

Evaluation of Prezygotic Barrier Using Laboratory Crosses Between Three Species of Blue Mussels of The Genus *Mytilus*, Present Along the Chilean Coast

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ABSTRACT

The genus *Mytilus*, is composed by 5 species; *M. californianus* (Conrad 1837), *M. edulis* (Linnaeus 1758), *M. galloprovincialis* (Lamarck 1819), *M. trossulus* (Gould, 1850) and recently the *Mytilus chilensis* (Hupé, 1854) species have been confirmed as a true species. In the Chilean coast until 2005 the only *Mytilus* species present was *M. chilensis*, however, during the last 15 years two new exotic mussel species have been described (*M. edulis* and *M. galloprovincialis*). The scientific literature has described evidence that natural hybridization between the *Mytilus* species have been occurring in several hybrid zones around the world. If this phenomenon occurs in Chile, could cause a tremendous impact on the biodiversity of marine life and also on the aquaculture industry based on *M. chilensis* in Chile. The aim of the present study was to determine, under laboratory conditions; the percentage of fecundation using a factorial crosses design with the three species of *Mytilus* present in Chile.

The results obtained, showed that even do significant differences exist among the percentage of fecundation among crosses, there were no significant differences between pure species crosses and the hybrid crosses. Therefore, no prezygotic barrier was found in any of the interspecies crosses. More studies are needed to establish the fitness of larvae and juveniles obtained by each of these crosses, because, even if there is no reproductive barrier between species the viability and survivorship of the juveniles can be affected if these mussels are expose to different environmental conditions.

Introduction

Marine mussels represent an important component of the intertidal and subtidal communities and estuaries, in terms of the number of individuals and of biomass and production and are also of considerable economic importance to aquaculture in many regions of the world [1,2]. The genus *Mytilus* is composed of at least five morphologically similar but genetically distinct species, *Mytilus edulis* Linnaeus, 1758 (in eastern U.S.A. and Canada, northern Europe, Southern Argentina, the Falkland Islands, and Kerguelen Island), *Mytilus galloprovincialis* Lamarck, 1819 (in the Mediterranean, western Australia, Tasmania, New Zealand, sympatrically with *M. edulis* in parts of Great Britain, Ireland and France, and accidentally introduced into: Japan, Hong Kong, South Africa, southern California, Chile and Brasil), *M. trossulus* Gould,

1850 (in the northern Pacific from Siberia to central California, the Canadian Atlantic provinces, and the Baltic Sea), *Mytilus chilensis* (Southern Chile) and *Mytilus californianus* (Pacific coast of USA).

The *M. chilensis* aquaculture began in 1943 in Chiloé Island, southern Chile, and the aquaculture production increased rapidly from 3,864 t in 1993 to 398,274 t in 2019 [3]. The issue with regard to the recently published evidence of the presence and potential dissemination of alien mussel species in the Chilean coast could cause a tremendous damage to the Chilean indigenous mussel (*M. chilensis*) aquaculture growing industry. In Chile the scientific literature described the presence of the blue mussel *M. galloprovincialis* in Tongoy Bay [4], central [5] and in southern coast of Chile [6]. The presence of *M. edulis* has been described in

the inner coast of the Chiloé Island [7] and in the Straits of Magellan. Unfortunately, among some of the main features of these *Mytilus* species is the well-known capability to naturally hybridize among them in locations where their distributions overlap [8,9], therefore, these alien mussel species could have also the potential for natural hybridization with the native mussel *M. chilensis*.

In fact, there is clear evidence for a high fitness of hybrids mussels resulting in the F1 from the interbreeding between *M. chilensis* and *M. galloprovincialis*, produced under controlled laboratory conditions [10]. *M. galloprovincialis* is well known to be a very drastic invasive species and is recognized as one of the 100 worst invasive species in the world [11,12]. Therefore, to understand the ecological and evolutionary processes that promote the invasion is of fundamental importance to elucidate the fecundity success and/or the presence of prezygotic barriers between crosses under laboratory controlled factorial designs.

