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Steinhausia mytilovum in *Mytilus galloprovincialis* (The Case of Atlantic Northwest Africa-Morocco)

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Steinhausia mytilovum (Field, 1924) is a microsporidian parasite that infects female individuals of Mediterranean mussel, *Mytilus galloprovincialis*. The aim of this work was to evaluate the incidence and histopathological effect of the microsporidian in the host from the Atlantic coast of northwest Africa. Samples were collected monthly during two years (March 2009 - March 2011) at three sites; north, middle and south, along the Moroccan coast. The prevalence showed to be the highest in the north (0% - 26.66%) and south (0% - 75%), respectively, while mussels from the middle sampling site showed no infection of *S. mytilovum*. The seasonality of *S. mytilovum* was investigated as well, although no significant variation of its incidence was observed in respect to season. The most usual histopathological characteristic of the *S. mytilovum* infection was hemocytic infiltration in gonads, showing a statistically significant relationship to the *S. mytilovum* infection ($P<.001$). Further investigation is recommended to study influences of the biological and physical parameters on the infections of *S. mytilovum* in the natural populations of the Mediterranean mussel along the Moroccan coast.

Keywords: Steinhasusia mytilovum; Atlantic coast of Morocco; Mytilus galloprovincialis; histology; seasonal dynamic; hemocytic infiltration.

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1. INTRODUCTION

Steinhausia mytilovum (Chitridiopsidae, Microsporidia) is an intracellular parasite of bivalve oocytes that develops on either the surface or interior of its nucleus [1]. It can damage the mussel female gametes (Mussel Egg Disease) but unlikely is lethal to the host [2]. The microsporidian could be observed in mussels from early stage of gametogenesis to spawning [3].

Firstly described in the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 along the Italian coast [4], it has a larger geographical distribution recognized today [5]; over the European Atlantic coasts from Norway [6] to Greece [7].

Previously, *S. mytilovum* has been observed in oocytes of *Eurhomalea lenticularis* from Chile, showing no evidence of cell damage in oocyte of the host [8]. It was also in the mussel *Mytella guyanensis* from the Amazon river estuary [9], as well as in ova of mussels from Atlantic and west coasts of USA [10,11]. It affected the condition index of infected *Mytilus galloprovincialis* from Greece and induced a strong hemocytic infiltration within affected gonadal follicles [7].

With the exception of approximately dozen tonnes of harvested *Cassostrea gigas* (oyster) and *Mytilus galloprovincialis* (mussels), there are almost no products of marine aquaculture in Morocco [12]. To bridge this gap, the fishery sector in Morocco developed in 2009 a strategy plan called "Halieutis" [13,14], integrated by coupling in the institutional frame of cooperation with the European Union [13].

Therefore, research of pathologies occurring in natural wild populations is necessary to collect fundamental information to secure future sustainable aquaculture.

The aim of this study was to evaluate incidence and effect of *S. mytilovum* in oocytes of the Mediterranean mussel along the Moroccan Atlantic coast, focusing on three natural biotopes of the host; Moulay Bousselham in the north, Cap Beddouza in the middle and Imessouane in the south.

2. MATERIALS AND METHODS

The samples were collected from three natural beds of mussel *Mytilus galloprovincialis*: Moulay Bousselham (34°53'N; 6°17'W), Cap Beddouza (32°32'N; 9°16'W) and Imessouane (30°50'N; 9°49'W) (Fig. 1), according to their socio-economic potential and lack of any industrial or agricultural activity.

Monthly in two years (March 2009 - March 2011), 30 bivalves with shell length equal or over 40 mm measured from the umbo, were sampled and shipped on ice to the laboratory, where they were processed immediately. The sex of mussel was determined with histological inspection. Gonads were cut at the central portion of the mantle, fixed in Davidson's solution for 24 hours, dehydrated in ascending ethanol concentrations and embedded in paraffin blocks. Histological sections (2 µm) were stained in hematoxylin-eosin Y and studied under light microscope (Leica DM2500).

In total, 720 bivalves were studied by histology at Cap Beddouza. Samples from Imessouane site of July 2009 and August 2009 were discarded because of unsatisfying fixation procedure (N=660), while samples from Moulay Bousselham in January 2010, January 2011 and March 2011 were not taken due to environmental conditions (N=630). The overall sample including the three sites was 2010 individuals.

