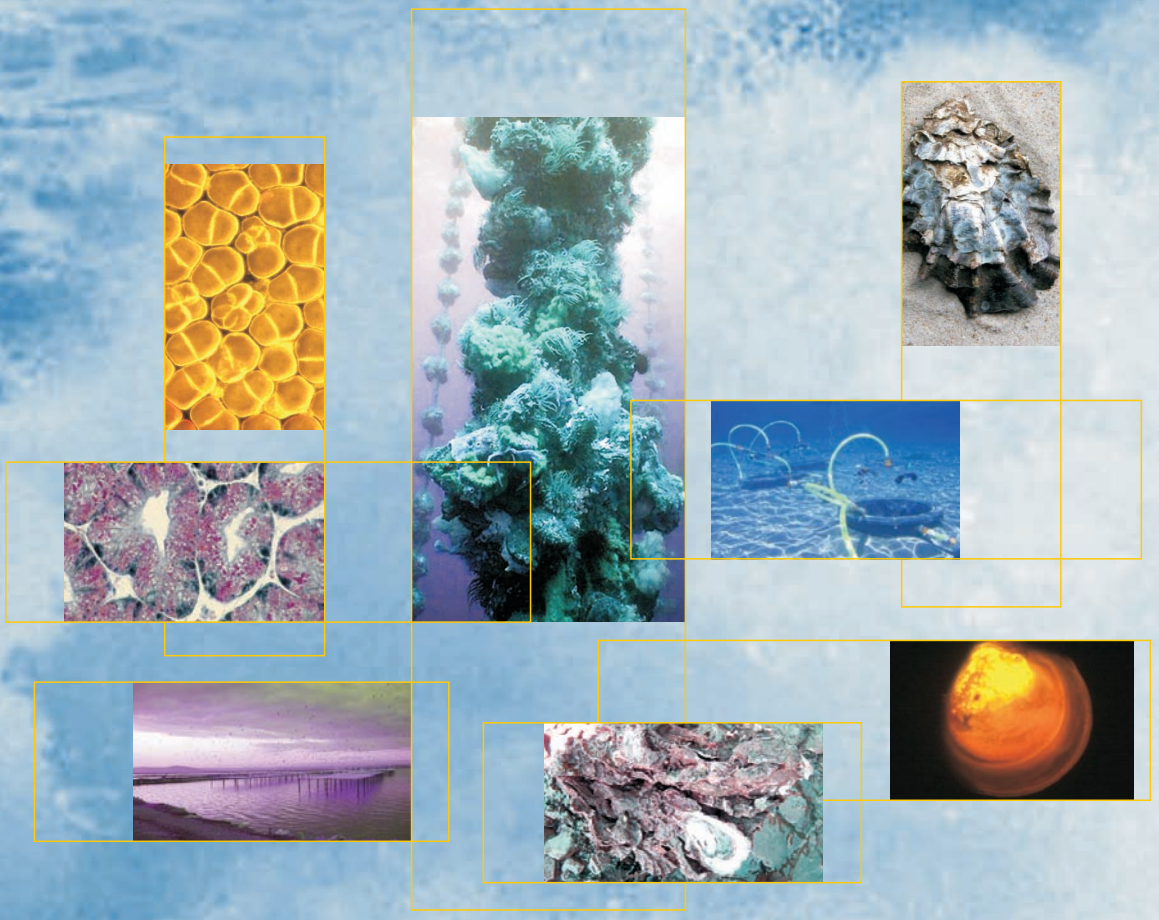
Physiomar 08

Physiological aspects of reproduction, nutrition and growth

"Marine molluscs in a changing environment"



Book of abstracts

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PHYSIOMAR 08

"Marine molluscs in a changing environment"

2nd marine mollusc physiology conference

Brest, France, September 1-4, 2008

Compiled by : M. Gaasbeek and H. McCombie

Editor : Ifremer

Welcome to Physiomar 2008

Welcome to the Physiomar 2008 conference and to Brest!

*This second mollusc physiology meeting is being organized jointly by **LEMAR** (**IUEM:** European Institute for Marine Studies) and **LPI** (**Ifremer**: French Institute for the Exploitation of the Sea), as both laboratories conduct research in this field.*

Physiomar 08 follows the very successful workshop 'Physiological aspects of reproduction and nutrition in marine mollusks' held in La Paz (Mexico) in November 2006 under the guidance of Maria Concepcion Lora Vilchis and Elena Palacios Metchenov from CIBNOR.

*This year's meeting will focus on **Reproduction**, **Growth**, **Nutrition** and **Genetics and genomics of marine molluscs**, and include a specific session on the effects of climate change on mollusc physiology: **Global change**.*

This conference will serve as a forum for the communication of recent advances in all aspects of the physiology in molluscs. Researchers, students, stakeholders and mollusc farmers will have the opportunity to communicate personally in a friendly environment with leaders in the field. This meeting aims to provide a vehicle for continuing exchanges, cooperation, and collaboration in the field of the physiology of marine molluscs to better understand their adaptation ability in a changing environment, their ecological significance, and to promote their sustainable culture.

Contents:

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Conference overview

Sunday 31 August		
Starting 17.00	Registration	Espace Vauban
18.30 - 23.00	Welcome buffet	Espace Vauban
Monday 1 September		
09.00 - 09.30	Introduction	IUEM
09.30 - 12.30	Nutrition	IUEM
12.30 - 14.00	Lunch	Restaurant Universitaire Plouzane
14.00 - 16.15	Genetics and genomics	IUEM
16.15 – 17.45	Poster session	IUEM
18.00 – 19.00	Welcome Aperitif	Ifremer
Tuesday 2 September		
08.45 - 10.30	Genetics and genomics	IUEM
11.00 - 12.30	Gobal change	IUEM
12.30 - 14.00	Lunch	Restaurant Universitaire Plouzane
14.00 - 16.30	Global change	IUEM
16.30 - 17.30	Poster session	IUEM
18.00 - 19.30	Welcome Aperitif	City Hall
Wednesday 3 September		
08.45 - 12.30	Growth	IUEM
12.30 - 14.00	Lunch	Restaurant Universitaire Plouzane
14.00 - 16.15	Growth	IUEM
16.15 - 17.30	Poster session	IUEM
20.00 - 23.00	Gala dinner	ARMEN Restaurant
Thursday 4 September		
09.00 - 12.30	Reproduction	IUEM
12.30 - 14.00	Lunch	Restaurant Universitaire Plouzane
14.00 - 15.00	Reproduction	IUEM
15.00	Conclusions	IUEM

The grey colour indicates activities in Brest city centre.

Oral presention abstracts

Abstracts of all presentations are listed below (all subject sessions together) in alphabetical order of the first author's last name. For papers with several authors, the name of the presenting author is underlined.

Preliminary spat recruitment of fan mussel (*Pinna nobilis*, Linnaeus 1758) on mesh bag collector in Karantina Island, Aegean Sea Turkey

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Keywords: Pinna nobilis, fan mussel, spat recruitment, collector, Aegean Sea

The fan mussel *Pinna nobilis* is a particularly important species in the Mediterranean Sea. The population of *Pinna nobilis* has been greatly reduced recently in Turkey as the other countries. It has been listed as an endangered species in the Mediterranean. For this reason, studies on *Pinna nobilis* are important for supporting populations in natural areas. Studies cover aspects such as spat recruitment, planting young individuals, reproductive cycle in selected areas, etc.

The aim of this study was to determine the most suitable time to attach fan mussel spat from natural populations. We evaluated experimental collection of *Pinna nobilis* spat on mesh bag substrates in Karantina Island, Aegean Sea, Turkey during a year. Spat collectors were deployed at 8 m deep every month at the study area. Spat-set were estimated as number of fan mussel per square meter of mesh bag after a month. Environmental parameters (temperature, salinity, particulate organic matter, particulate inorganic matter and chlorophyll-a) were recorded during the experiment .The average number of fan mussel spat was the highest 122 spat/m^2 in July. Spat data showed important variations between outside and inside of each mesh bag collector and also deployment time ($P < 0.05$). According to the results of spat recruitment we can say that reproductive period of fan mussel in Izmir Bay is in the summer months.

Reproductive patterns of the queen conch, *Strombus gigas* in various sites from the Caribbean region: management measures for its fishery

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Keywords: Strombus gigas, wild population reproductive cycle, management measures

The queen conch, *Strombus gigas* is a species of primary economic importance in the Caribbean region. Its importance comes from local demand for inner consumption and the tourist market as well as from export to USA and French Caribbean islands which consume most of the production exported by several Caribbean countries. Its populations have been depleted in many areas and seriously diminished in others. Therefore, various measures have been taken to regulate exploitation, which include minimum catch size, based on shell length, catch quotas, temporal or permanent fishing bans. However the bans not all the time correspond to the reproductive period. This is the same situation for the minimum catch size; this management measure was established using the shell length in most countries. Few countries have used the shell lip presence as a management measure. Frenkiel et al (2007) demonstrated a relationship between the first gonad development and the lip thickness for *S.gigas*. In order to analyze the reproductive pattern of the species and to determine the variability of its reproductive cycle under diverse environmental conditions in the Caribbean, data of the reproductive cycle were analyzed for Alacranes reef on the Gulf of Mexico coast of Yucatan, Mexico; San Pedro bank, Belize; San Andrès Island, Colombia and Guadeloupe, French West Indies. Data showed that the minimal catch size does not appear as mandatory; the existence of a thin shell lip is neither an efficient criteria of sexual maturity. Only conchs with a lip thickness >5 mm appear as reproductive organisms. Therefore, it is necessary to introduce this criterion to regulate the stock of this species. Finally different management measures for *S. gigas* were analyzed versus its reproductive biology. Based on this study, a temporal ban from 1st May to 30 September is proposed and a minimum catch size of 6mm of lip thickness which seems to be well fitted to protect the main reproduction period and to improve the reproductive stock. This study showing that conchs from 30-40 m depth have a normal reproductive cycle, in order to protect this reproductive stock it will be necessary to prohibit fishing with scuba gear and nets. Nets catching adults and juveniles appear as especially noxious. To be efficient these regulations have to be applied in all Caribbean countries and to be included in the CITES regulations for exportation. However, the level of protection will depend on a strict enforcement of regulations to control exploitation and reduce illegal fishing.

Development of functional genomics tools for the selection of QPX-resistant hard clam stocks

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Keywords: clam, parasite, SSH, cDNA libraries, microarray

The success or failure of shellfish restoration and aquaculture efforts largely depends upon the resistance of livestock to parasites and diseases. The hard clam or northern quahog, *Mercenaria mercenaria*, is one of the most commercially important bivalves in the United States. Over the last decade, several northeastern states have suffered severe losses in wild and aquacultured hard clam stocks due to a fatal disease caused by Quahog Parasite Unknown (QPX). Recent studies demonstrated variations in the susceptibility toward QPX among different hard clam stocks suggesting a genetic origin of clam resistance. Further progress in understanding factors affecting QPX disease development is limited by the lack of information regarding clam immune response toward the infection. Specifically, genetic information available on clams (all species combined) in public databases is extremely limited, representing only about 1% of information available on oysters. In the past few years, we have been actively working toward the identification of clam immune-related genes using functional genomics approaches. Differential expression techniques (subtractive suppressive hybridization or SSH) and standard methodologies (cDNA libraries from clam hemocytes) were used to identify and quantify differentially expressed genes in infected clams. These investigations allowed the sequencing of 2,720 cDNA clones and generated genetic information on 984 unique genes that were used to develop the first hard clam expression microarray. These studies already allowed the identification of several genes that are modulated by the exposure to QPX or that represent promising potential markers of clam resistance toward the parasite. Sequencing effort is ongoing to generate additional genetic information from SSH and hemocytes libraries and to increase the number of sequences spotted on the microarray. This approach is expected to allow the discovery of biomarkers and molecular mechanisms characteristic of both resistance to, and dysfunctions caused by, QPX; and will likely lead to the development and validation of test systems that are cost- and time-efficient for the screening and selection of clam stocks.

Clams in hot water: effect of temperature on surfclam, *Spisula solidissima*, reproduction and recruitment

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Keywords: global change, ecophysiology, reproduction, nutrients

The Atlantic surfclam, *Spisula solidissima*, is a commercially important bivalve in Northeastern Unites States that supports a multimillion dollar fishery. Recent surveys realized in federal waters found a significant decline in surfclam biomass off Delaware, Maryland and Virginia and in Southern New Jersey. Moreover, state surveys within three miles of the coastline, especially along the New Jersey coasts, also found dramatic declines. Recent studies suggested that increasing temperatures in the Mid-Atlantic Bight associated with global temperature rise might significantly influence surfclam growth and mortality. Temperature would be responsible for physiological stress resulting in low tissue weights and slow growth rates. Our study focused on surfclam population in New York State waters, off the southern coast of Long Island. Results of surfclam population surveys showed that the population dropped from 18.6 million industry bushels in 2002 to 10.1 in 2005 and only 9.5 million in 2006. Further analysis showed a significant decrease of juvenile clams from the population, indicating poor recruitments. Our preliminary results also show negative correlation between year-class strength and water temperature during spring. Thus, seawater temperatures exceeding 7°C in spring were associated with poor recruitments. Moreover, histopathological analysis of surveyed clams revealed the presence of significant levels of anomalous gonadal development. Specifically, unusually high levels of ova resorption were observed in clams that did not fully mature, did not spawn, or in those in early spawning phases. This abnormal development of gonad is characterized by an intense hemocytes infiltration into gonad tissues and the presence of degenerating (atretic) gametes in affected follicles. This condition appeared in clams collected in late spring and increased significantly during summer months (up to 67% of females). Both field and lab experiments are ongoing to assess direct (effect on clam's physiology) and indirect (through alterations of phytoplankton quality and quantity) effects of temperature on surfclam fitness and reproductive success.

Effect of flow rate on growth, biochemical composition of the mussel *Mytella strigata* and the penshell *Atrina maura*

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Keywords: flow, filtration, growth rate, biochemical composition

The growing interest in finding suitable bivalves for aquaculture along the Mexican Pacific coast has given some attention to the effect of temperature, salinity and seston concentration on growth and survival, but little attention has been given to influence of the flow rate on such species. Flow is a variable with a profound effect on growth and survival in filter and deposit feeders. Depending on species, environmental conditions and seston concentration, increases in speed enhance filtration at very low speeds up to a maximum, beyond which speed increase has no further effect on filtration because other processes limit it. At higher speeds, flow begins to have a negative effect on the filtration process, suppressing it by mantle edge closure or withdrawal of siphons in infaunal bivalves.

The effect of three duplicated water flow rates (3.1, 7.3, and 12.1 cm s^{-1}) on growth, biochemical composition, and mortality of the mussel *Mytella strigat*a and the penshell *Atrina maura*, was evaluated over 24 days placed a multiflume apparatus using water from the gate of a tidal pond. Biometric parameters were measured at the beginning and end of the experiment. Environmental parameters (temperature, salinity, total particulate, organic (POM) and inorganic (PIM) particulate material, POM/PIM ratio, suspended particle concentration and size, chlorophyll *a* and ammonium) were measured daily at the inlet and the outlet of the channels of each treatment. Biochemical composition was determined in crude extracts from frozen tissues (-80ºC) and lyophilized. Data were statistically analyzed using a one way ANOVA, *post-hoc* analysis (Scheffe) were used at $P < 0.05$ significance level.

Both species showed significant differences in shell growth among flow rates ($P<0.05$). Growth rates were a maximum at 3.1 cm s^{-1} for *M. strigata* and at 7.3 and 12.1 cm s^{-1} for *A. maura*. There were statistical differences ($P < 0.05$) between initial and final data of almost all biometric parameters measured in both species. *M. strigata* grew significantly ($P < 0.05$) faster at 3.1 cm s-1 than at the other flow rates studied when measured using shell dimensions. However, the experimental time (24 days) was not long enough to produce significant differences in wet and dry tissue mass. In *A. maura*, growth was significantly ($P < 0.05$) faster at 7.3 cm s-1 and 12.1 cm s^{-1} in all biometric parameters measured, except in dry tissue mass where differences between treatments were not significant ($P > 0.05$). Mortality average was 3 ± 0.6% in *M. strigata* and 9.5 ± 4.6% in *A. maura*, no differences were detected between flow rates ($P > 0.05$).

The preferential flow rates were 3.1 cm s^{-1} for *M. strigata* and 7.3 cm s^{-1} and 12.1 cm s^{-1} for *A. maura*. Both species are able to withstand high seston loads and temperatures typical of mangrove swamps and channels of tropical regions. *A. maura* may be able serve as a biofilter for shrimp-farm effluents because this species withstands high seston concentrations, temperature, and flow speeds. *M. strigata* used digestive gland proteins, carbohydrates, and glycogen to support somatic growth at a preferred flow rate of 3.1 cm s^{-1}, whereas *A. maura* used digestive gland, adductor muscle, and mantle proteins and total lipids for growth at the preferred flow rates of 7.3 cm s^{-1} and 12.1 cm s^{-1}.

Secretion of byssal threads in *Mytilus galloprovincialis*: quantitative and qualitative values after spawning stress

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Keywords: Mytilus galloprovincialis, byssogenesis, attachment strength, spawning

Mussels have the ability to secrete byssal threads through their foot organs that ensure a secure attachment point to the substratum in nature. Secretion of byssus represents a dynamic process widely described and may represent up to up 8-15% of total energy expenditure. The whole thread structure is mainly collagenous but the distal part has a supplementary composition in alanine and glycine that make it similar to silk fibroin whereas proximal section has additional components similar to those encountered in elastin. Both proximal and distal sections have common histidine-rich residues at their terminal flanking domains and specifically in the case of the byssal collagens, metal chelate complexes joining Zn^{2+}, Cu^{2+} and Fe^{2+} represent a significant cross-link alternative involving mainly histidine (among others) that gives integrity and strength to the byssus apparatus.

Byssogenesis process in mussels is influenced by a number of both exogenous and endogenous factors. Initially, emphasis was focused on the importance of abiotic factors, specifically the environmental hydrodynamics. However, other factors may also help to explain such variability by establishing a link with the animal's energetic status and the quality of the secretion.

Spawning events in mussels may cause of a number of perturbations in several physiological rates because of the abrupt change in the soft tissues state by gamete release. Under such stress, mussels still need to renovate the byssal apparatus permanently due to thread's ageing in order to keep an optimal attachment strength. We have tested under laboratory controlled conditions the hypothesis that byssus secretion of *M. galloprovincialis* and its attachment strength are negatively affected by the spawning in laboratory. Accordingly, we have followed both quantitative and qualitative aspects of byssus secretion.

Mussels forced to spawn showed a significant lower byssogenesis rates ($P<0.05$) as compared to unspawned individuals and this result was obtained regardless feeding regimes used. Surprisingly, force needed to dislodge mussels did not follow an exact pattern of that of thread's secretion number. Attachment force varied within a narrow range (1.7-1.8 N) and the only exception was a significant drop in recently spawned and kept unfed mussels (1.1 N; $P<0.001$) most likely as consequence of a slight but significant lower thread's thickness value for the latter experimental group (73.6µm) as compared to the rest of experimental individuals (80-83 µm).

Qualitative analysis concerning to the amino acid composition of the byssus highlighted that the basic residues histidine and lysine represented the residues with the major differences between experimental groups. Threads secreted by recently spawned and normally fed mussels were characterised by significantly higher percentage of both histidine and lysine residues ($P<0.01$) as compared to unspawned fed animals. Both amino acid residues dropped also significantly with the non-feeding period of individuals but such decrease was stronger in spawned individuals. Histidine and lysine residues are clearly associated to the formation of cross-links, joining two or more molecules by a covalent bond and specifically histidine has a functionality with a pronounced effect on metal chelation and/or cross-link ability to stabilise the integrity of the byssus structure. Results obtained here highlighted the importance of the qualitative analyses of the byssus secreted by the mussels when subjected to stress i.e. spawning events in order to understand plasticity of this secretion to eventually maintain an optimal attachment force values. Quantitative values of byssus secretion were clearly related to the state of the soft tissues of the individuals whereas amino acid composition of threads pointed out that certain changes in the proportion of specific residues may infer the actual potential to ensure optimal attachment strength under endogenous stress.

Effects of two oils on immune parameters and on the expression of immune related genes in the Pacific oyster, *Crassostrea gigas*.

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Keywords: Crassostrea gigas; gene expression; immune parameters, refined oil products

During the last decades, oil spills have been observed on the Atlantic coast, like the *Erika* tanker accident which spilt 20 000 tonnes of its Heavy Fuel Oil (HFO) into the sea or the *Prestige* accident in November 2002 with a cargo of 63 000 tonnes of HFO. In the immediate aftermath of the spills, damages attributed to oil were measured in several bivalve species like immune functions, which are described by some authors as being modulated by hydrocarbons. In this context, the effects of two types of refined products, HFO and light cycle oils (LCOs), were researched on the immune system of Pacific oysters, *Crassostrea gigas*. In a first contamination experiment, HFO (652 $ng.L^{-1}$) was applied *in vivo* to oysters for seven days, then a recovery period of two weeks took place. For a second experiment, LCOs (1 100 $ng.L^{-1}$) were tested with the same experimental design. The *in vivo* effects of the soluble fraction obtained were investigated on two haemocyte parameters (cell mortality and phagocytosis activity) by flow cytometry and on one haemolymphatic parameter (phenoloxidase activity) by spectrophotometry. Moreover, expression of immune related genes (laccase, macrophage express protein, myeloïd differentiation factor and molluscan defence precursor) was monitored by real time RT PCR. The HFO exposure induced a modulation of each haemocyte and haemolymphatic parameter and of the expression of three genes. The LCO exposure induced only the modulation of phagocytosis percentage and phenoloxidase activity; nevertheless modification of the expression of all genes tested was noted. A comparison between both types of refined products will be discussed according to the hydrocarbons found. To conclude, modulation of immune-related parameters was demonstrated using three different methods (flow cytometry, spectrophotometry and gene expression) in Pacific oysters after contact with two different oil types. However, demonstration of the link between modulation of immune-related parameters and immuno-competency requires experiments including combined exposure to pollutants and pathogens.

The cardiac activity of two species of Bivalvia in long-term experiment in the field.

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Keywords: heart rate; M. edulis; M. modiolus; oscillation; sublittoral

The cardiac activity of marine invertebrates has been studied extensively over the last 30 years. However, few attempts to study the heart function in the field have been made. It is suggested that lab conditions may mask the physiological functions due to stress. Besides, it is extremely interesting to check the heart activity and, consequently, the functions of the whole organism under severe winter conditions. The method of non-invasive recording of cardiac activity and the unique conditions in the study area (the White Sea) help cast light on these issues.

The dummy species *Mytilus edulis* L. and the typical subtidal mollusc *Modiolus modiolus* L. were selected for the study. The temperature and salinity during the experiment was –1.2±0.2 and 25.0±0.5 ppt, respectively. Recordings of heartbeat traces (duration 2 min, sampling frequency 12.5 Hz) were obtained for each mollusc at 30 min intervals (limit of method) during 3 days of the experiment. To estimate the oscillation of heart rate and cardiac signal amplitude, individual heartbeat traces were subjected to the Fast Fourier Transform (FFT) and Continuous Wavelet Transform (CWT).

Unique data on the cardiac activity of the animals were obtained. The absence of cardiac arrest was shown for both species for the first time. Another interesting point was that the animals were able to maintain the heart function on a relatively high level – 3.49±1.01 and 5.26±0.78 beats per min in *M. modiolus* and *M. edulis*, respectively. If we compare the heart rates of the animals at a temperature of 10^{o}C (9.0 and 15.0 beats per min), then it becomes clear that in spite of a drastic decrease in ambient temperature in the winter season, the heart activity declines only approximately three-fold. Also found for the first time was a significant negative correlation between heart rate and total shell length in *M. edulis*, but not for the *M. modiolus*.

