



Abstracts: Sustainable Abalone Aquaculture Workshop

SUDEVAB final results,

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Summary of presentations



Assessment of uptake of food contamination organisms by abalone (*Haliotis tuberculata*) in actual production areas and in comparative laboratory tests.

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Regulation EC 854/2004 requires competent authorities to classify bivalve mollusc production areas. The Regulation by Annex identifies the application 'by analogy' to live echinoderms, live tunicates and live marine gastropods'. Additionally, ISO 6887-3 provides methods for the preparation of gastropod samples for microbiological examination. In practice European countries have regarded gastropods and by inclusion abalone as low risk although little evidence existed to back that view. As a consequence classified areas for abalone do not currently exist in the direct sense and the onus is on producers to maintain 'end quality standards'. The experimentation undertaken was in two forms. Firstly back to back sampling of product from two physically different farms (Jersey, Channel Islands, open seawater, and the Fal Estuary, Cornwall) and using the standard sampling procedures for designated waters with the species reared on those farms. The abalone consistently recorded less than 8% of the contamination recorded in 125 samples of co-cultured, *C.gigas*, *O.edulis* and *M.edulis*. The highest single sample was 42 *E.coli*/100g. The 'in field' sample assessment is that abalone have not been shown to exceed Category A designation even if placed in an environment that would put bivalves for consumption at the upper boundary of Category B (<4200 *E.coli* /100g). The second was a comparative challenge test between *C.gigas*/*H.tuberculata*/*M.edulis* using known initial concentrations of *E.coli* and Norovirus. The *E.coli* uptake and discharge of a 72hr period mirrored the 'in field' sampling data, with abalone consistently an order of magnitude lower than the bivalves. Virus was accumulated very quickly by oysters, reaching a plateau within 5 hours and possibly as quickly as 30mins. Mussels also accumulated very quickly but peaked much later at around 9 hours and concentrations were much lower than in oysters. Abalone appeared to accumulate much more slowly and no significant amounts of virus could be detected until 9-hour exposure. These results indicate that the competent authority general approach to gastropods and abalone specifically is justifiably based on lower health risks than with bivalves.

Key-words: *abalone*, *Haliotis tuberculata*, *food contamination*, *E coli*, *Norovirus*



Genetic variability and selection of abalone (*Haliotis tuberculata*) strains for a better resistance to their specific pathogens: polymorphism diagnostics in environmental studies on marine species

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Since 1998, episodic mass mortality of the abalone *Haliotis tuberculata* has been observed along the northern Brittany coast of France caused by a complex interaction among the host, pathogen and environmental factors. In the present study, abalones were submitted to two successive infections with the pathogen *Vibrio harveyi* under controlled conditions. During the first challenge, infection by *V. harveyi* resulted in 64% mortality of mature abalone. After a second infection of those surviving the first challenge, only 44% mortality was observed. Physiological variability in the host response appears to be a major determinant in susceptibility to *V. harveyi*. In order to isolate differentially expressed genes in *H. tuberculata* challenged with this bacterium, suppression subtractive hybridization (SSH) cDNA libraries were constructed from muscle of moribund abalone (susceptibles), surviving individuals (apparently resistant to the bacterium) and control (unexposed) animals. Of the 1,152 clones sequenced, 218 different partial cDNA sequences were obtained and represented 69 known genes. Of these, 65 were identified for the first time in *H. tuberculata*. Using real-time PCR, a time-course study was conducted on 19 of the genes identified by SSH. A majority of differentially expressed transcripts were down-regulated in susceptible individuals as compared to their resistant counterparts. Bacterial challenge of abalone resulted in the up-regulation of three transcripts (encoding ferritin, Heat Shock Protein HSP84 and Fatty Acid Binding Protein FABP) in those that survived exposure to *V. harveyi*.

Genetic markers have been developed for *H. tuberculata* genotyping by using a recent technique, High Resolution DNA Melting Analysis (HRMA), that moreover has never been used before this study for marine organisms. This method consists simply in a high flow screening and genotyping method based on melting curves analysis of amplicons, mutations modifying the curves compared to references. The entire procedure we proposed of HRMA in combination with qPCR is completed within 1h20 min as a single closed-tube assay that allowed in the same time to monitor PCR quality and to perform a scanning of more than 300 samples on two 384- or four 96-well plates on a LightCyclerTM 480 Instruments and will be a very useful technique to identify some spots of resistant populations in the field.

