mesh are attached. The main characteristics and technical specifications (dimensions and materials) of the five "wallet-lines" that were apprehended in the Ria Formosa lagoon by the fishery authorities are compiled in Table 1 and the respective technical drawing containing the most relevant characteristics of the fishing gear is illustrated in Figure 2.

Through the detailed inspection and measurement of the "wallet-lines" it was possible to observe that the artisanal fishing gears were slightly variable in terms of materials and dimensions (Table 1). With the exception of a "wallet-line" partially built with natural materials (gangions of sisal), the remaining fishing gears were constructed with synthetic materials (main lines of interlaced polyamide - PA and gangions of twisted polyethylene -

PE). In the main line, the gangions are assembled to avoid entanglements among adjacent "wallets" and therefore were separated between each other by approximately 1.8 m (around one roughly measured fathom) and measured an average of 63.8 ± 19.4 cm in length. Each "wallet-line" comprised an average of 85 ± 14 "wallets" and this variability in the number of "wallets" per fishing gear (range: 76 - 109) is mainly due to the loss of some gangions or "wallets" during the fishing operations (detected through the atypical and excessive spacing in the main line between consecutive gangions). The measurement of half of the "wallets" in each fishing gear (in a total of 215 "wallets") showed that these roughly square bags measured an average of 13.5 ± 1.1 cm in length and 11.8 ± 0.7 cm in width (with a square mesh size of around 1 cm in diagonal) (Table 1). The "wallet-line" is set in the bottom of relatively shallow (2-3 m depth) channels of the lagoon, with a weight (usually a stone of around 1 kg) and a floating device (generally a small buoy made of cork or plastic) in each end of the fishing gear.

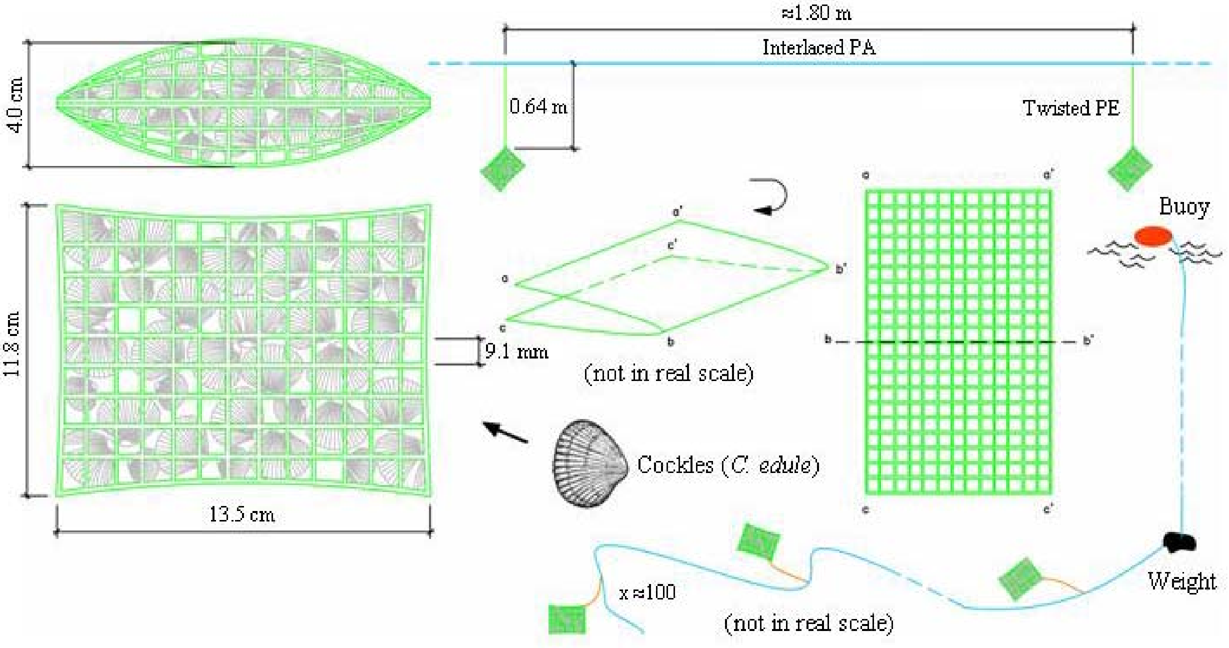


Figure 2. Technical drawing with the most relevant characteristics of a standard "wallet-line" used in the fishery for *Hexaplex (Trunculariopsis) trunculus* and *Bolinus brandaris* in the Ria Formosa lagoon [design modified from Carneiro *et al.* (2006) with the measurements made in the present study)].

Table 1. Main characteristics and technical specifications (dimensions and materials) of the artisanal fishing gear ("wallet-line") used in the fishery for *Hexaplex (Trunculariopis) trunculus* and *Bolinus brandaris* in the Ria Formosa lagoon (Algarve coast - southern Portugal).

	Main lines		**Gangions**		**"Wallets"**			**"Wallet" fastening cables**		**"Wallet" mesh sizes**		
	length* (m)	width (mm)	length (cm)	width (mm)	number	length (cm)	width (cm)	length (cm)	width (mm)	length (mm)	width (mm)	diagonal (mm)
Mean ± S.D.	≈ 1.8	4.0 ± 0.7	63.8 ± 19.4	1.5 ± 0.2	85 ± 14	13.5 ± 1.1	11.8 ± 0.7	27.4 ± 5.2	1.4 ± 0.3	9.1 ± 0.3	9.1 ± 0.5	11.0 ± 0.4
(min - max)	(≈ 1 fathom)	(2.1 - 5.0)	(26.5 - 124.0)	(1.1 - 2.6)	(76 - 109)	(11.5 - 16.5)	(9.0 - 14.0)	(11.0 - 44.0)	(1.0 - 2.6)	(8.4 - 10.3)	(8.2 - 10.4)	(10.3 - 12.1)
Material	Polyamide (interlaced PA)		Polyethylene (twisted PE)		Rigid plastic (square mesh)			Polyethylene (twisted PE)		-		

* - Approximate distance (spacing) between consecutive gangions (and "wallets") in the main lines.

At the beginning of every fishing survey, these “wallets” are filled with live cockles (*C. edule*), generally commercially under-sized individuals caught by the fishermen. Several predator and scavenger species are attracted to the bait and attach to the external surfaces of the “wallets” while handling, attacking or ingesting the bait, including the target species of this fishery, the banded murex (*T. trunculus*) and the purple dye murex (*B. brandaris*) (Figure 3). Subsequently, fishermen periodically retrieve the fishing gears (at intervals of approximately 24 hours), remove the target species, discard the by-catch species and simultaneously redeploy again the “wallet-lines” on the fishing ground (during three to five consecutive days, depending on the bait efficiency and resultant fishing yield).

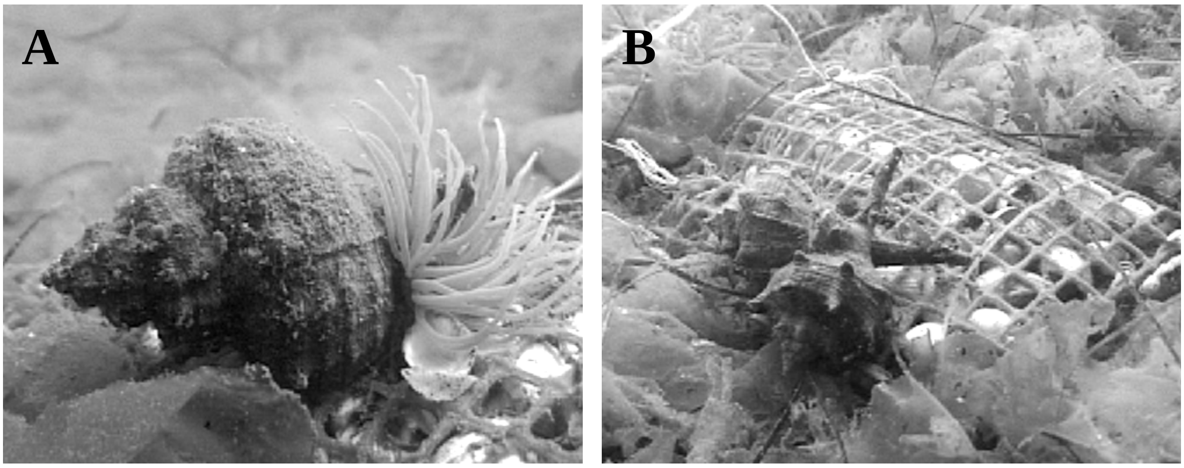


Figure 3. Under-water photographs of the target species attached to the external surfaces of the “wallets” baited with cockles (*Cerastoderma edule*): **a)** *Hexaplex (Trunculariopsis) trunculus*. **b)** *Bolinus brandaris*.

The duration of the experimental fishing operations (time needed for setting the gear, retrieving the catches, hauling the gear and retrieving the catches of a “wallet-line” with 100 “wallets”) during the three consecutive fishing days in the Ria Formosa lagoon are presented in Figure 4. The main feature of this kind of fishing operations is the rapid handling of the artisanal fishing gear. Actually, fishermen took an average of three minutes for setting the gear (day 0) and six minutes both for retrieving the catches (days 1 and 2) and retrieving the catches and hauling the "wallet-line" (day 3). Nevertheless, a high variability was observed in the duration of these fishing operations (between 2 and 20 minutes), because the handling depends on the amount of target-species caught and by-catch species discarded. Furthermore, under adverse weather conditions (particularly strong winds that difficult to manoeuvre the boat), fishermen took as much as 20 minutes both for setting and hauling the "wallet-line" (Figure 4).

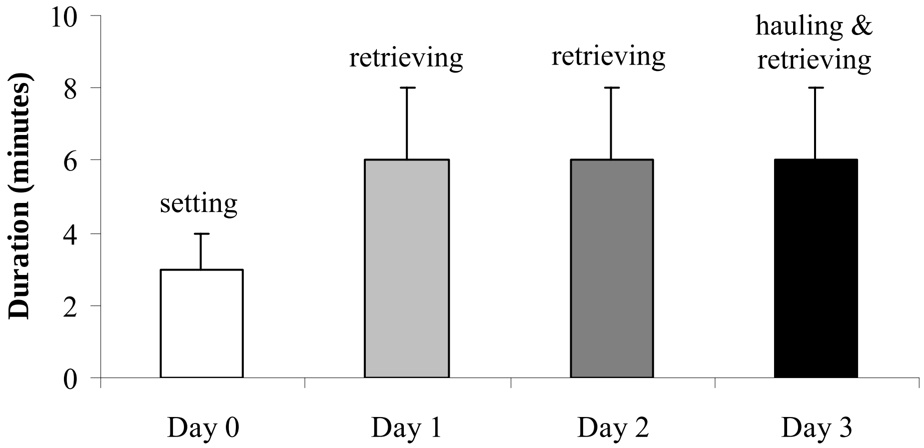


Figure 4. Average duration of the fishing operations (setting the gear, retrieving the catches, hauling the gear and retrieving the catches) undertaken with the "wallet-lines" in the experimental fishing surveys in the Ria Formosa lagoon. Data standardised for one fishing gear with 100 "wallets".

The relationship between the bait mortality (percentage of dead cockles at the end of the third fishing day) and the surface seawater temperature in the Ria Formosa lagoon during the experimental fishing surveys with the "wallet-lines" is illustrated in Figure 5. With few exceptions, a close relationship between seawater temperature and the bait mortality rate was detected, with increasing temperature leading to higher cockle mortality inside the "wallets". Indeed, bait mortality was lower during autumn and winter (namely between October 2005 and March 2006), markedly increasing during spring and summer (reaching the highest values in August and September 2005) (Figure 5).

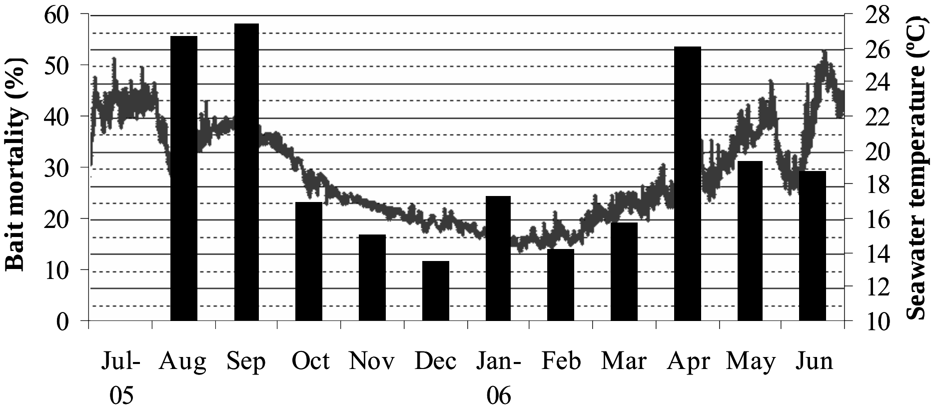


Figure 5. Relationship between bait mortality (percentage of dead cockles in the "wallet-line" at the end of three fishing days) and surface seawater temperature in the Ria Formosa lagoon during the experimental fishing surveys.

Catches of target and by-catch species

The main descriptive statistics of the catches of target species (banded murex and purple dye murex) in the experimental fishing surveys carried out with the "wallet-lines" in the Ria Formosa lagoon are summarised in Table 2. Overall, 1620 specimens of *T. trunculus* and 3306 specimens of *B. brandaris* were caught, corresponding to total catches of 19.74 kg and 31.24 kg, respectively. The specimens of both target species presented a broad size (*T. trunculus*: 9.10 - 77.14 mm SL; *B. brandaris*: 15.73 - 88.96 mm SL) and weight ranges (*T. trunculus*: 0.06 - 40.41 g TW; *B. brandaris*: 0.17 - 36.09 g TW) (Table 2).

Table 2. Descriptive statistics (mean ± standard deviation, range) of the catches of target species, *Hexaplex (Trunculariopis) trunculus* and *Bolinus brandaris*, in the experimental fishing surveys with the "wallet-line" in the Ria Formosa lagoon (Algarve coast - southern Portugal).

	Hexaplex trunculus	***Bolinus brandaris***
Number	1620	3306
Shell length (mm)	49.94 ± 10.15 (9.10 - 77.14)	53.82 ± 11.47 (15.73 - 88.96)
Total weight (g)	12.19 ± 6.67 (0.06 - 40.41)	9.45 ± 6.10 (0.17 - 36.09)
Total catch (kg)	19.74	31.24

Table 3. Abundance of by-catch species caught in the experimental fishing surveys using the "wallet-line" in the Ria Formosa lagoon (Algarve coast - southern Portugal).

Taxa	**No.**	*Taxa*	**No.**
Phylum Cnidaria		**Class Polyplacophora**	
Class Anthozoa		**Family Ischnochitonidae**	
Anthozoa n.d.	10	*Acanthochitona* sp.	4
Subclass Opistobranchia		*Chaetopleura angulata* (Spengler, 1797)	7
Order Nudibranchia		**Phylum Sipuncula**	
Nudibranchia n.d.	10	**Class Sipunculidae**	
Phylum Mollusca		Sipunculida n.d.	1
Class Gastropoda		**Phylum Arthropoda**	
Family Cerithiidae		**Class Thecostraca**	
Bittium reticulatum (Linnaeus, 1778)	322	**Superorder Eucarida**	
Cerithium vulgatum Bruguière, 1792	7	**Order Decapoda**	
Family Columbellidae		**Family Diogenidae**	
Columbella rustica (Linnaeus, 1758)	4	*Clibanarius erythropus* (Latreille, 1818)	3
Family Hydrobiidae		*Diogenes pugilator* (Roux, 1829)	9
Hydrobia ulvae Pennant, 1777	8	**Family Leucosiidae**	
Family Muricidae		*Ebalia edwardsii* (Costa, 1838)	1
Nassarius (Telasco) cuvieri (Payraudeau, 1826)	249	**Family Paguridae**	
Nassarius (Gussonea) pfeifferi (Philippi, 1844)	1	*Pagurus* sp.	2
Nassarius (Gussonea) corniculus (Olivi, 1792)	2	**Family Pilumnidae**	
Nassarius (Hima) incrassatus (Stroem,1768)	88	*Pilumnus hirtellus* (Linnaeus, 1761)	1
Nassarius (Hinia) reticulatus (Linnaeus, 1758)	391	**Family Porcellanidae**	
Nassarius spp.	60	*Pisidia longicornis* (Linnaeus, 1767)	1
Ocenebra erinaceus (Linnaeus, 1758)	3	**Family Portunidae**	
Ocinebrina aciculata (Lamarck, 1822)	32	*Carcinus maenas* (Linnaeus, 1758)	3
Cyclope (Cyclope) neritea (Linnaeus, 1758)	17	*Liocarcinus arcuatus* (Leach, 1814)	6
Family Naticidae		**Family Xanthidae**	
Naticidae n.d.	63	*Xantho incisus* (Leach, 1814)	1
Family Rissoidae		**Superclass Pycnogonida**	
Rissoidae n.d.	48	Pycnogonida n.d.	1
Family Trochidae		**Phylum Echinodermata**	
Gibbula spp.	6	**Order Asteroidea**	
Gibbula pennanti (Philippi, 1846)	8	**Family Asteriidae**	
Gibbula umbilicalis (da Costa, 1778)	9	*Coscinasterias tenuispina* (Lamarck, 1816)	23
Gibbula umbilicaris (Linnaeus, 1758)	268	**Family Asterinidae**	
Jujubinus striatus (Linnaeus, 1758)	183	*Asterina gibbosa* (Pennant, 1777)	120
Family Turritellidae		**Order Echinoidea**	
Mesalia brevialis (Lamarck, 1822)	3	**Family Echinidae**	
		Paracentrotus lividus (Lamarck, 1816)	3
		Total	**1978**

The taxonomic identification and abundance of the by-catch species caught in the experimental fishing surveys undertaken with the "wallet-line" in the Ria Formosa lagoon are compiled in Table 3. Overall, 1978 by-catch specimens belonging to 39 *taxa* were caught, with gastropods being clearly dominant (89.6% of the by-catch) particularly due to *Nassarius (Hinia) reticulatus* (n = 391), *Bittium reticulatum* (n = 322), *Gibbula umbilicaris* (n = 268), *Nassarius (Telasco) cuvieri* (n = 249) and *Jujubinus striatus* (n = 183). Several specimens from other taxonomic groups (Phyla Cnidaria, Sipuncula, Arthropoda and Echinodermata) were also caught, with echinoderms being the most abundant group (7.4% of the by-catch) mainly due to the sea stars *Asterina gibbosa* (n = 120) and *Coscinasterias tenuispina* (n = 23) (Table 3).

The comparison between the overall number of target specimens (*T. trunculus* and *B. brandaris*) and by-catch specimens caught by the "wallet-lines" during the experimental fishing surveys revealed that the target species corresponded to 71.3% of the total catches in number (unequally distributed between *T. trunculus* - 23.4% and *B. brandaris* - 47.9%), with by-catch species accounting for only 28.7% of the total catches in number.

The monthly variation of the target species CPUE (total weight/ 100 "wallets"/ 24 hours) and by-catch species CPUE (total number/ 100 "wallets"/ 24 hours) during the experimental fishing surveys with the "wallet-line" in the Ria Formosa lagoon is presented in Figure 6. The higher CPUE's in weight of *T. trunculus* occurred in September and November 2005, January and May 2006, whereas the higher CPUE's in weight of *B. brandaris* were registered in September and October 2005, between February and April 2006, and in June 2006. The average CPUE of *T. trunculus* was 105.70 ± 134.85 g (maximum of 821.10 g) and of *B. brandaris* was 166.71 ± 200.18 (maximum of 836.44 g). Except in November 2005, January and May 2006, the CPUE's in weight of *B. brandaris* were larger than for *T. trunculus*. The most significant CPUE's in number of by-catch specimens were observed in January, April and May 2006. Overall, the average CPUE's of both target and by-catch species were highly variable during the study period (even between consecutive monthly fishing surveys), without a discernible seasonal pattern (Figure 6).

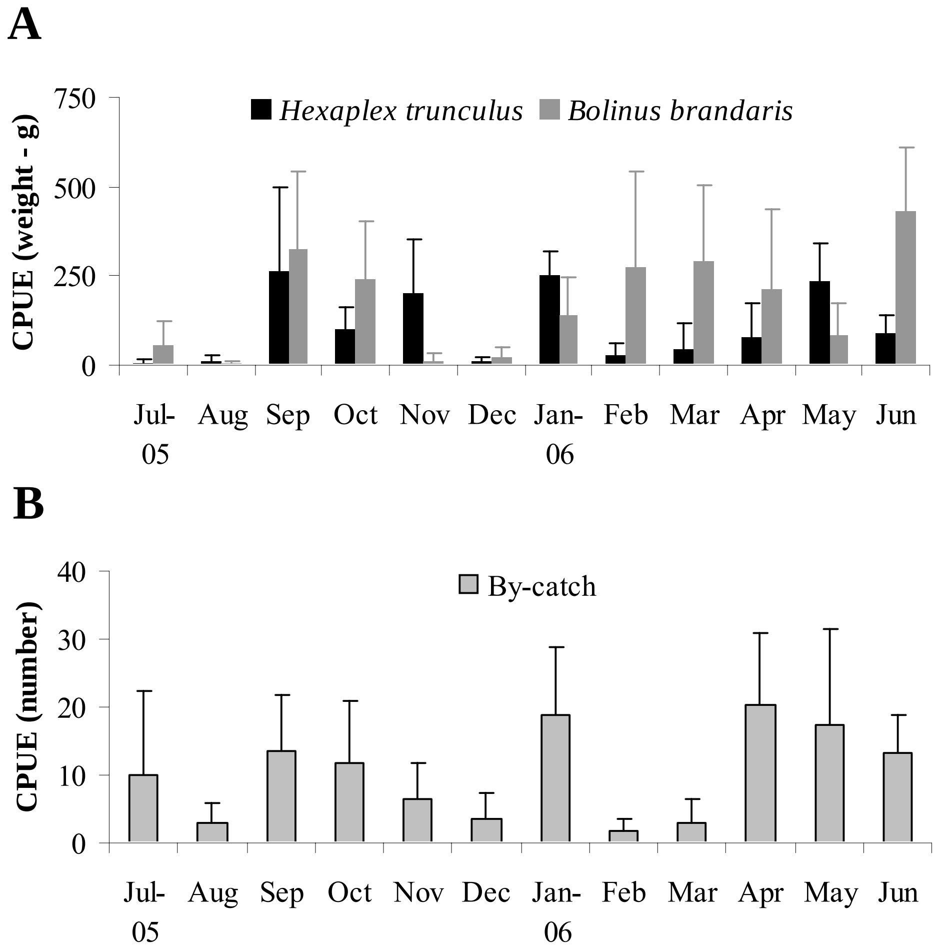


Figure 6. Monthly variation of the catch per unit effort (CPUE - weight or number / 100 "wallets" / 24 hours) with the "wallet-lines" during the experimental fishing surveys undertaken in the Ria Formosa lagoon: **a)** Target species - *Hexaplex (Trunculariopsis) trunculus* and *Bolinus brandaris*. **b)** By-catch species.

Variation in the average CPUE of target species (total weight/100 "wallets"/24 hours) and by-catch species (total number/100 "wallets"/24 hours) during three consecutive fishing days with the "wallet-line" in the Ria Formosa lagoon is illustrated in Figure 7. Independently of the fishing day, the CPUE in weight of *T. trunculus* was invariably lower than that of *B. brandaris*. Between consecutive fishing days, a marked decreasing trend was observed in the CPUE of *T. trunculus* and *B. brandaris* (CPUE in weight), as well as on the by-catch species (CPUE in number) (Figure 7).

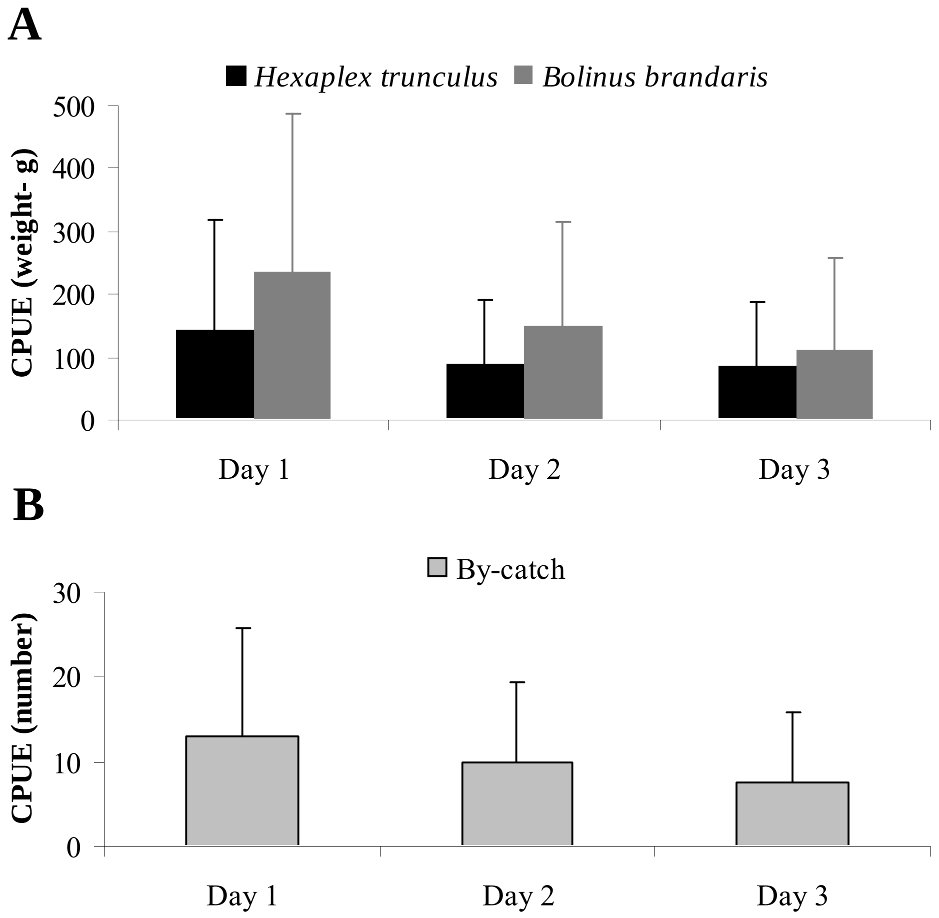


Figure 7. Variation of the average catches per unit effort (CPUE - weight or number / 100 "wallets" / 24 hours) during three consecutive fishing days with the "wallet-lines" in the Ria Formosa lagoon: **a)** Target species - *Hexaplex (Trunculariopsis) trunculus* and *Bolinus brandaris*. **b)** By-catch species.

The size-frequency distribution of the catches of the target species (*T. trunculus* and *B. brandaris*) and the proportion of individuals below and above the MLS stipulated for these species (*T. trunculus* = 50 mm SL and *B. brandaris* = 65 mm SL) is illustrated in Figure 8. During the overall fishing surveys, the "wallet-lines" caught specimens of both target species with a broad size range and with significant proportions of commercially under-sized individuals relatively to the MLS, namely 47.2% of *T. trunculus* smaller than 50 mm SL and 81.9% of *B. brandaris* smaller than 65 mm SL (Figure 8).

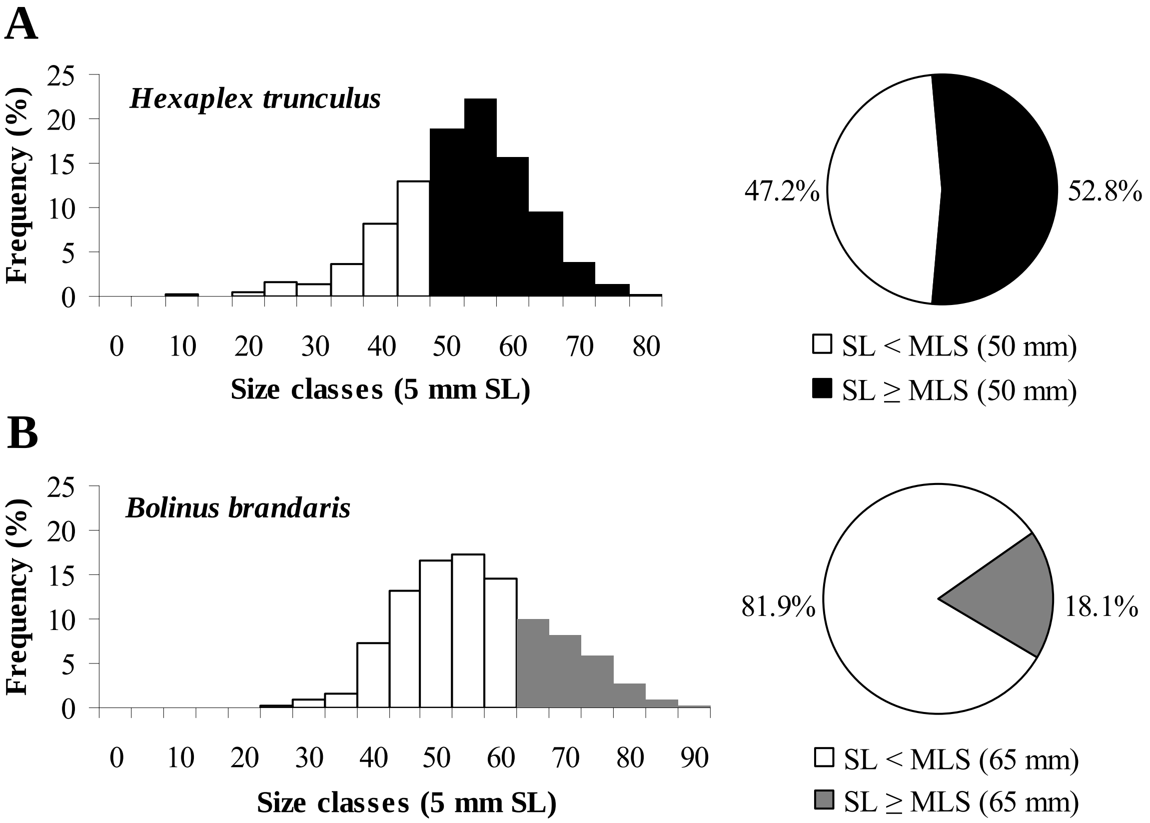


Figure 8. Size-frequency distribution of the catches of target species and respective proportion of individuals below and above the minimum landing size (MLS) of the species: **a)** *Hexaplex (Trunculariopsis) trunculus* (MLS = 50 mm SL). **b)** *Bolinus brandaris* (MLS = 65 mm SL).

DISCUSSION

Fishing gear and fishing operations

The "wallet-lines" are typical artisanal fishing gears, characterised by a high variability in their overall dimensions and size of specific fishing gear components (main lines, gangions and "wallets"), as well as by the usage of diversified materials in their construction. In fact, sometimes the "wallet-lines" are constructed with remains of other fishing gear (namely cables for assembling main lines and gangions, and rigid plastic mesh for constructing "wallets"). As confirmed through the measurement of five fishing gears apprehended by the fishery authorities in the Ria Formosa lagoon, each "wallet-line" is originally constructed to accommodate approximately 100 “wallets”, which is considered a reasonable number to facilitate the handling on-board and minimising the economical losses when a fishing gear is destroyed, apprehended or stolen (obliging for the repair or manufacture of new fishing gears).

Local fishermen generally use simultaneously three or four “wallet-lines” (Carneiro *et al.*, 2006), but some professional fishermen can operate up to ten fishing gears (Marques and Oliveira, 1995) in an almost permanent fishery (except during the period necessary for baiting the “wallets” with cockles), as confirmed by the inquiries made to fishermen from the Culatra Island involved in this fishing activity. Usually, this illegal fishing activity is a complement to various legal fisheries (*e.g.*, nets, long-lines, traps, etc.) and therefore the rapid handling of the “wallet-lines” is highly advantageous, allowing for the possibility of setting the gear, retrieving the catches and/or hauling the gear in the intervals available from other fishing activities.

The inquiry made to fishermen involved in the present experimental fishing surveys revealed that professional fishermen generally operate the “wallet-lines” during three consecutive days (with a soaking time of approximately 24 hours), exceptionally reaching five consecutive fishing days when the bait survival and attraction allows for an extraordinarily high and stable fishing yield. Usually, the “wallets” are baited with commercially under-sized cockles (*C. edule*) caught by the own fishermen, because they are easier to introduce and accommodate inside the “wallets”, and also due to their lower commercial value (Marques and Oliveira, 1995). In the present study, a close relationship between seawater temperature and cockle mortality was detected, with lower mortality rates during autumn and winter and with increasing temperature leading to higher mortality rates during spring and summer. The few exceptions to this general trend could be eventually related with particular features of the bottom sediments (*e.g.*, excessively muddy sediments that might obstruct the bivalve gills) or lower condition indices of the cockles (that might reduce their resistance to higher seawater temperatures and consequent lower levels of dissolved oxygen).

The muricid gastropods (*T. trunculus* and *B. brandaris*) targeted in this artisanal fishery are very active predators and generalist species. The banded murex is a non-selective carnivore that feeds on virtually any available food item (Rilov *et al.*, 2004), since its broad diet (both in the natural habitat and in the laboratory) includes various bivalves, gastropods, hermit crabs, barnacles, tunicates, bryozoans, tube and vermetid worms, and other types of prey (Fainzilber, 1978; Spanier, 1981; Lorenzini and Orlando, 1997; Muzavor and Morenito, 1999; Anon., 2001; Alyakrinskaya, 2002; Rilov *et al.*, 2004; Marin and López Belluga, 2005; Mejri *et al.*, 2005; Peharda and Morton, 2006). Additionally, *T. trunculus* supplements its diet by scavenging (mainly on dead fish) (Spanier, 1981, 1986; Anon., 2001; Rilov *et al.*, 2004; Mejri *et al.*, 2005) and cannibalism by drilling the shells of conspecifics (particularly under artificial conditions, when kept for a long period without an alternative food supply) (Spanier, 1981, 1986; Basedow, 1994, 1996; Rilov *et al.*, 2004; Mejri *et al.*, 2005). However, the most relevant feature of *T. trunculus* feeding behaviour is the remarkable ability to attack live bivalves, either by holding, handling and opening their valves by the mechanical action of the foot and the toothed labral lip of the shell, or by drilling their shells by the mechanical/chemical action of the radula and the accessory boring organ (ABO) (Spanier and Karmon, 1987; Lorenzini and Orlando, 1997; Alyakrinskaya, 2002; Peharda and Morton, 2006). The information available on the feeding behaviour and diet of *B. brandaris* is scarcer and more generic. The purple dye murex is also carnivorous and feeds mainly on bivalves (Anon., 2001; Ramón and Amor, 2001; Ramón and Flos, 2001; Mejhri *et al.*, 2005), other gastropods (Ramón and Amor, 2001), limpets and barnacles (Anon., 2001), and also supplements its diet through scavenging and cannibalism (Mejhri *et al.*, 2005). The non-selective carnivorous feeding habits of both species were probably known in ancient times, because historical sources, such as Aristotle and Pliny, described a method of catching muricid gastropods (namely *T. trunculus*, *B. brandaris*) using baited wicker baskets for subsequent extraction of the purple dye by the ancient dye industries (Spanier and Karmon, 1987). Similarly, the artisanal fishing gear ("wallet-line") baited with cockles (*C. edule*) used in the fishery for the banded murex and the purple dye murex in the Ria Formosa lagoon, reflects the empiric knowledge of local fishermen on the drilling behaviour of these muricid gastropods, particularly over bivalve species.

Catches of target and by-catch species

In the whole experimental fishing surveys 1620 specimens of banded murex (*T. trunculus*) and 3306 specimens of purple dye murex (*B. brandaris*) were caught. This disproportion in the catches of target species (more than double the number of *T. trunculus* compared to *B. brandaris*) was rather unexpected and might possibly reflect the intense fishing effort directed towards the banded murex, which has been gradually driven to lower abundances and catches of this species in the Ria Formosa lagoon (Marques and Oliveira, 1995). The information obtained previously during enquiries made to professional fishermen involved in this activity drew attention to the potential occurrence of high inter-annual variability in the abundance of banded murex and purple dye murex in the Ria Formosa. However, since no reliable official statistics are available on the catches of both species, it is virtually impossible to ascertain if during the study period abnormally low catches of *T. trunculus* (or unusually high catches of *B. brandaris*) occurred in the Ria Formosa lagoon.

Although the target species (*T. trunculus* and *B. brandaris*) represented the vast majority of total catches (71.3% of the catches in number: 23.4% of *T. trunculus* and 47.9% of *B. brandaris*), by-catch species accounted for 28.7% of total catches in number. Just for comparison, in hauls made with dredging gear (”rastell”) specially designed to target purple dye murex off the Catalan coast (Spain) and considered an efficient fishing gear, *B. brandaris* constituted 21% of the total catch and 73% of the commercial catch (Martín *et al.*, 1995). In the present experimental fishing surveys, among the high variety of organisms caught (39 *taxa*), the by-catch was largely dominated by gastropods (89.6%), followed by echinoderms (7.4%), revealing that the “wallet-line” is not a species-specific fishing gear since besides *T. trunculus* and *B. brandaris*, the bait (cockles) has a broad spectrum of attraction to many other predator and scavenger species. Fortunately, in the commercial fishery the vast majority of by-catch species are discarded on-board (alive and frequently undamaged) during the retrieving of the catches and hauling of the fishing-gear, and therefore the mortality rate of these discards is presumably negligible. The only exception occurs with very small specimens that penetrate through the “wallet” mesh (mainly gastropods), whenever after hauling the gear in the last fishing day the remaining bait in the “wallets” is not removed on-board and discarded into the lagoon. Nevertheless, compared with the amount of by-catch specimens discarded on-board, the proportion of by-catch specimens enclosed inside the “wallets” at the end of the fishing operations is insignificant (<1% of the overall by-catch, as detected in the “wallets” examined to estimate the bait mortality rate).

During the whole experimental fishing surveys with the “wallet-lines”, the average CPUE of both target species (*T. trunculus* and *B. brandaris*) and by-catch species presented a high monthly variation (even between consecutive monthly fishing surveys), without any evident seasonal pattern and apparently lacking any discernible relationship with the seawater temperature in the Ria Formosa lagoon or particular features of the biological cycle of the species (*e.g.*, reproduction and spawning). Firstly, the normal activity and behaviour of banded murex are apparently affected by the seawater temperature, since field and laboratory studies indicate that *T. trunculus* burrows shallowly into soft sediments (with siphon tips in contact with the water), trying to avoid both the unfavourable low water temperatures (hibernation) and high water temperatures (aestivation) (Spanier, 1981, 1986), being often observed partially buried in the sand (Spanier, 1986; Rilov *et al.*, 2004). However, in the present experimental fishing surveys, although some of the lowest CPUE's of *T. trunculus* occurred both in winter (*e.g.*, December 2005 and February 2006) and summer months (*e.g.*, July and August 2005), it was impossible to identify a clear trend of decreasing catches during the coldest and warmest seasons. Similarly, the lowest CPUE's of *B. brandaris* obtained in the present experimental fishing surveys were registered in July, August, November and December 2005, but also without an evident relationship between the catches and the seawater temperature in the Ria Formosa lagoon. By contrast, in the fishery off the Catalan coast (Spain), *B. brandaris* catches showed a pronounced seasonality (a characteristic shared with other fishing resources exploited in the Mediterranean Sea), being lowest in summer (June-July) and highest in late autumn and winter, which was attributed to changes in the species abundance throughout the year (Martín *et al.*, 1995).

Additionally, both under natural and laboratory conditions, the banded murex reduces its normal activity during the reproductive season and *T. trunculus* females even interrupt feeding during the spawning period (Dulzetto, 1946, 1950; Vasconcelos *et al.*, 2004a), therefore minimizing their attraction to baited fishing gears (such as the "wallet-line") and contributing to the occurrence of male-biased sex-ratios in the catches (Vasconcelos *et al.*, submitted). However, during the present experimental fishing surveys, unpredictably not evident trend for decreasing catches during the spawning periods of the banded murex and the purple dye murex were detected. Actually, in the Ria Formosa lagoon *T. trunculus* has a spawning peak in May-June and minor and partial spawning events in October-November, in agreement with the spawning season reported for Mediterranean populations of this species, which generally occurs in late spring - early summer (more frequently between May and July)

(Vasconcelos *et al.*, submitted). Also in the Ria Formosa lagoon, observations in the field revealed the simultaneous deposition of collective spawns in *T. trunculus* and *B. brandaris*, sometimes with females of both species depositing egg-capsules in the same spawn (P. Vasconcelos, personal observation). In several locations in the Mediterranean Sea, *B. brandaris* presented a lengthy spawning season (Martín et al., 1995), with the main spawning periods occurring mainly in spring (Bartolome, 1985) and early summer, namely in May-June (Anon., 2001; Ramón and Flos, 2001), May-July (Barash and Zenziper, 1980; Tirado *et al.*, 2002), June-July (Ramón and Amor, 2002) or August (Martín et al., 1995).

Finally, it is worth emphasising that the variability in the catches of the banded murex and the purple dye murex might be hypothetically due to an apparent patchy distribution of these species in the Ria Formosa lagoon (evidenced by the frequent occurrence of very dissimilar fishing yields between adjacent “wallet-lines”). Similarly, in the purple dye murex fishery off the Catalan coast (Spain), the lack of significant correlations between the duration of the hauls and the hourly fishing yields suggested that the distribution of *B. brandaris* is not homogeneous in that fishing areas of the north-western Mediterranean (Martín *et al.*, 1995). Furthermore, both field and laboratory observations detected the frequent occurrence of group foraging in *T. trunculus* (Peharda and Morton, 2006).

An evident decreasing tendency was detected in the CPUE of both banded murex and purple dye murex and of the by-catch species during the three consecutive fishing days. Since *T. trunculus* and *B. brandaris* are relatively slow moving gastropods, decreasing CPUE might indicate a temporary local depletion of these target species in the vicinities of the fishing gear, worsened by the declining bait attraction due to the progressive cockle deterioration and mortality during the three fishing days. Furthermore, the decreasing fishing yields of both target species in the experimental fishing surveys explains why local professional fishermen generally operate the “wallet-lines” during three consecutive fishing days (only exceptionally reaching a maximum of five consecutive fishing days during winter, because of the lower seawater temperatures) and then they haul the fishing gears, remove the previous bait and refill the “wallets” with recently caught cockles (*C. edule*) for further fishing operations.

The size-frequency distribution of the catches of both target species revealed that the “wallet-lines” caught significant proportions of commercially under-sized individuals relative to the minimum landing sizes (MLS) legally stipulated for these species (*T. trunculus*: 47.2% < 50 mm SL and *B. brandaris*: 81.9% < 65 mm SL). This information reveals that, besides not being species-specific, the “wallet-line” is also not a size-selective fishing-gear and therefore the size composition of the catches depends exclusively on the attainment of the MLS by the fishermen (through sorting of the catches and discarding the target species below the legal commercial size). Fortunately, during the fishing operations, professional fishermen generally sort on-board (select by size) the target species and roughly discard the majority of commercially under-sized *T. trunculus* and *B. brandaris* (which are immediately returned to the lagoon). Nevertheless, usually the MLS is not strictly adhered to (principally in the case of *B. brandaris*, the species with the highest MLS) and in addition fishermen sometimes retain some smaller individuals of both target species for their own consumption. Just for comparative purposes, in almost 2500 *T. trunculus* specimens from the Ria Formosa lagoon bought in the local seafood market for routine biological sampling, 37.8% of the individuals were below the MLS (50 mm SL), but less than 5% were smaller than 40 mm SL and less than 0.5% were inferior to 30 mm SL (Vasconcelos *et al.*, 2004b). In the overall catches of *B. brandaris* with the “wallet-lines”, specimen size ranged between 15.73 and 88.96 mm SL and the size-frequency distribution of the whole catches revealed 39.8% of the individuals smaller than 50 mm SL and 10.0% inferior to 40 mm SL. Similarly, in the fishery for the purple dye murex off the Catalan coast (Spain), in the total catches specimen size was between 16 and 90 mm SL, with 38.7% of the specimens ranging between 46 and 54 mm SL. In the culled catch (the marketed fraction of the whole catch) specimen size ranged between 28 and 90 mm SL, 60.3% of the individuals were included in the interval 46 - 54 mm SL and small-sized *B. brandaris* (< 42 mm SL) were practically absent (Martín *et al.*, 1995). Accordingly, commercial sizes of *B. brandaris* from the Catalan coast (Spain) ranging from 50 to 90 mm SL were reported by Ramón and Flos (2001).

Fishery management measures

Fishing activity with the "wallet-lines" targeting the banded murex (*T. trunculus*) and the purple dye murex (*B. brandaris*) is a complementary fishery, since fishermen hold other fishing licenses (nets, long-lines, traps, etc.) and generally only use this illegal fishing gear during particular periods of the year as an alternative and secondary source of income. Furthermore, because the fishery with "wallet-lines" is carried out inside the Ria Formosa (frequently in inner and sheltered channels of the lagoon), it is not greatly affected by adverse weather conditions, therefore allowing for many fishing days per year compared to other fishing metiers. These gastropod species are sold in local seafood markets and consumed by the resident population and tourists in restaurants and seafood festivals, with a noticeable increasing demand during the summer season (which obviously increases the market price). Recently, the banded murex reached prices of approximately 10-15 €/kg and the purple dye murex reached prices around 20 €/kg (both for first sale), confirming that even daily catches of a few kilograms of *T. trunculus* and *B. brandaris* represent an interesting profit for fishermen.

The baseline information gathered in this study (complemented by other works of relevant aspects on the target species biology, namely growth and the reproductive cycle), allows for the proposal to the fisheries administration of several management measures to be implemented in this traditional fishing activity, which could support the legalisation of this artisanal fishery in the near future. If effectively implemented, these specific management measures might ultimately contribute to the long-term sustainable exploitation of this locally important fishing resource.

Firstly, the fishing licenses (provisional and renewable every year) should only be given to boats already licensed to operate with other fishing gears in the Ria Formosa lagoon and preferentially to fishermen that can attest to previous sales of *T. trunculus* or *B. brandaris* in the past. The annual renewal of the fishing license for operating the “wallet-line” should be conditional on the accomplishment by fishermen of a minimum number of yearly sales or minimum amounts sold per year of *T. trunculus* and/or *B. brandaris* in wholesale auction. Fishermen should report relevant data on this fishing activity (*e.g.*, areas, effort, lost fishing gears, catches, value, etc.) during at least five years using confidential log-books specifically created for this purpose, allowing for the development of a data base on this gastropod fishery. In particular, it should be stipulated that failure to regularly deliver log-book information or report incorrect data would imply the loss of the fishing license.

Additionally, to control fishing effort and to prevent over-development of the fishery before sufficient knowledge is gathered to permit effective conservation, the number of fishing licenses should be restricted, the number and size of fishing gears per boat should be limited to a maximum of 10 “wallet-lines” (the highest number operated by some fishermen), each one limited to a maximum of 100 “wallets”. In the present experimental fishing surveys with the “wallet-lines”, the fishing yield of both target species was extremely variable and the average CPUE (standardised for 100 “wallets”/24 hours) was roughly 100 g of *T. trunculus* and slightly higher than 150 g of *B. brandaris*. These yields imply that operating with 1000 “wallets” corresponds to an average daily catch of around 1 kg of banded murex and approximately 1.5 kg of purple dye murex. However, these numbers refer to the fishing yield of the total catches, *i.e.*, including specimens of the target species below the MSL (*T.*

trunculus = 47.2% < MLS and *B. brandaris* = 81.9% < MLS). By discarding the commercially under-sized specimens of both target species, the culled catches in number (commercial fraction of the catches) are reduced to 52.8% of *T. trunculus* individuals and 18.1% of *B. brandaris* individuals, implying a consequent reduction in weight to 73.6% and 37.7% of the original and unsorted total catches of *T. trunculus* and *B. brandaris*, respectively. Considering the commercial value of these gastropod species in the local seafood market (*T. trunculus* = 10-15 €/kg and *B. brandaris* = 20 €/kg, both for first sale), these culled catches correspond to an income of around 20 Euros per fishing day (excluding expenses with boat and engine maintenance, insurance, licenses, fuel costs, fishing gear construction/repair, etc.), which is an acceptable earning for an alternative/complementary fishing activity that can be undertaken almost year-round.

Each fishing gear should be clearly identified with the respective boat registration number and labelled with conspicuous buoys (besides the buoys at each end of the fishing gear, the adoption of an additional float in the middle of the main line could help reduce the number of “wallet-lines” inadvertently cut by boat propellers and lost to the bottom). This limitation in the number of “wallets” per fishing gear and technical improvement of the “wallet-line” could be also beneficial for reducing the environmental impact due to lost fishing gears. Actually, mostly due to conflicts between fishing gears (some of them also illegal, such as the beam trawl) for the exploitation of the same fishing grounds in the Ria Formosa lagoon, the destruction, partial or even total loss of “wallet-lines” is a relatively common phenomenon. Nevertheless, it is worth emphasising that being a static/passive, closed and baited fishing gear, ghost fishing does not occur in lost “wallet-lines” and therefore is not an ecological concern to take into account with this particular type of fishing gear.

Since the “wallet-line” is not size-selective, the rigorous accomplishment of the MLS stipulated for these species (*T. trunculus* = 50 mm SL and *B. brandaris* = 65 mm SL) is crucial for the sustainable exploitation of the fishing resource. By this reason, the degree of compliance with the MLS by professional fishermen should be assessed by regularly monitoring the gastropod catches (sampling and analysing the length frequency distribution of the landings). In addition, since the “wallet-line” is not a species-specific fishing gear, by-catch species should be discarded immediately on-board and the removal of the bait from the “wallets” should be made in the fishing areas (releasing the surviving cockles and minimising the mortality of by-catch specimens that might have penetrated through the “wallet” mesh). Furthermore, the capture of cockles for baiting the “wallet-lines” should only be made with legally authorised fishing gears (namely the hand knife) and the use of commercially under-sized *C. edule* (below the MLS = 25 mm SL) should be avoided (or at least highly restricted).

Due to the apparent patchy distribution of *T. trunculus* and *B. brandaris*, further studies should be conducted to identify preferential distribution areas and ascertain the influence of depth, type of bottom and seagrass coverage (*Zostera* spp.) in the distribution of these species in the Ria Formosa lagoon. To sustain local gastropod populations, exploitation in these areas should be restricted, through the establishment of permanent closed areas or conservation zones (which also contribute to control fishing effort). Additionally, limiting the fishing season through the implementation of seasonal closures coincident with the reproductive and spawning periods of the target species (Vasconcelos *et al.*, submitted) may prove highly beneficial for successful recruitment.

Finally, it is worth emphasising that the success of most of these fishery management measures depends on their effective implementation by the fisheries administration, rigorous inspection of the fishery and control of the landings, as well as a understanding of their importance by the professional fishermen (who should be informed of the vulnerability of these fishing resources through a specific awareness campaign), therefore requiring a proximate and continuous relationship among the staff of fisheries administration, inspection, research and the local fishing community.

ACKNOWLEDGMENTS

The authors would like to thank the Portuguese Directorate-General for Fisheries and Aquaculture (DGPA) for authorising the experimental fishing operations. Special recognition is due to Sílvia Padinha (Associação de Moradores da Ilha da Culatra) for her commitment in involving the fishing community of the Culatra Island in this study, and particularly to the skippers of the fishing boats (Cristiano Mestre Artífice, Jorge Humberto do Carmo, Rui Alberto do Carmo, José Carlos Pacheco, João Luís Quintino, Celestino Patrício dos Santos and José Patrício dos Santos) who voluntarily carried out the fishing surveys. Thanks are also due to Rogélia Martins and Miguel Carneiro (IPIMAR) for making available the technical drawing of the "wallet-line" and to the technical staff of IPIMAR - CRIPSul for their help in the laboratory sampling procedures. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001) and by the Small-Scale Fisheries Program (IPIMAR).

REFERENCES

Alyakrinskaya, I.O., 2002. Morphofunctional properties of nutrition of certain predatory gastropods. *Biology Bulletin*, 29: 589-600.

Anon., 2001. *Especies de Interés Pesquero en el Litoral de Andalucía. Vol. II - Invertebrados*. Junta de Andalucía, Consejería de Agricultura y Pesca, Sevilla.

Bartolome, C., 1985. *Contribution à l'étude du gastéropode Murex brandaris (Linnaeus, 1758) dans le Golfe du Lion*. Diplôme d'Etudes Approfondies de Sciences de l'Eau et Aménagement, Académie de Montpellier, Université des Sciences et Techniques du Languedoc.

Barash, A.; Zenziper, Z., 1980. Egg masses of Mollusca from Mediterranean waters of Israel and notes on reproduction of the freshwater species *Theodoxus jordani* and *Melanoides tuberculata*. *Veliger*, 22: 299-317.

Basedow, T., 1994. Analysis of shells on beaches and rocky coasts of Europe led to new findings on nutritional and population ecology of predatory marine gastropods (Muricidae and Naticidae). *Zoologische Beiträge*, 36: 29-48.

Basedow, T., 1996. Contributions to the nutritional and population ecology of *Murex trunculus* (L. 1767) (Gastropoda, Muricidae), derived from findings at the north coast of Crete (Eastern Mediterranean), with remarks on two species of Naticidae (Gastropoda). *Zoologische Beiträge*, 37: 157-170.

Carneiro, M.; Martins, R.; Rebordão, F.R., 2006. Contribuição para o conhecimento das artes de pesca utilizadas no Algarve. *Publicações Avulsas do IPIMAR*, 13: 76 p.

Dalla Via, G.-J.; Tappeiner, U., 1981. Morphological and functional correlates with distribution of *Murex trunculus* L. and *Murex brandaris* L. (Mollusca, Gastropoda) in the northern Adriatic. *Bollettino di Zoologia*, 48: 191-195.

D.R., 1990. Regulamento da pesca na Ria Formosa. Portaria Nº 560/90 de 19 de Julho. Diário da República, Iª Série, Nº 165: 3010-3012 pp.

Dulzetto, F., 1946. Osservazioni sulla deposizione di *Murex trunculus* L. *Atti della Accademia Nazionale dei Lincei*, 8: 1356-1361.

Dulzetto, F., 1950. Sull'origine e la struttura delle capsule ovifere di *Murex trunculus*. *Archivio Zoologico Italiano*, 35: 83-99.

Fainzilber, M., 1978. An observation on *Trunculariopsis trunculus* feeding on *Conus mediterraneus*. *Levantina*, 16: 182.

Gaillard, J.M., 1987. Gastéropodes. In: *Fiches FAO d'Identification des Espèces pour les Besoins de la Pêche. Vol. I. - Méditerranée et Mer Noire* (Fischer, W.; Bauchot, M.L.; Schneider, M., Eds.), FAO, Rome.

Gharsallah, I.H.; Zamouri-Langar, N.; Missaoui, H.; El Abed, A., 2004. Étude de la croissance relative et de la biomasse d'*Hexaplex trunculus* dans la lagune de Bizerte. *Bulletin de la Société Zoologique de France*, 129: 427-436.

Houart, R., 2001. *A review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Ed. Evolver, Rome.

I.H., 2005-2006. Instituto Hidrográfico (Portuguese Hydrographical Institute - WWW Data base). URL: http://www.hidrografico.pt.

Leiva, G.E.; Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of the catches, management and the Chilean experience. *Reviews in Fish Biology and Fisheries*, 11: 283-300.

Lorenzini, G.; Orlando, E., 1997. Osservazioni sul comportamento alimentare di *Hexaplex trunculus* (Mollusca, Gastropoda, Muricidae). *Biologia Marina Mediterranea*, 4: 405-406.

Marin, A.; López Belluga, M.D., 2005. Sponge coating decreases predation on the bivalve *Arca noae*. *Journal of Molluscan Studies*, 71: 1-6.

Marques, M.R.; Oliveira, J.S., 1995. A pesca das "carteiras" na Ria Formosa. In: *Actas das Comunicações do 8º Congresso do Algarve*. Vilamoura, Portugal, 7-9 Abril 1995, 905-909 pp.

Martín, P.; Sánchez, P.; Ramón, M., 1995. Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research*, 23: 319-331.

Mejri, H.; Trigui El Menif, N.; Ben Souissi, J.; Ben Salem, M.; Zaouali, J., 2005. Ethologie alimentaire des Muricidae *Hexaplex trunculus* (Linnaeus, 1758) et *Bolinus brandaris* (Linnaeus, 1758). In: *Résumés de Communications du Sixième Congrès Maghrébin des Sciences de la Mer*, Monastir, Tunisie, 17-21 Décembre 2005, 16 pp.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Peharda, M.; Morton, B., 2006. Experimental prey species preferences of *Hexaplex trunculus* (Gastropoda: Muricidae) and predator-prey interactions with the Black mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). *Marine Biology*, 148: 1011-1019.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Ramón, M.; Amor, M.J., 2001. Increasing imposex in populations of *Bolinus brandaris* (Gastropoda: Muricidae) in the north-western Mediterranean. *Marine Environmental Research*, 52: 463-475.

Ramón, M.; Amor, M.J., 2002. Reproductive cycle of *Bolinus brandaris* and penis and genital duct size variations in a population affected by imposex. *Journal of the Marine Biological Association of the United Kingdom*, 82: 435-442.

Ramón, M.; Flos, R., 2001. First trials to cultivate the muricid gastropod *Bolinus brandaris* (Linnaeus). *European Aquaculture Society Special Publication*, 29: 219-220.

Rilov, G.; Benayahu, Y.; Gasith, A., 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? *Marine Ecology Progress Series*, 282: 193-204.

Spanier, E., 1981. Behavioral ecology of the marine snail *Trunculariopsis (Murex) trunculus*. In: *Developments in Arid Zone Ecology and Environmental Quality* (Shuval, H., Ed.), Balaban ISS, Philadelphia, 65-70 pp.

Spanier, E., 1986. Cannibalism in muricid snails as a possible explanation for archaeological findings. *Journal of Archaeological Science*, 13: 463-468.

Spanier, E.; Karmon, N., 1987. Muricid snails and the ancient dye industries. In: *The Royal Purple and the Biblical Blue: Argaman and Tekhelet* (Spanier, E., Ed.), Keter Publishing House Jerusalem Ltd., Jerusalem, 179-192 pp.

Tirado, C.; Rodríguez de la Rúa, A.; Bruzón, M.A.; López, J.I.; Salas, C.; Márquez, I., 2002. *La Reproducción de Bivalvos y Gasterópodos de Interés Pesquero en Andalucía*. Junta de Andalucía, Consejería de Agricultura y Pesca, Huelva.

Vasconcelos, P.; Gaspar, M.B.; Castro, M.; Monteiro, C.C., 2002. The artisanal fishery of *Hexaplex trunculus* L. (Mollusca: Gastropoda) in the Ria Formosa lagoon (Algarve - southern Portugal): Development of an integrated study for resource management. In: *Resúmenes del II Congreso Internacional de las Sociedades Malacológicas Europeas* (Rolán, E.; Troncoso, J.S., Eds.), Vigo, España, 9-13 Septiembre 2002, 210-211 pp.

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias, D.; Castro, M., 2004a. Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2004b. Morphometric relationships of the shell of *Hexaplex trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve - southern Portugal). In: *Resúmenes del XIII Simposio Ibérico de Estudios del Bentos Marino* (Lorenzo, J.M.; Pajuelo, J.G.; Ramos, A., Eds.), Las Palmas de Gran Canaria, España, 21-24 Septiembre 2004, 97-98 pp.

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar, M.B. Gametogenic cycle of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of the Marine Biological Association of the United Kingdom* (submitted).

SECTION 3

AGE AND GROWTH OF *HEXAPLEX (TRUNCULARIOPSIS) TRUNCULUS* IN THE RIA FORMOSA LAGOON

CHAPTER 3.1

Growth rate estimation of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast - southern Portugal)

Vasconcelos, P.; Gaspar, M.B.; Pereira, A.M.; Castro, M., 2006. Growth rate estimation of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of Shellfish Research*, 25 (1): 249-256.