Three types of oscillations for both heart rate and amplitude signal with wavelengths of 3-6, 9-16 and 18-25 hours were identified for both *M. edulis* and *M. modiolus* using FFT and CWT. It has been shown for both species that the peaks (maxima) of the periodic constituents of heart rate and amplitude signal of 3-6 h arise at ca. 3 h intervals. The oscillations were about 1 h long with an interval of approximately 1 h between them. These amplitude and frequency peaks coincided in time and arose 1-1.5 h before and 1-1.5 h after high tide (the same applies to low tide). Thus, in statu nascendi of 3-6 hour oscillation flow velocity varies from 6 to 8 cm/sec (Babkov, 1982). The heart rate and amplitude oscillations do not coincide in statu nascendi, except for 3-6 hour oscillations. Consequently, it is at these moments that the heartbeat is best coordinated. Obviously, it is during these periods of time that the heart supplies the largest volumes of hemolymph. It should be added that all other oscillations did not coincide with tidal or diurnal rhythms.

Physiological responses of the Manila clam, *Ruditapes philippinarum*, to the toxic dinoflagellate *Heterocapsa circularisquama*: feeding, respiration and growth

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Keywords: Ruditapes philippinarum, Heterocapsa circularisquama, feeding, respiration, scope for growth

Heterocapsa circularisquama is a novel toxic dinoflagellate species, forming massive and recurrent outbreaks along the western Japanese coasts, since 1988. These outbreaks have been associated with mass mortalities of at least 14 species of marine bivalves in a species-specific manner, occurring over a wide range of environmental conditions, notably temperature. Despite the huge economic loss caused by this harmful alga, studies on its physiological effects on marine bivalves are scarce and limited to the impacts on the clearance rate. Consequently, the aim of the present work was to assess feeding, respiration and growth of the Manila clam, *Ruditapes philippinarum*, exposed to relatively low concentrations of *H. circularisquama*, at three different temperatures.

Adult clams (N = 405; SL = 31.34 ±1.76 mm; DW = 0.25 ± 0.08 g), collected monthly from Tokyo Bay from May to July 2007, were acclimated for 7-10 days to the experimental conditions prior to measurements. Only the experimental temperature varied (15℃, 20℃ and 25℃), whereas other factors were set to 33‰, 8.00, and continuous illumination, for salinity, pH and light condition, respectively. For each temperature, the specific clearance rate (CRs, l h^{-1} g^{-1}), the specific respiration rate (RRs, mgO_2 h^{-1} g^{-1}), the absorption efficiency (AE, %), and the scope for growth (SFG, J $h^{-1}g^{-1}$) were estimated under exposure to *H. circularisquama* (5, 50, 500, 10^4 cells ml^{-1}) in mixture with *Isochrysis galbana.*Under control conditions, the CRs showed no significant difference at 15℃ and 20℃, but was significantly decreased at 25℃. On the other hand, the RRs increased significantly from 15℃ to 20℃, and a further increase in temperature had no effects on it. The AE was high at 15℃ and 20℃ and equal to 84% and 94%, respectively. At 25℃ however the AE was significantly decreased to less than 31%. The SFG was the highest at 15℃ and was especially decreased to negative values at 25℃ indicating a physiological stress at this temperature.

At 15℃, the feeding, respiration and food absorption were not affected by the presence of the toxic alga. However, the SFG was significantly decreased for cell concentrations of 250 cells ml^{-1} and above. For 20℃ and 25℃, the CRs and the RRs were significantly decreased, starting from a cell density of 5 cells ml^{-1}. At both temperatures, the AE became null starting form 250 cells ml^{-1}. The SFG was significantly reduced at 20℃ for all toxic alga concentrations. The SFG was negative for exposure at 25℃.

Decreased feeding and respiration imply gill damage. The cessation of food absorption at temperature exceeding 15℃ does not necessarily imply gastro-intestinal damage, and could be related to a malfunctioning of food processing through the damaged gills. Decreased feeding, respiration and food absorption may lead to a decrease in the energy reserves and thus a general weakening of the clams with possible death.

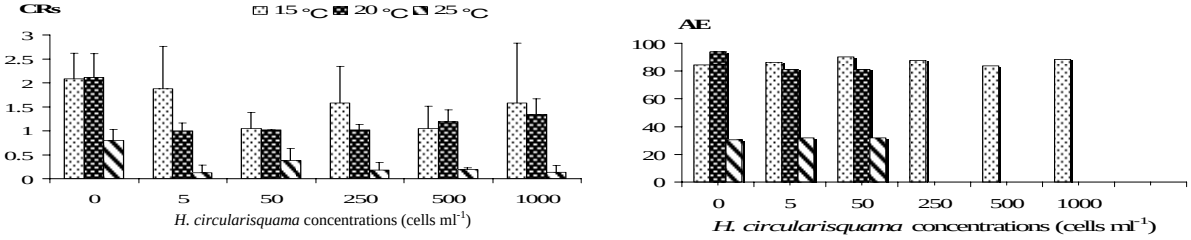

Fig. 1. Specific clearance rate (CRs, l h^{-1} g^{-1}) and absorption efficiency (AE, %) of adult *R. philippinarum* exposed to *H. circularisquama* at three different temperatures.

Short-term storage of tetraploid *Crassostrea gigas* spermatozoa to sustain commercial triploid spat production

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Keywords: triploid, sperm storage, fertilising ability, diagnosis, antibiotics, quantitative PCR

A large increase of triploid oyster spat production has been observed the last five years in France. This development was sustained by the use of large-scale hatchery techniques for fertilisation with sperm collected from tetraploid males. The aim of the present study was to test the possibility to defer tetraploid sperm collection from fertilization, in order to improve the broodstock management and the production protocol. Sperm from tetraploid pacific oysters was stored at 4°C in an appropriate medium, and maintenance of its motility, fertilizing ability, and sanitary quality was assessed upon storage time.

First, simple and objective methods were set up to reliably evaluate the main characteristics of sperm: motility, ploidy, sperm concentration and sperm fertilising ability. A standard procedure for experimental incubation of embryos was used to measure the capacity of each sperm sample to produce D larvae. Fertilization ability of fresh tetraploid oyster spermatozoa was lower than that of diploid males, as shown by the lower rate of triploid D larvae obtained.

Secondly, the storage condition at 4°C was set up in the perspective of its use at a commercial production scale. Packaging, spermatozoa concentration, storage medium and fertilisation protocol were tested to increase the larval rate at hatching after a 7-days storage period. The mean motility of spermatozoa decreased from 50 to 10 % after 7 days at 4°C. D-larval rates were not significantly different either between fresh and stored sperm samples over a 7-days storage or between sperm collected from diploid and tetraploid males.

Thirdly, the effectiveness of an antibiotic treatment to limit bacterial contaminations and proliferation in sperm samples was evaluated. A mixture of gentamicin and flumequin at 25 mg/L prevented samples from bacterial proliferation during storage at 4°C. We observed a bacteriostatic effect but not a total bactericidal effect of the antibiotic combination, even at doses as high as 100 mg/L. Sperm exposure to this antibiotic combination did not alter its fertilizing ability and did not affect early developmental quality of oyster larvae.

To evaluate the sanitary status of the stored spermatozoa samples and avoid the dissemination of pathogenic agents through gametes sample transfers, quantitative PCR protocols were developed for the detection of cupped oyster pathogens, *ie* Ostreid Herpevirus 1 (OsHV-1) and two *Vibrio* species (*V. splendidus* and *V. aestuarianus*). No significant correlation was found between OsHV-1 positive broodstock detection and the corresponding gametes among one hundred and fifty individuals tested. *Vibrio splendidus* and *V. aestuar*ianus were not detected in antibiotic treated samples contrarily to untreated controls.

Fourth, the use of stored tetraploid sperm was tested at commercial scale production by fertilising 150 millions oocytes batches in a commercial hatchery. The hatching rate observed in triploid larvae (48%) was significantly higher than the diploid control one (30%).

The methodology for sperm conservation developed in this work proved to be well adapted to large scale aquaculture production. It could improve the actual procedure by adding biological guarantees in terms of sperm quality and diagnosis control. This would sustain triploid Pacific oyster production.

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Heat shock protein expression is associated to allozyme genetic variability in juveniles of the mollusc *Concholepas concholepas*

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Keywords: Heat shock proteins, thermal stress, allozymes, heterozygosity

Organisms cope physiologically with extreme temperature by induction of heat shock proteins (HSPs). Specifically, the expression of Hsp70 enhances thermal tolerance, and it is a key strategy used by ectotherms to tolerate elevated temperatures in nature. The synthesis of these proteins, together with other physiological mechanisms to compensate thermal stress, involves a considerable expenditure of energy. A positive association between multiple and single locus heterozygosity (MLH and SLH) and traits associated to individual fitness have been widely demonstrated. In molluscs, MLH can decrease routine metabolic rates and improve the availability of energy for other requirements. We propose that for intertidal mollusks that are constantly exposed to thermal stress, allozyme heterozygosity should be positively correlated with higher basal and induced levels of hsp70. Higher hsp70 levels would increase thermal tolerance and thus survival chances of more heterozygous intertidal molluscs. We evaluated the effect of allozyme MLH and SLH on the basal and induced levels of the Hsp70, in juveniles of *Concholepas concholepas* that live in the intertidal zone. Juveniles (n=200) were acclimated at 16 ºC for 2 weeks; then 100 animals were exposed to 24 ºC (stress temperature) and 100 were kept at 16 ºC (control = habitat mean temperature) for 2 and 7 days (in two different experiments). For all animals, the variability of 20 loci was analyzed by starch gel electrophoresis. For SLH effects we used 7 polymorphic loci. We quantified expression of Hsp70 by Western blot analyses. The levels of hsp70 increased markedly (~ 90 %) when juveniles of *C. concholepas* were exposed to stress temperature (24 ºC). Basal levels of hsp70 were similar between animals exposed during 2 and 7 days to 16 ºC (2.6 μg × mg protein^{-1} and 2.9 μg × mg protein^{-1}, respectively). Also the increase of hsp70 was very similar for both, juveniles exposed during 2 and 7 days to 24 ºC, reaching the levels of 5.22 and 5.04 μg × mg protein^{-1}, respectively. In juveniles exposed during 2 days, temperature and the degree of MLH affected significantly the basal (16 ºC) and induced (24 ºC) levels of hsp70 ($P< 0.001$), however, the interaction between factors was not significant. Independently of temperature, the expression of the basal and induced hsp70 incremented lineally (r^2 = 0.7 and 0.9, respectively) with the increment of MLH, reaching the maximal levels in juveniles with intermediate and high MLH levels (2 and 3 loci), but decreased in those juveniles with the highest MLH (≥ 4 heterozygous loci). For juveniles exposed during 7 days, only the increase in temperature affected significantly the hsp70 levels ($P< 0.001$). The degree of MLH and the interaction of both factors did not affect the levels of basal and induced hsp70. For the juveniles that were exposed during 2 days to control (16 ºC) and stress (24 ºC) temperatures, we observed a significant effect of the SLH on 3 of the 7 loci analysed. Those individuals heterozygous for the *PGD**, *PGM-1** and *PEP-F** locus, showed higher levels of hsp70 than the homozygous ones. Particularly, the highest hsp70 levels were present in the juveniles that had the allele *PGD*^{147} (basal and induced), *PGM-1*^{162} (basal) and *PEP-F*^{85} (induced). For the juveniles that were exposed during 7 days, we also observed a significant effect of the allozyme SLH and the genotype on hsp70 levels, but not in the same loci that for the 2 days experiment. The heterozygous for the *IDH-2** and *LAP**, showed the highest levels of induced hsp70. The presence of the *IDH-2*^{139} and *LAP*^{159} was associated with the highest hsp70 levels. Our results show that temperature stress increase hsp70 levels markedly in juvenile *C. concholepas*, and that it is able to maintain the same induced levels of hsp70 over a long period (7 days). Most studies of intertidal molluscs have measured the induction of hsp70 during short term exposition (1-4 h) to thermal stress and found similar level of induction that we found, however, it is quite surprising that these animals are capable to maintain these high induced levels during such a long period. Therefore, the induction of hsp70 may be an important strategy to tolerate long term temperature stress. Moreover, when these molluscs are exposed to thermal stress for 2 days, those with intermediate and high multi-locus heterozygosity were better able to better tolerate elevated temperatures, as they have higher levels of hsp70 under both normal and stress temperature conditions.

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Annual dynamics of reproductive effort of *Pteria sterna* (Gould, 1851) at Bahía de La Paz, B.C.S., Mexico

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Keywords: pearl oysters, reproductive effort, bioenergetics

The rainbow-lip pearl oyster has been cultivated for commercial pearl production ventures since 1994. The method for inducing pearl formation has been related with reproductive period, since seeding of beds is generally undertaken when the gonads are empty. At this moment, however, the oysters are energetically exhausted and become highly vulnerable to manipulation. We investigated the relationship between stored energy in somatic tissues (adductor muscle, digestive gland, and mantle tissue) and changes in energy content of the gonad. These values were also correlated with variations in energy content of available food (seston). Twenty adult oysters were sampled monthly from a commercial farm at Bahía de La Paz from May 2006 through April 2007. The energy content from somatic and germinal tissues was directly determined through calorimetric estimations, which were further related with volumetric fractions of all tissues by means of stereological methods. Reproductive effort in *P. sterna* was estimated to be as 400% energy increase previous to spawning. The caloric participation of somatic tissues for channelling reproduction came, in decreasing order, from adductor muscle, digestive gland, and mantle tissue. Despite its position, an important role of mantle tissue during gonad development was observed. At the onset of gametogenesis in winter, *P. sterna* follows a conservative strategy using energy previously stored in somatic tissues when productivity is highest. In spring, in contrast, the species follows an opportunistic strategy from energy from food intake. We recommend that during commercial pearl culture practices with this species, grafting operations for inducing pearl formation are done from early autumn to spring time.

Molecular identification and expression study of differentially regulated genes in the abalone *Haliotis tuberculata* in response to a bacterial challenge by *Vibrio harveyi*

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Keywords: abalone, Haliotis tuberculata, Vibrio harveyi, bacterial challenge, Suppresive Substraction Hybridization (SSH)

Wild abalone stocks are declining worldwide due to over-exploitation or diseases affecting several *Haliotis* species including the ormer, *Haliotis tuberculata*, the single European abalone species. *Vibrio carchariae* was isolated from *H. tuberculata* during a mass mortality that occurred in France during a period of elevated seawater temperatures in 1998. This *Vibrio* species is synonymous with *Vibrio harveyi*, and is a known pathogen of species of shark and shrimp. Under controlled laboratory conditions, infections with the strain of *Vibrio harveyi* isolated from affected *H. tuberculata* resulted in 80% of mortality of 1 year old, reproductively mature *H. tuberculata* held at a constant temperature of 19°C. Disease and mortality appear to result from an interaction between environmental factors (temperature and presence of the bacterium) and physiological processes (reproductive maturation).

Successive bacterial infection experiments allowed us to select resistant and susceptible abalones. However, physiological processes involved in the response of *H. tuberculata* to *Vibrio* infection are still unknown. A suppression subtractive hybridization (SSH) method was used to identify infection-regulated genes (over-expressed and inhibited) from resistant and susceptible abalone under experimental conditions. The goal of this genomic study is to:

1) to increase our knowledge of the metabolic pathways affected by a bacterial challenge and

2) to identify the genomic basis of resistance and susceptibility of abalone to summer mortalities.

SSH libraries revealed ESTs similar to genes potentially involved in the different physiological functions: general and energetic metabolism, muscular activity, stress response, immune system, and others. Expression patterns of some genes identified by SSH were analysed by real time PCR in muscle over the time course of infection to validate their use as biomarkers.

Future efforts will focus on the search for functional polymorphism in some of these genes in abalone resistant and susceptible families after a bacterial infection to develop genetic markers of resistance to summer mortality.

Comparative growth, condition, and survival of juvenile oysters *Crassostrea gigas* and *C. corteziensis* during extreme conditions of temperature

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Keywords: Crassostrea.gigas, Crassostra corteziensis, growth, condition index, temperature

This study reports the influence of environmental variability of a semiarid subtropical coastal lagoon (Las Guásimas, Sonora) on growth, condition and survival of juveniles of *Crassostrea gigas* and *C. corteziensis*. The juveniles were produced in controlled conditions and cultivated in parallel during two seasons presenting extreme values of temperature; summer (July-September) and winter (December-February). Oysters were sampled every 15 days while six sampling stations were established to determine variation of temperature, salinity, seston, chlorophyll-a, oxygen, and pH in the coastal lagoon. Water samples were taken separately to identify phytoplankton groups and their abundance (H´). Significant changes of salinity, pH, oxygen, and PIM were detected between seasons, but phytoplankton abundance and temperature were the variables presenting the strongest trends; H´ = 6365 and 32.7°C in summer, and H´ = 1686 and 12.7°C in winter. Food availability (POM, including the phytoplankton fraction) was not a limiting factor, while temperature was the parameter affecting the most the oysters. It appears that summer conditions drive *C. gigas* to a physiological stress reflected in a limited ability to acclimatise its metabolic rates to high temperatures with negative effects on growth, condition and survival. In contrast, *C. corteziensis* seems to have a large ability to adapt its metabolic functions to temperature variation with no differences of growth rates and condition indices between seasons (mortality; 9.4% in summer and 0.5% in winter). *C. gigas* exhibited a great adaptation to variations of conditions in sites as Las Guásimas but high temperature will be always a potential limiting factor for cultivation. Changes in traditional aquaculture practices and the incorporation of *C. corteziensis* as an alternative for diversification may improve aquaculture production in northwestern Mexico.

A north-south study of Norwegian *Pecten maximus* broodstock

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Keywords: great scallop, local populations, morphology, Norway, Pecten maximus

The great scallop, *Pecten maximus*, reaches its northernmost distribution in northern Norway (68 °N). Along the Norwegian western coast scallop culture occurs from 59 °N to 65 °N comprising different climatic and oceanographically areas. Information about population characteristics is essential both for management of wild stocks and to the development of breeding and hatchery programs for establishing a sustainable aquaculture industry. Such information of great scallops in Norway is scarce and questions we have raised are if more than one distinct population exists and if a north-south variation between populations could be identified based on shape and weight characters. It has been speculated if intraspecific geographic variations in reproductive cycle and growth traits are genetically based or related to latitudinal distribution. Previously, the existence of separate *P. maximus* populations along the Norwegian coast has been suggested based on different timing of the reproductive cycle events in scallops from 60 °N and 64 °N (Strand & Nylund 1991). However, genetic studies have not supported this assumption up to now (Igland & Nævdal 1995, Ridgway & Dahle 2000). Environmental conditions may therefore explain local growth differences as indicated in a daily growth ring study of scallops from the 60 °N and 64 °N populations (Chauvaud & Strand 1999).

Adult *P. maximus* of suitable size (9-15 cm shell-height) and age (≥4 years) for use as hatchery broodstock were collected by divers from five geographical areas along the western coast of Norway. The scallops were taken from locations at 59 °N (Kvitsøy), 61 °N (Askvoll), 62 °N (Sandøy), 63 °N (Frøya) and 65 °N (Sømna) and transferred to sea-conditions at Scalpro AS hatchery (60 °N) in March and November-December 2004 and in December-January 2005-06. Sub-samples of 15-30 scallops were measured at several points during a two years period. Shell dimensions (height, length and depth), total mass before the scallops were dissected and wet-mass of the shell (both valves together), muscle, gonad and other soft tissues (including mantle, gills, digestive system and foot) were measured, and single character and multivariate comparisons of scallops from the different areas carried out.

Knowledge of population specific characters is of great relevance to the scallop industry as it will affect the spat production in hatcheries and juvenile performance after transfer to growout locations. This study complements a presentation about reproduction cycle and gonad maturation (Magnesen & Christophersen: Broodstock conditioning strategies of great scallop from different localities in Norway) given at the forerunner to Physiomar 08 (Physiological aspects of reproduction and nutrition in mollusks, CIBNOR international workshop 2006, Mexico). Differences in timing of gametogenesis and response to conditioning methods revealed dissimilar patterns suggesting three different populations, while the present study on morphological relationships indicates that the scallops belonged to two different groups.

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Activation of Oyster (*Crassostrea gigas*) Sperm Motility

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Keywords: Crassostrea gigas, spermatozoa, movement, quality index

Oyster (*Crassostrea gigas*) spermatozoa are initiated to flagellar motility right after shedding in sea water, but they show erratic movements with poorly efficiency translation. While exploring various changes in surrounding sea water conditions, we observed that several chemicals, belonging to the cAMP signaling process, were efficiently active to induce forwardly progressing movement. Short term incubation with drugs such as dibutyryl cAMP, theophilline, caffeine or serotonin do initiate progressive movement at high velocity. The effects of these chemicals are time dependent and active in a concentration dependent manner. These observations suggest a maturation process of spermatozoa along the genital tract.

Results: When sperm are collected in testis by scarification, then transferred by dilution in sea water (SW) at 12-25°C, they are not active. It is only after few minutes that spermatozoa show flagellar twitching with low beat frequency (BF) of a few Hz, then few minutes later, twitching raises up to 10-20 Hz.

When sperm collected from testis is transfered in SW containing 10 mM serotonine, a few minutes later their flagella reach a BF of about 40 Hz with flagella developing sine waves leading to flagellar movements similar to those of sea urchins spermatozoa and allowing directed translation of the majority of sperm cells. Short term incubation with drugs like dibutyryl cAMP, theophilline or caffeine also initiate progressive movement of spermatozoa, in a time dependent manner (5 to 15 min incubation in SW) and depending of concentration.

Serotonin is also efficient when injected directly in gonads (100 µL of a 1 mM solution) : after 1 to 10 min. at 19.5 °C, ejaculation occurs through the gonadal pore located near muscle. Spermatozoa shed this way become active (nearly 100%) few min. later. They acquire a high progressivity with BF up to 40 Hz.

Conclusion: in oysters, the maturation process occuring during the transit of spermatozoa along the genital tract appears crucial for potent motility. This maturation step is probably missing when intratesticular sperm is collected by scarification.

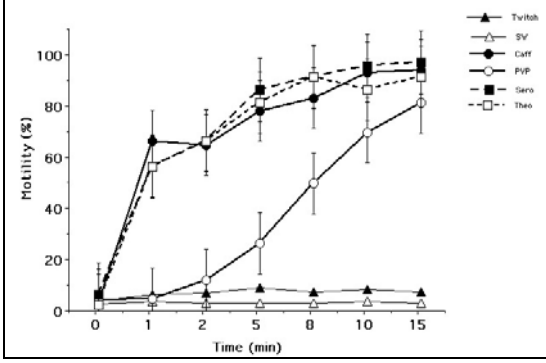

Figure legend: the percentage of motile spermatozoa is plotted versus time of incubation in SW containing either 10 mM caffeine (Caff) or 0.5% Polyvinyl-pyrolidone (PVP) or 5 mM serotonine (Sero) or 10 mM theophylline (Theo). The control in sea water is plotted as percentage of twitching (non progressive) cells (Twitch) and percentage of forwardly progressing cells (SW)

The partial sterility of triploid oyster investigated at cellular and molecular levels

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Keywords: gametogenesis, gene expression, oyster, reproduction, triploid

Triploidy has become increasingly important for aquaculture of *C. gigas* as it provides quicker growth and, most of the time, better survival over their diploid counterparts. This is probably due to the high level of infertility (Nell, 2002). Additionally, triploidy provides a beneficial taste profile for human consumption during the active gametogenesis period. In recent years, some studies have revealed that part of the triploid stocks were able to produce gametes (Normand et al., in press), potentially limiting their commercial value and raising environmental issues. In our study, we therefore conducted a comparative study to describe the dynamic of gametogenesis in diploid and triploid *C. gi*gas.

