Journal of Invertebrate Pathology 112 (2013) 253–259

Contents lists available at SciVerse ScienceDirect

Journal of Invertebrate Pathology

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

A histopathological survey of the razor clam *Ensis macha* (Pharidae) along the Patagonian Argentina coast

Nuria Vázquez, Elizabeth Perez Bruno, Federico Márquez, Silvina Van der Molen, Carmen Gilardoni, Florencia Cremonte *

Centro Nacional Patagónico, Boulevard Brown 2915 (U9120ACD), Puerto Madryn, Chubut, Argentina

ARTICLE INFO

Article history:
Received 21 May 2012
Accepted 7 December 2012
Available online 24 December 2012

Keywords:
Ensis macha
Health status
Patagonian coast
Parasites
Pathologies

ABSTRACT

This is the first study performed to determine the health status of the razor clam, *Ensis macha*, including six different populations along Argentina Patagonian coast and one of Chile. The parasites and pathologies affecting *E. macha* were analyzed and their prevalence and mean intensity values were calculated. To establish which factors affect the presence and intensity of infection, Generalized Linear Models (GLMs) were applied. Basophilic inclusions, ciliates, coccidians protozoans and turbellarians were found. We report an Aporocotylidae digenean and hemocyte infiltrations. None of the parasites is OIE (World Organisation for Animal Health) notifiable, and none seemed to be pathogenic, with the exception of the digenean. The prevalence of the parasites was affected mainly by environmental factors (such as site of sampling and season) instead of intrinsic conditions of the clam (such as size, condition index, sex and gonadal stage). On the other hand, the maximum intensity of parasites was not only related with cold seasons but also with the partially spawned gonadal stage of *E. macha*. During this stage, the clams would need to store energy for the next gametogenesis cycle, might be more susceptible to infection by the parasites.

1. Introduction

The razor clam *Ensis macha* (Molina, 1782) (Pharidae) is distributed along the coast of Southwest Atlantic from the Beagle Chanel (55°S) to north of San Matías Gulf (40°S) (Lasta et al., 1998; Márquez and Van der Molen, 2011), and on the Pacific coast from Caldera (27°S) to the Magellan Strait (52°S) (Osorio, 2002). It is one of the razor clam species that belong to soft bottom marine fauna, and lives burrowed in sand, silt, or mud substrates. Recent work has demonstrated that the beds from the northern Patagonian gulfs (Argentina, 42–43°S) have a potential for exploitation. The interest in the exploitation has increased after samples sent to Chile, Asia and domestic markets were found acceptable for marketing (Lasta et al., 1998). In Spain, during the last decade, the exploitation of the razor clams (*Ensis siliqua*, *Ensis arcatus* and *Solen marginatus*) has shown a significant increase (Montes Pérez, 2008). In Chile, *E. macha* is one of the most important razor clam species in volume of captures (Sernapesca, 2000), where beds have begun to show clear signs of overexploitation (Bustos et al., 1999).

Although in Chile and Europe there are studies regarding different aspects of the razor clams' biology, little is known about its health status. Montes Pérez (2008) and López Gómez et al. (2008) described the presence of parasites and pathologies in *E. siliqua*, *E. arcatus* and *S. marginatus*; López and Darriba (2006) and Ceschia et al. (2001) reported infection of *Marteilia* sp. in *S. marginatus* from Galicia, Spain and in *Ensis minor* from Italy, respectively. The disease caused by *Marteilia refringens* is currently listed by the World Organisation for Animal Health (OIE, 2011) as a notifiable disease. In Argentina, there are only two studies regarding morphometry, growth and reproduction aspects of *E. macha* (Barón et al., 2004; Robledo, 2009) and nothing about its health status. Therefore, this study was performed to determine a histological health status of the razor clam *E. macha* from Patagonian Argentina coast. Furthermore, to evaluate the factors affecting the presence and intensity of infection, the seasonal and geographical variations of prevalence values as well as their relationship with the condition index, size, sex and gonadal stages were analyzed in two populations in the northern Patagonian gulfs.