ABSTRACT

This study reports growth rates estimates for *Hexaplex (Truncülariopsis) trunculus* (Gastropoda: Muricidae) from mark/recapture experiments carried out in the Ria Formosa lagoon (Algarve coast - southern Portugal). A total of 726 specimens (shell length and total weight ranging between 20.65-58.36 mm and 0.86-19.89 g, respectively) were marked with Dymo® tape tags and released into a fish culture earth pond. During the marking process, no adverse effects on the whelks' health and behaviour or immediate post-marking mortality were detected. Periodical recapture operations were undertaken using a traditional fishing gear designated locally as "wallet-line" and by hand gathering by scuba divers. A total of 170 whelks were caught, equivalent to 216 recaptures (shell length and total weight ranging between 36.22-65.97 mm and 4.42-27.35 g, respectively), thus corresponding to a recapture rate of 29.8%. Despite the loss of one tag, all remaining tags were intact and easily readable. Marked individuals presented low monthly growth rates, both in terms of shell length (1.0 mm/month or 2.3%/month), shell perimeter (2.0 mm/month or 3.1%/month) and total weight (0.7 g/month or 10.8%/month), which were highly variable between individuals and higher in smaller specimens. Data were used to estimate the von Bertalanffy growth parameters (length and weight) (K=0.41, L_∞=82.76, W_∞=49.97 and t_0=-0.05). The growth rate of *T. trunculus* was compared with results obtained in similar studies with other gastropod species in order to evaluate its potential for molluscan aquaculture.

INTRODUCTION

Marking methods have been readily applied to several species of gastropods, mainly due to the presence of an external shell on which marks or tags can be attached with little or no adverse effects on the animals (Jones, 1979; Gosselin, 1993). Such experiments with gastropods have been used in diverse types of studies, to assess movement (Eversole and Anderson, 1988; Himmelman, 1988), fishing rate (Hancock, 1963), field of attraction and effective fishing area of baited traps (Himmelman, 1988; McQuinn *et al.*, 1988; Sainte-Marie, 1991), species' behaviour towards baited traps (Ito *et al.*, 1980), population size and mortality (Hancock, 1963) and growth rate (Hancock 1963, Eversole and Anderson, 1988).

Various marking techniques have been employed in gastropods, such as painting the shell spire with quick drying paints (Hancock and Urquhart, 1959; Hancock, 1963; Jones, 1979; Ito *et al.*, 1980), applying colour coded nail polish to the shell (Laxton, 1970; Gosselin, 1993), attaching rubber bands or nylon lines with tags around the shell (Appeldoorn, 1988; Himmelman, 1988; McQuinn *et al.*, 1988; Sainte-Marie, 1991), gluing a variety of tags to the shell (Hancock and Urquhart, 1959; Smith, 1987; Eversole and Anderson, 1988; Amos and Purcell, 2003), scrubbing the shell lip free from the periostracal layer (Hancock and Urquhart, 1959; Hancock, 1963), drilling and wiring the shell (Laxton, 1970; Weil and Laughlin, 1984; Kideys and Nash, 1993; Kideys, 1994), chemically marking the shell and operculum with fluorescent dyes (Kideys and Nash, 1993) and using underwater metal detectors to recapture specimens marked with aluminium tags (Crowe *et al.*, 2001).

The muricid gastropod *Hexaplex (Trunculariopsis) trunculus* (Linnaeus, 1758) is a common inhabitant of the subtidal and intertidal areas of the Ria Formosa lagoon (Algarve coast - southern Portugal), where it is subjected to a locally important artisanal fishery. This activity is traditionally undertaken both by manual harvesting during low tide and with an illegal and artisanal fishing gear known as "wallet-line", but more recently this species has also been caught by scuba divers operating illegally inside the lagoon. Furthermore, due to a growing demand for gastropods in the seafood market, the commercial value of *T. trunculus* in Portugal has increased markedly in recent years (reaching prices of 10-15 €/kg for first sale), which has been generating high expectations in terms of its potential as a new species for molluscan aquaculture.

Integrated in a general study on the biology, ecology and fishery of *T. trunculus* in the Ria Formosa lagoon, this work aimed to assess the growth rate of this species by mark/recapture experiments in a fish culture earth pond. To the authors' knowledge, this was the first experiment of this kind ever made with *T. trunculus* and the first growth data (growth rates and growth parameters) available for this species. Moreover, the information gathered in this study will be most useful for assessing the potential of *T. trunculus* for molluscan aquaculture, as well as for establishing adequate management measures for this artisanal fishery.

MATERIAL AND METHODS

Mark/Recapture Experiments

Marking experiments were carried out with commercial samples of *T. trunculus* from the Ria Formosa lagoon (Algarve coast - southern Portugal). Prior to marking, colonising algae and/or encrusting organisms (mainly polychaetes) were removed from the shell with a hard brush and a small area of the largest whorl of the shell was carefully smoothed with sandstone, cleaned and dried with absorbent paper. Subsequently, the specimens were tagged with Dymo® tape with two alphanumeric characters. The margins of these tags were cut with scissors, ensuring a tag of ≈6 x 4 mm size and facilitating adherence to the rounded and irregular surface of the gastropod shell. The tags were fixed with cyanoacrylate glue and covered with epoxy glue (Figure 1a), to diminish abrasion of the tag caused by the burrowing movements of the whelks into the sediment and the settlement of encrusting organisms over the tags, which could jeopardise mark retention and readability. The tags were generally fixed between the two most recently deposited growth bands of the last whorl of the shell (Figure 1a), to prevent eventual dropping of excessive glue from the shell onto the soft body of the gastropods. Finally, after allowing the glue to dry for approximately half hour, marked individuals were rinsed in seawater to avoid potential contamination problems provoked by glue residues, and maintained overnight in an aquarium with running seawater, where they were examined for general condition (comparison of behaviour between marked and unmarked specimens).

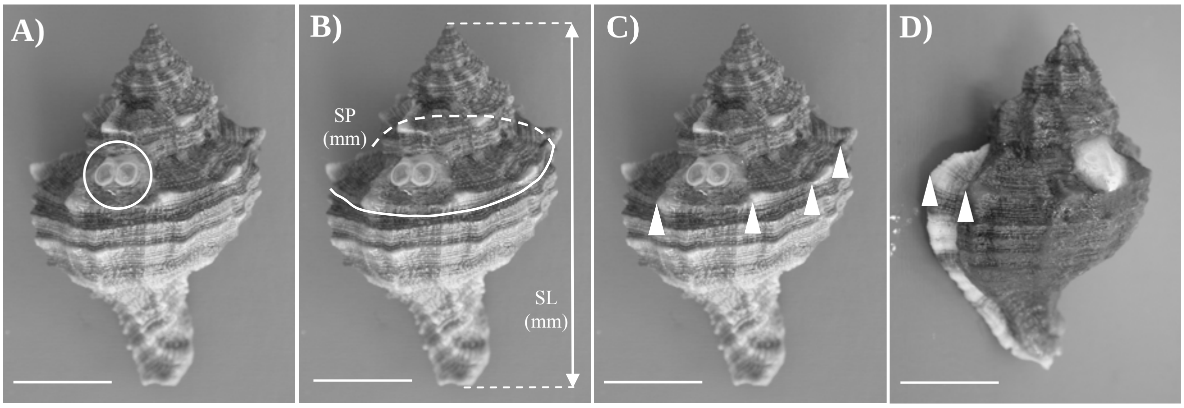


Figure 1. a) *Hexaplex (Truncilariopsis) trunculus* marked with a Dymo® tape tag (circle). **b)** Schematic representation of the shell length (SL - mm) and shell perimeter (SP - mm) measurements. **c)** Location of the shell growth bands (arrows). **d)** *T. trunculus* recaptured with a recently deposited growth band (arrows); (scale bar = 10 mm).

Mark/recapture experiments were undertaken in a fish culture earth pond of the Olhão Fish Culture Experimental Station, which was previously limited by a plastic net fence (area≈100 m^2; maximum depth≈2 m). This earth pond receives water directly from the adjacent Ria Formosa lagoon and closely resembles the surrounding natural environment in terms of sediment type, water quality and food availability. Seawater temperature and dissolved oxygen in the earth pond were monitored daily with a multi parameter monitor (Yellow

Springs Incorporated® - YSI 6820). Periodic recaptures were undertaken monthly, using a traditional fishing gear designated locally as “wallet-line” baited with cockles (*Cerastoderma edule*), and by hand gathering by scuba divers (whenever water visibility allowed for diving recapture operations).

Both during the marking process and immediately after the recapture operations, specimens were measured for shell length (SL - mm) (Figure 1b) with a digital calliper (MITUTOYO® Digimatic: CD-15D; precision = 0.01 mm), shell perimeter (SP - mm) of the last whorl of the shell (Figure 1b) and weighed for total weight (TW - g) on a top loading digital balance (AND®: HF-2000G; precision = 0.01 g). Particular care was taken to drain as much water as possible from the mantle cavity before weighing. On both occasions, the position of the tag on the gastropod shell was also registered in order to verify the degree of shell deposition (tag position at recapture *versus* tag position at marking) (Figure 1c).

Estimation of Growth Rates

Monthly growth rates in terms of unit/month and percentage/month (mm and %SL/month, mm and %SP/month, g and %TW/month, no. and %growth bands/month) were estimated with the following equations:

$$GR = \frac{(S_r - S_m)}{(t_r - t_m)} * 30 \quad \text{and} \quad \%GR = \frac{GR}{S_m} * 100$$

where: GR is the monthly growth rate (unit/month); %GR is the monthly growth rate (%/month); S_r is the size at recapture (SL - mm, SP - mm, TW - g or tag position); S_m is the size at marking (SL - mm, SP - mm; TW - g or tag position); t_r is the day of recapture; and t_m is the day of marking.

In the case of multiple recaptures, the size increment used (length, perimeter, weight and tag position) was derived from initial size and last recapture.

The relationships between the individuals' size (shell length at marking process, grouped in 5 mm SL classes) and the respective monthly growth rates (unit/month and percentage/ month) were assessed by regression analysis (least squares method) and the degrees of association between variables were estimated by the correlation coefficient (r).

Estimation of Growth Parameters

Whenever the period at liberty (interval between marking and recapture) is small, growth rates may be related to the mean length between marking and recapture (S_m+S_r/2), using the Gulland-Holt plot (Gulland and Holt, 1959). Because the present mark/recapture data lacked small individuals (<20 mm SL), data on growth rates of individuals hatched at the end of June 2004 in the Tavira Molluscan Aquaculture Experimental Station were pooled with data on growth rates obtained in mark/recapture experiments. Data were converted into weekly growth rates to increase the number of points in the plot and thus enhance the accuracy of this graphical method. Additionally, recaptured individuals that did not grow during the study period were removed from this estimation of growth parameters.

Growth parameters (K and L_∞) were estimated from the Gulland-Holt plot by the following relations (Gulland and Holt, 1959):

$$K = -b \qquad \text{and} \qquad L_\infty = \frac{-a}{b}$$

where: K is the growth coefficient; L_∞ is the asymptotic shell length (SL - mm); a is the linear regression intercept on the Y-axis; and b is the linear regression slope.

The theoretical age at shell length zero (t_0) cannot be obtained from mark/recapture data alone (without specific size-at-age information). For the present purposes t_0 was assumed to correspond to the shell length of *T. trunculus* at hatching (L_t=1.64±0.22 mm SL, n=100) (Vasconcelos *et al.*, 2004a) and was calculated from the following expression (von Bertalanffy, 1938):

$$t_0 = t + \frac{1}{K} \ln \frac{(L_\infty - L_t)}{L_\infty}$$

Subsequently, shell growth of *T. trunculus* was modelled by applying all these growth parameters (K, L_∞ and t_0) in the von Bertalanffy growth in length function, expressed by the following equation (von Bertalanffy, 1938):

$$L_t = L_\infty \left[1 - e^{-K(t-t_0)}\right]$$

where: L_t is the shell length at age t (SL - mm); L_∞ is the maximum asymptotic shell length (SL - mm); K is the von Bertalanffy growth coefficient; and t_0 is the theoretical age at shell length zero (years).

After applying the value estimated for maximum asymptotic shell length (L_∞) in the weight/length relationship ($TW = a * SL^b$) for this species, the maximum asymptotic total weight (W_∞) was obtained. Finally, growth in total weight of *T. trunculus* was modelled by the von Bertalanffy growth in weight function, by using the following expression (von Bertalanffy, 1938):

$$W_t = W_\infty \left[1 - e^{-K(t-t_0)}\right]^n$$

where: W_t is the total weight at age t (TW - g); W_∞ is the maximum asymptotic total weight (TW - g); K is the von Bertalanffy growth coefficient; t_0 is the theoretical age at total weight zero (years); and n is the slope (b) of the weight/length relationship ($TW=a*SL^b$).

RESULTS

Mark/Recapture Experiments

A total of 726 whelks were marked and released between March 2003 and June 2004. These marked specimens had an average shell length of 44.32±5.34 mm and average total weight of 8.94±3.06 g (ranges 20.65-58.36 mm and 0.86-19.89 g, respectively). A careful inspection of the condition of marked individuals held in the aquarium did not reveal any adverse effects on the gastropods health and behaviour or immediate post-marking mortality.

Daily monitoring of temperature and dissolved O_2 in the earth pond (Figure 2) showed that the average temperature was relatively high (20.7±4.9 ºC), varying between a minimum of 11.0 ºC in December 2003 and a maximum of 29.4 ºC in August 2003. The average dissolved O_2 was 7.2±1.1 mg/l, showing an opposite trend relative to water temperature, with a maximum in May 2004 (11.4 mg/l) and an unexpected minimum in February 2004 (4.2 mg/l).

In the mark/recapture study, 170 whelks were caught (216 recaptures) with an average shell length of 49.36±4.92 mm and average total weight of 11.89±4.08 g (ranges 36.22-65.97 mm and 4.42-27.35 g, respectively). Overall, these 216 recaptures corresponded to a recapture rate of 29.8%. In this context, it is worth emphasising that most recaptures were caught only once (133 individuals = 78.2%), 30 whelks were recaptured twice (17.7%), six were caught three times (3.5%), and one was recaptured four times (0.6%). In all, 107 dead whelks were recovered from the earth pond during diving operations, corresponding to a mortality rate of

14.7%. After 16 months from the beginning of the study, only one specimen was recaptured without a tag. All other recaptured specimens had their tags intact and easily readable, most of them presenting recently deposited growth bands (Figure 1d).

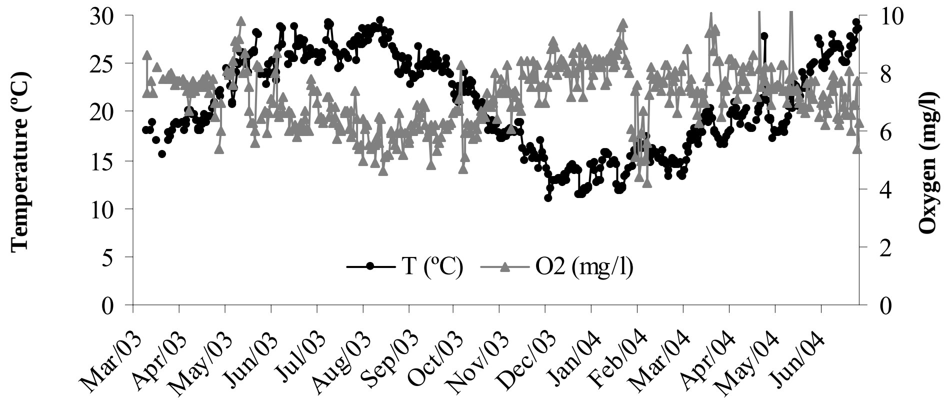


Figure 2. Seawater temperature and dissolved oxygen in the earth pond where the marked specimens of *Hexaplex (Trunculariapsis) trunculus* were released.

Estimation of Growth Rates

The recaptured whelks presented average monthly growth rates of 1.0±1.0 mm SL (2.3±2.6% SL/month), 2.0±1.6 mm SP (3.1±2.8% SP/month) and 0.7±0.6 g TW (10.8±11.3% TW/month). However, growth rates were highly variable among individuals, with some whelks simply not growing (both in terms of shell length, shell perimeter and/or total weight), while the fastest growing specimens exhibited much higher growth rates, *i.e.*, 4.0 mm SL/month (13.4% SL/month), 7.4 mm SP/month (15.7% SP/month) or 3.4 g TW/month (59.0% TW/month) (Table 1).

Table 1. Monthly growth rates (shell length, shell perimeter and total weight) of the *Hexaplex (Trunculariopsis) trunculus* specimens recaptured in the earth pond.

Monthly growth rate	**Shell length (SL - mm)**		**Shell perimeter (SP - mm)**		**Total weight (TW - g)**	
(N = 216)	mm/month	%/month	mm/month	%/month	g/month	%/month
Mean ± S.D. (Min. - Max.)	1.0 ± 1.0 (0.0 - 4.0)	2.3 ± 2.6 (0.0 - 13.4)	2.0 ± 1.6 (0.0 - 7.4)	3.1 ± 2.8 (0.0 - 15.7)	0.7 ± 0.6 (0.0 - 3.4)	10.8 ± 11.3 (0.0 - 59.0)

Individually, growth rates varied with size (shell length at marking process), being highly variable between individuals, and generally higher in smaller than in larger individuals. In fact, individual monthly growth rates displayed a decreasing trend during ontogeny, a phenomenon that was more evident in the relationships between growth in shell length, shell perimeter and shell deposition as a function of shell length (Figure 3a,b,c,d,g,h), than in the relationships between growth in total weight in function of shell length (Figure 3e,f).

Because of the high inter-individual variability observed in monthly growth rates, individual data were grouped in size classes (5 mm SL) and subjected to regression analyses (Figure 4). After pooling data into size classes, the inverse relationship between size (shell length at marking) and growth rates became even more evident, further strengthening the decreasing trend in growth during ontogeny. This was particularly evident in the relationships SL/month in function of shell length (Figure 4a,b) and SP/month in function of shell length (Figure 4c,d), while the relationships TW/month in function of shell length (Figure 4e,f) presented poorer fittings. Furthermore, the relationships established between shell deposition rate (growth bands/month) and shell length (Figure 4g,h) perfectly illustrate the remarkable decline in shell deposition with specimens' size (*i.e.*, during specimens growth), reflected by the high correlation coefficients (*r*) registered in these regressions.

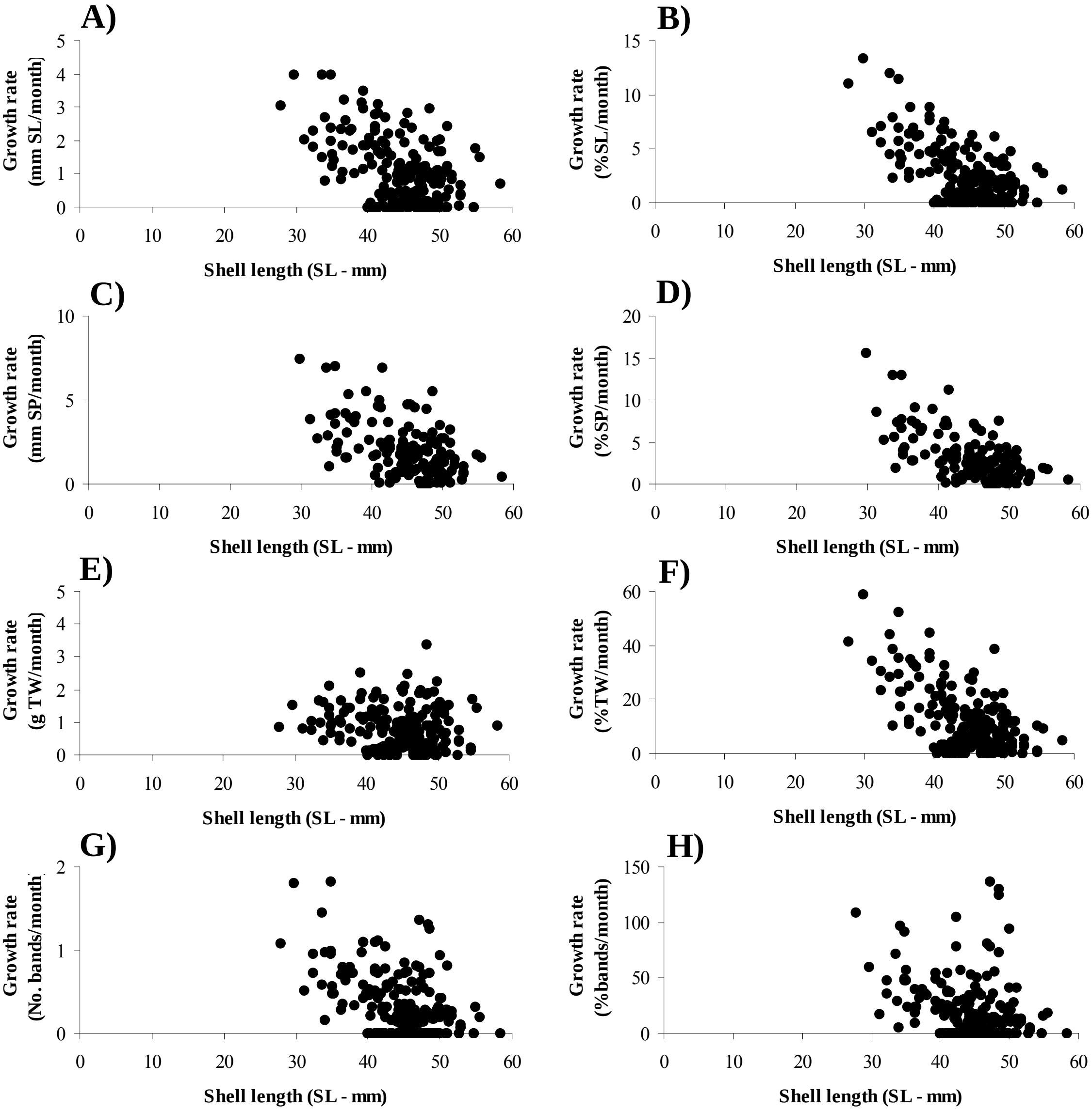


Figure 3. Individual monthly growth rates of recaptured specimens of *Hexaplex (Trunculariopsis) trunculus* in terms of shell length, shell perimeter, total weight and shell deposition: **a)** SL/month. **b)** %SL/month. **c)** SP/month. **d)** %SP/month. **e)** TW/month. **f)** %TW/month. **g)** no. growth bands/month. **h)** %growth bands/month.

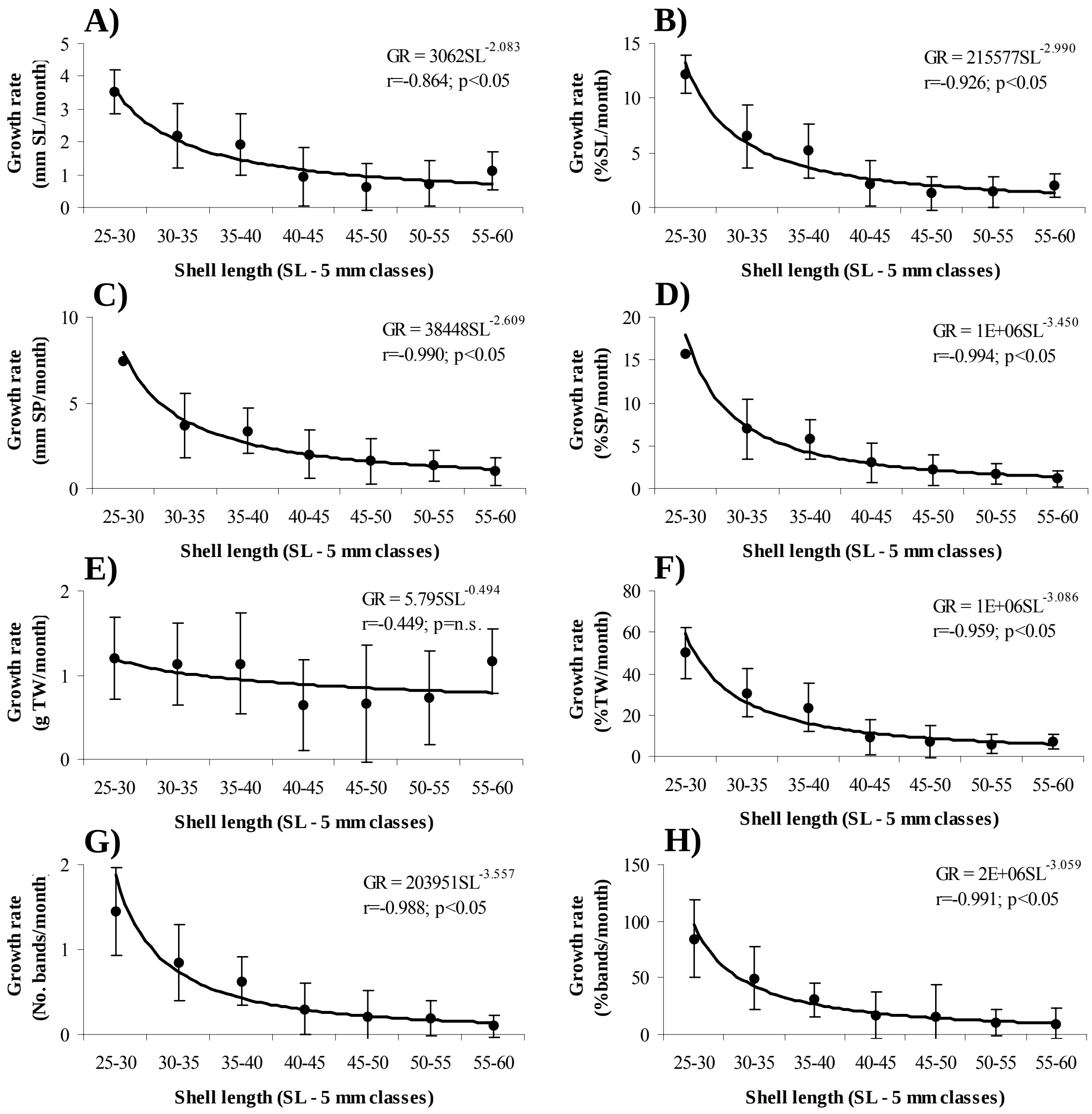


Figure 4. Monthly growth rates of recaptured specimens of *Hexaplex (Trunculariopsis) trunculus* (grouped in 5 mm SL classes) in terms of shell length, shell perimeter, total weight and shell deposition: **a)** SL/month. **b)** %SL/month. **c)** SP/month. **d)** %SP/month. **e)** TW/month. **f)** %TW/month. **g)** no. growth bands/month. **h)** %growth bands/month.

Estimation of Growth Parameters

The Gulland-Holt plot of weekly growth rate *vs*. mean shell length (with pooled data from mark/recaptured gastropods and from laboratory hatched gastropods) is illustrated in Figure 5a. After applying this graphical method, the estimated growth parameters were K = 0.008/week (0.41/year) and L_∞ = 82.76 mm SL. The calculation of t_0, assuming the shell length of *T. trunculus* at hatching of L_t = 1.64 mm SL, produced a value of –0.05 years. The von Bertalanffy growth function for *T. trunculus* in terms of shell length ($L_t=82.76[1–e^{-0.41(t+005)}]$) is presented in Figure 5b.

After employing the weight-length relationship ($TW=0.00008SL^{3.022}$; n=2401; r=0.975; $p<0.05$) estimated by Vasconcelos *et al.* (2004b) for *T. trunculus* from the Ria Formosa lagoon (Figure 5c), the von Bertalanffy growth function for this species in terms of total weight ($W_t=49.97[1–e^{-0.41(t+005)}]^{3.022}$) was obtained (Figure 5d).

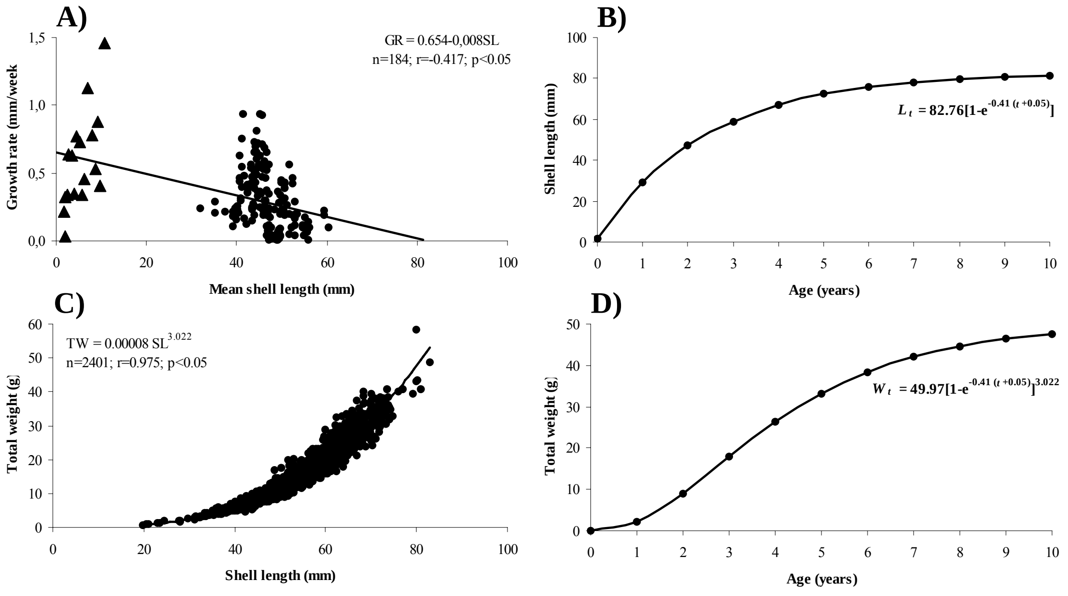


Figure 5. a) Gulland-Holt plot of weekly growth rate *vs*. mean shell length for *Hexaplex (Truncularìopsis) trunculus* (● - data from marked/recaptured gastropods, ▲- data from laboratory hatched gastropods). **b)** von Bertalanffy growth function in terms of shell length for *T. trunculus*. **c)** weight-length relationship for *T. trunculus* (data from Vasconcelos et al. 2004b). **d)** von Bertalanffy growth function in terms of total weight for *T. trunculus*.

DISCUSSION

Mark/Recapture Experiments

Marked gastropods are frequently disturbed and stressed by tagging procedures (Himmelman, 1988) that may result in quite different behaviour from undisturbed specimens (Sainte-Marie, 1991), often exhibiting a period of stress-induced inactivity during release after extended tagging manipulations (McQuinn *et al.*, 1988). In the present study, the marking process had no detectable adverse effects on the marked whelks' health and behaviour. When returned to the aquarium, marked whelks soon started crawling (within a maximum of 5-10 minutes), and no behavioural differences were apparent between marked and unmarked individuals. This was probably because *T. trunculus* quickly withdraws into the shell when removed from seawater, preserving most of the inner moisture.

The recapture rate obtained during these experiments (29.8%) was low, considering that specimens were released inside a fenced area on an earth pond, virtually without potential predators. In the present case, the high proportion of uncaught individuals might have been due to the type of bottom in the earth pond (muddy sediment), which favoured the burrowing movements of *T. trunculus* and decreased underwater visibility during recapture operations by hand gathering by scuba divers.

An unexpectedly high mortality rate was registered (14.7%). Besides natural mortality, this might have been due to other circumstances, namely a possible weakness and fragility of some marked individuals. Indeed, the fact that specimens were obtained from commercial samples meant that neither date of capture nor the conditions in which captured whelks were held were known. Additionally, despite water temperature in the earth pond closely reflecting the environmental conditions in the adjacent Ria Formosa lagoon, dissolved oxygen was low during some periods and might have also contributed to the mortality of marked specimens.

However, this high mortality rate is probably slightly underestimated, since dead individuals could only be recovered from the earth pond bottom by diving, frequently in poor visibility. Moreover, the burrowing behaviour of *T. trunculus*, coupled with the high sedimentation rate inside the earth pond, might have also made difficult the detection of all dead specimens, thus probably contributing to an underestimation of total mortality rate.

Estimation of Growth Rates

The monthly growth rates estimated in the present study, both in terms of shell length (1.0 mm/month or 2.3%/month), shell perimeter (2.0 mm/month or 3.1%/month) and total weight (0.7 g/month or 10.8%/month) were considered somewhat low, taking into consideration that *T. trunculus* is supposed to be a species with a short/medium life-span. Nevertheless, this growth rate is common among muricid species, since according to Spight *et al.* (1974) a typical young muricid grows only 1-2 mm/month. As expected, monthly growth rates decreased markedly during ontogeny, with smaller specimens growing more rapidly than larger ones. This was particularly evident in growth in terms of shell length, shell perimeter and shell deposition rates, but less noticeable in terms of total weight growth rate. This phenomenon was probably influenced by the seasonal fluctuations in physiological condition and total weight normally associated with the reproductive cycle of the species, being accentuated by the fact that it was virtually impossible to assure the complete removal of water from the shells, thus contributing to some variability in weighing both at marking and recapture operations.

Moreover, monthly growth rates were highly variable among individuals and some whelks did not grow between marking and recapture. The same high inter-individual variability in growth rates has been noted in several gastropod species and has been attributed to diverse factors (*e.g.* Tallmark, 1980; Smith, 1987 and references therein). In fact, most muricid adults simply do not grow (Spight *et al.*, 1974), and in some experiments with different species, larger specimens (presumably adults) did not grow at all during the study period, after becoming reproductively active, even though they were substantially smaller than their maximum shell length (*e.g.* Hancock, 1959; Laxton, 1970; Fotheringham, 1971; Spight *et al.*, 1974; Tallmark, 1980; Appeldoorn, 1988). However, in the present case, despite reduction or even cessation of growth in shell length, *T. trunculus* continued slowly growing in shell perimeter, total weight and shell deposition, with monthly growth rates which were invariably higher than shell length growth rates.

However, these growth estimates are most likely underestimated, since despite the inclusion of some juveniles in the experiments, both marked and recaptured gastropods were dominated by adult specimens (consequently with a much lower growth rate), which obviously induced a significant underestimation of the overall growth rate of the species. Furthermore, notwithstanding the use of a non size-selective fishing gear in the recapture operations, the samples caught through snorkelling and scuba diving could be size-biased, since bigger whelks were more conspicuous and more easily detected than smaller individuals, especially in a burrowing species and under low water visibility conditions. In addition, many animals were recaptured less than one year after being marked and during winter and spring, when lower water temperatures and less food availability in the earth-pond might have slowed their metabolism and decreased growth rates. For all these reasons, the present growth rates of *T. trunculus* should be interpreted with some caution and considered as average values for the size range employed in this study (dominated by adults). Extrapolation of the data to juveniles is especially problematic. *T. trunculus* individuals hatched at the end of June 2004 and maintained until now in the Tavira Molluscan Aquaculture Experimental Station presented a much higher growth rate, hatching with

1.64±0.22 mm SL and reaching 11.66±2.05 mm SL after four months (end of October 2004), therefore corresponding to a monthly growth rate of 2.5 mm SL/month during the first four months of life (Vasconcelos *et al.*, 2004a).

The comparison of the growth rates of *T. trunculus* obtained in the present study with those obtained for other gastropod species (namely some muricids with high commercial value) was rather difficult, since most studies had different objectives, different experimental designs (e.g. field *vs*. laboratory, with *vs*. without additional food supply) and variable size ranges of the individuals used for growth rate estimations (e.g. juveniles *vs*. adults) (Table 2). The average monthly growth rate of *T. trunculus* (1.0 mm/month) compared favourably with those obtained for *Ocenebra lurida*, *Urosalpinx cinerea*, *Busycon carica* and *Turbo sarmaticus*, being equal to other growth rates estimated for *B. carica*, *Busycon canaliculatum* and *Ocenebra interfossa*. In contrast, the present growth rate was lower than those obtained for *B. carica*, *Ceratostoma foliatum* and *Cerithium nodulosum*, and much lower than the growth rates of *Ocenebra lumaria*, *Eupleura caudata*, *Bolinus brandaris*, *Concholepas concholepas*, *Chicoreus virgineus*, *Strombus gigas* and *Chicoreus ramosus*. In this context, it is worth emphasising that most of these studies used newly hatched specimens or small juveniles during growth studies, thus growth rates are presumably overestimated considering the overall size ranges (juveniles + adults) of the natural populations of these species. Moreover, some of these species have a maximum size much bigger than *T. trunculus*. Comparisons of growth rates (mm SL/month) between gastropod species are complicated and should be carefully interpreted.

Table 2. Comparison of the growth rate of *Hexaplex (Truncalariopsis) trunculus* obtained through mark/recapture experiments with growth rates reported for other gastropod species.

Species	**Location**	**Size range (SL - mm)**	**Growth rate (SL - mm)**	**Author(s)**
Hexaplex trunculus	Ria Formosa, Portugal	20-58 mm	1.0 mm/month	* present study
Ocenebra lurida	Washington, U.S.A.	12-22 mm	0.4 mm/month	Spight *et al.* (1974)
Busycon canaliculatum	South Carolina, U.S.A.	-	1.0 mm/month	Eversole and Anderson (1988)
Busycon carica	South Carolina, U.S.A.	-	1.0 mm/month	Eversole and Anderson (1988)
Ocenebra interfossa	Washington, U.S.A.	12-21 mm	1.0 mm/month	Spight *et al.* (1974)
Ceratostoma foliatum	Washington, U.S.A.	juveniles	<2.0 mm/month	Spight *et al.* (1974)
Cerithium nodulosum	Guam, Mariana Islands	28-75 mm (juveniles)	2.3-4.6 mm/month	Yamaguchi (1977)
Ocenebra lumaria	Asamushi, Japan	-	3.7 mm/ month	Luckens (1970)
Eupleura caudata	Virginia, U.S.A.	-	4.0 mm/month	Mackenzie (1961)
Bolinus brandaris	Catalonia, Spain	>5 mm (juveniles)	4.3 mm/month	Ramón and Flos (2001)
Concholepas concholepas	Chile	juveniles	4.7 mm/month	Lara and Montes (1988)
Chicoreus virgineus	Cuddalore, India	70-85 mm	3.0-5.0 mm/month	Ramesh *et al.* (1992)
Strombus gigas	Los Roque, Venezuela	30-190 mm	4.0-15.0 mm/month	Weil and Laughlin (1984)
Chicoreus ramosus	Phuket, Thailand	25-45 mm (juveniles)	11.4 mm/month	Bech (1992)
Urosalpinx cinerea	England, U.K.	juveniles	3.0 mm/year	Hancock (1959)
Busycon carica	North Carolina, U.S.A.	juveniles	10.2 mm/year	Magalhães (1948)
Turbo sarmaticus	Port Alfred, South Africa	42 mm (juveniles)	2.4-13.8 mm/year	Foster *et al.* (1999)
Busycon carica	Virginia, U.S.A.	juveniles	13.2 mm/year	Kraeuter *et al.* (1989)

Estimation of Growth Parameters

The von Bertalanffy growth function in terms of shell length estimated for *T. trunculus* ($L_t=82.76[1–e^{-0.41(t+0.05)}]$) further confirmed that the growth of this gastropod species is relatively slow (*K*=0.41). According to these data the minimum landing size of *T. trunculus* (MLS = 50 mm SL) is only attained at 2.20 years old. Furthermore, at 95% of its theoretical maximum shell length (95% L_∞=82.76 mm), the von Bertalanffy growth equation yielded an estimated age of 7.24 years for *T. trunculus*, which can be considered a relatively short/medium life span for a gastropod species.

The estimated maximum asymptotic shell length (L_∞=82.76 mm) closely approached the maximum size attained locally by *T. trunculus*. During a general study on the biology, ecology and fishery of this species in the Ria Formosa lagoon, the largest specimen sampled measured 82.84 mm. Nevertheless, specimens of this size were quite exceptional, since amongst 2484 *T. trunculus* measured during a one-year sampling program, only one individual was bigger than the asymptotic shell length estimated in this study (L_∞=82.76 mm), which was very similar to the maximum size attained by this species in the Ria Formosa lagoon (85 mm SL) (Muzavor and Morenito, 1999) and included in the maximum size range reported for *T. trunculus* by other authors, between 80 mm SL (Poppe and Goto, 1991) and 90 mm SL (Macedo *et al.*, 1999).

The von Bertalanffy growth function in terms of total weight estimated for *T. trunculus* ($W_t=49.97[1–e^{-0.41(t+005)}]^{3.022}$) indicated that at 2.20 years old (age corresponding to the minimum landing size of *T. trunculus* - MLS = 50 mm SL) specimens achieved 10.86 g in total weight. The estimated maximum asymptotic total weight (W_∞=49.97 g) was lower than the maximum total weight registered in samples from the Ria Formosa lagoon (58.20 g), but yet again this was an exceptionally heavy individual, because amongst 2401 *T. trunculus* weighed during a one-year sampling program, only one specimen was heavier than the asymptotic total weight estimated in the present study (W_∞=49.97 g).

Therefore, and although the von Bertalanffy growth model has been criticised for not adequately representing growth of the entire size range of organisms, in the present case both size (shell length) and weight (total weight) data fitted the growth model satisfactorily.

Aquaculture and Fisheries Considerations

Despite being slightly low and underestimated due to the dominance of adult specimens in the samples, the growth rate estimated through mark/recapture experiments for *T. trunculus* (1.0 mm/month) may still create some expectations in terms of the potential of this species for molluscan aquaculture, considering that laboratory hatched specimens grow at a much higher rate (2.5 mm/month during the first four months of life) and that the von Bertalanffy growth function indicated an age of 2.20 years to attain the minimum landing size for this species (50 mm SL *i.e.*, 10.86 g TW).

Since environmental and feeding requirements of *T. trunculus* are not demanding, the culture of this gastropod species could become profitable in the near future, when the crucial phases of the species reproductive cycle (spawning and post-hatching development) are better known. Moreover, due to an increasing demand for gastropods in the seafood market, the commercial value of *T. trunculus* has been continuously rising (reaching values of 10-15 €/kg for first sale) and its abundance in the Ria Formosa lagoon apparently has been decreasing over the last years, probably due to illegal fishing and consequent overexploitation of the resource.

ACKNOWLEDGMENTS

The authors would like to acknowledge Pedro Pousão-Ferreira for allowing these mark/recapture experiments in an earth pond of the Olhão Fish Culture Pilot Station, as well as their collaborators for monitoring the seawater parameters. Thanks are also due to João Tata-Regala for his help in fencing the earth pond, and Susana Carvalho, Rita Constantino and João Cúrdia for their assistance during the recapture operations. The authors are indebted to Dr. Scoresby Shepherd and Dr. Fred E. Wells, whose suggestions greatly improved the manuscript. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001) and by the DIVERAQUA Project (Interreg IIIA).

REFERENCES

Amos, M.J.; Purcell, S.W., 2003. Evaluation of strategies for intermediate culture of *Trochus niloticus* (Gastropoda) in sea cages for restocking. *Aquaculture*, 218: 235-249.

Appeldoorn, R.S., 1988. Age determination, growth, mortality and age of first reproduction in adult queen conch, *Strombus gigas* L., off Puerto Rico. *Fisheries Research*, 6: 363-378.

Bech, M., 1992. Sediment preference, hiding behaviour and growth rate: Paws in the game of survival of juvenile *Chicoreus ramosus* (Linnaeus, 1758). *Phuket Marine Biological Center Special Publication*, 11: 79-82.

Crowe, T.P.; Dobson, G.; Lee, C.L., 2001. A novel method for tagging and recapturing animals in complex habitats and its use in research into stock assessment of *Trochus niloticus*. *Aquaculture*, 194: 383-391.

Eversole, A.G.; Anderson, W.D., 1988. Growth and movement of offshore populations of *Busycon carica* and *B. canaliculatum* evidenced by mark and recapture results. *Journal of Shellfish Research*, 7: 157.

Foster, G.G.; Hodgson, A.N.; Balarin, M., 1999. Effect of diet on growth rate and reproductive fitness of *Turbo sarmaticus* (Mollusca: Vetigastropoda: Turbinidae). *Marine Biology*, 134: 307-315.

Fotheringham, N., 1971. Life history patterns of the littoral gastropods *Shaskyus festivus* (Hinds) and *Ocenebra poulsoni* Carpenter (Prosobranchia: Muricidae). *Ecology*, 52: 742-757.

Gosselin, L.A., 1993. A method for marking small juvenile gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 73: 963-966.

Gulland, J.A.; Holt, S.J., 1959. Estimation of growth parameters for data at unequal time intervals. *Journal du Conseil International pour l'Exploration de la Mer*, 25: 47-49.

Hancock, D.A., 1959. The biology and control of the American whelk tingle *Urosalpinx cinerea* (Say) on English oyster beds. *Fisheries Investigation (London)*, 22: 1-66.

Hancock, D.A., 1963. Marking experiments with the commercial whelk (*Buccinum undatum*). *Special Publication - International Commission for the Northwest Atlantic Fisheries (ICNAF)*, 4: 176-193.

Hancock, D.A.; Urquhart, A.E., 1959. Methods for marking whelks (*Buccinum undatum* L.). *Journal du Conseil International pour l'Exploration de la Mer*, 24: 494-496.

Himmelman, J.H., 1988. Movement of whelks (*Buccinum undatum*) towards a baited trap. *Marine Biology*, 97: 521-531.

Ito, H.; Wakui, T.; Dimon, Y., 1980. Methods for marking sea snails, Genus *Buccinum*. *Bulletin of the Hokkaido Regional Fisheries Research Laboratory*, 45: 65-74.

Jones, R., 1979. Materials and methods used in marking experiments in fishery research. *FAO Fisheries Technical Paper*, 190: 134 p.

Kideys, A.E., 1994. Effect of tagging on the growth of the neogastropod, *Buccinum undatum* in the laboratory. *Fisheries Research*, 20: 283-289.

Kideys, A.E.; Nash, R.D.M., 1993. A note on the mortality associated with fluorescent marking in a gastropod. *DOGA - Turkish Journal of Zoology*, 17: 175-178.

Kraeuter, J.N.; Castagna, M.; Bisker, R., 1989. Growth rate estimates for *Busycon carica* (Gmelin, 1971) in Virginia. *Journal of Shellfish Research*, 8: 219-225.

Lara, E.; Montes, E., 1988. Estado del conocimiento en el cultivo del loco *Concholepas concholepas*. *Investigaciones Pesqueras (Chile)*, 35: 103-107.

Laxton, J.H., 1970. Shell growth in some New Zealand Cymatiidae (Gastropoda: Prosobranchia). *Journal of Experimental Marine Biology and Ecology*, 4: 250-260.

Luckens, P.A., 1970. Predation and intertidal zonation at Asamushi. *Bulletin of the Marine Biological Station of Asamushi*, 14: 33-52.

Macedo, M.C.C.; Macedo, M.I.C.; Borges, J.P., 1999. *Conchas Marinhas de Portugal (Seashells of Portugal)*. Editorial Verbo, Lisboa.

MacKenzie, C.L., 1961. Growth and reproduction of the oyster drill *Eupleura caudata* in the York River, Virginia. *Ecology*, 42: 317-338.

Magalhães, H., 1948. An ecological study of snails of the Genus *Busycon* at Beaufort, North Carolina. *Ecological Monographs*, 18: 379-409.

McQuinn, I.H.; Gendron, L.; Himmelman, J.H., 1988. Area of attraction and effective area fished by a whelk (*Buccinum undatum*) trap under variable conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, 45: 2054-2060.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa. Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Poppe, G.T.; Goto, Y., 1991. *European Seashells. Vol. I (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Ramesh, M.X.; Raghunuthan, C.; Ayyakkannu, K., 1992. Laboratory observations on food consumption and growth of *Chicoreus virgineus* - a pilot study. *Phuket Marine Biological Center Special Publication*, 11: 94-95.

Ramón, M.; Flos, R., 2001. First trials to cultivate the muricid gastropod *Bolinus brandaris* (Linnaeus). *European Aquaculture Society Special Publication*, 29: 219-220.

Sainte-Marie, B., 1991. Whelk (*Buccinum undatum*) movement and its implications for the use of tag-recapture methods for the determination of baited trap fishing parameters. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 751-756.

Smith, B.D., 1987. Growth rate, distribution and abundance of the introduced topshell *Trochus niloticus* Linnaeus on Guam, Mariana Islands. *Bulletin of Marine Science*, 41: 466-474.

Spight, T.M.; Birkeland, C.; Lyons, A., 1974. Life histories of large and small murexes (Prosobranchia: Muricidae). *Marine Biology*, 24: 229-242.

Tallmark, B., 1980. Population dynamics of *Nassarius reticulatus* (Gastropoda, Prosobranchia) in Gullmar Fjord, Sweden. *Marine Ecology Progress Series*, 3: 51-62.

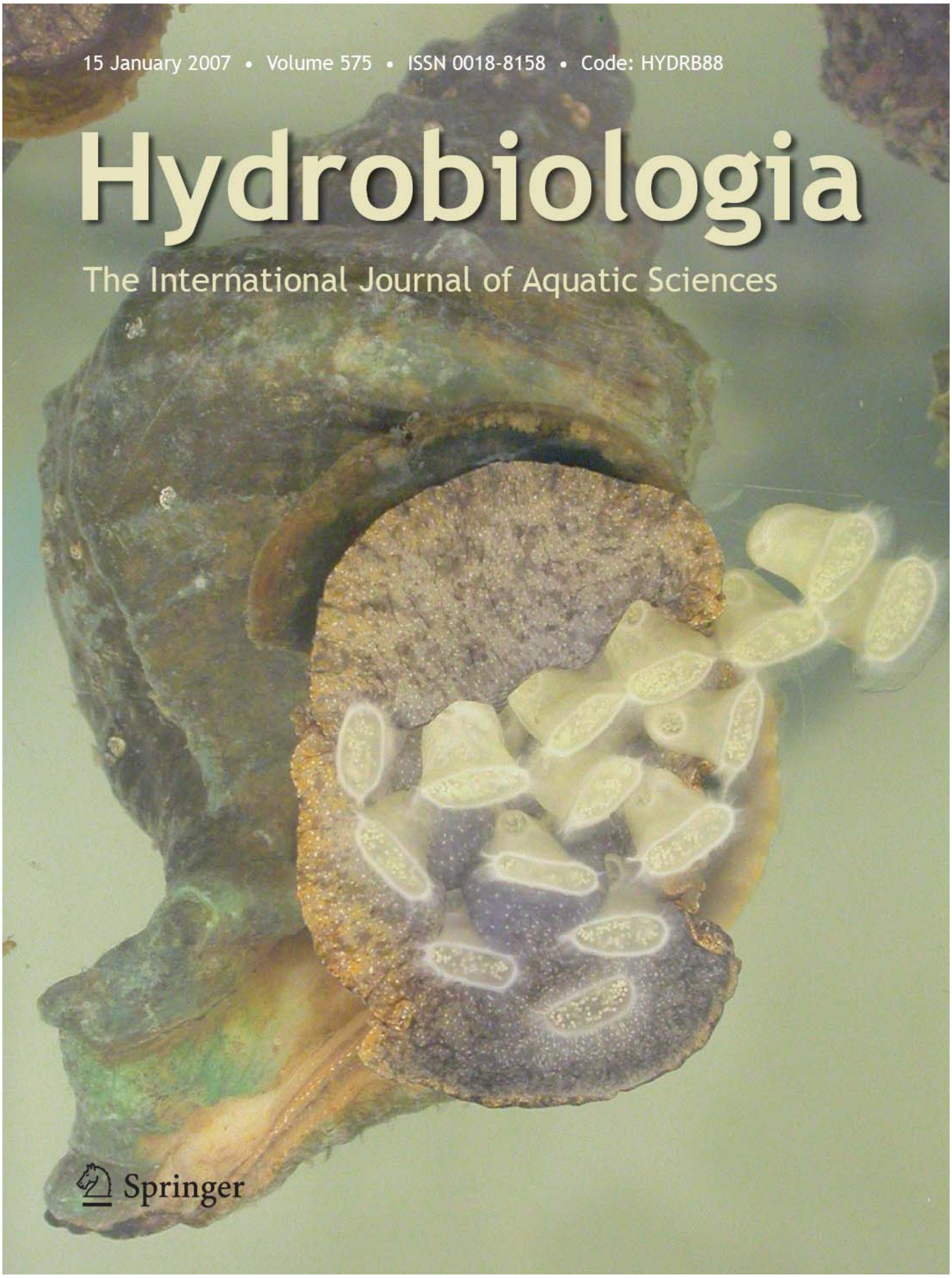
Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias D.; Castro, M., 2004a. Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2004b. Morphometric relationships of the shell of *Hexaplex trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve - southern Portugal). In: *Resúmenes del XIII Simposio Ibérico de Estudios del Bentos Marino* (Lorenzo, J.M.; Pajuelo, J.G.; Ramos, A., Eds.), Las Palmas de Gran Canaria, España, 21-24 Septiembre 2004, 97-98 pp.

Von Bertalanffy, L., 1938. A quantitative theory of organic growth. *Human Biology*, 10: 181-213.

Weil, E.; Laughlin, R., 1984. Biology, population dynamics, and reproduction of the queen conch *Strombus gigas* Linné in the Archipiélago de los Roques National Park. *Journal of Shellfish Research*, 4: 45-62.

Yamaguchi, M., 1977. Shell growth and mortality rates in the coral reef gastropod *Cerithium nodulosum* in Pago Bay, Guam, Mariana Islands. *Marine Biology*, 44: 249-263.



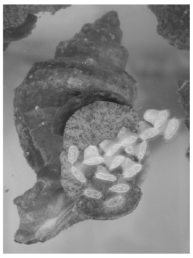
15 January 2007 • Volume 575 • ISSN 0018-8158 • Code: HYDRB88

Hydrobiologia

The International Journal of Aquatic Sciences

Springer

Hydrobiologia
The International Journal of Aquatic Sciences



Cover illustration: Individual spawn of *Hexaplex (Truncularìopsis) trunculus* in laboratory aquarium. See article by P. Vasconcelos et al., pp. 161-172. Photo credit: IPIMAR-CRIPSul (Miguel B. Gaspar / Paulo Vasconcelos).

ISSN 0018-8158

Printed in the Netherlands

CHAPTER 3.2

The shell of *Hexaplex (Truncularioposis) trunculus* (Gastropoda: Muricidae) as a mobile hard substratum for epibiotic polychaetes (Annelida: Polychaeta) in the Ria Formosa (Algarve coast - southern Portugal)

Vasconcelos, P.; Cúrdia, J.; Castro, M.; Gaspar, M.B., 2007. The shell of *Hexaplex (Truncularioposis) trunculus* (Gastropoda: Muricidae) as a mobile hard substratum for epibiotic polychaetes (Annelida: Polychaeta) in the Ria Formosa (Algarve coast - southern Portugal). *Hydrobiologia*, 575: 161-172.

ABSTRACT

This work reports and illustrates secondary colonisation of *Hexaplex (Truncylariopsis) trunculus* shells by polychaetes in the Ria Formosa lagoon (Algarve coast - southern Portugal). Information is presented in terms of qualitative and quantitative data on epibiotic polychaetes, fouling frequency, degree of epibiosis, preferential areas for colonisation and incidence of shell damages. A total of 2880 polychaetes belonging to 10 families were identified. Besides Spirorbidae (not quantified), the most abundant families were Serpulidae (67.5%), Sabellidae (10.2%), Sabellariidae (9.6%) and Cirratulidae (8.5%). From a total of 2264 *T. trunculus* individuals analysed for epibiosis, 28.4% were non-fouled, 66.8% were intermediately fouled and 4.8% were heavily fouled. The fouling frequency, degree of epibiosis and incidence of damages in the shell spire increased markedly with *T. trunculus* shell length. Some epibiotic polychaetes were located preferentially on/or nearby particular features of *T. trunculus* shells, which probably facilitate settlement and tube construction, in addition to provide some protection against tube damage. The main consequences of epibiosis (benefits and disadvantages) for the gastropod basibiont (*T. trunculus*) are discussed. The present study apparently indicates that besides burrowing into soft bottom substrates, sporadic inter-tidal exposure and mutual predation on epibiosis, *T. trunculus* lacks other typical antifouling defence adaptations.

INTRODUCTION

In most marine habitats, the dispersal stages (planktonic larvae) of sessile invertebrates depend on hard substrata to pursue ontogenesis (settlement and growth) (Wahl and Mark, 1999; Creed, 2000), and thousands of species with representatives from most marine phyla have adopted this mode of life for at least one ontogenetic phase (Wahl and Mark, 1999). Consequently, in the marine environment, any solid, exposed and undefended surface will sooner or later become fouled by a variety of hard bottom organisms (Wahl, 1989; Buschbaum and Reise, 1999). The fouling pressure varies with season, latitude, depth and local ecological factors (Wahl, 1989), but to escape fouling, any exposed surface must be ephemeral, regularly disturbed or defended against fouling (Wahl and Sönnichsen, 1992). By these reasons, the colonisation of abiotic and biotic surfaces by sessile micro- and macro-organisms is an extremely common phenomenon in the sea, where available hard substrata may easily become a limiting resource in an otherwise favourable environment (Wahl, 1989; Wahl and Mark, 1999; Creed, 2000). Therefore, in densely populated marine environments where competition for space is high, the advantage of colonising an unoccupied surface is probably the main reason for the fouling of living substrata (Wahl, 1989).

Recruitment onto living surfaces leads to epibiosis, which is basically the colonisation of a living substrate by sessile animals (epizoans) or plants (epiphytes) (Wahl *et al.*, 1997). By definition, epibiosis is an interspecific, non-symbiotic and facultative association, whereby one organism lives on the surface of another substrate organism (Wahl, 1989; Wahl *et al.*, 1997). Epibiosis is one of the spatially closest interspecific associations, including both direct and indirect interactions between the basibiont (host or substrate organism) and epibiont (fouling organism) (Wahl, 1996; Wahl and Mark, 1999). This association can be temporary, but often lasts for the entire lifespan of at least one of the organisms (Wahl, 1996; Warner, 1997; Buschbaum and Reise, 1999; Chan and Chan, 2005). Epibiosis creates a new interface between the host organism and its environment (Laudien and Wahl, 1999), replacing the basibiont/water interface by a basibiont/epibiont(s)/water interface (Wahl *et al.*, 1997). This can substantially change the basibiont's surface features and eventually influence some (or all) of its biological properties (such as size, shape, surface-volume ratio, surface topography, wettability and elasticity) (Wahl, 1996; Laudien and Wahl, 1999). By this reason, the presence of an epibiotic community can modify several interactions between the basibiont and the abiotic or biotic components of the system (Wahl, 1996; Wahl *et al.*, 1997; Wahl and Mark, 1999), with potential effects on the species involved and on community dynamics (Wahl and Mark, 1999).

An epibiotic association creates a complex network of direct and indirect interactions between basibiont and epibiont (Wahl, 1996), potentially causing a multitude of benefits and disadvantages for both colonised and colonising organisms (see review by Wahl, 1989). In each case, the relative importance of the positive and negative effects of epibiosis depends on environmental factors and on the biology and life history of basibionts and epibionts (species-specific factors) (Wahl, 1989; Wahl and Sönnichsen, 1992), and generally epibiosis cannot be classified globally as either "beneficial" or "detrimental" for either partner (Wahl, 1989). Any potential basibiont may adapt to epibiosis through at least three behavioural patterns: tolerance, avoidance and defence (Wahl, 1989). Since uncontrolled fouling is deleterious to most basibionts, many species have developed antifouling strategies and mechanisms to limit or prevent colonisation by epibionts (Wahl, 1989). Most of these antifouling defence systems are of multilevel nature (Wahl and Mark, 1999), comprising a multitude of mechanical, physical, chemical, behavioural, associational and/or life history defences, which may be found singly or in combination (Wahl, 1989; Wahl and Mark, 1999; Bers and Wahl, 2004).

The banded murex, *Hexaplex (Truncularioposis) trunculus* (Linnaeus, 1758), is distributed mainly in the Mediterranean Sea and adjacent Atlantic Ocean, from the Portuguese south coast, southward to Morocco and to the Madeira and Canary Archipelagos (Poppe and Goto, 1991; Houart, 2001). This species occurs in the inter-tidal and infra-littoral zones up to 100-120 metres depth (Poppe and Goto, 1991; Muzavor and Morenito, 1999), being more frequent at 0.3 - 30 metres depth (Houart, 2001). Inhabits both hard and soft substrates, from rocky shores (Houart, 2001) to sandy, sandy-muddy and preferentially muddy bottoms (Poppe and Goto, 1991; Macedo *et al.*, 1999; Muzavor and Morenito, 1999). Specimens attain a size comprehended between 80 mm (Poppe and Goto, 1991) and 90 mm (Macedo *et al.*, 1999; Muzavor and Morenito, 1999), exceptionally reaching a maximum size of 108 mm in shell length (Houart, 2001). *T. trunculus* is fished for human consumption in the Mediterranean Sea (Poppe and Goto, 1991; Anon., 2001; Houart, 2001) and in the Atlantic Ocean, namely in the Ria Formosa lagoon (Algarve coast - southern Portugal), where this species is subjected to a locally important artisanal fishery due to its high commercial value (Muzavor and Morenito, 1999).