We first examined gametogenesis by histology. Both diploids and triploids showed active gametogenetic cellular patterns. However, in triploids, the gonad occupation rate, analysed by quantitative histology, showed a mean 50% reduction compared with diploids. Germ cell development is characterized by a high individual variability. Indeed, about 70% of triploids stayed at early stages of gonadic development while the latest 30% showed complete gonadal maturation. Gametogenesis of most triploids appears characterized by the persistence of early stage germ cells.

The recent characterization of a first small set of candidate genes has brought new insights into the reproductive mechanisms of oysters, such as the *oyster vasa-like gene (Oyvlg*): a specific marker of germline cells in *C. gigas*, which allowed the characterization of the origin and development of germ cells in oyster (Fabioux et al., 2004), or the *oyster-gonadal-TGFβ-like* (*og-TGFβ-like*), specifically expressed in the somatic part of the gonad and supposed to act in the maturation of germ cells (Fleury et al., 2008). We therefore used these two genes to compare reproduction in diploid and triploid oysters at the molecular level, by studying their expression by in situ hybridization (ISH) and real time PCR. Results of ISH corroborate histological results: the development of germinal stem cells and gonia occurs in all triploid oysters as well as in diploids but most of triploids do not go past the gonial proliferation step. As a result, the origin of the reduced fecundity of most triploids is not found in the first stages of gametogenesis but rather at the point when spermatogonia/oogonia differentiate into spermatocytes/oocytes. Concerning real time PCR results, a significantly higher level of *Oyvlg* mRNA was observed in triploid compared to diploid oysters, suggesting that the transcription level of this gene is directly proportional to number of gene copy (additive effect). However, a very strong variability of *Oyvlg* mRNA level was observed between triploid oysters. Further experimentation is currently underway on triploids to assess if a correlation exists between the individual level of *Oyvlg* expression and the gametogenetic pattern. This would provide predictive tools to distinguish fertile and sterile triploid oysters.

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Identification of new genes associated with summer survival in the oyster *Crassostrea gigas* using cDNA microarray

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Keywords: Crassostrea gigas, microarray, reproduction, gene expression, survival

The examination of *Crassostrea gigas* mortality in France (in the framework of the Morest program) has suggested that it results from a complex interaction between the host, the environment and opportunistic pathogens. A strong genetic basis was shown to exist for observed variation in resistance to summer mortality. This has opened up possibilities of improvement by selection and has in turn allowed production of oysters that are 'resistant' (R) and susceptible (S) to summer mortalities. Due to the role that an animal's physiological state plays in mortality, the selected genetic character is likely to be connected with one or more oyster functions.

In the European program called Aquafirst, suppression subtractive hybridizations have been realized between R and S oyster lines to produce 8064 ESTs differentially expressed between the two lines. These sequences have been assembled with the ESTs characterized in the Marine Genomic Europe network of excellence and the public ESTs in a unique annotated database (9272 contigs; http://www.sigenae.org/aquafirst/).

A cDNA microarray, containing 9059 unigenes, was produced by the MPI (Berlin) and then used to conduct a genome-wide expression profiling of R and S families during a temporal survey around the mortality event, in order to characterize genes involved in the molecular pathways associated with summer survival in *C. gigas*.

A total of 423 non-redundant genes, differentially expressed between R and S, were identified from microarray SAM analysis. Within the identified clusters of correlated temporal expression profiles, significant enrichments in some Gene Ontology terms have suggested a few biological trends potentially affected during the mortality event.

A subset of genes found to be differentially expressed was independently validated using real-time PCR. Microarray results were also validated using known genes (*og-TGFβ-like*) previously found differentially expressed between R and S (Fleury et al., Gene 2008). This gene, expressed in the somatic part of the gonad, was hypothesized to be involved in germ cell maturation. In addition to the higher gonad investment in S, the higher expression of *og-TGFβ-like* in R compared to S might suggest its negative effect in gonad development. This underlines the hypothesis that reproductive effort may cause the higher rate of summer mortality of the S line. Some new relevant candidates, such as Neuropeptide Y, were also found to be differentially expressed between R and S, strengthening the hypothesis that the causes of mortality are linked to the cost of reproduction, interfering with the energy balance of the oyster.

Finally, the identity and ontology of identified genes provided significant hints that could result in a better understanding of the mechanisms associated with summer mortality. To evaluate these genes as potential candidate markers of resistance to summer mortality, they will be next studied "one by one" to evaluate their function with promising new tools (in development). They would also be used as valuable markers in the search for Quantitative Trait Loci.

Qualification of simple tests for assessing vitality of mollusc bivalves: muscular strenght, shell hermeticity, uprighting ability and survival response to standard stress.

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Keywords: Bivalves, quality index, muscular strength, hermeticiy, uprighting, survival test

Both researchers and farmers regularly need to assess the physiological condition of mollusc bivalves. The aim of this study has been to appraise simple tests in order to compare various qualities of cupped oysters, (*Crassostrea gigas*) or blue mussels (*Mytilus edulis*). The choice of the tests has focused on evaluations of the weakening of animals expressed by **muscle, mantle or byssus loosening**. Three methods have been compared for oysters: the **muscle strength recording,** the **animal hermeticity in freshwater** and the **survival response over a range of thermal shocks** ; an additional one for mussels has been the **uprighting ability** (role of byssus). Various batches of cupped oyster spat (3 g) or blue mussel juveniles (3 to 5 g) have been faced to a gradient of stressing factors: increasing durations in warm water at 30°C (0, 1, 2, 4 or 9 days), in air at 20°C (0, 3, or 7 days) and in freshwater at 16°C (0, 4, 8 or 11 days).

These animals were then submitted to the studied tests. Muscular strength was recorded out of the water with a force gauge (Fleury *et al*, 2005) for 5 mn per animal, especially maximal strength (tonic muscle) and sum of peaks (striated muscle contractions). The bivalve hermeticity was tested in warm freshwater (40°C for oysters, 30°C for mussels) by recording the increasing salinity due to loss of intervalvar water (Lukanin *et al*, 1979). Uprighting ability of mussels was recorded during 24 hours. Survival tests in response to a range of thermal shocks (Maguire *et al*, 1999) were carried out by immersion of 6 batches of 30 oysters in warm seawater (40°C for oysters, 35°C for mussels) during 0 hour (no immersion actually), 30 mn, 1 h, 1h30, 2 h and 2h30, then counting of surviving animals after 24 hours in air at ambient temperature (about 20°C).

The four tests were very easy and quick to perform, within a single day. A main difference is that muscle strength measurement and uprighting ability required individual recordings. The latest is also a non-lethal test whilst animals exposed to strength recording were more or less wounded by the hooks opening.

With regards to results, all tests provided coherent decreasing responses as a result of increasing stressing factors. In the oysters experiment, muscular strength and survival responses exhibited significant differences between most batches. The less sensitive test was hermeticity: nature of stressing factors (freshwater, thermal shocks at 30°C) could generate artefacts or acclimatization of oysters. On the contrary, in the mussels experiment, the most performant tests were hermeticity and uprighting ability.

This may be due, for a part, to the main role of muscle and the strong hermeticity in monomyaire bivalves (oysters and scallops) and opposite pattern in dimyaire bivalves (such as mussels).

In conclusion these experiments have shown that simple and quick tests can be performed by scientists or farmers in order to evaluate physiological abilities of bivalves facing to stressing factors, and then estimate their physiological condition (vitality). Possible applications are comparisons of animals issued from different sites, different suppliers, different rearing conditions, different genetic patterns, etc.

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Impact of brown ring disease on the energy budget of the Manila clam, *Ruditapes philippinarum*.

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Keywords: Ruditapes philippinarum, Vibrio tapetis, Manila clam, brown ring disease, energy budget

Brown ring disease (BRD) in the Manila clam, *Ruditapes philippinarum*, is a disease caused by the bacterium *Vibrio tapetis*. This disease induces a brown deposit on the inner shell of infected clams and can be associated with mass weight loss and a loss of glycogen reserves suggesting that the disease affects the energy budget.

In order to asses the effect of the disease on the energy budget an experiment was performed in order to asses the effect of the disease clearance and respiration rates. Measurements showed that the clearance rate of severely diseased clams was significantly decreased by both a decrease in filtration capacity and a reduction of the time spent on filtration activity. Thus one primary way of modification of the energy balance is a decrease in the food intake.

A starvation experiment was performed in uninfected and infected clams and indicated that in highly infected clams weight loss was higher than in uninfected one. This allowed to indicate that the energy balance was modified by the disease independently of the effect on filtration activity. These data highlight a second way of degradation of the energy balance: an increase in maintenance cost associated with energy needed for immunity and lesion repair. In order to asses the effect of the diseas on these maintenance costs, a model based on Dynamic Energy Budget theory, that provide rule of allocation of energy to maintenance was developed. Coupling the model simulations and starvation observations provides a quantitative and dynamic evaluation of the effect of BRD on maintenance costs and indicated that BRD development could be associated with an important increase in the maintenance cost.

Cellular and molecular characterization of early stages of spermatogenesis in the pacific oyster *Crassostrea gigas.*

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Keywords: Crassostrea gigas, spermatogenesis, reproductive cycle, germ cell characteristics

The Pacific oyster, *Crassostrea gigas*, is a successive and irregular protandrous hermaphrodite mollusc. Reproduction in this species is seasonal and timing of each step of germ lineage development is broadly affected by environmental conditions. The gonad is consists in gonadal tubules invaginated in a connective storage tissue where haemolymphatic vessels are numerous. The volume of the gonadal tubules is directly related to the developmental stage of gametogenesis from autumn (reproduction arrest followed by spermatogonial mitosis) to summer (ripe gonad). During early gmetogenetic stages, the sex of animals is undetectable and no molecular specific markers of spermatogonia and/or spermatocyte are actually disposable. That is why we have undertaken a cellular and molecular characterization of early spermatogenetic stages using different complementary approaches.

First, the ultrastructural characterisitics of the gonad were explored and a specific interest was devoted to the main cellular features of spermatogonia and spermatocytes. The gonadal tubules are surrounded by myoepithelial cells associated with an acellular matrix delimiting the outer part of the tubule; the inner part is composed by intragonadal somatic cells and germinal cells. Two types of spermatogonia were identified, where type I spermatogonia are large, scarce and pale cells leaned against the base of the tubule (nuclear diameter 5.5 ± 0.5µm). Type II spermatogonia are clustered and dark cell which appear smaller than type I (nuclear diameter 4.3 ± 0.3µm). The aspect of nuage-like material in cytoplasm is described from pale spermatogonia to primary spermatocytes (nuclear diameter 3.6 ± 0.3µm), while no structure related to chromatoid body could be observed in oyster spermatocytes and spermatids. Suspension of dissociated germ cells were also prepared and fractionated over density gradient and/or FACS (Fluorescence-activated cell sorting) in order to isolate spermatogonia and primary spermatocytes. Cells suspensions were validated according to structural/ultrastructural properties to develop a differential proteomic approach leading to characterize new molecular markers of spermatogonia. Laser microdissection (LMD) was also considered as an alternative technique to isolate islets of gonadal tubules at different stages of gametogenesis. The expression of genes known to be potentially involved in the mitotic activity of spermatogonia was measured by real time PCR in gonad and connective cells in order to identify pertinent markers of early spermatogenesis. The fine identification of the cells expressing these genes of interest could then be investigated by *in situ* hybridization. The characterization of specific molecular markers of the early stages of spermatogenesis is a first step in the study of gametogenesis regulation in a Lophotrochozoan.

Detachment of scallop *Pecten maximus* post-larvae from different diet treatments in relation to flow velocity in benthic biological flumes

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Keywords: Nutrition, attachment, Pecten maximus, benthic boundary layer, flume

Several studies investigated the effects of flow velocity on the attachment strength of Mytilidae, but at our knowledge this information is limited for Pectinidae. Thus, this experiment was carried out in a benthic biological flume (HYCOBENTHOS) to determine the effect of u_* (shear velocity) and the attachment period on the percentage of detachment of *Pecten maximus* post-larvae of different sizes reared and supplied with two experimental diet. PTC (*Pavlova lutheri* (P), *Isochrysis galbana* (T), *Chaetoceros calcitrans* (C)) served as a reference diet, commonly used in bivalve hatcheries and nurseries, and the second PTCR was the standard diet (PTC) with an addition of *Rhodomonas salina* (R) microalgae. Our results indicated that the detachment of post-larvae fed with PTCR showed significant interaction between length, attachment period and u_*. For PTC fed post-larvae, the u_* factor has a significant effect on the percentage of detachment of post-larvae without any interaction with other factors. For both diets, post-larvae previously attached during 12 h had lower levels of detachment comparatively to those attached during 30 min. The exposure of juveniles in currents of various flume velocities showed a higher percentage of detachment for post-larvae reared with PTCR diet. This result could be related with differences in biochemical composition of the diets and have been discussed.

Seasonal response of aerobic and anaerobic capacity in the adductor muscle of suspension-cultured *Crassostrea virginica* .

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Keywords: Crassostrea virginica, addutor muscle, metabolism, season, carrying capacity

In New Brunswick (Canada), *Crassostrea virginica* is cultured in floating bags from May to October. During the winter, the bags are sunk on the bottom to protect the bivalves from being crushed by the ice. Over a year, oysters thus experience temperatures ranging from -1.8 to 25 ^{o}C. In these conditions, we can expect an acclimatation response of the metabolic capacity of the animals in order to partly offset the effect of temperature on the metabolic rate. In the context of estimating the carrying capacity of a site for oyster farming, it is essential to have a thorough understanding of the metabolic responses of *C. virginica* to temperature. Even though bivalves may strongly rely on anaerobic pathways for the production of ATP, the anaerobic metabolism of the animals is seldom included in models. Our objective was to describe the seasonal variation in both the aerobic and the anaerobic capacity of the adductor muscle in the American Oyster *C. virginica*. Suspension-cultured oysters were sampled from May to October 2006. A winter sampling was also undertaken in March 2007, when oysters were still submerged under a 50 cm ice cover. The activity of strombine dehydrogenase (SDH), lactate dehydrogenase (LDH), citrate synthase (CS) and cytochrome c oxidase (CCO) was measured to assess the anaerobic and the aerobic capacity in the phasic and the tonic parts of the adductor muscle.

SDH, CCO and CS activity was higher in the phasic than in the tonic muscle (paired-samples t tests, $P < 0.001$), which confirms that the metabolic capacity is highest in the phasic muscle. Our results also show that in *C. virginica* the anaerobic metabolism mostly relies on SDH (annual mean of 6.23 ± 1.52 international units/ g wet weight in the phasic muscle and 3.83 ± 1.50 in the tonic muscle). In contrast, the activity of LDH was *circa* 0.14 iu / g ww in both parts of the adductor muscle. No acclimatation response to temperature was noted from May to October, as neither the aerobic nor the anaerobic capacity in the phasic and the tonic adductor muscle significantly varied during this period (Bonferroni tests, $P > 0.05$), except for the SDH activity in the tonic muscle which decreased from 4.15 ± 1.53 iu / g ww in September to 2.65 ± 0.88 in October. This contrasts with other bivalve species such as *Mytilus edulis* in which the aerobic metabolism clearly acclimates with temperature. Nevertheless, significant adjustments in the anaerobic capacity occurred in winter as levels of SDH increased by ~ 1.5 in the phasic muscle but decreased by ~ 1.3 in the tonic muscle, as compared with the levels observed during the growing season (Bonferroni tests, $P < 0.001$).

Our results also suggest that when physiological variables such as metabolic capacity are included in a carrying capacity model for oyster aquaculture, they can be reliably parameterized without requiring measurement of the metabolic capacity of the animals at different times during the growing season. In order to estimate the *in situ* metabolic rate of oysters at a particular period it would be sufficient to know 1- the typical metabolic capacity of the population under study 2- the Q10 value for the metabolic rate in the range of water temperature that prevails during the growing season and 3- the water temperature at the time of interest.

Biochemical, physiological and genomic responses of larvae from American oysters (*Crassostrea virginica*) during a mass mortality event.

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Keywords: Crassostrea.virginica, larvae, massive mortality, physiology, genomic

In mollusc aquaculture, pathogens are the principal factor influencing the efficacy of larval production. Bivalve larvae often experience high mortalities during their protracted larval development due to outbreak of opportunistic bacteria. Physiological impact of massive mortality during larval stages was few studied. Therefore it is important to develop tools allowing a better understanding of physiological changes induced by pathogenic infection. We have focused on the physiological and genomic responses of batches of American oysters larvae (*Crassostrea virginica*) submitted to a massive mortality event. Larval rearings were realized in two parallel conditions: 1) without specific water treatment and 2) with presence of a non selective antibiotic (chloramphenicol). This study examines the performance of American oyster from eggs to pediveliger stages in relation to the development of bacterial community in rearing tanks. Performance of larvae was characterized by growth, mortality, feeding rate, lipids dynamic, energetic metabolic activity, oxidative stress and gene expression. Lipid characteristics consisted on an estimation of major classes lipids (triacylglycerols , phospholipids, sterols, …) and fatty acids content in neutral and polar fractions. Energetic metabolism was approached by the study of key enzymes of energy production (citrate synthase and cytochrome oxidase). Oxidative stress was estimated by the measure of lipid peroxidation level (MDA) and antioxidant enzymes activity (catalase, glutathione peroxidase, superoxide dismutase). To establish relation with gene expression differences we chose several genes regrouped in three functional groups: immunity, stress, and metabolism. Gene expression was estimated by real-time PCR.

Chloramphenicol addition has strongly limited mortality in this treatment. Mortality during the planktonic larval period occurred primarily during late veliger stages (d13 to 20) as previously observed with other bivalve species. Groups of larvae reared without antibiotic showed massive mortality presumably reflecting by the emergence of pathogenic bacteria. DGGE analyses have shown strong changes in bacterial community structure during massive mortality emergence. In larvae submitted to massive mortality remodelling of lipid classes profile have been observed but not in fatty acids composition. Moribund larvae were also characterized by metabolic depression and higher oxidative stress. Finally, correlations were found between the biochemical and physiological responses to pathogenic infection and gene expression levels.

The effect of energetical reserves and cryoprotectants on overwintering mortality in *Mercenaria mercenaria notata* (Say, 1822) at two tidal levels.

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Key words: Energy reserves, growth, Mercenaria mercenaria notata, overwintering mortality, tidal levels

The aim of this study is to identify the possible causes of the high winter mortality noted in juvenile *Mercenaria mercenaria notata* in Eastern Canada. The percentage of mortality, the shell growth, the concentration of energetic reserves and the production of cryoprotectant molecules, were compared between notata and native quahogs kept at intertidal and subtidal levels. Overwintering mortality of the notata strain reached 47.2% and was 3 to 9 times higher than that of the native strain. Shell increment was higher in the native than in the notata strain and also at the intertidal than at the subtidal level. The quahogs from the subtidal zone had a higher concentration of protein than those from the intertidal zone in August and April while the opposite was noted in February. The notata strain had a lower concentration of lipids and glucose (34.9 and 0.21 mg/ g dry weight) than the native strain (42.2 and 0.28 mg/ g dry weight). Thermal hysteresis was detected in none of the quahog groups. High winter mortality in the notata strain thus seems to be caused in part by a lower capacity to stock energetic reserves as compared to the native strain. This lower capacity probably results from the higher metabolic rate of this strain.

Identification of *Pecten maximus* populations in Norway by muscle fatty acid profiles

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Keywords: Scallop, fatty acids, adductor muscle, local populations, Norway

The existence of genetically different Norwegian *Pecten maximus* populations has been suggested based on observed differences in timing of reproductive cycle events related to geographical location. The characteristics of the reproductive cycles remained after transplantation to new environment at different latitude and also after conditioning in hatchery. In general there is little evidence of substantial genetic differentiation of *P. maximus* populations throughout its range. The exception is the Mulroy Bay population in Ireland and possibly the Norwegian populations (Beaumont 2006). No study has so far been able to discriminate between the influence of environmental and genetic factors on the phenotypic variation in Norwegian scallop populations. In this study juvenile scallops originating from five geographical areas along the western coast of Norway were raised in the same environment, giving a unique possibility to study if tissue fatty acid (FA) composition was regulated by environmental factors i.e. diet, temperature, salinity, or is due to genetics.

Broodstock sized *P. maximus* were sampled from 5 locations along the Norwegian coast from 59 °N to 65 °N and translocated to natural sea conditions by the Scalpro AS hatchery near Bergen. The locations where selected to represent a coast line of more than 800 km. At the hatchery scallops were spawned in February 2006. The postlarvae were raised in indoor nursery before transfer to outdoor landbased nursery and finally to plastic trays on longline system at sea. Ten individuals from each population were sampled in May 2007 for lipid (FA) analysis.

Preliminary results from multivariate statistics indicate that the two first PCA coordinates describe 83% of the total variance. The total lipid content of the muscles were low (5.7 ± 0.3 mg/g tissue, determined as total amount of FA), and no significant difference was found among the five populations. The polyunsaturated FA made up close to 60% of the total, with 20:5n3 and 22:6n3 dominating. The saturated FA was also present in relatively large amounts (ca. 30%), while the monounsaturated FA was less abundant. The FA composition of the muscles of the five populations was apparently quite similar. In the 10 pairwise t-test constellations 41 significant differences were found among the 25 identified FA. Multivariate, supervised learning method PLS, showed that one population was rather distinct from the others, which apparently were mutually indistinguishable. Testing each pair separately showed that only two scallops were wrongly classified. The significance for population identification will be discussed.

Changes in shell-valve activities of *Crassostrea gigas* exposed to *Alexandrium minutum*.

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Keywords: shell-valve activities, Crassostrea gigas, Alexandrium minutum

Some harmful algae are recognized to have important effects on bivalve behavior, including reduced filtration and feeding rates, decreases in byssus production and oxygen consumption, and stimulation of shell valve closure. *Alexandrium* spp. are known to produce paralytic toxins and to have effects on bivalve behavior. The aim of this work was, thus, to assess the effect of *A. minutum* exposure upon oyster behavior, i.e., shell valve activities.

An original system using lightweight impedance electrodes was used to measure valve movements under experimental conditions. Sixteen oysters were equipped with electrodes and acclimated for 2 weeks with T-*Isochrysis*. After acclimation, 8 oysters were exposed for 7 days to a toxin-producing dinoflagellate, *A. minutum*, with a continuous flow of 14 ml min^{-1} of 5 X 10^3 cells ml^{-1}. As non-toxic controls, 8 oysters were maintained with T-*Isochrysis* with a continuous flow of 14 $ml.min^{-1}$ of 10^5 cells ml^{-1} (eq. 1/5 biovolume to 5 X 10^3 cells ml^{-1} *A. minutum*). Characteristics of *C. gigas* valve behavior (daily valve opening duration, gape amplitude, and microclosure frequency) were monitored continuously during acclimation and exposure.