Methods

Ripe blue mussels (35-64 mm shell length) were collected from the wild from Tumbes (36°38'S ;73°05'O); Yaldad (42°29'S; 73°46'O) and Buque Quemado (52°19S; 69°29'O), corresponding to *M. galloprovincialis*, *M. chilensis* and *M. edulis* respectively [6]. Mussels were held in ambient running seawater until required for spawning. A factorial design was carried out to permit full reciprocal crosses. Fifty-five mussels from each species were exposed to air for about 1h, then placed in filtered seawater at ambient temperature, one mussel per beaker, for induction of spawning using thermal shock. Eggs and sperm were held for about 45m at 18°C before use, to allow as many mussels as possible to spawn, thereby helping to synchronize the egg stage [13]. Eggs from each female were rinsed with filtered (1mm) UV-irradiated seawater (FSW) and then resuspended in 2000mL FSW at 18°C for fertilization. The sperm-egg ratio used for fertilization was approximately 100:1 in all trials. Eggs from each cross and each pure line were rinsed after 10m to remove excess sperm and resuspended in two 3L glass flasks (replicates) containing 2000 mL at a density 90-120 eggs mL [6].

The larval cultures were then maintained at 17°C and after 30 and 60 minutes, three samples of 15mL were taken from each culture and fixed with 95% ethanol. The fixed sample was later examined in order to determine the percentage of eggs which had developed into a embryo. Three samples from each replicate flask were used to count the numbers of embryos larvae within one field of view (100 magnification). Evaluations of fecundity (three for each cross) were carried out using a Petroff-Hausser counting chamber. The spawned mussels were typed to confirm the species identification, using two PCR-based markers: Me15/16 [14] and COIXba [15]. The fecundity percentage in each cross, were analyzed using first the Cochran's Q test for heterogeneity and then general linear models were applied.

Results

Fertilization occurred in all crosses and fecundation (cleavage) was observed within 25 to 60 m following mixing of eggs and sperm. Between 72 and 100% of fecundation at 30 m and 90% to 100% at 60m were detected. Nested ANOVAs at 30 m and 60m showed significant differences ($P<0.05$) among all crosses in the percentage of fecundity, however not significant differences between replicates were found. Similar statistical analysis showed no significant differences ($P>0.05$) between pure crosses (same species) and hybrids crosses (between species) with regard to the percentage of eggs developed into embryos (Table 1). A few abnormal larvae (less than 1%) were detected by direct observation in the hybrid crosses, but the proportion of normal embryos during the early stages of growth showed no significant differences between pure and hybrid crosses.

Table 1: Results of Nested ANOVA for fecundity among *Mytilus* laboratory crosses.

Factor	Source	df	SS	F-ratio
Fecundity	Cross	8	3714.66	90.848*
	Replicate [cross]	9	5.11	0.704NS
All crosses at 60 minutes alter fertilization:				
Factor	Source	df	SS	F-ratio
Fecundity	Cross	8	397.92	10.331*
	Replicate [cross]	9	43.33	0.747NS
Between pure and hybrids crosses:				
Factor	Source	df	SS	F-ratio
Fecundity	Cross	2	62.29	1.34NS
	30 vs 60m	1	342.51	7.39NS
	Replicate [cross]	5	8.23	0.18NS
	Cross x minutes	1	18.96	0.41NS
	Error	99	46,33	

Discussion

The results of this study provide evidence that there are no differences in the percentage of fertilized eggs between pure line crosses and hybrid crosses between the three blue mussels belonging to the *Mytilus* genus found in coastal Waters of southern Chile. These results suggest that there is little or no evidence of prezygotic barriers to fertilization between these species. Therefore, there is a great possibility that the two exotic mussels present in the Chilean coasts may interbreed with the native Chilean mussel, causing a tremendous damage to the aquaculture industry in Chile. According to [16] there should be a prezygotic barrier between *M. chilensis* and *M. galloprovincialis* because a difference in the sperm morphology of the latter mussel. However, this study showed that the interbreeding between these two blue mussels is completely possible in both reciprocal crosses. Further investigations should be done, including larval abnormalities and juvenile mortality

caused by incompatibilities between mitochondrial and nuclear genomes, that may be causing a higher frequency of abnormalities and lower survival among F1 hybrid larvae [17].

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