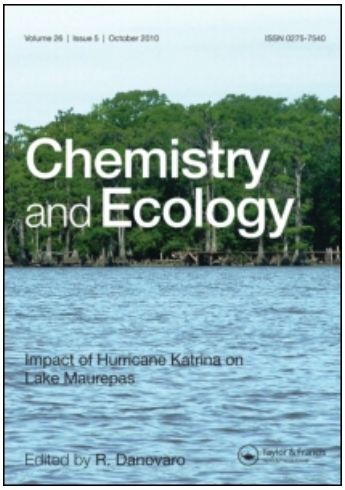

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Reproduction trials under experimental conditions of Mediterranean abalone (*Haliotis tuberculata, L.* 1758)

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Reproduction trials under experimental conditions of Mediterranean abalone (*Haliotis tuberculata,* L. *1758*)

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The aim of this paper is to show early results obtained during reproduction trials of *Haliotis tuberculata* under experimental conditions. Laboratory experiments were carried out at the CISS of the University of Messina (Italy). Two hundred adult abalones were collected, separated by sex and put in 20-litre tanks. In order to induce spawning, temperature was gradually increased. Cultures of eggs and sperm were allowed to develop for 4 h. The egg fertilisation, the formation of trocophora as well as the evolution of such larval stage in veliger were achieved. The adhesion to substrate remains up to now to be optimised. The success in obtaining the earlier stages of the life cycle of *H. tuberculata* is important considering the possible economic effects due to the industrialisation of the production process of this species.

Keywords: abalone; *Haliotis tuberculata*; reproduction; spawning; trochophora; veliger

1. Introduction

Abalone farming is expanding worldwide increasing the scientific interest towards such molluscs [1]. The genus *Haliotis* has been severely over-exploited as it is one of the highest-priced shellfish in the world. Moreover, the juvenile hatchery production could contribute to reconstitute the natural stocks. Despite the high technical and scientific level gained in extra-European abalone industry [2,3], data available on the Mediterranean species [4], particularly its reproduction [5], are actually sporadic and partial. The optimal sperm concentration and time of fertilisation have been studied for different extra European abalone species [6–8] as well as for the Mediterranean ormer [9]. In European countries, the production of *Haliotis tuberculata* is present [10], but the market demand is not yet satisfied. In Italy the farming of such species is completely absent, in spite of the presence of wild stocks of such species and of the presence of a wide coastal surface. In our previous paper [11] we underlined the meaning of some diseases as a limiting factor for the farming development. However the main problem for this industry is the reproduction and

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the rearing of larval stages. The aim of this paper is to show early results obtained during an attempt to reproduce *H. tuberculata* in tanks. These data could be used to evaluate the possibility of introducing aquaculture of such gastropod molluscs in Italian euryhaline fish farms, creating integrated aquaculture systems.

2. Materials and methods

Laboratory experiments were carried out over a 2-month period, July and August 2006, at CISS of the University of Messina (Italy). Two hundred adult abalones, belonging to the local species *Haliotis tuberculata*, were collected in the harbour area of Villa S. Giovanni (RC), after previous authorisation, when the ambient sea water temperature was about 20°C. The subjects, 5 to 9 cm in size, were reared in cold tanks (19–20°C) containing about 500 litres of filtered marine water for each one. In all experiments we used the same salinity of ambient sea water. After a period of acclimatising, the larger molluscs were selected and separated by sex. A total number of 40 abalones, subdivided into four groups, were used for this experiment (Figure 1). Each group was constituted of 10 subjects, 2 males (20%) and 8 females (80%). The differentiation of males and females was carried out on the basis of a simple visual analysis, as the gonads were fully mature during this period of the year. The male has a milk-white testicle, while the fully matured female shows a greenish-brown gonad. Following a period of 30 minutes out of water, at a temperature of 26°C, they were put in 20-litre tanks. Because thermal shock has been demonstrated the only method used for *H. tuberculata* [6], in these tanks the temperature was gradually increased from 20°C to 25°C, over a period of 4–6 hours, in order to induce spawning. When the male abalones had ejaculated sperms for an hour or so, the sperm suspension was moved to another container to estimate its concentration. This was calculated under a microscope using a haemocytometer. The correct concentration of sperm is 5×10^5 sperms/ml^{-1} [5]. Once gamete emission had been obtained after thermal shock, all the procedures needed to achieve successful fertilisation were carried out. A sperm/egg contact time of about 15 min was used. The percentage of eggs fertilised was assessed, using a compound microscope, by the presence of normal or abnormal cleavage in 100 undamaged eggs that were randomly sampled (Figure 2). Successfully fertilised eggs sank to the bottom of the tank; so we removed the upper water layer with abnormal and unfertilised eggs

Figure 1. *Haliotis tuberculata*. An adult subject reared in one of the tanks in the laboratory during the acclimatising time.