Prevalence was evaluated according to Bush et al. [15]. Further, due to its location in the oocytes, *Steinhausia mytilovum* infects only females or hermaphrodites (Table 1). Thus, its prevalence was calculated only from female and hermaphrodite data [2,5]. The confidence intervals were calculated using R (R 2.15.2 software, R. Core Team).

$$Prevalence = \frac{number\ of\ hosts\ infected}{number\ of\ females\ examined} * 100$$

Statistical analysis was done to compare the total prevalence of the *S. mytilovum* infection between the three sampling sites using Fisher Exact Test. Potential difference in seasonal distributions from each sampling site was conducted by using Chi^2 test, and logistic regression was used to quantify the association between infection and the risk of hemocytic infiltration in gonads from Imessouane (N =336) (R 2.15.2 software, R. Core Team). The presence or absence of hemocytic infiltration in the gonad was observed by means of histological sections under light microscope (Leica DM2500).

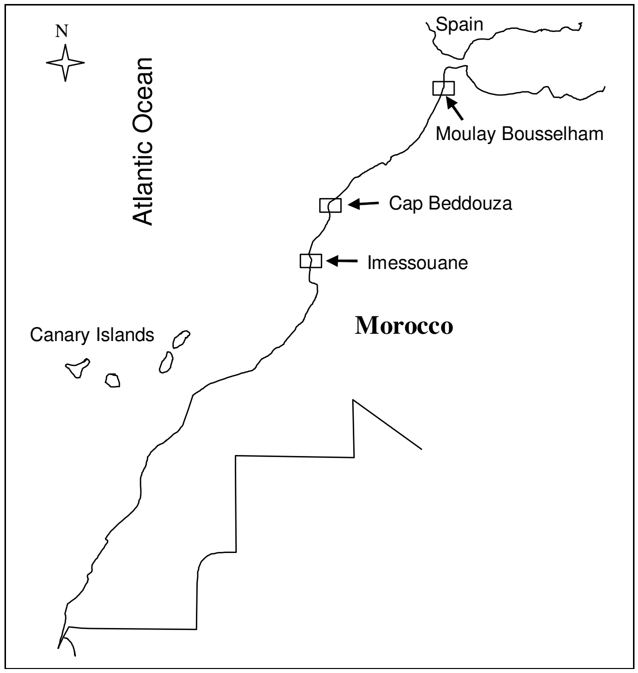

Fig. 1. Geographic distribution of the three sampling sites (in small rectangles); Bousselham (34°53'N; 6°17'W), Cap Beddouza (32°32'N; 9°16'W) and Imessouane (30°50' N; 9°49'W)

Table 1. Number of females *Mytilus galloprovincialis* identified in each sample from the sampling sites (MB= Moulay Bousselham; CB= Cap Beddouza; IM= Imessouane) between March 2009 and March 2011

	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
MB	**2009**			14	15	13	9	15	17	15	15	15	10
(N=317)	**2010**		20	14	14	16	12	16	11	20	20	10	14
	2011		12										
CB	**2009**			18	16	19	15	12	7	12	12	14	10
(N=347)	**2010**	16	15	15	13	8	11	16	14	20	15	14	14
	2011	10	17	14									
IM	**2009**			17	12	9	16				14	15	20
(N=336)	**2010**	9	16	16	12	14	13	11	19	12	14	15	16
	2011	18	18	14									

3. RESULTS

Steinhausia mytilovum was recorded in females and hermaphrodites *Mytilus galloprovincialis* from samples collected in North and South of Atlantic Moroccan coast.

Hermaphroditism were considered as females (Table 1). The total prevalence of *Steinhausia mytilovum* was 11.67% in Moulay Bousselham (95% IC 8.35 - 15.72 %), while throughout the study period, the overall sampled mussels in Cap Beddouza showed no cases of infection by this oocytic parasite. In Imessouane, the total prevalence was 23.51% (95% IC 19.06 - 28.41%). Fisher Exact Test results showed that the presence and absence of the parasite was significantly dependent upon the sites (*P*<.001).

Infected mussel females showed no gross pathology by visual inspection.

The parasite was observed inside a sporoform vacuole, measuring 7.5 and 12.5µm (mean= 10µm, SD±1.76, N=9), delineated by a membrane of variable thickness from the oocyte nucleus, sometimes difficult to intercept. The vacuole was cylindrical or subspherical, containing a number of variable spores, from 1 - 40 (Fig. 2) localized in the cytoplasm or inside the nucleus of pedicular or free vitellogenical oocytes. A single sporoform vacuole formed in the oocyte, rarely up to five.