Rocky substrata are very scarce in the Ria Formosa lagoon, where most parts of the seafloor are covered with soft-bottom substrata (sandy and muddy sediments). In these rather unstable habitats, sessile and sedentary benthic invertebrates depend on biogenic hard substrates to settle and grow, thus epibiosis becomes an alternative colonisation mechanism and valuable survival strategy. In the Ria Formosa lagoon, the shells of several epibenthic molluscs provide available and suitable hard substrata for the settlement of planktonic larvae, and therefore *T. trunculus* shells are usually colonised by a variety of epibiota, mostly polychaetes. The establishment of fouling communities on a wide variety of substrata has been investigated thoroughly and the resulting literature is vast (Wahl, 1989), with thousands of epibiotic associations reported from marine and freshwater habitats (Wahl and Mark, 1999). However, in the particular case of *T. trunculus*, besides generic descriptions (*e.g.* Spanier, 1981; Spanier and Karmon, 1987; Poppe and Goto, 1991; Anon., 2001) or single specific associations (*e.g.* Stachowitsch, 1980; Corriero and Pronzato, 1987), there is no detailed information available on the colonisation of this gastropod species by polychaetes. In this context, this work reports and illustrates the secondary colonisation of *T. trunculus* shells from the Ria Formosa lagoon, describing the composition, abundance and colonisation level of epibiotic polychaetes and analysing their spatial distribution on the basibiont shell. Finally, the main consequences of epibiosis (benefits and disadvantages) for the gastropod basibiont and the influence of some behavioural characteristics of *T. trunculus* on polychaete epibiosis are discussed.

MATERIAL AND METHODS

Based on a monthly sampling program (March 2003 - February 2004) in the Ria Formosa lagoon (Algarve coast - southern Portugal) (Figure 1), *T. trunculus* individuals from commercial samples caught in the vicinity of the Culatra Island were analysed for epibiosis.

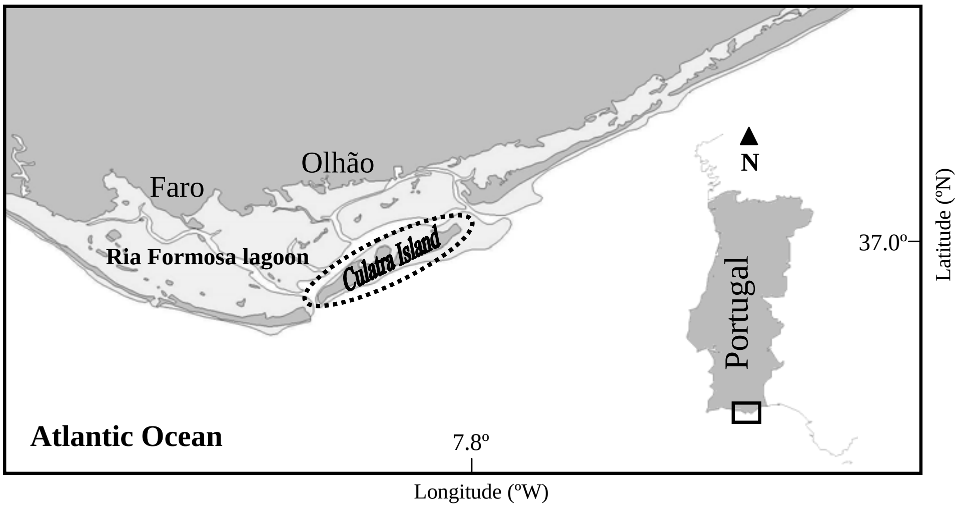


Figure 1. Geographical location of the Ria Formosa lagoon (Algarve coast - southern Portugal), with emphasis on the Culatra Island, where *Hexaplex (Truncularíopsis) trunculus* specimens were caught.

Initially, *T. trunculus* specimens were measured for shell length (SL - mm) with a digital calliper and subjected to visual inspection of the shell. Subsequently, their shell fouling level by polychaetes (degree of epibiosis) was estimated by eye with the following colonisation scores (Figure 2):

- score 0 = "non-fouled/colonised" (0% colonised shell surface coverage);
- score 1 = "intermediately fouled/colonised" (<25% colonised shell surface coverage);
- score 2 = "heavily fouled/colonised" (≥25% colonised shell surface coverage).

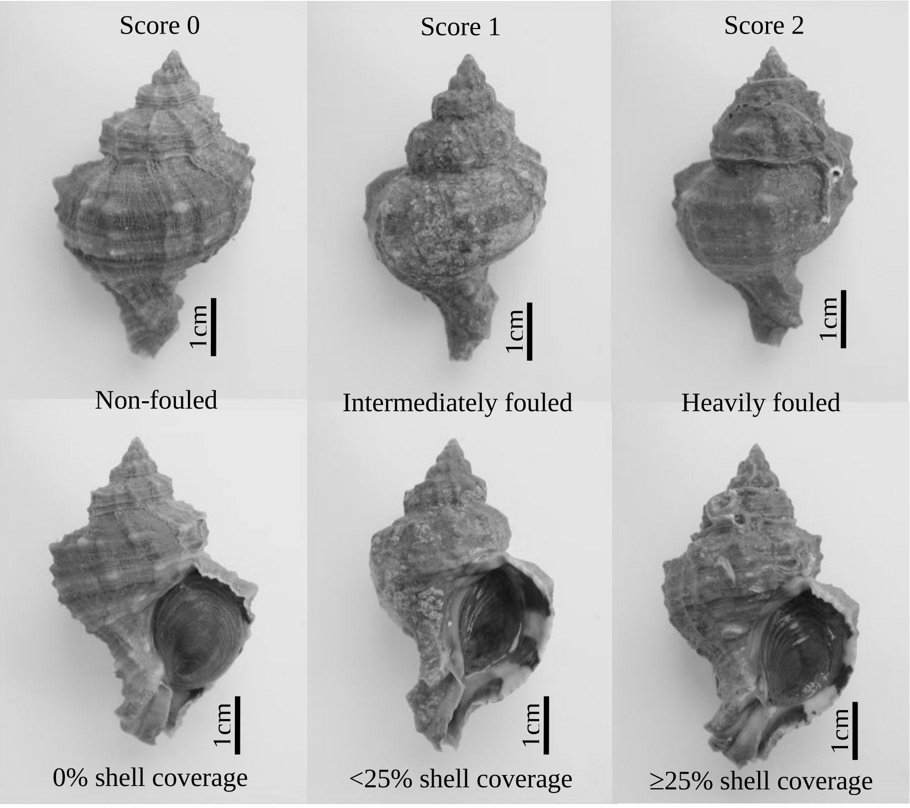


Figure 2. Illustration of the colonisation scores applied to estimate the fouling level (degree of epibiosis) of *Hexaplex (Trunculariopsis) trunculus* shells by epibiotic polychaetes.

The preferential areas/locations for colonisation of *T. trunculus* shells by some of the more abundant and conspicuous polychaete families were also registered. Simultaneously, the incidence of damages in the shell border, shell spire and/or siphonal canal was also assessed, and individuals were classified as "intact" or "damaged" in each of these parts of the shell.

Subsequently, all encrusting organisms were scrapped off and removed (with a dissection needle, stiletto or spatula depending on their type and size), fixed and preserved in 4% buffered formalin-seawater. The vast majority of these epibionts were polychaetes, but a few other colonisers also occurred on *T. trunculus* shells (namely unidentified algae, sponges, bryozoans, hydrozoans and ascidians, not considered in this study due to their low abundance and variety). Finally, polychaetes were identified to the lowest possible taxonomical level (which depended on whether specimens were intact or damaged, making unfeasible their absolute identification). Due to the very small size and high fragility of spirorbid tubes, it was virtually impossible to guarantee their complete removal from the gastropod shells, therefore were excluded from quantitative estimations (abundance and relative proportion), being only included in qualitative evaluations (fouling incidence, degree of epibiosis and preferential areas for colonisation).

RESULTS

A total of 2264 *T. trunculus* individuals were analysed for epibiosis by polychaetes, with an average shell length of 53.71 ± 8.58 mm (with a broad size range of 20.65 - 82.84 mm SL). Overall, 643 shells (28.4%) were non-fouled, while the remaining 1621 shells (71.6%) were colonised by polychaetes. Among these, 1513 shells (66.8%) were intermediately fouled, whereas 108 shells (4.8%) were heavily fouled by epibiotic polychaetes (Figure 3a). In terms of basibiont size, on average non-fouled *T. trunculus* individuals were smaller (50.16 ± 8.80 mm SL) than fouled specimens (55.12 ± 8.07 mm SL) (*Z*-test = 12.38; $p<0.05$), with intermediately fouled shells being also significantly smaller (54.77 ± 8.02 mm SL) than heavily fouled shells (60.06 ± 7.15 mm SL) (*Z*-test = 7.37; $p<0.05$) (Figure 3b).

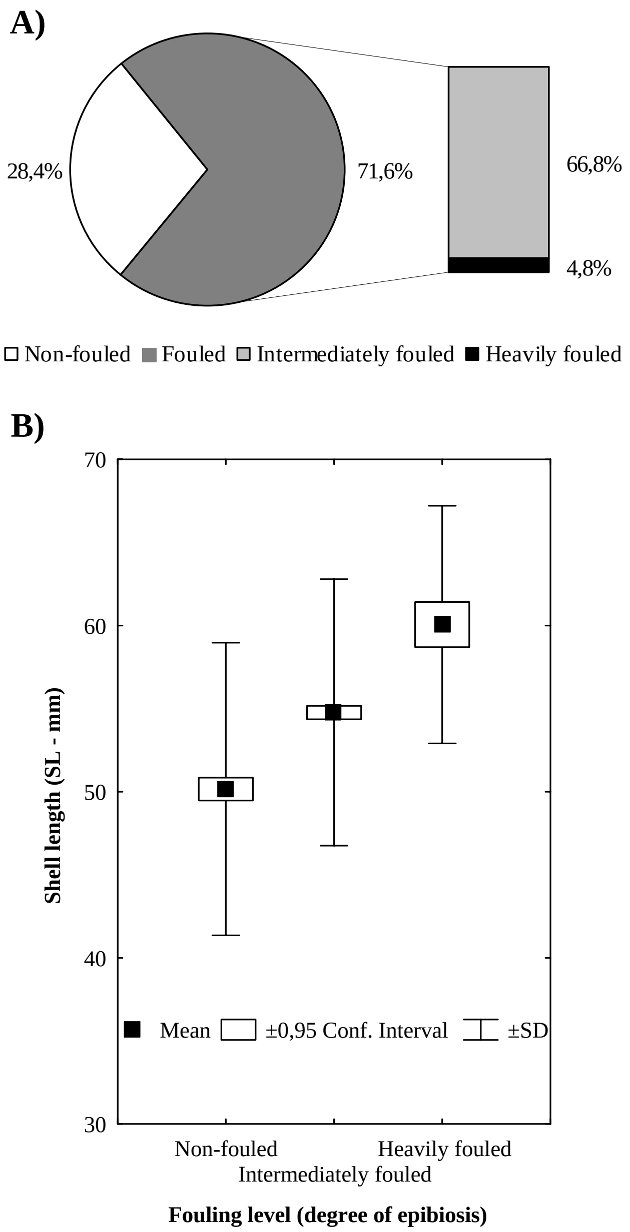


Figure 3. Colonisation of *Hexaplex (Trunculariopsis) trunculus* by epibiotic polychaetes, according to the fouling level of the shell (degree of epibiosis): **a)** Fouling incidence (percentage of colonised shells). **b)** Basibiont size (mean shell length, ± standard deviation, ± 95% confidence interval).

In order to assess the influence of basibiont size on the frequency and intensity of colonisation by epibiotic polychaetes, *T. trunculus* specimens were grouped into size classes (10 mm SL). The fouling incidence (percentage of colonised shells) and respective degree of epibiosis (mean colonisation score - MCS) in function of *T. trunculus* shell length classes are illustrated in Figure 4.

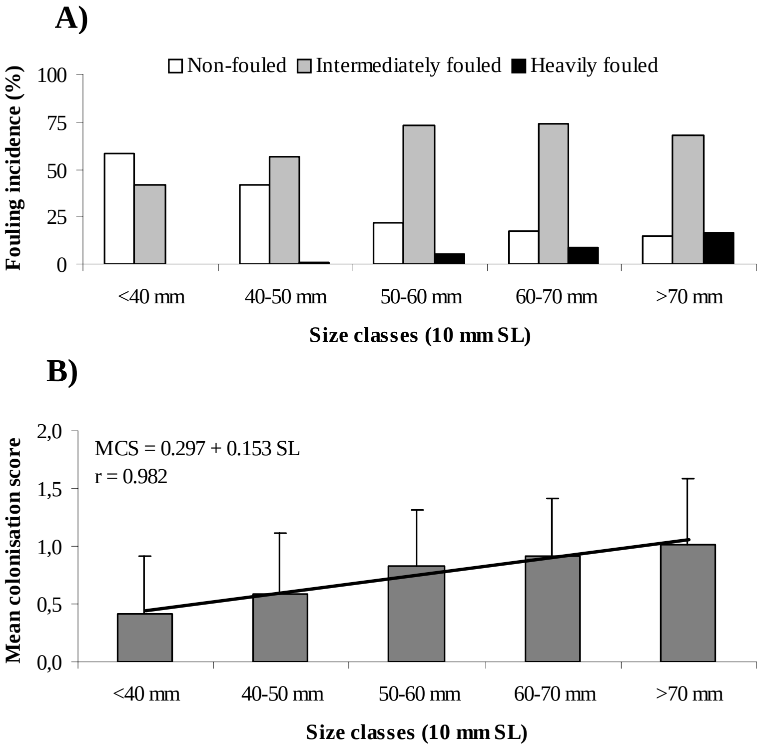


Figure 4. Frequency and intensity of fouling of *Hexaplex (Truncalariopsis) trunculus* shells by epibiotic polychaetes in function of the basibiont size classes (10 mm SL): **a)** Fouling incidence (percentage of colonised shells). **b)** Degree of epibiosis (mean colonisation score).

The frequency of fouled shells increased with basibiont size, with intermediately fouled shells varying from 42.2% in the smaller specimens (<40 mm SL) to 68.2% in the larger specimens (≥70 mm SL). Heavily fouled shells were not found among smaller individuals (<40 mm SL), but their frequency also increased in the remaining size classes, from 1.2% (40-50 mm SL) to 16.7% (≥70 mm SL) (Figure 4a). Simultaneously, an increasing trend was also registered between basibiont size and the respective shell epibiosis degree (MCS), *i.e.*, besides being less frequently fouled, smaller shells were also less intensely colonised (<40 mm SL: MCS = 0.42 ± 0.50) than larger *T. trunculus* shells (≥70 mm SL: MCS = 1.02 ± 0.57). This intimate relationship between basibiont size and epibiosis degree was further emphasised by the high correlation coefficient (*r* = 0.982) obtained in the linear regression established between shell length classes and the respective mean colonisation scores (Figure 4b).

The epibiotic polychaetes removed and identified in the shells of *T. trunculus* are listed in Table 1. A total of 2880 polychaetes were identified, belonging to 10 families and 25 polychaete *taxa*. Spirorbids (not quantified due to the reasons explained above) were by far the most abundant polychaete colonisers of *T. trunculus* shells, followed by the families Serpulidae (67.5%) represented mostly by *Pomatoceros lamarckii*, (1722 individuals), Sabellidae (10.2%) represented mainly by *Perkinsiana* sp. (214 individuals) and *Chone* cf. *acustica* (77 individuals), Sabellariidae (9.6%) represented exclusively by *Sabellaria* cf. *spinulosa* (278 individuals), and Cirratulidae (8.5%) represented only by *Aphelochaeta* sp. (246 individuals) (Table 1).

The spatial distribution of epibiotic polychaetes on shells of *T. trunculus* varied among the different areas/locations of the shell, but usually the dorsal surface was more fouled than the ventral surface. Generally, the most frequently and heavily colonised parts were the spire and the siphonal canal, although sporadically some smaller specimens were also lodged into a small hole formed between a varix and the siphonal fasciole. Nevertheless, even in heavily fouled specimens, the shell surfaces in permanent contact with the soft parts of the organism (internal surface and borders of the shell) were invariably maintained clean of epibiotic polychaetes. The preferential areas/locations for colonisation of *T. trunculus* shells by some of the more frequent and conspicuous polychaete families (*e.g.* Spirorbidae, Sabellariidae and Spionidae) are schematically illustrated in Figure 5.

Table 1. Taxonomic identification, abundance and relative proportion of the epibiotic polychaetes removed from the shells of *Hexaplex (Truncularioptsis) trunculus*.

Family	**Species**	**Number**	**Family proportion (%)**	**Species proportion (%)**
Spionidae	*Polydora hoplura* Claparède, 1870	77	2.7%	2.7%
Cirratulidae	*Aphelochaeta* sp.	246	8.5%	8.5%
Capitellidae	*Notomastus* sp.	1	0.1%	0.0%
	Capitella spp.	2		0.1%
Syllidae	*Exogone verugera* (Claparède, 1868)	14	1.2%	0.5%
	Sphaerosyliis pirifera Claparède, 1868	5		0.2%
	Syllis sp. I	1		0.0%
	Syllis gracilis Grübe, 1840	13		0.4%
	Pionosyllis morenoae San Martín, 1984	1		0.0%
Nereidae	*Nereis caudata* (Delle Chiaje, 1825)	1	0.1%	0.0%
	Ceratonereis costae (Grübe, 1840)	1		0.0%
Dorvilleidae	*Ophryotrocha* sp.	3	0.1%	0.1%
Sabellariidae	*Sabellaria* cf. *spinulosa* Leuckart, 1849	278	9.6%	9.6%
Sabellidae	*Branchiomma* sp.	1	10.2%	0.0%
	Perkinsiana sp.	214		7.4%
	Chone cf. *acustica* (Claparède, 1870)	77		2.7%
	Fabricia sp.	1		0.0%
Serpulidae	*Pomatoceros lamarckii* (Quatrefages, 1865)	1722	67.5%	59.8%
	Pomatoceros triqueter (Linnaeus, 1767)	7		0.2%
	Serpulidae spp.	152		5.3%
	Serpula vermicularis Linnaeus, 1767	20		0.7%
	Vermiliopsis striaticeps (Grübe, 1862)	41		1.4%
	Hydroides elegans (Haswell, 1883)	2		0.1%
	Hydroides stoichadon Zibrowius, 1971	1		0.0%
Spirorbidae	Spirorbiidae spp.	*	*	*
Total		**2880**	**100.0%**	**100.0%**

* Polychaetes belonging to family Spirorbidae were excluded from calculations.

Most polychaete families revealed an apparently random distribution on *T. trunculus* shells, phenomenon that was particularly noticeable with family Spirorbidae, whose tubes were arbitrarily disseminated all over the shell surface (Figure 5a). Contrarily, other polychaete families made use of some shell features to facilitate their settlement and tube construction, phenomenon that was particularly evident with families Sabellariidae (represented by *S. spinulosa*) and Spionidae (represented by *Polydora hoplura*). Actually, the sandy tubes of *S. spinulosa* were mainly constructed horizontally around the shell spire and/or vertically on the shell varices or around the siphonal canal (Figure 5b), whereas *P. hoplura* settled preferentially on/or nearby the shell spire, around the siphonal canal and on the siphonal fasciole (Figure 5c), also occurring along the edge of other polychaete tubes.

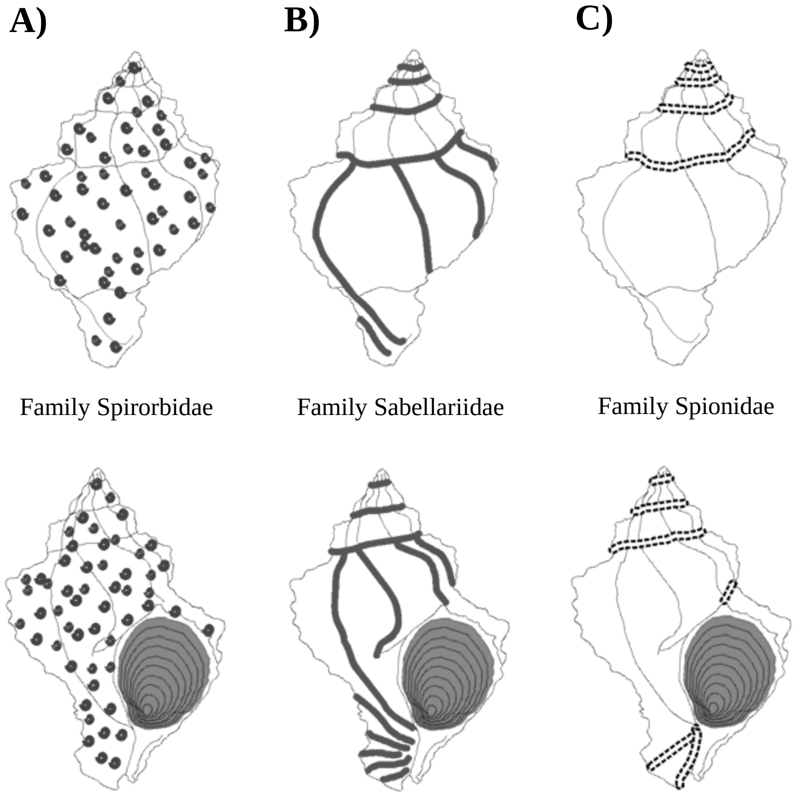


Figure 5. Schematic representation of the preferential areas/locations of the shell for the colonisation of *Hexaplex (Truncilariopsis) trunculus* by some of the more abundant and conspicuous polychaete families: **a)** Family Spirorbidae. **b)** Family Sabellariidae (represented by *Sabellaria* cf. *spinulosa*). **c)** Family Spionidae (represented by *Polydora hoplura*). Designed for illustration purposes, basibiont shells and polychaete tubes are not necessarily represented in the same dimensional scale.

Concerning shell damages, among the 2264 *T. trunculus* specimens analysed for epibiosis by polychaetes, 558 (24.6%) were damaged in the shell border, 609 (26.9%) were broken in the siphonal canal and 68 (3.0%) were damaged/eroded in the shell spire. However, while the individuals damaged in the shell border and siphonal canal were apparently broken during fishing operations and handling, on the opposite, the damages in the shell spire (eroded spire and/or truncated apex), where most spionid polychaetes settled, were probably caused by boreholes of *P. hoplura*, the only boring polychaete identified on *T. trunculus* shells. Moreover, whereas the frequency of damages in the shell border and siphonal canal were apparently independent of basibiont size, on the contrary, damages in the shell spire clearly increased with basibiont shell length.

DISCUSSION

Influence of basibiont size on polychaete epibiosis

Fouling is evenly likely to be a function of time (Wahl, 1989), therefore shell cover by epibiota depends on shell age, *i.e.*, the longer the shell has been available for settlement (longer exposure duration), the more likely it is to be colonised (Warner, 1997). In the present case, the fouling frequency or incidence (percentage of colonised shells), fouling intensity or degree of epibiosis (mean colonisation score) and incidence of damages in the shell spire (attributable to the boring polychaete *P. hoplura*), increased markedly with shell length (and age), revealing that fouling of *T. trunculus* shells by epibiotic polychaetes is a time-dependent phenomenon that increases during basibiont ontogenesis. Just for illustrative purposes, the von Bertalanffy growth function estimated for *T. trunculus* from the Ria Formosa lagoon (Vasconcelos *et al.*, 2006) revealed that the sizes 40, 50, 60 and 70 mm SL (the limits of the size classes used in this study) are reached at approximately 1.6, 2.2, 3.1 and 4.5 years old, respectively. Creed (2000) also found that larger cerith shells (*Cerithium atratum*) had a higher abundance of epizoites, including the tube-forming polychaete *Hydroides plateni*. However, whether this was because larger hosts provided a greater colonisable area or represented older organisms whose surface has been available for colonisation for a longer period was not clear (Creed, 2000).

Additionally, since shell age influences its surface characteristics, the shells of smaller gastropods are less colonised by epifauna than those of larger gastropods also because they are less attractive for settlement, since the shell laid down during the early life-stages is smoother and has a greater proportion of the surface covered by periostracum (Warner, 1997). However, this phenomenon can also be attenuated by shell erosion and scouring over time, with older shells being smoother than younger shells (Bell, 2005). Moreover, in this particular case, the burrowing behaviour of *T. trunculus* into soft-bottom substrates (Spanier, 1981, 1986; Spanier and Karmon, 1987; Rilov *et al.*, 2004) certainly increases the abrasion of the shell, smoothing its surface texture and potentially destroying some of the less resistant polychaete tubes.

Assemblage of epibiotic polychaetes

The shells of *T. trunculus* are generally colonised by various epiphytes and epizoans, namely algae (Spanier, 1981; Spanier and Karmon, 1987; Anon., 2001), calcareous growths (Poppe and Goto, 1991), sponges (*e.g. Clathria* sp.) (Corriero and Pronzato, 1987; Anon., 2001), bryozoans and hydrozoans (Anon., 2001), anemones (*e.g. Calliactis parasitica*) (Stachowitsch, 1980), barnacles (Spanier, 1981; Spanier and Karmon, 1987; Rilov *et al.*, 2004), polychaetes and tube worms (Spanier, 1981; Spanier and Karmon, 1987; Anon., 2001; Rilov *et al.*, 2004).

Polychaetes are among the most frequent and abundant marine metazoans in benthic environments (Fauchald and Jumars, 1979), therefore the establishment of associations with other marine invertebrates is a rather common phenomenon (Martin and Britayev, 1998). The present study revealed that in the Ria Formosa lagoon, *T. trunculus* acts as a mobile hard substratum for polychaetes secondary colonisation, congregating a diversified group of polychaete species, and to the authors' best knowledge this is the first detailed information available on the fouling of this gastropod species by epibiotic polychaetes.

This diverse assemblage of epibiotic polychaetes intimately reflects the variety of habitats occupied by *T. trunculus* in the Ria Formosa lagoon, inhabiting sandy, sandy-muddy and preferentially muddy sediments (Poppe and Goto, 1991; Macedo *et al.*, 1999; Muzavor and Morenito, 1999). By this reason, *T. trunculus* shells presented epibiotic polychaetes from distinct habitats, namely typical inhabitants of sandy substrates (*e.g.* Sabellariidae), organic matter enriched bottoms (*e.g.* Cirratulidae and Capitellidae) and rocky substrates (*e.g.* Sabellidae and Serpulidae). This was also reflected in the occurrence of epibiotic polychaetes with distinct degrees of mobility, such as sessile (*e.g.* Sabellariidae, Sabellidae, Serpulidae and Spirorbidae), discretely motile (*e.g.* Spionidae) and completely motile species (*e.g.* Cirratulidae, Capitellidae, Syllidae and Dorvilleidae) (Fauchald and Jumars, 1979), as well as with different life-styles, such as boring polychaete species (*e.g. P. hoplura*). These findings contradict the observations made on *T. trunculus* from the Gulf of Trieste (North Adriatic Sea) by Stachowitsch (1980), who found that living specimens were virtually epizoite-free and never contained endolithic species, namely *Polydora* sp.

Distribution of epibiotic polychaetes on the basibiont shell

In the shells of *T. trunculus*, the dorsal surface was generally more fouled by epibiotic polychaetes than the ventral surface, probably due to the fact that this is a hydrodynamically favourable position that ensures higher water flow rates, and therefore a greater food supply and a more efficient waste evacuation for epibionts (Wahl, 1989). In addition, surface texture is also important for the settlement of encrusting organisms (Bell, 2005), and a rough and uneven surface texture is known to attract more settlement than a smooth surface (Warner, 1997). In the present case, despite having low pronounced shell ornamentation, some shell features of *T. trunculus* still provided a variety of refuges for small polychaetes, namely into a hollow formed by the varix and the siphonal fasciole. This has also been reported for *Hexaplex nigritus*, whose ornamentation provides micro-habitats of protection for juvenile phases of mobile individuals, contributing to the defence against predators (Olabarria, 2000).

Although most types of polychaete tubes were randomly distributed on *T. trunculus* shells (*e.g.* Spirorbidae), some others were located on/or nearby particular shell features (*e.g.* Sabellariidae and Spionidae). In the case of *S. spinulosa*, the sandy tubes were mainly constructed horizontally around the shell spire and/or vertically on the shell varices or around the siphonal canal, while in the case of *P. hoplura*, the tubes and galleries were preferentially positioned on/or nearby the shell spire, around the siphonal canal and in the siphonal fasciole, also occurring along the edge of other polychaete tubes. In both cases, these preferential locations nearby those shell features are probably related to an easier larvae settlement, less effort invested in tube construction, and also stronger support and protection against tube damage or abrasion/erosion during basibiont burrowing into soft-bottom sediments.

The preferential location of spionid polychaetes in the spire zone corresponded to the most eroded and damaged area in *T. trunculus* shells. Actually, some specimens (mostly aged individuals) presented shells with eroded spire and truncated apex, presumably due to the boring activity of *P. hoplura*. The spire zone is the older, thinner and weaker part of the shell, because the shell laid down during the rapid growth characteristic of the early life-stages is smooth and with a thin periostracum (Warner, 1997). According to Stachowitsch (1980), the spines and siphonal canal of *T. trunculus* were frequently damaged or missing due to shell damaging species, but despite being found all over the shell, *Polydora* sp. was especially common in the shell apex. Infestations and similar shell damages caused by spionid polychaetes on gastropod shells have been reported for *P. ciliata* inhabiting *Littorina littorea* (Warner, 1997), *P. hoplura* and *Boccardia knoxi* colonising *Haliotis rubra* and *H. laevigata*

(Lleonart *et al.*, 2003), and *P. rickettsi* parasiting *Crepidula fecunda* (Bertrán *et al.*, 2005). The alternative location of spionid polychaetes along the edge of serpulid tubes (where the tube made an angle with the surface of the shell), has also been reported for *P. ciliata* colonising *L. littorea* shells and was attributed to the additional irregularities provided on the shell surface and distortions at the suture, which increase the settlement of *P. ciliata* by attracting settling larvae (Warner, 1997).

Consequences of polychaete epibiosis for the basibiont

Numerous consequences of epibiosis have been reported for both basibionts and epibionts (see review by Wahl, 1989). In the present case, shell colonisation by epibiotic polychaetes may also entail some benefits and disadvantages (both theoretical and practical) for *T. trunculus*, but it is worth emphasising that some considerations on this subject are just hypothetical and should be confirmed experimentally.

Some molluscs possess specific adaptations that allow them to survive in the air for a long time and to retain internal water even under high temperatures, avoiding extreme dehydration of the soft tissues. In gastropods, a solid shell is an essential morphological adaptation to drying (Alyakrinskaya, 2004). *T. trunculus* has a considerably solid and thick shell, as revealed by its relative shell weight, comprised on average between 61.85% (P. Vasconcelos, unpublished data), 66.4% (Dalla Via and Tappeiner, 1981) and 65.76% to 66.70% of total weight (Alyakrinskaya, 2004, 2005). However, despite this apparently efficient protection that could attenuate heating and drying under unfavourable environmental conditions, *T. trunculus* has a relatively low resistance to desiccation (50% of mortality after 60 hours of exposure to warm air) (Rilov *et al.*, 2004). Possibly by this reason, under high temperatures in the Mediterranean Sea, this species was only detected sub-tidally in shallow protected areas, in deeper water in wave exposed shores, or off-shore at greater depths (Rilov *et al.*, 2004).

Contrarily, in the inter-tidal areas of the Ria Formosa lagoon, *T. trunculus* is occasionally found during low tide predating bivalves in aquaculture beds (although not under severe drying conditions). In this case, shell coverage by epibiotic polychaetes should contribute for protecting from harmful heat and desiccation. In fact, among the most important benefits of epibiosis for a temporarily emersed basibiont, should be the insulating effect and protection against desiccation and harmful irradiation or desalination (Wahl and Sönnichsen, 1992; Wahl, 1996; Wahl and Mark, 1999), namely through water-retaining epibionts that slow down desiccation of intertidal organisms during low tide (Wahl, 1989). For instance, fouled *Batillaria zonalis* had lower shell and body temperatures than non-fouled specimens, indicating that epibionts could prevent this gastropod from absorbing heat from solar radiation during summer low tides (Chan and Chan, 2005). Similarly, during air exposure under laboratory conditions, fouled shells of *T. trunculus* kept moisture longer than non-fouled shells, mostly in the shell areas nearby water-retaining polychaete tubes (P. Vasconcelos, personal observation).

T. trunculus is a very active, non-selective carnivore and scavenger species (Spanier and Karmon, 1987; Rilov *et al.*, 2004) that supplements its generalist diet with cannibalism (Spanier, 1981, 1986; Spanier and Karmon, 1987; Basedow, 1994, 1996; Rilov *et al.*, 2004). However, laboratory observations indicated that cannibalism by drilling the shells of conspecifics only occurred under artificial conditions, when specimens were kept together for a considerable time without alternative food supply (Spanier, 1981, 1986; Spanier and Karmon, 1987; Rilov *et al.*, 2004). Under these circumstances, shell fouling may be advantageous to *T. trunculus*, since cannibalistic drilling is affected by the colonisation level of the shell, with a preference of *T. trunculus* for drilling conspecifics with little or no fouling rather than those heavily colonised (87% *vs.* 54% of the shells), where the chance of a successful drilling (*i.e.*, reaching the flesh) is greater (Spanier, 1981; Spanier and Karmon, 1987).

Fouling may also involve several disadvantages for the basibiont, but in the present case the degree of epibiosis by polychaetes on *T. trunculus* shells apparently is not enough to cause highly detrimental impacts on the basibiont. Nevertheless, in more heavily fouled gastropods the increased weight and drag provoked by larger polychaetes (*e.g.* serpulid and spirorbid tubes) may still affect their mobility, agility and ability to burrow into soft bottom sediments. Actually, there are several examples of this kind of negative impacts of epibiosis on diverse gastropod species, such as restricted mobility and lower crawling speed in *L. littorea* (Buschbaum and Reise, 1999), and reduced locomotion (lower crawling speed and movement tortuosity) in *B. zonalis* (Chan and Chan, 2005).

Epibiosis in gastropods is known to enhance the risk of shell destruction by mechanical and chemical damages caused by boring organisms (Wahl, 1989, 1996; Wahl and Sönnichsen, 1992; Bers and Wahl, 2004). A large portion of the burrowing polychaetes belongs to the family Spionidae, particularly to the genus *Polydora*, endolithic polychaetes that on calcareous substrata (such as shells) bore into the shell, create extensive galleries and provoke great damage to the gastropod shells (Bertrán *et al.*, 2005). In this context, the most evident disadvantage of epibiosis on *T. trunculus* shells is the colonisation by the boring polychaete *P. hoplura*, because it leads to the gradual destruction of the gastropod shell, possibly affecting its condition and growth and increasing vulnerability to predators. For instance, infestation by polydoran worms reduced the condition and growth of *Haliotis rubra* and *H. laevigata* (Lleonart *et al.*, 2003), and significantly weakened the shell structure of *L. littorea* that became more easily cracked by predators (Buckley and Ebersole, 1994).

Basibiont behaviour and polychaete epibiosis

T. trunculus apparently lacks typical antifouling adaptations, *i.e.*, mechanical, physical and chemical defences seem to be negligible or nonexistent in this species. However, this assumption is restricted to the size range analysed for epibiosis (20.65 - 82.84 mm SL) and therefore should not be extrapolated to very young specimens (<20 mm SL, not available for this study). Nevertheless, some behavioural characteristics of *T. trunculus* may still contribute to keep shell fouling at a tolerable level.

Field and laboratory studies indicate that *T. trunculus* burrows shallowly into soft sediments (with siphon tips in contact with the water), trying to avoid both the unfavourably low (hibernation) and high water temperatures (aestivation) (Spanier, 1981, 1986; Spanier and Karmon, 1987), being often observed partially buried in the sand (Spanier, 1986; Rilov *et al.*, 2004). In burrowing species, this friction between the sediment and the body surface may reduce the degree of fouling (Wahl, 1989). This burrowing behaviour of *T. trunculus* induces a temporary shielding from planktonic settlers, may provoke suffocation and/or erosion of colonising organisms by sand (Rilov *et al.*, 2004), and therefore, epibiotic colonisers of *T. trunculus* shells must be able to survive periodical burial (Stachowitsch, 1980). However, and as occurs with other species, it should also be considered that fouling might progressively reduce the gastropod ability to burrow into the sediments (Chan and Chan, 2005).

Mobile gastropod basibionts may expose epibionts to environmental conditions that are harmful for polychaete colonisers (Olabarria, 2000). Tidal emersion is probably a primary factor controlling epibionts on gastropod shells, as they will be periodically exposed to unfavourable environments at low tide, and thus may experience heat, oxygen stress and desiccation (Bell, 2005; Bertrán *et al.*, 2005). Most polychaete species that colonise *T. trunculus* shells are largely tolerant to aerial exposure and moderate desiccation (*e.g.* sabellarids, sabellids and serpulids retract into their tubes to avoid excessive water loss), but some species might be negatively affected by the sporadic inter-tidal exposure of *T. trunculus* during low tide in the Ria Formosa lagoon (*e.g. P. hoplura*). In fact, the boring polychaetes (*Polydora* spp.) inhabiting gastropod shells are considered to be both sedimentary and filter feeders and therefore are limited to feeding during immersion (Bertrán *et al.*, 2005).

Finally, because the generalist diet of *T. trunculus* includes polychaetes (Spanier, 1981; Spanier and Karmon, 1987), eating these worms on the shells of conspecifics (mutual predation on epibiosis) might also contribute to control the profusion of epibiotic polychaetes and reduce shell fouling (Rilov *et al.*, 2004). Moreover, since the presence of specific epibionts could deceive or deter further colonisers (Wahl, 1989), this phenomenon may also help keeping *T. trunculus* shell fouling at a tolerable level.

ACKNOWLEDGEMENTS

The authors would like to thank the technical staff of IPIMAR - CRIPSul for their help in the laboratory sampling procedures. Thanks are also due to Dr. Paula F. Pereira and MSc. Fábio Pereira for their assistance in the taxonomic identification of polychaetes. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001).

REFERENCES

Alyakrinskaya, I.O., 2004. Resistance to drying in aquatic mollusks. *Biology Bulletin*, 31: 299-309.

Alyakrinskaya, I.O., 2005. Functional significance and weight properties of the shell in some molluscs. *Biology Bulletin*, 32: 397-418.

Anon., 2001. *Especies de Interés Pesquero en el Litoral de Andalucía*. Junta de Andalucía. Consejería de Agricultura y Pesca, Sevilla.

Basedow, T., 1994. Analysis of shells on beaches and rocky coasts of Europe led to new findings on nutritional and population ecology of predatory marine gastropods (Muricidae and Naticidae). *Zoologische Beiträge*, 36: 29-48.

Basedow, T., 1996. Contributions to the nutritional and population ecology of *Murex trunculus* (L. 1767) (Gastropoda, Muricidae), derived from findings at the north coast of Crete (Eastern Mediterranean), with remarks on two species of Naticidae (Gastropoda). *Zoologische Beiträge*, 37: 157-170.

Bell, J.J., 2005. Influence of occupant microhabitat on the composition of encrusting communities on gastropod shells. *Marine Biology*, 147: 653-661.

Bers, A.V.; Wahl, M., 2004. The influence of natural surface microtopographies on fouling. *Biofouling*, 20: 43-51.

Bertrán, C.; Vargas, L.; Quijón, P., 2005. Infestation of *Polydora rickettsi* (Polychaeta: Spionidae) in shells of *Crepidula fecunda* (Mollusca: Calyptraeidae) in relation to intertidal exposure at Yaldad Bay, Chiloe, Chile. *Scientia Marina*, 69: 99-103.

Buckley, W.J.; Ebersole, J.P., 1994. Symbiotic organisms increase the vulnerability of a hermit crab to predation. *Journal of Experimental Marine Biology and Ecology*, 182: 49-64.

Buschbaum, C.; Reise, K., 1999. Effects of barnacle epibionts on the periwinkle *Littorina littorea* (L.). *Helgoland Marine Research*, 53: 56-61.

Chan, D.H.L.; Chan, B.K.K., 2005. Effect of epibiosis on the fitness of the sandy shore snail *Batillaria zonalis* in Hong Kong. *Marine Biology*, 146: 695-705.

Corriero, G.; Pronzato, R., 1987. Epibiontic sponges on the bivalve *Pinna nobilis*. *Marine Ecology Progress Series*, 35: 75-82.

Creed, J.C., 2000. Epibiosis on cerith shells in a seagrass bed: correlation of shell occupant with epizoite distribution and abundance. *Marine Biology*, 137: 775-782.

Dalla Via, G.-F.; Tappeiner, U., 1981. Morphological and functional correlates with distribution of *Murex trunculus* L. and *Murex brandaris* L. (Mollusca, Gastropoda) in the northern Adriatic. *Bollettino di Zoologia*, 48: 191-195.

Fauchald, K.; Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanography and Marine Biology. An Annual Review*, 17: 193-284.

Houart, R., 2001. *A Review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Ed. Evolver, Rome.

Laudien, J.; Wahl, M., 1999. Indirect effects of epibiosis on host mortality: seastar predation on differently fouled mussels. *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 20: 35-47.

Lleonart, M.; Handlinger, J.; Powell, M., 2003. Spionid mudworm infestation of farmed abalone (*Haliotis* spp.). *Aquaculture*, 221: 85-96.

Macedo, M.C.C.; Macedo, M.I.C.; Borges, J.P., 1999. *Conchas Marinhas de Portugal (Seashells of Portugal)*. Editorial Verbo, Lisboa.

Martin, D.; Britayev, T.A., 1998. Symbiotic polychaetes: review of known species. *Oceanography and Marine Biology. An Annual Review*, 36: 217-340.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Olabarria, C., 2000. Epibiont molluscs on neogastropod shells from sandy bottoms, Pacific coast of Mexico. *Journal of the Marine Biological Association of the United Kingdom*, 80: 291-298.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Rilov, G.; Benayahu, Y.; Gasith, A., 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? *Marine Ecology Progress Series*, 282: 193-204.

Spanier, E., 1981. Behavioral ecology of the marine snail *Trunculariopsis (Murex) trunculus*. In: *Developments in Arid Zone Ecology and Environmental Quality* (Shuval, H., Ed.), Balaban ISS, Philadelphia, 65-70 pp.

Spanier, E., 1986. Cannibalism in muricid snails as a possible explanation for archaeological findings. *Journal of Archaeological Science*, 13: 463-468.

Spanier, E.; Karmon, N., 1987. Muricid snails and the ancient dye industries. In: *The Royal Purple and the Biblical Blue: Argaman and Tekhelet* (Spanier, E., Ed.), Keter Publishing House Jerusalem Ltd., Jerusalem, 179-192 pp.

Stachowitsch, M., 1980. The epibiotic and endolithic species associated with the gastropod shells inhabited by the hermit crabs *Paguristes oculatus* and *Pagurus cuanensis*. *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 1: 73-101.

Vasconcelos, P.; Gaspar, M.B.; Pereira, A.M.; Castro, M., 2006. Growth rate estimation of *Hexaplex (Truncularioptsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast, southern Portugal). *Journal of Shellfish Research*, 25: 249-256.

Wahl, M., 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series*, 58: 175-189.

Wahl, M., 1996. Fouled snails in flow: potential of epibionts on *Littorina littorea* to increase drag and reduce snail growth rates. *Marine Ecology Progress Series*, 138: 157-168.

Wahl, M.; Sönnichsen, H., 1992. Marine epibiosis. IV. The periwinkle *Littorina littorea* lacks typical antifouling defences - why are some populations so little fouled? *Marine Ecology Progress Series*, 88: 225-235.

Wahl, M.; Mark, O., 1999. The predominantly facultative nature of epibiosis: experimental and observational evidence. *Marine Ecology Progress Series*, 187: 59-66.

Wahl, M., Hay, M.E.; Enderlein, P., 1997. Effects of epibiosis on consumer-prey interactions. *Hydrobiologia*, 355: 49-59.

Warner, G.F., 1997. Occurrence of epifauna on the periwinkle, *Littorina littorea* (L.), and interactions with the polychaete *Polydora ciliata* (Johnston). *Hydrobiologia*, 355: 41-47.

SECTION 4

REPRODUCTIVE CYCLE OF *HEXAPLEX (TRUNCULARIOPSIS) TRUNCULUS* IN THE RIA FORMOSA LAGOON

CHAPTER 4.1

Gametogenic cycle of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal)

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar, M.B. Gametogenic cycle of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of the Marine Biological Association of the United Kingdom* (submitted).

ABSTRACT

The gametogenic cycle of the banded murex, *Hexaplex (Trunculariopsis) trunculus*, from the Ria Formosa lagoon (Algarve coast - southern Portugal) was studied through macro- and microscopic analyses of male and female gonads during a 1-year study period (March 2003 - February 2004). Simultaneously, the macroscopic aspect of other organs of the female reproductive system (sperm-ingesting gland and capsule gland) was also observed to determine their significance for the assessment of sexual maturity and reproductive stage. The overall samples presented a balanced sex-ratio, with males dominating among smaller individuals and females predominating in larger size classes. The gametogenic cycle of *T. trunculus* in the Ria Formosa lagoon covered an extensive period of gonadal activity, with the simultaneous occurrence of distinct developmental stages of gametogenesis in both sexes almost year-round, and with males invariably presenting gonads in more developed stages of gametogenesis than females. The microscopic analysis of histological sections of the gonads and the calculation of the mean gonad index (MGI) revealed that spawning occurred mainly between May and June 2003 (apparently triggered by the rising seawater temperature during spring), followed by another spawning event in October and November 2003. Due to the extensive period of gonadal activity, *T. trunculus* exhibited a short resting phase that occurred simultaneously in both sexes in July and August 2003 and coincided with the period of warmest seawater temperatures. Taking into consideration these findings, the main repercussions for the management of the artisanal fishery and for the assessment of the potential of *T. trunculus* for molluscan aquaculture are discussed.

INTRODUCTION

The banded murex, *Hexaplex (Truncülariopsis) trunculus* (Linnaeus, 1758), is distributed in the Mediterranean Sea and adjacent Atlantic Ocean, from the Portuguese coast southward to Morocco and to the Madeira and Canary Archipelagos (Poppe and Goto, 1991; Houart, 2001). This species occurs in the inter-tidal and infra-littoral zones up to 100-120 metres depth (Poppe and Goto, 1991; Muzavor and Morenito, 1999), being more frequent at 0.3 - 30 metres depth (Houart, 2001). It inhabits both hard and soft substrates, from rocky shores (Houart, 2001) to sandy, sandy-muddy and preferentially muddy bottoms (Poppe and Goto, 1991; Macedo *et al.*, 1999; Muzavor and Morenito, 1999). Generally, specimens attain a maximum size between 80 mm (Poppe and Goto, 1991) and 90 mm (Macedo *et al.*, 1999; Muzavor and Morenito, 1999), only exceptionally reaching a maximum shell length of 108 mm (Houart, 2001).

The banded murex is fished for human consumption both in the Mediterranean Sea (Poppe and Goto, 1991; Houart, 2001) and in the Atlantic Ocean, namely in the Ria Formosa (Algarve coast - southern Portugal), where *T. trunculus* is the target species of a locally important fishery in the subtidal areas of the lagoon, traditionally undertaken with an illegal and artisanal fishing gear (baited with cockles), locally known as “wallet-line”. The commercial value of the banded murex in Portugal has increased markedly in recent years (reaching prices of 10-15€/kg for first sale), both due to the growing demand for gastropod species in the Portuguese seafood market and to the declining catches of *T. trunculus* in the Ria Formosa lagoon.

Some literature exists on diverse aspects of the reproduction of *T. trunculus*, namely descriptions of the spawning behaviour and egg-laying pattern, egg-masses and egg-capsules, embryonic and early post-metamorphic development (Fischer and Raffy, 1933; Dulzetto, 1946, 1950; Wondrak, 1974; Bandel, 1975; Barash and Zenziper, 1980; Vasconcelos *et al.*, 2004; Lahbib *et al.*, 2006). However, the most studied subject with consequences in the reproduction of *T. trunculus* is undoubtedly the imposex phenomenon (genital abnormalities caused by the development and superimposition of male secondary sexual characters - penis and vas deferens - onto females, ultimately inducing sterility and reproductive failure). Imposex was first described in this species by Martoja and Bouquegneau (1988) and since then has been monitored in many populations from the Mediterranean Sea and Atlantic Ocean, including the Ria Formosa lagoon (southern Portugal) (Gibbs *et al.*, 1997; Langston *et al.*, 1997; Coelho *et al.*, 1998; Vasconcelos *et al.*, 2006). Comparatively, the information available on the gametogenesis and gonadal cycle of *T. trunculus* is scarcer and restricted to Mediterranean populations of this species, disseminated in studies published by Schitz (1920), Bolognari *et al.* (1979, 1981), Albanese *et al.* (1983), Martoja and Bouquegneau (1988), Tirado *et al.* (2002) and Lahbib *et al.* (2004).

In this context, the aim of the present work was to study the reproductive dynamics and gametogenic cycle of *T. trunculus* from the Ria Formosa lagoon (Algarve coast - southern Portugal), through macro- and microscopic analyses of male and female gonads. In the eventuality of the legalisation of this fishing activity in the near future, this kind of information would allow for the implementation of management measures based on the species reproductive cycle (namely seasonal closures of the fishery) that could ultimately contribute to the long-term sustainable exploitation of this fishing resource. Additionally, this kind of information is also fundamental for improving the knowledge available on the potential of *T. trunculus* for molluscan aquaculture.

MATERIALS AND METHODS

During a 1-year study period (March 2003 - February 2004), approximately 100 specimens of *Hexaplex (Trunculariapsis) trunculus* from commercial samples caught in the vicinity of Culatra Island (Algarve coast - southern Portugal) (Figure 1) were sampled monthly. In order to assess eventual gonadal changes during maturation associated with seasonal temperature fluctuations throughout the year, seawater temperature was monitored daily with a multi parameter monitor (Yellow Springs Incorporated® - YSI 6820) at the Olhão Fish Culture Experimental Station, which receives water directly from the adjacent Ria Formosa lagoon and intimately resembles the surrounding natural environment in terms of seawater temperature.

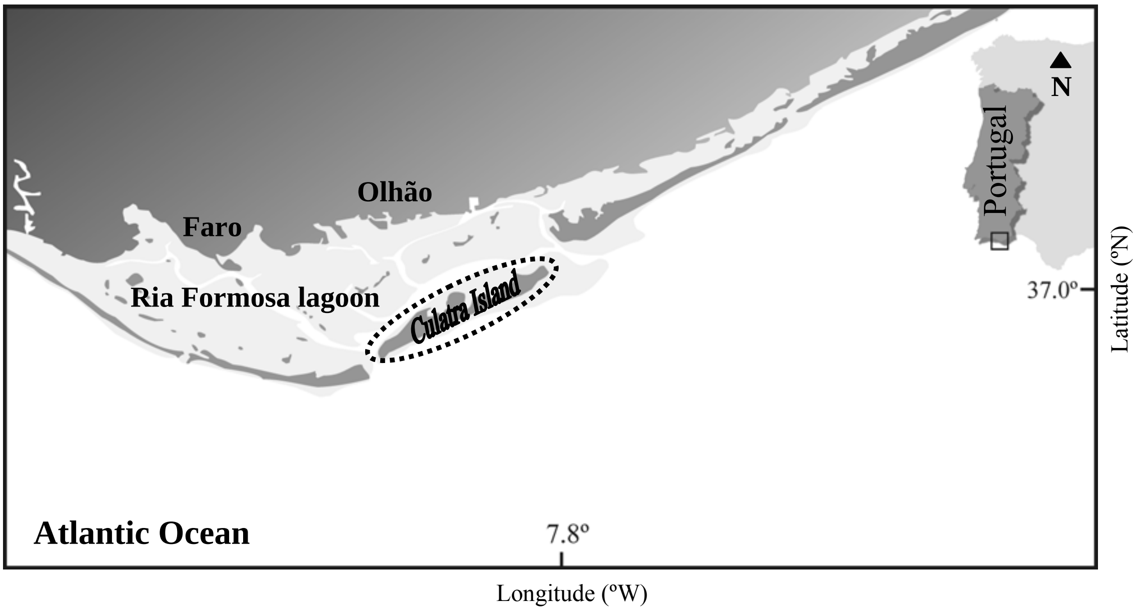


Figure 1. Geographical location of Culatra Island (dotted ellipse) in the Ria Formosa lagoon (Algarve coast - southern Portugal), where the specimens of *Hexaplex (Trunculariapsis) trunculus* were caught.

Initially, *T. trunculus* specimens (Figure 2a) were measured for shell length (SL - mm) with a digital calliper (Mitutoyo® Digimatic: CD-15D; precision of 0.01 mm) and weighed for total weight (TW - g) on a top-loading digital balance (AND®: HF-2000G; precision of 0.01 g). Specimens' size (SL and TW) was compared between sexes using a *t*-test (H_0: $\mu_M = \mu_F$; H_A: $\mu_M \neq \mu_F$), with statistical significance considered at $P < 0.05$ (Zar, 1996).

Since up to the present stage of research, no external sexual dimorphism has been detected in *T. trunculus*, sexes could not have been distinguished from shell morphological characters and therefore the soft body was totally extracted from the shell. For this purpose, specimens' shells were broken in a bench vice to allow for the removal of the soft parts of the organism, exposition of the mantle cavity and observation of the sexual organs (Figure 2b). However, due to the occurrence of imposex in *T. trunculus* in the studied area (for details see Vasconcelos *et al.*, 2006), individuals could not have been sexed simply by the presence or absence of penis. Consequently, males were identified by the presence of a penis and lack of a capsule gland, whereas females were identified by the presence of a vagina and capsule gland. The sex-ratio (expressed as the proportion of females:males) was determined and statistically significant deviations from a balanced sexual proportion of 1:1 were assessed by the χ^2-test, with statistical significance considered at $P < 0.05$ (Zar, 1996).

The gonad of *T. trunculus* lies on the dorsal side of the digestive gland to which it is intimately associated, constituting the visceral coil (digestive gland and gonad complex) (Figure 2c). Since the gonadal material of both sexes was normally distinguishable from the brownish colouration of the adjacent digestive gland, the external appearance and colour of the gonads were examined macroscopically to evaluate the degree of individual maturation, and whenever the gonads could not be distinguished from the digestive gland, individuals were considered as immature. Simultaneously, the macroscopic aspect of other organs of the female reproductive system, namely the sperm-ingesting gland (receptaculum seminis) and the capsule gland, was also observed to determine their significance for the assessment of sexual maturity and reproductive stage.

Because the gonad is intimately associated with the digestive gland, neither the testis nor the ovary could be precisely dissected from the visceral mass to employ standard histological methods (20 individuals per month). Therefore, in order to avoid excessive damages to the gonadal tissue, this assemblage of tissues (digestive gland and gonad complex) was fixed in Davidson solution for 24-48 hours, preserved in 70% ethanol and only then a transversal cut (2-3 mm thick section) was made on the medial zone of the visceral coil, immediately behind the gastric caecum (comprising both the digestive gland and the gonad) (Figure 2c).

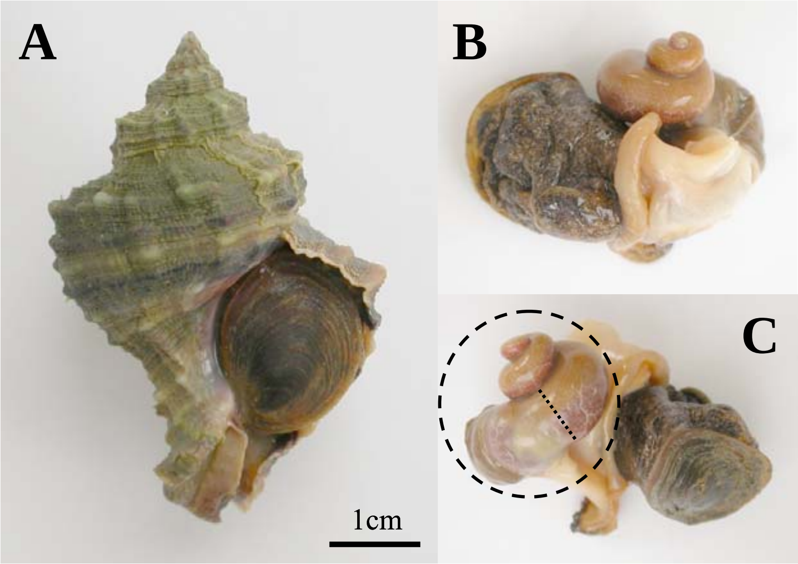


Figure 2. a) The banded murex, *Hexaplex (Truncualriopsis) trunculus*. **b)** Shell breakage for extraction of the soft part of the organism, exposure of the mantle cavity and sexual identification of the specimens. **c)** Location of the digestive gland and gonad complex (dotted circle), illustrating the transversal cut (dotted line) made for histological purposes on the medial zone of the visceral coil (behind the gastric caecum).

Subsequently, samples were dehydrated with serial dilutions in a graded ethanol series using an automatic tissue processor (Leica® TP1020), infiltrated and embedded in paraffin wax (Pronalab®) using a paraffin embedding station (Leica® EG 1140H). The gonads were cut into 7 µm thick sections using an automatic rotary microtome (Leica® RM2155), stained with Harris's haematoxylin-eosin, mounted between a glass slide and a cover slip with synthetic resin (D.P.X.: BDH®) and examined under an optical microscope (Zeiss® Axiovert 135) connected to an imaging system (Zeiss® KS100: release 3.0).

The identification of the different developmental stages of gametogenesis through the microscopic observation of histological sections of the gonads of both sexes was based on a maturation scale (briefly described in Table 1) adapted from the works by Albanese *et al.* (1983), Barroso and Moreira (1998) and Tirado *et al.* (2002). Whenever multiple gametogenic stages were observed simultaneously within each gonad histological section, the individual was assigned to the prevalent maturation stage (the condition in the majority of the section). Finally, the quantitative analysis of the reproductive cycle was performed by calculating the mean gonad index (MGI) (Brown, 1982) for each monthly sample. This index broadly defines the reproductive condition of the population at any particular time, with an increasing index in successive samples indicating gonadal development and a decreasing index indicating spawning in progress. The MGI was calculated through the following equation:

$$\mathrm{MGI} = \frac{\sum\left[(1 * \mathrm{no.\,ind.\,stage\,1}) + (2 * \mathrm{no.\,ind.\,stage\,2}) + \ldots + (\mathrm{n} * \mathrm{no.\,ind.\,stage\,n})\right]}{\mathrm{no.\,ind.\,analysed\,monthly}}$$

Table 1. Maturation scale used to assess the gametogenic development of the gonads and respective scores employed in the calculation of the mean gonad index (MGI) for the banded murex, *Hexaplex (Truncolariopsis) trunculus*.

Maturation stage	**MGI score**	**Brief description of the most relevant features of the gonad**
I - immature	0	The gonad presents little detailed internal structure; small groups of primary germ cells are dispersed in a loose matrix of connective tissue.
II - pre-active	1	The gonad has few separate follicles embedded in connective tissue; the first gametogenic stages appear near the thicker follicle wall.
III - active	2	The gonad progressively increases in size, the follicles become more closely packed in a condensed connective tissue; in males, a few spermatozoa appear in the lumen of some follicles; in females, small and half-grown oocytes appear at the periphery of the follicles.
IV - ripe	3	The gonad greatly increases in size, the follicles are more grouped and highly compressed, and the connective tissue disappears; in males, the follicles are distended and occupied by clearly visible spermatozoa in the lumen of the follicles (although earlier stages of spermatogenesis still occur near the follicle wall); in females, the follicles are packed with fully developed oocytes in the lumen of the follicle (although half-grown oocytes still occur near the follicle wall).
V - partially spent	-2	The gonad progressively reduces in size because spawning is in progress and the lumen of the follicle becomes emptier and surrounded by some connective tissue; the lumen of most follicles still contains spermatozoa or ripe oocytes (although gametogenesis decreases near the follicle wall).
VI - spent	-1	The gonad further reduces in size because all follicles have released the gametes (varying from an earlier stage of large empty follicles to a later stage of small empty follicles dispersed in a loose matrix of fully developed connective tissue).

RESULTS

Sexual proportion

A total of 1183 specimens of *Hexaplex (Truncularioopsis) trunculus* (621 males and 562 females) were sampled between March 2003 and February 2004 for the study of the reproductive cycle. Specimens presented a broad size range, both in terms of shell length (40.17 - 82.84 mm) and total weight (5.28 - 48.84 g), and on average females were significantly larger and heavier (57.84 ± 6.30 mm and 18.11 ± 6.91 g, respectively) than males (55.64 ± 5.50 mm and 16.86 ± 5.69 g, respectively) (*t*-test; $P < 0.05$).