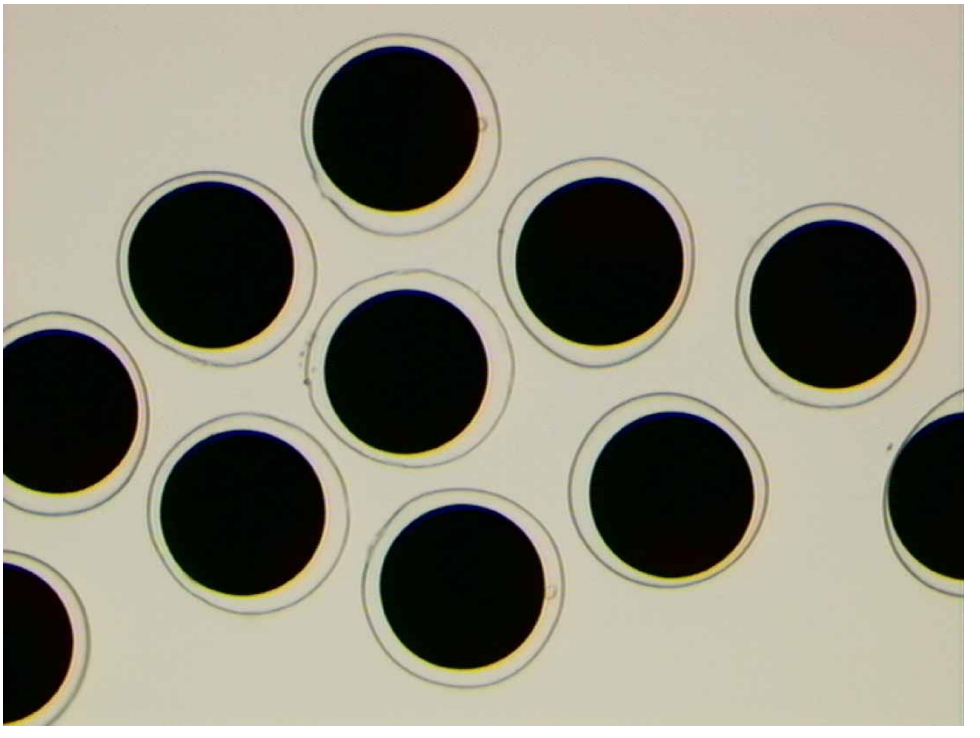

Figure 2. *Haliotis tuberculata*. Eggs obtained from wild female after thermal shock.

by suction. Cultures of eggs and sperm were allowed to develop for 4 h. The eggs fertilised were collected using a sieve rinsed with 1.6 μm-filtered seawater, and then transferred to plastic tanks with a capacity of 100 litres at a temperature of 19°C.

3. Results and discussion

Gametes were obtained 4 to 6 hours after thermal stimuli. The sperm were viable and showed high vitality in fresh samples observed under a light microscope (Figure 3). The egg fertilisation was shown by the evidence of polar body emission peripherally located in the perivitellinic space (Figure 4). An average fertilisation success of 80% was obtained. Twenty four hours after egg fertilisation, the formation of trocophore was seen, first ciliate larval stage with the dimensions of about 60 μm (Figure 5). The free swimming larvae were seen in the water surface, whereas damaged ones lay on the tank bottom. Each day the actively swimming trocophore were rinsed, separated by the egg and larval residuals, and transferred to clean water. Almost 50% hatch out was obtained. After a further 24 hours the evolution of selected trocophore larvae in veliger was demonstrated (Figure 6). Such larval stage is characterised by a foot, by the first appearance of the shell and a ciliate velum expanded in two large lateral wings to swim. No food was provided during larval rearing. The adhesion to the substrate remains to be investigated.

Reproduction under controlled conditions of *H. tuberculata* is today the main problem limiting the introduction of such species among the farmed ones. For this reason, the success in obtaining the earlier stages of the life cycle of *H. tuberculata* is really important by the scientific point of view also considering the possible economic effects due to the industrialisation of the production process of this species, not yet farmed and scarcely available in the market at very high prices.