Exposure to *A. minutum* resulted in a marked impact on oyster valve behavior. Daily valve opening duration (Figure 1, parameter 1) for oysters exposed to *A. minutum* was nearly double (69.8 ± 2.4 % opening) that of oysters exposed to T-*Isochrysis* (42.7 ± 2.1 % opening), but gape amplitude (Figure 1, parameter 3) was lower in *A. minutum*-exposed oysters (open mode 30-40 % *vs* 80-90 % with T-*Isochrysis*). Also, very brief and incomplete valve closures (less than 1.6 sec) were observed frequently in oysters (Figure 1 parameter 2) exposed to *A. minutum* (180-200 microclosures during open time).

From this study, high frequency monitoring of oyster valve behavior appeared as a good tool to assess the impact of harmful algae on bivalve behavior and biology. This study is currently being continued by establishing relationships with other physiological parameters (filtration, ingestion, bio-deposition) and by testing other toxic algae.

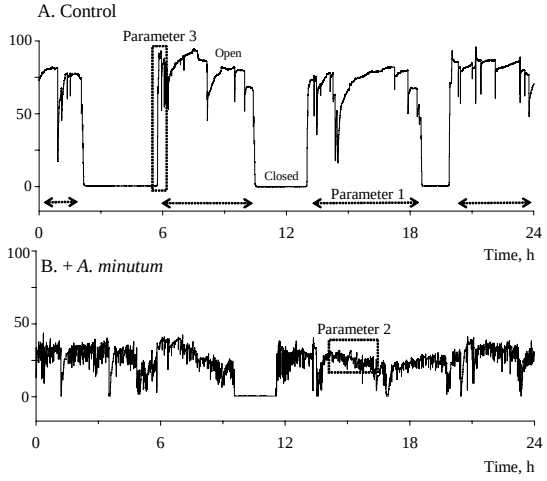

Figure 1: valve activity record of non exposed oysters (A) and *A. minutum* exposed oysters (B)

Magnetic resonance image (MRI) as a mean to assess the growth and the gonad development of *Crassostrea gigas* oyster

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Keywords: Crassostrea gigas, oyster, growth, gonad, magnetic resonance image, MRI

Standard methods for measuring the growth of juvenile and adult oysters, *Crassostrea gigas*, whole body or organs, or for evaluating the gonad development, are based upon observations and measures on random samples; most of the methods for these observations and measures are destructive. This makes difficult or impossible individual tracking. The variability observed within each sample is due to different evolutions of individuals according to time or to different amplitudes of variations. This study is a follow-up of the works of Pouvreau et al. (2006): its first aim is to explore possibilities and limits of magnetic resonance imaging (MRI) for measuring the growth of the oyster *C.gigas* soft parts at the individual level. The second aim is to compare the evolution of three different groups of oysters maintained in the same environmental conditions during a year. One group comprises diploids and the two others triploids; all are borne in April 2006 and the offspring of the same group of females. This comparison is based upon the measures by means of MRI 0.2T and MRI 4.7T performed on five dates, from February 27th till October 16th 2007. The evolution of the volume of the soft body and the mature gonad is measured on 67 oysters by means of the first apparatus on the five dates. The evolution of the volume of the soft body, the abductor muscle, the gills, the whole digestive gland - gonad is measured on twelve of these 67 oysters by means of MRI 4.7T.

These methods allow an individual tracking. Under the rearing conditions of this assay, while the shell growth is homogeneous within each group, the growth of the soft parts and the maturation of the gonad is variable among individuals: they can be either precocious or late, and either small or large.

Fully mature gametes are visible in the diploids and the triploids gonads, without significant signs of spawning for the last ones. The majority of diploids spawn between May 30th and July 11th and the other ones show no sign of significant spawning between July 11th and October 16th.

Seasonal biochemical composition, lipid classes, sterols and fatty acids of *Crassostrea corteziensis* in relation to reproductive cycle

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Keywords: mollusk, Oyster, HUFA, maturation

Crassostrea corteziensis is highly cultivated for human consumption in northwest of Mexico. However, nutritional aspects for maturation and reproduction in captivity are scarce in comparison to other mollusk species. An approach to increase the knowledge of their nutritional aspects, mainly in lipids, the female reproductive annual cycle of *C. corteziensis* and their seasonal biochemical composition was analyzed in a wild population collected from April 2005 to April 2006 in Ceuta lagoon in Sinaloa, Mexico. The highest values of wet tissue weight were recorded in April 2006 and the lowest in November–December 2005. Gonad development was analyzed using digitalized images (4×) from histological slides and reported as gonad coverage area (GCA). Mature oysters were observed throughout the sampled period, with two reproductive peaks, one in April 2005 and a second less intense in July 2005. A decrease in GCA, probably indicating spawning events, occurred in June and September 2005, when sea surface temperature reached 25 and 30°C, respectively. Lowest GCA values, indicating a resting period were observed in winter and coincide with lower sea surface temperature (20°C) and higher chlorophyll *a* levels (12.5–15.5 mg m^{-3}). Total protein, carbohydrate and lipid concentrations were significantly affected by the sampling date. Carbohydrates were significantly positively correlated with lipids (r=0.97, $P<0.05$), with bigger accumulation in spring and lower in winter. Proteins values were higher in spring and decreased after spawning in summer. Triacylglyceride concentration analyzed by TLC–FID had a significant positive correlation with total lipids (r=0.63, $P<0.05$), meanwhile free sterols showed an inverse pattern (r= –0.80, $P<0.05$). Phospholipid concentration was higher in April and December 2005, and lower in February 2006. Cholesterol was the major sterol in the free and esterified fraction, followed by brassicasterol, isofucosterol, and other in minor proportions. Total sterols from the free and esterified fraction were higher in April, July and September 2005, while lower in November 2005. Highly unsaturated fatty acids (HUFA) concentration from lipid reserves was higher in April–May 2005 and from January to April 2006, and lower in November 2005. HUFA in the phospholipids of membrane showed an inverse pattern, with higher values in November–December 2005, and lower in July 2005 and April 2006. From principal components analysis (PCA), we concluded that increase of postvitellogenic oocytes was positively correlated with concentration of 20:5n–3 and 22:6n–3 (from triacylglycerides), total lipids, triacylglycerides, and esterified sterols. High positive correlation between lipid and carbohydrates concentration suggested an independent accumulation process, even from protein accumulation. Additionally, a second PCA was performed using fatty acids (%) from triacylglycerides as a food quality imprint. The results showed a higher correlation of 18:4n–3 and carotenoids in oyster tissues with chlorophyll *a*, which suggested that phytoplankton peak (winter–spring) might be mainly composed by dinoflagellate species, meanwhile strong correlation of 14:0, 16:1n–7 and 20:5n–3 with postvitellogenic oocytes, suggested that diatom species were dominant during this period (spring–summer) in Ceuta lagoon.

Biochemical defences of *Pomacea canaliculata* eggs. Ovorubin as an antinutritive perivitelin

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Keywords: Gastropods, perivitellins, protease inhibitor, pH stability, protein structure

The reproductive strategy of many species of the freshwater genus *Pomacea* involves cementing brightly coloured egg clutches on different substrata above the water line (Figure), thus exposing the embryos to sunlight, high temperatures and predators. The conspicuous coloration of *Pomacea canaliculata* eggs is thought to be a warning signal, but there is not evidence of the defensive compounds. It has been proposed that their perivitellins, besides the usual role as reserve proteins, are also involved in the adaptation of the embryo to the aerial conditions. The major egg protein is Ovorubin, a 300 KDa thermostable multifunctional protein, with protease inhibitor activity. This glyco-lipo-carotenoprotein also contains the carotenoid astaxanthin as a prosthetic group, a potent antioxidant responsible for the pink-reddish egg color.

Here we report (1) the effect of pH on ovotubin stability, using small angle X-ray scattering (SAXS), electron microscopy (EM), circular dichroism, fluorescence and absorption spectroscopy and (2) pH stability in relation to a potential role of ovorubin in the egg defence against predators evaluating a simulated gastrointestinal digestion.

Results showed that the protein has an axial length of 69 Å and a radius of 43 Å, a very large protease inhibitor compared to those of most animals or plants. The quaternary structure was only affected at pH values below 4 showing a reduction in size and loss of tertiary structure. Astaxanthin binding was not affected at pH values between 2 - 12. Taken together these results indicate a high structural stability in a wide pH range, showing a disassembling, but not an unfolding of the oligomer only at low pH values.

Considering the high structural stability in a wide pH range and the previously reported protease inhibitor function, we speculated that ovorubin was actively involved in the chemical defence of embryos by limiting the predator's ability to digest and use essential nutrients from the eggs. To test this hypothesis we subjected ovorubin to a simulated *in vitro* gastrointestinal digestion (4h with pepsin, pH 2.5, followed by 4 h with trypsin pH 7.5) Electrophoretic and spectrophotometric analysis showed that ovorubin resisted gastrointestinal digestion and retained its serine protease inhibiting activity. In addition, partial aminoacid sequencing showed that ovorubin belongs to the Kunitz-type serine protease inhibitors´ family.

We therefore show evidence that ovorubin may withstand the passage through the digestive tract of potential predators affecting trypsin activity, thus rendering the eggs antinutritive to predators. This is a novel function of perivitellins in the chemical defence of embryos.

Optimal feeding of *Mytilus edulis* and *Cerastoderma edule* seed for land-based culture

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Keywords: Mytilus edulis, Cerastoderma edule, spat, food availability, growth

Recently, a new development has started in shellfish production in the Netherlands. Shellfish species that are traditionally fished as seed (the mussel *Mytilus edulis*) or as adults (the cockle *Cerastoderma edulis*) are now being cultured on land. This happens at an experimental scale in hatcheries, nurseries and grow-out ponds. Hatchery and nursery knowledge is present for species such as *Crassostrea gigas* and *Tapes philippinarum*. However, mussels and cockles are new species for this type of culture. Thus, optimal feeding regimes need to be defined. To make sure no energy is wasted in pseudofaeces production, the concentration at which pseudofaeces production starts was determined. In addition, the uptake efficiency of different algal species was measured to ensure optimal supply of algae. After that, growth of seed fed different diets of algal species and mixes of species was tested. Large-scale on-land culture of shellfish involves water discharge into the surrounding waters. For this reason, native algal species need to be found that give sufficient shellfish growth. Algal species that are native, easy to culture and of suitable size and fatty acid composition as feed for shellfish were identified. The species tested were: *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Thalassiosira pseudonana*, *Dunaliela tertiolecta*, *Pyramimonas parkeae* and *Tetraselmis* sp. Results on pseudofaeces thresholds for the different algal species and for different sizes of seed will be presented. In addition, growth rates of seed fed different diets will be shown.

Trophic relationship between sea squirt *Halocynthia roretzi* and Pacific oyster *Crassostrea gigas* under co-cultures in suspension in the southern coast of Korea

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Keywords: Halocynthia roretzi, Crassostrea gigas, trophic relationship, co-culture, food partitioning

Of the two most important aquaculture species in Korea are the edible sea squirt, *Halocynthia roretzi*, and the Pacific oyster, *Crassostrea gigas*. Since 1980th when the longline method was expanded, the production of sea squirt peaked with 42,800 tons in 1994, and declined to 4,500 tons in 2004 and 10,819 tons in 2006. The decline in sea squirt production is attributed to the decrease of growth rate and the increase of mass mortality event. Causes of ubiquitous mass mortalities of sea squirts in Korean coasts are unknown yet. Recently, fishermen developed a co-culturing method, by which one line attached by sea squirts is hung and another following line is attached by oysters. With the expansion of this method, mortality of *Halosynthia rorezi* was less pronounced under the condition of co-cultures of ascidians and oysters. In contrast, during that time, the mass mortalities happened much more frequently under monoculturs of ascidians.

In order to examine trophic relationship between co-cultured suspension feeders, *Halocynthia roretzi* and *Crassostrea gigas*, in the southern coast of Korea, their $\delta^{13}C$ and $\delta^{15}N$ were determined from February 2005 to December 2006, and compared with their mono-cultured and natural counterparts. $\delta^{13}C$ and $\delta^{15}N$ of 2 fractions of suspended particulate organic matter (coarse, > 20 μm, CPOM; fine, < 20 μm, FPOM) were also analysed to identify seasonal dynamic of isotopic composition of their dietary sources. $\delta^{13}C$ values of CPOM were slightly more variable with a range of –21.9 to –15.4 ‰ than those of FPOM with a range of –23.6 to –19.7 ‰ over the sampling period. In contrast, $\delta^{15}N$ values of CPOM were less variable with a range of 6.2 to 9.7 ‰ than those of FPOM with a range of 3.3 to 8.6 ‰. While co- and mono-cultured sea squirts were less variable than co-cultured oysters in $\delta^{13}C$, the formers were more variable than the latter group in $\delta^{15}N$ over the sampling period. The $\delta^{13}C$ and $\delta^{15}N$ values of co-cultured sea squirts were consistently lighter (ranges = –21.6 to –19.4 ‰ and 8.0 to 10.9 ‰, respectively) than those of co-cultured oysters over the sampling period (–20.2 to –16.5 ‰ and 10.3 to 13.6 ‰, respectively). More pronounced difference in $\delta^{13}C$ value between co-cultured stocks was attributed to a striking isotopic change in oysters during the autumn-winter when they showed fast growth. While $\delta^{13}C$ of sea squirts were different between co-, mono- and natural habitats, no significant difference in $\delta^{13}C$ between co- and mono-cultured oysters was found. This result suggests that the indiscriminate feeding habit of sea squirts and the availability of food resources may lead to their feeding plasticity. In addition, considering the known trophic fractionation in $\delta^{13}C$ and $\delta^{15}N$ between prey-predator, isotopic values of co-cultured sea squirts and oysters indicate that these suspension feeders can utilize different food resources (e.g. different size fractions of POM) in the same habitat. The marked ^{13}C-enrichment in oyster tissues, particularly during their autumn-winter growing period, may result from their strong selectivity on diatoms. By contrast, both ^{13}C- and ^{15}N-depleted values in co-cultured sea squirts indicate the importance of pico-/nano-size fractions as their diets. Food partitioning due to species-specific differences in qualitative selection capacity as shown in the literatures might account for such isotopic differences between these two species.

Development of an immunological probe to measure reproductive effort of the Suminoe oyster *Crassostrea ariakensis*

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A polyclonal antibody specific to egg protein of the Suminoe oyster, *Crassostrea ariakensis* developed in this study to assess reproductive effort of the oysters. New Zealand White rabbit was immunized with the purified oyster egg. After two month of immunization, the antiserum exhibited a weak but recognizable cross-reaction to the non-gonadal tissues in the double immuno-diffusion and in enzyme-linked immuno-sorbent assay (ELISA). After eliminating the cross-reacting antibody with an immuno-absorbent prepared from somatic tissue protein of the oyster, a strong egg-specific antibody-antigen reaction was confirmed in ELISA. In an indirect sandwich ELISA, the rabbit anti-oyster egg IgG detected 0.25-10 μg/ml oyster egg protein. To test the sensitivity of the probe, known amount of the oyster egg was mixed with known quantity of the oyster somatic tissue and assessed using the probe. The result indicated that the immuno probe developed in this study was sensitive enough to measure as little as 1% of the oyster egg presented in the oyster tissue. In an attempt to measure the reproductive effort of the Sumino oysters, 40 oysters were collected monthly from January to July in 2007 from an estuarine area on the south coast of Korea. Sex and reproductive condition of each oyster was then assessed from histological preparation of a cross-section removed in the middle of the oyster body. Histology indicated that the gametogenesis initiated in January and in the middle of July all the female oysters were ripe and ready for spawning. The percentage of eggs in an individual oyster in April ranged 0.7 to 14.0, when most female oysters were in early to developing stage. In July, the GSI ranged 17.5 to 67.0, with a mean of 47.7. Fecundity of the ripe oyster was also estimated by dividing the amount of egg estimated in ELISA with a mean dry weight of single egg, 13 ng. The fecundity ranged from 117 to 796 million eggs. A positive correlation was found between the GSI and size of the oyster.

Effect of reproduction on escape responses, metabolic rates and muscle mitochondrial properties in the scallop *Placopecten magellanicus*

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Keywords: mitochondria, reproduction, escape response, metabolic rate, cytochrome c oxidase, phospholipids, fatty acids, Placopecten magellanicus.

Gametogenesis represents a period of high-energy demand, and when external food supplies are limited, gamete production occurs at the expense of biochemical components in somatic tissues. In scallops, muscle is one of the most affected tissue, with reserves decreasing and mobilized to support gametogenesis and reproduction activity. This mobilization decreases muscle metabolic capacities with scallops showing a weaker escape response and recuperating slower from exhausting burst exercise (1,2). Interestingly, this response or recuperation from an exhausting escape response appeared to be related to modifications in oxidative capacities of mitochondria isolated from the adductor muscle (1,3).

In the present study, we first assessed the impact of the reproductive cycle on muscle metabolic capacities and compared the escape response and capacity for recuperation from exhausting exercise in terms of number of valve claps until exhaustion, rate of clapping and recovery after recuperation in adult giant scallops, *Placopecten magellanicus,* sampled at different stages in the annual reproductive cycle (mature, after spawning, and reproductive quiescent). In parallel, we measured the standard metabolic rate before exhaustion (VO_{2min}) and the maximal metabolic rate after exhaustion (VO_{2max}). Further, the oxidative capacities and cytochrome *c* oxidase (CCO) activity of mitochondria isolated from the adductor muscle were compared. The changes demonstrate a marked effect of reproduction on the escape responses, VO_{2min} /VO_{2max} and functional properties of isolated muscle mitochondria in scallops. Shifts in maintenance requirements (VO_{2min}/VO_{2max}) and aerobic scope provided the best explanation for the impact of reproduction on escape response performance.

Further, we sought to identify potential mechanisms by which mitochondrial oxidative capacities changed. Specifically, an important level at which adjustments can occur is in the phopholipid composition of membranes with marked shifts in classes and fatty acid composition accompanying seasonnal cycles of gametogenesis and spawning. To test this hypothesis, we examined the modifications of phospholipid and fatty acid compositions concomitantly to contents of adenylate nucleotide translocase, cytochromes and proteins membrane of adductor muscle mitochondria. Structural changes in membrane phospholipids contrast with the limited modifications of the membrane protein components and support the concept of modifications in specific membrane lipid classes composition modulating mitochondrial protein capacities.

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Biotic and abiotic influences on the attachment strength of blue mussel (*Mytilus edulis*) from suspension-culture

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Keywords: mussel, attachment, seasonal variation, byssus, spawning

Mussels can attach themselves to solid substrata by producing byssal threads. Mussels need to produce new threads regularly to replace the decaying ones and maintain their attachment to the substratum. Nevertheless, their attachment strength varies with time. Up to now, studies on mussels attachment strength have used two complementary approaches. Most studies were performed in laboratory conditions and looked at the influence of single factors on the secretion of new byssal threads. The underlying assumption of these studies is that higher number of threads equals stronger attachment. Other studies were performed in field conditions with intertidal mussels living on rocky shores and they looked at temporal variation in attachment strength based on monthly samplings.

Mussels in suspension-culture may fall off the sleeves during the summer. Losses could be important when large clumps of 2-yr-old cultured mussels slip from the sleeves. These fall-offs are probably the consequence of a weaker attachment of mussels to the culture substrate. However, no previous studies were performed on attachment strength of suspension-cultured mussels. The available information from laboratory studies and field studies with intertidal mussels from rocky shores cannot be transposed to suspension-culture conditions.

This study examined on a weekly basis, from late May to mid-October, the temporal variation in attachment strength of 2-year-old cultured mussels (*Mytilus edulis*) from submerged longlines in a semi-enclosed lagoon. This study also looked at some possible factors influencing the attachment strength. Environmental factors (temperature, food availability, wind velocity and hydrodynamic conditions like current velocity, turbulence and waves height) and reproductive condition were measured concurrently. Attachment strength was measured directly on mussels attached from sleeves with a dynamometer. The mass spawning in early summer (late June) seemed to be synchronized with a quick and major decrease (-32%) in attachment strength. Then, attachment strength varied twofold from summer to fall, a difference related not only to the number of byssal threads but also to their individual strength. Water temperature (negative relationship) and turbulence (positive relationship) were the most important factors explaining the variation in mussel attachment strength after spawning. In contrast to previous studies, no trade-offs were observed between reproduction and attachment strength.

Use of probabilistic way of thinking as an alternative to the problem of the high interindividual variability in bivalves.

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Keywords: bivalve, interindividual variability, toxic phytoplankton, immunological parameters.

All researchers working with mollusks and especially bivalves have been faced one day or another with the high interindividual response of animals according to experimental conditions, either looking at physiological, immunological or biological parameters.
As a consequence, observation of significant changes according to various experimental conditions becomes unlikely or observed differences are so small that it is difficult to interpret them in a biological way. Finally, interindividual variability is often suggested as a confounding factor to explain the lack of differences between experimental conditions leading to an unwanted effect, the deletion from data set of values considered as abnormal.
However, this is not satisfactory and alternative approaches to counter the "individual variability" can be proposed: taking care of the role of micro-environment, increasing the sample size, use of families as experimental model to reduce potential genetic variability…
These approaches could be relevant when using the deterministic way of thinking. Determinism is the philosophical proposition that every event is causally determined by an unbroken chain of prior occurrences. Determinism may also be defined as the thesis that there is at any instant exactly one physically possible future. In other words, under a given constraint, there is only one response, even if the intensity of the response is varying. When doing experimental design to study response of bivalves to one or another constraint, we are waiting to one response (a mean response), and if the constraint is positive, the mean response will be significantly different from the control for a given parameter.
There is however another way of thinking, named probabilism, which considers that chance is a part of the response. As a consequence a same constraint can lead to multiple responses, especially in cases of complex phenomena. So the conclusion of an experiment will be the following: X % of animals gave response A to the constraint, Y % the response B and maybe Z % the contrary. The question is not to know if the constraint is effective, as in deterministic thinking, but to know the proportion of individuals in the population with the A or B type response in order to estimate the real impact of the constraint in the population.
As an example, analysis of a data set concerning immunological responses of bivalve hemocytes to toxic algae extract will be done and discussed according to deterministic or probabilistic way of thinking (Figure 1). data set from Ford, S.E., Bricelj, M., Lambert, C. and Paillard, C. (2008) Deleterious effect of a non-PSP bioactive compound of *Alexandrium tamarense* on bivalve hemocytes. *Marine Biology* (in press)].

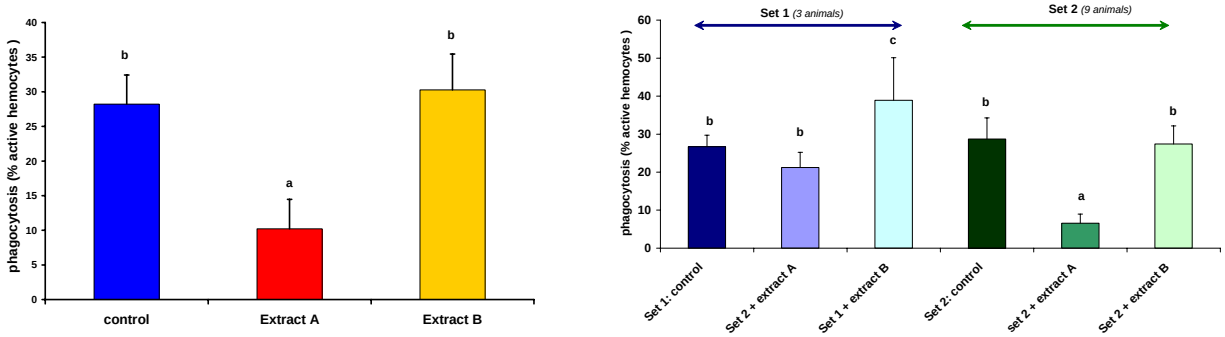

Fig. 1: Mean level of hemocyte phagocytosis by bivalve hemocytes after contact or not (+ seawater = control) with toxic algae (A or B) extracts. **Left**: analysis done using deterministic way of thinking (n=12, + CI, p=0.05); **Right**: analysis using probabilistic thought (Set 1, n=3 ; Set 2, n=9, + CI, p=0.05; letters indicate significant differences ANOVA, p<0.00001).