Key-words: *Haliotis tuberculata*, pathogens, *Vibrio harveyi*, gene variability, resistance



Identifying pathogens in farmed stocks

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Analyses based on histological examination, bacteriology and molecular techniques were carried out on juvenile and adult abalone during the course of SUDEVAB project. The objective was to define the health status of animals before making transfers in the different SME partners farming facilities. Sample examination allowed to report the presence of *Candidatus xenohaliotis californiensis* in abalone. This prokaryote was detected by PCR and its detection constituted the first evidence in French abalone. No other pathogen including *Vibrio harveyi* was observed in analysed samples. The susceptibility of abalone to *Perkinsus olseni* was investigated. No mortality was observed in abalone in contact with *P. olseni* during the course of the experiment. Thioglycollate analysis indicated that abalone appeared not infected. In comparison to *V. harveyi* ORM4, 6 strains of *Vibrio* isolated from French moribund oysters *Crassostrea gigas* presented a low pathogenicity to *H. tuberculata*. Mortality rates significantly different were observed between abalone injected with the OsHV-1 suspension and with the control suspension. However, to the view of mortality rates observed in control batches, the virus OsHV-1 seems not to be highly pathogenic to *H. tuberculata*. No significant mortality was evidenced in abalone incubated in presence of moribund oysters infected by OsHV-1. For specific detection of pathogenic strains of *V. harveyi* a real time PCR technique has been developed.

Key-words: *Haliotis tuberculata*, pathogens, *Candidatus xenohaliotis californiensis*, *Vibrio harveyi*, experimental infection, diagnostic, real time PCR



Polychaete infestation in farmed and wild abalones

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The abalone *Haliotis tuberculata* is a slow growing gastropod, so that epibionts can develop easily on its shell surface. For example, when the shell is colonized by polychaetes worms, the growth and health of the abalone may be threatened. The epibiont becomes a parasite and drastic measures need to be applied including good practices of farming, allowing to maintain acceptable growth rate and a good quality of abalone production. Polychaetes are marine worms, either predators or filter-feeding species. Filter-feeding polychaetes are sedentary worms belonging to the benthic fauna: they live in galleries that they burrow into calcareous rocks or mollusc shells. The burrowing activity of the worms can lead to serious damages of the shell, that make the abalone more vulnerable to external contaminants.

Key words: abalone *Haliotis tuberculata*, polychaetes infestation, shell damage, farming ,



Abalone shell damages caused by polychaetes worms.



Abalone cell cultures as tools for *in vitro* pathogenicity assays

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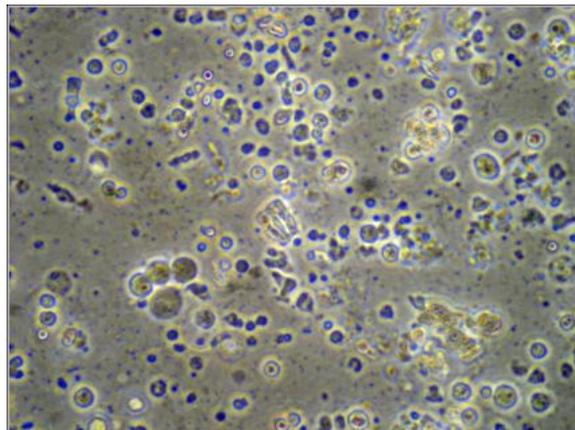
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Cell cultures provide alternative and controlled experimental models for fundamental studies as well as for various applications in developmental biology, physiology and host-pathogen studies. Here we report the development of primary cell cultures from abalone *Haliotis tuberculata* tissues and their use for studying pathogenicity at a cellular level. Primary cell cultures were developed from the gill which is a target tissue for two abalone parasites, *Vibrio harveyi* bacteria and the Ostreid Herpes Virus OsHV1. Cells were obtained using the explants culture method as previously described for abalone tissue culture. First, our investigations focused on cell characterization, intra-cellular metabolism and viability of cultured cells. Cytological staining as well as flow cytometry analysis was used to characterize the cell populations. The results showed that gill cells could be maintained in sub-cultures over 13 days with a significant metabolic activity. In a second part, we report the use of gill cell cultures to investigate the mode of action of both pathogens at the cellular level. The results showed that in the presence of *V. harveyi* bacteria, phenol-oxydase activity of gill cells increased during the four first hours of contact and strongly decreased afterwards. Flow cytometry experiments are in progress to investigate furthermore the interaction between gill cells and abalone pathogens.

To conclude, primary short-term cultures derived from target tissues are suitable tools for *in vitro* investigations and open the way to innovative biotechnological assays for abalone aquaculture.

Key-words: *abalone, Haliotis tuberculata, gill cell culture, Vibrio harveyi, OsHV1, in vitro assays*



Four-day-old explant primary culture of abalone gills.