2. Materials and methods

2.1. Sample collection

During 2007, 480 razor clams of 124.42 ± 11.87 mm (mean ± SD) were seasonally collected at 10 m depth at Puerto Lobos (42°00′S, 65°05′W – San Matías gulf) and at Fracasso Beach (42°25′S, 64°07′W – San José gulf) (Fig. 1) (60 clams collected

* Corresponding author. Tel./fax: +54 280 4451543.
E-mail address: fcremont@cenpat.edu.ar (F. Cremonte).

0022-2011/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jip.2012.12.004

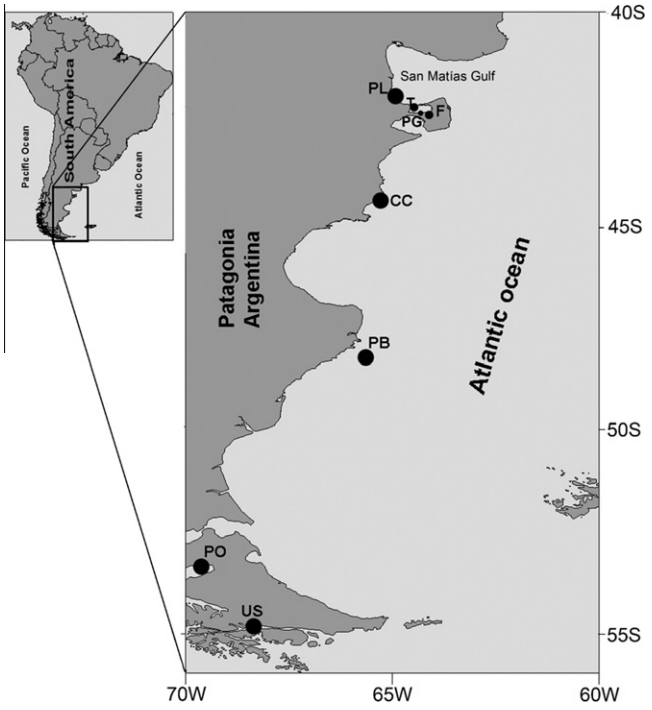

Fig. 1. *Ensis macha* sampling populations: Puerto Lobos (PL), La Tapera (T), Punta Gales (PG), Fracasso Beach (F), Caleta Carolina (CC), Punta Buque (PB), El Porvenir (PO), Ushuaia (US).

during each season and at each population). The clams were collected by scuba diving using a water jet pump and transported to aquaria with aerated seawater, maintained for 24 h until processing. Moreover, from 2006 to 2009, one sample of approximately 30 clams were taken of each of the following populations: La Tapera (42°3$\acute{3}$S, 64°5$\bar{5}$W) (n = 30), Caleta Carolina (44°54′S, 65°35′W) (n = 30), Punta Buque (48°2′S, 65°55′W) (n = 28), El Porvenir (53°24′S, 69°54′W) (Chile) (n = 21) and Ushuaia (54°48′S, 68°15′W) (n = 28) (Fig. 1).

2.2. Histological processing

Maximum shell length of each specimen was measured; shell and flesh were weighed separately to calculate the condition index, as the ratio of the wet flesh weight to shell weight × 100 (Lucas and Benninger, 1985). Soft parts were fixed in Davidson's fixative (Shaw and Battle, 1957) for 24 h. Two oblique transverse 5 mm thick sections, containing gill, digestive gland, mantle, nephridia and gonad (Fig. 2) were taken from each clam. Tissue samples were embedded in paraffin and then 5 μm sections were stained with haematoxylin and eosin. Histological sections were examined under a light microscope for presence of parasites and pathological alterations. For each tissue section, sex and gonadal stages were recorded. A 6 stage gametogenic scale was determined after examining the oocytes and espermatocytes developmental pattern, following Robledo (2009): (1: early active, 2: late active, 3: ripe, 4: partially spawned, 5: spawned, 6: restoration). For the hemocyte infiltration a qualitative scale was established based on the connective tissues area covered by hemocytes: low (1), moderate (2) and heavy (3).