Evidence of disseminated neoplasia in the Bay of Arcachon and associated modifications of membrane lipids.

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Keywords: Cerastoderma edule, disseminated neoplasia, lipid and fatty acids composition

Disseminated neoplasia (DN) is characterised by the proliferation of large and anaplastic circulating cells in the hemolymph. These cells have an abnormal ploidy, a nucleus containing one or more prominent nucleoli and a high nucleus to cytoplasm ratio. DN has been reported with prevalence exceeding 90% and can result in mass mortality. This disease is proliferative and can be transmitted to uninfected individuals, indicating an infectious etiology. Neoplasia has been previously described in cockle (*Cerastoderma edule*) populations in Galicia, Ireland and Brittany, but never before in the Bay of Arcachon. The present work gives information about the presence of this disease in the study area.

Diagnosis of DN was realized by hemocytology. Abnormal cells were observed, which were larger, less complex and had a higher nucleus to cytoplasm ratio than hemocytes. Histological study of tissues of diseased cockles showed abnormal cells in all organs. This permitted to clearly establish that cockles from the Bay of Arcachon are affected by DN. A one-year survey was carried out in 2007, with sampling performed every 45 days to study the prevalence and the intensity of the disease in this area. Diagnosis technique estimating the ploidy of the hemolymph cells by flow cytometry was used to classify cockles in 3 stages of disease severity, function of the percentage of hyperploid cells (4N) to normal hemocytes (2N) : neoplastic > 80% ; 20% < intermediate < 80% and low and non-diseased < 20%. The prevalence of the disease at its maximum intensity was low and varied from 0% (June and December) to 2.4% (September). Prevalence of intermediate stages was registered with values comprised between 0% (February) and 4.3% (July). No clear relation between the season and the disease stages could be established. No mass mortality was associated to DN, although some risk of mortality may exist when DN prevalence is higher than 4% (6.4% observed in July).

To determine how structure and composition of individual membrane lipids of circulating cells are affected by neoplasia, the fatty acid composition and the proportion of lipid classes from normal and neoplastic cells were analyzed. Significant differences appeared between diseased and healthly animals. Total plasmalogen content of phospholipid classes was lower in neoplastic cells. Plasmalogens (1-O-alk-1'-enyl-2-acyl glycerophospholipids) constitute a sub-class of phospholipids characterised by the presence of a vinyl-ether bond at the *sn-1* position of the glycerol backbone. Concerning the fatty acid composition of the different lipid classes, non-methylene-interrupted (NMI) fatty acids tend to decrease in neoplastic cells. The plasmalogen form of phosphatidylethanolamine is the class where largest modifications were observed with a decrease of NMI concomitantly to an increase of 22:5(n-3) and 22:6(n-3). Some other differences appeared in the other classes, but at a lower level. Several functions have been attributed to plasmalogens but their exact physiological role(s) still remain elusive. Bivalve's neoplastic cells (leukemia *like* cells) would allow the development of an original approach investigating functions and properties of these important membrane phospholipids that are ubiquitous among living animals. The present results ask the question of the role of plasmalogens and their specific fatty acid composition in cell signalling pathways such as cell death signalling.

The Transforming Growth Factor-β (TGF-β) signalling pathways in the Lophotrochozoan Protostome *Crassostrea gigas* (Mollusca Bivalvia)

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Keywords: Crassostrea.gigas, TGF-beta, activin, BMPs

The Transforming Growth Factor-β (TGF-β) is a widely distributed superfamily of signalling molecules involved in the regulation of numerous functions such as cell proliferation and differentiation, development, reproduction and immunity. The TGF-β signalling pathway is activated by the binding of dimeric ligands to a type II serine threonine kinase receptor dimeric complex. Subsequently this complex interacts with two type I serine threonine kinase receptors to form a tetrameric complex which allows phosphorylation and activation of type I receptors. This activated complex thus becomes capable to activate the Smad nuclear effectors by phosphorylation.

This pathway appears well conserved in the animal kingdom with several TGF-β components being identified in Deuterostomes and Protostomes, essentially in model organisms. Generally, the TGF-β superfamily is divided into four major families including: TGF-β sensu stricto, Activins, BMPs and divergent TGF-β. In our lophotrochozoan animal model, the Pacific oyster *Crassostrea gigas*, we have characterized conserved orthologous ligands for each family as well as five receptors and five nuclear effectors. Four ligands have been identified by an RT-PCR approach using degenerated primers (Cg-mGDF1, Cg-TGF-β, Cg-Dorsalin and Cg-Gbb). Recently and through two Expressed Sequence Tag (EST) sequencing projects in *Crassostrea gigas*, three new ligands were also characterized (Cg-Ogtbl and two activin orthologues) together with various extracellular regulators. The identification of these vertebrate orthologous molecules in molluscs suggests a strict conservation of the TGF-β pathways in lophotrochozoans and reveals an early diversity of ligands before the divergence between Ecdysozoans and Lophotrochozoans, but also between Deuterostomes and Protostomes.

To understand the involvement of the ligands in the physiology of oyster, first approaches have been developed like transcriptional expression analyses by real time RT-PCR and *in situ* hybridization. Expression of Cg-mGDF1, Cg-Dorsalin and Cg-Gbb suggests their implication during the development (in early events such as axis formation and organogenesis). These ligands are ubiquitously expressed in adult tissues in contrast to the gonadal specific Cg-ogtβl which is specifically expressed in the somatic cells surrounding the germinal cells. In other respects, Cg-TGF-β has been shown to work as a cytokine in adult tissues in case of Gram$^-$ bacterial infections.

Though structural homologies and spatio-temporal expression of ligand encoding genes is a mean to appreciate their functions and to gain insight into their physiological involvement, other functional studies are currently developed. Molecular interactions between oyster TGF-β components is explored by co-transfection of plasmids encoding oyster TGF-β ligands and TGF-β receptors in mammalian cells. Formation of molecular complexes is measured by the activation of distinct TGF-β pathways through specific responsive elements that control the expression of the Firefly luciferase. Results show the wide conservation of the TGF-β pathways in oyster and confirm the orthology of the genes encoding oyster TGF-β components.

Molecular characterization and expression analysis of a novel vitellogenin from the Pacific oyster, *Crassostrea gigas*

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Keywords: Crassostrea gigas, vitellogenin, oogenesis, reproduction, gene expression.

Vitellogenesis involves a selective transport of proteins and lipids into growing oocytes. This function has been attributed to vitellogenins, which are proteins grouped into the Large Lipid Transfer Protein (LLTP) superfamily. Among bivalves, there is one full-length sequence of vitellogenin reported in the GenBank database (http://www.ncbi.nlm.nih.gov/), isolated previously from *Crassostrea gigas* (CgVTG, access BAC22716), and some partial sequences of similar messengers isolated in Pectinidae and Mytilidae species. However, some evidence obtained in vertebrates and insects suggest that more than one vitellogenin gene could actively participate in vitellogenesis of many groups of metazoan, due to an ancient diversification of LLTP superfamily. This has been supported also by a genomic survey on public and private Expressed Sequence Tags (ESTs) databases, in where a particular contig (partial sequence resulted from overlapping of various different and consensus ESTs) presents significant similarity to insect and fish vitellogenins in conceptual translation by protein BLAST search algorithm. Surprisingly, no significant similarity was found with CgVTG; therefore, this could be the first report of homologous vitellogenin genes on a lophocotrozoan species. Full-sequencing of cDNA ends and phylogenetic analysis, currently under development, will provide information about the homology nature of two vitellogenins (i.e. orthology, paralogy), protein family relationships, and a better understanding of evolution and diversification of the LLTP superfamily in bivalve mollusks.

We are also interested to determine the expression profile over a complete gametogenic cycle. Preliminary analysis by end-point RT-PCR on gonad tissues shows a narrowly correlated expression between both messengers, related also with the oogenic developmental stage, and non-detectable expression on male gonads, suggesting a non-divergent evolutionary trait between both gene products. Quantitative technique of Real Time-PCR is under development, and will allow a more accurate description of temporal expression of each vitellogenin messengers during oogenesis.

Reproductive cycle and biochemical composition of flat oyster (*Ostrea edulis*, Linnaeus, 1758) in Turkey

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Keywords: Ostrea edulis, flat oyster, reproductive, biochemical composition, Aegean Sea

The European flat oyster, *Ostrea edulis* (Linnaeus, 1758) is a commercially important oyster that is distributed along the coast of Turkey. This species is considered a potential fishery in Aegean Sea. Despite its biological and economical importance in Europe and exported to European countries from Turkey, there is limited information on it until now. Nowadays the interest on oyster culture and harvesting from natural stocks increases in Turkey. Therefore studies on *Ostrea edulis* are important to know its population characteristics.

This study was carried out to describe the reproductive cycle and biochemical composition of flat oyster in Aegean Sea, Turkey and its relation to the environmental parameters such as temperature, salinity and food availability.

In order to determine the reproductive cycle and biochemical composition of flat oyster, *Ostrea edulis*, samples of adult oysters were collected monthly in Aegean Sea, Western Turkey. The samples were processed using the technique of embedding in paraffin and staining with hematoxilin-eosin for reproductive cycle. Biochemical analyses (total lipid, protein and carbohydrate) were performed on dried samples.

Five developmental stages were determined in the study area during a one-year observation. Sex ratio was also observed, with male individuals being dominant. Biochemical composition is important for reproductive cycle. Results indicated that glycogen storage was the highest during the reproductive period but the lowest except.

Brooding of egg capsules in *Crepidula fornicata*: physiological and morphological consequences of egg capsule hypoventilation in the egg, larvae and juveniles

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Keywords: Brooding, intracapsular development, ventilation, hatching

Eggs of *Crepidula fornicata* do not survive if capsules are removed from the mantle cavity of the female. Ventilation, nourishment, antibiosis, and physical protection against predators are some of the roles that are believed to play adult limpets in caring their egg masses. In this paper some of these and other functions were investigated. As egg capsule survival depends on the presence of the mother, it was necessary to investigate the ventilation effect on eggs by reducing pumping rates of the adult. One-week starvation was found to reduce pumping rate from 32 to 13 mL h^{-1} in adult *C. fornicata*. With this results a larval quality index (total Ca/dry tissue weight) was determined on self-hatched veligers incubated by fed and starved adults during the whole capsular development (15 days). Results indicate a reduction in larval quality index from 537 ngCa μg^{-1} $larva^{-1}$ in adult-fed treatment to 125 ngCa μg^{-1} $larva^{-1}$ in adult-starved treatment. These results were confirmed by subjecting freshly-laid egg capsules to different flows (16, 103, 197 and 811 mL h^{-1}) of water at 88% O_2 saturation, produced by an artificial ventilation apparatus. Normal egg capsules were considered those showing well defined embryos and crystalline egg capsule fluid. Larvae from all capsules at 197 mL h^{-1} reached the ready-to-hatch veliger stage in 15 days while only 90 % from capsules incubated at 103 mL h^{-1}. More than 50 % of the egg capsules from treatments 16 and 811 mL h^{-1} already contained dead embryos at days 5 and 11, and were all dead by days 12 and 16 respectively. Although this experiment was extended for 25 days, larvae from 103 and 197 mL h^{-1} treatments were unable to hatch, which indicates that under natural conditions, the participation of the parent is essential in the hatching process.

Regarding morphology, "naked" *C. fornicata* larvae and juveniles from hypoventilated egg capsules were studied by scanning electron microscopy. Naked organisms were produced by subjecting embryos and larvae to hypoxia when were still developing within their egg capsules in the mantle cavity of the mother. Hypoxia was produced when starvation of the brooding parent produced a drop in ventilation rates and oxygen diffusion to the egg capsule fluid, thus subjecting the embryos and larvae to chronic hypoxic conditions. As hypoxia makes mollusks to shift into anaerobic metabolism, carbonate molecules originally destined to shell formation are used to neutralize the end products from anaerobiosis, preventing calcification. The critical stage at which this treatment must be applied is at the onset of shell formation. This treatment prevented calcification and eventually the loss of the larval shell without affecting survival and development rate. Treated specimens allowed to follow for the first time morphological changes occurring in larvae and juveniles, and to closely examine kidneys, operculum, osphradium, gill filaments, foot, velum, mantle edge, tentacles, and other structures.

Evolution and formation of *Nautilus macromphalus* nacre: biochemistry and proteomic of the shell organic matrix

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Keywords: Biomineralization, organic matrix, evolution, glycosylation, mass spectrometry

In mollusks, the shell formation is a genetically controlled process handled by the calcifying mantle cells. One of the most studied shell texture is the nacre, also called mother-of-pearl, the lustrous aragonitic layer that constitutes the internal part of the shell of several bivalves, a few gastropods and one cephalopod, the nautilus. Like other shell texture, nacre contains a minor organic fraction, which displays a wide range of functions in relation with the biomineralization process. Here, we have characterized biochemically the nacre matrix of the cephalopod *Nautilus macromphalus*.

The acido-soluble matrix contains a mixture of polydisperse and discrete proteins, glycoproteins and proteoglycans, which inhibits the *in vitro* precipitation of $CaCO_3$, interacts with the formation of calcite crystals, and binds Ca^{2+} ions in solution. Although the saccharidic moieties are not involved in binding calcium, they participate to the modulation of the shape of calcite crystals grown *in vitro*. On 2-DE, the different components of the nacre matrix migrate either at very acidic or at very basic p*I*. In addition, we have used a 'shellomic' approach (proteomics applied to the shell matrix) on the acetic soluble and acetic insoluble matrices, as well as on spots obtained after 2-D electrophoresis. Our data demonstrate that the so-called insoluble and soluble matrices contain numerous shared peptides. Furthermore, while most of the obtained partial sequences do not fit with known molluscan shell proteins, few of them partly match with shell proteins of bivalvian origin.. These findings have implications in the knowledge of the macro-evolution of molluscan shell matrices.

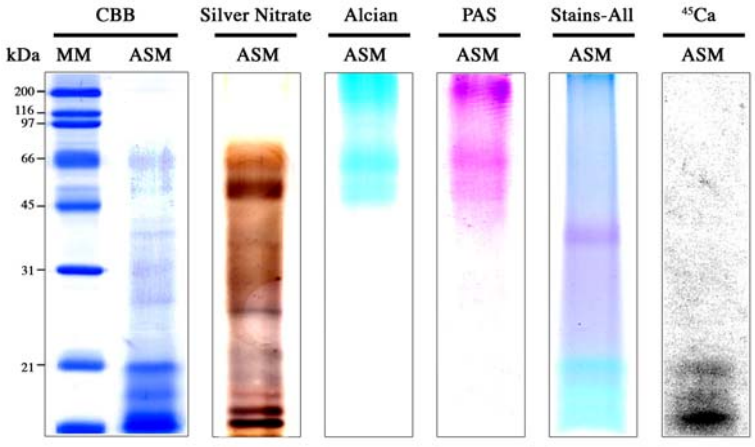

Figure 1: SDS-PAGE analysis of the ASM of *Nautilus macromphalus* nacre. 12% acrylamide gels were stained with CBB, silver nitrate, Alcian blue, PAS, Stains-All, from left to right. The last right lane corresponds to the result of the calcium overlay test (^{45}Ca). MM, molecular mass markers.

In situ expression of *Cg-DMl*, a factor of the DM family, in the pacific oyster *Crassostrea giga*s

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Keywords: Crassostrea gigas, Dmrt1, Dmrt4, Dmrt5, sex determination

The Pacific oyster *Crassostrea gigas* is a successive, not systematic, protandric hermaphrodite. Its gonad is a diffuse, non permanent organ: it regresses during the autumn, period of the sexual rest; it growths and develops during the winter and the spring till it reaches the mature stage before the spawning period, in the summer. Moreover, in *C. gigas*, there are no distinguishable sexual chromosomes. Therefore the early determination of oyster's sex during the adult gametogenetic cycle is very difficult, which is a problem for the control of the genitor sex-ratio and thus for spat production in nurseries. The pacific oyster has an indirect development with different larval stages (trocophore, D-veliger, umbo-veliger, eyed-veliger, pediveliger) and a metamorphosis leading to metamorphosed larvae and spats stages who reach sexual maturity between 4 and 10 months old. Then the precise moment and factors responsible of the gonadic differentiation during the adult gametogenetic cycle but also during the development are unknown. According to some authors, the oyster's sex determinism might be under some paternal genetic control but also under an environmental influence (epigenetic determinism). Then a genetic dose-dependant determinism initiated by some environmental factor might be hypothesized. Whatever is the sex determinism, a series of molecular activations/inactivations govern the gonadic differentiation. To date, little has been published on sex determinism among Invertebrates. So our approach was based on the comparison of this gene cascade among Vertebrates and Invertebrates, and the study of a potential ortholog of a conserved factor in *C. gigas*. It led us to identify, for the first time in Molluscs, a gene called *Cg-DMl* (*Crassostrea gigas* DM like) belonging to the DM family. Its expression, studied by in *situ* hybridization and immunohistochemistry with a heterologous antibody, was located in specific cells in different tissues. The potential role of this factor in the oyster is discussed in relation to the role of other factors of the DM family.

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Genetic polymorphism of an invasive species, the Pacific oyster *Crassostrea gigas*, along the English Channel-Atlantic Ocean coasts

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Keywords: Crassostrea gigas, invasion, polymorphism, microsatellites, SSCP

The Pacific oyster *Crassostrea gigas* is the most commercially important marine species in France. Oysters, essentially cultivated over extensive areas essentially, form one large genetic pool because of important natural and human-mediated gene flow between populations. The wide spreading of this species during the last decade quickly led to the development of "wild" populations mainly on rocky intertidal zones. *C. gigas* now is considered as an invasive species in various part of the world. In fact, mechanisms of adaptation strategies of this invasive species need to be better understood. The purpose of this work was to study genetic variability in some European populations to identify an effect of selection. Microsatellites were used to assess neutral effects like genetic drift or the Wahlund effect. In parallel, enzymes and DNA polymorphisms were studied to measure potential selection effects that might modify genetic variability and structure. Enzyme polymorphisms involved in general and energetic metabolism were studied using allozymes. DNA polymorphism, investigated on coding sequences of some genes previously identified by suppression subtractive hybridization (SSH) methods, was involved in different physiological functions: general and energetic metabolism, stress response, and others. Genetic structure and variability were assessed and discussed at different spatial and temporal scales: European, regional French scales and at low and high tidal heights on adult oysters and also in different cohorts from 2005 to 2006.

Protein synthesis, protein degradation and protein retention: mechanisms of indeterminate growth in cephalopods

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Keywords: squid, lifetime growth, protein synthesis, protein accretion

This study examined the underlying process of growth in a cephalopod to ascertain the mechanism by which indeterminate is achieved in this live-fast, die-young group of animals. Individuals were held in controlled facilities with ad libitum access to food, animals of different ages were terminal sampled following injection of 3H phenylalanine to measure rates of protein synthesis. Rates of protein accretion were estimated from the concentration of protein in the tissues. This is the first study that has estimated rates of protein synthesis and growth of squid from 7-140 days old, providing an understanding of both the pattern and process of growth throughout the lifetime of a squid species. Younger and smaller individuals had greater rates of protein synthesis, greater protein retention efficiency, and more RNA than older and bigger individuals. Variation in growth rates among older and larger individuals was a function of individuals with faster growth rates having greater protein retention efficiency but also greater concentrations of protein. The retention of synthesis protein being retained for growth (efficiency) remained relatively high in older animals (Fig 1). Critically growth did not cease in the adults and with on average 10% of protein synthesised being retained, the mechanism to support the non-asymptotic growth model of cephalopods is provided.

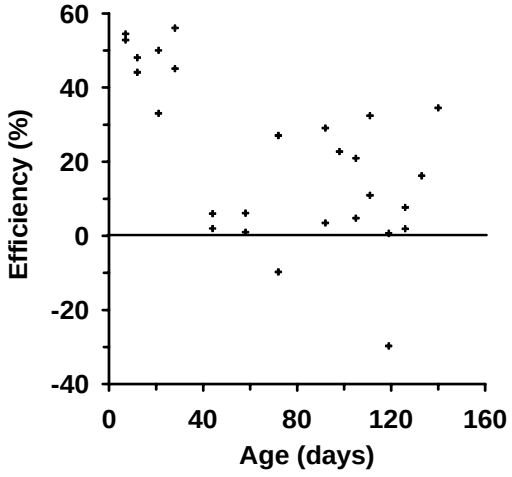

Figure 1: The relationship between the percentage of synthesised protein retained for growth (efficiency) and age of dumpling squid.

Influence of bacteria assocaited with algae on larval growth of bivalve

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Keywords: algae, bacteria, larvae, bivalve, scallop, growth

Generally microalgae are cultured with particular attention to prevent contamination with other ciliates. However the bacterial flora naturally associated with these algal cultures has never been tested and its impact on algae as well as on larvae has not yet been estimated. In a previous study (Nicolas et al, 2004) we determined the main groups of bacteria growing in several types of algae collected from some commercial hatcheries in Europe. We hypothesized that some of them could be detrimental to bivalve larvae.

To demonstrate this hypothesis, scallop *(Pecten maximus*) larvae were fed with axenic algae including, *Pavlova lutheri, Isochrysis affinis galbana*. The control larvae received the same non axenic algae. The diatom, *Skeletonema costatum,* was distributed into all batches to complement the diet and antibiotic was added to the treated control.

Scallop larvae fed with axenic algae grew faster than non treated larvae fed with standard algae. At day 20 they reached 217 ± 23µm in length, versus 202 ± 19µm for the control (see Figure), and metamorphosis started 4 days before that of the control larvae. The detrimental effect of bacterial flora was reduced by the antibiotic treatment (Treated control). The mortality rate did not exceed 15% at the metamorphosis stage in any of the batches.

Besides probiotic bacteria *(Phaeobacter sp*, X34 X129, or 04/149 strains) were associated with axenic algal cultures where they grew until 10^7/ml, about the same concentration of algal cells. These combinations distributed to larvae, enhanced their performances as the axenic algae (even slightly better). These associations allow protecting the "monoxenic" algae against the contamination, and also producing and supplying probiotics to larvae without additional cost. However this method requires the use of photo-bioreactor which can be autoclaved and can remain sterile during working. Indeed even if the probiotic exhibits antibacterial activity it can not prevent any contamination.

The next objective is to replace antibiotics, generally added to sequential scallop larval rearing, by these co-cultures to prevent mortality. They could also be used for any bivalve species in order to improve their performances and to decrease the load of pathogenic vibrios in early stages.