In Moulay Bousselham, *Steinhausia* was observed during all seasons with monthly prevalence varying between 0% and 26.66% (Fig. 3). The highest presence of this parasite was detected in September 2009, although there was no difference between the seasons (Chi ^{2} Test, *P*>.05). Prevalence ranged between 7.25% (95% IC 0.26 - 14.56%) recorded in spring and 15.83% (95% IC 9.94 - 25.90%) in fall.

In Imessouane site, *S. mytilovum* was more frequently observed during the two years of study (Fig. 4). The monthly prevalence was ranged between 0% and 75%, the highest prevalence (up to 50%) was calculated in November 2009, March 2010 and April 2010. The seasonal prevalence was ranged between 19.58% (95% IC 12.22 - 28.88%) recorded in winter and 38.37% (95% IC 28.08 - 49.48%) recorded in fall, but showed no statistical difference (Chi ^{2} Test, *P*>.05).

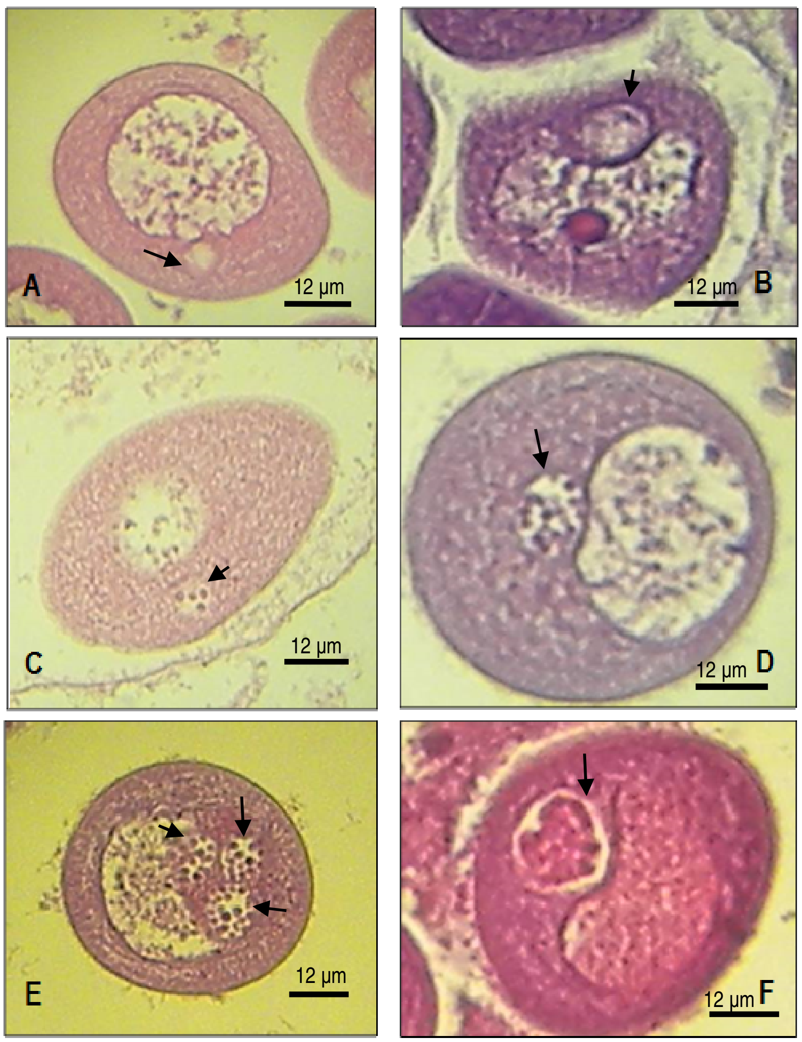

Fig. 2. *Steinhausia mytilovum* in the oocytes of *Mytilus galloprovincialis* (magnification x400)
A: oocyte with an empty sporoform vacuole (arrow); B: sporoform vacuole with a single microsporidian cell (arrow); C: sporoform vacuole with four microsporidia cells (arrow); D: sporoform vacuole with over 40 microsporidia cells (arrow); E: Three sporoform vacuole within oocyte cytoplasm (three arrows); F: sporoform vacuole containing several large microsporidian cells