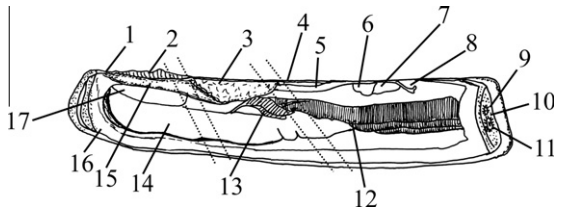

Fig. 2. Diagram of the two oblique cuts of 3–5 mm thick (dotted line) through the visceral mass of *Ensis macha*, which were included in the biopsy cassette. Abbreviations: 1: hinge; 2: ligament; 3: digestive gland; 4: pericardial cavity; 5: rectum; 6: posterior adductor muscle; 7: foot retractor muscle; 8: anus; 9: pallial tentacles; 10: inhalant siphon; 11: exhalant siphon; 12: gills; 13: labial palps; 14: foot; 15: gonad; 16: robe; 17: anterior adductor muscle.

2.3. Statistical analyses

Clams collected from all populations were examined for parasites and pathologies, and Prevalence (P) and Mean Intensity (I) of the different parasites were calculated. Mean intensity was calculated as the number of parasites per total parasitized hosts (Bush et al., 1997). The intensity was estimated by counting the number of parasites in each histological section of 5 μm using a magnification of 400×. Only the samples from Puerto Lobos and Fracasso Beach were included in the following statistical analyses.

To evaluate different factors affecting the presence and intensity of parasites, Generalized Linear Models (GLMs) were applied. Presence-absence of parasites (binary response) was evaluated by GLMs with binomial distribution with a logit link function, and intensity of parasites (count data) was evaluated with Poisson distribution of response variable with a log link function (Agresti, 2007). Different models were used to test these variables with regard to the following explanatory variables: site (Puerto Lobos, Fracasso Beach), season (1: summer, 4: autumn, 7: winter, 10: spring), sex (1: male, 2: female), gonadal stages (1 to 6), shell size and condition index.

The Akaike information criterion (AIC) was used to determine the best model for the analyzed data set. Model selection was performed with an Information Theory (IT) approach using Akaike's information criterion (AIC) and Model averaging (Burnham and Anderson, 2002; Grueber et al., 2011). The AIC values (Akaike, 1973) and the AIC for small samples (AICc) (Hurvich and Tsai, 1989) were calculated for each model. From the AICc differences (Δi), where Δi = AICC (i) – AICC (min), Akaike weights (wi) (Akaike, 1978) were obtained for all candidate models. For each data set, the models were ranked by their wi values; the model with the highest wi was considered the one with the best supporting data (Burnham and Anderson, 2002). Model averaging was calculated using candidate models, which together account for 95% confidence interval. The top model set was averaged using the zero method (Symonds and Moussalli, 2011), where the best AIC model was not strongly weighted. The results are expressed in terms of odd ratio. The odds are calculated as the exponential of the coefficient of each parameter corresponding to the averaging model.

All statistical analyses were performed in R (*R* Development Core Team 2011). The standardized function to input variables is available within the arm package (Gelman et al., 2009). Model selection and averaging were calculated with the MuMIn package (Barton, 2009).

3. Results

A summary of the main characteristics (sex ratio, shell size and condition index) of *E. macha* from Puerto Lobos and Fracasso Beach, and the results of the histological examinations (parasites, pathologies, mean prevalence and mean intensity) from all sampling populations are presented in Tables 1 and 2 respectively.

Intracellular inclusions caused by prokaryote-like organisms in the digestive gland epithelium, ciliates in gills, turbellarians of the genus *Paravortex* Wahl, 1906 in the intestine lumen and different