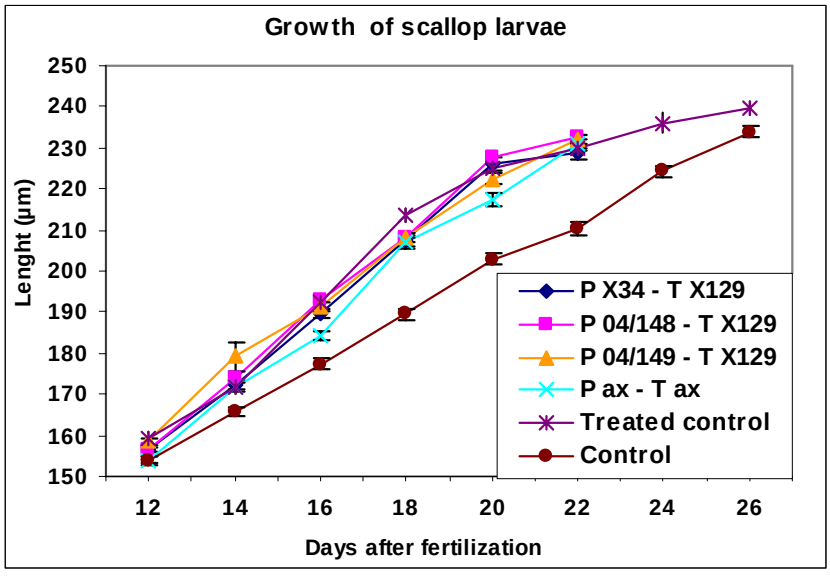

Figure: Growth of scallop larvae fed on axenic algae (*Pavolva lutheri, Isochrysis affinis galbana* (T-iso) or on « monoxenic » algae associated with a probiotic (*Phaeobacter* sp X34 or X129, 04/179 strains) in comparison with control batches with or not treated by antibiotic. Experiment was performed in 2L bottles.
Legends: P: Pavolva lujyjeri, T: T-iso, ax: axenic

Genetic basis of gametogenesis in diploid and triploid Pacific oysters, *Crassostrea gigas*.

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Keywords: gametogenesis, quantitative genetics, oyster, triploidy, parentage analysis

Today, triploidy is the most common method used to genetically improve marine bivalve aquaculture production. In the Pacific oyster *Crassostrea gigas*, triploidy affects many traits. The most prominent one is reproductive effort, which is greatly reduced compared to diploids. The combined effects of physiological trade-offs between survival, growth and reproduction together with the reduction of reproductive effort in triploids explains the global enhancement of yield observed. However, the sterility of triploid oysters is only partial and some triploid individuals can show normal gametogenesis (*i.e.* similar to diploids), especially under warm conditions. In diploid oysters, the heritability of reproductive effort has been shown to be relatively low, but a clear relationship between survival during summer (a period of extreme mortality), a trait with high heritability, and allocation to reproduction has been demonstrated.

Three specific issues related to triploidy can be identified. Firstly, as demonstrated in various species from crops to invertebrates, triploidization is known to directly affect the average performance (i.e. phenotypic mean), regardless of the breeders genetic values. Indeed, depending on the trait considered and possibly the age of the individuals, triploid Pacific oysters show lower (e.g. reproductive effort), or higher (e.g. growth) mean performances compared to diploids. Secondly, triploidy interacts with the transmission of additive genetic values between parents and their progeny. This is due to the unbalanced contribution of male and female parents, as demonstrated in triploid rainbow trout triploids in which 2/3 of the triploid genome comes from the dam, and 1/3 from the sire. Thirdly, triploidization can increase non-additive genetic effects (notably due to the interaction between three alleles at each locus), as shown for flowering time in *Brassica napus*. All these ploidy-related characteristics strongly influence the possibility to select diploids on their breeding value to obtain improved triploid progenies, notably depending on the relative importance of additive and non-additive genetic variance for the selected traits.

In order to further examine (1) the genetic basis of the observed variation in reproductive effort, both in diploid and triploid oysters, and (2) the relationship between reproductive effort in diploid and triploid sibs, a quantitative genetics experiment was set up. A full-factorial cross was performed by mating 2 sets of 16 males (16 diploids and 16 tetraploids) with 6 diploid females. This generated 3 experimental groups comprising of 96 full-sib families: a diploid group and 2 triploid groups obtained either by crossing tetraploid and diploid broodstock ("natural" triploids: 3nn) or by second polar body retention ("chemically induced" triploids: 3nc). These were reared together (i.e. mixed-family approach) to avoid environmental bias. Reproductive effort (estimated by image analysis of histological cross sections) and growth performances were first measured on 300 individuals/group of 6 month-old spat, in parallel with parentage analysis assisted by microsatellite markers. Analyses are currently in progress. The results that will be presented deal with (1) the effects of the ploidy level (2n *versus* 3n) and of the method of triploid production ("natural" *versus* "chemically induced") on phenotypic means and variance for growth and reproduction; and (2) the variance between families and the resulting estimated genetic parameters (heritability, genetic correlations) for these two traits. Our results will be discussed in light of the trade-off between growth and reproduction and the opportunities for selective breeding of diploid broodstock to produce improved triploid progenies will also be covered.

Influence of seawater acidification on larvae of the European flat oyster *Ostrea edulis*

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Keywords: Ostrea edulis, larvae, ocean acidification, growth

The emissions of carbon dioxide into the atmosphere have risen strongly since the beginning of the industrialisation. About 48% of the anthropogenic CO_2 emissions are taken up by the world's oceans. The excess carbon dioxide interferes with the ocean's capacity to buffer changes in pH, the carbonate system, and causes the acidification of the seawater. A decrease in the pH value of the ocean has negative consequences for calcifying metazoans, e.g. bivalves, because their calcification process may be negatively influenced. Larval stages are assumedly more sensitive to declining pH values than adults, but until now, there are only few studies on larvae.

In the present study, the effects of acidified seawater on growth and survival of larvae of the European flat oyster (*Ostrea edulis* L.) were investigated. The seawater was acidified by addition of hydrochloric acid, and five pH values below the recent pH (in this case 8.22) were created, decreasing in steps of 0.25 units. These were 7.97, 7.72, 7.47, 7.22 and as lowest 6.97.

One treatment was left at the natural condition as a control; another treatment was alkalised to simulate a pre-industrial pH value (8.47). Three replicates per pH treatment were set up. The experiment was performed in 2 liter glass bottles with separate aeration for each treatment. Air from the headspaces of the bottles was pumped into the water, thus a mixing with outside air was prevented.

Acidified seawater had a significantly negative effect on larval shell length. The smallest shells and slowest growth were found in larvae cultivated at the lowest pH value whereas the highest growth rate and biggest shells by far occurred in the highest pH treatment. Survival rates were not significantly affected by the pH value, although larvae in the alkalised treatment survived seven days longer than in all the other treatments. In addition, the morphology and appearance of the larval shells within the most acidified bottles seemed to differ from the shell morphology of normally growing oyster larvae. Larvae cultivated under a "pre-industrial" pH scenario showed a transparent "rim" along the shell edges. It can be concluded that seawater acidification will have a detrimental effect on the shell development and growth of European oyster larvae.

The sweet relationship between microalgae and *Crassostrea virginica*: implication of carbohydrate and lectin interactions in particle selection in suspension feeding bivalves

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Keywords: ecophysiology, feeding, selection, bivalve, mucus

Despite advances in the study of particle selection in suspension feeding bivalves, the mechanisms upon which bivalves rely to discriminate among particles have not been elucidated. In order to test the hypothesis that particle sorting in suspension feeding bivalves could be based on a biochemical recognition mechanism, the mucus that covers the feeding organs of *Crassostrea virginica* was investigated and its effect on particle sorting was evaluated. Results showed that mucus was able to agglutinate several microalgae species and red blood cells, demonstrating the presence of lectins. Hemagglutination (HA) and HA inhibition assays, used to determine the carbohydrate specificity of the lectin activity, showed that mucus contains mannose-binding lectins. The presence of carbohydrates moieties, potential ligands for lectins, on the surface of several microalgae species were detected using FITC-labeled lectins. In addition, the incubation of microalage with pallial mucus significantly reduced the binding of FITC-labeled lectins to their surface ligands suggesting that lectins present in mucus competitively blocked binding sites. Results of particle selection studies with *C. virginica* strongly suggest the involvement of lectin/carbohydrate interactions in the mechanisms of microalgae recognition. In conclusion, this work confirms the presence of lectins in mucus that covers the feeding organs of oysters and emphasizes their implication in particle sorting.

p53, p73 and mortalin gene expression associated with haemic neoplasia in soft-shell clam *Mya arenaria*

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The molecular mechanisms by which haemocytes in clams became neoplastic remain by far unknown. The aim of the study is to understand the role of p53 and its related gene p73 and mortalin in the molecular mechanism involved in haemic neoplasia in the soft-shell clams *Mya arenaria*. Haemic neoplasia was diagnosed using ploidy status of clam haemocytes. For this purpose, clams were sampled in North River at Prince Edward Island. The ploidy status for each clam was assessed using propidium iodide protocol while gene expression of p53, p73 and mortalin were quantified using real time quantitative RT PCR. Clams with low (0-5%), moderate (5-15%), high (15-50%) and diseased (>50%) tetraploid level were grouped. Data on gene expression show that the variation in expression levels of p53/p73 and mortalin are higher in clams with percentage of tetraploid haemocytes ranging between 15 and 50%. However the level of these transcripts remains low for the other groups. Interestingly, a correlation are found between p53 and mortalin expression (r^2=0.68, p<0.01). Furthermore, no p53 mutation was found on analyzed clams using RFLP protocol. Based on these data, it could be believed that p53/p73 and mortalin are involved in haemocyte tetraploid process in the soft-shell clams.

Quantifying the growth rate of ectotherms after dormancy periods as an intermittent non-steady-state compensatory process

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Keywords: Pecten maximus, daily growth rate, ecophysiology, transitory phase, compensatory growth

Generally, growth rates of living organisms are considered to be steady-state functions of the environmental variability. For example, growth rates are described as function of light for plants and food resources for animals, and are regulated or not by temperature and other factors. What are the consequences if this is not the case? This question can be asked for organisms presenting phases of dormancy. In order to describe precisely this critical growth phase, it is necessary to describe more accurately the growth pattern of poïkilotherms at high frequency. This smaller scale of growth description, which must be correspond to what individuals experience in term of food supply and temperature fluctuations, should permit to investigate, in addition, possible growth reaction of environmental stress. This study presents a dynamical perspective for the short-term quantification of the shell growth of *Pecten maximus* using a high-resolution determination of the growth rate of individuals (daily increments).

The Great Scallop (*Pecten maximus L.*) is largely distributed along the European coast, from Norway to Portugal. The growth stops during winter for a duration which vary as a function of the latitude. During the period of metabolic activity, environmental conditions (photoperiod, temperature, food availability) controlled the shell growth by modulating the size of each daily increment but not as a steady-state process. The dynamics of the Mean Daily Growth Rate (MDGR) was measured from several sets of juveniles over 12 years (1987 to 1998), and our results suggest that the MDGR variations after the growth restart are described by a 43 days periodic oscillation. The growing season appears to be structuring by a succession of increase and decrease phases of growth rate during which the MDGR trajectory converges asymptotically toward an equilibrium. This dynamic of the MDGR was simulated by a forced damped oscillation and for 8 years out of 12, the observed MDGR variation pattern was simulated accurately by our model. The damped oscillating component could be interpreted as a transitory phase of the growth dynamics and might be attributed to the dynamics of energy allocation to somatic growth or maybe to the dynamics of energy transfer between organs. This is also consistent with the prevailing notion that an organism is able to manage its internal energy fluxes during growth (Kooijman 2000). The synthesis of our results suggests in addition, a possible explanation of the compensatory-growth processes mentioned in the literature (refers to the ability of an organism to grow at an accelerated rate following a period of food shortage or a decline in reproductive weight), and second, the possibility of a trade-off between a fast growth and a high capacity to compensate. This implies that the concept of maximization of energy acquisition and allocation for growth, the so-called “bigger is better”, appears too simplistic.

Are temperature and food important for sexual maturation in blue mussels (*Mytilus sp.)* and the soft shell clam (*Mya arenaria)*?

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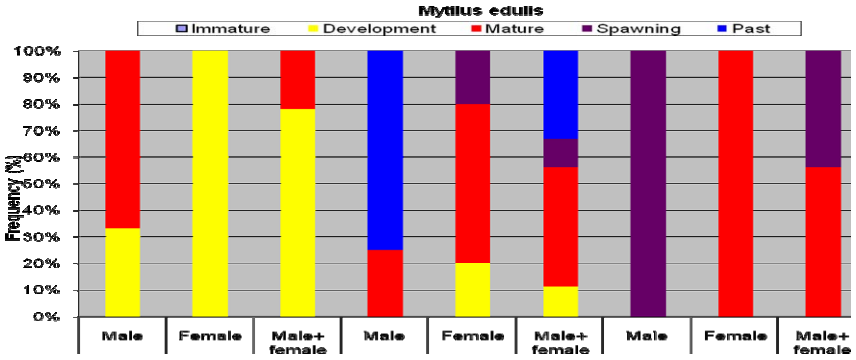
Keywords: molluscs, mussels, sexual maturation, nutrients, site effects

Choice of good culture sites for bivalves is necessary to assure efficient production for farmers and increased economic activities. However, many stresses originating from environmental factors like temperature and variations in food quantity can impair survival, reproduction, growth and quality of the product. Understanding the effects of these factors could eventually help farmers to choose sites with the best water quality. We first carried out an experimental protocol to validate the hypothesis that food was a main factor to induce sexual maturation in the blue mussel and the soft shell clam. The experiment was realized in duplicate aquaria using continuous seawater flow (1 L▪ min $^{-1}$) with 30 mussels and 30 clams in each aquarium. Gonadosomatic indices were calculated for both species at the beginning and at the end of the experiment as well as the determination of the gametogenesis stages of the mussels by standard histological procedures. Gonadosomatic indices were not significantly different (ANOVA; p= 0.052 > 0.05) between the two concentrations of spirulin when compared to control mussels. Clams gonadosomatic indices were however significantly different (Kruskall-Wallis p=0.011 < 0.05) with lower values in the highest concentration of spirulin probably due to colmated gills or to increased organic matter in sediment where clams were burrowed. A χ^2 statistical test (P = 0,007 < 0,05) showed that increased spirulin concentrations accelerated sexual maturation in mussels (graph shown below).



Control **1 g spirulin** **5 g spirulin**

Seasonal monitoring of transplanted Gaspé mussels in Gaspé Bay, Carleton and Havre-St-Pierre mussels from different farm sites of Eastern Quebec allowed us to validate these findings. A positive influence of phytoplankton cell concentration and temperature on the reproductive cycle was discovered even if no significant relationship has been showed because of the fragmented pattern of gametogenesis. Since partial spawning occurred throughout the sampling and commercialisation periods, and was not significantly related to phytoplankton concentrations, it is not possible at this stage of the research to determine a threshold of food abundance sufficient to support both reproductive cycle and growth energetic needs. It appeared however that good inputs of nutrients in farm sites could help to have more synchronized spawning periods helping farmers to plan their production. In clams further studies are needed to assess precise gametogenic stages according to changes in temperature and nutrients.

Genetic diversity and population structure of Southern Portugal *Ruditapes decussatus* (Mollusca Bivalvia) populations detected bu RAPD markers

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Keywords: Ruditapes decussatus, RAPDs, genetic diversity, population differentiation.

Genetic studies of marine invertebrate species, such as bivalves, have greatly increased in recent years. The clam *Ruditapes decussatus* is a commercially important bivalve in the south of Portugal, however to date, there are no records of population studies performed in Portuguese populations of this species. The random amplified polymorphic DNA (RAPD) technique was applied to assess genetic diversity and population structure of two Portuguese populations of *Ruditapes decussatus*. Twenty five individuals from two Portuguese populations of *Ruditapes decussatus* of different geographic origin (Alvor and Faro), were investigated by RAPD profiles. Of the twenty primers screened, ten primers amplified clear and reproducible bands. Within population polymorphism as measured by percentage of polymorphic RAPDs, varied between 87.5% and 85% (criterion 95%) respectively in Alvor and Faro population. Genetic variability was measured using Shannon's information index and genetic diversity of Nei´s (1973), respectively 0.4105 and 0.2501 for Alvor population and 0.4765 and 0.3125 for Faro population. Overall, genetic variation among *Ruditapes decussatus* populations was high. The total genetic diversity was explained by a low variation between populations (mean G_{st}=0.145), which is consistent with high gene flow between them (N_m=2.9). The values of Nei's (1972) genetic distance and Nei's (1978) unbiased genetic distance, respectively 0.0881 and 0.0828, and the low value of Theta p (=F_{st}=0.2393) between populations explained the low degree of population differentiation, despite the geographic separation. The results obtained from this study can be of great interest among others to stock enhancement or restoking programs.

Biochemical and physiological adaptation of marine bivalves to temperature

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Keywords: Crassostrea.virginica, Mytilus edulis, temperature, membrane lipid

Blue mussels *Mytilus edulis* and eastern oysters *Crassostrea virginica* are two bivalves widely distributed along the east coast of North America. *M. edulis* ranges from Baffin Island to North Carolina, whereas *C. virginica* is mainly found in the southern part of North America, from the Gulf of St Lawrence to the Gulf of Mexico. In the Gulf of St Lawrence, *C. virginica* is restricted to warm shallow bays and estuaries whereas *M. edulis* is found almost everywhere. This reflects the thermal preferences of the two species: maximal and optimal temperatures for *M. edulis* are much lower than those of *C. virginica*. *Crassostrea virginica* is of primary interest for cold-water aquaculture because of its high commercial value compared to that of *M. edulis*. However, variability in growth rates among individuals and sporadic juvenile overwintering mortalities complicate the commercial exploitation of this species.

We compared lipid dynamics and the physiological responses of *M. edulis*, a cold-adapted species, and *C. virginica*, a warmer-water species, during simulated overwintering and passage to spring conditions. Mussels used digestive gland TAG stores for energy metabolism or reproductive processes during the winter, whereas oysters did not accumulate large TAG stores prior to overwintering. Discrepancies in TAG dynamics between mussels and oysters during overwintering partly reflect differences in feeding rate. We also found that both bivalves underwent a major remodelling of membrane phospholipids: their unsaturation index (the number of double bonds per 100 fatty acid chains) decreased with increasing acclimation temperature, principally due to 22:6n-3 and 20:5n-3. Interestingly, we found that interspecific differences in metabolic rates correlate with unsaturation index of membrane lipids.

Then, we tested two hypotheses: first, that intraspecific growth variations in *C. virginica* are correlated with physiological (basal metabolic rate and scope for growth) and biochemical (membrane lipids) characteristics, and, second, that this bivalve shows intraspecific variations in physiological and biochemical adaptations to temperature. We found that a lower basal metabolic rate and lower unsaturation index of membrane lipids coincides with higher growth rates and a higher scope for growth in oysters. A perfect negative relationship was observed between the acclimation temperature and the unsaturation index of membrane lipids in oysters. However, the pattern of biochemical compensation in response to temperature in this species shows intraspecific variation.

In conclusion, an important component of the cold response is the increase in membrane lipid unsaturation, and this has been linked to an enhanced resistance to cold. Given that membrane adaptation is now viewed as a central contributor to low temperature survival of all organisms, this trait may be of particular interest for the coldwater aquaculture of marine invertebrates.

Heart function and respiratory haemolymph circulation in the abalone, *Haliotis iris*

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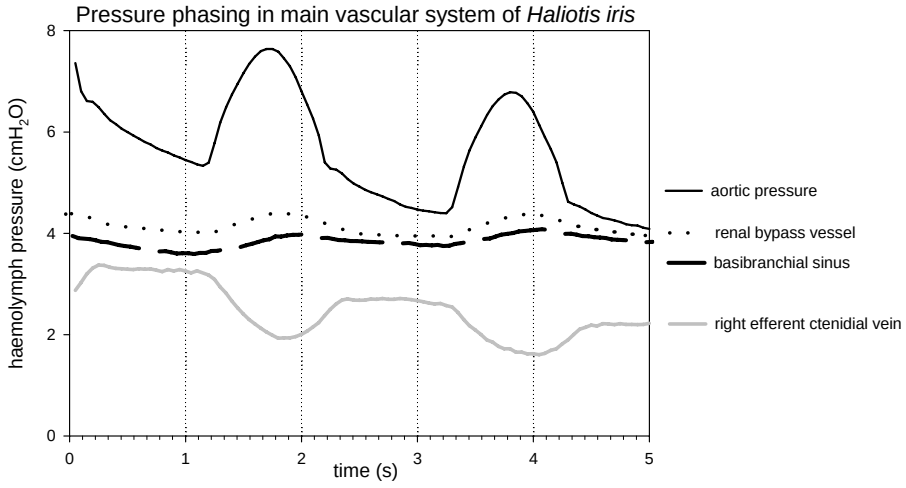
Keywords: Haliotis iris, pressure, heart, gills, respiration

The small abalone heart must generate sufficient pressure to overcome vascular resistance throughout the body while still perfusing the large gills at the end of the venous path, adjacent to the heart. Here we examine the mechanisms that support gill perfusion and transfer of oxygenated haemolymph to respiring tissue in the blackfoot abalone, *Haliotis iris*. Detailed anatomical studies (corrosion casting, histology, dissection) revealed several previously undescribed vascular spaces, including a major vessel bypassing the extensive right kidney, potentially creating a low resistance path to the gills ('renal bypass vein'). Experimental animals were prepared by cutting small shell windows above target regions; one cannula was fitted to the aorta and a second inserted into either the renal bypass vein, basibranchial sinus (entering gills) or right efferent ctenidial vein (leaving gills). Cannulae were connected to pressure transducers and impedance coupler leads mounted either side of the pericardium monitored heart activity. Mean aortic pressure (4.56cmH_2O; 5.92cmH_2O systolic, 3.69cmH_2O diastolic) fell slightly to 3.50cmH_2O in the bypass vein, which showed no further significant decline to the basibranchial sinus (3.30±0.22 cmH_2O). The latter were weakly pulsatile in phase with the aorta, implying a very low resistance route from the aorta to the gills via the bypass vein and the possibility of lateral pressure transmission across the walls of adjacent vessels. A distinct pressure drop was measured across the gills, with mean efferent ctenidial pressure of 1.16 cmH_2O in the region where post-branchial haemolymph is collected and enters the heart. This is below the mean circulatory filling pressure of 1.95 cmH_2O and 180º out of phase with pre-branchial vessels, effectively confirming Ramsay's (1952) constant volume hypothesis for the molluscan heart, where pressure reduction during ventricular systole is harnessed to actively suck blood into the auricles. Circulatory design in *H. iris* appears poised to maximise haemolymph flow across the gills, presumably at the cost of reduced flow to other regions. The high oxygen binding affinity of abalone haemocyanin and relatively low oxygen tensions around respiring tissues may complement this design, retaining bound oxygen in circulation until target tissues are reached.



The conflicting requirements of feeding an important but potentially toxic diatom, *Chaetoceros calcitrans*, to Greenshell™ mussel larvae

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Keywords: Chaetoceros calcitrans forma pumilum, toxicity, nutrition, Perna canaliculus larvae

Study at Cawthron Institute's research hatchery, Glenhaven Aquaculture Centre, Nelson, New Zealand, showed that the diatom *Chaetoceros calcitrans* forma *pumilum* ('Cc'), is a valuable dietary component for cultured Greenshell™ mussel larvae, *Perna canaliculus*. Unfortunately, this diatom is also known to be potentially detrimental to the health of shellfish larvae; possibly due to direct toxicity, mechanical damage caused by the frustules or an association with pathogenic bacteria.

Here we considered simple methods for reducing potential toxicity by manipulating residence time in batch culture, and then considered the relationship between larval performance and potential risk in the development of a feeding model. Cc (20 L) was batch cultured in Conway medium by up-scaling through 250 mL and 2500 mL steps. The flagellate, *Isochrysis galbana* T-Iso Clone ('Iso'), was also grown, using continuous culture in hanging 40 L bags. Greenshell™ mussel larvae were reared in purpose-built 2.5 L assay tanks at 18ºC. Larvae were held in this system from first D-veliger formation to pediveliger (~215µm shell height, foot and eyespot indicating readiness to settle).