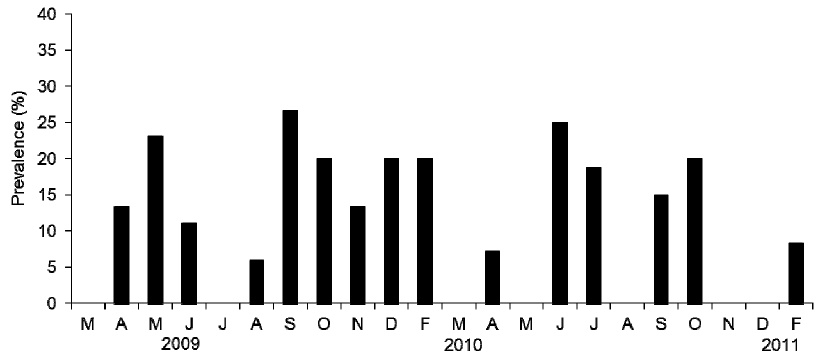

Fig. 3. Prevalence of *Steinhausia mytilovum* in oocytes of *Mytilus galloprovincialis* in Moulay Bousselham sampling site, between March 2009 and March 2011

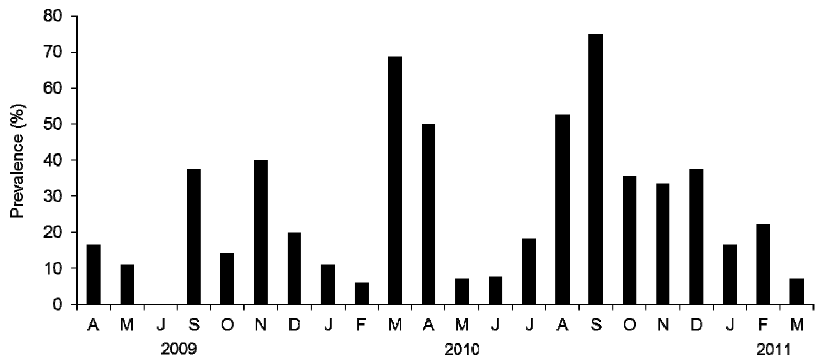

Fig. 4. Prevalence of *Steinhausia mytilovum* in oocytes of *Mytilus galloprovincialis* in Imessouane sampling site, between March 2009 and March 2011

In order to determine the side effect of *Steinhausia mytilovum* on infected gonads, histological sections were inspected for the presence of hemocytic infiltration. Females from Imessouane site revealed heavy hemocytic infiltrations in the gonad, in the connective tissue and epithelia of gonadal follicles of several infected mussels. This condition was observed in some individuals and could not be linked to any detectable infected oocyte. (Table 2), revealed the result of logistic regression method in the case of the dependant variable (presence or absence of hemocytic infiltration) according to the descriptive variable (presence or absence of Steinhausia). Logistic regression showed that the existence of microsporidian infection had a relationship with the presence of hemocytic infiltration; the infection by Steinhausia implied that there was hemocytic infiltration (*P*<.001) (Table 2).

Table 2. Results of logistic regression showing the relationship between the observation of the hemocytic infiltration in the female gonad from Imessouane and the presence of *Steinhausia mytilovum* in *Mytilus galloprovincialis* (N = 336). z value was the measure of the divergence of the individual status from the most probable result. Pr (>|z|) was the *P*-value used in comparison to a critical value, .05 to determine if the model was statistically significant

	Coefficient				
	Estimate	**Std. Error**	**Z value**	**Pr(>|z|)**	
Intercept	-1.5232	1.63E-01	-9.361	< 2E-16	***
S. mytilovum	+1.9076	2.81E-01	+6.787	1.15E-11	***

*Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1*

4. DISCUSSION

The increase of aquaculture industrialization provoked the frequent apparition of epizooties in marine bivalves [16,17] resulting in serious propagation, as witnessed by the disappearance of certain bivalve populations. The parasites are major disease-causing agents of marines bivalves [16], because bivalves facing permanent parasite infection have compromised immunity system [18,19,20,21].

In this study, the presence of *Steinhausia mytilovum* in female of mussels sampled during two years of monitoring was assessed for the first time along the Atlantic coasts of Morocco. Using histopathological approach systematic sampling of mussels from north (Moulay Bousselham), middle (Cap Beddouza) and south (Imessouane) of Atlantic Moroccan coast with systematic sampling allowed us to investigate geographic and seasonal dynamics of the Mussel egg disease.

The microsporidian *S. mytilovum*, intra-oocytic parasite of the mussel was identified in Morocco, which widens the area of its distribution along the Northwest Atlantic coast extending from Norway [6] to the northwest of Africa (Morocco).