Globally, samples presented a balanced sex-ratio (F:M = 1:1.10; χ^2-test, $P < 0.05$), despite some monthly samples being significantly dominated by females (June 2003) or males (May 2003, November 2003 and January 2004) (χ^2-test, $P < 0.05$) (Figure 3a). In order to detect variations in the sexual proportion as a function of specimen size, data were grouped into size classes (5 mm SL). Males dominated the smaller individuals (below 55 mm SL), both sexes were statistically balanced in intermediate size classes (between 55 and 65 mm SL), while females predominated in larger size classes (above 65 mm SL) (χ^2-test, $P < 0.05$) (Figure 3b). The decreasing proportion of males with increasing specimen size is further illustrated by the linear regression ($r = 0.985$) established between the shell length size classes (SL - 5 mm classes) and the respective sex-ratios (F:M) (Figure 3b).

Macroscopic observation of the sexual organs

In *T. trunculus*, the dimensions, appearance and colouration of the gonads of both sexes varied during the reproductive cycle, and the macroscopic aspect of other organs / structures of the female reproductive system (the sperm-ingesting gland and the capsule gland) gave additional information on the reproductive stage and sexual maturity of the specimens.

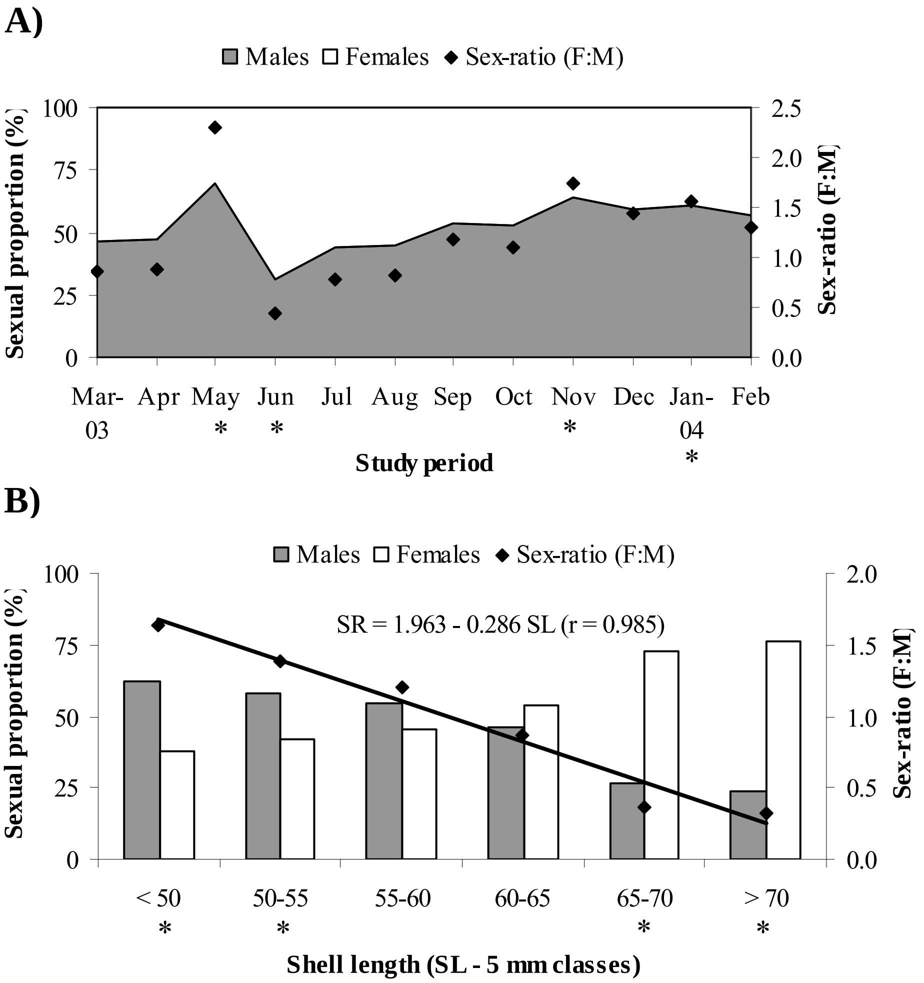


Figure 3. Sexual proportion (%) and sex-ratio (F:M): **a)** Variation during the study period (March 2003 - February 2004). **b)** Variation according to specimen size (grouped in shell length size classes - 5 mm SL). Asterisks denote monthly samples with unbalanced sexual proportions (χ^2-test, $P < 0.05$).

The dimensions of the gonads of both sexes varied according to the reproductive stage of *T. trunculus*, reaching a substantial volume during the reproductive season and considerably reducing its size in the periods of lower reproductive activity. The testis and the ovary were easily distinguishable from the brownish digestive gland due to their different colouration, except in very young (immature) individuals and in older specimens in absolute gonadal regression (rest phase), both presenting vestigial or undifferentiated gonads with greyish or brownish colouration. The external appearance and colouration of the ovary and testis were also fairly variable depending on age and sexual maturation, with females presenting palecreamy yellow, light yellow, orange or salmon gonads, and males presenting light yellow, dark yellow, mustard yellow or light brown gonads (the later sometimes quite similar to the brownish digestive gland).

In females, behind the complex capsule gland / albumen gland, is located the inconspicuous sperm-ingesting gland (including its whitish duct, the seminal receptaculum or receptaculum seminis, visible at the base of the pallial oviduct), and is responsible for storing the sperm obtained during copulation. However, being frequently almost unnoticeable, the examination of its general aspect was not useful and practical as a descriptor of the specimen's reproductive stage. Nevertheless, the sperm-ingesting gland occasionally presented a visible whitish mass, indicative of the occurrence of copulation and subsequent storage of sperm.

Located in the pallial oviduct, the capsule gland presents a characteristic vase-shaped and bi-lobed structure, being circular in transverse section and broadest in the middle section. The capsule gland and the albumen gland form an almost continuous structure, being only distinguishable among each other due to a small difference in opacity. The dimensions and overall aspect (colouration and opacity) of *T. trunculus* capsule gland varied considerably during maturation, growing in size (length and volume), evolving from a whitish to a yellowish colouration, and increasing its opacity and viscosity before the spawning season.

Microscopic analysis of the gonads

The most relevant features of gametogenesis observed in the histological sections of *T. trunculus* male and female gonads at each maturation stage are illustrated in Figures 4 and 5, respectively. The histology of the gonads revealed that all specimens analysed were gonochoristic (hermaphrodites or sex reversals were not detected) and with a standard gametogenic cycle (normal development of the gonadal activity and gametogenesis).

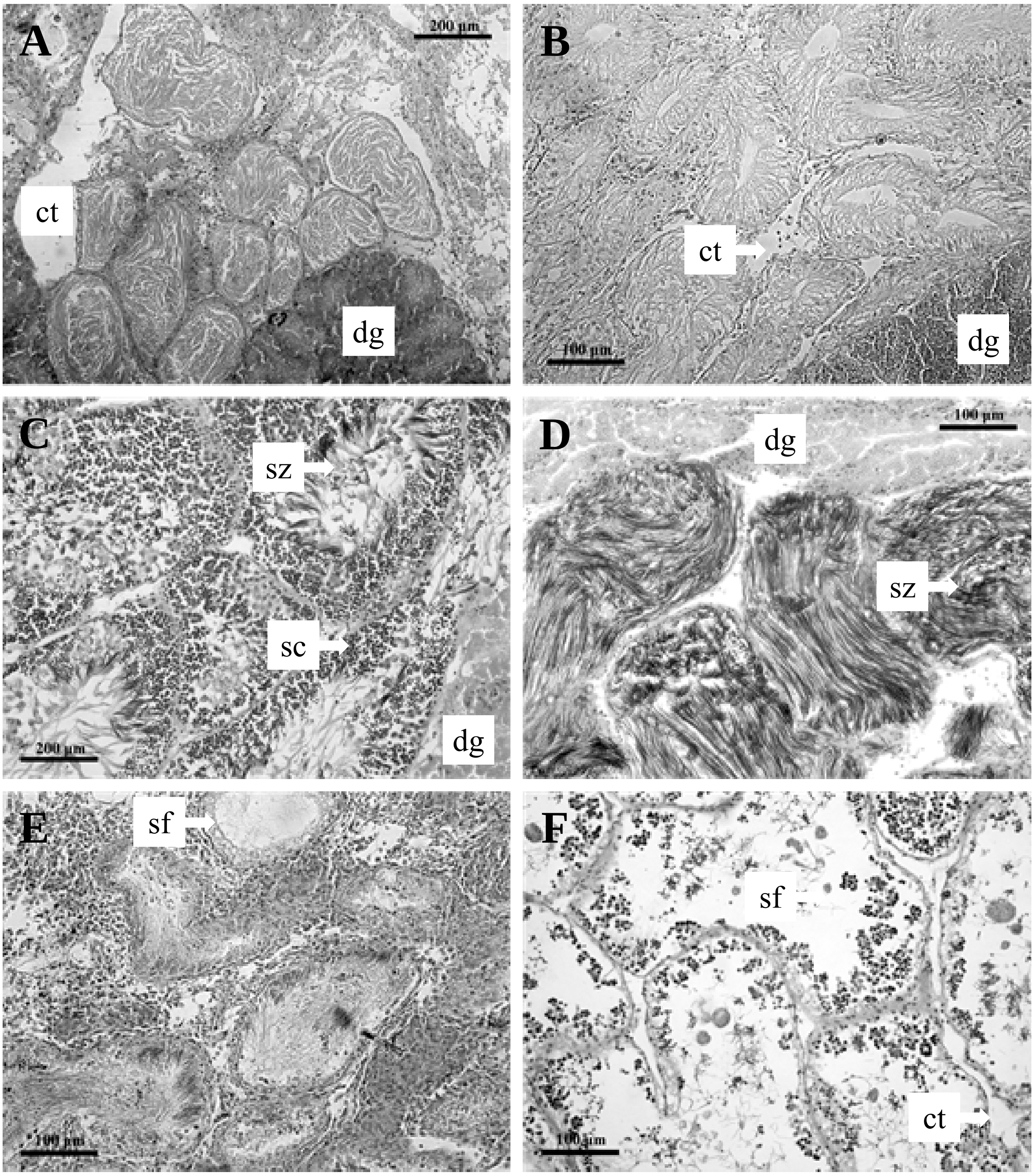


Figure 4. Photographs of histological sections showing the distinct developmental stages of spermatogenesis in *Hexaplex (Trunculariopsis) trunculus*: **a)** Stage I - immature. **b)** Stage II - pre-active. **c)** Stage III - active. **d)** Stage IV - ripe. **e)** Stage V - partially spent. **f)** Stage VI - spent. Abbreviations: ct - connective tissue; dg - digestive gland; sc - spermatocytes; sf - empty spent follicle; sz - spermatozoa.

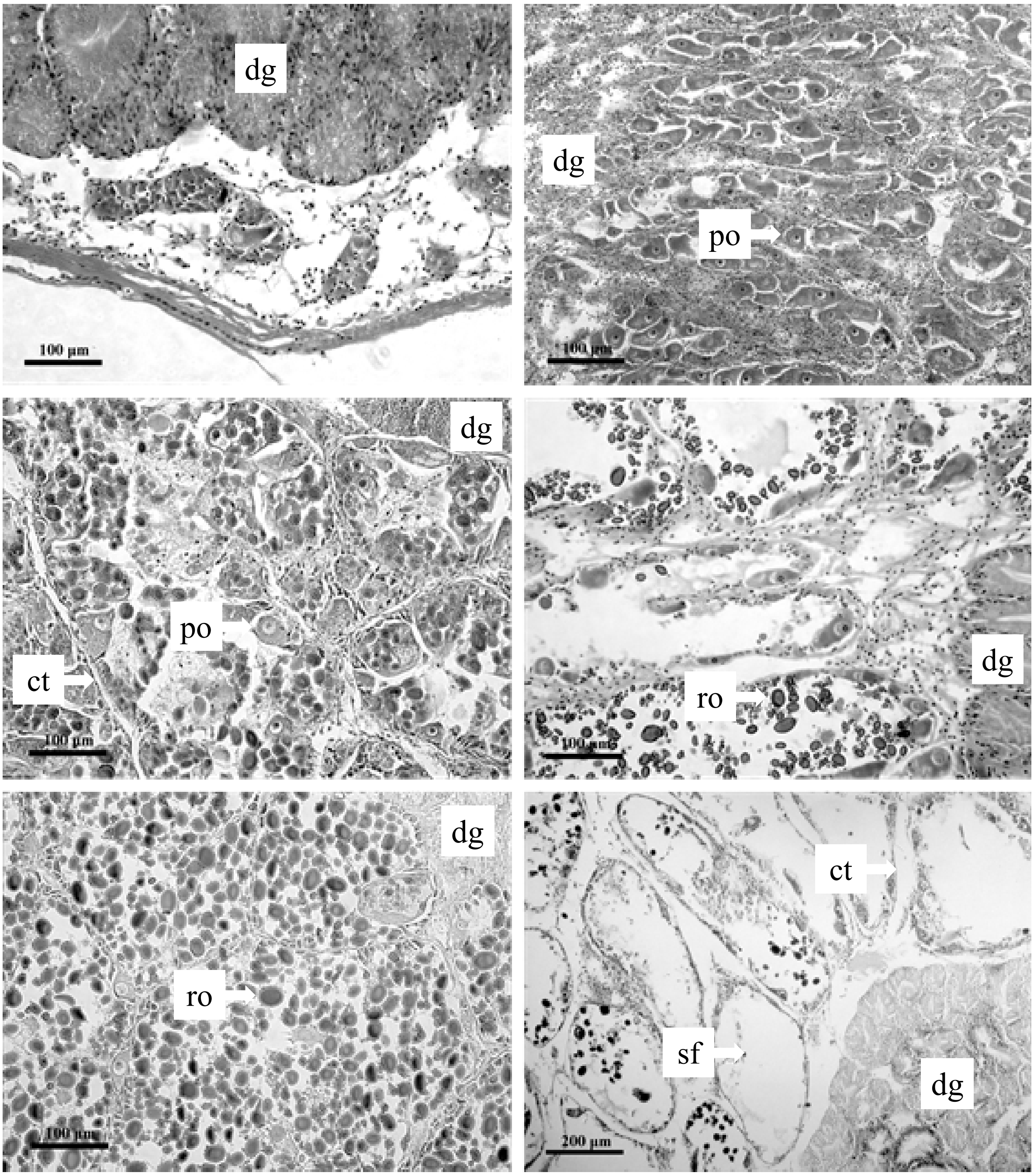


Figure 5. Photographs of histological sections showing the distinct developmental stages of oogenesis in *Hexaplex (Trunculariopsis) trunculus*: **a)** Stage I - immature. **b)** Stage II - pre-active. **c)** Stage III - active. **d)** Stage IV - ripe. **e)** Stage V - partially spent. **f)** Stage VI - spent. Abbreviations: ct - connective tissue; dg - digestive gland; po - previtellogenic oocyte; ro - ripe oocyte; sf - empty spent follicle.

Moreover, females did not present modifications in the ovary nor any signs of sexual change (with suppression of oogenesis and development of spermatogenesis), which constitute interferences with the normal sexual development generally associated with the highest degrees of imposex.

The monthly percentages of occurrence of *T. trunculus* males and females at each maturation stage during the study period (March 2003 - February 2004) in the Ria Formosa lagoon are presented in Figure 6. The most evident feature of the gametogenic cycle of this species is the simultaneous occurrence of distinct developmental stages of gametogenesis in almost every month, reaching a maximum of six different stages of spermatogenesis in June 2003 (Figure 6a) and five different stages of oogenesis in November 2003 (Figure 6b). Males presented reproductive activity almost year-round, with active, ripe or partially spent gonads being found during all sampling periods. The highest frequencies of ripe male gonads were observed from March to June 2003 and from October 2003 to January 2004 (Figure 6a). Females presented a more intense reproductive activity in certain months of the year, with active, ripe or partially spent gonads being found in whelks from March to May 2003 and from September 2003 to February 2004 (ripe gonads were only observed from March to May 2003 and from November 2003 to February 2004). Mature oocytes were observed both in ripe and partially spent gonads (during the period from March to May 2003 and from November 2003 to February 2004). A phase of lower reproductive dynamics was detected from June to August 2003, with the prevalence of immature, pre-active and spent gonads (Figure 6b).

Due to the frequent simultaneous occurrence of the most developed stages of gametogenesis (III - active + IV - ripe + V - partially spent) in each gonad during the greater part of the study period, the percentage of occurrence of these maturation stages were grouped together in order to better discriminate the periods of highest reproductive activity of *T. trunculus* males and females (Figure 7). During the study period, males presented an higher frequency of active, ripe and partially spent gonads, varying from a minimum of 57% in June 2003 and a maximum of 100% from March to May 2003, from September to November 2003 and from January to February 2004. Females exhibited a markedly lower frequency of active, ripe and partially spent gonads, with higher percentages found between March and May 2003 (>80%) and from December 2003 to February 2004 (>70%), separated by a period between June and August 2003 with no gonads in the most developed stages of gametogenesis (active + ripe + partially spent gonads = 0%) (Figure 7).

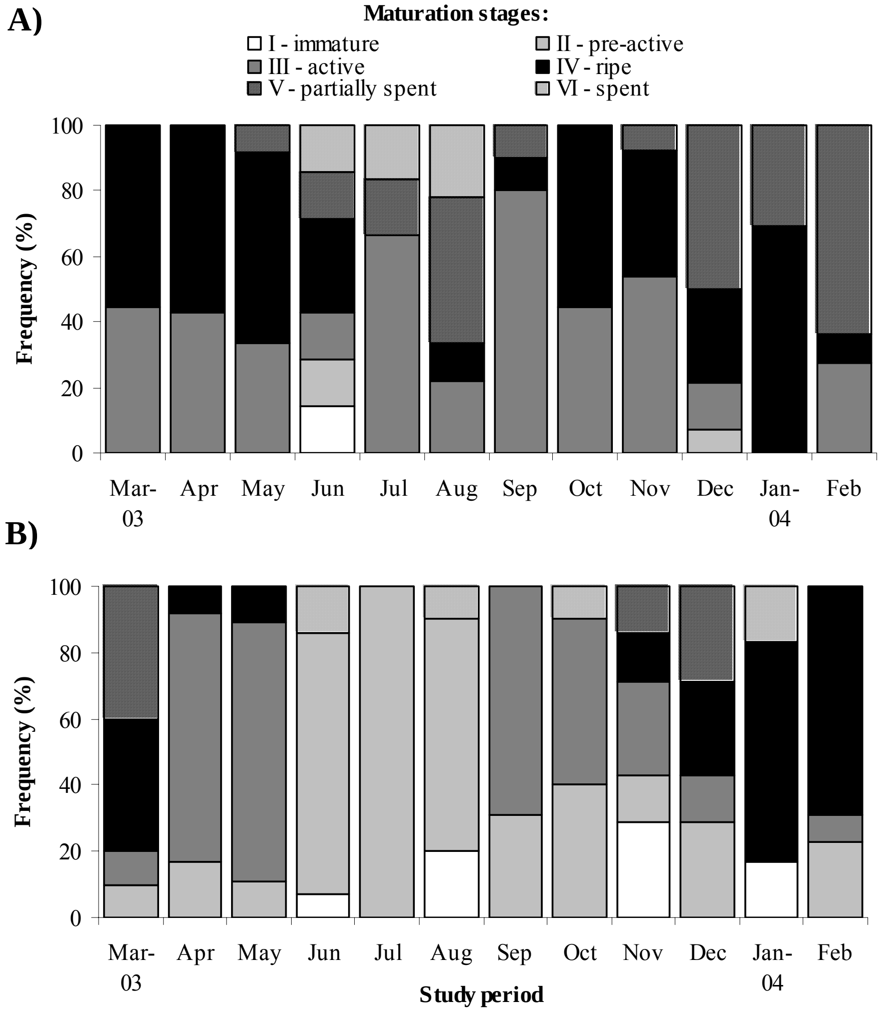


Figure 6. Percentage occurrence of different developmental stages of gametogenesis in *Hexaplex (Truncilariopsis) trunculus* during the study period: **a)** Frequency of males at each spermatogenic stage. **b)** Frequency of females at each oogenic stage.

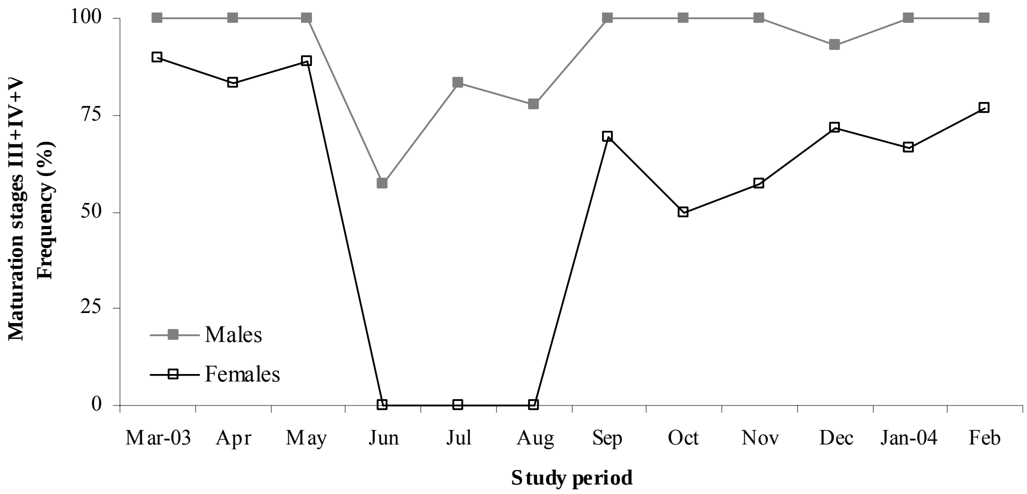


Figure 7. Percentage occurrence of the most developed stages of gametogenesis (III - active + IV - ripe + V - partially spent) in males and females of *Hexaplex (Truncულariopsis) trunculus* during the study period.

The relationship between the mean gonad index (MGI) of males and females of *T. trunculus* and the seawater temperature in the Ria Formosa lagoon during the study period is illustrated in Figure 8. The MGI showed that both sexes presented a high reproductive condition almost year-round, with males presenting a higher reproductive condition than females from March to June 2003 and from October to November 2003. Gametogenesis (indicated by an increasing MGI in consecutive months) and spawning (indicated by a decreasing MGI in consecutive months) was roughly synchronous in both sexes. This index revealed that female spawning occurred mainly in May and June 2003, followed by another spawning event in October and November 2003. A phase of lower reproductive condition (corresponding to the resting period) occurred simultaneously in both sexes in July and August 2003 (Figure 8). During the study period, seawater temperature ranged from a minimum of 13.4 ± 1.0ºC in December 2003 and a maximum of 26.0 ± 1.5ºC in August 2003, corresponding to an average temperature of 20.2 ± 4.7ºC. The first periods of gametogenesis and spawning in both sexes (between March and June 2003) were apparently triggered by the rising seawater temperatures during spring. The lowest MGI values (lower reproductive condition indicative of the resting period) coincided with the period of highest seawater temperatures during summer (namely in July and August 2003) (Figure 8).

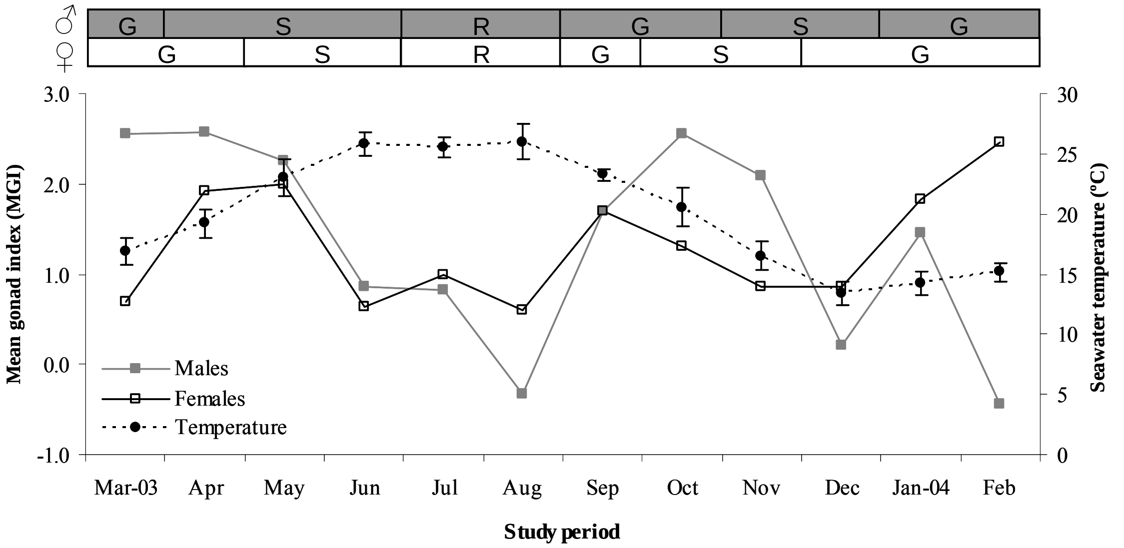


Figure 8. Relationship between the mean gonad index (MGI) of male and female *Hexaplex (Truncularioppsis) trunculus* and seawater temperature in the Ria Formosa lagoon during the study period. G - gametogenesis; S - spawning; R - resting.

DISCUSSION

Sexual proportion

Like most prosobranch gastropods, *Hexaplex (Truncularioppsis) trunculus* is gonochoristic (separate sexes). As in other works with this species, in the present study a balanced sex-ratio (F:M = 1:1.10) was found in the overall samples, but some monthly samples presented biased sexual proportions, being dominated by females (June 2003) or males (May 2003, November 2003 and January 2004), which is in agreement with the study by Tirado *et al.* (2002).

Independently of the sampling period, males dominated among smaller individuals, while females predominated in larger size classes. Generally, females are more common in older populations of gonochoristic molluscs (Fretter and Graham, 1964), and this increasing proportion of females with increasing specimen size is probably explained by differential growth between the sexes, with females allocating higher energy expenditure towards growth than for reproduction. Moreover, in imposex-affected *T. trunculus* females, reduced reproductive effort leads to increased somatic growth, *i.e.*, less energy is shifted towards reproduction (lower reproductive capability) than towards growth (higher somatic growth) (Axiak *et al.*, 1995; Rilov *et al.*, 2000). Besides the differential growth between sexes, it is also possible to speculate on the occurrence of higher natural mortality in males than in females, possibly caused by their stronger reproductive effort throughout the year. Finally, some unbalanced sex ratios may be hypothetically attributed to different practices of fishing and collecting *T. trunculus* in the Ria Formosa lagoon and also influenced by the season of the year. For instance, samples caught by hand-harvesting in the vicinity or directly in collective spawns (in the low tide or by scuba diving) are most likely dominated by females, whereas samples caught with the traditional fishing gear ("wallet-line") during the spawning season of this species are probably dominated by males, because females are occupied with spawning and interrupt their feeding during the deposition of the spawn (therefore decreasing their attraction to baited fishing gears).

Macroscopic observation of the sexual organs

Despite the colouration of the ovary and testis being frequently distinguishable from the brownish adjacent digestive gland (excepting in immature individuals and in older specimens in resting phase), the assessment of sexual maturation based exclusively on the colouration of *T. trunculus* gonads could be fairly subjective and/or inaccurate. Actually, with the exception of the dark yellow or mustard yellow gonads (mature and ripe males) and orange or salmon gonads (mature and ripe females), some yellowish and brownish gradations (the later sometimes hardly distinguishable from the brownish digestive gland) might difficult the evaluation of the maturation stage. Moreover, besides the fact that very young individuals present vestigial gonads (immature specimens with incipient gonads), the reproductive cycle of *T. trunculus* also comprises an absolute gonadal regression (rest phase with undifferentiated gonads) that makes unfeasible and/or inaccurate the assessment of sexual maturation simply by macroscopical observation, therefore requiring histological examination of the gonads (Martoja and Bouquegneau, 1988; Tirado *et al.*, 2002). Furthermore, and as stated by Martoja and Bouquegneau (1988), in some individuals of both sexes occurs a phenomenon of “mixture” of activity and neutral zones in the gonads, which present a “mosaic” colouration. On the other hand, because the volume of the gonads of both sexes presented clear oscillations throughout the reproductive cycle, almost disappearing during gonadal regression (rest phase) and markedly filling up during sexual maturation, the dimensions of the gonads have a remarkable potential for usage as a reproductive condition index, particularly for monitoring the reproductive season (Vasconcelos *et al.*, submitted).

Being a characteristic of neogastropods, *T. trunculus* reproduction involves copulation and internal fertilisation. In females, the sperm-ingesting gland is the structure responsible for storing the sperm obtained from copulation during long periods (with the spermatozoids oriented and nourished by the female) (Fretter and Graham, 1994; Tirado *et al.*, 2002). In fact, *T. trunculus* can store viable sperm for a considerable period of time (Spanier and Karmon, 1987), as confirmed by circumstantial evidences of females that spawned in the laboratory two months after being isolated from congeners (P. Vasconcelos, personal observation) to two years after being kept without companions (Wondrak, 1974).

The capsule gland is responsible for the formation of the egg capsules that enclose the eggs and developing embryos. In *T. trunculus* females, besides some differentiation in colouration, opacity and viscosity, the dimensions (length and volume) of the capsule gland varied markedly during the reproductive cycle, reaching a substantial size in the reproductive season and considerably reducing its dimensions immediately after the spawning season. For this reason, together with the gonads of both sexes, the dimensions of the capsule gland present a high potential for usage as a female reproductive condition index, specifically for monitoring the spawning season (Vasconcelos *et al.*, submitted).

Microscopic analysis of the gonads

In gastropod populations highly affected by imposex, the masculinising effect of TBT may not be confined to the modification of the reproductive tract but also extend to gametogenesis and induce complete sexual change (with suppression of oogenesis and development of spermatogenesis) (*e.g.*, Gibbs *et al.*, 1988, 1990, 1991). In fact, previous studies with *T. trunculus* confirmed that advanced imposex was associated with histopathological damages in the ovary, that in the most severe cases protogyne sex change occurred in young and sexually immature specimens, and that in some cases oogenesis was suppressed and sperm-like structures were found in the ovaries (Axiak *et al.*, 2003). Fortunately, our analysis of ovary histological sections revealed that all specimens were gonochoristic (without hermaphrodites or sex reversals) and have not detected interferences with normal sexual development (gonadal activity and oogenesis developed normally), indicating that for the moment imposex apparently does not highly affect the population dynamics of *T. trunculus* in the Ria Formosa. Accordingly, imposex surveys in this *T. trunculus* population did not find females sterilised by oviduct structure modification (Gibbs *et al.*, 1997; Vasconcelos *et al.*, 2006) and observations both in the natural environment and in laboratory revealed a normal spawning activity, with deposition of egg capsules in individual and collective spawns (Vasconcelos *et al.*, 2004).

One of the most evident features of the gametogenic cycle of this species was the simultaneous occurrence of distinct developmental stages of gametogenesis in both sexes almost year-round (Figure 6) (sometimes in each gonad and even in different follicles of the same histological section). This asynchrony in the gonadal development of the population (with the coexistence of specimens in distinct gametogenic stages) is apparently typical of this species, since it was also found in *T. trunculus* populations from Marbella - Spain (with individuals in at least three maturation stages in almost all samples) (Tirado *et al.*, 2002) and from Bizerte - Tunisia (with gonads presenting sexual cells in different developmental stages almost all year-round) (Lahbib *et al.*, 2004).

The gametogenic cycle of *T. trunculus* in the Ria Formosa lagoon involved an extensive period of gonadal activity, with males presenting gonads with higher frequency of more developed stages of gametogenesis (active + ripe + partially spent gonads) than females during the whole study period (Figure 7). An extended period of gametogenic activity was also detected in *T. trunculus* populations from Marbella - Spain (Tirado *et al.*, 2002), Corsica - France (Martoja and Bouquegneau, 1988), Sicily - Italy (Albanese *et al.*, 1983) and Bizerte - Tunisia (Lahbib *et al.*, 2004), also with males generally presenting longer periods of gonadal activity and more developed gametogenic stages than females (males: October - July and females: January - July, Albanese *et al.*, 1983; males: early autumn - late spring and females: late winter - mid spring, Tirado *et al.*, 2002).

In the present study, *T. trunculus* males and females from the Ria Formosa lagoon undertook a roughly synchronous gonadal development (Figure 8), a phenomenon that has also been observed in populations of this species from Marbella - Spain (Tirado *et al.*, 2002), Corsica - France (Martoja and Bouquegneau, 1988) and Bizerte - Tunisia (Lahbib *et al.*, 2004). Nevertheless, as mentioned by Tirado *et al.* (2002), since *T. trunculus* has internal fecundation some particular features of the reproductive cycle of this species should also be considered. In this context, the remarkable ability of *T. trunculus* females to store viable sperm in the sperm-ingesting gland during long periods and afterwards fertilise the oocytes and spawn during the environmentally most favourable periods (namely in terms of seawater temperature and food availability), might eventually contribute to the slight asynchrony in the gametogenic development and spawning periods between sexes (Figure 8) and also explain the relatively common occurrence of episodes of partial spawning in specimens held in captivity (P. Vasconcelos, personal observation).

The earlier phases of gametogenesis and spawning in *T. trunculus* from the Ria Formosa lagoon were apparently triggered by the rising seawater temperatures during spring (Figure 8). Generally, the breeding activity of *T. trunculus* begins between early spring and the beginning of summer, being probably activated by the increasing seawater temperature and not day length (in the laboratory reproduction started at ambient temperature and constant light regime) (Spanier and Karmon, 1987). In fact, in the Israel coast, as well as in laboratory aquaria, *T. trunculus* reproductive activity started when the seawater temperature reached 19ºC (Spanier, 1981; Spanier and Karmon, 1987).

Due to the extensive period of gonadal activity of *T. trunculus* in the Ria Formosa lagoon, the MGI revealed a short phase of lower reproductive condition (corresponding to the resting period) that occurred simultaneously in both sexes in July and August 2003 and coincided with the period of warmest seawater temperatures (Figure 8). Similarly, short resting phases were detected in *T. trunculus* populations from Marbella - Spain (females in July and males in July and August (Tirado *et al.*, 2002) and in Sicily - Italy (August and September in both sexes) (Albanese *et al.*, 1983), whereas a longer resting phase was observed in Corsica - France (during half of the year, from August to January) (Martoja and Bouquegneau, 1988).

In *T. trunculus* from the Ria Formosa lagoon, the MGI indicated that spawning occurred mainly in May and June 2003, followed by another spawning event in October and November 2003 (Figure 8). Actually, according to local fishermen, *T. trunculus* spawns may be found in the Ria Formosa lagoon between February and June, but are more frequent and abundant in May and June (Vasconcelos *et al.*, 2004). Furthermore, *T. trunculus* specimens maintained in captivity spawn regularly between late April and early June (Vasconcelos *et al.*, 2004), but sporadic and weaker spawning events have already occurred in October - November (P. Vasconcelos, personal observation). The information available on the spawning season of *T. trunculus* from various localities throughout the distributional range of the species (Atlantic Ocean and Mediterranean Sea) is summarized in Table 2. As reported in several studies undertaken with populations from the Mediterranean Sea, the spawning season of *T. trunculus* in the natural environment generally occurs in late spring - early summer (more frequently between May and July). Similarly, but under laboratory conditions resembling the environmental temperature regime, the spawning events of *T. trunculus* occurred approximately in the same period. Nevertheless, in some localities in the Mediterranean Sea *T. trunculus* exhibited more than one spawning period or even an exceptionally broad spawning season. In fact, in Tunisian waters three periods of spawning were identified (varying according to the sampling site) (Lahbib *et al.*, 2004), whereas along the Israel coast (which has the warmest seawater temperature within the distributional range of this species) spawns cast ashore are especially abundant in May - July, but are commonly found all year-round (Barash and Zenziper, 1980) (Table 2).

Table 2. Spawning season (grey), peak of spawning season (black) and spawning events under laboratorial conditions (stripes) observed in different localities throughout the distributional range of the banded murex, *Hexaplex (Trunculariopsis) trunculus*.

	Spawning												
Location	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Reference**
Portugal (Ria Formosa) (f)					█	█				▒	▒		Present study
Portugal (Ria Formosa) (f, i)		▒	▒	▒	█	█							Vasconcelos *et al.* (2004)
Portugal (l)				▨	▨	▨							Vasconcelos *et al.* (2004)
Portugal (l)										▨	▨		P. Vasconcelos (pers. obs.)
Spain (Marbella) (f)				▒	▒	▒	▒						Tirado *et al.* (2002)
France (l)						▨							Fischer and Raffy (1933)
France (l)					▨	▨							Bandel (1975)
France (Corsica) (f)							▒						Martoja and Bouquegneau (1988)
Italy (f)					▒								Dulzetto (1946)
Italy (l)							▨						Dulzetto (1950)
Italy (l)					▨	▨							Terlizzi *et al.* (1999)
Croatia (l)					▨	▨							Wondrak (1974)
Israel (f)	▒	▒	▒	▒	█	█	█	▒	▒	▒	▒	▒	Barash and Zenziper (1980)
Israel (f)					▒								Spanier (1981)
Tunisia (Bizerte lagoon) (f)	▒	▒	▒	█	█	█	█	▒	▒				Lahbib *et al.* (2004)
Tunisia (Bizerte channel) (f)		▒	▒	▒	▒		▒	▒	▒	▒			Lahbib *et al.* (2004)
Tunisia (l)			▨										Abidli *et al.* (2006)
Tunisia (l)				▨	▨	▨	▨						Lahbib *et al.* (2006)
Tunisia (l)		▨	▨	▨	▨	▨	▨						Trigui El-Menif *et al.* (2006)

(f), field / natural environment; (i), information from inquiries made to local fishermen; (l), laboratory conditions with temperature regime resembling the natural environment.

Implications for fishery management and molluscan aquaculture

In the last five years there has been some pressure from local fishermen involved in the fishery for the banded murex in the Ria Formosa lagoon to legalise the use of the "wallet-line". For this reason, a monitoring survey of the fishery was undertaken, complemented by studies of diverse aspects of the species biology (including the present study on *T. trunculus* gametogenic cycle). The overall information gathered in this integrated study is essential for the proposal to the fisheries administration of adequate management measures for this fishing activity, including specific technical measures for the protection of the crucial phases of *T. trunculus* reproductive cycle. In particular, due to the slight asynchrony in the gametogenic development and spawning between sexes (probably related to the functioning of the female sperm-ingesting gland), management measures should focus mainly on the protection of the female broodstock and collective spawns. For this purpose, a closed season in the fishing activity should be implemented during the main spawning period of female *T. trunculus* (May and June). Despite the positive effect of reducing the fishing effort, the implementation of an additional closed season in the secondary and apparently more unpredictable spawning period (October and November) could be rather problematic and unsuccessful. In fact, whenever variation occurs in the timing of spawning, seasonal closures to protect spawning stocks are unlikely to be highly effective (Ward and Davis, 2002).

The knowledge on the reproductive dynamics of commercially exploited species is a basic requirement for the establishment of successful aquaculture programmes. In terms of the assessment of the potential of *T. trunculus* for molluscan aquaculture, the data gathered on the main periods of maturation and spawning constitute valuable baseline information for establishing the most appropriate phase and process (manipulation of seawater temperature) for the artificial induction of spawning in captivity. This achievement would be highly advantageous both for the commercial production of this species and for stock enhancement of the apparently over-exploited populations of *T. trunculus* from the Ria Formosa lagoon.

ACKNOWLEDGEMENTS

The authors would like to thank the technical staff of IPIMAR - CRIPSul for their valuable collaboration in the laboratory sampling procedures. Thanks are also due to Pedro Pousão-Ferreira for kindly providing the data on the seawater temperature monitored at the Olhão Fish Culture Experimental Station and to João Cúrdia for his valuable help in photographing the histological sections of the gonads. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001) and by the research project PROMAR (Interreg III-A: Cooperación Algarve - Andalucía para la Promoción de Recursos Aquícolas Marinos en el Litoral Suratlántico).

REFERENCES

Abidli, S.; Trigui El-Menif, N.; Boumaiza, M., 2006. Variation de la concentration du TBT sur la fécondité et l'émission gamétique d'*Hexaplex trunculus*. *Résumés des Communications du Troisième Congress Franco-Tunisien de Zoologie*, Tabarka, Tunisie, 3-7 Novembre 2006, 73 pp.

Albanese, M.P.; Calabrò, C.; Acosta Pomar, M.L.C., 1983. Ciclo biológico di *Truncalariopsis trunculus* L. (Moll. Gast. Pros.) vivente nel Lago di Faro. *Memorie di Biologia Marina e di Oceanografia*, 13: 133-147.

Axiak, V.; Vella, A.J.; Micallef, D.; Chircop, P.; Mintoff, B., 1995. Imposex in *Hexaplex trunculus* (Gastropoda; Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, 121: 685-691.

Axiak, V.; Micallef, D.; Muscat, J.; Vella, A.; Mintoff, B., 2003. Imposex as a biomonitoring tool for marine pollution by tributyltin: some further observations. *Environment International*, 28: 743-749.

Bandel, K., 1975. Das embryonalgehäuse mariner prosobranchier der region von Banyuls-sur-Mer. *Vie et Milieu*, 25: 83-118.

Barash, A.; Zenziper, Z., 1980. Egg masses of Mollusca from Mediterranean waters of Israel and notes on reproduction of the freshwater species *Theodoxus jordani* and *Melanoides tuberculata*. *Veliger*, 22: 299-317.

Barroso, C.M.; Moreira, M.H., 1998. Reproductive cycle of *Nassarius reticulatus* in the Ria de Aveiro, Portugal: Implications for imposex studies. *Journal of the Marine Biological Association of the United Kingdom*, 78: 1233-1246.

Bolognari, A.; Albanese-Carmignani, M.P.; Zaccone, G.; Calabrò, C., 1979. Correlazioni citochimiche e citoenzimologiche tra il nucleolo e i globuli di vitello negli ovocito del Mollusco *Murex trunculus* L. *Acta Embryologica Experimentalis*, 3: 370-371.

Bolognari, A.; Albanese-Carmignani, M.P.; Calabrò, C.; Zaccone, G., 1981. Structural and cytochemical features of the yolk globules and of the nucleolus in the growing oocytes of the mollusc *Murex trunculus* L. *Basic and Applied Histochemistry*, 25: 159-167.

Brown, R.A., 1982. Reproduction of *Abra nitida* (Müller) (Bivalvia) in the Southern Skagerrak. *Sarsia*, 67: 55-60.

Coelho, M.R.; Bebianno, M.J.; Gibbs, P.E., 1998. Imposex in neogastropod snails from southern Portugal. *Cuadernos de Investigación Biológica*, 20: 273.

Dulzetto, F., 1946. Osservazioni sulla deposizione di *Murex trunculus* L. *Atti dell'Accademia Nazionale dei Lincei*, 8: 1356-1361.

Dulzetto, F., 1950. Sull'origine e la struttura delle capsule ovifere di *Murex trunculus*. *Archivio Zoologico Italiano*, 35: 83-99.

Fischer, P.H.; Raffy, A., 1933. La ponte du *Murex trunculus* L. *Bulletin de l'Institut Océanographique (Monaco)*, 618: 1-4.

Fretter, V.; Graham, A., 1964. Reproduction. In: *Physiology of Mollusca. Vol. 1.* (Wilbur, K.M., Ed.), Academic Press, New York, 127-164 pp.

Fretter, V.; Graham, A., 1994. *British Prosobranch Molluscs: Their Functional Anatomy and Ecology*. The Ray Society, London.

Gibbs, P.E.; Pascoe, P.L.; Burt, G.R., 1988. Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 68: 715-731.

Gibbs, P.E.; Bryan, G.W.; Pascoe, P.L.; Burt, G.R., 1990. Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributyltin-induced imposex. *Journal of the Marine Biological Association of the United Kingdom*, 70: 639-656.

Gibbs, P.E.; Pascoe, P.L.; Bryan, G.W., 1991. Tributyltin-induced imposex in Stenoglossan gastropods: pathological effects on the female reproductive system. *Comparative Biochemistry and Physiology*, 100C: 231-235.

Gibbs, P.E.; Bebianno, M.J.; Coelho, M.R., 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution, with notes on a species (*Columbella rustica*) lacking the imposex response. *Environmental Technology*, 18: 1219-1224.

Houart, R., 2001. *A Review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Ed. Evolver, Rome.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2004. Données sur le cycle reproducteur du mollusque gastéropode *Hexaplex trunculus* (Linné, 1758) de la Lagune de Bizerte (Tunisie). *Bulletin de la Société Zoologique de France*, 129: 407-418.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2006. La ponte et la croissance absolue d'*Hexaplex trunculus* réalisée au laboratoire. *Résumés des Communications du Troisième Congrès Franco-Tunisien de Zoologie*, Tabarka, Tunisie, 3-7 Novembre 2006, 64 pp.

Langston, W.J.; Gibbs, P.E.; Livingstone, D.R.; Burt, G.R.; O'Hara, S.; Bebianno, M.J.; Coelho, M.R.; Porte, C.; Bayona, J.; McNulty, M.; Lynch, G.; Keegan, B., 1997. Risk assessment of organotin antifoulings on key benthic organisms of European coastal habitats (BOATS). MAS2-CT-94-0099, Final Report.

Macedo, M.C.C.; Macedo, M.I.C.; Borges, J.P., 1999. *Conchas Marinhas de Portugal (Seashells of Portugal)*. Editorial Verbo, Lisboa.

Martoja, M.; Bouquegneau, J.-M., 1988. *Murex trunculus*: un nouveau cas de pseudo-hermaphrodisme chez un gastéropode prosobranche. *Bulletin de la Société Royale de Liège*, 57: 45-58.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Rilov, G.; Gasith, A.; Evans, S.M.; Benayahu, Y., 2000. Unregulated use of TBT-based antifouling paints in Israel (Eastern Mediterranean): high contamination and imposex levels in two species of marine gastropods. *Marine Ecology Progress Series*, 192: 229-238.

Schitz, V., 1920. Sur la spermatogenèse chez *Murex trunculus* (L), *Aporrhais pes pelicani* (L), *Fusus* sp. et *Nassa reticulata*. *Archives de Zoologie Expérimentale et Générale*, 59: 477-508.

Spanier, E., 1981. Behavioral ecology of the marine snail *Truncularìopsis (Murex) trunculus*. In: *Developments in Arid Zone Ecology and Environmental Quality* (Shuval, H., Ed.), Balaban ISS, Philadelphia, 65-70 pp.

Spanier, E.; Karmon, N., 1987. Muricid snails and the ancient dye industries. In: *The royal Purple and the Biblical Blue: Argaman and Tekhelet* (Spanier, E., Ed.), Keter Publishing House Jerusalem Ltd., Jerusalem, 179-192 pp.

Terlizzi, A.; Geraci, S.; Gibbs, P.E., 1999. Tributyltin (TBT)-induced imposex in the Neogastropod *Hexaplex trunculus* in Italian coastal waters: morphological aspects and ecological implications. *Italian Journal of Zoology*, 66: 141-146.

Tirado, C.; Rodríguez de la Rúa, A.; Bruzón, M.A.; López, J.I.; Salas, C.; Márquez, I., 2002. *La Reproducción de Bivalvos y Gasterópodos de Interés Pesquero en Andalucía*. Junta de Andalucía, Consejería de Agricultura y Pesca, Huelva.

Trigui El-Menif, N.; Lahbib, Y.; Le Pennec, M.; Flower, R.; Boumaiza, M., 2006. Intensity of the imposex phenomenon - impact on growth and fecundity in *Hexaplex trunculus* (Mollusca: Gastropoda) collected in Bizerta lagoon and channel (Tunisia). *Cahiers de Biologie Marine*, 47: 165-175.

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias, D.; Castro, M., 2004. Spawning of *Hexaplex (Truncularioρsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006. Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin*, 52: 337-341.

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar, M.B. Comparison of indices for assessing the reproductive condition of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Aquatic Living Resources* (submitted).

Ward, D.W.; Davis, A.R., 2002. Reproduction of the turban shell *Turbo torquatus* Gmelin 1791 (Mollusca: Gastropoda), in New South Wales, Australia. *Marine and Freshwater Research*, 53: 85-91.

Wondrak, G., 1974. Laichverhalten und gelegebildung bei *Murex trunculus* L. (Gastropoda: Prosobranchia). *Archiv fuer Molluskenkunde*, 104: 115-119.

Zar, J.H., 1996. *Biostatistical Analysis*. 3rd Edition. Prentice-Hall International Inc., New Jersey.

CHAPTER 4.2

Comparison of indices for assessing the reproductive condition of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae)

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar, M.B. Comparison of indices for assessing the reproductive condition of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae). *Aquatic Living Resources* (submitted).

ABSTRACT

The aim of the present study was to compare the ability of conventional condition indices (based on weighing of the whole organism, flesh or shell) and visual quantitative condition indices (based on measurement of sections of the digestive gland, gonad and capsule gland) for assessing the reproductive condition of *Hexaplex (Truncularíopsis) trunculus*. Compared to conventional indices, the visual quantitative indices (gonadosomatic index - GSI and capsule gland index - CGI) revealed a remarkable ability to follow the seasonal variations of the species reproductive dynamics and clearly identified broad changes in reproductive development (being highly accurate in detecting the periods of gonad maturation and spawning). Consequently, these visual quantitative indices (GSI and CGI) constitute simple, practical and efficient methods for the routine assessment of the reproductive condition of *T. trunculus*, providing a useful and reliable alternative to several conventional condition indices and to laborious, time-consuming and expensive histological techniques. The results gathered in the present study strongly encourage the use of similar techniques and analogous visual quantitative indices for assessing the reproductive condition of other gastropod species.

INTRODUCTION

The banded murex, *Hexaplex (Truncularioposis) trunculus* (Linnaeus, 1758), is exploited by commercial fisheries both in the Mediterranean Sea (Poppe and Goto, 1991; Houart, 2001) and in the Atlantic Ocean, particularly in the Ria Formosa lagoon (Algarve coast - southern Portugal), where it is subjected to a locally important artisanal fishery (Marques and Oliveira, 1995; Muzavor and Morenito, 1999). In this context, the availability of information on the reproductive dynamics of the target species is essential for establishing adequate fishery management measures, which could ultimately contribute to the long-term sustainable exploitation of the fishing resource. For this reason, the gametogenic cycle of *T. trunculus* has been studied (through macro- and microscopic analyses of the gonads) in populations from the Mediterranean, namely in Marbella - Spain (Tirado *et al.*, 2002), Sicily - Italy (Albanese *et al.*, 1983) and Bizerte lagoon - Tunisia (Lahbib *et al.*, 2004), and more recently in an Atlantic population of this species from the Ria Formosa lagoon - Portugal (Vasconcelos *et al.*, submitted).

The histological micro-examination of the gonads is unanimously considered the most consistent method for detecting seasonal trends in reproduction (Seed and Suchanek 1992). However, although highly accurate, histology requires a series of specialised, laborious and time-consuming tasks (dissection, fixation, dehydration, embedding, sectioning, staining and observation of gonad sections) and therefore is not a very practical technique for routine sampling and assessment of the general reproductive condition. Moreover, through histological analyses gonads are generally classified into distinct developmental stages (*e.g.*, resting, developing, mature, spawning and spent), a classification method that despite being fairly subjective is widely used to identify broad trends in the reproductive cycle (Buchanan, 2001). Alternatively, in order to simplify procedures and allow for practical routine usage, several condition indices have been developed to summarize the physiological activity of the organisms and have been successfully applied to the assessment of the reproductive condition in several molluscan species (*e.g.*, see reviews by Mann, 1978; Lucas and Beninger, 1985; Davenport and Chen, 1987; Crosby and Gale, 1990).

However, like in some other gastropod species, some particular features of the banded murex make inappropriate or inaccurate the use of some conventional reproductive condition indices. The gonad of *T. trunculus* is located on the dorsal side and in close contact with the digestive gland, constituting the visceral coil (digestive gland and gonad complex). However, despite being easily distinguishable from the digestive gland due to different colouration, neither testis nor ovaries can be precisely dissected and separated from the rest of the soma without significant damages and losses of gonadal tissue, making impracticable the use of gonadosomatic indices that employ gonad weight to estimate allocation of resources to reproductive versus somatic tissue. Additionally, *T. trunculus* has a voluminous digestive gland, whose contents may interfere considerably in the weight of the digestive gland and gonad complex (Tirado *et al.*, 2002), making less sensitive the condition indices that employ the visceral coil weight (digestive gland and gonad complex weight) because variations in weight may not be attributable to changes in gonadal activity.

In order to overcome these difficulties, it would be highly advantageous identifying alternative solutions to several conventional condition indices and to histological techniques for assessing the gonadal development and monitoring the reproductive dynamics of this species. In a recent study of the gametogenic cycle of *T. trunculus* from the Ria Formosa lagoon, it was observed that the seasonal variation in the dimensions of the gonads of both sexes and of the female capsule gland could possibly have a remarkable potential for usage as reproductive condition indices, particularly for monitoring the maturation and spawning periods (Vasconcelos *et al.*, submitted). For this reason, a simple method for assessing the reproductive condition could eventually be achieved through visual quantitative techniques based on the dimensions of these organs, which would allow describing changes in the reproductive condition. In this context, this study aimed to compare the ability of several conventional condition indices (based on weighing of the whole organism, flesh or shell) and visual quantitative condition indices (based on measurement of sections of the digestive gland, gonad and capsule gland) for assessing the reproductive condition of *T. trunculus*.

MATERIALS AND METHODS

Specimens of banded murex from commercial samples caught in the Ria Formosa lagoon (Algarve coast - southern Portugal) were analysed monthly during a one-year study period (March 2003 - February 2004). Initially, *T. trunculus* individuals (Figure 1a) were measured for shell length (SL) with a digital calliper (to the nearest 0.01 mm), slightly blotted dry (to remove surface fluids and drain as much water as possible from the mantle cavity) and weighed for total weight (Tw) on a top-loading digital balance (precision of 0.01 g). Routine sexual identification was made in de-shelled specimens (after breaking the shells in a bench vice) and due to the occurrence of imposex in *T. trunculus* in the studied area (Vasconcelos *et al.*, 2006), males were identified by the presence of penis and lack of capsule gland, whereas females were identified by the presence of vagina and capsule gland.

Conventional condition indices

For this comparative approach, six conventional condition indices incorporating weight data (wet weight, dry weight or ash-free dry weight) of the whole specimen, flesh or shell were considered. Initially, individuals with damaged shells were systematically discarded and only undamaged specimens were weighed for total weight (Tw), after removing the colonising algae and/or encrusting organisms (mainly polychaetes) with a hard brush. After de-shelling each specimen, the soft-body (flesh excluding the operculum) was removed, drained and blot-dried onto absorbent paper to eliminate the extra-visceral water, and weighed for wet weight (Fww). Particular care was taken to collect all broken fragments of the shell, which were also weighed for wet weight (Sww). All these measurements were made on the top-loading digital balance (precision of 0.01 g). Subsequently, the samples of flesh and shell were dried at 80ºC during 24 hours, cooled in a desiccator for approximately 10-20 minutes and weighed for dry weight (Fdw and Sdw). Finally, the flesh samples were incinerated in a muffle furnace at 500ºC during 6 hours, cooled in a desiccator for approximately 1 hour and weighed for ash-free dry weight (Fafdw). These procedures were repeated until no further weight reduction was detectable (constant weight) and all weighings were made on a high-precision digital balance (precision of 0.001 g).

A total of 10 specimens were sampled monthly and only individuals in the 60-70 mm SL interval were analysed. The calculation of the indices for standard-sized specimens aimed to suppress the influence of growth (minimising the interference of the increase in somatic weight during ontogeny) (Cledón *et al.*, 2005) and therefore emphasise the seasonal variation in the accumulation or loss of organic matter associated with reproduction (Beninger and Lucas, 1984). The equations used for the calculation of these conventional condition indices are compiled in Table 1 (further details on the preparation of samples and calculation of indices may be found elsewhere).

Visual quantitative indices

For comparison purposes, two visual quantitative indices were considered, the gonadosomatic index (GSI) and the capsule gland index (CGI). Initially, in order to facilitate the dissection of the soft-body tissues (digestive gland / gonad complex and capsule gland), specimens were preserved in buffered 4% formaldehyde-seawater (formalin) during at least

24-48 hours and then carefully washed in running water to remove fixative residues from the tissues before further procedures. In both sexes, a transversal cut was made in the medial zone of the visceral coil (digestive gland and gonad complex) (Figure 1b) and a section (2-3 mm thick) of this assemblage of tissues immediately behind (posterior position) the gastric caecum was cut and placed on an overhead transparency film. Simultaneously, the capsule gland was carefully removed, measured in its maximum extension (capsule gland length: CGl) with the digital calliper (to the nearest 0.01 mm), a transversal cut was made in the medial zone of this organ (Figure 1c) and a section (2-3 mm thick) of tissue was cut and also placed on the overhead transparency film. Finally, these sections of tissues were scanned using a previously calibrated scanner (HP® Scanjet 5530), the outlines of the digestive gland and overlying gonad (Figure 1d) and of the bi-lobed capsule gland (Figure 1e) were traced with a computer mouse in the digitised image and their areas (digestive gland and gonad complex area - DGGCa; digestive gland area - Dga; gonad area - Ga; capsule gland area - CGa) measured with a digital imaging system (Zeiss® KS100: release 3.0).

The gonadosomatic index (GSI) was estimated in approximately 60-80 specimens of both sexes per month and calculated as the relative proportion of the gonad cross-sectional area in the total cross-sectional area of the digestive gland and gonad complex. The capsule gland index (CGI) was estimated in all females present in the samples (approximately 30-40 specimens per month) and calculated through a proxy of the overall dimensions of this organ, simply by multiplying the length (CGl) and area (CGa) of the capsule gland. The equations used for the calculation of these visual quantitative indices (GSI and CGI) are also presented in Table 1.

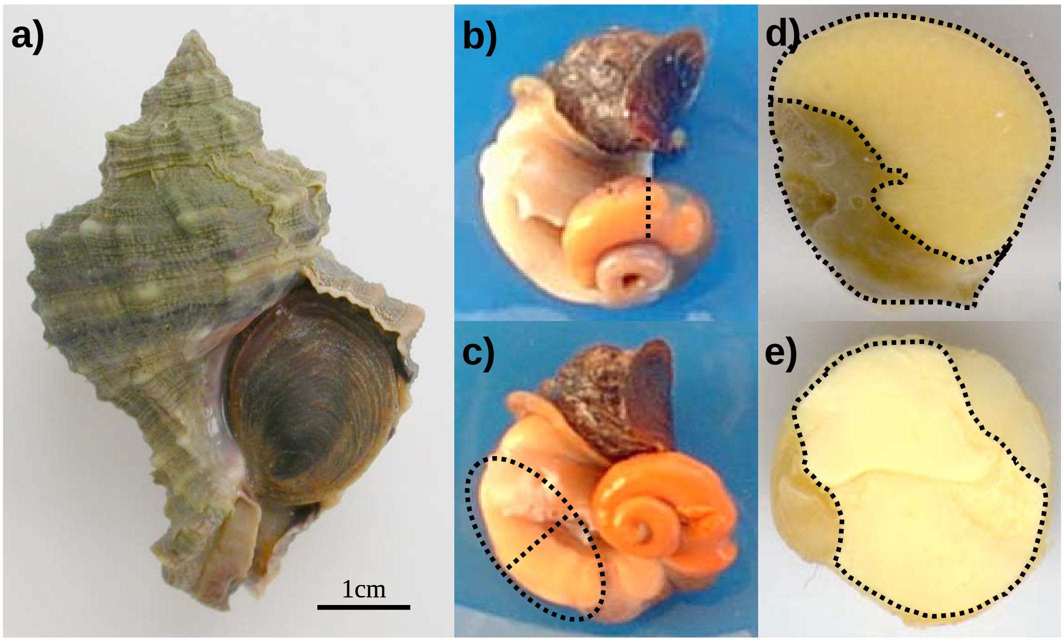


Figure 1. Illustration of the sequential procedures for the assessment of visual quantitative indices of reproductive condition (GSI and CGI) of *Hexaplex (Truncularіopsis) trunculus*: **a)** Live specimen (ventral view). **b)** Location of the transversal cut (dotted line) made in the medial zone of the visceral coil (digestive gland and gonad complex). **c)** Location of the transversal cut (dotted line) made in the medial zone of the capsule gland. **d)** Outlines (dotted lines) traced in the digitised image during the measurement of the gonad and digestive gland areas for the estimation of the gonadosomatic index (GSI). **e)** Outline (dotted line) traced in the digitised image during the measurement of the capsule gland area for the estimation of the capsule gland index (CGI).