The hypothesis that age of Cc batch culture influenced the deleterious effects on larvae was tested by growing the diatom for 2, 3, 4, 5 and 6 day periods in 20 L carboys before feeding the larvae a 2:1 ratio of Cc: Iso (6 replicates). Microalgae were continuously added to the filtered seawater flowing into the larval cultures (3.2% replacement min^{-1}) at a rate sufficient to maintain a feed environment of 40 cells μL^{-1} in the outflow water. It was found that larval performance declined with increasing Cc culture age. Pediveligers were dominant at 23 days post hatching. At this point, animals fed 2 day old Cc had a mean shell height of 236 ±4µm, were eating 34,760 ±3,950cells $larva^{-1}$ day^{-1}, and had a survival rate of 59 ±3%; whilst corresponding values for 6 day old Cc were 214 ±2µm, 16,880 ±1,260cells $larva^{-1}$ day^{-1} and 46 ±7% survival. The performance of larvae fed 3, 4 or 5 day-old Cc were similar to each other, occupying an intermediate level between 2 and 6 day Cc. Effects persisted post-metamorphosis, reflected in a 5-fold difference in spat survival between 2 and 6 day old Cc, assessed 10 days after settlement.

Subsequent experiments examined the effects of varying the amount of Cc available to larvae, either as total ration (cells μL^{-1}) or the fraction of Cc in the diet. Cc was offered as 0, 5, 66, 95 and 100% of the incoming diet, with Iso making up the balance to a level of 40 cells μL^{-1}. The 66%Cc diet was also offered at 3, 20, 60 and 120 cells μL^{-1}. Higher Cc fractions sustained faster growth, with 95% and 100% Cc producing 23 day-old pediveligers of 240 ±15µm and 228 ±5µm respectively, compared to just 212 ±7µm in the 66%Cc treatment (n=6). However, high Cc treatments were more volatile, causing higher mortality and greater predisposition towards population crashes. Similarly, reduced survival was observed in the high feed rate of 120 cells μL^{-1}. This was reflected in a final pediveliger yield of 29 ±6%, which compared to 47 ±3% at 60 cells μL^{-1} and 36 ±7% at 40 cells μL^{-1}. The 5%Cc and 20 cells μL^{-1} treatments showed signs of nutrient limitation, while 0%Cc or 3 cells μL^{-1} starved the larvae, which failed to reach metamorphosis. The extent to which nutrient limitation versus potential toxicity influenced this outcome will be discussed.

Methods for the commercial-scale production of Cc in continuous bag culture have now been developed by the Cawthron Institute. In addition to greatly facilitating bulk production, continuous culture provides a far more sensitive tool for the manipulation of cell quality. Applications for the optimization of larval performance and minimization of toxic impact will also be discussed.

Blue Mussels and Pacific Oysters in Belgian coastal harbours as test organisms for environmental stress

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Keywords: Mytilus edulis, Crassostrea gigas, growth, gonad development, pollution

Harbours receive, as semi-enclosed structures, high loads of chemical substances through river inputs, direct discharges (oil spills) as well as by indirect ways such as shipping traffic, atmospheric deposition, wastewater, etc. This means that organisms living in harbours are exposed to a wide range of pollutants which have the potential to cause stress by disturbing the normal functioning of the biological organisation.

The risk of micropollutants is studied for the Belgian coastal zone in three Belgian coastal harbours (Nieuwpoort, Oostende & Zeebrugge) and in a sluice dock in Oostende. Cage experiments with blue mussels (*Mytilus edulis*) and pacific oysters (*Crassostrea gigas*) are conducted to study the susceptibility of these bivalves to the prevealing stress. Growth, condition index and gonad development are recorded. Soft tissue is saved for chemical concentration measurements. Abiotic parameters are recorded fortnightly.

The results from June 2007 till October 2007 showed significant differences in growth and condition indeces between three of the sites. The shell length increment and condition indices decreased significantly according to a spatial gradient from outer harbour towards inner harbour and towards the sluice dock. Biometric differences of the bivalves between the different sampling stations are evaluated in relationship with the prevealing stress.

A more elaborated cage experiment is conducted from February 2008 till July 2008, on five stations, of which four harbour locations and one location at open sea. Results about growth, condition indices, gonad development and possible correlations with body burdens and/or with abiotic factors will be discussed and coupled to the gradient of environmental stress.

Funding: Belgian Science Policy: INRAM project: Integrated Risk Assessment and Monitoring of micropollutants in the Belgian coastal zone. Dec 2006 – Jan 2009.

Importance of turbulent kinetic energy on dispersion and transport of clams (*Mya arenaria)* of different sizes

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Keywords: Clams dispersion, substratum erosion, laminar currents, turbulence, ADV, flume

Soft-shell clams, *Mya arenaria*, live in soft substrates in the tidal zone and burial depth is correlated to clam size. Thus, small clams are buried near the surface and prone to passive dispersal. The purpose of this study was to measure the effects of hydrodynamic conditions, substratum and clam size on dispersal in a flume in relation to characterization obtained in the field. Firstly, unidirectional currents with increasing velocities (0 to 0.6 m s^{-1}) were applied upon three substrates (medium- and large-grain sand and sandy mud) in which were clams from one of three size classes (10, 15 and 20 mm). Then, the effects of turbulent kinetic energy on the erosion of medium-grain sand and clams from the three size-classes were examined. Turbulent energy was created with a home-made device acting on the unidirectional currents. Nearly 95% of buried clams (all substrates and size-classes together) resisted to transport and dispersion with unidirectional currents up to 0.60 m s^{-1} but only 10% with turbulent kinetic energy of 10.1 J m^{-3}, a level lower that one measured in the field during autumnal storm. The transport and dispersion of clams is related directly to level of substratum erosion.

Age-related tolerance to osmotic stress: implications for reproduction and recruitment ecology of *Mesodesma donacium* in the Humboldt Current Ecosystem

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Keywords: fresh-water input, surf clams, performance, ecophysiology

The surf clam, *Mesodesma donacium*, is an ecologically and economically important species in sandy beach ecosystems of Chile and Peru. The massive mortality of this clam after strong El Niño events causes a significant reduction of the northern edge of geographic distribution, revealing a low ability to recover that might be related to reproduction and recruitment constraints. From an integrative data analysis comprising eight independent studies (including our own two-year data) revering to seven sandy beaches covering the entire geographic distribution, we determined the spatial-temporal patterns of the reproductive cycle and evaluated potential environmental factors explaining the observed patterns by using a multivariate analysis. During a one-month laboratory study we determined the effect of temperature (17.5, 23.5º C) and salinity (10, 20, 35 psu) on body condition, growth and mortality of this clam.The reproductive cycle of *M. donacium* showed a marked geographic pattern. Northern populations (16 - 32º S) showed an austral-spring spawning and southern (42 - 43º S) populations showed an austral-autumn spawning, with transitional populations in Mehuin (39º S), which exhibit a biannual, spring and autumn spawning seasons. Sea surface temperature variability showed a consistent seasonal latitudinal pattern, which could only explain the northern pattern of the reproductive cycle. In contrast, fresh-water input (river inflow and coastal precipitation) seems to explain better the observed pattern, as seasonality was not the same: pluvial, mixed and nival river regimes exhibit different seasonal patterns along latitude.

A preliminary analysis showed that performance of *M. donacium* was not affected at low salinity (20 psu) but is negatively affected by extreme low salinity (10 psu) and anomalous high temperature (23.5º C). When analyzed by age groups, juveniles showed faster growth rates, higher body condition and lower mortality than adults at low salinities (10, 20 psu). They also seem to perform better than adults at anomalous high temperature. Although we used clams from the only place having apparently no influence of fresh-water input, *M. donacium* (notably juveniles) seems to be tolerant to osmotic stress. We discuss this finding and the fact that six out of seven of the studied populations are directly influenced by river inflow, as an adaptation of this species, to find suitable areas for settlement and recruitment. This is supported by previous observations showing that i) interspecific interactions play a critical role on the recruitment ecology of *Mesodesma* spp and *Donax* spp (species with a similar ecological role in sandy beaches); ii) *M. donacium* shows a definite spatial distribution pattern with age classes segregation along gradients of distance to river mouths and iii) former populations disappeared after closings of river input to the coastal zone. The availability of suitable places for settlement and recruitment may play a critical role in explaining the recovery ability of *M. donacium* after El Niñoty given the distribution of rivers flowing to the coast in Chile and Peru, with the Atacama dessert region acting as a geographic barrier to the northward distribution of this species.

This study was financed and conducted in the frame of the EU project CENSOR (Climate Variability and El Niño Southern Oscillation: Implications for Natural Resources and Management, contract 511071) and Programa bicentenario de Ciencia y Tecnología Proyecto CENSOR-RUE02.

Dynamic Energy Budget (DEB) model for *Crassostrea gigas* larvae

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Keywords: Dynamic Energy Budget, Crassostrea gigas, bivalve larvae, ecophysiology, growth

Dynamic Energy Budget (DEB) theory aims to quantify the energetic framework of an individual organism as a dynamic model from the uptake of food to the utilisation for metabolic processes (maintenance, growth, development and reproduction). The only difference between species lies in a different set of parameters values used in this energetic model. The purpose of the present paper is to build an energetic model for the Pacific oyster *Crassostrea gigas* in its larval life stage. We described the methodological procedure to obtain the DEB parameters from available laboratory data in terms of ingestion and growth for larvae related to phytoplankton densities (lean, restricted and *ad libitum* diets) and temperature (17, 22, 25, 27, 32°C) as forcing variables in the simulations for the model. Increases in temperature and food density supported significantly faster physiology rates in larvae. At highest temperatures (27 and 32 °C), high larval growth and metamorphosis performances were reached in less than 15 days. Low temperatures (17°C) strongly inhibited ingestion during whole larval period. Ingestion and growth rate at higher algal densities displayed a linear increase up to a maximum with 40 cells μl^{-1} around the fed larvae. All calculated parameters for oyster larvae followed the physiologic statements of the DEB theory. The simulations of the DEB larval model allowed us to predict properly length growth at controlled conditions providing a more extensive description of the energetic cost in the *C. gigas* larvae. These simulation data led to propose several promising perspectives of application for this model in post larval life stage for this species.

Genomic of adaptation of the pacific cupped oyster, *Crassostrea gigas*, in the context of its geographic expansion

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Keywords: Crassostrea.gigas, genome scan, AFLP, invasive, Pacific oyster

Originating from the north eastern Asia, *Crassostrea gigas* has been introduced and translocated, mainly for aquaculture purposes, into several European countries (from Norway to Portugal and in the Mediterranean Sea) (1). Although highly variable, the invasiveness pattern of *C. gigas* has been demonstrated in several countries and therefore considered as a pest or a noxious species in those areas (2 and 3).

Our project aims at identifying the characteristics of such a flourishing species: can its success be explained by chance and/or global warming only or does it exhibit a greater adaptation potential than other species? Therefore we developed a population genomics approach, known as "genome scan". It corresponds to the study of numerous loci spread through the genome, in order to quantify the part of the genome affected by a molecular signature that takes the form of a valley of diversity centred on the selected mutation (selective sweep) (see example in Figure 1a) and/or the form of an unexpected level of population differentiation (see example in Figure 1b). The genome scan is performed with AFLPs (Amplified Fragment Length Polymorphisms) as this technique allows obtaining a considerable number of loci over a large number of genomic regions which is a pre-requisite for studying the genetic basis of adaptation using genome scan method. We are genotyping different "colonizing" populations (Charente Maritime, Brittany and Herault – France, Denmark, Netherlands, Sweden), and a "native" population (Japan). Equilibrium models are then used to evaluate outliers to help focus research on genomic regions of interest. In addition, we are mapping on a reference population the candidate outliers and quantifying the part of the genome affected by a loss of diversity. We will present and discuss the first results.

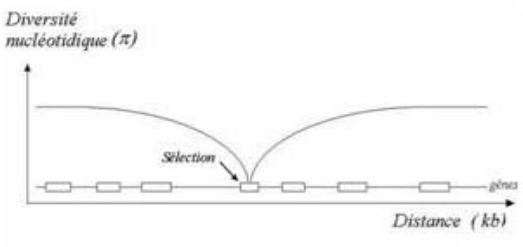

Fig 1a. Diversity decrease where selection had fixed a advantageous allele (4)

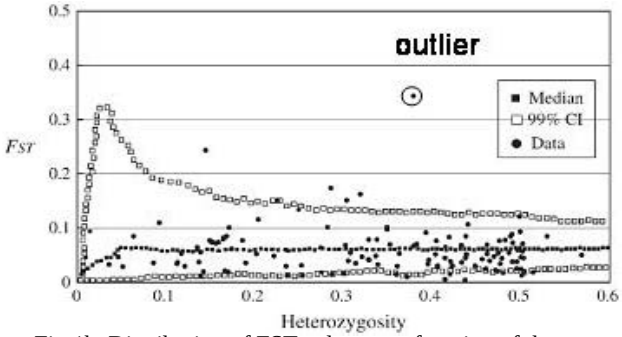

Fig 1b. Distribution of FST values as a function of the new heterozygosity. (5)

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Application of the DEB model on *Mytilus edulis* in a Low Seston Environment

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Keywords: DEB, mussel, energetic, low seston, growth

There is a need for modeling tools to understand ecological interaction and processes of relevance for estimations of carrying capacity in shellfish culture. The application of the DEB-model to study bio-energetics and growth of several molluscan species has received increasing interest, and the model has been successfully applied to simulate growth and reproduction in Pacific oyster (Bacher & Gangnery 2006, Pouvreau et al. 2006). The implementation of DEB on the mussel *Mytilus edulis* requires data from contrasted environmental conditions in order to test, validate and refine the existing parameter set. Most of parameters have been estimated in independent studies (van der Veer et al., 2006). The main parameters that have been adjusted here for refinement are the half-saturation coefficient (i.e. the food density at which ingestion rate is half the maximum), which might be site-specific and/or related to food quality, the fraction (kappa, K) of energy utilized for somatic growth and reproductive growth. Also, the representation of food that estimates the available energy for assimilation has been examined in relation to refining the half-saturation coefficient.

We will present results from the mussel DEB-model applied on data from two sites in Norway; Flødevigen on the southern coast and Austevoll on the western coast. The sites represent fjords and coastal waters considered as low seston environments compared to sites where most studies on mussel feeding on natural seston have been carried out. Low seston environments do not only occur under natural oligotrophic conditions, but may also take place where high bivalve culture densities cause seston depletion. Bivalve culture in such conditions may suffer low growth or tissue wasting due to reduced feeding and negative net energy balance. Environmental data used as driving forces include chlorophyll a and phytoplankton (numbers and biomass estimates). Mussel growth data are collected in 2007-8 from both sites and data on feeding physiology are presented to support parameter adjustments. The DEB-model has been applied to simulate growth of *M. edulis* and to validate modeled growth trajectories with observed data from the sites in Fødevigen and Austevoll. The model will be used in combination with ecosystem and farm models to assess the carrying capacity of Norwegians fjords

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Interactive effects of temperature and diet on growth and biochemical composition of hatchery-reared *Pinctada mazatlanica* spat

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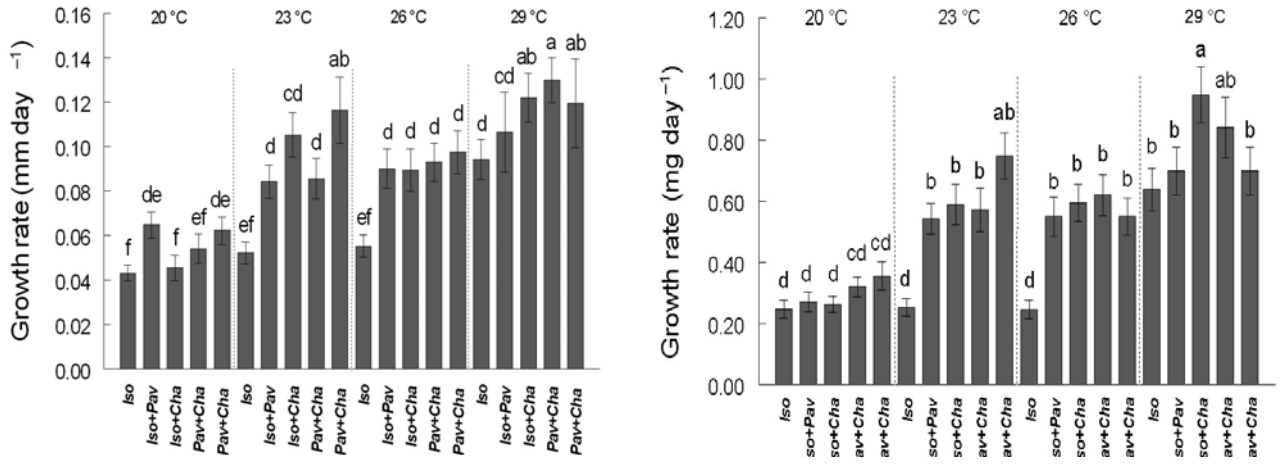
Keywords: *diet, biochemical composition, hatchery, Pinctada mazatlanica, spat*

The combined effects of temperature and diet on growth and biochemical composition of spat of the pearl oyster *Pinctada mazatlanica* during late-nursery care at the hatchery were investigated. Specimens were acclimated for 48 h at 25 °C and then subjected to one of 20 treatments consisting of a combination of four temperatures (20, 23, 26, and 29 °C) with five microalgal diets each one. Dietary treatments included *Isochrysis galbana* (*Iso*) alone, three binary mixes of *Iso*+ *Pavlova salina* (*Pav*), *Iso*+ *Chaetoceros muelleri* (*Cha*), and *Pav*+*Cha*, and a ternary mixture of *Iso*+*Pav*+ *Cha*. Shell and wet weight growth rates were estimated by the difference between final and initial values, divided by the experimental time (days). Microalgae and spat samples were used for analyses of carbohydrate, protein, and total lipid composition. Increase of shell height occurred in a linear pattern, while wet weight gain fitted an exponential model. Temperature, more than diet, exerted a stronger influence on growth and condition of the specimens. In general, the binary combinations supported faster growth than the *Iso*+*Pav*+*Cha* diet, which yielded better results only at 23 °C. The combination of 29 °C with *P. salina* + *C. muelleri* led to fastest growth in shell height, while the combination of 29 °C with *I. galbana* + *C. muelleri* led to highest wet biomass. *Chaetoceros* was the common factor in both cases. In contrast, spat grew significantly less in shell height and wet weight at 20 °C, regardless of the diet. Biochemical composition of tested specimens (carbohydrates and total lipids) reflected that of their diets, with the only exception being protein levels that were three to five times greater in soft body tissues than in the diets. This result suggests that young animals lacking sexual organs and having no need to channel energy for gametogenesis, instead undergo alternate metabolic routes for converting energy-rich ingested compounds, such as carbohydrates, into protein substrates needed to promote somatic growth. Additionally, biochemical composition of body tissues showed an inverse relation with temperature, suggesting a conservative use of available energy below the optimum thermal range for growth of juveniles observed in this study (29 °C) and that for respiration and excretion of adults reported from previous studies (23 °C). The only exception to this trend occurred with the monodiet of *Iso*, which favored at all temperatures, significantly highest levels of carbohydrate, protein, and lipid reserves within soft body tissues. This appears to be a strategy of young animals to grow less under unfavorable conditions for reaching a better physiological condition that allows them to deal possible stressful events.



Mapping QTL for resistance to summer mortality in the Pacific oyster

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Keywords: Crassostrea gigas, summer mortality, QTL mapping, SNPs, herpes virus

Summer mortality, described for decades in the Pacific oyster *Crassostrea gigas,* is responsible for important economic loss in this major aquaculture species. A complex combination of environmental and biological parameters has been suggested as the cause of this phenomenon (1). One way to overcome this problem would be to select oysters more tolerant to summer mortality, as this trait was shown to be highly heritable (2). Resistant and susceptible lines were selected for, allowing the identification of genes differentially expressed between them (3) and the identification of QTLs, using the available SSR-based linkage map (4), recently developed SNPs (5) and SSR-EST derived markers (6). These markers were notably characterized in several genes involved in the immune response and energetic pathways, testing for the colocalization of QTLs and some of these genes (3).

Linkage maps were built for three F2 segregating oyster families showing a strong differential response during a summer mortality event in experimental facilities in 2006. Crimap was used to build a consensus linkage map based on the analysis of three F2 families of 300 individuals each. The map is composed of 80 markers forming 10 linkage groups, which matches the haploid number of chromosomes. The average genome coverage was estimated to 76.4% and the sex specific maps showed a higher recombination rate in females than in males (1.37:1) as previously described (4).

In addition to survival, the viral load in Herpes Virus (OsHV1) was individually quantified by Q-PCR and these two traits were analysed. QTL detection was successful for the two studied traits. Over the three F2s, five QTL regions were identified in Linkage Groups V, VI, VII and IX. These explained 49% and 32.9% of mortality and viral load variances respectively. Most of the QTL co-localized for the two traits which supports the fact that the genetic architectures of the two traits are very similar in our experiment. These results are very promising for potential Maker Assisted Selection, but need to be confirmed by QTL experiments in the field, as well as experiments to link the selection to summer mortality and Herpes Virus. Furthermore, this approach will be complemented by the localization of genes differentially expressed in resistant and sensitive lines (7).

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Distribution of carpet shell clam (*Tapes decussatus*, Linnaeus 1758) larvae in Çakalburnu Lagoon, Izmir-Turkey

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Keywords: Tapes decussatus, carpet shell clam, larvae, distribution, Aegean Sea

Most of marine benthic invertebrates, including bivalves, have complex life histories with benthic and planktonic phases and it has long been recognized that larval recruitment processes influence the bivalve population dynamics. So this study was conducted for determining abundance of clam larvae in Izmir Bay.

The carpet shell clam *Tapes decussatus* is commercially very important in Turkey due to foreign trade. *T. decussatus* has been found abundantly in Aegean Sea, especially in Izmir Bay where the main fishery grounds are located.

Çakalburnu Lagoon (150 ha acreage) in Izmir Bay, Aegean Sea was selected for the study site, located at the south part of Izmir Bay. The study was conducted 5 stations at the outside part of the lagoon and 3 stations at the inside. Field sampling was carried out monthly during a year. Environmental parameters such as temperature, salinity, dissolved oxygen, chlorophyll-a and total particulate matter were measured.

Minimum and maximum temperature values were recorded in February and August, respectively. During study chlorophyll-a concentration changed between 0.69 µg/l and 64.66 µg/l.

As a result of this study larval abundance fluctuated according to stations and the amount of larvae in outside of lagoon was significantly higher than inside.

Bivalve filtration and nutrient turnover

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Keywords: metabolism, biodeposition, nutrient stoichiometry, nutrient regeneration

Filtration of particles by the bivalves results in a flow of material of a certain nutrient stoichiometry from the water column to the bivalves. Pre-ingestive selection and pseudofaeces production directs a particle flow to biodeposits. Nutrient composition of pseudofaeces may differ from water column stoichiometry. Digestion and absorption are different for the various food components; metabolic faecal loss will differ for various nutrients, and these processes result in differentiation of nutrient composition of absorbed material and faeces and biodeposits. In the metabolic processes carbon is accumulated or lost through respiration, direct excretion of bivalves consist mainly of nitrogen, resulting in a C/N ration that differs from water column ratios. Faeces and pseudofaeces will accumulate on or in the sediment, will resuspend and will be mineralized at a certain rate.