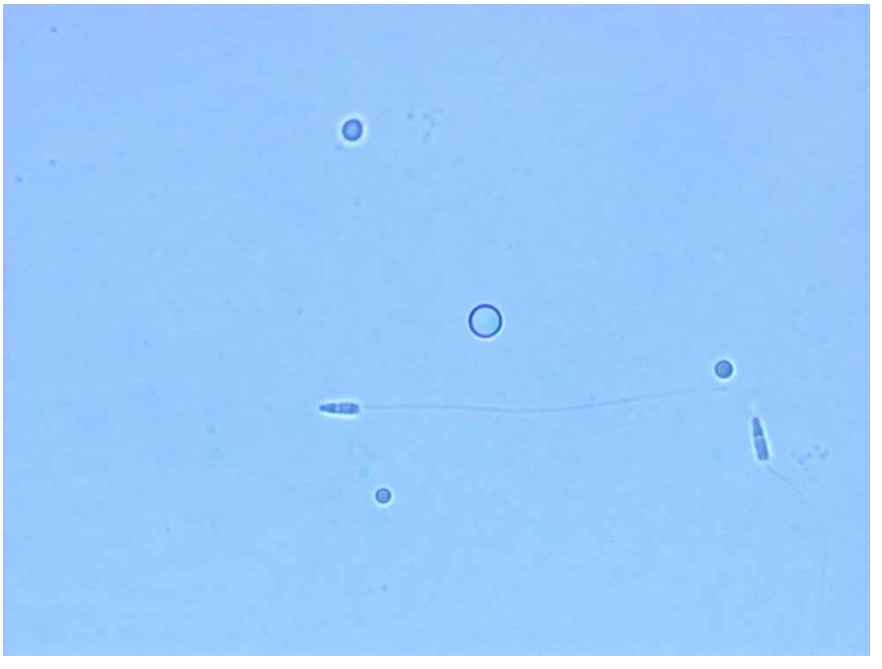

Figure 3. Abalone sperm showing the long tail characteristic of the species.

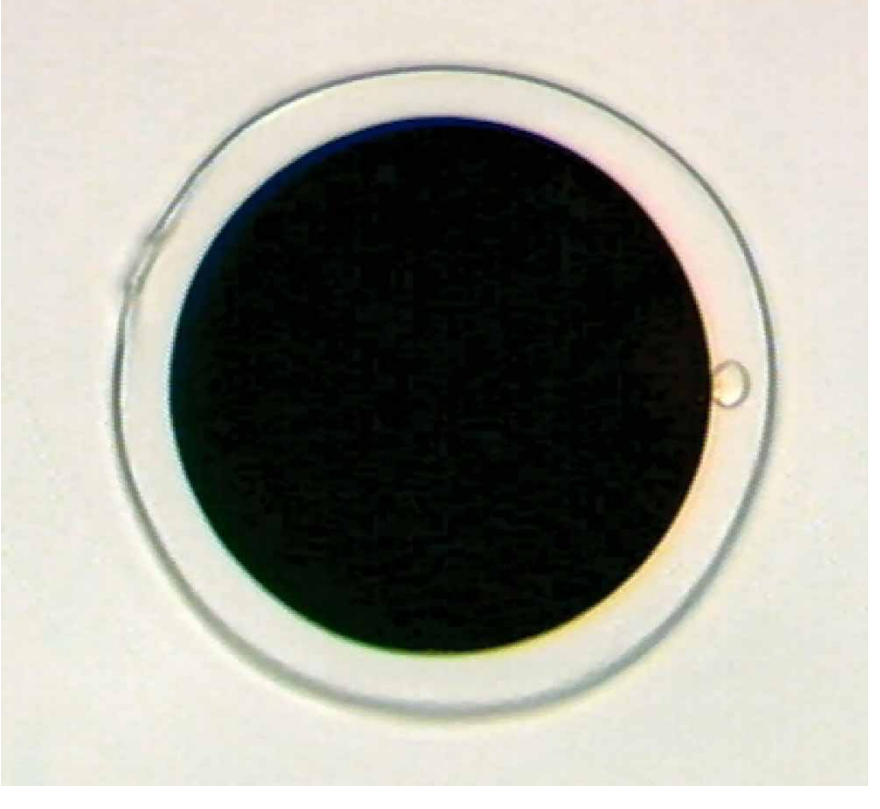

Figure 4. An abalone egg after fertilisation showing the polar body peripherally located in the perivitellinic space.