RESULTS

Conventional condition indices

A total of 120 standard-size specimens (50 males and 70 females) were sampled for the estimation of the conventional condition indices, varying between 60.12 and 68.71 mm SL (mean SL = 64.40 ± 2.19 mm). The variation of the diverse conventional indices of reproductive condition of *T. trunculus* during the study period is compiled in Figure 2.

Table 1. Equations employed in the calculation of the conventional and visual quantitative indices used to assess the reproductive condition of *Hexaplex (Trunculariopsis) trunculus*. Fww - flesh wet weight; Tw - total weight; Sww - shell wet weight; Fdw - flesh dry weight; Sdw - shell dry weight; Fafdw - flesh ash-free dry weight; Ga - gonad area; DGGCa - digestive gland and gonad complex area; CGl - capsule gland length; CGa - capsule gland area.

Conventional condition indices	**Source**
Indices employing wet weight:	
Fww / Tw	Lucas and Beninger (1985)
Fww / Sww	Lucas and Beninger (1985)
Indices employing dry weight:	
Fdw / Fww	Lucas and Beninger (1985)
Fdw / Sdw	Lucas and Beninger (1985)
Fdw / (Tw - Sdw)	Crosby and Gale (1990)
Indices employing ash-free dry weight:	
(Fdw - Fafdw) / Sdw	Walne and Mann (1975)
Visual quantitative indices	**Source**
Gonadosomatic index (GSI)	
(Ga / DGGCa) * 100	Poore (1973)
Capsule gland index (CGI)	
CGl * CGa	Present study

The two condition indices employing only wet weight data (total weight - Tw; flesh wet weight - Fww or shell wet weight - Sww) (Figure 2a,b) displayed a similar trend, with a slight and analogous oscillation in both sexes during the study period. In general, females presented higher condition indices than males, increasing between March and May 2003 and then decreasing in June 2003. After a short period of stabilisation during July and August 2003, a minor increase occurred again in September and October 2003, followed by another stabilisation during the rest of the study period (Figure 2a,b).

Similarly, the other four condition indices employing dry weight data (flesh dry weight - Fdw or shell dry weight - Sdw) (Figure 2c-e) or ash-free dry weight data (flesh ash-free dry weight - Fafdw) (Figure 2f) also exhibited comparable tendencies between each other, equivalent variations in both sexes during the study period, and generally females with higher condition indices than males. However, these condition indices presented great oscillations during the study period, without a clearly discernible seasonal pattern (strong monthly variation, with successive increases and decreases in consecutive months) (Figure 2c-f).

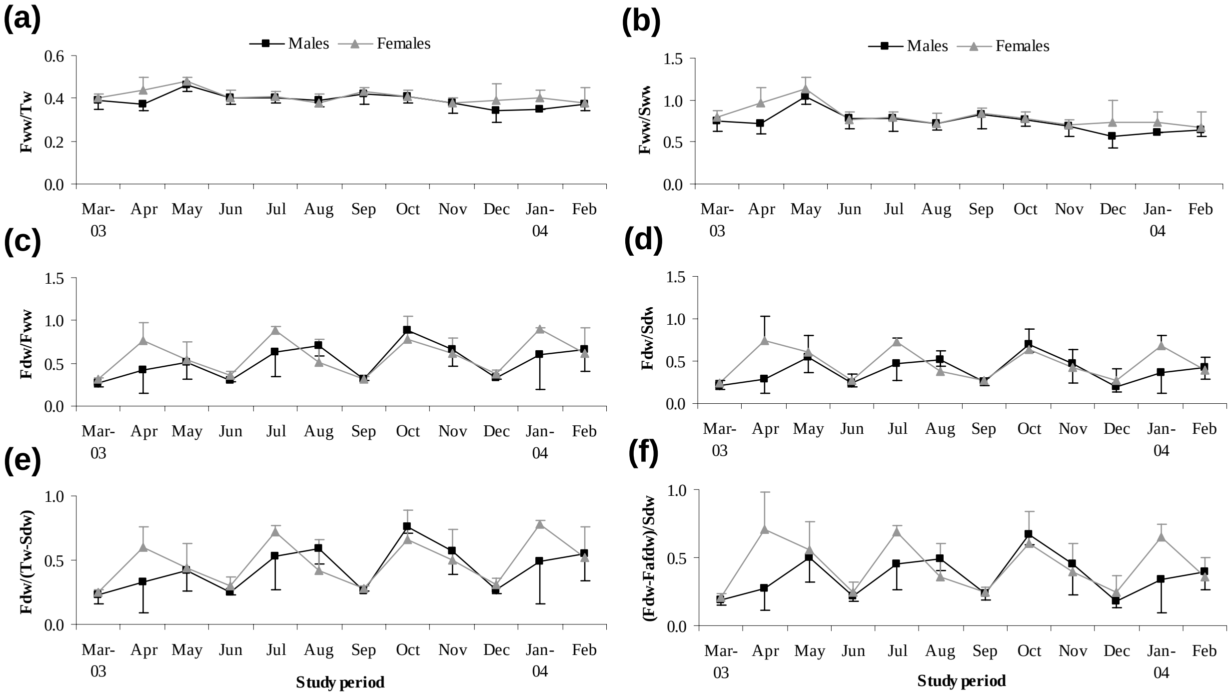


Figure 2. Variation of the conventional indices of reproductive condition of *Hexaplex (Truncularìopsis) trunculus* during the study period in the Ria Formosa lagoon (Algarve coast - southern Portugal): **a,b)** Indices employing wet weight data (Tw - total weight; Fww - flesh wet weight; Sww - shell wet weight). **c-e)** Indices employing dry weight data (Fdw - flesh dry weight; Sdw - shell dry weight). **f)** Index employing ash-free dry weight data (Fafdw - flesh ash-free dry weight).

Visual quantitative indices

A total of 903 *T. trunculus* specimens (501 males and 402 females) were sampled for the estimation of the gonadosomatic index (GSI). Overall, these specimens had a size range of 40.17 - 79.84 mm SL and on average females were larger (57.42 ± 6.21 mm SL) than males (54.87 ± 5.54 mm SL). All females were also sampled for the estimation of the capsule gland index (CGI), with specimen size comprehended between 43.03 and 79.84 mm SL. The variations of these visual quantitative indices (GSI and CGI) in *T. trunculus* during the study period are illustrated in Figure 3.

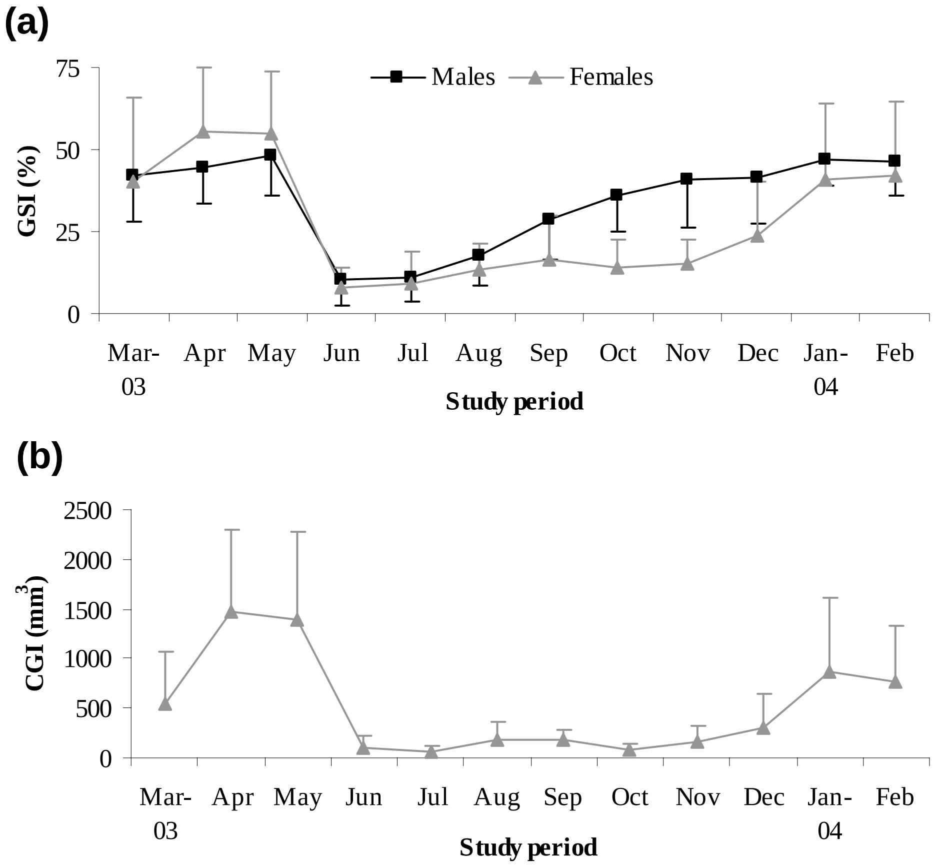


Figure 3. Variation of the visual quantitative indices of reproductive condition of *Hexaplex (Truncularioposis) trunculus* during the study period in the Ria Formosa lagoon (Algarve coast - southern Portugal): **a)** Gonadosomatic index (GSI). **b)** Capsule gland index (CGI).

Females presented a higher GSI between March and May 2003, whereas males exhibited greater GSI in the remaining study period (June 2003 - February 2004). In both sexes, the GSI increased between March and May 2003 followed by a sharp decrease in June 2003. Afterwards, males GSI showed a continuous and steady increase during the rest of the study period (from July 2003 to February 2004), while females GSI presented a slight increase in August and September 2003, followed by another marked increase from November 2003 to February 2004 (Figure 3a). Similarly, the CGI displayed a noticeable increase between March and April 2003, stabilised in May 2003 and then sharply decreased in June 2003. Subsequently, a small increase in the capsule gland volume occurred during August and September 2003, followed by another marked increase in the CGI in the remaining study period (namely between November 2003 and January 2004) (Figure 3b).

In order to better demonstrate the seasonal evolution of *T. trunculus* reproductive organs (gonads of both sexes and female capsule gland) and consequently clarify the periods of higher reproductive dynamics and effort, the monthly frequencies of individuals with gonad area (Ga) and capsule gland area (CGa) greater than the digestive gland area (DGa) are illustrated in Figure 4.

A clear seasonal trend was detected in the percentage of specimens with gonad area bigger than digestive gland area. A great proportion of female gonads were larger than the digestive gland between March and May 2003, and then this proportion steadily increased again from December 2003 to February 2004. Between June 2003 and November 2003 occurred a period of lower reproductive dynamics, with almost all female gonads smaller than digestive glands. Male gonads bigger than digestive glands were only sampled between March and May 2003 and then from September 2003 to February 2004 (separated by a phase of lower reproductive effort between June and August 2003, without male gonads greater than digestive glands). From March to May 2003 and from January to February 2004 more females than males presented gonads larger than digestive glands, whereas from September to December 2003 more males than females had gonads bigger than digestive glands (Figure 4a). Concerning the other organ considered as an additional measure of female reproductive condition, a great proportion of individuals exhibited a capsule gland greater than the digestive gland between March and May 2003. After a period without any specimens with capsule gland bigger than the digestive gland (phase of lower female reproductive effort between June and July 2003), a small fraction of females exhibited some development of the capsule gland in August and September 2003, followed by a gradual increase in this proportion from November 2003 to February 2004 (Figure 4b).

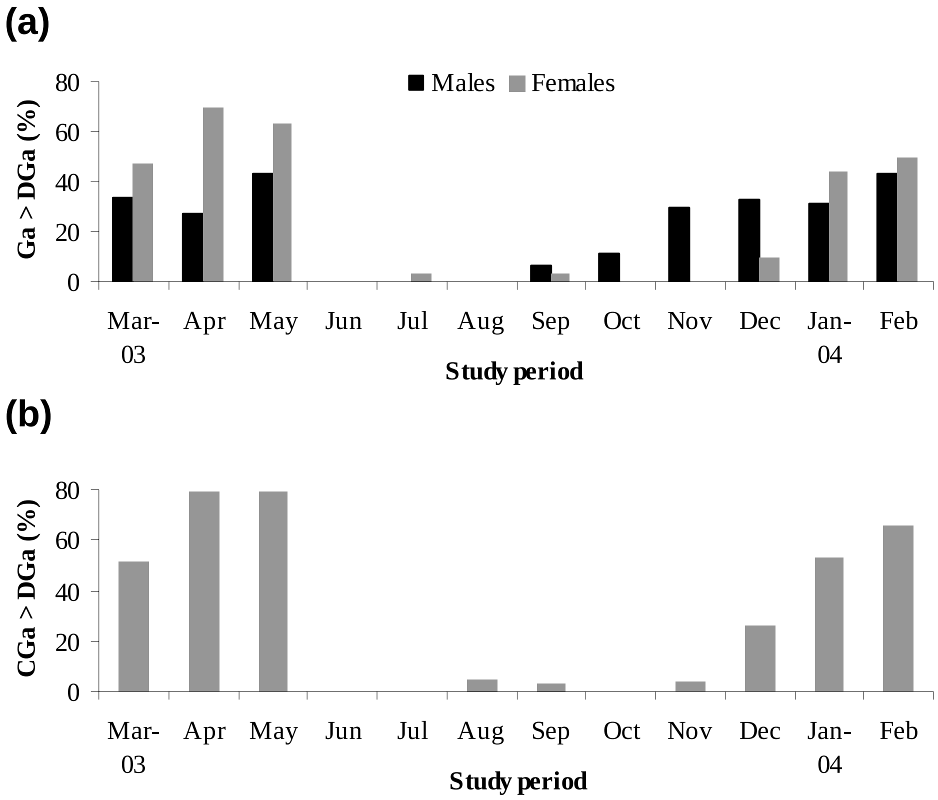


Figure 4. Seasonal evolution in the monthly frequency of *Hexaplex (Truncularìopsis) trunculus* specimens with: **a)** Gonad area (Ga) greater than the digestive gland area (DGa). **b)** Capsule gland area (CGa) greater than the digestive gland area (DGa).

DISCUSSION

There are a great number of condition indices in the specialised bibliography and generally there is no consensus about the best index to employ for a determined species, what makes difficult the comparison of results both intra- and inter-specifically. For this reason, and also because some particular features of *T. trunculus* make inappropriate or inaccurate the use of several conventional reproductive condition indices (namely gonadosomatic indices that employ gonad weight or digestive gland and gonad complex weight), six conventional indices (based on weighing of the whole organism, flesh or shell) and two visual quantitative indices (based on measurement of sections of the digestive gland, gonad and capsule gland) were compared in terms of their ability for assessing the reproductive condition of the banded murex.

Conventional condition indices

The two conventional condition indices employing only wet weight data (Tw, Fww or Sww) presented the same trend and slight fluctuation during the study period (which was more evident in the index: Fww / Sww) (Figure 2a,b). Nevertheless, despite not being much accentuated, the seasonal variation of these condition indices still roughly reflected the phases of higher reproductive dynamics of this species. In fact, both indices presented two periods of decreasing values, one more significant between May and June 2003 and another less noticeable between September and October 2003, which nearly correspond to the main spawning seasons (main spawning period in May and June 2003, followed by a secondary spawning event in October and November 2003) detected through the histological analysis of the gonads of *T. trunculus* from the Ria Formosa lagoon (Vasconcelos *et al.*, submitted). All the remaining four conventional condition indices, employing dry weight data (Fdw or Sdw) or ash-free dry weight data (Fafdw) (Figure 2c-f), followed each other quite closely and displayed great oscillations during the study period [which were more evident in the indices: Fdw / (Tw - Sdw) and (Fdw - Fafdw) / Sdw)] (Figure 2e,f), but without an evident seasonal pattern that could be definitely associated with the species reproductive cycle.

In general, there are several constraints in the sampling and analytic procedures that might introduce inaccuracies in the calculation of these conventional condition indices (particularly during routine work), mostly related to some imprecision that may occur during weighing of the specimen total weight, shell weight and/or flesh weight. Firstly, due to the difficulty to standardise the blot-drying procedures (removal of surface fluids and drainage of the mantle cavity) and to the variability in the amount of water retained inside the shell (normally sealed by the operculum), the weighing of specimen total weight (Tw) can be fairly imprecise. Additionally, the accuracy of shell weighing might also be impaired by particular characteristics of the shell of some gastropod species (*e.g.*, damages, erosion, fouling) that probably make somewhat inaccurate the use of conventional condition indices that employ shell weight. Furthermore, being the secretory product of the organism metabolism, the shell represents cumulative growth (Lucas and Beninger, 1985), and therefore inter-individual variation in shell weight among similar size individuals might be also due to energy investment in repairing previous shell damages. In the particular case of *T. trunculus*, fishing operations and handling procedures frequently cause shell damages (mainly in the shell border and siphonal canal) and fouling by epibiotic polychaetes (including boring/drilling species, namely *Polydora hoplura*) weakens and erodes some shells (mostly in the shell spire) (Vasconcelos *et al.*, 2007), therefore contributing for discrepancies in shell weight among individuals with the same shell length (Vasconcelos *et al.*, 2004a). Similarly, due to strong oscillation in shell weight among similar size individuals caused by the presence of epibiotic *Lithophaga* mussels and drilling polychaetes, the conventional condition index: flesh wet weight / shell wet weight was not the best proxy to evaluate the reproductive condition of *Adelomelon brasiliana* (Cledón *et al.*, 2005). Still concerning shell weighing, even with the strict usage of intact specimens for the estimation of conventional condition indices (like in the present study), during shell breakage in a bench vice it is virtually impossible to guarantee the complete collection of all shell fragments (as well as the absolute removal of the smallest remains of shell from the flesh), which might introduce further imprecision to shell weighing (and also to flesh weighing). Moreover, the notions of “wet” shell and “drained” flesh are physiologically vague and practically very difficult to standardise (Lucas and Beninger, 1985), potentially also inducing additional inaccuracy in the estimation of conventional condition indices that employ these wet weight parameters (Fww and Sww). Overall, these constraints can affect the performance of conventional indices that employ shell weight, thus whenever possible their use should be avoided in gastropod species whose shell must be meticulously broken in a bench vice and that might be affected by damages, erosion or fouling (such as *T. trunculus*).

Normally, drying the samples contributes for standardising weighing and eliminating the bias due to water content fluctuations of the whole tissue, reason why flesh dry weight (Fdw) and shell dry weight (Sdw) have been widely employed in conventional condition indices and are recommended for most routine work (Lucas and Beninger, 1985). Furthermore, the condition indices that employ flesh ash-free dry weight (*e.g.*, Walne and Mann, 1975) are easily standardised and theoretically more precise, being considered the best static condition indices available (Lucas and Beninger, 1985), namely because the ash-free dry weight is a superior indicator of flesh weight since errors due to the unintentional incorporation of shell fragments and variation in the meat mineral content are avoided (Gabbott and Walker, 1971). Unexplainably, in the present study, neither dehydration nor incineration of the samples improved the overall ability of the conventional condition indices to reflect the reproductive dynamics of this species. On the contrary, the four indices that have in common the use of dry weight (Fdw or Sdw) or ash-free dry weight (Fafdw) presented an analogous and continuous oscillation during the study period, without clarifying any discernible seasonal trend attributable to gonad maturation or spawning (Figure 2c-f). Possibly, these conventional condition indices are more sensitive to other physiological activities (*e.g.*, somatic growth, shell secretion) and highly influenced by the prevailing environmental conditions (*e.g.*, seawater temperature, food availability), which might eventually mask their seasonal trend and obscure the fluctuations related to the species reproductive cycle.

Visual quantitative indices

In *T. trunculus*, the gonads of both sexes were easily distinguishable from the brownish digestive gland due to their different colouration (excepting the vestigial gonads of immature individuals and the undifferentiated gonads of individuals in absolute gonadal regression), but neither testis nor ovary could be precisely dissected from the digestive gland, making unfeasible the use of gonadosomatic indices that employ gonad weight. This impossibility of exactly separating the gonads from the visceral mass and therefore estimating gonadosomatic indices also occurs in other gastropod species, for instance in *A. brasiliana* from Argentina (Cledón *et al.*, 2005).

Nevertheless, in a preceding study of the reproductive cycle of *T. trunculus* from the Ria Formosa lagoon (Vasconcelos *et al.*, submitted) it was detected that the dimensions of the gonads of both sexes presented significant oscillations throughout the reproductive cycle (almost disappearing during the resting period and reaching a considerable volume during the reproductive season), therefore possessing a remarkable potential to be employed in visual quantitative condition indices. The present study perfectly confirmed this assumption, since the seasonal fluctuation in the GSI clearly reflected the reproductive cycle of *T. trunculus*

(Figure 3a), therefore constituting a useful tool for monitoring the gonad maturation and the reproductive season of this species. Indeed, compared to conventional condition indices that better reflected the reproductive dynamics of this species (Fww / Tw and Fww / Sww) (Figure 2a,b), both GSI and CGI displayed more evident and abrupt variations during the gonad maturation and spawning seasons. Similar gonadosomatic indices (based on the relative proportion of the gonad in the digestive gland and gonad complex) have also been successfully employed for assessing the reproductive condition of other gastropod species, namely *Haliotis* spp. from New Zealand (Poore, 1973) and *Concholepas concholepas* from Chile (Ramorino, 1975).

Observations both in the natural environment and in the laboratory revealed that during the spawning season *T. trunculus* females get together in massive aggregations depositing collective spawns (Vasconcelos *et al.*, 2004b). Being the organ that provides substances for the encapsulation of eggs and developing embryos within the capsules that constitute the spawn, the capsule gland could eventually constitute an additional indicator of female reproductive condition in *T. trunculus*. Indeed, a previous study of the reproductive cycle of *T. trunculus* from the Ria Formosa lagoon revealed that the overall dimensions of the capsule gland varied markedly during the reproductive cycle (reaching a substantial volume during the reproductive season and considerably reducing its size immediately after the spawning period), consequently possessing an interesting potential to be employed in visual quantitative condition indices (Vasconcelos *et al.*, submitted). Once again, the present study completely corroborated this expectation, since the seasonal oscillation in the CGI noticeably reflected the reproductive dynamics of female *T. trunculus* (Figure 3b), therefore representing an additional helpful tool for monitoring the spawning season. Similarly, the capsule gland has formerly been considered as a secondary sexual character in female *Zidona dufresnei* from Argentina, its relative change in size being used as a sexual maturity criterion monitored through a capsule gland index (CGI = body total weight / capsule gland weight) (Giménez and Penchaszadeh, 2003).

Even adopting a more direct and simplistic approach (basically by analysing the evolution in the percentage of specimens with gonad area or capsule gland area greater than the digestive gland area), it was possible to detect a clear seasonal trend in the periods of higher reproductive dynamics of this species, namely in terms of the evolution of gonadal maturation in both sexes (Figure 4a) and the female spawning season (with particular emphasis on the evident peak of female spawning) (Figure 4b). The visual comparison of the gonad area (Ga *vs.* DGa) is very practical to be used as reproductive condition index in species whose gonads reach a considerable size at maturation (such as *T. trunculus*), contrarily to what happens in other gastropod species whose gonad is only a thin layer around the digestive gland (namely *A. brasiliana*), as reported by Cledón *et al.* (2005). In addition, since *T. trunculus* females can store viable sperm in the sperm-ingesting gland for a considerable period of time (Wondrak, 1974; Spanier and Karmon, 1987), potentially contributing for slight asynchrony in the maturation and spawning periods between sexes and for partial spawning events (Vasconcelos *et al.*, submitted), the visual comparison of the capsule gland area (CGa *vs.* DGa) is highly useful for monitoring the spawning peak and detecting the eventual occurrence of episodes of partial spawning. Altogether, these findings further confirm the biological and physiological validity of the parameters considered in this study (measurements of the digestive gland, gonad and capsule gland), their ability to reflect the reproductive dynamics of the species and their efficacy as visual quantitative indices for assessing the reproductive condition of *T. trunculus*.

CONCLUSION

Due to their remarkable ability to follow the seasonal variations of the species reproductive dynamics and clearly identify broad changes in reproductive development (highly accurate in detecting the periods of gonad maturation and spawning), these visual quantitative indices (GSI and CGI) constitute simple, practical and efficient methods for the routine assessment of the reproductive condition of *T. trunculus*, providing a useful and reliable alternative to several conventional condition indices and to laborious, time-consuming and expensive histological techniques. Furthermore, the results gathered in the present study strongly encourage the use of similar techniques and analogous visual quantitative indices for assessing the reproductive condition of other gastropod species, whose reproductive organs and other particular features might also represent a constraint for the use of conventional condition indices, and ultimately also as an alternative to histology in routine sampling and analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the technical staff of IPIMAR - CRIPSul for their help in the laboratory sampling procedures. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001) and by the research project PROMAR (Interreg III-A: Cooperación Algarve - Andalucía para la Promoción de Recursos Aquícolas Marinos en el Litoral Suratlántico).

REFERENCES

Albanese, M.P.; Calabrò, C.; Acosta Pomar, M.L.C., 1983. Ciclo biológico di *Trunculariopsis trunculus* L. (Moll. Gast. Pros.) vivente nel Lago di Faro. *Memorie di Biologia Marina e di Oceanografia*, 13: 133-147.

Beninger, P.G.; Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams and Reeve). *Journal of Experimental Marine Biology and Ecology*, 79: 19-37.

Buchanan, S., 2001. Measuring reproductive condition in the Greenshell^{TM} mussel *Perna canaliculus*. *New Zealand Journal of Marine and Freshwater Research*, 35: 859-870.

Cledón, M.; Arntz, W.; Penchaszadeh, P.E., 2005. Gonadal cycle in an *Adelomelon brasiliana* (Neogastropoda: Volutidae) population of Buenos Aires province, Argentina. *Marine Biology*, 147: 439-445.

Crosby, M.P., Gale, L.D., 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research*, 9: 233-237.

Davenport, J.; Chen, X., 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). *Journal of Molluscan Studies*, 53: 293-297.

Gabbott, P.A.; Walker, A.J.M., 1971. Changes in the condition index and biochemical content of adult oysters (*Ostrea edulis* L.) maintained under hatchery conditions. *Journal du Conseil International pour l'Exploitation de la Mer*, 34: 99-106.

Giménez, J.; Penchaszadeh, P.E., 2003. Size at first sexual maturity in *Zidona dufresnei* (Caenogastropoda: Volutidae) of the south-western Atlantic Ocean (Mar del Plata, Argentina). *Journal of the Marine Biological Association of the United Kingdom*, 83: 293-296.

Houart, R., 2001. *A Review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Ed. Evolver, Rome.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2004. Données sur le cycle reproducteur du mollusque gastéropode *Hexaplex trunculus* (Linné, 1758) de la Lagune de Bizerte (Tunisie). *Bulletin de la Société Zoologique de France*, 129: 407-418.

Lucas, A.; Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture*, 44: 187-200.

Mann, R., 1978. A comparison of morphometric, biochemical and physiological indexes of condition in marine bivalve molluscs. In: *Energy and Environmental Stress in Aquatic Systems* (Thorp, J.H.; Gibbons, I.W., Eds.), *DOE Symposium Series*, 48: 484-497.

Marques, M.R.; Oliveira, J.S., 1995. A pesca das "carteiras" na Ria Formosa. *Actas das Comunicações do 8º Congresso do Algarve*, 7-9 Abril 1995, Vilamoura, Portugal, 905-909 pp.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Poore, G.C.B., 1973. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda): 4. Reproduction. *New Zealand Journal of Marine and Freshwater Research*, 7: 67-84.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Ramorino, L.M., 1975. Ciclo reproductivo de *Concholepas concholepas* en la zona de Valparaíso. *Revista de Biología Marina (Valparaíso)*, 15: 149-177.

Spanier, E.; Karmon, N., 1987. Muricid snails and the ancient dye industries. In: *The Royal Purple and the Biblical Blue: Argaman and Tekhelet* (Spanier, E., Ed.), Keter Publishing House Jerusalem Ltd., Jerusalem, 179-192 pp.

Seed, R.; Suchanek, T.H., 1992. Population and community ecology of *Mytilus*. In: *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture* (Gosling, E., Ed.), Elsevier, Amsterdam, 87-169 pp.

Tirado, C.; Rodríguez de la Rúa, A.; Bruzón, M.A.; López, J.I.; Salas, C.; Márquez, I., 2002. *La Reproducción de Bivalvos y Gasterópodos de Interés Pesquero en Andalucía*. Junta de Andalucía, Consejería de Agricultura y Pesca, Huelva.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2004a. Meat yield of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae), an important fishing resource from the Ria Formosa lagoon (Algarve - southern Portugal). In: *Resúmenes del XIII Simposio Ibérico de Estudios del Bentos Marino* (Lorenzo, J.M.; Pajuelo, J.G.; Ramos, A., Eds.), Las Palmas de Gran Canaria, España, 21-24 Septiembre 2004, 143 pp.

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias, D.; Castro, M., 2004b. Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006. Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin*, 52: 337-341.

Vasconcelos, P.; Cúrdia, J.; Castro, M.; Gaspar, M.B., 2007. The shell of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) as a mobile hard substratum for epibiotic polychaetes (Annelida: Polychaeta) in the Ria Formosa (Algarve coast - southern Portugal). *Hydrobiologia*, 575: 161-172.

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar M.B. Gametogenic cycle of *Hexaplex (Truncolariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of the Marine Biological Association of the United Kingdom* (submitted).

Walne, P.; Mann, R., 1975. Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: *Proceedings of the 9th European Marine Biology Symposium* (Barnes, H., Ed.), Oban, Argyll, 2-8 October 1974. Aberdeen University Press, Aberdeen, 587-607 pp.

Wondrak, G., 1974. Laichverhalten und gelegebildung bei *Murex trunculus* L. (Gastropoda: Prosobranchia). *Archiv fuer Molluskenkunde*, 104: 115-119.

CHAPTER 4.3

Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias, D.; Castro, M., 2004. Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46 (2-3): 125-138.

ABSTRACT

Some authors have studied and described different aspects of the reproductive cycle of *Hexaplex (Truncolariopsis) trunculus*, but most data are quite ancient and fragmented, lacking information in important respects on the reproductive cycle of this species. Based on several individual and collective spawns deposited in laboratory aquaria, this study provides additional and more detailed information on the spawning behaviour and egg-laying pattern, description of the general morphology and dimensions of the spawns, egg capsules, eggs, embryos and early post-metamorphic juveniles, as well as the first data available on the growth rate of *T. trunculus* hatchlings and juveniles (until 4 months old). Females deposited an average of 118 ± 89 tongue-shaped egg capsules per individual spawn, measuring on average 5.5 mm length x 4.7 mm width x 2.6 mm thickness. These egg capsules contained 723 ± 66 eggs with an average diameter of 240 ± 8 μm. *T. trunculus* is a direct developer species (metamorphosed hatchlings) with an incubation period of approximately 1 month. At hatching, individuals measured 1.64 ± 0.22 mm shell length and presented a growth rate of 2.5 mm/month at the end of four months. The breeding habits, embryonic development, hatchling and juvenile growth rates are discussed in terms of their implications for the management of the artisanal fishery for *T. trunculus* in the Ria Formosa lagoon and the assessment of the potential of this muricid species for molluscan aquaculture.

INTRODUCTION

The Family Muricidae constitutes a very diverse and important component of marine communities, comprising more than 1150 confirmed species distributed worldwide (Vokes, 1996). *Hexaplex (Trunculariopsis) trunculus* (Linnaeus, 1758) is distributed in the Mediterranean Sea and adjacent Atlantic Sea, from the Portuguese coast to Madeira and Canaries Archipelagos (Poppe and Goto, 1991; Macedo *et al.*, 1999). This species occurs in the inter-tidal and infra-littoral zones up to 100 metres depth, inhabiting sand, sandy-mud or preferentially mud substrates (Poppe and Goto, 1991).

The muricid *T. trunculus* is a common inhabitant of the sub-tidal and inter-tidal areas of the Ria Formosa lagoon (Algarve coast - southern Portugal), where is subjected to a locally important artisanal fishery. Due to the decrease in the abundance of this species in the Ria Formosa in recent years and the growing demand for gastropods in the seafood market, the commercial value of *T. trunculus* in Portugal increased markedly in the latest years (reaching values of 10-15€/kg for first sale), which has been generating some expectations in terms of its potential as a new species for molluscan aquaculture.

According to fishermen that catch this species in the lagoon, the spawning period of *T. trunculus* in the Ria Formosa lagoon generally occurs between February and June. During this period, females get together in massive agglomerations depositing collective spawns, locally known as “sponges” due to their morphology. According to Muzavor and Morenito (1999), the bigger communal egg masses can measure one metre in length and a half meter in width. The larger agglomerations of females during communal spawning (that can reach several hundred individuals in larger spawns) are subjected to hand collecting by fishermen during low tide, due to the easy capture and consequent high fishing yield (that can amount to some tens of kilos in larger spawns). Fishermen mark the location of these communal spawns in order to periodically collect the individuals that are spawning (Muzavor and Morenito, 1999).

This collecting practice could have contributed to the decrease in the abundance of this species in recent years.

The most studied aspect of the biology of *T. trunculus* is the sexual abnormality known as imposex (superimposition of male sexual characters onto females), which was described in this species by Martoja and Bouquegneau (1988) and has been pursued by several workers on Mediterranean Sea populations (*e.g.* Axiak *et al.*, 1995; Terlizzi *et al.*, 1998, 2004). However, some authors have studied and described different aspects of the reproductive cycle, namely gametogenesis (Schitz, 1920), histology of the gonadal cycle (Albanese *et al.*, 1983), spawning behaviour and egg-laying pattern (Wondrak, 1974), egg masses and egg capsules (Fischer and Raffy, 1933; Dulzetto, 1946, 1950; Wondrak, 1974; Bandel, 1975; Barash and Zenziper, 1980), and embryonic and early post-metamorphic juveniles (Bandel, 1975; Barash and Zenziper, 1980; Sabelli and Tommasini, 1982).

Nevertheless, the knowledge available on embryonic and larval development in the Family Muricidae is still very limited (Romero *et al.*, 2004) and, in the particular case of *T. trunculus,* some data are quite ancient and fragmented, lacking information on important aspects of the reproductive cycle of this species. In this context, the aim of the present work is to provide additional and more detailed information on the spawning behaviour and egg-laying pattern, and to describe the general morphology and dimensions of egg masses, egg capsules, eggs, embryos and early post-metamorphic juveniles. Additionally, to the author’s best knowledge, this study reports the first data available on the growth rates of *T. trunculus* hatchlings and juveniles (until 4 months-old).

MATERIAL AND METHODS

Specimens of *T. trunculus* were maintained in two laboratory glass aquaria (45 L) without substrate (to minimize maintenance and consequent disturbance to the individuals), with oxygenated seawater and under natural photoperiod. During the study period, seawater temperature and salinity were monitored periodically. Seawater temperature ranged between 18ºC and 20ºC and salinity was maintained approximately constant at 35‰ by adding diluted seawater to aquaria to compensate for excessive evaporation. Specimens were provided with abundant food supply, namely live bivalves of species that constitute their natural prey in the bivalve growth banks of the Ria Formosa lagoon (cockle, *Cerastoderma edule*; grooved carpet shell, *Ruditapes decussatus*; giant cupped oyster, *Crassostrea gigas*; and common mussel, *Mytilus edulis*) (P. Vasconcelos, personal observation).

Both at the end of April (aquarium 1) and May 2004 (aquarium 2) females started depositing individual and collective spawns. Spawning behaviour and egg-laying pattern were photographed and filmed. The rhythm of egg mass deposition was assessed only for individual spawns, by counting periodically (4–12 h interval) the number of egg capsules deposited, but only in the early stages of the spawning process (while the number of egg capsules in each spawn could be accurately counted). Immediately after they finished spawning, females were measured for shell length (SL - mm) with a digital calliper.

In the present work, the terms "*embryos*" refer to all stages prior to release from egg capsules (oothecae) and "*early post-metamorphic juveniles*" or "*hatchlings*" to those stages that have undergone metamorphosis and hatched from egg capsules. Excapsulated embryos are those stages that have been artificially removed from egg capsules.

During the incubation period, egg capsules were removed periodically (daily during the first week and weekly during the remaining incubation period) from a selected individual spawn, in order to count the eggs and describe the embryonic development. After carefully opening the egg capsules with small scissors, eggs and/or excapsulated embryos were counted, photographed and measured. Excapsulated embryos were then preserved in 70% alcohol to prevent erosion of the protoconch. Counting and measurements (egg diameter: ED - µm; shell length: SL - mm) were made with a calibrated ocular micrometer under a stereo-microscope.

The number of egg capsules per individual egg mass was estimated by weight, using a top loading digital balance. 100 egg capsules from a collective egg mass were found to weigh 2.69 g and this value was then compared with the total weight. These egg capsules were preserved in 4% buffered seawater formalin to maintain their shape. The length (CL - mm), width (CW - mm) and thickness (CT - mm) of each egg capsule were measured with a digital calliper. Egg capsule measurements followed the criteria defined by D'Asaro (1970, 1986), with length being the greatest distance between the basal membrane and the apex, width the greatest distance between lateral edges at right angles to length, and thickness the greatest distance from convex side to concave side at right angles to length and width.

Immediately after hatching, early post-metamorphic individuals were carefully removed with pipettes from aquaria and transferred to an open water tank circuit (running seawater and oxygenation) in the Tavira Molluscan Aquaculture Experimental Station. Separate groups of hatchlings were installed in different tanks according to their date of hatching, inside floating plastic sieves (500 µm mesh size) where they were provided with food "*ad libitum*" (a mixed diet of fish ration in pellets and bivalves soft tissue: cockle - *Cerastoderma edule*, white clam - *Spisula solida* and giant cupped oyster - *Crassostrea gigas*).

In order to describe hatchling development, early post-metamorphic juveniles (1 day after hatching = 1 DAH) were photographed and measured with a calibrated ocular micrometer under a stereo microscope, and this procedure was repeated weekly until gastropods were 1 month-old. To allow for hatchling and juvenile growth rates estimation, 100 individuals from a group of gastropods hatched in the same day were measured for shell length (SL - mm) almost daily until 1 month old and weekly until now (4 months old). Initially, measurements were made with a calibrated ocular micrometer under a stereo microscope, until specimens reached a suitable dimension (approximately 10 mm SL) to be accurately measured with a digital calliper.

RESULTS

Spawning behaviour and egg-laying pattern

Spawning started both at the end of April (aquarium 1: 23/04/2004) and May (aquarium 2: 20/05/2004), with females depositing asynchronously both individual (Figure 1a-f) and communal egg masses (Figure 1g). Initially, egg capsules were attached to the walls of the aquaria and afterwards also to the bottom of the aquaria. At the beginning of spawning, the slow deposition of egg capsules through the pedal gland was clearly visible on the glass walls of the aquaria (Figure 1a,b). Apparently, egg-laying followed a relatively similar pattern among the several females that spawned during the experiment, starting with the vertical movement of the foot (up-down direction) and deposition of a few egg capsules (10-20) (Figure 1c-e) that provided a suitable base for the deposition of several upper layers of egg capsules, slowly forming voluminous egg masses of irregular shape (Figure 1f). Some unattached egg capsules (<10) were occasionally detected in the bottom of the aquaria, and the deposition of a few egg capsules (<5) on the shell of another spawning female was observed on a single occasion.

Frequently, both previously deposited spawns and egg masses being deposited were used by other females to start spawning on their periphery, thus constituting collective egg masses. This type of communal spawning also resulted when individual egg masses that were deposited in close proximity grew in size due to continuous egg capsule deposition. Egg masses in communal spawns were very difficult (normally impossible) to assign to individual females. Nevertheless, due to slightly different colorations of egg capsules (between pasty yellow and pale orange), individual egg masses in collective spawns could occasionally be differentiated (Figure 1g).

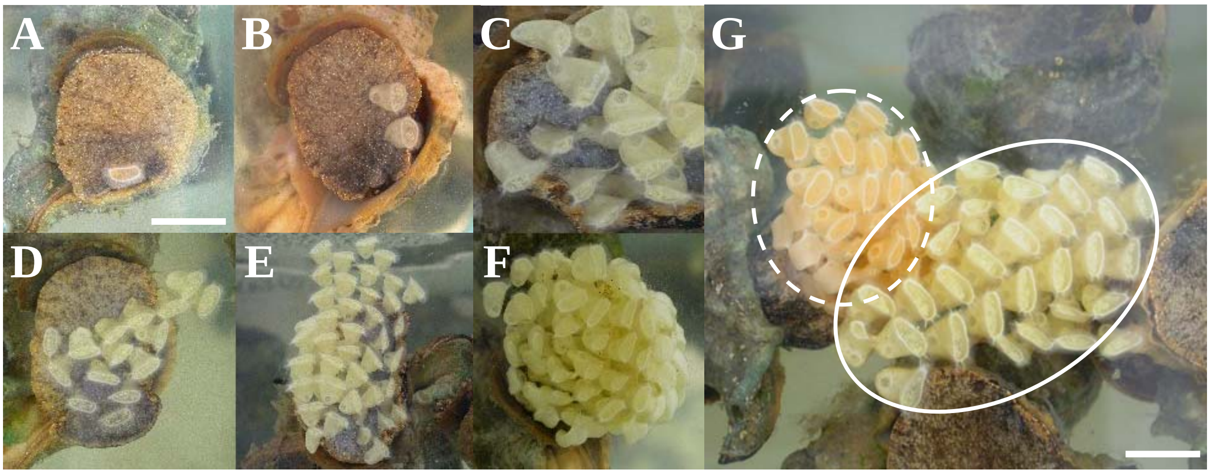


Figure 1. Spawns of *T. trunculus* in the laboratory aquarium, with reference to spawning behaviour and egg-laying pattern: **a-b)** Detail of the pedal gland during oviposition. **c-f)** Sequential images of the deposition of egg capsules on the aquarium wall, with the foot covering the last deposited egg capsules. The small eggs can be seen through the transparent basal membrane of the egg capsules. **g)** Communal spawning group of *T. trunculus*, showing the different coloration of egg capsules, from yellowish white (brighter spawn on the right, limited by a continuous line) to pale orange (darker spawn on the left, limited by a dotted line) (scale bars = 1 cm).

During the study period, a total of seven individual egg masses and nine communal spawns (from an unknown number of females, but generally of two to five females) were deposited in the aquaria. Spawning females measured between 55.07 and 75.21 mm SL (64.70 ± 7.31 mm SL). The spawning duration was highly variable between the seven individually spawning females, with an average duration of 1.8 ± 1.6 days, but varying between a minimum of a few hours and a maximum of five consecutive days (Table 1).

Table 1. Summary of the main characteristics of *T. trunculus* spawning in the laboratory (spawning behaviour, egg capsules, eggs, embryos and hatchlings), with comparison to similar data available from previous studies.

		Previous studies					
	Present study	**Fischer and Raffy (1933)**	**Dulzetto (1946)**	**Wondrak (1974)**	**Bandel (1975)**	**Barash and Zenziper (1980)**	**Sabelli and Tommasini (1982)**
Spawning season (field/laboratory)	F: February - June L: late April - early June	L: mid June	F: May	F: May - June	L: May - June	F: May - July	
Spawning duration (days)	1.8 ± 1.6 days (few hours - 5 days)	≈ 2*					
Spawning rhythm (no. capsules/hour)	0.9 ± 0.6 (0.3 - 2.0)		≈ 3*				
Number of egg capsules per spawn	118 ± 89 (17 - 242)	100			90		
Size of egg capsules (mm)	CL: 5.5 ± 0.5 (4.4 - 7.9) CW: 4.7 ± 0.4 (3.5 - 6.2) CT: 2.6 ± 0.3 (1.5 - 3.2)	CL: 6 CW: 4 - 5	CL: 3 - 5 CW: 3 - 5			CL: 5 - 6 CW: 4 - 4.5	
Proportions of egg capsules (%)	CW / CL: 85.3 ± 7.8 (54.3 - 98.4) CT / CL: 48.1 ± 6.9 (28.8 - 61.9) CT / CW: 56.5 ± 7.8 (35.1 - 79.3)						
Number of eggs per capsule	723 ± 66 (641 - 804)		111 - 168** 418 - 849**				
Egg diameter (μm)	240 ± 8 (216 -264)	≈ 250	168 - 223				
Incubation period (days)	30 - 32				≈ 30*		
Number of embryos per capsule		10 - 20	5 - 10		15		
Hatchling size (mm SL)	1.64 ± 0.22 (1.10 - 2.30)				1.1 - 1.3		
Number of protoconch whorls	1.5 - 2.5				2.5	2	1¾ - 2.5
Growth rate (mm SL/month)	2.5 (4 months)						

F - field; L - laboratory; SL - shell length; CL - capsule length; CW - capsule width; CT - capsule thickness; * - units modified from original data; ** - according to collecting site.

Accordingly, the spawning rhythm (estimated in the early stages of deposition of individual spawns) was also very inconsistent among females, varying between 0.3 and 2.0 egg capsules deposited per hour (mean, 0.9 ± 0.6 egg capsules) (Table 1). The spawning rhythm apparently increased in speed during the night. In a few cases, without being disturbed, females interrupted spawning soon after depositing only a small number (<10) of egg capsules; these were excluded from estimations of spawning duration and rhythm.

During the spawning period, females stopped feeding, irrespectively of prey species and size. This was confirmed by the periodic inspection of bivalve prey, which were always alive and showed no signs of predation attempts (damaged borders or drilled holes on the shell).

Description of egg masses and egg capsules

Both size and shape of egg masses varied considerably, generally forming an irregular mass supported by a more or less wide base of egg capsules attached to each other. Individual egg masses usually presented a more uniform and rounded/spherical shape, while collective spawns were more heterogeneous and asymmetrical in shape. Nevertheless, within each egg mass (either individual or collective, and irrespectively of their size and shape), egg capsules were generally positioned with concavities adjacent to convexities, with most capsules in each layer oriented in the same direction and with escape apertures oriented towards the interior. The few exceptions to this organisation were attributed to female activity during communal spawning. Immediately after oviposition, egg capsules were whitish and fairly transparent, but slowly became slightly darker and opaque, normally yellowish-white and occasionally pale orange, which was attributed to the degree of development of the embryos. An example of the morphology, size and proportions of a collective egg mass deposited in the laboratory aquaria is presented in Figure 2a.

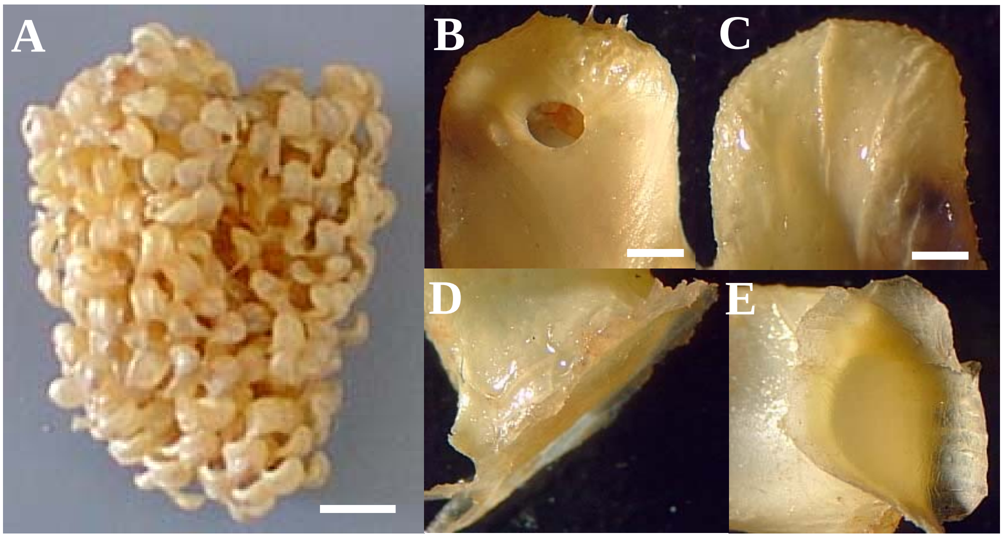


Figure 2. a) Collective egg mass of *T. trunculus* deposited in the laboratory aquarium (dimensions: 71.6 mm length x 49.6 mm width x 57.1 mm height; total weight = 30.36 g, estimated number of egg capsules = 1129) (scale bar = 1 cm). **b)** Concave side of an egg capsule, with an embryo seen through the rounded escape aperture. **c)** Convex side of an egg capsule, showing the capsular ridges. **d-e)** Details of the basal membrane of the egg capsules. Images photographed under a stereo microscope (magnification: 6.5x) (scale bars = 1 mm).

The estimated number of egg capsules per individual spawn reflects the high variability in terms of egg mass size. The average number was 118 ± 89, varying between 17 and 242 (Table 1). Communal egg masses, from an undetermined number of spawning females, had between 111 and 1813 egg capsules per spawn.

During oviposition, egg capsules were deposited by the pedal gland, while the foot covered the last spawned capsules. Egg capsules were softly curved and tongue-shaped (lingulate), with a rounded apex and with a convex side (with irregular longitudinal ridges and smaller interconnecting ridges) (Figure 2c) and a concave side (with a more regular surface) (Figure 2b). The rounded escape aperture was located near the apex, on a large longitudinal depression of the concave side (Figure 2b), being covered by a thin and slightly transparent membrane before hatching. Egg capsules were closely (and strongly) attached to the aquaria walls and bottom by a large and branched basal membrane (Figure 2d,e), or deposited on each other, normally being attached to adjacent egg capsules by two or rarely three peduncles of the basal membrane. In recently deposited spawns, eggs were clearly visible through the translucent capsule wall, being generally located on the bottom of basal membrane.

The size (length, width and thickness) and relative proportions of the egg capsules are presented in Table 1. Egg capsules were longer than wider (capsule width corresponded to 85% of capsule length) and thicker (capsule thickness corresponded to 48% capsule length). On average (n = 100), egg capsules measured 5.5 mm length x 4.7 mm width x 2.6 mm thickness, but dimensions were highly variable among egg capsules, with capsules ranging in length between 4.4 mm and 7.9 mm (Table 1).

Intracapsular development

Five capsules deposited in the aquaria on day 1 contained an average of 723 ± 66 eggs per capsule, varying between 641 and 804 (Table 1).

Such recently spawned eggs were spherical and enclosed in a fine vitelline membrane (Figure 3a). Egg coloration was whitish and opaque due to the large amount of vitelline material contained, which is typical of gastropod species with direct development (non-planktotrophic development). The measurement of 100 eggs from each of those five egg capsules (n = 500) revealed that they had an average diameter of 240 ± 8 µm, ranging from 216 µm to 264 µm) (Table 1).

The excapsulation of the eggs allowed the initial stages of early embryonic development to be followed, as illustrated in Figure 3a-f. These stages were characterised by a marked asynchrony both throughout the egg mass and within individual egg capsules, with different developmental phases and embryos with distinct sizes being present inside the same egg capsule. Besides these normally developed eggs, many others had not developed or showed an atypical development (e.g., arrest or abnormal cleavage, or the development of prominent malformations). These eggs developed no further, but provided nutrition for the developing embryos.

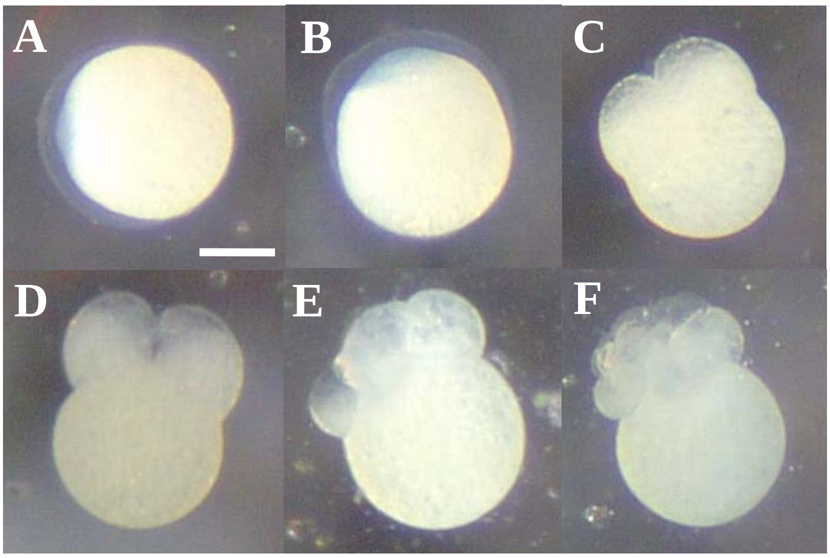


Figure 3. Eggs and different phases of early embryonic development of *T. trunculus*: **a)** Fertilised egg (average diameter: 240 µm). **b)** Beginning of deutoplasmic segregation prior to polar body formation. **c)** Beginning of the oblique constriction in the upper third of the egg, generating unequal blastomeres. **d)** Latitudinal division with the completion of the C blastomere and formation of an undifferentiated D blastomere. **e)** Longitudinal cleavage of the AB blastomere with the formation of an embryo with four blastomeres and a large polar body. **f)** Dextral development of the first quartet of micromeres prior to absorption of the polar lobe by the D blastomere and the formation of the 16-cell stage. Images photographed under a stereo microscope (magnification: 50x) (scale bar = 100 µm).

The most advanced phases of embryonic development were characterised by the continuous absorption of vitelline material, development and subsequent degeneration of the velum and gradual formation of the foot and protoconch (Figure 4). Initially, the embryo contained an enormous amount of yellow vitelline material, the velum was small and approximately rounded in shape, and the organism was enclosed in a very fragile membrane-like shell (Figure 4a). Subsequently, almost all vitelline material was absorbed, the lobed velum bordered by cilia increased in size and activity, but the shell was still transparent and very fragile (Figure 4b). At this stage the lobed velum was very active and the embryos moved rapidly via ciliary action (mostly with rotational movements), but were unable to swim since this intracapsular veliger never attained the size characteristic of swimming veligers. Afterwards, the velar lobes progressively regressed in size as the velum gradually degenerated, accompanied by the development of the foot (Figure 4c,d). The pulsating embryonic heart and the eyespots were barely visible through the thin and clear protoconch, which gradually developed a pale yellow-brownish pigmentation.

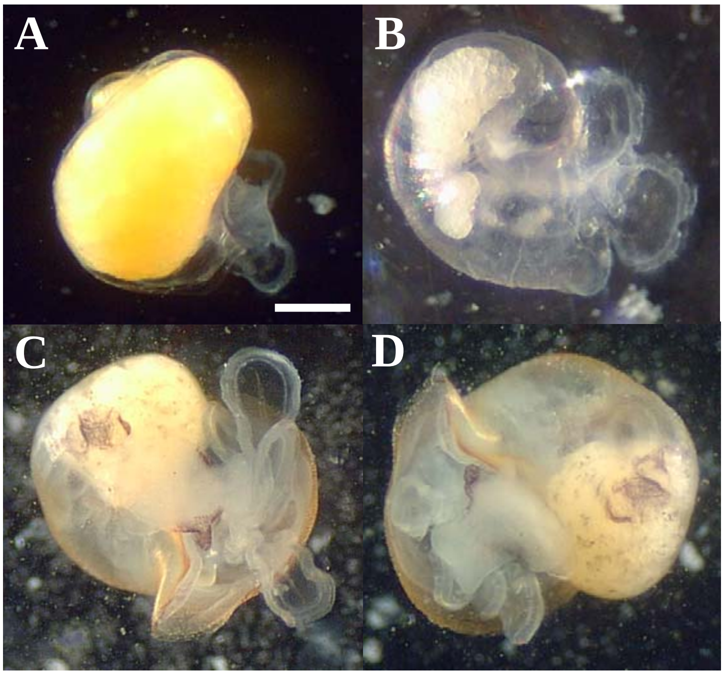


Figure 4. Excapsulated embryos of *T. trunculus* in different phases of intracapsular development: **a)** Embryo with abundant vitelline material, at the beginning of the formation of a small velum and a very thin and transparent shell. **b)** Embryo completing the absorption of vitelline material, with a visible lobed velum and an incipient and transparent protoconch. **c)** Ventral view of the pediveliger stage, still with a large lobed velum and beginning the development of the foot. **d)** Progressive degeneration of the lobed velum and further development of the foot, accompanied by the strengthening and darkening of the protoconch. Images photographed under a stereo microscope (magnification: 6.5x) (scale bar = 100 µm).

At the end of intracapsular development (3-4 weeks of incubation), the lobed velum completely disappeared, the mantle and foot gained a brown pigmentation and became actively used for locomotion. When turned over, the embryos always righted themselves by extending the foot to make contact with the surface. The eyes became darkly pigmented and supported on short stalks, which progressively grow to extend beyond the shell. The smooth brown protoconch began to darken, assuming an increasingly translucent brownish coloration, and growth in shell length was clearly seen by the elongation of the siphonal canal and the deposition of a thin new layer on the outer lip (forming the first varix). Once again, an asynchronous embryonic development was observed inside each egg capsule, with embryos varying in their developmental stages and shell sizes.

Hatchling and juvenile development

The incubation period lasted approximately one month (30 days in aquarium 1 and 32 days in aquarium 2) (Table 1), after which benthic early post-metamorphic individuals started hatching continuously from the egg capsules during the following week. Hatching was not synchronous in the same spawn, since frequently the same egg mass contained both full and empty egg capsules. Completely metamorphosed hatchlings abandoned the egg capsules at the crawling stage. Many hatchlings readily dispersed at the surface of the egg capsules and in the interstitial spaces between egg capsules. Simultaneously, some completely developed embryos remained inside the egg capsules, despite the escape aperture being opened through the dissolution of the covering membrane.

At hatching, the early post-metamorphic protoconch was smooth and with a regular surface, presenting some variation in the number of shell whorls, varying between approximately 1.5 in smaller to 2.5 whorls in bigger hatchlings (Table 1). Individuals hatched with an average shell length of 1.64 ± 0.22 mm (Figure 5a). The hatchling size was also highly variable (between 1.10 and 2.30 mm shell length) (Table 1), reflecting the differential intracapsular development (number of protoconch whorls) and the consequent disparity in the embryos size (protoconch length).

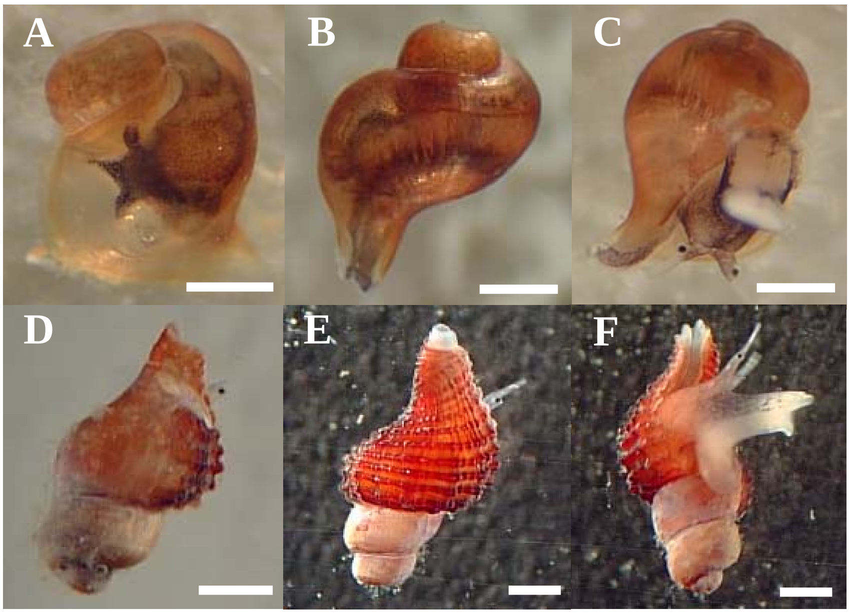


Figure 5. Laboratory-hatched specimens of *T. trunculus*: **a)** Recently hatched individual (1 DAH) with its characteristic smooth and regular surface (average shell length = 1.64 ± 0.22 mm). **b)** Dorsal view of 1 week-old hatchling, with the elongation and folding of the siphonal canal (average shell length = 1.76 ± 0.22 mm). **c)** Ventral view of 1 week-old hatchling, with increasing pigmentation of the foot. **d)** Dorsal view of 2 weeks-old hatchling, with the teleoconch with recently deposited striae on the shell outer lip (average shell length = 1.89 ± 0.26 mm). **e)** Dorsal view of 1 month-old juveniles, with the characteristic darkening of the brown pigmentation of the teleoconch striae (average shell length = 2.63 ± 0.44 mm). **f)** Ventral view of 1 month-old juveniles, showing foot and eye stalks movements. Images photographed under a stereo microscope (magnification: 6.5x) (scale bars = 500 μm).