Genetic resources of European abalone

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The aim of the work was to study genetic diversity and structuration of the European abalone (*Haliotis tuberculata*) populations using molecular markers (mitochondrial gene, microsatellite and nuclear markers). 21 populations of this species from Canary Islands to Normandy and from the Mediterranean sea were collected and analyzed. By using different analysis a phylogeographic scenario of evolution was proposed taking into account the variation of the level of the sea and the currents changes during the Pleistocene. The COI results evidenced the presence of two groups of sequences which did not fit with the two subspecies defined by morphological traits and geographical localization; *H.t. tuberculata* in Northern Europe and *H. t. coccinea* in the Canary Islands. The existence of a mitochondrial introgression was characterized in the two subspecies. The results obtained with the microsatellites markers evidenced the presence of three genetic clusters located in Northern Atlantic, Canary Islands and Mediterranean sea. In this later region, especially in Banyuls, a recent mixture with Atlantic cluster was demonstrated whereas, in the Adriatic sea, an isolation of the population was evidenced. These results were confirmed by using another nuclear marker: a lysin protein gene which presented a specific signature. These results will be useful for further aquaculture improvement programs.

Key-words: European abalone, *Haliotis tuberculata*, genetic resources, diversity, structuration



The European Abalone: first steps of a MAS (Marker Assisted Selection) program

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As the European abalone becomes of high economical importance in Europe and especially in France, the aim of our work was to initiate a MAS (Marker Assisted Selection) program for this marine gastropod. As we worked on farming conditions, we disposed of ten families issued from different crosses, supposedly being full-sib families. The first step was to determine the parentage of the descents by using microsatellite markers. Three families were showed to have a pluri-parental origin, and seven families were compound of full-sib individuals. We used these seven families to study different traits of interest and obtain preliminary results concerning weight, size, and color. These traits presented a unimodal distribution revealing a polygenic determinism. Concerning the size parameter, we evidenced a high heritability for length, width and weight (0.37, 0.29 and 0.40 respectively) with an important correlation between them, but a low heritability for condition factor and length/width (0.12 and 0.02 respectively). Concerning the shell color trait, a medium to high heritability was evidenced for the red color and hue (0.33 and 0.20 respectively) with a high correlation between them. No correlation was evidenced between size and color traits. To obtain more precise results concerning these traits, 14 new full-sib families were created and will permit to study more precisely the different characters.

Key-words : European abalone, Haliotis tuberculata, selection, heritability



First step of genetic selection in France

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First technical results of the production of 14 selected families are presented. These are the first step towards the domestication of European abalone. The main technical difficulties were identified and technical solutions were provided. The first assessment of the families during the first 90 days after settlement provided information about the influence of the culture environment on the performances. The future directions to give to the program are discussed.



Seaweeds, food for thought !

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The increasing demand from the industry for macroalgae biomass as a source of bioactive molecules, animal feed and/or for biofuel production, is helping to develop intensive sea and tank cultivation of various macroalgae species all over Europe. Seaweed cultivation trials of relevance to abalone feed were performed in Ireland (long-line cultivation) and Gran Canaria (tank cultivation). In Ireland at the reproductive season for each species, seaweeds were brought to the laboratory, spores were released and after a few weeks seeded long-lines were brought at sea. Seaweeds were placed next to salmon cages. In Gran Canaria, four trials were carried out evaluating the biological indexes of the seaweeds *Gracilaria cornea* J. Agardh, *Hypnea spinella* (C. Agardh) Kützing and *Ulva rigida* J. Agardh under different rearing conditions. Maximum growth rate and yield were obtained at a low initial stocking density and high water exchange. Production was significantly highest in *G. cornea* whereas *U. rigida* showed the highest growth rate. The present study examined the nutritional variability of the macroalgae by analyzing the biochemical composition as well as the fatty acid profiles of, fresh and dried, macroalgal species present in all the partners' states. Differences between, drying method, seasons, locations, nutrients enrichment and species were studied. Proximate biochemical compositions as well as fatty acids profiles were characteristic of each macroalgal classes and were influenced by seasons, nutrient enrichment and drying processes.

Key-words : Seaweed production, long-line, biofilters, growth rate nutrients enrichment, proximate biochemical composition, fatty acids





Development of various seaweed-based diets and their suitability for European abalone *Haliotis tuberculata* spp.

