Journal of Experimental Marine Biology and Ecology 407 (2011) 337–344

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Study of *Pinna nobilis* growth from inner record: How biased are posterior adductor muscle scars estimates?

Jose R. Garcia-March [a,*], Ana Marquez-Aliaga [b], You-Gan Wang [c], Donna Surge [a], Diego Kurt Kersting [d]

[a] Department of Geological Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA
[b] Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Departamento de Geología, Universitat de Valencia, Valencia, Spain
[c] Centre for Applications in Natural Resource Mathematics, School of Mathematics and Physics, The University of Queensland, Queensland 4072, Australia
[d] Columbretes Islands Marine Reserve, Castellón, Spain

ARTICLE INFO

Article history:
Received 14 June 2011
Received in revised form 19 July 2011
Accepted 20 July 2011
Available online 17 August 2011

Keywords:
Bivalvia
Endangered species
Mediterranean
Sclerochronology
Spain

ABSTRACT

Previous studies have shown that the external growth records of the posterior adductor muscle scar (PAMS) of the bivalve *Pinna nobilis* are incomplete and do not produce accurate age estimations. We have developed a new methodology to study age and growth using the inner record of the PAMS, which avoids the necessity of costly *in situ* shell measurements or isotopic studies. Using the inner record we identified the positions of PAMS previously obscured by nacre and estimated the number of missing records in adult specimens with strong abrasion of the calcite layer in the anterior portion of the shell. The study of the PAMS and inner record of two shells that were 6 years old when collected showed that only 2 and 3 PAMS were observed, while 6 inner records could be counted, thus confirming our working methodology. Growth parameters of a *P. nobilis* population located in Moraira, Spain (western Mediterranean) were estimated with the new methodology and compared to those obtained using PAMS data and *in situ* measurements. For the comparisons, we applied different models considering the data alternatively as length-at-age (LA) and tag-recapture (TR). Among every method we tested to fit the Von Bertalanffy growth model, we observed that LA data from inner record fitted to the model using non-linear mixed effects and the estimation of missing records using the calcite width was the most appropriate. The equation obtained with this method, $L = 57.3^{*}(1-e^{-0.16(t-0.02)})$, is very similar to that calculated previously from *in situ* measurements for the same population.

1. Introduction

The bivalve mollusc *Pinna nobilis* is an endangered Mediterranean endemic included in the European lists of species for which specific conservation strategies should be implemented (ANNEX II of Barcelona Convention) and surveillance of the conservation status undertaken (ANNEX IV of Habitats Directive). This large bivalve, which can reach more than 1 m of shell length, has attracted the interest of Mediterranean inhabitants since the times of the ancient Greeks. Its first description dates to the times of Aristotle and his zoological writings (Voultsiadou and Vafidis, 2007). In the Hellenic period, the species was harvested for its byssus threads, which were considered a very rare and valuable fabric (Laufer, 1915). During the last decades of the past century, the interest in this species, mainly for souvenir purposes, overcame its capacity to recover from anthropogenic impacts and the populations suffered a strong decline (Vicente and Moreteau, 1991). Factors as the destruction of its main habitat (*Posidonia oceanica* meadows), the deterioration of Mediterranean water quality (Vicente, 1990), and accidental killing by anchoring and trawling (Katsanevakis, 2007) have surely contributed to this problem. Strikingly, despite the long relationship of this bivalve with humans and the benefits humans got from it in the past, the ecological knowledge on this species is still vague (García-March et al., 2007a; Katsanevakis, 2007; Richardson et al., 1999).

One of the causes for this lack of knowledge is the low population densities of *Pinna nobilis*, typically around 1–10 individual/100 m^2 (Moreteau and Vicente, 1982; Vicente and Moreteau, 1991; Zavodnik et al., 1991) (although in some sheltered areas higher densities of up to 104 individual/100 m^2 have been reported (Galinou-Mitsoudi et al., 2006; Katsanevakis, 2005)). The low densities of individuals make it difficult and expensive to perform the necessary repeated surveys to study the species ecology (Richardson et al., 2004). Therefore, aspects as age and growth are poorly documented in the literature, and only a handful of studies report such data (Galinou-Mitsoudi et al., 2006; García-March et al., 2007b; Moreteau and Vicente, 1982; Rabaoui et al., 2007; Richardson et al., 1999, 2004; Siletic and Peharda, 2003). Studying the age and growth rates of the populations is fundamental to evaluate the stock and resilience of this species and to make decisions for its protection (Richardson et al., 2004). Because measuring animals *in situ* is extremely time consuming, several

* Corresponding author. Tel.: +1 9192606134.
E-mail address: jrafa@unc.edu (J.R. Garcia-March).

0022-0981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2011.07.016

authors proposed methods to estimate growth parameters from empty shells. Moreteau and Vicente (1982) used the posterior adductor muscle scars (PAMS) to estimate growth parameters assuming a similar time lapse between each of them. Later, Richardson et al. (1999) used oxygen isotope ratios to demonstrate that the PAMS were deposited annually and calculated growth parameters from different populations. Richardson et al. (2004) analyzed Mg/Ca and Sr/Ca ratios to infer the positions of the oldest PAMS. García-March and Márquez-Aliaga (2007), however, demonstrated that many old PAMS could be obscured by nacre in the anterior part of old specimens and that some of the new records could be deposited underneath another confounding the age counts from PAMS. Furthermore, sometimes inner records could be deposited without forming a PAMS externally. Therefore, these authors proposed to use the inner record to identify the position and real number of PAMS. Recently, Garcia-March et al. (2011) confirmed that the inner records are deposited annually.