During the following weeks of hatchling development, teleoconch growth is mainly due to a marked elongation of the siphonal canal, which became increasingly folded and pointed to the exterior of the shell (Figure 5b,c). Progressively, the deposition of teleoconch became more evident due to its most characteristic feature, namely the sequential deposition of irregular striae and lamellae on the shell outer lip (Figure 5d). During this phase, the shell assumed a gradually darker coloration due to the brownish pigmentation of the teleoconch layers (Figure 5e,f).

Despite being very active (crawling quite rapidly in comparison to their size) these hatchlings apparently did not feed during the initial weeks of development, refusing all types of food available in the tanks (fish ration in pellets and bivalves soft tissue) and probably subsisting with the residual energetic reserves from the vitelline material. Immediately after this phase, but whilst specimens were still not ingesting the available food sources, extensive cannibalism occurred, with the larger hatchlings predating smaller hatchlings (confirmed by the large number of drilled and empty shells found in the tanks) (Figure 6a,b). Additionally, some hatchlings apparently died from dehydration, due to crawling above the water surface in the aquaria and tanks. Altogether, these factors resulted in a marked increase in the mortality rate. Then after approximately 1 month, when cannibalism by juveniles ceased and they started to feed on crushed bivalves and drilling live prey (small bivalves) (Figure 6c,d), the mortality rate decreased markedly.

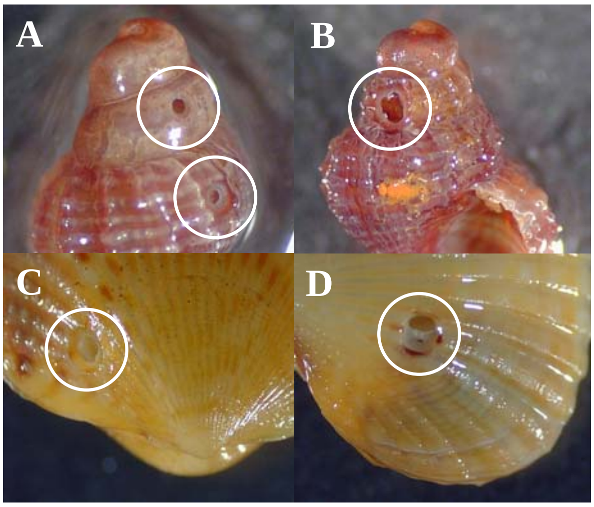


Figure 6. Drilling behaviour of *T. trunculus*: **a,b)** Detail of drilled shells of *T. trunculus* due to juvenile cannibalism (white circles). **c,d)** Detail of drilled shells of live prey (small cockles, *Cerastoderma edule*) (white circles). Images photographed under a stereo microscope (magnifications: 6.5x and 10x).

The growth curve and monthly growth rates of hatchlings and juveniles during the first four months of life are represented in Figure 7. The growth was characterised by the fact that specimens were still in a phase of exponential growth, with an increasing inter-individual variability in shell length (Figure 7a), and by a continuous increase in monthly growth rates (mm SL/month) (Figure 7b). In fact, growth rate was 1.0 mm/month during the first month (2.63 ± 0.44 mm SL), gradually increasing to 2.0 mm/month during the first two months (5.58 ± 0.91 mm SL), 2.2 mm/month during the first trimester (8.28 ± 1.32 mm SL) and 2.5 mm/month over the first four months (11.66 ± 2.05 mm SL). These monthly growth rates resulted in an increase of 60% in shell length during the first month, 240% during the first 2 months, 405% in the first trimester and 610% at the end of 4 months.

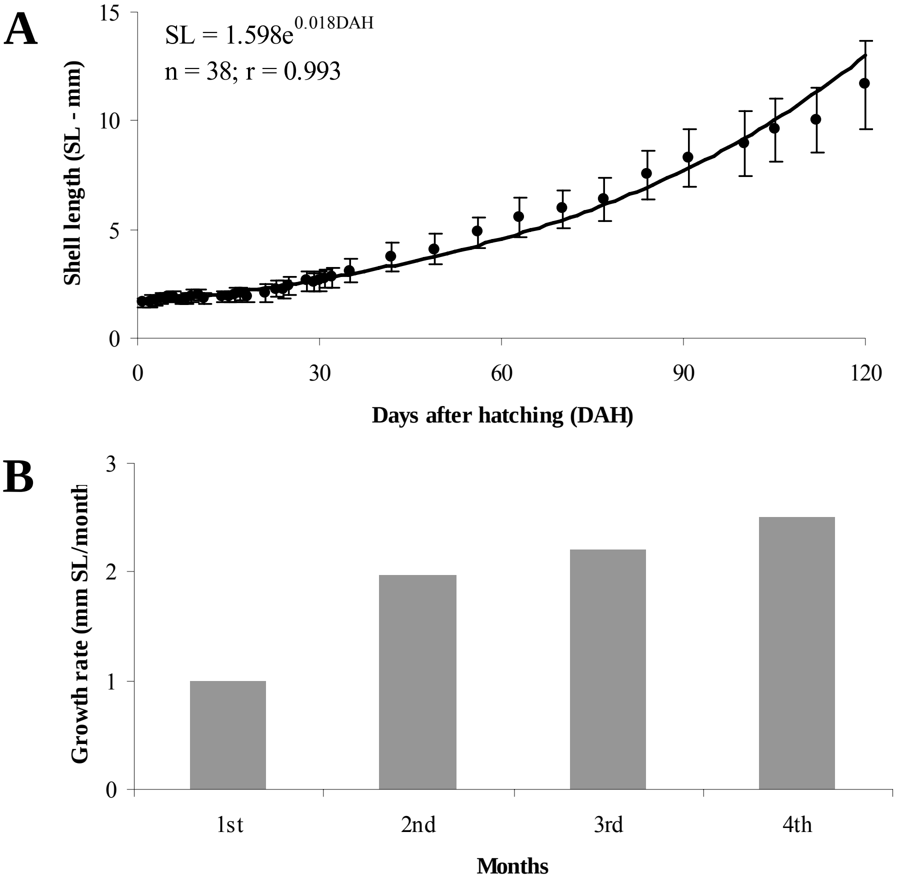


Figure 7. Growth features of *T. trunculus* hatchlings and juveniles maintained in the tank circuit during the first 4 months of life: **a)** Growth curve. **b)** Monthly growth rates at the end of each month.

DISCUSSION

Like most neogastropods, *T. trunculus* is gonochoristic and fertilisation is internal. According to fishermen, the spawning season in the Ria Formosa lagoon usually occurs between February and June; similarly, spawning periods in May (Dulzetto, 1946), May-June (Wondrak, 1974) and May-July (Barash and Zenziper, 1980) have been reported in relation to different areas of the Mediterranean Sea (Table 1). In the present study, spawning in laboratory aquaria started both at the end of April and end of May and extended until early June, which corroborates other information under these conditions that specifies mid-June (Fischer and Raffy, 1933) and May-June (Bandel, 1975) (Table 1).

During the spawning period females stopped feeding, a phenomenon confirmed by the fact that bivalve prey in the aquaria were invariably alive and without any signs of attempted predation. It is known from experimental studies on muricid predation (under non-spawning circumstances) that under crowded conditions specimens may change their behaviours (*e.g.*, lower activity levels, lower prey consumption rates, change in type of attack) (Gregory Herbert, personal communication). In the present case, although specimens were not housed individually in separate containers, conditions in the aquaria were definitely not crowded. Furthermore, since suspension of feeding during spawning has been observed previously (Dulzetto, 1946, 1950), it should perhaps be considered normal behaviour.

In the field *T. trunculus* generally builds collective spawns, sometimes of substantial dimensions (Fischer and Raffy, 1933), with many females depositing hundreds of capsules (Bandel, 1975). However, the dimensions of these collective egg masses are highly variable, as reported by Barash and Zensiper (1980) (3.5 to 16 cm length) and Dulzetto (1946) (8 to 25 cm length, according to collecting site). The largest spawns of *T. trunculus* in the Ria Formosa lagoon can reach approximately 1 meter in length by 0.5 meters in width (Muzavor and Morenito, 1999), which due to the number of spawning females are obviously much bigger than the collective egg masses deposited in laboratory aquaria in the present study. Nevertheless, spawns produced in aquaria may be rather different from spawns collected in the field, namely in morphology and the number of egg capsules (D'Asaro, 1970). According to Dulzetto (1946, 1950), egg masses deposited in laboratory aquaria are generally smaller than those in the field.

In laboratory aquaria, the spawning duration of *T. trunculus* was highly variable between females, with an average period of 1.8 days and thus similar to the spawning duration of approximately 48 hours reported by Fischer and Raffy (1933) (Table 1). The spawning rhythm in the early stages of oviposition also varied among females, with an average of almost one egg capsule per hour (0.9 ± 0.6 egg capsules), a figure slightly lower than that observed by Dulzetto (1946), of one capsule per 20-25 minutes (Table 1).

Because of their size and conspicuous appearance, muricid egg masses have been studied and illustrated by many authors. The collective spawns of *T. trunculus* are very similar to those of *Chicoreus ramosus* (D'Asaro, 1970), *Phyllonotus erythrostomus* (D'Asaro, 1991) and *Phyllonotus pomum* (D'Asaro, 1970, 1991; Moore and Sander, 1978), with the egg capsules positioned in the typically muricine manner, with concavities adjacent to convexities (D'Asaro, 1986) and assembled into a massive protective mass (D'Asaro, 1991). Just as in other muricids, the bulky communal egg masses of *T. trunculus* doubtless promote survival. Since egg masses are preyed upon by other gastropods, crustaceans and fishes, the formation of a voluminous and multi-layered egg mass constitutes a physical barrier to many predators, which can penetrate the outer layers but are unable to proceed further, due to the resistance of the tough and spongy mass (D'Asaro, 1970). This was evidenced by some egg masses of *T. trunculus* collected in the Ria Formosa, which showed signs of predation of the external capsules, but without damage to the protected interior (P. Vasconcelos, personal observation).

At least four distinct capsular shapes occur in the genus *Murex*, with the curved and tongue-shaped (lingulate) egg capsules of *T. trunculus* corresponding to the third type described by D'Asaro (1970). The morphology of these egg capsules is quite similar to those of *Siratus senegalensis* (D'Asaro, 1970), *Phyllonotus erythrostomus* (D'Asaro, 1991) and *Phyllonotus pomum* (D'Asaro, 1970, 1991; Moore and Sander, 1978). Higher prosobranchs that employ the pedal moulding mechanism produce egg capsules that are shaped into morphologically distinct structures of varying complexity, which may be valuable in recognising taxonomic relationships (being attributable to specific genera, or less frequently, to specific families) (D'Asaro, 1986). However, the taxonomic value of these features remains to be determined, perhaps when more complete descriptions become available. In fact, intrageneric differences in capsular shape and structure are slight in most cases (D'Asaro, 1970), but a minority can include extreme divergence (D'Asaro, 1986).

Within the family Muricidae, egg capsules are typically composed of 3-4 discrete laminae: an outer protective lamina that seals the whole capsule including the escape aperture; a thick middle lamina that comprises the internal skeleton of the capsule wall and is composed of multiple fibrous layers; and one or two inner laminae that enclose the developing embryos and intracapsular fluid within a transparent chamber (D'Asaro, 1988; Rawlings, 1995). The tough and thick-walled egg capsules of *T. trunculus* apparently provide high physical protection to the eggs and developing embryos. Additionally, the small ridges on the external surface of *T. trunculus* egg capsules apparently serve to further reinforce and increase the resistance of the egg capsule walls, thus enhancing the protection of eggs and embryos. Furthermore, the orientation of the escape apertures, which are directed towards the interior of the egg mass, also suggests that during oviposition females position capsules to provide protection from predators that may attack via the escape apertures (D'Asaro, 1991). Nevertheless, this physical protection alone may not be sufficient to protect the reproductive material, since the egg masses are composed primarily of proteins and polysaccharides, making them highly suitable for the settlement of microorganisms and potentially vulnerable to microbial infection in the marine environment (Benkendorff, 2001b). In this context, several gastropod species have evolved some form of chemical protection for their eggs and encapsulated embryos, and this is the case for *T. trunculus*, whose egg capsules contain chemical compounds that inhibit the activity of marine pathogenic bacteria (Benkendorff, 2001a,b).

In the present study, *T. trunculus* deposited in aquaria an average of 118 egg capsules per individual spawn, which is similar to the values reported for this species by other authors, namely, 90 capsules (Bandel, 1975) and 100 capsules per egg mass (Fischer and Raffy, 1933) (Table 1). These egg capsules measured an average of 5.5 mm length x 4.7 mm width x 2.6 mm thickness, but dimensions were highly variable among egg capsules, varying between 4.4 and 7.9 mm capsule length (Table 1). Similar capsular dimensions have been reported previously: 6 mm length x 4-5 mm width (Fischer and Raffy, 1933) and 5-6 mm length x 4-4.5 mm width (Barash and Zenziper, 1980). Dulzetto (1946) reported smaller dimensions - 3-5 mm length x 3-5 mm width (Table 1), although these were highly variable between different collecting sites (Dulzetto, 1946, 1950).

Spawning females deposited an average of 723 eggs per capsule in the aquaria (minimum of 641 and maximum of 804 eggs per capsule) (Table 1). Dulzetto (1946) detected a high variability in the number of eggs per capsule according to collecting site, varying between 111-168 eggs in capsules from one site and 418-849 eggs in capsules from another (the latter are similar to the potential fecundity registered in the present study) (Table 1). On average, *T. trunculus* egg diameter was 240 μm (minimum 216 μm and maximum 264 μm), which corroborates the size of approximately 250 μm measured by Fischer and Raffy (1933), but is markedly larger than the 168-223 μm egg diameter referred by Dulzetto (1946) (Table 1).

These eggs are characterised by their large size and possession of a large amount of vitelline material, which is typical of species with direct development (non-planktotrophic development) (Spight, 1975; Lima and Lutz, 1990). Developmental times (times required by embryos to complete development) are also correlated with egg size, with smaller eggs developing more rapidly than larger ones (Spight, 1975). In the present case, the incubation period lasted approximately one month (30-32 days), which agrees perfectly with the duration of about one month reported by Bandel (1975) (Table 1).

In this study, metamorphosed individuals hatched with an average shell length of 1.64 mm (highly variable between a minimum of 1.10 and maximum of 2.30 mm shell length), a significantly greater size than the 1.1-1.3 mm shell length of recently hatched gastropods previously reported by Bandel (1975) (Table 1). Reflecting this variability in size, the early post-metamorphic protoconch of recently hatched *T. trunculus* also presented some variation in the number of shell whorls (between 1.5 and 2.5 whorls) (Table 1). Sabelli and Tommasini (1982) also detected this phenomenon, reporting marked variability in the number of whorls in the protoconch of this species, varying between 1¾ and 2.5 whorls. However, other authors have referred to only a single number of 2 whorls (Barash and Zenziper, 1980) or 2.5 whorls (Bandel, 1975) (Table 1).

The size of the hatchling shell has been reported to reflect egg diameter, which is a function of the amount of yolk material contained in the egg (Lima and Lutz, 1990). Furthermore, higher variability in protoconch size and number of whorls has been considered to be characteristic of species that have direct development and feed on nurse eggs inside the capsules (Moore and Sander, 1978). In the case of *T. trunculus*, this variability has been attributed to different developmental conditions (affecting the ratio of embryos:nurse eggs) that vary among egg capsules and spawns (Sabelli and Tommasini, 1982). Nevertheless, this variation does not correspond to variability in protoconch microsculpture, which is always characterised by the presence of small granules that are widely dispersed and irregularly positioned (Sabelli and Tommasini, 1982). Additionally, in *T. trunculus* the contribution of nurse eggs during embryonic development also produces more irregular protoconchs and larger embryonic shells in comparison to closely related species that lack additional nutrition (Bandel, 1975). Altogether, these phenomena reduce the taxonomic value of protoconch analysis (Sabelli and Tommasini, 1982), at least for comparisons between species with and without direct development and/or nurse eggs.

As predictable for a muricacean from this latitude (37ºN) and habitat (shallow water soft bottoms) (Spight, 1977), *T. trunculus* is a direct developer species (with metamorphosed hatchlings). This reproductive pattern is intimately connected to species life-history strategy. It provides enhanced protection of eggs and embryos against physical stresses and predation, and allows for supplementary mechanisms of embryo nutrition (nurse eggs), whilst reducing the potential dispersion of hatchlings (Gallardo, 1989). A relatively complete understanding of breeding habits and type of development is necessary to properly develop and improve gastropod fisheries (D'Asaro, 1970). This species is subjected to an artisanal fishery in the Ria Formosa, where communal spawns are subjected to hand collecting during low tide and also by scuba divers fishing illegally inside the lagoon. Moreover, from each egg capsule only a small number of hatchlings apparently emerge: 5-10 (Dulzetto, 1946), 15 (Bandel, 1975) or 10-20 (Fischer and Raffy, 1933) (Table 1), and these are then probably subjected to a high natural mortality during growth. For all these reasons, fishery management measures should be implemented in order to preserve this crucial phase of the species life cycle, namely by protecting spawning females and their egg masses through the establishment of a fishery closure (closed season) during the spawning period of *T. trunculus*.

The abundance of *T. trunculus* in the Ria Formosa lagoon has apparently decreased in recent years (probably due to overfishing) and, consequently, its commercial value has increased markedly (10-15 €/kg for first sale). These developments have raised expectations about the potential of this species for molluscan aquaculture, both for commercial production (under policulture regime, with low costs and simple maintenance) and for restocking the lagoon. In this respect, the direct development and the high growth rates of hatchlings and juveniles of *T. trunculus* (2.5 mm/month after four months) are very advantageous features.

Nevertheless, the relatively high mortality and prevalence of cannibalism during the first month of hatchling growth should be a matter of concern. Feeding behaviours of recently hatched muricids are not well documented and, to our knowledge, no other such information is available for *T. trunculus* hatchlings. Cannibalism among hatchlings is apparently a common feature in laboratory studies with muricids (*e.g,,* see DiSalvo and Carriker, 1994) and it may well be considered a natural phenomenon for *T. trunculus*, since observations on shell drill-holes in the Mediterranean Sea revealed that the rate of cannibalism in juvenile and adult of this species amounts to 20%, with congeners being the third most preferred prey species (Basedow, 1996). However, observations on shell drill-holes in the Mediterranean (Mallorca and Crete) suggest that juvenile and adult *T. trunculus* prey upon 14 gastropod species (including their own) and 3 bivalve species (Basedow, 1994, 1996). Thus, in the present case, the initial refusal of early post-metamorphic juveniles to ingest any of the types of food provided could possibly be due to an unsuitable diet (the provision of unnatural prey). Furthermore, studies of other muricids indicate that during the initial phases of development and growth some species may be herbivorous or feed on non-bivalve prey. For example, *Concholepas concholepas* hatchlings are not carnivorous initially, but instead graze on encrusting algae and diatoms for a period of several days, after which they begin boring into small barnacles (DiSalvo and Carriker, 1994). Similarly, *Urosalpinx cinerea* hatchlings initially feed on bryozoans before switching to carnivory, when they prey on barnacles and bivalves (Franz, 1971).

Besides amplifying knowledge available on the reproductive biology of *T. trunculus*, this work provides the first data on the growth rates of hatchlings and juveniles of this species. Altogether, this kind of information could be most helpful for establishing effective management measures for this fishery and for assessing the potential of this species for molluscan aquaculture. Further developments of this study will be focused on estimating the potential fecundity of *T. trunculus*, through the relationships between female size and spawn dimensions, number of egg capsules, eggs per capsule, nurse eggs, embryos and hatchlings, and on continuing the assessment of hatchling and juvenile feeding requirements and growth rates.

ACKNOWLEDGEMENTS

The authors would like to acknowledge João Teixeira for installing the tanks circuit in the Tavira Molluscan Aquaculture Experimental Station. Gratitude is owed to Margarete Ramos and David Fernandes for their enthusiasm and dedication to this project and for the daily work related to the maintenance of hatchlings and juvenile gastropods. Sincere thanks are also due to Dr. Gregory Herbert and another anonymous referee whose suggestions greatly improved the manuscript and to Dr. Helena Fortunato for her tireless help with the literature review. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001) and by the DIVERAQUA Project (Interreg IIIA).

REFERENCES

Albanese, M.P.; Calabrò, C.; Acosta Pomar, M.L.C., 1983. Ciclo biológico di *Truncularioipsis trunculus* L. (Moll. Gast. Pros.) vivente nel Lago di Faro. *Memorie di Biologia Marina e di Oceanografia*, 13: 133-147.

Axiak, V.; Vella, A.J.; Micaleff, D.; Chircop, P.; Mintoff, B., 1995. Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, 121: 685-691.

Bandel, K., 1975. Das embryonalgehäuse mariner prosobranchier der region von Banyuls-sur-Mer. *Vie et Milieu*, 25: 83-118.

Barash, A.; Zenziper, Z., 1980. Egg masses of Mollusca from Mediterranean waters of Israel and notes on reproduction of the freshwater species *Theodoxus jordani* and *Melanoides tuberculata*. *Veliger*, 22: 299-317.

Basedow, T., 1994. Analysis of shells on beaches and rocky coasts of Europe led to new findings on nutritional and population ecology of predatory marine gastropods (Muricidae and Naticidae). *Zoologische Beiträge*, 36: 29-48.

Basedow, T., 1996. Contributions to the nutritional and population ecology of *Murex trunculus* (L. 1767) (Gastropoda, Muricidae), derived from findings at the north coast of Crete (Eastern Mediterranean); with remarks on two species of Naticidae. *Zoologische Beiträge*, 37: 157-170.

Benkendorff, K.; Bremner, J.B.; Davis, A.R., 2001a. Indole derivatives from the egg masses of muricid molluscs. *Molecules*, 6: 70-78.

Benkendorff, K.; Davis, A.R.; Bremner, J.B., 2001b. Chemical defense in the egg masses of benthic invertebrates: an assessment of antibacterial activity in 39 mollusks and 4 polychaetes. *Journal of Invertebrate Pathology*, 78: 109-118.

D’Asaro, C.N., 1970. Egg capsules of prosobranch mollusks from South Florida and the Bahamas and notes on spawning in the laboratory. *Bulletin of Marine Science*, 20: 414-440.

D’Asaro, C.N., 1986. Egg capsules of eleven marine prosobranchs from Northwest Florida. *Bulletin of Marine Science*, 39: 76-91.

D’Asaro, C.N., 1988. Micromorphology of neogastropod egg capsules. *Nautilus*, 102: 134-148.

D’Asaro, C.N., 1991. Gunnar Thorson’s world-wide collection of prosobranch egg capsules: Muricidae. *Ophelia*, 35: 1-101.

DiSalvo, L.H.; Carriker, M.R., 1994. Planktonic, metamorphic, and early benthic behavior of the Chilean loco *Concholepas concholepas* (Muricidae, Gastropoda. Mollusca). *Journal of Shellfish Research*, 13: 57-66.

Dulzetto, F., 1946. Osservazioni sulla deposizione di *Murex trunculus* L. *Atti della Accademia Nazionale dei Lincei*, 8: 1356-1361.

Dulzetto, F., 1950. Sull’origine e la struttura delle capsule ovifere di *Murex trunculus*. *Archivio Zoologico Italiano*, 35: 83-99.

Fischer, P.H.; Raffy, A., 1933. La ponte du *Murex trunculus* L. *Bulletin de l'Institut Océanographique (Monaco)*, 618: 1-4.

Franz, D.R., 1971. Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. *Biological Bulletin*, 140: 63-72.

Gallardo, C.S., 1989. Patrones de reproducción y ciclo vital en moluscos marinos bénticos; una aproximación ecológico evolutiva. *Medio Ambiente*, 10: 25-35.

Lima, G.M.; Lutz, R.A., 1990. The relationship of larval shell morphology to mode of development in marine prosobranch gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 70: 611-637.

Macedo, M.C.C.; Macedo, M.I.C.; Borges, J.P., 1999. *Conchas Marinhas de Portugal (Seashells of Portugal)*. Editorial Verbo, Lisboa.

Martoja, M. ; Bouquegneau, J.M., 1988. *Murex trunculus*: un nouveau cas de pseudo-hermaphrodisme chez un gastéropode prosobranche. *Bulletin de la Société Royale de Liège*, 57: 45-58.

Moore, E.A.; Sander, F., 1978. Spawning and early life history of *Murex pomum* Gmelin, 1971. *Veliger*, 20: 251-259.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Rawlings, T.A., 1995. Direct observation of encapsulated development in muricid gastropods. *Veliger*, 38: 54-60.

Romero, M.S.; Gallardo, C.S.; Bellolio, G., 2004. Egg laying and embryonic-larval development in the snail *Thais (Stramonita) chocolata* (Duclos, 1832) with observations on its evolutionary relationships within Muricidae. *Marine Biology*, 145: 681-692.

Sabelli, B.; Tommasini, S., 1982. Osservazioni sulla radula e sulla protoconca di *Bolinus brandaris* (L., 1758) e *Phyllonotus trunculus* (L., 1758). *Bollettino Malacologico*, 18: 291-300.

Schitz, V., 1920. Sur la espermatogenèse chez *Murex trunculus* (L), *Aporrhais pespelicani* (L), *Fusus* sp. et *Nassa reticulata*. *Archives de Zoologie Expérimentale et Générale*, 59: 477-508.

Spight, T.M., 1975. Factors extending gastropod embryonic development and their selective cost. *Oecologia*, 21: 1-16.

Spight, T.M., 1977. Latitude, habitat, and hatching type for muricacean gastropods. *Nautilus*, 91: 67-71.

Terlizzi, A.; Geraci, S.; Minganti, V., 1998. Tributyltin (TBT) pollution in the coastal waters of Italy as indicated by imposex in *Hexaplex trunculus* (Gastropoda: Muricidae). *Marine Pollution Bulletin*, 36: 749-752.

Terlizzi, A.; Delos, A.L.; Garaventa, F.; Faimali, M.; Geraci, S., 2004. Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin*, 48: 188-192.

Vokes, E.H., 1996. One last look at the Muricidae. *American Conchologist*, 24: 4-6.

Wondrak, G., 1974. Laichverhalten und gelegebildung bei *Murex trunculus* L. (Gastropoda: Prosobranchia). *Archiv fuer Molluskenkunde*, 104: 115-119.

SECTION 5

IMPOSEX IN *HEXAPLEX (TRUNCULARIOPSIS) TRUNCULUS* FROM THE RIA FORMOSA LAGOON

CHAPTER 5.1

Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal)

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006. Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin*, 52 (3): 337-341.

BASELINE

The phenomenon known as imposex (Smith, 1971) or pseudohermaphroditism (Jenner, 1979) is the development and superimposition of male secondary sex characters (penis and vas deferens) onto females of prosobranch gastropods, resulting ultimately in sterile females and reproductive failure. This apparently irreversible abnormality is a morphological indicator of sub-lethal exposure to organotin compounds, namely tributyltin (TBT) and its derivative compounds, extensively applied as biocides in antifouling paints for boats and ships hulls (Terlizzi *et al.*, 2001).

Imposex is a widespread phenomenon that affects several coastal gastropod species, and more recently, also offshore gastropods (Ellis and Pattisina, 1990). At ambient concentrations of few nanograms per litre, TBT potentially induces genital abnormalities in several marine gastropod species, affecting at least 63 genera and 118 species (Fioroni *et al.*, 1991). For these reasons, the use of morphological parameters in imposex-affected gastropod species has been widely used to assess coastal and offshore TBT pollution.

Some studies have been undertaken on imposex in *Hexaplex (Trunculariopsis) trunculus*, mostly in the Mediterranean including Spain (El Hamdani *et al.*, 1998), France (Martoja and Bouquegneau, 1988), Italy (Terlizzi, 2000; Terlizzi *et al.*, 1997, 1998, 1999, 2004; Chiavarini *et al.*, 2003; Pellizzato *et al.*, 2004), Malta (Axiak *et al.*, 1995, 2000, 2003) and Israel (Rilov *et al.*, 2000). *T. trunculus* has proved to be a good sentinel species for TBT biomonitoring in coastal waters, being considered one of the most sensitive neogastropod species to TBT contamination, exhibiting initial stages of imposex at concentrations lower than 1 ng Sn/g dry weight (Axiak *et al.*, 1995). This species inhabits muddy, sandy-muddy and hard bottoms, being abundant in areas rich in organic matter and in the vicinity of ports (Šimunović, 1995). Comparative studies have shown that *T. trunculus* is more sensitive to TBT exposure than other gastropod species probably due to its lifestyle as a scavenger that burrows on muddy bottoms where the concentration of TBT is high (Coelho *et al.*, 1998; El Hamdani *et al.*, 1998). Furthermore, *T. trunculus* is a direct developer species (lacks a planktotrophic larva) and therefore is highly restricted in movement and capacity to colonise new areas, which explains its remarkable ability to detect point sources of pollution by TBT at a high spatial resolution (Axiak *et al.*, 2003).

Imposex data from Portuguese waters are relatively scarce, limited to a few gastropod species and mostly from the western coast of Portugal. To our best knowledge, the only studies reporting imposex in *T. trunculus* from the Ria Formosa lagoon (although with very few specimens), were published by Gibbs *et al.* (1997), Langston *et al.* (1997) and Coelho *et al.* (1998). This study aimed to assess the incidence and degree of imposex in *T. trunculus* and to provide additional data that could be used as a baseline for future monitoring programmes and for temporal trend surveillance related to TBT pollution in the Ria Formosa lagoon (southern Portugal).

Approximately 100 specimens of *T. trunculus* from commercial samples caught near Culatra Island (Figure 1) were examined monthly between March 2003 and February 2004. In the laboratory, specimens were measured (shell length) with a digital vernier calliper (precision of 0.01 mm) and weighed (total weight) on a top loading digital balance (precision of 0.01 g). In order to allow for gender identification, the shell was broken in a bench vice and the soft (edible) part of the organism was removed, exposing the mantle cavity and sexual organs. Males were identified by the presence of penis and lack of capsule gland, and females by the presence of vagina and capsule gland. Finally, the curved penises of both males and imposex-affected females were flattened and measured to the nearest 0.01 mm (total length - proximal and distal portions) under a stereo microscope with a calibrated eyepiece or with a digital calliper, depending on penis size.

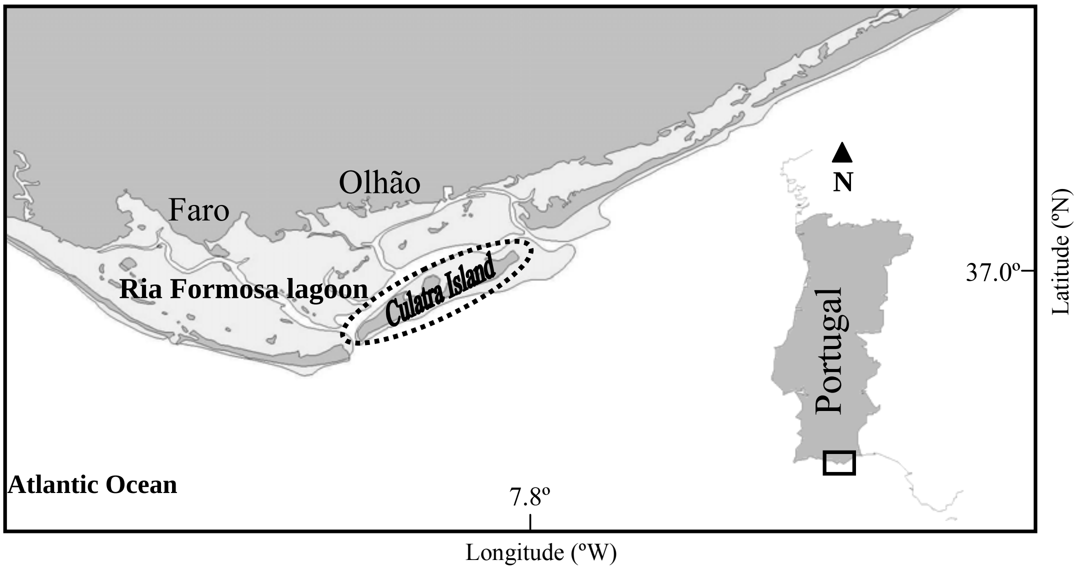


Figure 1. Geographical location of the Ria Formosa lagoon (Algarve coast - southern Portugal), with emphasis on Culatra Island (dotted ellipse) where the commercial samples of *Hexaplex (Trunculariopsis) trunculus* were caught.

The sex-ratio was expressed as the percentage of females and statistically significant deviations from a sex-ratio of 1:1 were assessed by means of the *chi*-square test. The incidence or frequency of imposex (I%) was expressed as the percentage of females showing any development of male secondary characters (vas deferens or penis). In order to allow for comparison with similar studies, the degree of imposex was determined using both the relative penis length index [RPLI = (female penis mean length / male penis mean length) x 100] and relative penis size index [RPSI = (female penis mean length)^3 / (male penis mean length)^3 x 100] (Gibbs *et al.*, 1987). Additionally, and following Ellis and Pattisina (1990), both RPLI and RPSI were calculated using data from all females (Population RPLI and RPSI) and excluding data from unaffected females, *i.e.*, evaluating the degree of imposex based only on those specimens in which the effect was actually measured (Incidence RPLI and RPSI). At 100% imposex frequency, the Population and Incidence RPLI and the Population and Incidence RPSI are the same.

Together with sample descriptive statistics, imposex data in terms of incidence (I%) and degree (Population and Incidence RPLI and RPSI) are presented in Table 1. A total of 1183 *T. trunculus* specimens were subjected to imposex analysis (40.17 - 82.84 mm shell length and 5.28 - 48.84 g total weight). Overall, specimens presented a balanced sex-ratio (F:M =1:1.10; *Chi*-square test, $P<0.05$) and 93.24% of the females were affected by imposex (Table 1). Female penises frequently appeared only as a small protuberance (vestigial or incipient penises) and a small proportion of females presented a vas deferens, but no penis (aphallic route) (Oehlmann *et al.*, 1996). On average, male penis total length (11.82 ± 1.68 mm) was more than twice longer than female penis total length (5.18 ± 2.04 mm). Accordingly, Population RPLI was 36.13 and Incidence RPLI was 43.82, while Population RPSI was 4.71 and Incidence RPSI was 8.42 (Table 1).

Table 1. Summary of imposex data for *Hexaplex (Truncularioposis) trunculus* from the Ria Formosa lagoon (southern Portugal) (N - number of specimens; S.D. - standard deviation; Min. - minimum value; Max. - maximum value).

	Sex	N	Shell length (SL - mm)	Total weight (TW - g)	Penis length (PL - mm)
Mean ± S.D. (Min. - Max.)	Males	621	55.64 ± 5.50 (40.17 - 82.84)	16.86 ± 5.69 (5.28 - 48.84)	11.82 ± 1.68 (6.48 - 17.10)
Mean ± S.D. (Min. - Max.)	Females	562	57.84 ± 6.30 (42.11 - 79.84)	18.11 ± 6.91 (5.44 - 43.04)	5.18 ± 2.04 (1.45 - 12.72)

Sex-ratio (F : M)	Females (%)	Imposex (%)	Population RPLI	Incidence RPLI	Population RPSI	Incidence RPSI
1 : 1.10	47.51	93.24	36.13	43.82	4.71	8.42

Data were grouped into size classes (5 mm shell length) to assess the influence of specimen size (and consequently age and exposure time to organotin) on the imposex incidence (I%) and degree (Population and Incidence RPLI and RPSI) (Figure 2). As it is typical in this species, males dominated among smaller individuals, while females predominated in larger size classes. This phenomenon was accompanied by a decreasing trend in both the frequency and degree of imposex with specimen size, *i.e.*, despite a shorter exposure time to TBT, younger individuals were more affected (I%) and at a higher level (Population and Incidence RPLI and RPSI) than older individuals (Figures 2a,b).

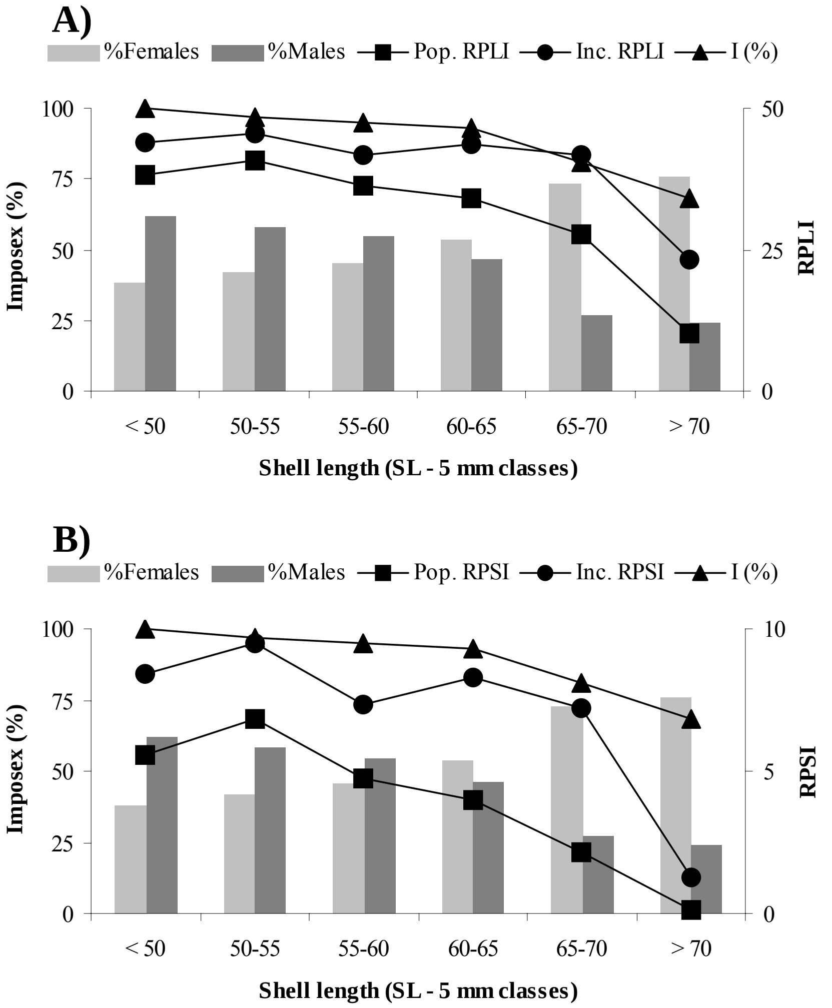


Figure 2. Variation of imposex incidence (I%) and degree (Population and Incidence RPLI and RPSI) according to specimen size and sex-ratio (grouped in shell length size classes - 5 mm SL).

Results revealed that this *T. trunculus* population from the Ria Formosa lagoon is highly affected by imposex (>90% of masculinised females), but at moderately low degrees (RPLI and RPSI), namely when compared with several locations in the Mediterranean Sea, such as

Italy (Terlizzi *et al.*, 1998, 2004; Pellizzato *et al.*, 2004), Malta (Axiak *et al.*, 1995, 2000, 2003) and Israel (Rilov *et al.*, 2000). The high prevalence of imposex in *T. trunculus* from the Ria Formosa is attributable to the intense boating activity in the lagoon. Although boat traffic is mostly dominated by small leisure and fishing boats, large fishing and commercial vessels also use the two major ports in the lagoon, Olhão (fishing harbour) and Faro (commercial harbour), the most important ports of the Algarve coast (southern Portugal). Analysis of water and sediments revealed that, although organotin levels were not excessively high in most of the lagoon, contamination at low levels was fairly widespread (Coelho *et al.*, 2002).

Some malformations of the penis were detected in both sexes. Two males presented excrescences in the penis, but bifurcated penises and aphallic males (Dumpton Syndrome: Gibbs, 1993) were not found in the sampled population. This malformation in the male genital system of *T. trunculus* has also been reported for other gastropod species and apparently has no consequences on reproduction. Additionally, five females presented a rounded-end penis (without the sharp tip), but bifurcated penises, advanced imposex-affected females lacking a penis and potentially sterile females (namely through the splitting of the capsule gland or presence of aborted capsules forming an amorphous dark mass filling the capsule gland), were also not found in the sampled population. For these reasons, at present, imposex apparently does not significantly affect the population dynamics of *T. trunculus* in the studied area, because overall samples did not show male-biased sex-ratios (that could be interpreted as sex selective mortality) and interferences with normal sexual development were also not detected. Oogenesis still develops normally in *T. trunculus* (P. Vasconcelos, unpublished data) and observations both in the laboratory and in their natural habitat revealed a normal breeding and spawning activity, with the formation of egg capsules and deposition of typical collective spawns (Vasconcelos *et al.*, 2004).

As previously described for other muricid species (e.g. *Nucella lapillus*: Gibbs *et al.*, 1988), the inverse relationship detected in this study between specimen size and imposex incidence and degree apparently reveals that *T. trunculus* juveniles are more sensitive to the effects of TBT exposure than the adults. This may be hypothetically due to a higher metabolic rate of juveniles (with consequent higher accumulation of TBT) or to an acclimation of the adults during a continuous and prolonged exposition to the pollutant (with subsequent attenuation of the sensitivity to the effects of imposex). Moreover, the female reproductive system appears to be more modified by imposex during the developing stages than when the genital system is fully developed and functional (Gibbs *et al.*, 1988).

The gross morphological changes and pathological effects of imposex depend on dose and duration of exposure, being apparently irreversible (Gibbs and Bryan, 1986; Gibbs *et al.*, 1991). For this reason, the present inverse relationship between specimen size and imposex incidence and degree apparently also indicates that despite the recent global TBT restrictions, organotin levels in the Ria Formosa lagoon have not decreased recently. An estimation of the growth rate of *T. trunculus* from the Ria Formosa (Vasconcelos *et al.*, 2006) suggests that most individuals sampled in the present study were 2-3 years old, revealing that imposex was caused by recent exposure to TBT. This may be due to the dredging operations periodically carried out to maintain the navigability of the main channels between the sea and the major ports, with the consequent resuspension of sediments and remobilisation of the organotin accumulated in highly contaminated muddy sediments.

Like most European countries, Portugal introduced in 1993 legislation to ban the use of TBT-based antifouling paints on small boats (<25 m) (EC directive 89/677/EEC), but these restrictions appear not to be fully effective at the present time. The International Maritime Organisation (IMO) has called for a total ban on further organotin applications on the global fleet in 2003 and for complete replacement of existing organotin coatings from boat hulls by

2008 (EC directive 2002/62/EC, now effective EU legislation). However, despite this legislation advocating removal of TBT from the global fleet it is possible that this environmental problem could increase over next few years before finally attenuating or disappearing (Champ, 2000). In this context, TBT contamination in the Ria Formosa lagoon is also unlikely to change for a considerable period of time (Coelho *et al.*, 2002).

The availability of this kind of baseline information on the imposex levels in *T. trunculus* from the Ria Formosa is important to assess the environmental consequences of the intense boating activity in the lagoon and of the dredging operations in the main channels and nearby major ports, and also to verify the effectiveness of the recent legislation in reducing the environmental impacts of organotin in the near future. Additionally, and as already mentioned by Cajaraville *et al.* (2000), a monitoring programme should be implemented to assess environmental pollution in the Iberian Peninsula, which should include the study of imposex in *T. trunculus* from ecosystems that must be especially protected, such as the Ria Formosa Natural Park. Moreover, continued surveillance of the imposex incidence and organotin contamination in *T. trunculus* might be important to assess the potential hazards and implications for human consumption of this gastropod species, subjected to a locally important artisanal fishery in the Ria Formosa lagoon, with high commercial value and greatly appreciated in Portugal.

ACKNOWLEDGMENTS

Thanks are due to the technical staff of IPIMAR - CRIPSul for their help in the laboratory sampling procedures. The authors are also grateful to the anonymous referees whose suggestions improved the final version of the manuscript. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001).

REFERENCES

Axiak, V.; Vella, A.J.; Micaleff, D.; Chircop, P.; Mintoff, B., 1995. Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, 121: 685-691.

Axiak, V.; Vella, A.J.; Agius, D.; Bonnici, P.; Cassar, G.; Cassone, R.; Chircop, P.; Micallef, D.; Mintoff, B.; Sammut, M., 2000. Evaluation of environmental levels and biological impact of TBT in Malta (central Mediterranean). *The Science of the Total Environment*, 258: 89-97.

Axiak, V.; Micallef, D.; Muscat, J.; Vella, A.; Mintoff, B., 2003. Imposex as a biomonitoring tool for marine pollution by tributyltin: some further observations. *Environment International*, 28: 743-749.

Cajaraville, M.P.; Bebianno, M.J.; Blasco, J.; Porte, C.; Sarasquete, C.; Viarengo, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *The Science of the Total Environment*, 247: 295-311.

Champ, M.A., 2000. A review of organotin regulatory strategies, pending actions, related costs and benefits. *The Science of the Total Environment*, 258: 21-71.

Chiavarini, S.; Massanisso, P.; Nicolai, P.; Nobili, C.; Morabito, R., 2003. Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). *Chemosphere*, 50: 311-319.

Coelho, M.R.; Bebianno, M.J.; Gibbs, P.E., 1998. Imposex in neogastropod snails from southern Portugal. *Cuadernos de Investigación Biológica*, 20: 273.

Coelho, M.R.; Bebianno, M.J.; Langston, P.E., 2002. Organotin levels in the Ria Formosa lagoon, Portugal. *Applied Organometallic Chemistry*, 16: 384-390.

El Hamdani, A.; Ferrer, J.M.; García Carrascosa, A.M., 1998. Imposex in prosobranch molluscs: an indicator of TBT pollution in the Valencian coast (Spain, Western Mediterranean). *Cuadernos de Investigacíon Biológica*, 20: 275-278.

Ellis, D.V.; Pattisina, L.A., 1990. Widespread neogastropod imposex: a biological indicator of global TBT contamination? *Marine Pollution Bulletin*, 21: 248-253.

Fioroni, P.; Oehlmann, J.; Stroben, J., 1991. The pseudohermaphroditism of Prosobranchs: morphological aspects. *Zoologischer Anzeiger*, 226: 1-26.

Gibbs, P.E., 1993. A male genital defect in the dog-whelk, *Nucella lapillus* (Neogastropoda), favouring survival in a TBT-polluted area. *Journal of the Marine Biological Association of the United Kingdom*, 73: 667-678.

Gibbs, P.E.; Bryan, G.W., 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 66: 767-777.

Gibbs, P.E.; Bryan, G.W.; Pascoe, P.L.; Burt, G.R., 1987. The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *Journal of the Marine Biological Association of the United Kingdom*, 67: 507-523.

Gibbs, P.E.; Pascoe, P.L.; Burt, G.R., 1988. Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 68: 715-731.

Gibbs, P.E.; Pascoe, P.L.; Bryan, G.W., 1991. Tributyltin-induced imposex in stenoglossan gastropods: pathological effects on the female reproductive system. *Comparative Biochemistry and Physiology*, 100C: 231-235.

Gibbs, P.E.; Bebianno, M.J.; Coelho, M.R., 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution, with notes on a species (*Columbella rustica*) lacking the imposex response. *Environmental Technology*, 18: 1219-1224.

Jenner, M.G., 1979. Pseudohermaphroditism in *Ilyanassa obsoleta* (Mollusca: Neogastropoda). *Science*, 205: 1407-1409.

Langston, W.J.; Gibbs, P.E.; Livingstone, D.R.; Burt, G.R.; O'Hara, S.; Bebianno, M.J.; Coelho, M.R.; Porte, C.; Bayona, J.; McNulty, M.; Lynch, G.; Keegan, B., 1997. Risk assessment of organotin antifoulings on key benthic organisms of European coastal habitats (BOATS). MAS2-CT94-0099, Final Report.

Martoja, M.; Bouquegneau, J.-M., 1988. *Murex trunculus*: un nouveau cas de pseudo-hermaphrodisme chez un gastéropode prosobranche. *Bulletin de la Société Royale de Liège*, 57: 45-58.

Oehlmann, J.; Stroben, E.; Fioroni, P., 1996. New facts about TBT-induced imposex in prosobranchs: general aspects. *Malacological Review*, Suppl. 6: 149-156.

Pellizzato, F.; Centanni, E.; Marin, M.G.; Moschino, V.; Pavoni, B., 2004. Concentrations of organotin compounds and imposex in the gastropod *Hexaplex trunculus* from the lagoon of Venice. *Science of the Total Environment*, 332: 89-100.

Rilov, G.; Gasith, A.; Evans, S.M.; Benayahu, Y., 2000. Unregulated use of TBT-based antifouling paints in Israel (Eastern Mediterranean): high contamination and imposex levels in two species of marine gastropods. *Marine Ecology Progress Series*, 192: 229-238.

Šimunović, A., 1995. Ecological study of Prosobranchiata (Gastropoda) in the eastern part of the Adriatic Sea and their relationship to benthic biocoenoses. *Acta Adriatica*, 36: 3-162.

Smith, B.S., 1971. Sexuality in the American mud snail *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London*, 39: 377-378.

Terlizzi, A., 2000. Imposex (pseudoermafroditismo) in molluschi gasteropodi mediterranei: aspetti morfologici e considerazioni ecologiche. *Bollettino Malacologico*, 36: 155-158.

Terlizzi, A.; Scuderi, D.; Faimali, M.; Minganti, V.; Geraci, S., 1997. Imposex in *Hexaplex trunculus* e *Stramonita haemastoma* (Gastropoda: Muricidae): prime segnalazioni per le acque costiere italiane. *Biologia Marina Mediterranea*, 4: 496-499.

Terlizzi, A.; Geraci, S.; Minganti, V., 1998. Tributyltin (TBT) pollution in the coastal waters of Italy as indicated by imposex in *Hexaplex trunculus* (Gastropoda: Muricidae). *Marine Pollution Bulletin*, 36: 749-752.

Terlizzi, A.; Geraci, S.; Gibbs, P.E., 1999. Tributyltin (TBT)-induced imposex in the Neogastropod *Hexaplex trunculus* in Italian coastal waters: morphological aspects and ecological implications. *Italian Journal of Zoology*, 66: 141-146.

Terlizzi, A.; Fraschetti, S.; Gianguzza, P.; Faimali, M.; Boero, F., 2001. Environmental impact of antifouling technologies: state of the art and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 11: 311-317.

Terlizzi, A.; Delos, A.L.; Garaventa, F.; Faimali, M.; Geraci, S., 2004. Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin*, 48: 188-192.

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias, D.; Castro, M., 2004. Spawning of *Hexaplex (Truncularíopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Pereira, A.M.; Castro, M., 2006. Growth rate estimation of *Hexaplex (Truncularíopsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of Shellfish Research*, 25: 249-256.

CHAPTER 5.2

Development of indices for nonsacrificial sexing of imposex-affected *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae)

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006. Development of indices for nonsacrificial sexing of imposex-affected *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae). *Journal of Molluscan Studies*, 72 (3): 285-294.

ABSTRACT

The muricid *Hexaplex (Truncularıopsis) trunculus* lacks external sexual dimorphism and is highly affected by imposex, which further complicates its sexual identification. In this context, the aim of this study was to develop sexual indices based on the dimensions of male and imposex-affected female penis, which could constitute a useful, simple and non-sacrificial tool for sexing live specimens of *T. trunculus*. The adoption of sexual indices consisting on the percentage of penis dimensions of both sexes relatively to individual size revealed a high accuracy in the sexual identification of sacrificed specimens (>95% correct sexing). Additionally, multivariate discriminant analysis allowed for correctly sexing 98.8% of the original 1053 sacrificed individuals, with accurate sexual identification being higher for males (99.5%) than for imposex-affected females (97.7%). An anaesthetization experiment was performed to investigate the effects produced by the anaesthetic ($MgCl_2$) on penis measurements, to test this non-sacrificial approach and to validate the previously developed sexual indices. The anaesthetic provoked an expected enlargement in penis dimensions, but despite this side effect, the sexual indices developed for sacrificed specimens were still highly successful in sexing anaesthetized *T. trunculus* (generally more than 95% correct sexual identification). The practical application and some limitations of developing and employing this kind of indices for the sexual identification of *T. trunculus* and other imposex-affected gastropod species are discussed.

INTRODUCTION

Sexual dimorphism is common in many animal *taxa*, but occurs only sporadically in the phylum Mollusca. Nevertheless, subtle differences in shell morphology between sexes occur in some prosobranch gastropods. Sexual dimorphism has been registered in some species of freshwater (e.g. Kantor and Sysoev, 1991; Brande *et al.*, 1996; Estebenet, 1998; Kurata and Kikuchi, 2000) and marine gastropods (e.g. Bernard, 1968; ten Hallers-Tjabbes, 1979; Castagna and Kraeuter, 1994; Kenchington and Glass, 1998). However, since most gastropod species do not present external sexual dimorphism, sexing generally requires the exposal of the soft body and the examination of sexual organs and/or gonads, which is frequently impossible without sacrificing the specimens (through shell breakage and with consequent death).

Furthermore, several prosobranch gastropod species are affected by the phenomenon known as imposex (Smith, 1971) or pseudohermaphroditism (Jenner, 1979), which is the development and superimposition of male sexual characters (penis and vas deferens) onto females. This abnormality is induced by tributyltin (TBT) and its derivative compounds, widely applied as biocides in antifouling paints of boats and ships hulls (Terlizzi *et al.*, 2001). Imposex became a widespread phenomenon that affects both coastal and offshore gastropod species (Ellis and Pattisina, 1990), involving at least 63 genera and 118 species (Fioroni *et al.*, 1991). In these imposex-affected species, sexual identification becomes even more complicated, because specimens cannot be sexed simply by the presence or absence of penis, instead requiring a further exhaustive inspection of other sexual organs.

Some literature exists on several aspects of the biology and ecology of *Hexaplex (Trunculariopsis) trunculus* (Linnaeus, 1758), but to the present authors best knowledge, no external signs of sexual dimorphism have yet been reported for this species. Several studies have been conducted on the imposex levels of *T. trunculus*, mostly in the Mediterranean and

Adriatic, namely in Spain (El Hamdani *et al.*, 1998), France (Martoja and Bouquegneau, 1988), Italy (Terlizzi *et al.*, 1997, 1998, 1999, 2004; Terlizzi, 2000; Chiavarini *et al.*, 2003; Pellizzato *et al.*, 2004; Garaventa *et al.*, 2006), Croatia (Garaventa *et al.*, 2006), Malta (Axiak *et al.*, 1995, 2000, 2003) and Israel (Rilov *et al.*, 2000). In Portuguese waters, the only works reporting the imposex levels in *T. trunculus* were performed in the Ria Formosa lagoon (southern coast of Portugal) by Gibbs *et al.* (1997), Langston *et al.* (1997) and Coelho *et al.* (1998) (although all with very few specimens), and more recently by Vasconcelos *et al.* (2006a).

This species is the target of a locally important artisanal fishery in the Ria Formosa lagoon (Algarve coast - southern Portugal), where it is being subjected to an integrated and multidisciplinary study on its biology, ecology and fishery. Since *T. trunculus* lacks external sexual dimorphism (sexes cannot be distinguished from shell characters), its sexual identification implies breaking the shell and sacrificing the organism. Additionally, because this species is highly affected by imposex, sexing is even further complicated and requires specific knowledge on the reproductive organs of the species.

In some circumstances, it may be advantageous to sex specimens without extracting them from their shells (Gibbs, 1999), namely for diverse biological, ecological and behavioural studies that require live specimens and the respective sexual identification. However, in several gastropod species, live sexing is only possible through anaesthetization. This technique of sexual identification by exposition of the pallial cavity and examination of the penis development under narcosis has already been applied to other muricid species, such as *Nucella lapillus* (Gibbs, 2005).

In this context, the aim of the present study was to develop indices for the sexual identification of live *T. trunculus*, using data on specimen size and penis dimensions of sacrificed males and imposex-affected females. For this purpose, an anaesthetization experiment (narcosis with magnesium chloride) was performed to investigate the effects produced by the anaesthetic on penis measurements. Subsequently, data from these anaesthetized specimens were applied on the sexual indices previously developed with sacrificed specimens, in order to validate the indices and to confirm whether they could constitute a useful, simple and non-sacrificial tool for sexing live and anaesthetized *T. trunculus*.

MATERIAL AND METHODS

For this study, approximately 100 specimens of *T. trunculus* (Figure 1a) from commercial samples caught near the Culatra Island (Ria Formosa lagoon - southern Portugal) were examined monthly between March 2003 and February 2004. In the laboratory, individuals were measured (shell length - SL) with a digital calliper (to 0.01 mm) and weighed (total weight - TW) on a top-loading digital balance (precision of 0.01g).

Subsequently, routine sexual identification was made in sacrificed de-shelled specimens. The shells were broken in a bench vice to allow the removal of the soft parts of the organism (Figure 1b). The mantle cavity was exposed and individuals were sexed: males by the presence of penis and lack of capsule gland, females by the presence of vagina and capsule gland. Finally, the curved penises of both males and imposex-affected females (Figure 1c) were flattened and measured to the nearest 0.01 mm (penis total length of proximal and distal portions - PTL; penis partial length of proximal portion - PPL; penis maximum width of proximal portion - PMW) (Figure 1d) under a stereo microscope with a calibrated eyepiece or with the digital calliper, depending on the penis size.

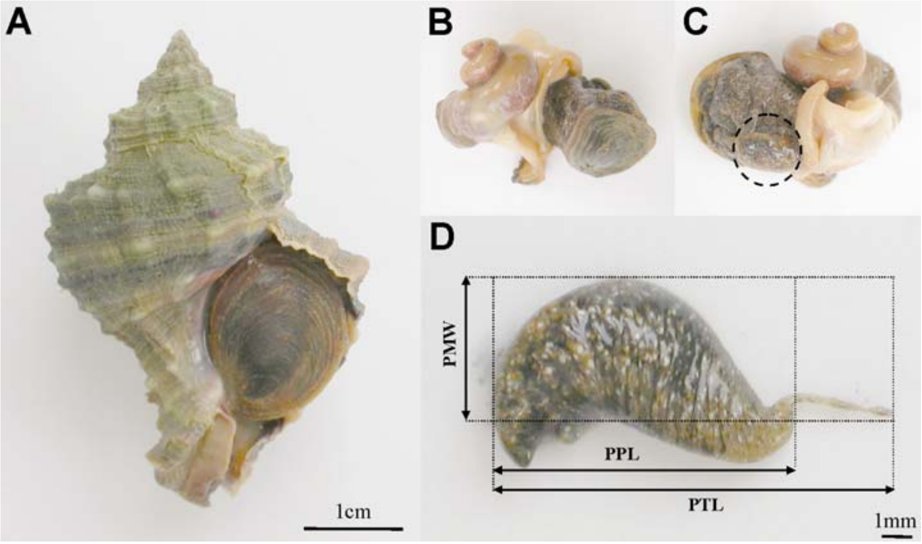


Figure 1. Illustration of the sequential procedures for the measurements of male and imposex-affected female penis of *Hexaplex (Trunculariopsis) trunculus*. **a)** Live specimen (ventral view). **b)** Soft parts of the organism after shell breakage. **c)** Location of the curved penis on the foot, adjacent to the ocular tentacles (eyestalks). **d)** Schematic representation of the flattened penis measurements. Abbreviations: PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion).

Penises dimensions (PTL, PPL and PMW) and relative proportions (PPL/PTL and PMW/PTL) were compared between sexes using a *t*-test (H_0: $\mu_M=\mu_F$; H_A: $\mu_M\neq\mu_F$ and H_0: $p_M=p_F$; H_A: $p_M\neq p_F$), with statistical significance considered for $P<0.05$. The morphometric relationships between penis dimensions (PTL, PPL and PMW) and the relationships between individual size (SL) and penis dimensions (PTL, PPL and PMW) for both sexes were obtained by linear regression analysis (least squares method), by adjustment of a linear function ($Y = a + bX$) to raw data. The degree of association between variables was assessed by the correlation coefficient (r) and the slopes of linear regressions for males and imposex-affected females were compared using a *t*-test (H_0: $\beta_M=\beta_F$; H_A: $\beta_M\neq\beta_F$) (Zar, 1996), with statistical significance at $P<0.05$.