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In order to develop a sustainable, ecobased, low-cost artificial diet, based on vegetable sources and matching abalone nutritional requirements, more than 70 publications related to the nutrition of various species of abalone in different countries have been reviewed. Results showed that the proximate composition of artificial diets tested averaged: 30% crude protein; 2-5% lipid content and 45% carbohydrate whereas the caloric content was generally around 4 Kcal.g⁻¹. The nutritional values of the seaweeds *Palmaria palmata*, *Laminaria digitata*, *Gracilaria* sp. and *Ulva* sp. were examined in order to estimate their potential inclusion in vegetal based artificial diets for abalone, *H. tuberculata* spp. Three isoproteic and isoenergetic, seaweed-based, diets, with proximate biochemical compositions matching the ones of other artificial diets according to the revision, were formulated and processed. These experimental diets are actually tested against two control diets; one mixed algal diet containing fresh *Gracilaria cornea* and *Ulva rigida*, and a commercial one. Biochemical composition and energy content of the diets have been analysed and will be related to survival, growth performance and feed utilization by the animals.

Key words: Macro-algae, growth performance, European abalone, *H. tuberculata* spp., *Gracilaria* sp., *Palmaria palmata*, *Laminaria digitata*, *Ulva* sp., artificial diets.





Commercial farming trials of abalone (*Haliotis tuberculata*) in offshore and intertidal sea cages: technical results

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In the context of SUDEVAB, four commercial cage trials were programmed to assess the economical and technical feasibility of abalone farming in four different European countries. Three types of cages were tested: lanterns, ORTAC and large offshore cage. Three trials were successfully set up in western France (Aber Wrac'h), South western England (Portland) and South of Jersey in the Channel. The set up of a fourth trial in Spain aborted in the early stage of the set up due to very high handling mortalities in the first 2 weeks. The trial lasted from 18 to 21 months. Mortality was mostly observed in the early stage of the experiment probably associated to initial handling stresses. It ranged from 0 to 73% mortality. Growth ranged from 0.23 to 1.90 mm per month with the best growth obtained in France. The biomass gained over the 21 months of experiment ranged from 0 to 2 kg of abalone per square meter of available habitat. At a given density of abalone, the best biomass gain per square meter of available habitat was obtained in ORTAC cages in an offshore set up. Lantern cage were rapidly disqualified because of their fragility unadapted to intertidal and offshore set up. Large offshore cages were unadapted to intertidal set up but were very efficient to reduce labour feeding cost in offshore set up. Despite the very variable results between experimental sites, it appeared quite clearly that experience and know how are a key to success. Production cycle to reach 45 to 50 gr abalone from 20mm spat was evaluated at 3 years. Abalone farming remains a very “technical” activity.

Key words: European abalone, Haliotis tuberculata farming trials, sea cages





European abalone marketing

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For aquaculture to be sustainable, a project has to be able to make money to repay its investment. To achieve this, a project has to be designed to function technically, but also to produce products that are in demand from the market. A project therefore should be a market led development – just because the technology exists to produce a product does not mean that a project will be economically successful unless the product it produces sells for a profit. Market analysis is there to ensure that the markets exist and that the demand and prices will provide the expected returns which will sustain the project development.

In the case of market analysis for abalone, the market data is difficult to obtain, clouded in secrecy, and has a large volume of “black” traded products being fished illegally due to high market prices. During SUDEVAB, extensive market data was collected to assess products available, prices in worldwide markets, volumes traded from aquaculture and wild production, and the future standards that have been developed by the WWF abalone dialogue for sustainable abalone production. In Work Package 8 an assessment was also undertaken to understand and estimate the potential demand for abalone in European markets and the type of products the European gastronomy sector desired. The marketing study concluded that there is a strong demand for “live” quality European abalone in Europe, and these products were also seen as premium products in the major Far Eastern markets.

Key words: European abalone, Haliotis tuberculata, aquaculture, wild production, market analysis, gastronomy



Technical and economical analysis of three types of abalone farming systems in Europe

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The technical and economic operation of three types of abalone farms were analyzed to better understand the potential of halioticulture in Europe. Slower growth are obtained by sea-farming compared to farmed land in semi-recirculating farms. The control of temperature and photoperiod allows for better growth performance and shorten the production cycle. However, the labour required for feeding and cleaning of tanks is more important in semi-recirculating systems. The rearing at sea requires the use of natural and live food, the algae that are more expensive but can also reduce maintenance time throughout the production cycle. The choice of farming system based on several technical and financial criteria such as the production site or the level of investment ... Abalone farming is a long-term economic adventure with many risks. The risks of long cycles that are often incompatible with the usual economic rhythms are also presented.

Key words: *European abalone, Haliotis tuberculata , farming systems, technical and economical analysis*