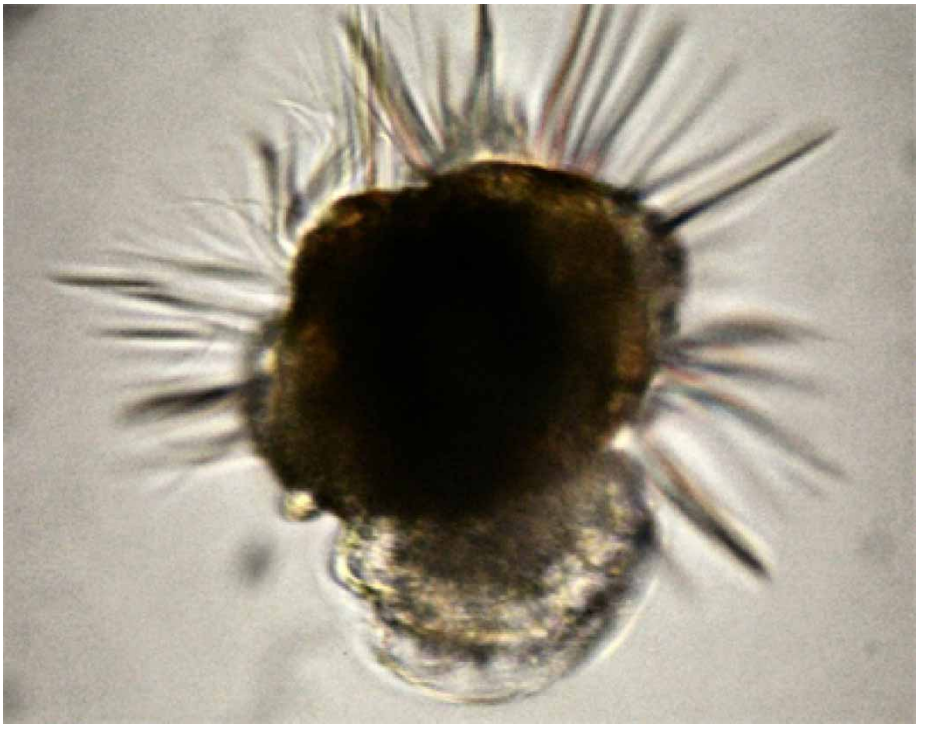

Figure 5. Trochophore larva.

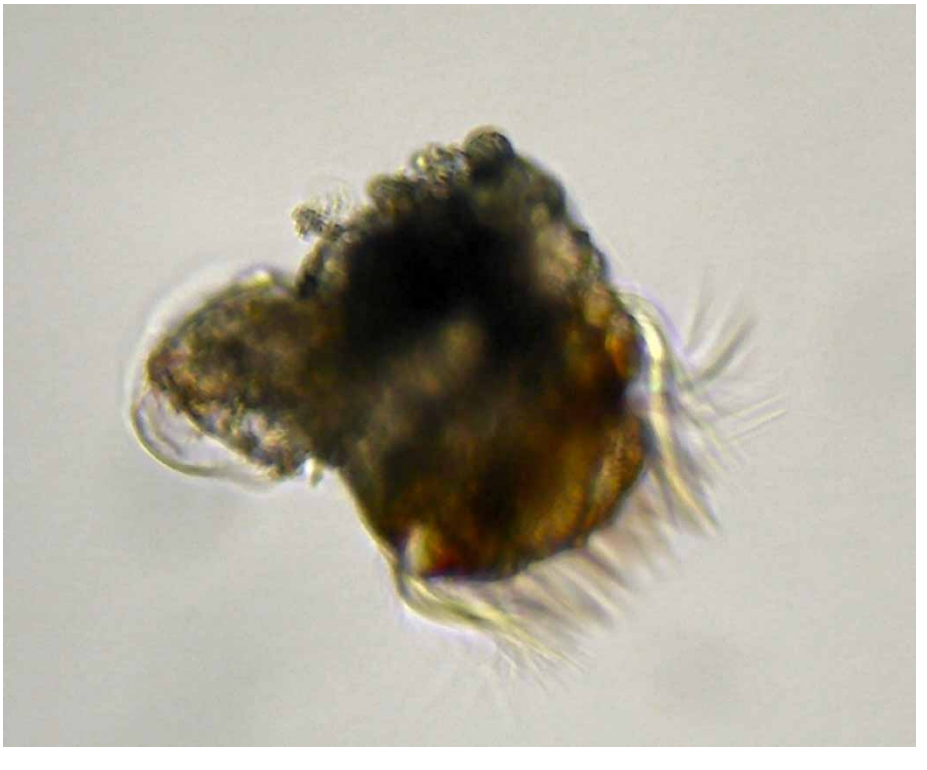

Figure 6. Veliger.

Moreover, we underline the possible impacts imaginable after future hypothesis of active repopulation actions with direct introduction of juveniles in the sea, considering we use wild adults as spawners, avoiding impact on genetic of local populations. Data reported in the present paper, even if only preliminary findings could be considered as a milestone in the development of the artificial reproduction of *H. tuberculata* in Italy. Further basic research is still needed particularly on the artificial induction of spawning in order to control both spawning time and fertilisation and to identify the factors controlling maturation and spawning of *H. tuberculata*. Up to now, we failed in obtain larval settlement. Already different settlement inducers, such as mucus, *Navicula* and mixed diatoms, have been proposed for other abalone species [12], even if a specific test must be performed to determine the most effective for *H. tuberculata* larvae. Larval settlements as well as artificial diets for use during on-growing of *H. tuberculata* juveniles will be the main themes for future studies aimed at developing this product in Italy.

Acknowledgements

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