Allometric relationships between penis dimensions (PTL, PPL and PMW) and between individual size (SL) and respective penis dimensions (PTL, PPL and PMW) were also estimated for both sexes. The allometry coefficient is expressed by the exponent *b* of the allometric relationship ($Y = a\ X^b$), which can also be expressed in its linearised logarithmic form ($\ln Y = \ln a + b \ln X$). In relationships between linear variables, the relationship reflects an isometric growth when b=1 (i.e., growth rates of both variables are identical during ontogeny). A *t*-test (H_0: b=1; H_A: $b \neq 1$) (Sokal and Rohlf, 1987) was applied to confirm if the slopes of allometric relationships were included in the isometric range (b=1) or allometric ranges (negative allometry: $b<1$ or positive allometry: $b>1$), with statistical significance considered at $P<0.05$.

Sexual indices consisting on the percentage of penis dimensions relatively to individual size (PTL/SL, PPL/SL and PMW/SL) of males and imposex-affected females were employed to standardise data and eventually facilitate the sexual identification. A Kruskal-Wallis *H*-test for multiple independent samples (H_0: $\text{month}_1 = \text{month}_n$; H_A: $\text{month}_1 \neq \text{month}_n$) (Zar, 1996) was used to detect differences in these sexual indices during the one-year study period. Monthly variations in these sexual indices between consecutive months were assessed with the Mann-Whitney *U*-test for two independent samples (H_0: $\text{month}_n = \text{month}_{n+1}$; H_A: $\text{month}_n \neq \text{month}_{n+1}$) (Zar, 1996). In both statistical tests, significance was considered at $P<0.05$.

Multivariate discriminant analysis was performed using the individual size (shell length - SL) and the respective penis dimensions (PTL, PPL and PMW) of both males and imposex-affected females. Discriminant functions were calculated and the percentages of cases that were correctly sexed by the linear function were established. Multivariate discriminant analysis was carried out using the data analysis software system Statistica® version 6.0 (StatSoft, Inc.).

For the non-sacrificial approach, an anaesthetization experiment was performed to investigate the effects produced by the narcotic on penis measurements and to test the validity of applying data from anaesthetized specimens in the sexual indices previously developed with data from sacrificed specimens. Firstly, 100 *T. trunculus* specimens were marked with Dymo® tape tags, fixed with cyanoacrylate glue and covered with epoxy glue (for marking details see Vasconcelos *et al.*, 2006b). The anaesthetization was performed following the protocol developed by Gibbs (1999) and successfully applied in *Nucella lapillus* by Gibbs (2005). Specimens were immersed during two hours in a solution of magnesium chloride ($MgCl_2.6H_2O$ - 75 g per litre of distilled water) and monitored for full relaxation. Those individuals that did not attain a completely relaxed state after this period were monitored at regular intervals of 15 minutes until full relaxation was achieved, allowing for being sufficiently eased from their shell, exposition of the anterior part of the pallial cavity and measurement of penis dimensions (PTL, PPL and PMW). Finally, and within 24-48 hours after having recovered from the anaesthetization, these same specimens were sacrificed (de-shelled in a bench vice), subjected to sexual identification and subsequent measurement of penis dimensions (PTL, PPL and PMW).

A *Z*-test (Zar, 1996) was applied to compare average penis dimensions obtained from these anaesthetized and later sacrificed specimens of both sexes. Furthermore, a paired-sample *t*-test (Zar, 1996) was applied to individually identify significant differences between penis measurements obtained from these narcotised and sacrificed specimens. Alternatively, the Wilcoxon signed rank test was employed whenever the normality test failed (Zar, 1996). The paired-sample *t*-test examines the changes that occur before and after a single experimental intervention on the same individuals to determine whether the treatment produced a significant effect. Examining the changes rather than the values observed before and after the treatment, removes the differences due to individual responses and produces a more sensitive and powerful test. In all these statistical analyses, significance was considered at $P<0.05$.

RESULTS

A total of 1183 *T. trunculus* specimens were subjected to imposex analysis (40.17 - 82.84 mm SL and 5.28 - 48.84 g TW), 621 males and 562 females (93.24% of which were affected by imposex) (Table 1). The dimensions of *T. trunculus* male and imposex-affected female penises are presented in Table 1. On average, male penis dimensions were significantly greater than female imposex-affected penis (*t*-test; $P<0.05$). In general, the male penis mean total length (PTL=11.82±1.68 mm), mean partial length (PPL=8.41±1.25 mm) and mean maximum width (PMW=3.39±0.59 mm), were more than two times larger than the same measurements of female imposex-affected penis (PTL=5.18±2.04 mm; PPL=3.53±1.58 mm; PMW=1.56±0.59 mm) (Table 1), which frequently appeared only as a small protuberance (vestigial or incipient penises).

Table 1. Specimen's size and penis dimensions of males and imposex-affected females of *Hexaplex (Trunculariopsis) trunculus*.

		Specimens size		Penis Dimensions		
		Shell length (SL - mm)	**Total weight (TW - g)**	**Total length (PTL - mm)**	**Partial length (PPL - mm)**	**Max. width (PMW - mm)**
Males	*n*	621	621	611	621	621
	mean ± SD	55.64 ± 5.50	16.86 ± 5.69	11.82 ± 1.68	8.41 ± 1.25	3.39 ± 0.59
	(min - max)	(40.17 - 82.84)	(5.28 - 48.84)	(6.48 - 17.10)	(3.89 - 12.35)	(1.67 - 5.18)
Females	*n*	562	562	442	465	466
	mean ± SD	57.84 ± 6.30	18.11 ± 6.91	5.18 ± 2.04	3.53 ± 1.58	1.56 ± 0.59
	(min - max)	(42.11 - 79.84)	(5.44 - 43.04)	(1.45 - 12.72)	(0.81 - 9.45)	(0.47 - 3.53)

Abbreviations: *n* - number; SD - standard deviation; min - minimum value; max - maximum value.

The relative proportions and morphometric relationships established between penis measurements are illustrated in Figure 2. Despite the marked differences in penis dimensions, the relative proportions (ratios: PPL/PTL and PMW/PTL) were similar between sexes (Figure 2a), revealing that male and female imposex-affected penis are morphologically alike and roughly comparable. Nevertheless, statistically significant differences between sexes (*t*-test; $P<0.05$) were detected in these relative proportions, with a higher ratio PPL/PTL in males and a higher ratio PMW/PTL in females, indicating that on average the proximal portion is slightly longer in male penis and wider in female imposex-affected penis. Moreover, the relative proportions of female imposex-affected penises were more variable than the relative proportions of male penises (Figure 2a).

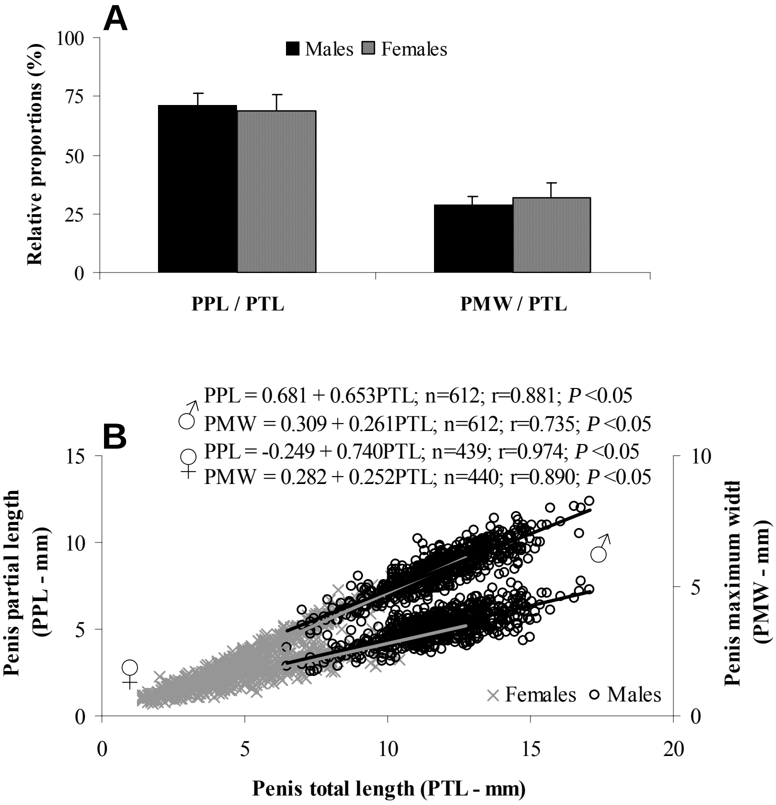


Figure 2. a) Relative proportions of penis measurements in *Hexaplex (Truncülariopsis) trunculus* males and imposex-affected females. **b)** Morphometric relationships established between penis measurements of males and imposex-affected females. Abbreviations: PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion).

The morphometric relationships established between penis measurements (PTL, PPL and PMW) (Figure 2b) highlighted the similarity in relative proportions and morphology of the penises of males and imposex-affected females. In fact, these morphometric relationships further confirmed that despite obvious differences in penis dimensions, there was an almost continuous morphometric relationship between male and female imposex-affected penises measurements. The morphological similarity between male and female imposex-affected penises was reinforced by the fact that only the slopes of the relationships PTL *vs.* PPL were significantly distinct between sexes (*t*-test; $P<0.05$), being the slopes of the relationships PTL *vs.* PMW statistically similar between male and female penises (*t*-test; $P<0.05$).

The morphometric relationships established between shell length (SL) and penis dimensions (PTL, PPL and PMW) of males and imposex-affected females are shown in Figure 3. In all these relationships, both the correlation coefficients (r) and the slopes (b) of linear regressions were higher in males than in females (*t*-test; $P<0.05$) (Figure 3). Despite the existence of some overlapping between the distributions of male and female imposex-affected penis dimensions (mainly due to a greater variability in female penis dimensions), this indicated that male penis size is more size-dependent (relatively to shell length) than female penis size.

The parameters of the allometric relationships established between penis dimensions (PTL, PPL and PMW) and between individual size (SL) and respective penis dimensions (PTL, PPL and PMW) of males and imposex-affected females are compiled in Table 2.

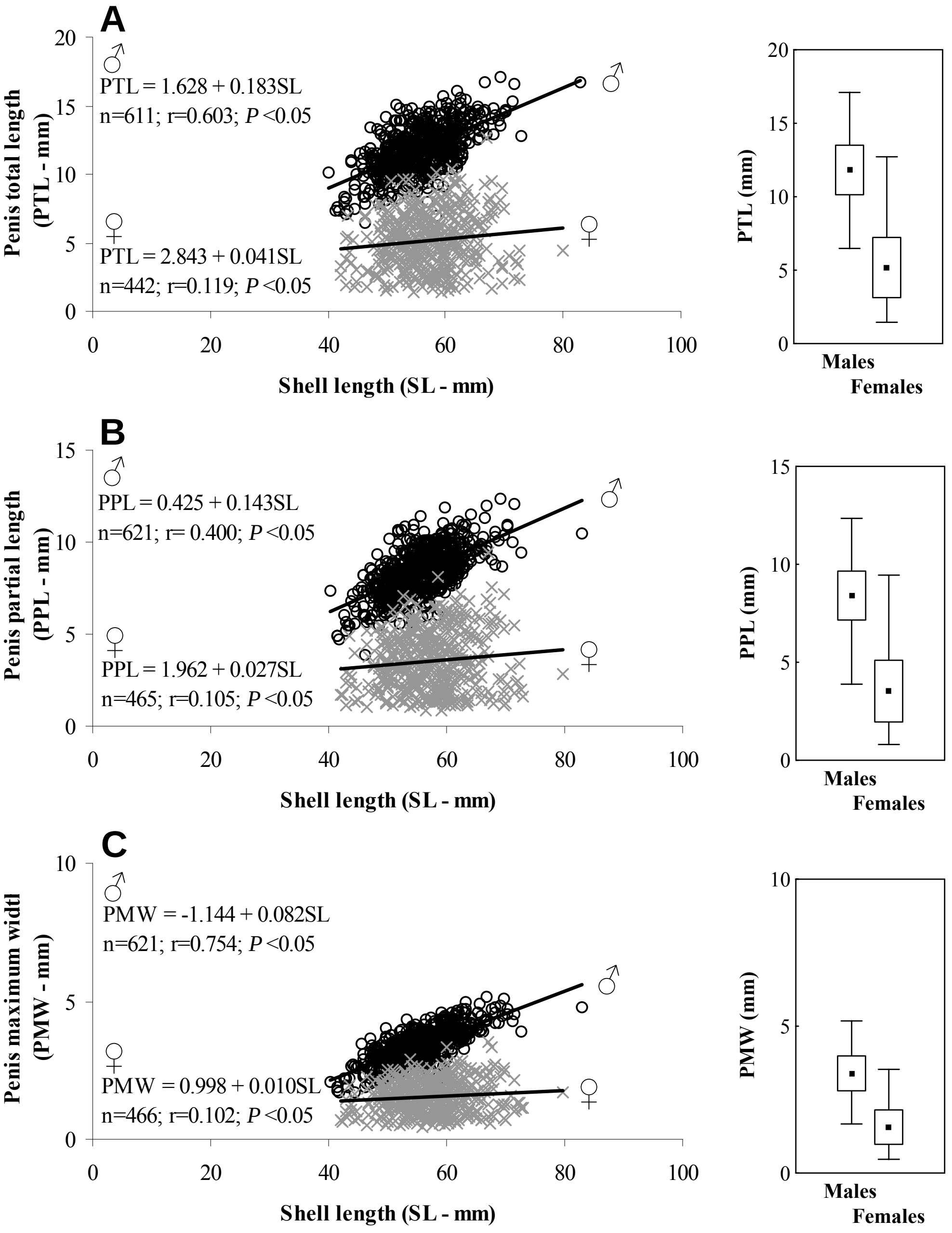


Figure 3. Morphometric relationships established between shell length and penis measurements of males and imposex-affected females of *Hexaplex (Truncululariopsis) trunculus*. **a)** Relationship shell length *vs.* penis total length. **b)** Relationship shell length *vs.* penis partial length. **c)** Relationship shell length *vs.* penis maximum width. Abbreviations: SL - shell length; PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion). Box-whisker graph: middle point = mean; box = ±standard deviation; whisker = minimum and maximum values.

Table 2. Estimated parameters of the allometric relationships established between penis dimensions (PTL, PPL and PMW) and shell length (SL) of males and imposex-affected females of *Hexaplex (Trunculariopsis) trunculus*.

Morphometric variables		**Parameters of the allometric relationships**						
Independent (*X*)	**Dependent (*Y*)**	**Sex**	***n***	***a'***	***b* ± SE (95% CI of *b*)**	***r***	***P-value***	**Growth type**
PTL	PPL	Males	612	0.840	0.932 ± 0.019 (0.894 - 0.970)	0.891	0.000	- Allometry
		Females	439	0.628	1.052 ± 0.012 (1.029 - 1.075)	0.974	0.000	+ Allometry
PTL	PMW	Males	612	0.333	0.938 ± 0.033 (0.873 - 1.003)	0.753	0.000	Isometric
		Females	440	0.413	0.816 ± 0.019 (0.778 - 0.854)	0.896	0.000	- Allometry
SL	PTL	Males	611	0.326	0.892 ± 0.048 (0.798 - 0.986)	0.602	0.000	- Allometry
		Females	442	0.953	0.398 ± 0.199 (0.006 - 0.789)	0.095	0.046	- Allometry
SL	PPL	Males	621	0.150	0.999 ± 0.048 (0.905 - 1.094)	0.640	0.000	Isometric
		Females	465	0.814	0.335 ± 0.215 (-0.087 - 0.757)	0.072	0.119 (ns)	-
SL	PMW	Males	621	0.012	1.393 ± 0.049 (1.297 - 1.489)	0.753	0.000	+ Allometry
		Females	466	0.347	0.352 ± 0.180 (-0.002 - 0.707)	0.090	0.051 (ns)	-

Abbreviations: *n* - number; *a'* - anti-logarithm of the linear regression intercept *a* ($a' = e^{\ln a}$); *b* - linear regression slope; SE - standard error; CI - confidence interval; *r* - correlation coefficient; *P*-value - statistical significance; ns - not significant.

All allometric relationships involving male penis dimensions (both between penis dimensions: PTL *vs.* PPL and PTL *vs.* PMW, and between shell size and penis dimensions: SL *vs.* PTL, SL *vs.* PPL and SL *vs.* PMW) were statistically significant ($P<0.05$) and presented high correlation coefficients. For imposex-affected females, the allometric relationships between penis dimensions (PTL, PPL and PMW) were also statistically significant ($P<0.05$) and presented high correlation coefficients as well, but among the allometric relationships between shell size and penis dimensions, only the relationship between shell length and penis total length (SL *vs.* PTL) was statistically significant ($P<0.05$), despite with a very low correlation coefficient. In the allometric relationships between penis dimensions (PTL, PPL and PMW), the type of growth was opposite between sexes, with a negative allometry (males) and a positive allometry (females) in the relationship PTL *vs.* PPL, and with an isometric growth (males) and a negative allometry (females) in the relationship PTL *vs.* PMW. The type of growth was also distinct in the allometric relationships between individual size (SL) and penis dimensions (PTL, PPL and PMW), being a negative allometry for SL *vs.* PTL (both sexes), isometric for SL *vs.* PPL (males) and a positive allometry for SL *vs.* PMW (males). Overall, only males presented isometric relationships, both among penis dimensions (PTL *vs.* PMW) and between shell length and penis measurements (SL *vs.* PPL) (Table 2).

The use of sexual indices (PTL/SL, PPL/SL and PMW/SL) for the sexual identification of males and imposex-affected females is illustrated in Figure 4. These sexual indices reduced the overlap between males and females. Furthermore, the definition of percent limits for the separation of sexes (PTL/SL<15% = females and PTL/SL≥15% = males; PPL/SL<10% = females and PPL/SL≥10% = males; PMW/SL<5% = females and PMW/SL≥5% = males) allowed for the classification of gender on the basis of penises measurements. The ratio PTL/SL identified correctly the sex of 97.2% of the specimens, PPL/SL properly sexed 96.0%

of the individuals and PMW/SL correctly classified 95.7% of the specimens. Correct classifications using PTL/SL and PPL/SL were higher for males (PTL/SL: 99.5% males and 94.1% females; PPL/SL: 99.8% males and 91.0% females), but were lower when applying PMW/SL (92.9% males and 99.4% females) (Figure 4).

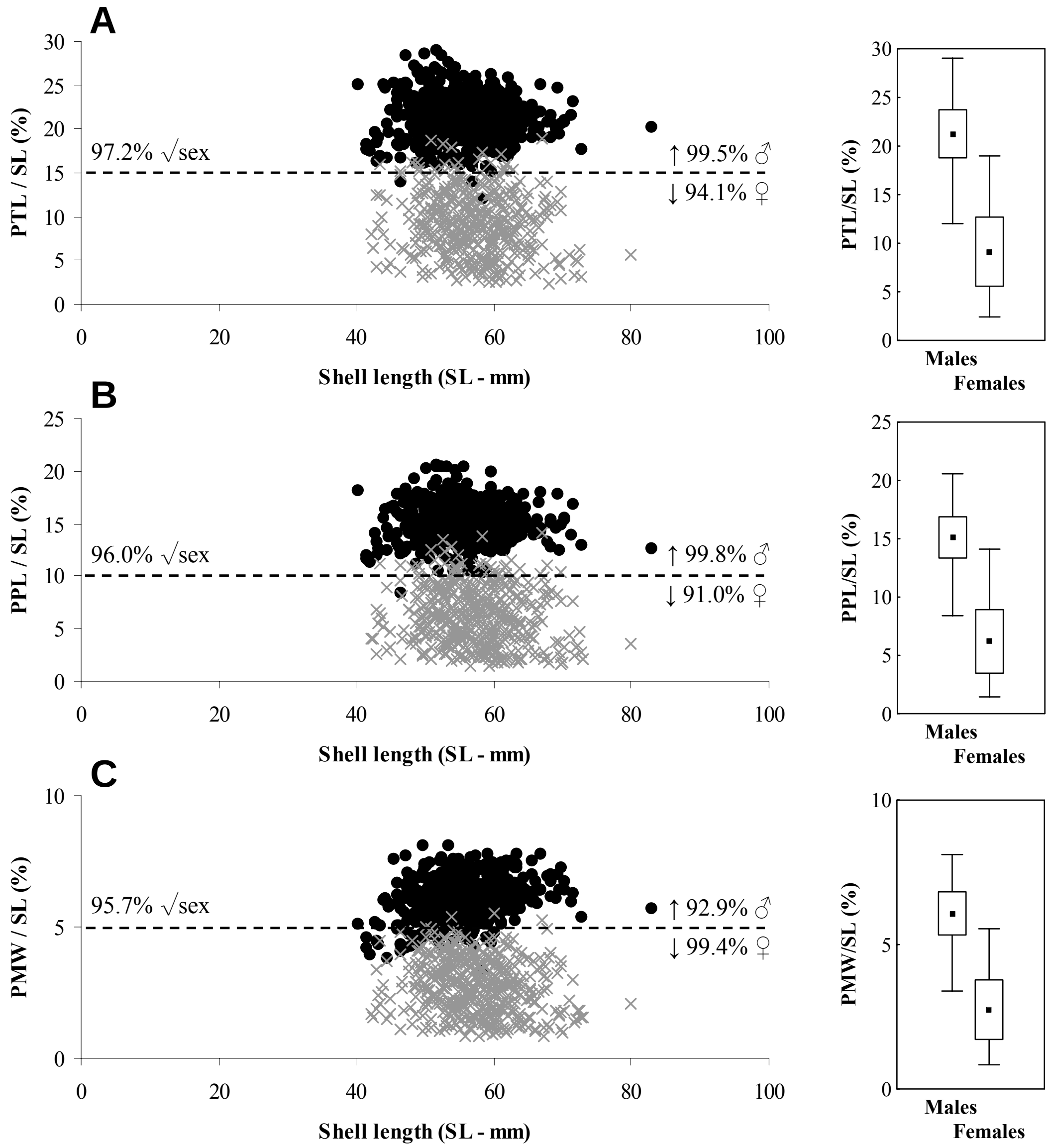


Figure 4. Sexual indexes adopted for the sexual identification of males and imposex-affected females of *Hexaplex (Truncularιopsis) trunculus*. **a)** Relationship shell length *vs.* PTL/SL. **b)** Relationship shell length *vs.* PPL/SL. **c)** Relationship shell length *vs.* PMW/SL. Abbreviations: SL - shell length; PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion). Box-whisker graph: middle point = mean; box = ±standard deviation; whisker = minimum and maximum values.

The monthly variation of these sexual indices (PTL/SL, PPL/SL and PMW/SL) during the one-year study period is illustrated in Figure 5. All sexual indices presented some oscillations all year-round (Kruskal-Wallis *H*-test, $P<0.05$), which were comparable between indices (PTL/SL, PPL/SL and PMW/SL) and with a similar trend between males and imposex-affected females. The Mann-Whitney *U*-test detected statistically significant differences ($P<0.05$) between several consecutive monthly samples of both sexes, mostly between April and October (Figure 5).

Multivariate discriminant analysis using both the individual size (shell length - SL) and the respective penis dimensions (PTL, PPL and PMW) confirmed the distinctness of the two sexes based on penis morphometrics. The linear equation that best discriminates between males and females was the following:

$$D = -0.385PTL - 0.305PPL - 0.421PMW + 0.072SL + 2.493$$

$$[\text{canonical } R = 0.924,\ F_{(4,1048)} = 1525.6\ (P<0.05)]$$

In this function, negative discriminant scores indicated males and positive discriminant scores designated females. Means of canonical variables were -2.05 for males and 2.83 for females (Figure 6a). The distinctness between sexes was further reinforced in the plot of the individual scores based on standardised canonical coefficients for the first two variates (1st and 2nd canonical variables) (Figure 6b). Applying this canonical discriminant function, 98.8% of the original 1053 individuals (611 males + 442 females) were assigned to the correct gender. Correct sexual identification was higher for males (608 specimens = 99.5%) than for females (432 specimens = 97.7%).

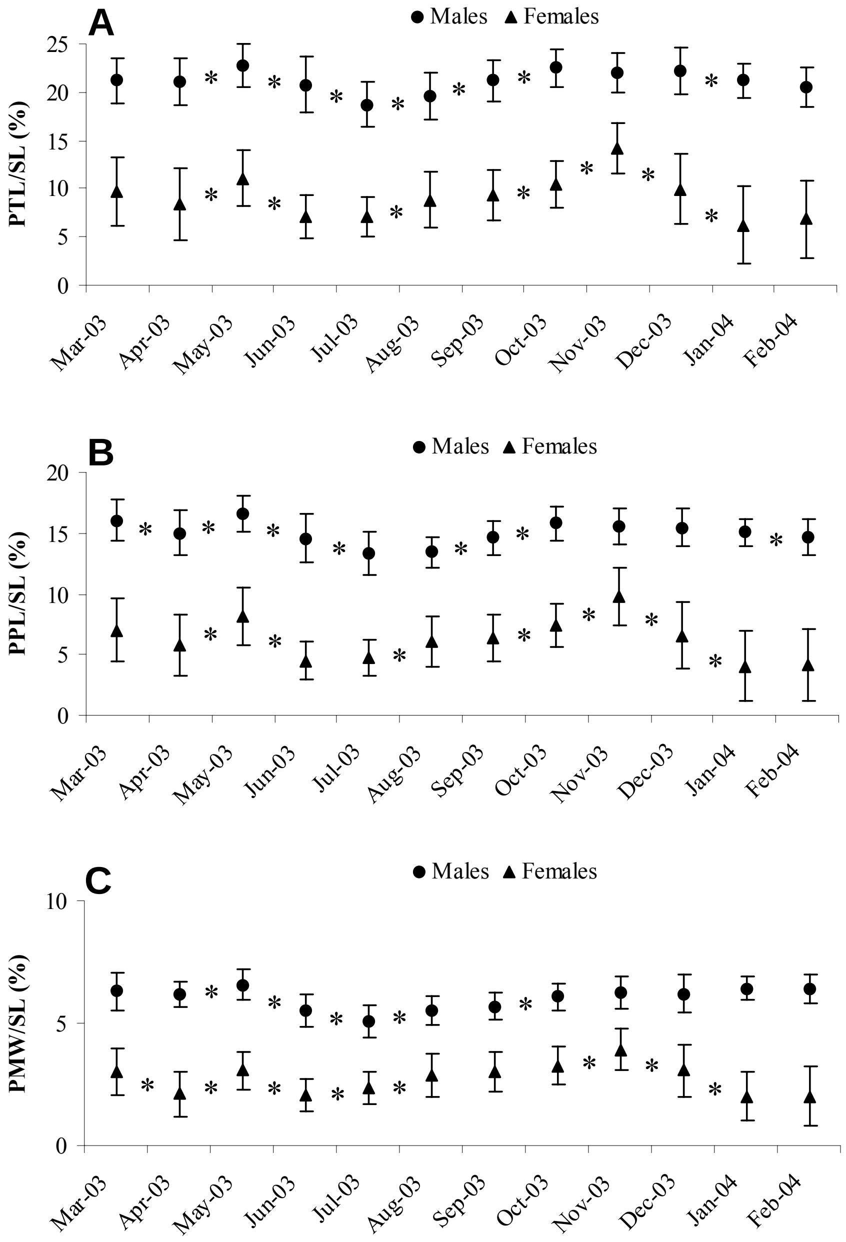


Figure 5. Monthly variation of the sexual indexes during the one-year study period. **a)** Sexual index PTL/SL. **b)** Sexual index PPL/SL. **c)** Sexual index PMW/SL. Abbreviations: SL - shell length; PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion). * = statistically significant differences between consecutive monthly samples (Mann-Whitney *U*-test, $P<0.05$).

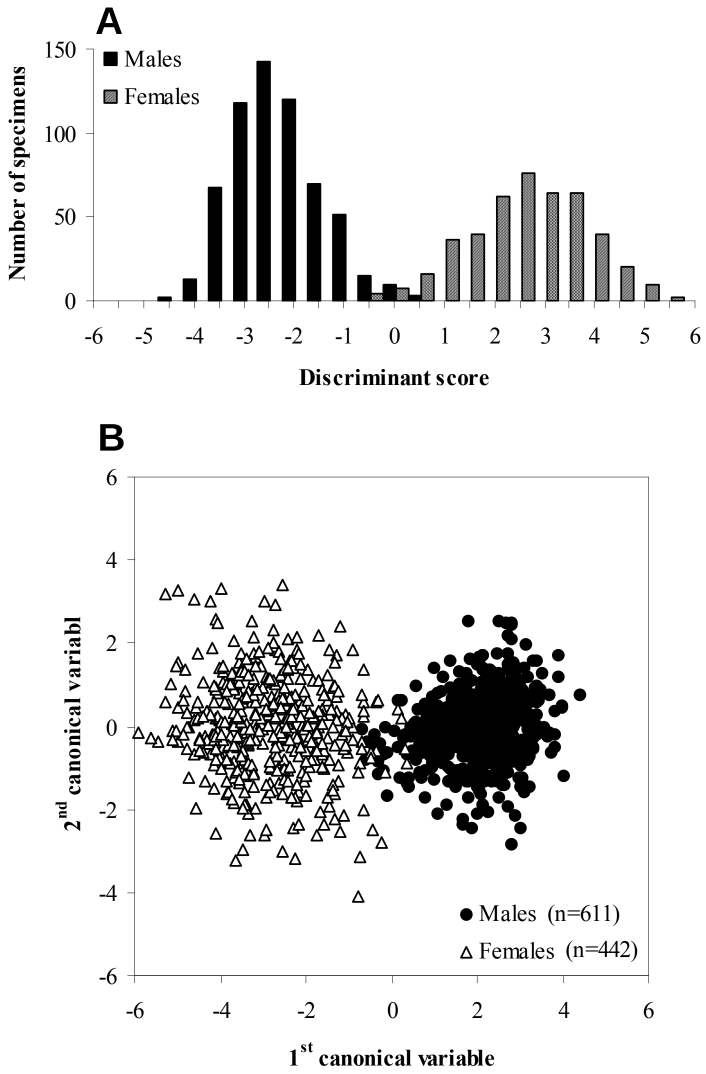


Figure 6. a) Frequency of male and female *Hexaplex (Trunculariopsis) trunculus* relatively to the discriminant scores calculated from the linear equation obtained by multivariate discriminant analysis. **b)** Plot of the individual scores for male and female *Hexaplex (Trunculariopsis) trunculus* in the first two canonical variables resulting from multivariate discriminant analysis.

The anaesthetization of *T. trunculus* with magnesium chloride ($MgCl_2.6H_2O$) was highly effective, since under full relaxation all specimens were sufficiently eased from their shells, allowing for the required exposition of the anterior part of the pallial cavity and measurement of the penis. Nevertheless, specimens took an average of 3:04±0:58 hours to become fully relaxed and the period required for the anaesthetic to produce full relaxation increased with specimen size (linear regression analysis - SL *vs.* time: $r = 0.445$; $P<0.05$). On the other hand, *T. trunculus* proved highly tolerant to the anaesthetization, since all specimens recovered completely from the narcosis treatment after being held overnight in laboratory aquaria with aerated seawater.

The anaesthetization invariably produced an enlargement of average penis dimensions (PTL, PPL and PMW) comparatively to the same penis measurements obtained from subsequently sacrificed specimens (PTL=1.49±1.19 mm; PPL=0.98±1.23 mm; PMW=0.20±0.27 mm). Furthermore, the longer the penis measurement, the higher the augmentation effect produced by the anaesthetic (PTL=18.55±14.60%; PPL=12.89±15.68%; PMW=10.01±12.50%). These differences in average penis dimensions were not statistically significant before and after the narcosis treatment (*Z*-test; $P<0.05$), but individually the anaesthetization produced a significant increase in all these penis measurements (PTL, PPL and PMW) (paired-sample *t*-test: $P<0.05$ or Wilcoxon signed rank test: $P<0.05$). The effect produced by the narcotisation treatment in the average penis dimensions (PTL, PPL and PMW) of males and imposex-affected females of *T. trunculus* is illustrated in Figure 7. On average, the anaesthetization only caused a significant increase in the male's penis total length (PTL) and partial length (PPL) (*Z*-test; $P<0.05$), but individually the narcotisation provoked a significant augmentation in all penis measurements (PTL, PPL and PMW) of both sexes (paired-sample *t*-test: $P<0.05$).

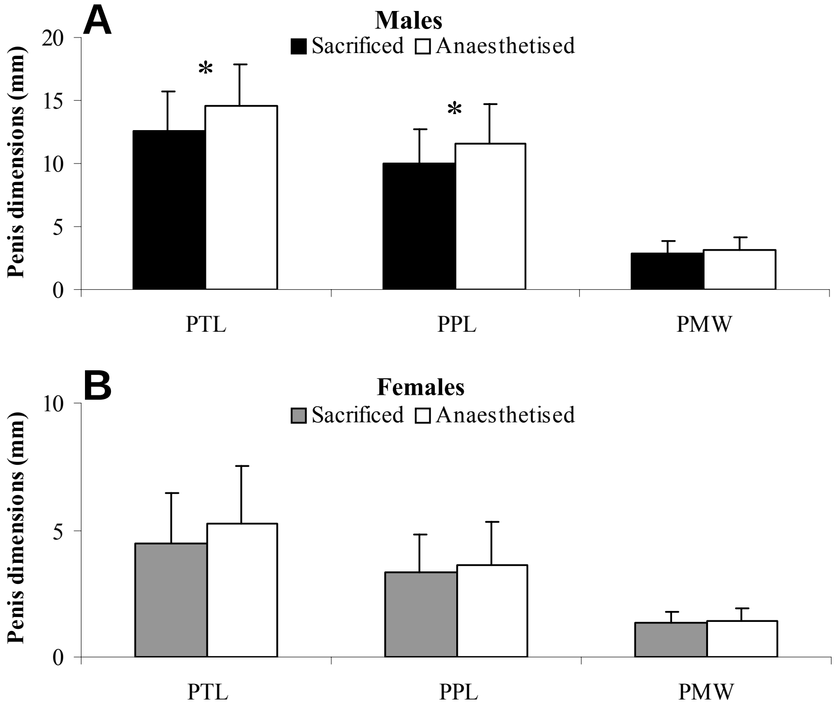


Figure 7. Effect produced by the anaesthetisation in the average penis dimensions (PTL, PPL and PMW) of males and imposex-affected females of *Hexaplex (Truncularioposis) trunculus*. Abbreviations: PTL - penis total length (proximal and distal portions); PPL - penis partial length (proximal portion); PMW - penis maximum width (proximal portion). * = statistically significant differences between average dimensions (*Z*-test, *P*<0.05).

In order to test this non-sacrificial approach and to validate the sexual indices, data on specimen size (SL) and penis dimensions (PTL, PPL and PMW) of these 100 anaesthetized and subsequently sacrificed specimens were applied on the sexual indices previously established from 1053 sacrificed *T. trunculus*. For this purpose, both raw data (anaesthetized and sacrificed: PTL, PPL and PMW) and data transformed by a conversion factor destined to attenuate the effects from the anaesthetization treatment, corresponding to the average penis enlargement provoked by the narcotic (anaesthetized: PTL-18.55%, PPL-12.89% and PMW-10.01%), were employed. The correct sexual identifications of both anaesthetized and sacrificed *T. trunculus* obtained through the application of these sexual indices are compiled in Table 3.

Table 3. Correct sexual identifications of males and imposex-affected females obtained through the application of sexual indices to 100 specimens of *Hexaplex (Truncupariopsis) trunculus* that were anaesthetized and subsequently sacrificed.

	Sacrificed			**Anaesthetized (raw data)**			**Anaesthetized (conversion factor)**		
Sexual indices	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
PTL / SL (%)	54 (100.0%)	45 (97.8%)	99 (99.0%)	54 (100.0%)	41 (89.1%)	95 (95.0%)	53 (98.1%)	44 (95.7%)	97 (97.0%)
PPL / SL (%)	54 (100.0%)	44 (95.7%)	98 (98.0%)	54 (100.0%)	40 (87.0%)	94 (94.0%)	54 (100.0%)	44 (95.7%)	98 (98.0%)
PMW / SL (%)	42 (77.8%)	46 (100.0%)	88 (88.0%)	47 (87.0%)	46 (100.0%)	93 (93.0%)	45 (83.3%)	46 (100.0%)	91 (91.0%)
Discriminant (*D*)	51 (94.4%)	45 (97.8%)	96 (96.0%)	52 (96.3%)	45 (97.8%)	97 (97.0%)	52 (96.3%)	46 (100.0%)	98 (98.0%)

Abbreviations: PTL - penis total length; PPL - penis partial length; PMW - penis maximum width; SL - shell length.

All sexual indices revealed a high accuracy in sexing *T. trunculus* (usually more than 95% correct identifications), independently of being applied on data from sacrificed or anaesthetized specimens (raw data or data transformed by the conversion factor). The indices PTL/SL and PPL/SL revealed more appropriate for correctly sexing males (almost always 100% correct classifications), while PMW/SL was more suitable for accurately sexing females (invariably 100% correct classifications). By integrating data both from specimen size (SL) and respective penis dimensions (PTL, PPL and PMW), the discriminant function (*D*) also revealed a very high precision in the gender identification (generally above 95% correct classifications), which was inclusively higher for imposex-affected females than for males (Table 3).

DISCUSSION

Despite being an organ resulting from an abnormal development, the penes of imposex-affected females of *T. trunculus* were visually very similar to male penes, with the exception of their coloration (identical pigmentation to the foot), which generally was slightly darker in males than in females. The morphological similarity between these organs was further reinforced by the comparison of their relative proportions (ratios: PPL/PTL and PMW/PTL) and by the morphometric relationships established between penis measurements (PTL, PPL and PMW). This similar morphology of male and imposex-affected female penises was already evidenced both externally and internally (histologically) (Martoja and Bouquegneau, 1988; Axiak *et al.*, 2003). Altogether, these similar characteristics between male and imposex-affected female penes make the sexual identification based solely on relative penis proportions and morphology not feasible.

The imposex-affected female average penis size obviously varies according to the degree of imposex in the population, but ultimately can reach (or even exceed) the average size of male penis. However, at the imposex incidence (I=93.24%) and degree (Population RPLI=36.13, Incidence RPLI=43.82, Population RPSI=4.71 and Incidence RPSI=8.42) detected in this population from the Ria Formosa lagoon (Vasconcelos *et al.*, 2006a), male penis average dimensions (PTL, PPL and PMW) were invariably more than two times greater than the same measurements in imposex-affected females.

Moreover, and because TBT apparently does not affect the male penis size in this gastropod species (Axiak *et al.*, 1995), the morphometric relationships established between shell length (SL) and penis dimensions (PTL, PPL and PMW) clearly demonstrated that male penis dimensions were more size-dependent (relatively to shell length), whereas female penis is an organ resulting from an abnormal development (imposex phenomenon), whose dimensions only depend on the duration of the exposure and intensity of contamination by TBT. Additionally, the allometric relationships estimated between shell length (SL) and penis dimensions (PTL, PPL and PMW) further confirmed this phenomenon. Statistically significant relationships revealed that male penis dimensions were intrinsically related to their body growth, while on the contrary, non-significant relationships (or with a low correlation coefficient) indicated that imposex-affected female penis dimensions were not directly connected to their age, instead depending on the deleterious effects of TBT pollution.

In the case of male penis, the SL *vs.* PPL isometric relationship perfectly confirmed the assumption that both variables (shell length and penis partial length) developed at the same growth rate. The other allometric relationships revealed that male penis total length grows relatively slower than shell length, possibly due to the decrease or damage of the penis extremity (distal portion) (negative allometric relationship SL *vs.* PTL), phenomenon that is accompanied by the progressive thickening of the penis during growth (positive allometric relationship SL *vs.* PMW). For the allometric relationships established between penis dimensions, the different types of growth detected in the relationships PTL *vs.* PPL between males and females also revealed the different features and characteristics of this organ between sexes. For the male penis, the negative allometry indicates that during penis development and growth, an extension of the penis distal portion (sharp tip) occurs, probably to facilitate copulation. On the contrary, in the case of female penis, the positive allometry reveals that during growth and development of this anomalous organ, the penis proximal portion increases at a higher rate than the penis distal portion (and consequently than the penis total length).

Despite some overlapping, the morphometric relationships established between individual size (shell length - SL) and penis dimensions (PTL, PPL and PMW) created some expectations that the use of these measurements could be highly valuable for distinguishing gender between males and imposex-affected females of *T. trunculus*. Subsequently, the adoption of sexual indices consisting on the percentage of penis dimensions relatively to individual size (PTL/SL, PPL/SL and PMW/SL) and the simple definition of percent limits for the separation between males and females revealed a very high accuracy in the sexual classification based on penis measurements. These sexual ratios allowed for the correct identification of gender of the vast majority of specimens (PTL/SL = 97.2%, PPL/SL = 96.0% and PMW/SL = 95.7%), being exact sexual recognition generally higher for males than for females.

These sexual indices (PTL/SL, PPL/SL and PMW/SL) presented slight oscillations during the one-year study period, with some significant variations between several consecutive monthly samples of both sexes, mostly between April and October. These oscillations were analogous between sexual indices and with a similar trend between males and imposex-affected females all year-round, thus they could not be attributed to the reproductive cycle of this species and to an eventual variation in male penis size during sexual maturity. Furthermore, imposex apparently does not show any seasonal variation during the reproductive cycle of *T. trunculus* (Martoja and Bouquegneau, 1988). This relative proportionality between penis dimensions of both sexes irrespectively of sampling season allowed for the employment of these sexual indices in this species all year-round.

Multivariate discriminant analysis using both the individual size (shell length - SL) and the respective penis dimensions (PTL, PPL and PMW) further confirmed the clear distinctness between sexes based on penis morphometrics. Applying the canonical function (linear equation) best discriminating between males and females allowed for the correct classification of gender for 98.8% of the specimens, which was slightly higher for males (99.5%) than for females (97.7%). Nevertheless, in this context it should be emphasised that using the same individuals for function estimation and testing could eventually have generated excessively optimistic estimates of the present sexual classification success.

Measurement of the penis is inevitably difficult to standardise and consequently is not a highly precise procedure, mainly because this organ is muscular and subject to contraction with tactile stimulation (Gibbs, 1999). Furthermore, the anaesthetization causes penial muscle tissue to relax and therefore induces an increase in penis measurements. The experimental anaesthetization of *T. trunculus* was very successful and this species proved highly tolerant to the narcosis treatment, but as expected, the anaesthetic ($MgCl_2$) provoked an enlargement in penis dimensions. In the present case, the longer the penis measurement the higher the augmentation effect produced by the anaesthetic (PTL>PPL>PMW). Nevertheless, despite this side effect from the narcotic, the sexual indices previously developed for sacrificed specimens (PTL/SL, PPL/SL, PMW/SL and discriminant function - *D*) were still highly successful in sexing anaesthetized *T. trunculus* specimens (generally more than 95% correct sexual identifications), using both raw data and transformed data (conversion factor).

Taking into account that *T. trunculus* lacks external sexual dimorphism and that gender identification implies breaking the shell and sacrificing the organism, the data gathered in this study are highly valuable for sexing live specimens with a previously known imposex degree. This procedure was easily performed by anaesthetizing the individuals (with $MgCl_2$), gently exposing and measuring penis dimensions, and finally applying sexual indices. This kind of information is a very useful and non-sacrificial tool for the sexual identification of live specimens and has a vast practical application in several types of biological, ecological and behavioural studies.

It is worth emphasising that these sexual indices can be successfully applied to all *T. trunculus* populations with an imposex degree equal or inferior to the one registered in this study and that at progressively lower imposex degrees sexual discrimination will be increasingly higher. Furthermore, with TBT levels dropping and consequent less severe imposex degrees, it is expected that the sexual recognition of *T. trunculus* will progressively present less complexity in the near future. Nevertheless, it may take longer than initially supposed, since despite the recent global TBT restrictions, it is possible that this environmental problem could even increase over the next few years before finally attenuating or disappearing (Champ, 2000). Therefore, TBT contamination in the Ria Formosa lagoon is also unlikely to change for a considerable period of time (Coelho *et al.*, 2002) and apparently has not decreased recently (Vasconcelos *et al.*, 2006a).

Similar sexual indices can be developed for other gastropod species without external sexual dimorphism and affected by imposex, only requiring baseline data on the imposex degrees of their populations. However, it should be emphasised that similar sexual indices should be cautiously applied to gastropod species whose male penis dimensions present significant seasonal fluctuations related to the sexual maturity, reproductive cycle and/or breeding season, phenomenon that has been detected in some muricid species, namely *Bolinus brandaris* (Ramón and Amor, 2001, 2002), *Nucella lapillus* and *Ocenebra erinaceus* (Stroben *et al.*, 1996), as well as in non-Muricidae species, such as *Ilyanassa obsoleta* (Bryan *et al.*, 1989), *Nassarius reticulatus*, *Nassarius incrassatus* and *Trivia arctica* (Stroben *et al.*, 1996). Nevertheless, in practice, this seasonal enlargement of the male penis size is only a minor source of error because many populations breed throughout the year (Gibbs, 1999).

ACKNOWLEDGMENTS

The authors would like to thank the technical staff of IPIMAR - CRIPSul for their help in the laboratory sampling procedures. Thanks are also due to the anonymous referee whose suggestions greatly improved the final version of the manuscript. This study was partially funded by a PhD grant from the Fundação para a Ciência e Tecnologia (FCT: SFRH/BD/5139/2001).

REFERENCES

Axiak, V.; Vella, A.J.; Micaleff, D.; Chircop, P.; Mintoff, B., 1995. Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, 121: 685-691.

Axiak, V.; Vella, A.J.; Agius, D.; Bonnici, P.; Cassar, G.; Cassone, R.; Chircop, P.; Micallef, D.; Mintoff, B.; Sammut, M., 2000. Evaluation of environmental levels and biological impact of TBT in Malta (central Mediterranean). *The Science of the Total Environment*, 258: 89-97.

Axiak, V.; Micallef, D.; Muscat, J.; Vella, A.; Mintoff, B., 2003. Imposex as a biomonitoring tool for marine pollution by tributyltin: some further observations. *Environment International*, 28: 743-749.

Bernard, F.R., 1968. Sexual dimorphism in *Polinices lewisi* (Naticidae). *Nautilus*, 82: 1-3.

Brande, S.; Turner, M.; Heller, J.; Ben Yehuda, O., 1996. Statistical discrimination of sex in *Melanoides tuberculata* (Gastropoda: Thiaridae). *Biological Journal of the Linnean Society*, 59: 87-112.

Bryan, G.W.; Gibbs, P.E.; Huggett, R.J.; Curtis, L.A.; Bailey, D.S.; Dauer, D.M., 1989. Effects of tributyltin pollution on the mud snail, *Ilyanassa obsoleta*, from the York River and Sarah Creek, Chesapeake Bay. *Marine Pollution Bulletin*, 20: 458-462.

Castagna, M.; Kraeuter, J.N., 1994. Age, growth rate, sexual dimorphism and fecundity of the knobbed whelk *Busycon carica* (Gmelin, 1791) in a western mid-Atlantic lagoon system, Virginia. *Journal of Shellfish Research*, 13: 581-585.

Champ, M.A., 2000. A review of organotin regulatory strategies, pending actions, related costs and benefits. *The Science of the Total Environment*, 258: 21-71.

Chiavarini, S.; Massanisso, P.; Nicolai, P.; Nobili, C.; Morabito, R., 2003. Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). *Chemosphere*, 50: 311-319.

Coelho, M.R.; Bebianno, M.J.; Gibbs, P.E., 1998. Imposex in neogastropod snails from southern Portugal. *Cuadernos de Investigación Biológica*, 20: 273.

Coelho, M.R.; Bebianno, M.J.; Langston, W.J., 2002. Organotin levels in the Ria Formosa lagoon, Portugal. *Applied Organometallic Chemistry*, 16: 384-390.

El Hamdani, A.; Ferrer, J.M.; García Carrascosa, A.M., 1998. Imposex in prosobranch molluscs: an indicator of TBT pollution in the Valencian coast (Spain, Western Mediterranean). *Cuadernos de Investigación Biológica*, 20: 275-278.

Ellis, D.V.; Pattisina, L.A., 1990. Widespread neogastropod imposex: a biological indicator of global TBT contamination? *Marine Pollution Bulletin*, 21: 248-253.

Estebenet, A.L., 1998. Allometric growth and insight on sexual dimorphism in *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Malacologia*, 39: 207-213.

Fioroni, P.; Oehlmann, J.; Stroben, J., 1991. The pseudohermaphroditism of prosobranchs: morphological aspects. *Zoologischer Anzeiger*, 226: 1-26.

Garaventa, F.; Pellizzato, F.; Faimali, M.; Terlizzi, A.; Medakovic, D.; Geraci, S.; Pavoni, B., 2006. Imposex in *Hexaplex trunculus* at some sites on the North Mediterranean Coast as a base-line for future evaluation of the effectiveness of the total ban on organotin based antifouling paints. *Hydrobiologia*, 555: 281-287.

Gibbs, P.E., 1999. Biological effects of contaminants: Use of imposex in the dogwhelk (*Nucella lapillus*) as a bioindicator of tributyltin pollution. *ICES Techniques in Marine Environmental Sciences*, 24: 1-29.

Gibbs, P.E., 2005. Male genital defect (Dumpton Syndrome) in the dog-whelk *Nucella lapillus* (Neogastropoda): Mendelian inheritance inferred, based on laboratory breeding experiments. *Journal of the Marine Biological Association of the United Kingdom*, 85: 143-150.

Gibbs, P.E.; Bebianno, M.J.; Coelho, M.R., 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution, with notes on a species (*Columbella rustica*) lacking the imposex response. *Environmental Technology*, 18: 1219-1224.

Jenner, M.G., 1979. Pseudohermaphroditism in *Ilyanassa obsoleta* (Mollusca: Neogastropoda). *Science*, 205: 1407-1409.

Kantor, Y.I.; Sysoev, A.V., 1991. Sexual dimorphism in the apertural notch of a new species of *Gemmula* (Gastropoda: Turridae). *Journal of Molluscan Studies*, 57: 205-209.

Kenchington, E.; Glass, A., 1998. Local adaptation and sexual dimorphism in the waved whelk (*Buccinum undatum*) in Atlantic Nova Scotia with applications to fisheries management. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 2237: 43 p.

Kurata, K.; Kikuchi, E., 2000. Comparisons of life-history traits and sexual dimorphism between *Assiminea japonica* and *Angustassiminea castanea* (Gastropoda: Assimineidae). *Journal of Molluscan Studies*, 66: 177-196.

Langston, W.J.; Gibbs, P.E.; Livingstone, D.R.; Burt, G.R.; O'Hara, S.; Bebianno, M.J.; Coelho, M.R.; Porte, C.; Bayona, J.; McNulty, M.; Lynch, G.; Keegan, B., 1997. Risk assessment of organotin antifoulings on key benthic organisms of European coastal habitats (BOATS). MAS2-CT-94-0099, Final Report.

Martoja, M.; Bouquegneau, J.-M. 1988. *Murex trunculus*: un nouveau cas de pseudo-hermaphrodisme chez un gastéropode prosobranche. *Bulletin de la Société Royale de Liège*, 57: 45-58.

Pellizzato, F.; Centanni, E.; Marin, M.G.; Moschino, V.; Pavoni, B., 2004. Concentrations of organotin compounds and imposex in the gastropod *Hexaplex trunculus* from the Lagoon of Venice. *Science of the Total Environment*, 332: 89-100.

Ramón, M.; Amor, M.J., 2001. Increasing imposex in populations of *Bolinus brandaris* (Gastropoda: Muricidae) in the north-western Mediterranean. *Marine Environmental Research*, 52: 463-475.

Ramón, M.; Amor, M.J., 2002. Reproductive cycle of *Bolinus brandaris* and penis and genital duct size variations in a population affected by imposex. *Journal of the Marine Biological Association of the United Kingdom*, 82: 435-442.

Rilov, G.; Gasith, A.; Evans, S.M.; Benayahu, Y., 2000. Unregulated use of TBT-based antifouling paints in Israel (Eastern Mediterranean): high contamination and imposex levels in two species of marine gastropods. *Marine Ecology Progress Series*, 192: 229-238.

Smith, B.S., 1971. Sexuality in the American mud snail *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London*, 39: 377-378.

Sokal, R.R.; Rohlf, F.J., 1987. *Introduction to Biostatistics.* 2nd Edition. W. H. Freeman and Company, New York.

Stroben, E.; Oehlmann, U.; Schulte-Oehlmann, U.; Fioroni, P., 1996. Seasonal variations in the genital ducts of normal and imposex-affected Prosobranchs and its influence on biomonitoring indexes. *Malacological Review*, Suppl. 6: 173-184.

ten Hallers-Tjabbes, C.C., 1979. Sexual dimorphism in *Buccinum undatum* L., *Malacologia*, 18: 13-17.

Terlizzi, A., 2000. Imposex (pseudoermafroditismo) in molluschi gasteropodi mediterranei: aspetti morfologici e considerazioni ecologiche. *Bollettino Malacologico*, 36: 155-158.

Terlizzi, A.; Scuderi, D.; Faimali, M.; Minganti, V.; Geraci, S., 1997. Imposex in *Hexaplex trunculus* e *Stramonita haemastoma* (Gastropoda: Muricidae): prime segnalazioni per le acque costiere italiane. *Biologia Marina Mediterranea*, 4: 496-499.

Terlizzi, A.; Geraci, S.; Minganti, V., 1998. Tributyltin (TBT) pollution in the coastal waters of Italy as indicated by imposex in *Hexaplex trunculus* (Gastropoda: Muricidae). *Marine Pollution Bulletin*, 36: 749-752.

Terlizzi, A.; Geraci, S.; Gibbs, P.E., 1999. Tributyltin (TBT)-induced imposex in the Neogastropod *Hexaplex trunculus* in Italian coastal waters: morphological aspects and ecological implications. *Italian Journal of Zoology*, 66: 141-146.

Terlizzi, A.; Fraschetti, S.; Gianguzza, P.; Faimali, M.; Boero, F. 2001. Environmental impact of antifouling technologies: state of the art and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 11: 311-317.

Terlizzi, A.; Delos, A.L.; Garaventa, F.; Faimali, M.; Geraci, S., 2004. Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin*, 48: 188-192.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006a. Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin*, 52: 337-341.

Vasconcelos, P.; Gaspar, M.B.; Pereira, A.M.; Castro, M., 2006b. Growth rate estimation of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of Shellfish Research*, 25: 249-256.

Zar, J.H., 1996. *Biostatistical Analysis.* 3rd Edition. Prentice-Hall International Inc., New Jersey.

SECTION 6

GENERAL DISCUSSION AND CONCLUSION

SECTION 6

General discussion and conclusion

GENERAL DISCUSSION

The present work aimed primarily to describe the artisanal fishery with the “wallet-line” targeting two commercially valuable muricid gastropods, the banded murex - *Hexaplex (Truncularіopsis) trunculus* and the purple dye murex - *Bolinus brandaris* and study relevant aspects of the biology and ecology (mainly age and growth, reproduction and imposex) of *T. trunculus* in the Ria Formosa lagoon. Overall, the ultimate objective of this study was to propose management measures for the implementation of specific regulations for this artisanal fishery and to contribute to the long-term sustainable exploitation of this locally important fishing resource.

Experimental fishing surveys with the “wallet-line” allowed gathering detailed information on the fishing operations, fishing gear, fishing yield, target species catch composition, by-catch species and discards (Chapter 2.1). The CPUE of both target species and by-catch species presented a high monthly variation (without an evident seasonal pattern), markedly decreasing during consecutive fishing days (mainly due to declining bait attraction). Despite not being a species-specific fishing gear, the “wallet line” captures essentially gastropods (besides *T. trunculus* and *B. brandaris* that accounted for 71.3% of the total catches, 89.6% of the by-catch corresponded to other 21 gastropod species). Furthermore, the “wallet-line” is also not a size-selective fishing gear, because the catches comprised a high proportion of commercially under-sized target species (47.2% of *T. trunculus* below the MLS = 50 mm SL; 81.9% of *B. brandaris* below the MLS = 65 mm SL). Nevertheless, the vast majority of the by-catch species and commercially under-sized target species are immediately discarded on-board and therefore mortality is presumably negligible.

Mark/recapture experiments in a fish culture earth pond in the Ria Formosa lagoon allowed an estimation of the first growth data (growth rates and von Bertalanffy growth parameters) available for *T. trunculus* (Chapter 3.1). Growth rates exhibited a high inter-individual variability and revealed that *T. trunculus* is a relatively slow-growing gastropod species (average growth rate = 1.0 mm SL/month). According to the von Bertalanffy growth function (L_t=82.76[1–$e^{-0.41(t+0.05)}$]), the minimum landing size (MLS) legally stipulated for *T. trunculus* (50 mm SL) is attained at 2.20 years-old. Since among other detrimental effects, epibiosis might affect gastropod growth, a complementary study aimed to describe the secondary colonisation of *T. trunculus* shells by epibiotic polychaetes, presenting information on fouling frequency, degree of epibiosis, preferential areas for colonisation and incidence of shell damage (Chapter 3.2). In general, the results indicated that besides burrowing into soft bottom substrates, sporadic inter-tidal exposure and mutual predation on epibiosis, *T. trunculus* apparently lacks other typical antifouling defences. Nevertheless, the present degree of epibiosis apparently is not enough to cause highly detrimental impacts on *T. trunculus* growth and fitness, except in terms of shell colonisation by burrowing/drilling polychaetes, which leads to the gradual destruction of the gastropod shell, possibly affecting somewhat its general condition and growth, and potentially also increasing vulnerability to predators.

The reproductive dynamics and gametogenic cycle of *T. trunculus* from the Ria Formosa lagoon were studied through macro- and microscopic analyses of male and female gonads (Chapter 4.1). The reproductive cycle of *T. trunculus* comprehended an extensive period of gonadal activity (with the simultaneous occurrence of distinct gametogenic stages in both sexes almost year-round) and a short resting phase during the period of warmest seawater temperatures (July and August). Spawning occurred mainly in May and June (apparently triggered by the rising of seawater temperature during spring), followed by a secondary spawning event in October and November. Since histological techniques are not very practical for routine assessment of reproductive condition, an additional study aimed to compare the ability of conventional condition indices and visual quantitative condition indices for assessing the reproductive condition of *T. trunculus* (Chapter 4.2). Overall, the visual quantitative condition indices (based on measurements of the digestive gland, gonad or capsule gland, and subsequent calculation of the gonadosomatic index - GSI or capsule gland index - CGI) exhibited a remarkable ability to follow seasonal variations in the species reproductive dynamics and identify broad changes in the reproductive development of *T. trunculus* (namely the periods of gonad maturation and spawning). Furthermore, since the information on some aspects of *T. trunculus* reproductive biology was relatively scarce, a complementary study aimed to describe the spawning behaviour and egg-laying pattern, the morphology and dimensions of the spawns, egg capsules, eggs, embryos and early post-metamorphic juveniles, and estimate the growth rate of *T. trunculus* hatchlings and juveniles (Chapter 4.3). The spawning behaviour of *T. trunculus* induces females to form massive agglomerations (sometimes comprising hundreds of specimens) and therefore this crucial phase of the reproductive cycle of this species should be carefully protected to ensure successful spawning (and subsequent recruitment). Altogether, besides increasing and detailing the knowledge available on these subjects, this study provided valuable information to support adequate fishery management measures, as well as helpful information for a preliminary assessment of the potential of *T. trunculus* as a new species for molluscan aquaculture (identifying both advantageous and unfavourable features of this species in terms of aquaculture production).

The incidence and degree of imposex were monitored monthly in a *T. trunculus* population from the Ria Formosa lagoon (Chapter 5.1). Fortunately, interferences with normal sexual development were not observed and potentially sterile females (through the splitting of the capsule gland or presence of aborted capsules in the capsule gland) were also not detected, revealing that presently imposex apparently does not significantly affect the population dynamics of *T. trunculus* in the studied area (which is highly relevant in a fishery-exploited gastropod species). Nevertheless, this study also revealed that recent global TBT restrictions appear not to be yet fully effective nowadays, since imposex levels in *T. trunculus* indicated that organotin contamination in the Ria Formosa lagoon have not decreased recently, therefore requiring for regular monitoring and surveillance in this highly important and vulnerable ecosystem. An additional study aimed to develop sexual indices based on the dimensions of male and imposex-affected female penis that could constitute non-sacrificial means for sexing *T. trunculus* (which usually requires breaking the shell and sacrificing the organism, due to the absence of external sexual dimorphism in this species) (Chapter 5.2). An anaesthetization experiment was performed to investigate the effects produced by the anaesthetic on penis measurements, to test this non-sacrificial approach and to validate the sexual indices. Overall, the sexual indices revealed a high accuracy in the sexual identification of anaesthetized individuals (generally more than 95% correct sexing) and therefore constitute a useful, simple and non-sacrificial tool for sexing *T. trunculus* (which is highly advantageous for diverse biological, ecological and behavioural studies that require live specimens and the respective sexual identification).