The inner record of the PAMS is formed by a nacre tongue deposited between couplets of opaque and translucent calcitic layers visible under transmitted light (or light and dark layers, respectively, visible under reflected light) (Fig. 3 of García-March and Márquez-Aliaga, 2007). We will refer to opaque and translucent layers throughout the rest of this paper. The three components are directed towards the posterior of the shell. Inner records are formed as a result of interruptions and resumption of the deposition of calcite and nacre in response to patterns of seasonal growth in the posterior part of the dorsal nacre lobe (DNL).

In the present work we describe a new methodology to estimate *Pinna nobilis* population growth parameters from empty shells using the inner record of the PAMS. This new methodology is free of the bias produced by using the PAMS and avoids the necessity of having to use stable isotopes to infer the position of the older growth records. It is applicable to any population of *P. nobilis* where empty shells can be collected. To develop the new methodology we have worked on 4 main aspects of the growth records: i) description of the formation of the inner record; ii) identification of the inner record in the most anterior part of the shell where nacre tongues are not present; iii) location of an homologous structure of the inner record to measure annual growth for all the records; and iv) identification of the number of years missing in adult specimens due to the anterior abrasion of the shell. We applied this methodology to study growth from empty shells in a population located in Moraira, Spain (western Mediterranean).

2. Methods

2.1. Sample collection and processing

We used 53 empty shells. Forty-nine of them were collected during different surveys in Moraira Bay (East Spain) in the period 1993–2003 from a depth range between 10 and 15 m (area of Population 1 of García-March et al. (2007b)) (Fig. 1). Four more shells were collected in l'Illa Grossa (Columbretes Islands, northeast Spain). Eighteen shells from Moraira were cut dorso-ventrally and the positions of their inner record and PAMS measured using Discover imaging software. The criteria used to select these 18 shells were the time of death (prioritizing shells for which the date of death was known or those that were collected early in our research; i.e. 1993), the size (including large and small shells), and the degree of preservation of the shell. Considering that the process of cutting would destroy the shells, we used the valve that was already more damaged by breakages, provided this did not affect our measurements of the inner record or PAMS, and conserved the other valve for future studies. We also cut 2 of the four shells from Columbretes Islands and studied their inner record and the PAMS. These shells were selected from juveniles recruited on artificial spat collectors in September

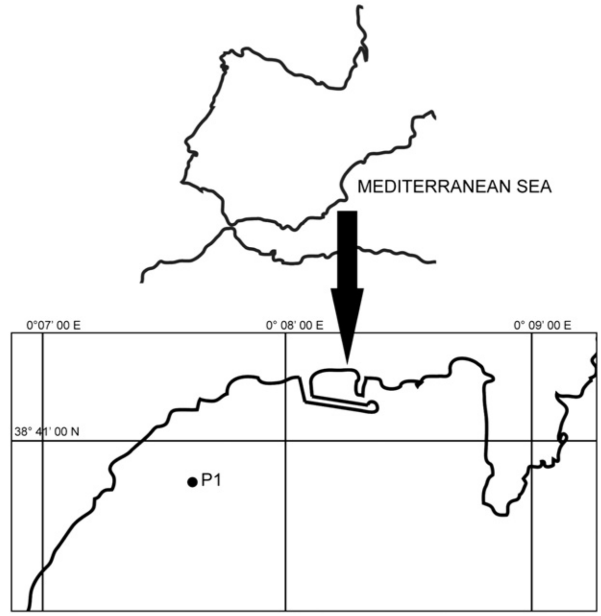

Fig. 1. Map of Moraira Bay (Eastern Spain), showing the position population 1 (P1) where most shells were collected for the present study.

2004. The two specimens had been grown *in situ* in protected cages from November 2004 to November 2007, and then transplanted to sand and protected with a mesh until summer 2010 (i.e., they were 6 years old when collected). The other two shells from Columbretes Islands were found dead in the same zone in 2008 and together with the two juveniles were used to estimate the relationship between total size and position of the PAMS for the population of l'Illa Grossa. The four shells lived at a depth range between 16 and 18 m.

We cut 8 cm long dorsal-to-ventral sections (portion lost in cut c. 0.7 mm) from the 20 shells (18 form Moraira and 2 from Columbretes Islands). Each piece was cut in radial sections across the records of the PAMS. One side of the cross sections was polished to 6 μm diamond suspension grit (Buehler), mounted on a glass slide, and a thick section (c. 300 μm) was cut using a precision sectioning saw designed for cutting various types of materials with minimal deformation (Buehler Isomet low-speed saw). The free surface of the slide was polished down to 1 μm diamond suspension grit (Buehler). We took digital pictures of each slide and described the process and timing of formation of the inner record using the date of death. With this information we created a standard method for establishing the position of measurement of the entire inner record in all shells and then measured the distance of the records to the anterior end of the shell.

We constrained the month/season of death of 12 of the 20 specimens to study the inner record (10 from Moraira and 2 from Columbrtes Islands). Five shells died in July 2000 (MG1EpMor to MG5EpMor), two in July 2010 (IG1 and IG2). One specimen (N1MG) died between August and November 1999. Specimens MG051103_7, MG150 and MG231 died between late October early November 2003, and one shell (MG44) died in December 1999.

2.2. Back-calculation of total sizes from growth records

To estimate the growth parameters we converted the positions of the inner record and PAMS to total sizes (Ht). We used the relationship between Ht and the length of the DNL because several authors have shown that there is a good linear relationship between these two dimensions (García-March and Márquez-Aliaga, 2007; Moreteau and Vicente, 1982; Richardson et al., 1999). We used the 49 shells from Moraira and the 4 shells from Columbretes Islands to fit an equation for