The parasite was more prevalent in south (Imessouane) than in north (Moulay Bousselham), while mussels from the middle sampling site (Cap Beddouza) showed no infection of *S. mytilovum*. The difference of prevalence between the sampling sites could be explained by the wide variability of environmental conditions for the three studied populations.

Various developmental forms of *S. mytilovum* observed during this study were identical to those described in other populations of parasitized mussels [22,23,24,10], and similar to the parasite found in oyster *Saccostrea commercialis* [25] and in cockle *Cerastoderma edule* [26].

The average size (10µm) of parasitic sporoform vacuole was superior to that reported by Anderson et al. [25] (1.7-4.1µm) and Sprague, 1965 (6-8µm), and was inferior (9-18) to that recorded by Ercolini et al. (2008). The number of sporocysts can increase up to four within the same oocyte as already indicated by numerous authors [2,5,10,26,27,28]. Anderson et al. [25] observed a single spore in the sporocyst, assuming that this was only a premature developmental stage of the parasite.

However, all three studied site in Morocco, showed a number of oocysts, cylindrical sporocysts without any track of spores. This shape could be considered as a premature stage or state of *Steinhausia* lysis. To avoid any doubt, these cases were not considered in our statistical analysis, moreover because they were not observed mussels studied along the Mediterranean coasts [22].

The highest prevalence of this parasite in the North of Morocco (26.66%) was lower than the one found in the females of *Mytilus galloprovincialis* in California [11], in Galicia (28.3%) [2] and in M'diq (76.5%) [22]. This maximum is also lower than that of the Moroccan south (75%). Previously, significant relationship between microsporidian prevalence and temperature or salinity was not found [22], possibly explaining the lack of seasonality of prevalence in Moulay Bousselham and Imessouane, although this coastal part of the Atlantic Moroccan coast is very dynamic between the seasons [29]. The temporal variation were studied in the Mediterranean coasts [22] and in the Atlantic [3]. [3] and [22] found a significant relationship between the infection of *S. mytilovum* and reproductive cycle of mussels, particularly strengthening during the spawning period.

The environmental conditions of water in a particular site would indirectly influence to the infections of *S. mytilovum* by determining the timing and intensity of reproduction. Indeed, the highest prevalence of *S. mytilovum* in mussels from Imessouane might be explained by the abundance of mature oocytes through entire seasons which was indicated by [30]. With regard to the environmental context in Imessouane, the temperature has a very dynamic rate of variations, with recesses in summer [30]. This region is subject to permanent upwelling entire seasons, which have the greatest impact on nutrient-enriched waters [29].

Furthermore, the histological effect of this parasite on infected tissue was investigated from samples collected in Imessouane, showing that hemocytic infiltrations in gonads was significantly related to the presence of the parasite (P<.001), suggesting that the existence of infected oocytes in mussel did multiply the risk of hemocyte infiltration at the gonads. This is accordance to most of previous reports [2,3,19], in contrast to [26]. In [23], intensive development of hemocytic infiltration limited the healthy growth of the other organs, which considering the potential risk upon the aquaculture in Imessouane, implicates the need for a continuous monitoring of the infection and impact of the *S. mytilovum*.

Finally, the absence of *S. mytilovum* at Cap Beddouza located in the middle of Moroccan coast, could have different yet unidentified reasons, related to host's immune status. Also, it should be noted that conversely to the mussels in north and in south, the spawning was periodic in this site (unpublished data). This point deserves further consideration by the study of the reproductive characteristics of each treated female and its conditions of life, in order to find an answer to this question.

In summary, this is a part of preliminary study aiming to a more detailed research of the extent of *S. mytilovum* along Moroccan coast: M'diq, Moulay Bousselham, Cap Beddouza and Imessouane.

5. CONCLUSION

During a 25-month period, we sought the presence of the parasite *Steinausia mytilovum* in *Mytilus galloprovincialis* in three sites *along the Moroccan Atlantic coast: Moulay Bousselham, Cap Bedouzza, Imessouane, was assessed. There was a clear differentiation of the presence at these three sites. At Moulay Bousselham, we found the presence of S. mytilovum throughout the study period* was evenly distributed over the seasons. At Cap Bedouzza, no cases of *S. mytilovum* were recorded. At Imessouane, the parasite was intensely present, seasonally uniform throughout the studied period. Moreover, its presence is strongly correlated with the gonad hemocytic infiltration.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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