The ultimate objective of the present work was to propose a specific management plan for the artisanal fishery with the “wallet-line” targeting muricid gastropods (*T. trunculus* and *B. brandaris*) in the Ria Formosa lagoon. In this context, the overall information gathered in this study allowed for the proposal to the fisheries administration of several management measures to be implemented in this traditional and locally important fishing activity, which could support the legalisation of this artisanal fishery in the near future.

PROPOSAL OF A MANAGEMENT PLAN FOR THE ARTISANAL FISHERY WITH THE "WALLET-LINE" IN THE RIA FORMOSA LAGOON

By integrating diverse information gathered in the previous sections of this study (particularly on the characterisation of the fishery and target species biology, namely growth and reproductive cycle), the following management measures and regulations are suggested for the artisanal fishery with the "wallet-line" in the Ria Formosa lagoon:

- Fishing licenses for operating the "wallet-line" in the Ria Formosa lagoon should be provisional and renewable every year. Furthermore, being a complementary activity to other artisanal fisheries (fishermen generally only use "wallet-lines" during particular periods of the year as an alternative and secondary source of income), fishing licenses should only be attributed to fishing boats already licensed to operate other fishing gears inside the lagoon and preferentially to fishermen that can attest sales of *T. trunculus* and/or *B. brandaris* in the past;

- The renewal of fishing licenses should be conditioned to the accomplishment by fishermen of a minimum number of yearly sales or minimum amounts sold per year of *T. trunculus* and/or *B. brandaris* in wholesale auction. Moreover, fishermen should report relevant data on the fishing activity (e.g., areas, effort, lost fishing gears, catches, value, etc.) on confidential log-books that will be developed specifically for this purpose, allowing for the development of a data basis on this gastropod fishery. Failure to regularly deliver log-book information or reporting incorrect data should imply the lost of the fishing license;

- The number of fishing gears per boat should be limited to a maximum of 10 "wallet-lines" (the highest number operated by some fishermen), each one restricted to a maximum of 100 "wallets". The length of the main line should be limited to a maximum of 200 metres, the length of the gangions should be less than half of the distance / spacing between consecutive gangions (and "wallets") in the main line, and the size of the "wallets" should be inferior to 15 cm length x 15 cm width (*i.e.*, 225 cm^2). Each fishing gear should be identified with the respective boat registration number and signalised with conspicuous buoys (besides the buoys in each end of the fishing gear, the adoption of an additional float in the middle of the main line could help reducing the number of lost “wallet-lines” accidentally cut by boat propellers);

- Because the “wallet-line” is not a species-specific fishing gear (by-catch specimens belonging to 39 *taxa* accounted for 28.7% of the total catches in number), by-catch species should be discarded immediately on-board and the removal of the bait from the “wallets” should be made in the fishing areas (releasing the surviving cockles and minimising the mortality of by-catch specimens that might have penetrated trough the “wallet” mesh);

- Because the “wallet-line” is not a size-selective fishing gear (commercially under-sized *T. trunculus* and *B. brandaris* accounted for 47.2% and 81.9% of the total catches in number, respectively) and since *T. trunculus* is a relatively slow-growing gastropod species (attaining the minimum landing size at approximately 2.20 years-old), the minimum landing sizes legally stipulated for these species (*T. trunculus* = 50 mm SL and *B. brandaris* = 65 mm SL) should be rigorous accomplished, by sorting the target species on-board and immediately discarding commercially under-sized specimens. Simultaneously, regular monitoring of the catches (including the length frequency distribution of the landings) should be implemented in order to oblige the compliance with the target species minimum landing sizes by fishermen. The results from the present study apparently indicate that the MLS stipulated for *T. trunculus* (50 mm SL) is well adapted to the main biological characteristics of this species, whereas in the case of *B. brandaris* further studies should be conducted to ascertain the suitability of the MLS established for this species (65 mm SL);

- To reduce fishing effort, the fishery with the “wallet-lines” should be restricted to working days, a limitation that should also be extended to the other fishing activities and collecting practices targeting *T. trunculus* and *B. brandaris* in the Ria Formosa lagoon. Additionally, to protect spawning females and collective spawns (consequently contributing for successful recruitment), the fishing season should be limited through the implementation of a seasonal closure coincident with the main reproductive and spawning periods of the target species in the Ria Formosa lagoon (May and June). Moreover, to sustain local populations and simultaneously control fishing effort, further studies should be conducted to identify preferential distribution areas of these target species in the Ria Formosa lagoon, in order to eventually restrict the exploitation in some particular zones through the establishment of permanent closed areas or conservation zones.

FINAL REMARKS AND FUTURE RESEARCH NEEDS

The present study allowed improving the knowledge available on the muricid gastropods fishery with the “wallet-line” in the Ria Formosa lagoon and on the respective target species (*T. trunculus* and *B. brandaris*), with emphasis on some relevant aspects of *T. trunculus* biology and ecology. Furthermore, some experiments provided the first data available on *T. trunculus* growth (particularly in terms of the estimation of age, growth rates and von Bertalanffy growth parameters) and the first information available on the reproductive cycle for an Atlantic population of this species. In addition, particular emphasis was given to the application and/or development of methodologies that could allow for practical and expedite procedures during routine sampling and analysis, such as the indices for non-sacrificial sexing of imposex-affected specimens and the visual quantitative condition indices (gonadosomatic index - GSI and capsule gland index - CGI) to assess reproductive condition of *T. trunculus*.

Nevertheless, in a study of this kind, typically there are differences between the original plan and the final work. Generally, some unanswered questions persist, others require further studies or regular monitoring, and usually new topics to be addressed in future studies arise. The present study highlighted the need for a continuous follow of the fishery for muricid gastropods in the Ria Formosa lagoon, through the implementation of a monitoring programme for assessing the evolution of the catches of target species, as well as the accomplishment and effects of the management measures proposed for this fishing activity. Future studies should focus on establishing the influence of various environmental parameters (depth, type of bottom substrata, seagrass coverage, density of bivalve beds, etc.) on the spatial distribution of *T. trunculus* and *B. brandaris* in the Ria Formosa lagoon. Furthermore, it would be very important gathering additional information on some aspects of the population dynamics of these species that could help improving fisheries management, and for instance it would be highly interesting to ascertain potential relationships between environmental parameters and recruitment of *T. trunculus* and *B. brandaris* in the Ria Formosa lagoon. Additionally, the baseline data gathered on the imposex phenomenon in *T. trunculus* accentuated the need for periodical surveys to follow the evolution of imposex in this species, and subsequently for the regular surveillance of the Ria Formosa lagoon environmental contamination by organotin compounds.

As local demand for sea products augments and the commercial value of *T. trunculus* and *B. brandaris* increases, these muricid species have been progressively considered as potential candidates for molluscan aquaculture (Ramón and Flos, 2001; Vela and Moreno, 2004; Lahbib *et al.*, 2004, 2006; Peharda and Morton, 2006). This study has also drawn the attention to some features of *T. trunculus* biology (*e.g.*, spawning, development, feeding, growth, mortality in aquaculture facilities) that improved the knowledge available on these subjects and constitute baseline information for a preliminary assessment of the potential of the banded murex as a new species for muriciculture. Results revealed that *T. trunculus* has several advantageous characteristics (*e.g.*, direct development, high growth rates of hatchlings and juveniles, low feeding requirements, simple maintenance), as well as some unfavourable features (*e.g.*, hatchling mortality by dehydration due to crawling above the water surface, cannibalism among hatchlings), and this information (together with some ongoing studies) further supported previous expectations about the potential of *T. trunculus* for mariculture. However, besides these initial steps that improved the knowledge available on some aspects, several complementary studies in this innovative field of study should be undertaken in the near future, in order to ascertain the technical and economical feasibility of rearing *T. trunculus* and *B. brandaris*, both for commercial production and for stock enhancement initiatives in the Ria Formosa lagoon.

REFERENCES

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2004. Données sur le cycle reproducteur du mollusque gastéropode *Hexaplex trunculus* (Linné, 1758) de la Lagune de Bizerte (Tunisie). *Bulletin de la Société Zoologique de France*, 129: 407-418.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2006. La ponte et la croissance absolue d'*Hexaplex trunculus* réalisée au laboratoire. *Résumés des Communications du Troisième Congrès Franco-Tunisien de Zoologie*, Tabarka, Tunisie, 3-7 Novembre 2006, 64 pp.

Peharda, M.; Morton, B., 2006. Experimental prey species preferences of *Hexaplex trunculus* (Gastropoda: Muricidae) and predator-prey interactions with the Black mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). *Marine Biology*, 148: 1011-1019.

Ramón, M.; Flos, R., 2001. First trials to cultivate the muricid gastropod *Bolinus brandaris* (Linnaeus). *European Aquaculture Society Special Publication*, 29: 219-220.

Vela, J.M.; Moreno, Ó., 2004. Posibilidades del cultivo de gasterópodos murícidos en la región suratlántica ibérica. *Resumos do Seminário Moluscicultura no Litoral Sul-Atlântico - Diversificação*. 19-20 Fevereiro 2004, Tavira, Portugal.

SECTION 7

LITERATURE CITED IN THE THESIS

SECTION 7

Literature cited in the thesis

LITERATURE CITED IN THE THESIS

Abidli, S.; Trigui El-Menif, N.; Boumaiza, M., 2006. Variation de la concentration du TBT sur la fécondité et l'émission gamétique d'*Hexaplex trunculus*. *Résumés des Communications du Troisième Congress Franco-Tunisien de Zoologie*, Tabarka, Tunisie, 3-7 Novembre 2006, 73 pp.

Albanese, M.P.; Calabrò, C.; Acosta Pomar, M.L.C., 1983. Ciclo biológico di *Truncularíopsis trunculus* L. (Moll. Gast. Pros.) vivente nel Lago di Faro. *Memorie di Biologia Marina e di Oceanografia*, 13: 133-147.

Alyakrinskaya, I.O., 2002. Morphofunctional properties of nutrition of certain predatory gastropods. *Biology Bulletin*, 29: 589-600.

Alyakrinskaya, I.O., 2004. Resistance to drying in aquatic mollusks. *Biology Bulletin*, 31: 299-309.

Alyakrinskaya, I.O., 2005. Functional significance and weight properties of the shell in some molluscs. *Biology Bulletin*, 32: 397-418.

Amos, M.J.; Purcell, S.W., 2003. Evaluation of strategies for intermediate culture of *Trochus niloticus* (Gastropoda) in sea cages for restocking. *Aquaculture*, 218: 235-249.

Anderson, W.D.; Eversole, A.G., 1984. Evolution of a *Busycon* fishery in the nearshore South Atlantic Bight. *Abstracts of Technical Papers presented at 1983 Annual Meeting of the National Shellfisheries Association*, Hilton Head Island, South Carolina, 6-9 June 1983. *Journal of Shellfish Research*, 4: 81.

Anderson, W.D.; Eversole, A.G., 1988. Managing a predator: decline of an offshore *Busycon* fishery. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 147.

Anderson, W.D.; Eversole, A.G., 1993. Over exploitation and signs of recovery: Analysis of an offshore whelk fishery. *Abstracts of the technical papers presented at the 85th Annual Meeting of the National Shellfisheries Association*, Portland, Oregon, 31 May - 3 June, 1993. *Journal of Shellfish Research*, 12: 138.

Anon., 2001. *Especies de Interés Pesquero en el Litoral de Andalucía. Vol. II - Invertebrados*. Junta de Andalucía, Consejería de Agricultura y Pesca, Sevilla.

Appeldoorn, R.S., 1987. Practical considerations in the assessment of queen conch fisheries and population dynamics. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 307-324 pp.

Appeldoorn, R.S., 1988. Age determination, growth, mortality and age of first reproduction in adult queen conch, *Strombus gigas* L., off Puerto Rico. *Fisheries Research*, 6: 363-378.

Appeldoorn, R.S.; Ballantine, D.L., 1983. Field release of cultured queen conch in Puerto Rico: implications for stock restoration. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 89-98 pp.

Axiak, V.; Vella, A.J.; Micaleff, D.; Chircop, P.; Mintoff, B., 1995. Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, 121: 685-691.

Axiak, V.; Vella, A.J.; Agius, D.; Bonnici, P.; Cassar, G.; Cassone, R.; Chircop, P.; Micallef, D.; Mintoff, B.; Sammut, M., 2000. Evaluation of environmental levels and biological impact of TBT in Malta (central Mediterranean). *The Science of the Total Environment*, 258: 89-97.

Axiak, V.; Micallef, D.; Muscat, J.; Vella, A.; Mintoff, B., 2003. Imposex as a biomonitoring tool for marine pollution by tributyltin: some further observations. *Environment International*, 28: 743-749.

Ballantine, D.L.; Appeldoorn, R.S., 1983. Queen conch culture and future prospects in Puerto Rico. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 57-63 pp.

Bandel, K., 1975. Das embryonalgehäuse mariner prosobranchier der region von Banyuls-sur-Mer. *Vie et Milieu*, 25: 83-118.

Barash, A.; Zenziper, Z., 1980. Egg masses of Mollusca from Mediterranean waters of Israel and notes on reproduction of the freshwater species *Theodoxus jordani* and *Melanoides tuberculata*. *Veliger*, 22: 299-317.

Barroso, C.M.; Moreira, M.H., 1998. Reproductive cycle of *Nassarius reticulatus* in the Ria de Aveiro, Portugal: Implications for imposex studies. *Journal of the Marine Biological Association of the United Kingdom*, 78: 1233-1246.

Bartolome, C., 1985. *Contribution à l'étude du gastéropode Murex brandaris (Linnaeus, 1758) dans le Golfe du Lion*. Diplôme d'Etudes Approfondies de Sciences de l'Eau et Aménagement, Académie de Montpellier, Université des Sciences et Techniques du Languedoc.

Basedow, T., 1994. Analysis of shells on beaches and rocky coasts of Europe led to new findings on nutritional and population ecology of predatory marine gastropods (Muricidae and Naticidae). *Zoologische Beiträge*, 36: 29-48.

Basedow, T., 1996. Contributions to the nutritional and population ecology of *Murex trunculus* (L. 1767) (Gastropoda, Muricidae), derived from findings at the north coast of Crete (Eastern Mediterranean), with remarks on two species of Naticidae (Gastropoda). *Zoologische Beiträge*, 37: 157-170.

Bech, M., 1992. Sediment preference, hiding behaviour and growth rate: Paws in the game of survival of juvenile *Chicoreus ramosus* (Linnaeus, 1758). *Phuket Marine Biological Center Special Publication*, 11: 79-82.

Bell, J.D.; Rothlisberg, P.C.; Munro, J.L.; Loneragan, N.R.; Nash, W.J.; Ward, R.D.; Andrew, N.L., 2005. Restocking and stock enhancement of marine invertebrate fisheries. *Advances in Marine Biology*, 49: 392 p.

Bell, J.J., 2005. Influence of occupant microhabitat on the composition of encrusting communities on gastropod shells. *Marine Biology*, 147: 653-661.

Béné, C.; Tewfik, A., 2003. Biological evaluation of marine protected area: evidence of crowding effect on a protected population of queen conch in the Caribbean. *Marine Ecology*, 24: 45-58.

Beninger, P.G.; Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams and Reeve). *Journal of Experimental Marine Biology and Ecology*, 79: 19-37.

Benkendorff, K.; Bremner, J.B.; Davis, A.R., 2001a. Indole derivatives from the egg masses of muricid molluscs. *Molecules*, 6: 70-78.

Benkendorff, K.; Davis, A.R.; Bremner, J.B., 2001b. Chemical defense in the egg masses of benthic invertebrates: an assessment of antibacterial activity in 39 mollusks and 4 polychaetes. *Journal of Invertebrate Pathology*, 78: 109-118.

Bernard, F.R., 1968. Sexual dimorphism in *Polinices lewisi* (Naticidae). *Nautilus*, 82: 1-3.

Bers, A.V.; Wahl, M., 2004. The influence of natural surface microtopographies on fouling. *Biofouling*, 20: 43-51.

Bertrán, C.; Vargas, L.; Quijón, P., 2005. Infestation of *Polydora rickettsi* (Polychaeta: Spionidae) in shells of *Crepidula fecunda* (Mollusca: Calyptraeidae) in relation to intertidal exposure at Yaldad Bay, Chiloe, Chile. *Scientia Marina*, 69: 99-103.

Bolognari, A.; Albanese-Carmignani, M.P.; Zaccone, G.; Calabrò, C., 1979. Correlazioni citochimiche e citoenzimologiche tra il nucleolo e i globuli di vitello negli ovocito del Mollusco *Murex trunculus* L. *Acta Embryologica Experimentalis*, 3: 370-371.

Bolognari, A.; Albanese-Carmignani, M.P.; Calabrò, C.; Zaccone, G., 1981. Structural and cytochemical features of the yolk globules and of the nucleolus in the growing oocytes of the mollusc *Murex trunculus* L. *Basic and Applied Histochemistry*, 25: 159-167.

Brande, S.; Turner, M.; Heller, J.; Ben Yehuda, O., 1996. Statistical discrimination of sex in *Melanoides tuberculata* (Gastropoda: Thiaridae). *Biological Journal of the Linnean Society*, 59: 87-112.

Brown, R.A., 1982. Reproduction of *Abra nitida* (Müller) (Bivalvia) in the Southern Skagerrak. *Sarsia*, 67: 55-60.

Brownell, W.N.; Stevely, J.M., 1981. The biology, fisheries, and management of the queen conch, *Strombus gigas*. *Marine Fisheries Review*, 43: 1-12.

Bryan, G.W.; Gibbs, P.E.; Huggett, R.J.; Curtis, L.A.; Bailey, D.S.; Dauer, D.M., 1989. Effects of tributyltin pollution on the mud snail, *Ilyanassa obsoleta*, from the York River and Sarah Creek, Chesapeake Bay. *Marine Pollution Bulletin*, 20: 458-462.

Buchanan, S., 2001. Measuring reproductive condition in the Greenshell^{TM} mussel *Perna canaliculus*. *New Zealand Journal of Marine and Freshwater Research*, 35: 859-870.

Buckley, W.J.; Ebersole, J.P., 1994. Symbiotic organisms increase the vulnerability of a hermit crab to predation. *Journal of Experimental Marine Biology and Ecology*, 182: 49-64.

Buschbaum, C.; Reise, K., 1999. Effects of barnacle epibionts on the periwinkle *Littorina littorea* (L.). *Helgoland Marine Research*, 53: 56-61.

Cadée, G.C.; Boon, J.P.; Fischer, C.V.; Mensink, B.P.; ten Hallers-Tjabbes, C.C., 1995. Why the whelk (*Buccinum undatum*) has become extinct in the Dutch Wadden Sea. *Netherlands Journal of Sea Research*, 34: 337-339.

Cajaraville, M.P.; Bebianno, M.J.; Blasco, J.; Porte, C.; Sarasquete, C.; Viarengo, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *The Science of the Total Environment*, 247: 295-311.

Carneiro, M.; Martins, R.; Rebordão, F.R., 2006. Contribuição para o conhecimento das artes de pesca utilizadas no Algarve. *Publicações Avulsas do IPIMAR*, 13: 76 p.

Castagna, M.; Kraeuter, J.N., 1994. Age, growth rate, sexual dimorphism and fecundity of the knobbed whelk *Busycon carica* (Gmelin, 1791) in a western mid-Atlantic lagoon system, Virginia. *Journal of Shellfish Research*, 13: 581-585.

Champ, M.A., 2000. A review of organotin regulatory strategies, pending actions, related costs and benefits. *The Science of the Total Environment*, 258: 21-71.

Chan, D.H.L.; Chan, B.K.K., 2005. Effect of epibiosis on the fitness of the sandy shore snail *Batillaria zonalis* in Hong Kong. *Marine Biology*, 146: 695-705.

Chiavarini, S.; Massanisso, P.; Nicolai, P.; Nobili, C.; Morabito, R., 2003. Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). *Chemosphere*, 50: 311-319.

Cledón, M.; Arntz, W.; Penchaszadeh, P.E., 2005. Gonadal cycle in an *Adelomelon brasiliana* (Neogastropoda: Volutidae) population of Buenos Aires province, Argentina. *Marine Biology*, 147: 439-445.

Coelho, M.R.; Bebianno, M.J.; Gibbs, P.E., 1998. Imposex in neogastropod snails from southern Portugal. *Cuadernos de Investigación Biológica*, 20: 273.

Coelho, M.R.; Bebianno, M.J.; Langston, P.E., 2002. Organotin levels in the Ria Formosa lagoon, Portugal. *Applied Organometallic Chemistry*, 16: 384-390.

Corriero, G.; Pronzato, R., 1987. Epibiontic sponges on the bivalve *Pinna nobilis*. *Marine Ecology Progress Series*, 35: 75-82.

Coulston, M.L.; Berey, R.W.; Dempsey, A.C.; Odum, P., 1987. Assessment of the queen conch (*Strombus gigas*) population and predation studies of hatchery reared juveniles in Salt River Canyon, St. Croix, U. S. Virgin Islands. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 294-306 pp.

Creed, J.C., 2000. Epibiosis on cerith shells in a seagrass bed: correlation of shell occupant with epizoite distribution and abundance. *Marine Biology*, 137: 775-782.

Crosby, M.P., Gale, L.D., 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research*, 9: 233-237.

Crowe, T.P.; Dobson, G.; Lee, C.L., 2001. A novel method for tagging and recapturing animals in complex habitats and its use in research into stock assessment of *Trochus niloticus*. *Aquaculture*, 194: 383-391.

D.R., 1990. Regulamento da pesca na Ria Formosa. Portaria Nº 560/90 de 19 de Julho. Diário da República, Iª Série, Nº 165: 3010-3012 pp.

D'Asaro, C.N., 1970. Egg capsules of prosobranch mollusks from South Florida and the Bahamas and notes on spawning in the laboratory. *Bulletin of Marine Science*, 20: 414-440.

D'Asaro, C.N., 1986. Egg capsules of eleven marine prosobranchs from Northwest Florida. *Bulletin of Marine Science*, 39: 76-91.

D'Asaro, C.N., 1988. Micromorphology of neogastropod egg capsules. *Nautilus*, 102: 134-148.

D'Asaro, C.N., 1991. Gunnar Thorson's world-wide collection of prosobranch egg capsules: Muricidae. *Ophelia*, 35: 1-101.

Dalla Via, G.-F.; Tappeiner, U., 1981. Morphological and functional correlates with distribution of *Murex trunculus* L. and *Murex brandaris* L. (Mollusca, Gastropoda) in the northern Adriatic. *Bollettino di Zoologia*, 48: 191-195.

Davenport, J.; Chen, X., 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). *Journal of Molluscan Studies*, 53: 293-297.

Davis, J.P.; Sisson, R.T., 1988. Aspects of the biology relating to the fisheries management of New England populations of the whelks, *Busycotypus canaliculatus* and *Busycon carica*. *Journal of Shellfish Research*, 7: 453-460.

Davis, M.; Hesse, C., 1983. Third world level conch mariculture in the Turks and Caicos Islands. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 73-82 pp.

Dicosimo, J., 1988. Commercial fisheries analysis of *Busycon* whelks in Virginia. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 155.

DiSalvo, L.H.; Carriker, M.R., 1994. Planktonic, metamorphic, and early benthic behavior of the Chilean loco *Concholepas concholepas* (Muricidae, Gastropoda. Mollusca). *Journal of Shellfish Research*, 13: 57-66.

Dulzetto, F., 1946. Osservazioni sulla deposizione di *Murex trunculus* L. *Atti della Accademia Nazionale dei Lincei*, 8: 1356-1361.

Dulzetto, F., 1950. Sull'origine e la struttura delle capsule ovifere di *Murex trunculus*. *Archivio Zoologico Italiano*, 35: 83-99.

Duncan, S.; Gilling, C.; Hughes, G.; Hawkins, S.J.; Nash, R.D.M., 1989. The viability of establishing a whelk fishery in Manx waters. Mimeo. University of Liverpool.

El Hamdani, A.; Ferrer, J.M.; García Carrascosa, A.M., 1998. Imposex in prosobranch molluscs: an indicator of TBT pollution in the Valencian coast (Spain, Western Mediterranean). *Cuadernos de Investigacíon Biológica*, 20: 275-278.

Ellis, D.V.; Pattisina, L.A., 1990. Widespread neogastropod imposex: a biological indicator of global TBT contamination? *Marine Pollution Bulletin*, 21: 248-253.

Estebenet, A.L., 1998. Allometric growth and insight on sexual dimorphism in *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Malacologia*, 39: 207-213.

Eversole, A.G.; Anderson, W.D., 1988. Growth and movement of offshore populations of *Busycon carica* and *B. canaliculatum* evidenced by mark and recapture results. *Journal of Shellfish Research*, 7: 157.

Fahy, E.; Yalloway, G.; Gleeson, P., 1995. Appraisal of the whelk *Buccinun undatum* fishery of the Southern Irish Sea with proposals for management strategy. *Irish Fisheries Investigations. Series B (Marine)*, 42: 26 p.

Fahy, E.; Masterson, E.; Swords, D.; Forrest, N., 2000. A second assessment of the whelk fishery *Buccinum undatum* in the southwest Irish Sea with particular reference to its history of management by size limit. *Irish Fisheries Investigations*, 6: 67 p.

Fainzilber, M., 1978. An observation on *Trunculariopsis trunculus* feeding on *Conus mediterraneus*. *Levantina*, 16: 182.

Fauchald, K.; Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanography and Marine Biology. An Annual Review*, 17: 193-284.

Fioroni, P.; Oehlmann, J.; Stroben, J., 1991. The pseudohermaphroditism of Prosobranchs: morphological aspects. *Zoologischer Anzeiger*, 226: 1-26.

Fischer, P.H.; Raffy, A., 1933. La ponte du *Murex trunculus* L. *Bulletin de l'Institut Océanographique (Monaco)*, 618: 1-4.

Foster, G.G.; Hodgson, A.N.; Balarin, M., 1999. Effect of diet on growth rate and reproductive fitness of *Turbo sarmaticus* (Mollusca: Vetigastropoda: Turbinidae). *Marine Biology*, 134: 307-315.

Fotheringham, N., 1971. Life history patterns of the littoral gastropods *Shaskyus festivus* (Hinds) and *Ocenebra poulsoni* Carpenter (Prosobranchia: Muricidae). *Ecology*, 52: 742-757.

Franz, D.R., 1971. Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. *Biological Bulletin*, 140: 63-72.

Fretter, V.; Graham, A., 1964. Reproduction. In: *Physiology of Mollusca. Vol. 1.* (Wilbur, K.M., Ed.), Academic Press, New York, 127-164 pp.

Fretter, V.; Graham, A., 1994. *British Prosobranch Molluscs: Their Functional Anatomy and Ecology*. The Ray Society, London.

Gabbott, P.A.; Walker, A.J.M., 1971. Changes in the condition index and biochemical content of adult oysters (*Ostrea edulis* L.) maintained under hatchery conditions. *Journal du Conseil International pour l'Exploitation de la Mer*, 34: 99-106.

Gaillard, J.M., 1987. Gastéropodes. In: *Fiches FAO d'Identification des Espèces pour les Besoins de la Pêche. Vol. I. - Méditerranée et Mer Noire* (Fischer, W.; Bauchot, M.L.; Schneider, M., Eds.), FAO, Rome.

Gallardo, C.S., 1989. Patrones de reproducción y ciclo vital en moluscos marinos bénticos; una aproximación ecológico evolutiva. *Medio Ambiente*, 10: 25-35.

Garaventa, F.; Pellizzato, F.; Faimali, M.; Terlizzi, A.; Medakovic, D.; Geraci, S.; Pavoni, B., 2006. Imposex in *Hexaplex trunculus* at some sites on the North Mediterranean Coast as a base-line for future evaluation of the effectiveness of the total ban on organotin based antifouling paints. *Hydrobiologia*, 555: 281-287.

Gendron, L., 1989. Reproductive biology of the whelk *Buccinum undatum* in the northwest Gulf of St. Lawrence. *Abstracts of Technical Papers presented at the 1990 Annual Meeting of the National Shellfisheries Association*, Williamsburg, Virginia, 1-5 April 1990. *Journal of Shellfish Research*, 8: 483-484.

Gendron, L., 1991. Gestion de l'exploitation du buccin *Buccinum undatum* au Québec: détermination d'une taille minimale de capture. *Rapport Technique Canadien des Sciences Halieutiques et Aquatiques*, 1833: 40 p.

Gendron, L., 1992. Determination of the size at sexual maturity of the waved whelk, *Buccinum undatum* Linnaeus, 1758, in the Gulf of St. Lawrence, as the basis for the establishment of a catchable size. *Journal of Shellfish Research*, 11: 1-7.

Gharsallah, I.H.; Zamouri-Langar, N.; Missaoui, H.; El Abed, A., 2004. Étude de la croissance relative et de la biomasse d'*Hexaplex trunculus* dans la lagune de Bizerte. *Bulletin de la Société Zoologique de France*, 129: 427-436.

Gibbs, P.E., 1993. A male genital defect in the dog-whelk, *Nucella lapillus* (Neogastropoda), favouring survival in a TBT-polluted area. *Journal of the Marine Biological Association of the United Kingdom*, 73: 667-678.

Gibbs, P.E., 1999. Biological effects of contaminants: Use of imposex in the dogwhelk (*Nucella lapillus*) as a bioindicator of tributyltin pollution. *ICES Techniques in Marine Environmental Sciences*, 24: 1-29.

Gibbs, P.E., 2005. Male genital defect (Dumpton Syndrome) in the dog-whelk *Nucella lapillus* (Neogastropoda): Mendelian inheritance inferred, based on laboratory breeding experiments. *Journal of the Marine Biological Association of the United Kingdom*, 85: 143-150.

Gibbs, P.E.; Bryan, G.W., 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 66: 767-777.

Gibbs, P.E.; Bryan, G.W.; Pascoe, P.L.; Burt, G.R., 1987. The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *Journal of the Marine Biological Association of the United Kingdom*, 67: 507-523.

Gibbs, P.E.; Pascoe, P.L.; Burt, G.R., 1988. Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 68: 715-731.

Gibbs, P.E.; Bryan, G.W.; Pascoe, P.L.; Burt, G.R., 1990. Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributyltin-induced imposex. *Journal of the Marine Biological Association of the United Kingdom*, 70: 639-656.

Gibbs, P.E.; Pascoe, P.L.; Bryan, G.W., 1991. Tributyltin-induced imposex in Stenoglossan gastropods: pathological effects on the female reproductive system. *Comparative Biochemistry and Physiology*, 100C: 231-235.

Gibbs, P.E.; Bebianno, M.J.; Coelho, M.R., 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution, with notes on a species (*Columbella rustica*) lacking the imposex response. *Environmental Technology*, 18: 1219-1224.

Gibson, J.; Strasdine, S.; Gonzales, K., 1983. The status of the conch industry in Belize. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 99-107 pp.

Giménez, J.; Penchaszadeh, P.E., 2003. Size at first sexual maturity in *Zidona dufresnei* (Caenogastropoda: Volutidae) of the south-western Atlantic Ocean (Mar del Plata, Argentina). *Journal of the Marine Biological Association of the United Kingdom*, 83: 293-296.

Gonzales, B.J.; Galon, W.M.; Becira, J.G., 2006. Community-based stock enhancement of topshell in Honda Bay, Palawan, Philippines. *Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern* (Primavera, J.H.; Quinitio, E.T.; Eguia, M.R.R., Eds.), Iloilo City, Philippines, 13-15 July 2005, 49-59 pp.

Gosselin, L.A., 1993. A method for marking small juvenile gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 73: 963-966.

Gros, P.; Santarelli, L., 1986. Méthode d'estimation de la surface de pêche d'un casier à l'aide d'une filière expérimentale. *Oceanologica Acta*, 9: 81-87.

Gulland, J.A.; Holt, S.J., 1959. Estimation of growth parameters for data at unequal time intervals. *Journal du Conseil International pour l'Exploration de la Mer*, 25: 47-49.

Gunnarsson, K.; Einarsson, S., 1995. Observations on the whelk populations (*Buccinum undatum* L., Mollusca: Gastropoda) in Breidifjördur, western Iceland. *ICES Council Meeting Papers*, ICES-CM-1995/K-20: 13 p.

Hancock, D.A., 1959. The biology and control of the American whelk tingle *Urosalpinx cinerea* (Say) on English oyster beds. *Fisheries Investigation (London)*, 22: 1-66.

Hancock, D.A., 1963. Marking experiments with the commercial whelk (*Buccinum undatum*). *Special Publication - International Commission for the Northwest Atlantic Fisheries (ICNAF)*, 4: 176-193.

Hancock, D.A.; Urquhart, A.E., 1959. Methods for marking whelks (*Buccinum undatum* L.). *Journal du Conseil International pour l'Exploration de la Mer*, 24: 494-496.

Hensen, R.R., 1983. Queen conch management and culture in the Netherlands Antilles. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 53-56 pp.

Higgs, C., 1987. Current status of queen conch (*Strombus gigas*) stocks and fisheries regulations in the Bahamas. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 377 pp.

Himmelman, J.H., 1988. Movement of whelks (*Buccinum undatum*) towards a baited trap. *Marine Biology*, 97: 521-531.

Houart, R., 2001. *A review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Ed. Evolver, Rome.

Hunt, J.H., 1987. Status of queen conch (*Strombus gigas*) management in the Florida Keys, U.S.A. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 376 pp.

I.H., 2005-2006. Instituto Hidrográfico (Portuguese Hydrographical Institute - WWW Data base). URL: http://www.hidrografico.pt.

Ito, H.; Wakui, T.; Dimon, Y., 1980. Methods for marking sea snails, Genus *Buccinum*. *Bulletin of the Hokkaido Regional Fisheries Research Laboratory*, 45: 65-74.

Iversen, E.S., 1983. Feasibility of increasing Bahamian conch production by mariculture. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 83-88 pp.

Jenner, M.G., 1979. Pseudohermaphroditism in *Ilyanassa obsoleta* (Mollusca: Neogastropoda). *Science*, 205: 1407-1409.

Jones, R., 1979. Materials and methods used in marking experiments in fishery research. *FAO Fisheries Technical Paper*, 190: 134 p.

Jory, D.E.; Iversen, E.S., 1983. Queen conch predators: not a roadblock to mariculture. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 108-111 pp.

Kantor, Y.I.; Sysoev, A.V., 1991. Sexual dimorphism in the apertural notch of a new species of *Gemmula* (Gastropoda: Turridae). *Journal of Molluscan Studies*, 57: 205-209.

Kaplan, I.M.; Boyer, B.C., 1992. Monitoring marine resources: Ecological and policy implications affecting the scientific collecting and commercial value of New England conch (*Busycon*). *Biological Bulletin*, 183: 379-380.

Kaplan, I.M.; Boyer, B.C.; Santos, K.A., 1988. Marine policy implications related to the commercial value and scientific collecting of the whelk *Busycon*. *Biological Bulletin*, 175: 312.

Kenchington, E.; Glass, A., 1998. Local adaptation and sexual dimorphism in the waved whelk (*Buccinum undatum*) in the Atlantic Nova Scotia with applications to fisheries management. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 2237: 43 p.

Kideys, A.E., 1994. Effect of tagging on the growth of the neogastropod, *Buccinum undatum* in the laboratory. *Fisheries Research*, 20: 283-289.

Kideys, A.E.; Nash, R.D.M., 1993. A note on the mortality associated with fluorescent marking in a gastropod. *DOGA - Turkish Journal of Zoology*, 17: 175-178.

Kraeuter, J.N.; Castagna, M.; Bisker, R., 1989. Growth rate estimates for *Busycon carica* (Gmelin, 1971) in Virginia. *Journal of Shellfish Research*, 8: 219-225.

Kurata, K.; Kikuchi, E., 2000. Comparisons of life-history traits and sexual dimorphism between *Assiminea japonica* and *Angustassiminea castanea* (Gastropoda: Assimineidae). *Journal of Molluscan Studies*, 66: 177-196.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2004. Données sur le cycle reproducteur du mollusque gastéropode *Hexaplex trunculus* (Linné, 1758) de la Lagune de Bizerte (Tunisie). *Bulletin de la Société Zoologique de France*, 129, 407-418.

Lahbib, Y.; Trigui El-Menif, N.; Le Pennec, M.; Boumaiza, M., 2006. La ponte et la croissance absolue d'*Hexaplex trunculus* réalisée au laboratoire. *Résumés des Communications du Troisième Congrès Franco-Tunisien de Zoologie*, Tabarka, Tunisie, 3-7 Novembre 2006, 64 pp.

Langston, W.J.; Gibbs, P.E.; Livingstone, D.R.; Burt, G.R.; O'Hara, S.; Bebianno, M.J.; Coelho, M.R.; Porte, C.; Bayona, J.; McNulty, M.; Lynch, G.; Keegan, B., 1997. Risk assessment of organotin antifoulings on key benthic organisms of European coastal habitats (BOATS). MAS2-CT-94-0099, Final Report.

Lanteigne, M.; Davidson, L.A., 1992. Aperçu des rendements par casier et de la taille à maturité sexuelle de buccins communs du Nord, *Buccinum undatum*, capturés le long de la côte est du Nouveau-Brunswick - 1992. *Rapport Technique Canadien des Sciences Halieutiques et Aquatiques*, 1896: 27 p.

Lapointe, V.; Sainte-Marie, B., 1992. Currents, predators, and the aggregation of the gastropod *Buccinum undatum* around bait. *Marine Ecology Progress Series*, 85: 245-257.

Lara, E.; Montes, E., 1988. Estado del conocimiento en el cultivo del loco *Concholepas concholepas*. *Investigaciones Pesqueras (Chile)*, 35: 103-107.

Laudien, J.; Wahl, M., 1999. Indirect effects of epibiosis on host mortality: seastar predation on differently fouled mussels. *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 20: 35-47.

Laughlin, R.A.; Weil, M.E., 1983. Queen conch mariculture and restoration in the Archipiélago de los Roques: preliminary results. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 64-72 pp.

Laxton, J.H., 1970. Shell growth in some New Zealand Cymatiidae (Gastropoda: Prosobranchia). *Journal of Experimental Marine Biology and Ecology*, 4: 250-260.

Leiva, G.E.; Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of the catches, management and the Chilean experience. *Reviews in Fish Biology and Fisheries*, 11: 283-300.

Lima, G.M.; Lutz, R.A., 1990. The relationship of larval shell morphology to mode of development in marine prosobranch gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 70: 611-637.

Lleonart, M.; Handlinger, J.; Powell, M., 2003. Spionid mudworm infestation of farmed abalone (*Haliotis* spp.). *Aquaculture*, 221: 85-96.

Lorenzini, G.; Orlando, E., 1997. Osservazioni sul comportamento alimentare di *Hexaplex trunculus* (Mollusca, Gastropoda, Muricidae). *Biologia Marina Mediterranea*, 4: 405-406.

Lucas, A.; Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture*, 44: 187-200.

Luckens, P.A., 1970. Predation and intertidal zonation at Asamushi. *Bulletin of the Marine Biological Station of Asamushi*, 14: 33-52.

Macedo, M.C.C.; Macedo, M.I.C.; Borges, J.P., 1999. *Conchas Marinhas de Portugal (Seashells of Portugal)*. Editorial Verbo, Lisboa.

MacKenzie, C.L., 1961. Growth and reproduction of the oyster drill *Eupleura caudata* in the York River, Virginia. *Ecology*, 42: 317-338.

Magalhães, H., 1948. An ecological study of snails of the Genus *Busycon* at Beaufort, North Carolina. *Ecological Monographs*, 18: 379-409.

Mann, R., 1978. A comparison of morphometric, biochemical and physiological indexes of condition in marine bivalve molluscs. In: *Energy and Environmental Stress in Aquatic Systems* (Thorp, J.H.; Gibbons, I.W., Eds.), *DOE Symposium Series,* 48: 484-497.

Marin, A.; López Belluga, M.D., 2005. Sponge coating decreases predation on the bivalve *Arca noae*. *Journal of Molluscan Studies*, 71: 1-6.

Marques, M.R.; Oliveira, J.S., 1995. A pesca das "carteiras" na Ria Formosa. *Actas das Comunicações do 8º Congresso do Algarve,* Vilamoura, Portugal, 7-9 Abril 1995, 905-909 pp.

Martin, D.; Britayev, T.A., 1998. Symbiotic polychaetes: review of known species. *Oceanography and Marine Biology. An Annual Review*, 36: 217-340.

Martín, P.; Sánchez, P.; Ramón, M., 1995. Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research*, 23: 319-331.

Martoja, M. ; Bouquegneau, J.M., 1988. *Murex trunculus*: un nouveau cas de pseudo-hermaphrodisme chez un gastéropode prosobranche. *Bulletin de la Société Royale de Liège*, 57: 45-58.

McQuinn, I.H.; Gendron, L.; Himmelman, J.H., 1988. Area of attraction and effective area fished by a whelk (*Buccinum undatum*) trap under variable conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, 45: 2054-2060.

Mejri, H.; Trigui El Menif, N.; Ben Souissi, J.; Ben Salem, M.; Zaouali, J., 2005. Ethologie alimentaire des Muricidae *Hexaplex trunculus* (Linnaeus, 1758) et *Bolinus brandaris* (Linnaeus, 1758). In: *Résumés de Communications du Sixième Congrès Maghrébin des Sciences de la Mer*, Monastir, Tunisie, 17-21 Décembre 2005, 16 pp.

Moore, E.A.; Sander, F., 1978. Spawning and early life history of *Murex pomum* Gmelin, 1971. *Veliger*, 20: 251-259.

Muñoz, L.; Alcolado, P.; Fraga, I.; Llorente, P., 1987. Status of populations and fisheries of *Strombus gigas* in Cuba, with some results of juvenile rearing in pens. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 353-361 pp.

Muzavor, S.; Morenito, P.M., 1999. *Roteiro Ecológico da Ria Formosa, Vol. IV - Moluscos Gastrópodos*. Universidade do Algarve, Faro.

Nasution, S.; Roberts, D., 2005. Laboratory trials on the effects of different diets on growth and survival of the common whelk, *Buccinum undatum* L. 1758, as a candidate species for aquaculture. *Aquaculture International*, 12: 509-521.

Naylor, E., 2005. Chronobiology: implications for marine resource exploitation and management. *Scientia Marina*, 69: 157-167.

Nichols, K.E., 1987. Conch management program - St. Lucia. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 379 pp.

Nicholson, G.J.; Evans, S.M., 1997. Anthropogenic impacts on the stocks of the common whelk *Buccinum undatum* (L.). *Marine Environmental Research*, 44: 305-314.

Oehlmann, J.; Stroben, E.; Fioroni, P., 1996. New facts about TBT-induced imposex in prosobranchs: general aspects. *Malacological Review*, Suppl. 6: 149-156.

Olabarria, C., 2000. Epibiont molluscs on neogastropod shells from sandy bottoms, Pacific coast of Mexico. *Journal of the Marine Biological Association of the United Kingdom*, 80: 291-298.

Peharda, M.; Morton, B., 2006. Experimental prey species preferences of *Hexaplex trunculus* (Gastropoda: Muricidae) and predator-prey interactions with the Black mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). *Marine Biology*, 148: 1011-1019.

Pellizzato, F.; Centanni, E.; Marin, M.G.; Moschino, V.; Pavoni, B., 2004. Concentrations of organotin compounds and imposex in the gastropod *Hexaplex trunculus* from the lagoon of Venice. *Science of the Total Environment*, 332: 89-100.

Poore, G.C.B., 1973. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda): 4. Reproduction. *New Zealand Journal of Marine and Freshwater Research*, 7: 67-84.

Poppe, G.T.; Goto, Y., 1991. *European Seashells, Vol. 1 (Polyplacophora, Claudofoveata, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, Wiesbaden.

Ramesh, M.X.; Raghunuthan, C.; Ayyakkannu, K., 1992. Laboratory observations on food consumption and growth of *Chicoreus virgineus* - a pilot study. *Phuket Marine Biological Center Special Publication*, 11: 94-95.

Ramón, M.; Amor, M.J., 2001. Increasing imposex in populations of *Bolinus brandaris* (Gastropoda: Muricidae) in the north-western Mediterranean. *Marine Environmental Research*, 52: 463-475.

Ramón, M.; Flos, R., 2001. First trials to cultivate the muricid gastropod *Bolinus brandaris* (Linnaeus). *European Aquaculture Society Special Publication*, 29: 219-220.

Ramón, M.; Amor, M.J., 2002. Reproductive cycle of *Bolinus brandaris* and penis and genital duct size variations in a population affected by imposex. *Journal of the Marine Biological Association of the United Kingdom*, 82: 435-442.

Ramorino, L.M., 1975. Ciclo reproductivo de *Concholepas concholepas* en la zona de Valparaíso. *Revista de Biología Marina (Valparaíso)*, 15: 149-177.

Rawlings, T.A., 1995. Direct observation of encapsulated development in muricid gastropods. *Veliger*, 38: 54-60.

Rilov, G.; Gasith, A.; Evans, S.M.; Benayahu, Y., 2000. Unregulated use of TBT-based antifouling paints in Israel (Eastern Mediterranean): high contamination and imposex levels in two species of marine gastropods. *Marine Ecology Progress Series*, 192: 229-238.

Rilov, G.; Benayahu, Y.; Gasith, A., 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? *Marine Ecology Progress Series*, 282: 193-204.

Romero, M.S.; Gallardo, C.S.; Bellolio, G., 2004. Egg laying and embryonic-larval development in the snail *Thais (Stramonita) chocolata* (Duclos, 1832) with observations on its evolutionary relationships within Muricidae. *Marine Biology*, 145: 681-692.

Rowley, R.J., 1994. Marine reserves in fisheries management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 4: 233-254.

Sabelli, B.; Tommasini, S., 1982. Osservazioni sulla radula e sulla protoconca di *Bolinus brandaris* (L., 1758) e *Phyllonotus trunculus* (L., 1758). *Bollettino Malacologico*, 18: 291-300.

Sainte-Marie, B., 1991. Whelk (*Buccinum undatum*) movement and its implications for the use of tag-recapture methods for the determination of baited trap fishing parameters. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 751-756.

Sainte-Marie, B.; Hargrave, B.T., 1987. Estimation of scavenger abundance and distance of attraction to bait. *Marine Biology*, 94: 431-443.

Santarelli, L., 1988. Estimación de la densidad poblacional de *Buccinum undatum* L. (Gasteropoda: Prosobranchia) por medio del modelo de extracción sucesiva de DeLury. *Anales del Instituto de Ciencias del Mar y Limnología*, 15: 65-72.

Santarelli, L.; Gros, P., 1984. Modélisation bioéconomique de la pêcherie de buccin (*Buccinum undatum* L.: Gastropoda) du port de Granville (Manche Ouest). Eléments de gestion de la ressource. *Revue des Travaux de l'Institut des Pêches Maritimes*, 48: 23-32.

Santarelli, L.; Gros, P., 1985. Détermination de l'âge et de la croissance de *Buccinum undatum* L. (Gasteropoda: Prosobranchia) à l'aide des isotopes stables de la coquille et de l'ornamentation operculaire. *Oceanologica Acta*, 8: 221-229.

Santarelli, L.; Véron, G.; Huet, J., 1986. Exploitation du buccin dans le golfe Normano-Breton. *La Pêche Maritime*, 1294: 48-52.

Schitz, V., 1920. Sur la espermatogenèse chez *Murex trunculus* (L), *Aporrhais pespelicani* (L), *Fusus* sp. et *Nassa reticulata*. *Archives de Zoologie Expérimentale et Générale*, 59: 477-508.

Seed, R.; Suchanek, T.H., 1992. Population and community ecology of *Mytilus*. In: *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture* (Gosling, E., Ed.), Elsevier, Amsterdam, 87-169 pp.

Siddall, S.E., 1983. Biological and economic outlook for hatchery production of juvenile queen conch. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 46-52 pp.

Šimunović, A., 1995. Ecological study of Prosobranchiata (Gastropoda) in the eastern part of the Adriatic Sea and their relationship to benthic biocoenoses. *Acta Adriatica*, 36: 3-162.

Sisson, R.T.; Wood, R.S., 1988. Observation on some life history aspects of a commercially exploited population of *Busycon canaliculatum* (Linne) in Narragansett Bay, Rhode Island. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 176.

Smith, B.D., 1987. Growth rate, distribution and abundance of the introduced topshell *Trochus niloticus* Linnaeus on Guam, Mariana Islands. *Bulletin of Marine Science*, 41: 466-474.

Smith, B.S., 1971. Sexuality in the American mud snail *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London*, 39: 377-378.

Sokal, R.R.; Rohlf, F.J., 1987. *Introduction to Biostatistics.* 2nd Edition. W. H. Freeman and Company, New York.

Spanier, E., 1981. Behavioral ecology of the marine snail *Truncculariopsis (Murex) trunculus*. In: *Developments in Arid Zone Ecology and Environmental Quality* (Shuval, H., Ed.), Balaban ISS, Philadelphia, 65-70 pp.

Spanier, E., 1986. Cannibalism in muricid snails as a possible explanation for archaeological findings. *Journal of Archaeological Science*, 13: 463-468.

Spanier, E.; Karmon, N., 1987. Muricid snails and the ancient dye industries. In: *The Royal Purple and the Biblical Blue: Argaman and Tekhelet* (Spanier, E., Ed.), Keter Publishing House Jerusalem Ltd., Jerusalem, 179-192 pp.

Spight, T.M., 1975. Factors extending gastropod embryonic development and their selective cost. *Oecologia*, 21: 1-16.

Spight, T.M., 1977. Latitude, habitat, and hatching type for muricacean gastropods. *Nautilus*, 91: 67-71.

Spight, T.M.; Birkeland, C.; Lyons, A., 1974. Life histories of large and small murexes (Prosobranchia: Muricidae). *Marine Biology*, 24: 229-242.

Stachowitsch, M., 1980. The epibiotic and endolithic species associated with the gastropod shells inhabited by the hermit crabs *Paguristes oculatus* and *Pagurus cuanensis*. *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 1: 73-101.

Stroben, E.; Oehlmann, U.; Schulte-Oehlmann, U.; Fioroni, P., 1996. Seasonal variations in the genital ducts of normal and imposex-affected Prosobranchs and its influence on biomonitoring indexes. *Malacological Review*, Suppl. 6: 173-184.

Tallmark, B., 1980. Population dynamics of *Nassarius reticulatus* (Gastropoda, Prosobranchia) in Gullmar Fjord, Sweden. *Marine Ecology Progress Series*, 3: 51-62.

ten Hallers-Tjabbes, C.C., 1979. Sexual dimorphism in *Buccinum undatum* L., *Malacologia*, 18: 13-17.

ten Hallers-Tjabbes, C.C.; Everaarts, J.M.; Mensink, B.P.; Boon, J.P., 1996. The decline of the North Sea whelk (*Buccinum undatum* L.) between 1970 and 1990: A natural or a human-induced event? *Marine Ecology - Pubblicazioni della Stazione Zoologica di Napoli I*, 17: 333-343.

Terlizzi, A., 2000. Imposex (pseudoermafroditismo) in molluschi gasteropodi mediterranei: aspetti morfologici e considerazioni ecologiche. *Bollettino Malacologico*, 36: 155-158.

Terlizzi, A.; Scuderi, D.; Faimali, M.; Minganti, V.; Geraci, S., 1997. Imposex in *Hexaplex trunculus* e *Stramonita haemastoma* (Gastropoda: Muricidae): prime segnalazioni per le acque costiere italiane. *Biologia Marina Mediterranea*, 4: 496-499.

Terlizzi, A.; Geraci, S.; Minganti, V., 1998. Tributyltin (TBT) pollution in the coastal waters of Italy as indicated by imposex in *Hexaplex trunculus* (Gastropoda: Muricidae). *Marine Pollution Bulletin*, 36: 749-752.

Terlizzi, A.; Geraci, S.; Gibbs, P.E., 1999. Tributyltin (TBT)-induced imposex in the Neogastropod *Hexaplex trunculus* in Italian coastal waters: morphological aspects and ecological implications. *Italian Journal of Zoology*, 66: 141-146.

Terlizzi, A.; Fraschetti, S.; Gianguzza, P.; Faimali, M.; Boero, F., 2001. Environmental impact of antifouling technologies: state of the art and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 11: 311-317.

Terlizzi, A.; Delos, A.L.; Garaventa, F.; Faimali, M.; Geraci, S., 2004. Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin*, 48: 188-192.

Tirado, C.; Rodríguez de la Rúa, A.; Bruzón, M.A.; López, J.I.; Salas, C.; Márquez, I., 2002. *La Reproducción de Bivalvos y Gasterópodos de Interés Pesquero en Andalucía*. Junta de Andalucía, Consejería de Agricultura y Pesca, Huelva.

Trigui El-Menif, N.; Lahbib, Y.; Le Pennec, M.; Flower, R.; Boumaiza, M., 2006. Intensity of the imposex phenomenon - impact on growth and fecundity in *Hexaplex trunculus* (Mollusca: Gastropoda) collected in Bizerta lagoon and channel (Tunisia). *Cahiers de Biologie Marine*, 47: 165-175.

Valentinsson, D.; Sjödin, F.; Jonsson, P.E.; Nilsson, P.; Wheatley, C., 1999. Appraisal of the potential for a future fishery on whelks (*Buccinum undatum*) in Swedish waters: CPUE and biological aspects. *Fisheries Research*, 42: 215-227.

Vasconcelos, P.; Gaspar, M.B.; Castro, M.; Monteiro, C.C., 2002. The artisanal fishery of *Hexaplex trunculus* L. (Mollusca: Gastropoda) in the Ria Formosa lagoon (Algarve - southern Portugal): Development of an integrated study for resource management. In: *Resúmenes del II Congreso Internacional de las Sociedades Malacológicas Europeas* (Rolán, E.; Troncoso, J.S., Eds.), Vigo, España, 9-13 Septiembre 2002, 210-211 pp.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2004a. Morphometric relationships of the shell of *Hexaplex trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve - southern Portugal). In: *Resúmenes del XIII Simposio Ibérico de Estudios del Bentos Marino* (Lorenzo, J.M.; Pajuelo, J.G.; Ramos, A., Eds.), Las Palmas de Gran Canaria, España, 21-24 Septiembre 2004, 97-98 pp.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2004b. Meat yield of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae), an important fishing resource from the Ria Formosa lagoon (Algarve - southern Portugal). In: *Resúmenes del XIII Simposio Ibérico de Estudios del Bentos Marino* (Lorenzo, J.M.; Pajuelo, J.G.; Ramos, A., Eds.), Las Palmas de Gran Canaria, España, 21-24 Septiembre 2004, 143 pp.

Vasconcelos, P.; Gaspar, M.B.; Joaquim, S.; Matias D.; Castro, M., 2004c. Spawning of *Hexaplex (TruncularIopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction and Development*, 46: 125-138.

Vasconcelos, P.; Gaspar, M.B.; Castro, M., 2006. Imposex in *Hexaplex (Truncularlopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin*, 52: 337-341.

Vasconcelos, P.; Gaspar, M.B.; Pereira, A.M.; Castro, M., 2006. Growth rate estimation of *Hexaplex (Truncularlopsis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast, southern Portugal). *Journal of Shellfish Research*, 25: 249-256.

Vasconcelos, P.; Cúrdia, J.; Castro, M.; Gaspar, M.B., 2007. The shell of *Hexaplex (Truncülariopsis) trunculus* (Gastropoda: Muricidae) as a mobile hard substratum for epibiotic polychaetes (Annelida: Polychaeta) in the Ria Formosa (Algarve coast - southern Portugal). *Hydrobiologia*, 575: 161-172.

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar M.B. Gametogenic cycle of *Hexaplex (Truncülariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Journal of the Marine Biological Association of the United Kingdom* (submitted).

Vasconcelos, P.; Lopes, B.; Castro, M.; Gaspar, M.B. Comparison of indices for assessing the reproductive condition of *Hexaplex (Truncülariopsis) trunculus* (Gastropoda: Muricidae) in the Ria Formosa lagoon (Algarve coast - southern Portugal). *Aquatic Living Resources* (submitted).

Vela, J.M.; Moreno, Ó., 2004. Posibilidades del cultivo de gasterópodos murícidos en la región suratlántica ibérica. *Resumos do Seminário Moluscicultura no Litoral Sul-Atlântico - Diversificação*. 19-20 Fevereiro 2004, Tavira, Portugal.

Villiers, L., 1981. Croissance, dynamique du "stock" et de la biomasse de *Turbo setosus* (Gastropode - Turbinidae). Atoll de Hao (Tuamotu, Polynésie Française). *Vie et Milieu*, 31: 3-13.

Vokes, E.H., 1996. One last look at the Muricidae. *American Conchologist*, 24: 4-6.

Von Bertalanffy, L., 1938. A quantitative theory of organic growth. *Human Biology*, 10: 181-213.

Wahl, M., 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series*, 58: 175-189.

Wahl, M., 1996. Fouled snails in flow: potential of epibionts on *Littorina littorea* to increase drag and reduce snail growth rates. *Marine Ecology Progress Series*, 138: 157-168.

Wahl, M.; Mark, O., 1999. The predominantly facultative nature of epibiosis: experimental and observational evidence. *Marine Ecology Progress Series*, 187: 59-66.

Wahl, M.; Sönnichsen, H., 1992. Marine epibiosis. IV. The periwinkle *Littorina littorea* lacks typical antifouling defences - why are some populations so little fouled? *Marine Ecology Progress Series*, 88: 225-235.

Wahl, M., Hay, M.E.; Enderlein, P., 1997. Effects of epibiosis on consumer-prey interactions. *Hydrobiologia*, 355: 49-59.

Walker, R.L., 1988. Intertidal populations of four species of whelks (*Busycon*) in Wassaw Sound, Georgia. *Abstracts of the technical papers presented at 1987 Annual Meeting of the National Shellfisheries Association*, Halifax, Nova Scotia, 9-13 August 1987. *Journal of Shellfish Research*, 7: 180.

Walne, P.; Mann, R., 1975. Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: *Proceedings of the 9th European Marine Biology Symposium* (Barnes, H., Ed.), Oban, Argyll, 2-8 October 1974. Aberdeen University Press, Aberdeen, 587-607 pp.

Ward, D.W.; Davis, A.R., 2002. Reproduction of the turban shell *Turbo torquatus* Gmelin 1791 (Mollusca: Gastropoda), in New South Wales, Australia. *Marine and Freshwater Research*, 53: 85-91.

Warner, G.F., 1997. Occurrence of epifauna on the periwinkle, *Littorina littorea* (L.), and interactions with the polychaete *Polydora ciliata* (Johnston). *Hydrobiologia*, 355: 41-47.

Weil, E.; Laughlin, R., 1984. Biology, population dynamics, and reproduction of the queen conch *Strombus gigas* Linné in the Archipiélago de los Roques National Park. *Journal of Shellfish Research*, 4: 45-62.

Wicklund, R.I.; Hepp, L., 1987. Ongoing population studies of juvenile queen conch, *Strombus gigas*, in the Exuma Cays, Bahamas. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 378 pp.

Wilkins, R.M.; Goodwin, M.H.; Reid, D.M., 1987. Research applied to conch resource management in St. Kitts/Nevis. *Proceedings of the Thirty-Eight Annual Gulf and Caribbean Fisheries Institute*, Trois-Islets, Martinique, November 1985, 370-375 pp.

Wondrak, G., 1974. Laichverhalten und gelegebildung bei *Murex trunculus* L. (Gastropoda: Prosobranchia). *Archiv fuer Molluskenkunde*, 104: 115-119.

Wood, R.S.; Olsen, D.A., 1983. Application of biological knowledge to the management of the Virgin Islands conch fishery. *Proceedings of the Thirty-Fifth Annual Gulf and Caribbean Fisheries Institute*, Nassau, Bahamas, November 1982, 112-121 pp.

Yamaguchi, M., 1977. Shell growth and mortality rates in the coral reef gastropod *Cerithium nodulosum* in Pago Bay, Guam, Mariana Islands. *Marine Biology*, 44: 249-263.

Zar, J.H., 1996. *Biostatistical Analysis*. 3rd Edition. Prentice-Hall International Inc., New Jersey.