The flow of nutrients through bivalves occurs at different rates: nutrients are either stored in biomass, shell material and biodeposits or regenerated and mobilized through remineralization. These processes result in different turnover rates of nutrients and will therefore change the nutrient stoichiometry in the water column. Filtration of phytoplankton and regeneration of nutrients at specific rates will influence phytoplankton composition and growth in bivalve dominated ecosystems. Accumulation of nutrients in bivalve biomass and biodeposits may result in immobilization of nutrients. In oligothrophic systems further nutrient limitation may be enhanced by bivalve harvesting. Removal of nutrients from systems through harvesting of bivalves can be used for water quality management and may be applied in nutrient trading.

Available data will be analyzed and the impact of bivalve filtration on nutrient turnovers will be discussed.

Response to oxidative stress of *Crassostrea gigas*: transcriptomic and physiological approach

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Keywords: Crassostrea gigas, oxidative stress, hypoxia, microarray, reactive oxygen species (ROS)

In recent decades coastal ecosystems have been subjected to increased anthropogenic pollution (hydrocarbons, pesticides, heavy metals, nutrient inputs) and other environmental stressors (hypoxia, temperature variations, salinity). At the cellular level, oxidative stress is an important response to pollutants via production of Reactive Oxygen Species (ROS). Some of these stressors are also known to induce modifications in the genetic structure of populations through differential mortality of individuals carrying specific genotypes. Of the environmental stressors, marine hypoxia has become a major ecological concern. Benthic communities are the most sensitive parts of the coastal ecosystem to eutrophication and resulting hypoxia. Moreover most of the invertebrate species that inhabit the intertidal zone, especially sedentary animals, have developed mechanisms for surviving twice daily oxygen deprivation during low tides. At the cellular level, hypoxia also favors a decrease in the generation of reactive oxygen species and, therefore, a decrease in the activity of antioxidant enzymes. Large-scale mortality events have been observed in Pacific oyster *Crassostrea gigas* on the west coast of France since the early 1980s, particularly during the summer. A complex combination of environmental and biological parameters has been suggested as the cause. In recent studies it has been shown that susceptibility or resistance to summer mortality in juvenile *C. gigas* has a strong genetic basis and a high heritability of the phenotype. Therefore, two groups of oysters were selected ('R' and 'S', resistant and susceptible, respectively). S oysters put more energetic effort in to reproduction and had higher ROS production than R oysters.

We investigated the response of the Pacific oyster, *C. gigas*, to hypoxia under experimental conditions using S and R families. A cDNA microarray analysis was performed on 56 samples, using RNA from digestive glands. The microarray containing 9056 sequences was produced in May 2007 as part of a European project (Marine Genomics Europe). We focused on the analysis of differential expression patterns of specific genes or gene networks associated with hypoxia stress. In addition to gene expression analyses, production of ROS by flow cytometry and enzymatic activity of oxidative stress proteins were performed in order to investigate hypoxia effects at the cellular level. The aim was to characterize genetic markers or metabolic pathways in the Pacific oyster, *C. gigas*, that could be linked with responses to hypoxia stress and to investigate different expression patterns between R and S families. This research will characterize potential genetic markers that could be used in future environmental monitoring and could identify previously unknown mechanisms of stress tolerance in marine mollusc species.

Feeding behaviour of *Pecten maximus* and *Mytilus edulis* in a Low Seston Environment

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Keywords: feeding physiology, feeding behaviour

Low seston environments occur under natural oligotrophic conditions and where high bivalve culture densities cause seston depletion. Bivalve culture in such conditions may suffer low growth or tissue wasting due to reduced feeding and negative net energy balance. A positive net energy balance in bivalves will require a certain level of seston consumption, which depends on seston concentration, composition and transport rate. Norwegian fjords and coastal waters are considered as low seston environments compared to sites where most studies on mussel feeding on natural seston have been carried out. Regulation or cessation in feeding activity at low seston concentration is likely to be related to optimising energy cost of food absorption. The principal objective of this study is to elucidate the relationship between feeding physiology and bioenergetic balance for *P. maximus* and *M. edulis* in low seston environment. Results from laboratory and field experiments on feeding physiology and behaviour in response to natural low seston concentrations will be presented.

Seasonal induction of P450scc and progesterone synthesis by nutritional levels in *Mytilus galloprovincialis* gland digestive

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Keywords: Mytilus galloprovincialis, P450scc, progesterone, steroidogenic pathways, cholesterol diet

Most organisms have an enzymatic capability to biosynthesize cholesterol in all the tissues, but the great part is synthesize in liver. From liver it is transported as low-density lipoprotein (LDL) cholesterol through the circulatory system to steroidogenic cells, which internalize it by endocytosis process. Into the mitochondria of these cells the cholesterol is transformed in pregnenolone by cholesterol side-chain cleavage cytochrome P450 enzyme (P450scc) and, subsequently the pregnenolone is carried to endoplasmic reticulum where it is transformed in progesterone. Both, pregnenolone and progesterone are precursors in the other steroid hormones.

In bivalves, as well as in other molluscs, the presence of sterols, including cholesterol, has been demonstrated, but the ability to synthesize cholesterol does not exist or is very low, and it is generally assumed that the cholesterol content is diet-dependent. We have shown that P450scc enzyme, which catalizes the first step in steroidogenic pathway from cholesterol, is only located in the basophilic cells of digestive gland from *Mytilus galloprovincialis*, mainly in microsomes. Therefore, in order to confirm these assumptions we studied the correlation between the seasonal variations of nutrientes availability, P450scc quanttity and progesterone levels in this tissue.

The nutrientes availability, quantified as concentration of chlorophyll *a*, evidences the phytoplankton blooms characteristics in the studied area (estuary of Vigo), which start in he spring and continue until mid-autumn. The seasonal variation of P450scc quantity in mitochondrial and post-mitochondrial fractions was analyzed by relative immunostaining intensity in slot blot. The results show seasonal differences statistically significant, with a relative quantity of P450scc until 2.5 times higher in April-October period than in November-March, which is positively correlated with the seasonal variation of chlorophyll *a* (Rho Spearman r_s= 0.51; $0.005<p<0.05$). The progesterone levels were measured by enzyme immunoassay. They varie in a parallel maner to P450scc with a positive correlation (Rho Spearman r_s= 0.625; $0.005<p<0.05$).

These results suggests that the high concentrations of nutrients lead to an increase in the cholesterol ingestion and subsequently to the translation induction of this enzyme, increasing then the bioshynthesis of steroid hormones in *Mytilus galloprovincialis*.

Modelling the growth and reproduction of *Mytilus edulis* in Mont Saint-Michel Bay, from a Dynamic Energy Budget and Satellite Chlorophyll

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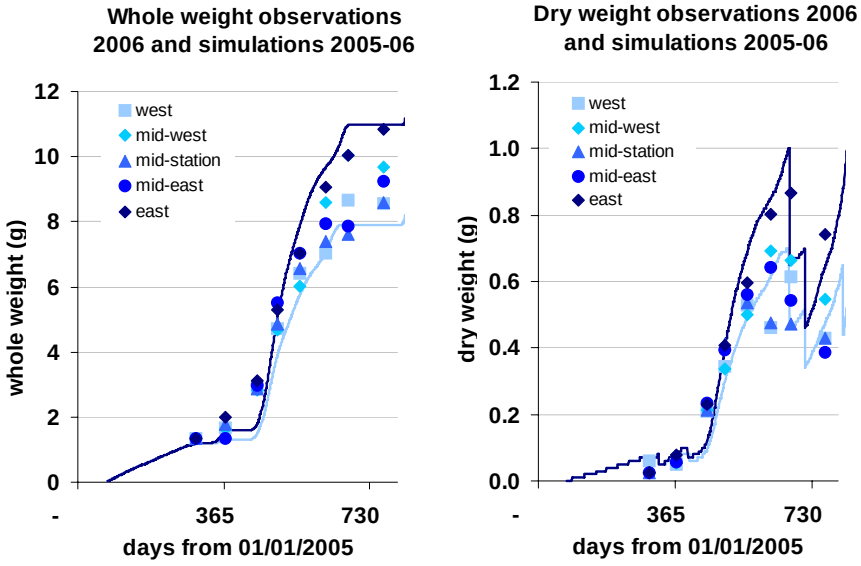
Keywords: Growth Model, Mytilus edulis, Mont Saint Michel, Dynamic energy budget, satellite

Modelling the growth of bivalves with a bioenergetic model allows to account for their response (growth, reproduction) and environmental impact (food depletion) in varying environments. The method proposed here consists of coupling a dynamic energy budget (last generation of generic growth model) with environmental parameters extracted from satellite images (chlorophyll concentration and temperature): such data, available on *http://www.ifremer.fr/nausicaa/* from different sensors (SeaWiFS, MODIS, MERIS), have the advantage over traditional measures of being extensive, validated and inexpensive. In this application, we developed a DEB model to estimate the responses of *Mytilus edulis* to a natural trophic gradient observed from West to East of Mont Saint Michel Bay, a major shellfish farming area of North Brittany, France.



After Van der Veer et al. (2006), this model takes into account the reproductive strategy of mussels, and proposes new estimations for key parameters like kappa (the fraction of energy allocated to growth). With simple assumptions, it succeeds in representing the growth and reproduction (period and effort of spawning) of *Mytilus edulis* from this site. The mean monthly Chlorophyll estimated from satellite images proves to be an acceptable descriptor of the trophic resource of these mussels. For a wider validation, the proposed model will be applied to other sites.

Van der Veer, HW; Cardoso, JFMF; Van der Meer, J. (2006) .The estimation of DEB parameters for various Northeast Atlantic bivalve species. Journal of Sea Research [J. Sea Res.]. Vol. 56, no. 2, pp. 107-124. 2006.

Match-mismatch between larval supply of *Mytilus edulis* L. and food quality: the incidence on recruitment

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Keywords: Mytilus edulis, larvae, seston quality, settlement rate, FA composition

Even if studies in hatcheries lead to a good knowledge of physiological requirements of bivalve larvae in controlled conditions, few data exist on larvae sampled in the field conditions. Classically, the accumulation of energetic reserves by larvae, and particularly essentials fatty acids (EFA), has been demonstrated to be fundamental for the settlement and the metamorphosis of bivalves species. Diatoms constitute a significant value diet for pelagic larvae due to their high content of particular EFA. In the Magdalen Islands (Qc, Canada), the culture of mussels is based on the collection of natural spat on rope collectors in a coastal lagoon where the dominant trophic pathway is a microbial food web punctuated by short diatom blooms. The aim of this study is thus to test if the settlement and metamorphosis success of wild blue mussels is relating to the match or mismatch of the larval supply with a trophic resource of high energetic value. From May to August 2007 (15 weeks), we have monitored twice-weekly the temporal evolution of pelagic larval densities of mussel and of seston and nutrients concentration in 8 stations of water pumping distributed in the coastal basin. The dynamic of mussels' settlement and metamorphosis was evaluated by sampling weekly 5 collector ropes, left 2 weeks in the water, in two separate sites of commercial collect. Moreover, the fatty acid (FA) compositions were determined in 1) natural competent larvae 2) post-larvae and 3) trophic resources. Neutral and polar fractions were separated in the 2 first cases. For technical reasons, the only criterion used to assess larval competence for metamorphosis consists of observation of the presence of the eye-spot on the shell. These larvae were sorted manually from the multi-specific pool at each date of sampling (200 ind. by sample). The post-larvae were sampled manually on the collectors used for the determination of settlement-metamorphosis rate.

The first D-veliger larvae were observed in May 17th (J_0) whereas eye-spoted and first post-larvae appeared at J_0+28. The evolution of densities of veliger and eye-spoted larvae follows the same pathway, with 3 distinct peaks, and the abundances of eye-spoted larvae reached a maximum value at J_0+49. Seston concentrations were homogeneous (2-3 mg/L) during the period of settlement, but a peak of particulate organic material (POM) was observed at J_0+67 and coincided with an increase of silicate nutrients in water column and an increase of EFA in seston. This period corresponded to the beginning of high rate of settlement-metamorphosis of mussels with values over 100 ind/m/day. In contrast to results obtained in hatcheries, non-methylene interrupted (NMI) FA constitute high level of fatty acid profiles in wild larvae and post-larvae. Lipids analysis revealed that the proportion of EFA (AA; EPA; DHA) were very low ($\leq$ 1,3 % of total FA) in both eye-spoted larval and post-larval samples whereas high levels of NMI FA were observed. No deficiency was observed since the mass of neutral and polar fractions were equivalent. The total lipids mass of competent larvae reached a maximal value of 0,12 µg per larva at J+45, but lower values were observed for the rest of the period (0,052-0,076 µg/larva). This coupling with the fact that the peak of abundance of competent larvae did not coincide with the maximal settlement rate, we assumed that these individuals could have delayed their metamorphosis. Consistent with the augmentation of POM and Si, the mass of total lipids in the seston reached a maximal value of 102,5 µg/L at J_0+67 including a significant increase of EPA (20:5n-3), DHA (22:6n-3) and SA (18:4n-3). Preceding the peak of settlement (J_0+68 to J_0+88), this switch of phytoplankton communities seemed to synchronise the settlement and metamorphosis of mussels' larvae.

Temperature dependent vibriosis of the European abalone, *Haliotis tuberculata*

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Keywords: Vibrio harveyi, spawning, mass mortalities, critical temperature, global warming

Vibrio harveyi strains, isolated from moribund abalone, have been shown to be responsible for episodic mass mortalities of *Haliotis tuberculata* (1998 – 2007, France). Mortalities were recorded in both natural and farm populations at the end of summer, when temperatures reached their maximum (19°C) and abalone were reproductively mature. In order to facilitate management of this recently emerged abalone disease, we investigated mechanisms of interaction between *V.harveyi* and *H. tuberculata*.

Conditions of disease development (temperature, sexual condition and age-size) were investigated in the laboratory. Abalone at various reproductive stages were challenged by bacterial immersion at 19°C. Mature abalone were more susceptible to bacterial infection (80% of mortalities within 12 days) than those spawned two months before (40% within 12 days) or immature abalone (0%).

Experimental infections conducted at different temperatures using mature abalone revealed an absence of mortality below 17°C, yet more than 80% died at 18° or 19°C after 4 days. These results support field observations and emphasize the importance of water temperature (and global warming) and host reproductive status in the susceptibility to this bacterium.

As all these parameters (temperature and reproductive status) are linked, a field survey was conducted that examined temperature, reproduction status, mortalities, and host immunity were studied over a 8 month period. This allowed us to define the susceptibility period centred around spawning time.

To further understand the pathogenic potential of this bacterial species, *H. tuberculata* were exposed to several strains of *V.harveyi* via both bath exposure and intramuscular injection. Interestingly, significant differences in disease development were detected: Only the strain isolated from moribund European abalone was pathogenic via bath exposure and also caused the highest losses via injection.

The recent mortalities of European abalone were thus caused by the emergence of a specific pathogenic *V.harveyi* in conjunction with host reproductive stress and elevated temperatures.

Characterization and description of stages of *Ruditapes phillipinarum* shell repair face front to the Brown Ring Disease explained with SEM and Raman spectroscopy

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Oral presentation

Keywords: Ruditapes phillipinarum, Vibrio tapetis, Scanning Electron Microscopy, RAMAN

The Brown Ring Disease (BRD) is a pathology caused by *Vibrio tapetis*, which affected the manila clam *Ruditapes phillipinarum*. In 1987, mass mortalities of cultured manila clams were noticed in Landeda (Brittany) north Finistere, which was the first production site in France (500t in 1987). In 1989, Paillard and Maes showed that mortalities were associated with the presence of a brown deposit on the inner surface of the valves.

A consequence of this disease is an inhibition of the normal process of shell biomineralization. Indeed, *Vibrio tapetis* colonizes the periostracum on the inner face of the shell. Then the clam defends itself in covering the *V. tapetis* with a brown organic matrix which often caused death by exhaustion. But some clams can recover. In these cases, a white layer entirely covers the brown matrix.
Stages of shell repair were already described based on binocular microscope observations which were not very precise.

The aim of this study is to understand the mechanism of repair using different tools like Scanning Electronic Microscopy (SEM) or RAMAN.

SEM allows us to study this mechanism on a smaller and more precise scale (x 10.000) than binocular microscope. SEM photographs, of the inner face of safe and diseased valves, are analysed. On safe shells, a crystals layer is covered by a thin organic layer. When clams are infected with *V. tapetis* but not repaired, a thick organic layer is observed on the inner face of the valve. On repairing clams, there is a gradient from isolate crystal deposit to a dense crystal layer, on the diseased brown organic matrix. At the end of the process, this crystal layer is covered by an organic layer, which seems different than safe or brown matrix because it is quite thick, bright and smooth. This matrix seems to be essential for clams' recovery.

We try to identify RAMAN signatures of the carbonate crystals and the three organic layers.
Safe and diseased shells are analysed on surface and on slices. In each case, carbonate signature corresponds to aragonite signal. The brown matrix shows a high luminescence. This luminescence is absented in safe shell and still present in repairing shell. The luminescence decreases with repaired stages. This luminescence was linked to the present of organic matrix.

This study shows that repair mechanism is caused by an accumulation of aragonite crystal calcium on the disease layer and that the organic matrix was particularly present.

Disclosing patterns of gene expression in mussels from the Venice lagoon (Italy)

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Keywords: Mytilus galloprovincialis, gene expression profiling, ecophysiology, pollution, sea-food quality

In the coastal waters, intertidal suspension feeders such as the *Mytilus* species are usually exposed to a variety of stress factors: from temperature and salinity changes to toxic chemicals and potential pathogens. Along the time scale of many generations, mussels living in chronically polluted sites could reveal interesting functional differences compared to marketable mussels grown in front of the open sea or off-shore. The large transition ecosystem surrounding the Venice town in the North Adriatic Sea displays a variety of microenvironments, defined by gradients of natural factors as well as gradients of chemical contaminants. Contamination sources refer to the watershed, industrial and urban wastewaters, erosion of contaminated soils, atmospheric fall-out and ship traffic. Actually, reduced heterozygosity at polymorphic loci and persistent exposure to genotoxic contaminants was previously demonstrated in native mussels of the petrochemical district (P. Marghera). Taking advantage of a cDNA microarray of *Mytilus galloprovincialis* sized 1.7k, and implementing the resulting gene expression profiles with qRT-PCR data, we studied native mussels from selected lagoon sites in three annual campaigns (early summer, 2005-2007). Total RNA was purified from the digestive mussel gland, well known for metabolic processes and toxicant bioaccumulation. Overall, gene transcription profiling appears an innovative experimental approach able to classify the sampled lagoon mussels and providing many candidate markers of functional status in *M. galloprovincialis*. In the lagoon mussels, changes related to specific gene categories and transcript sequences suggest complex functional modulations which interpretation proceeds in the time according to the increased number of sequence data in the public databases and relative functional annotations.

In the frame of the European Integrated Project FOOD-CT-2005-007103, additional EST sequencing from mussels subjected to different experimental challenges is increasing the non redundant transcript tag collection to about 7k size, with significant enrichment of immune-specific and immune-related ESTs. The definition of a new larger DNA microarray will allow a detailed study of innate immune responses and will expand the gene expression dataset of *M. galloprovincialis*, thus providing new hints for understanding physiological adjustments and genetic adaptation to the changing environment.

Influence of salinity and freshwater inflows on the seasonal and spatial variation in the reproduction and larval recruitment of oysters in three Southwest Florida (USA) estuaries.

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Keywords: oysters, reproduction, larval recruitment, salinity, restoration

Oysters, *Crassostrea virginica* are prolific in the estuaries along the east coast of the United States as well as the Gulf of Mexico including the southwest Florida coast. Alterations in freshwater inflow, resulting from watershed development and water management practices, have impacted salinity and water quality within southwest Florida estuaries. For example, in the Caloosahatchee estuary, where oyster abundances have declined precipitously from historic values, altered hydrology including unnatural high and low water deliveries to the estuary have been identified as key stressors. In addition, Southwest Florida estuaries encounter heavy rains during the summer months resulting in low salinities and little or no rain during the winter months resulting in very high salinities. Since oysters are benthic, sessile, filter feeding organisms, it is easy to make cause-and-effect relationships between the water quality and organism responses. This project has examined the seasonal and spatial variation of oyster reproduction and larval recruitment of natural oyster populations in three southwest Florida estuaries (Caloosahatchee estuary, Estero River, and Hendry Creek) along a salinity gradient. Reproduction was assessed using histological techniques and microscopy while larval recruitment was assessed using shell substrates. These responses were correlated with freshwater inflows and salinity.

Significant relationship exists between freshwater inflows and salinities at various points in all the three estuaries examined (R2 = 54 – 84% depending on the location and estuary). Oysters from all three estuaries spawn continuously between April – October, with peak spawning occurring between May – September. These results were corroborated by the larval recruitment, with recruitment occurring between April – November, with midstream – downstream locations encountering higher spat recruitment compared to the upstream locations. The peak spawning and recruitment in these estuaries coincides with regulatory freshwater discharges and/or watershed runoff due to seasonal rainfall resulting in flushing of larvae to downstream locations and/or creating low salinity conditions at the upstream locations that are unfavorable for the survival of oyster larvae. While downstream locations attract higher spat recruitment due to higher substrate availability and estuarine conditions during high flow summer-fall months, growth and survival of juveniles is poor during winter months when salinities are very high.

Depending on the estuary, flows between 50 - 3500 CFS into the estuaries, combined with the reduction of freshwater releases during peak spawning months will limit flushing of oyster larvae to downstream locations and create favorable salinity regime for spat recruitment, survival, and growth of oysters. Oyster responses including reproduction and recruitment are being used to set water quality targets in Southwest Florida estuaries, and as an indicator of restoration success of the Comprehensive Everglades Restoration Plan, which attempts to restore natural flows into the south Florida estuaries.

Apoptosis signaling by oxidized polyunsaturated fatty acids: possible roles in trophic interactions between bivalves and food particles

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Keywords: apoptosis, bivalve molluscs, polyunsaturated fatty acids

Research with vertebrate cells has identified "cytotoxic" activity, i.e., diminished survival and re-growth of cultured-cell lines, attributable to free fatty acids -- especially polyunsaturated fatty acids (PUFAs). In aquatic organisms, planktonic and attached algae have been reported to produce free PUFAs as grazing deterrents and allelopathic exudates. Further studies have implicated oxidation products of PUFAs, e.g., aldehydes and epoxides, in signaling pathways for apoptosis, or programmed cell death. This body of research stands in stark contrast to the literature on nutrition of suspension-feeding bivalve molluscs, wherein improvements in survival and growth are almost universally associated with diets high in PUFAs. How does one reconcile these divergent findings?

Our *in vivo* and *in vitro* experiments exposing bivalve molluscs or their cells to harmful algae or free PUFAs, and results from published studies, provide evidence that lipid characteristics and digestive processes are interactive. We propose the hypothesis that the PUFA lipid class (free or polar) and anatomical site of digestion (intracellular within digestive cells or before passing the epithelial barrier within the lumen of the alimentary canal) are determinant in the capability of a feeding bivalve to obtain nutritional benefits of PUFAs without suffering the consequences of cellular apoptosis initiation. Examples wherein these protective mechanisms are violated appear to include bivalve trophic interactions with certain harmful algae and feeding on some artificial PUFA supplements. We believe that this perspective may help to explain seemingly-contradictory results obtained with certain microalgal or artificial diets in shellfish-aquaculture settings.

Bleed it out

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Keywords : bivalve molluscs, haemolymph, hemolymph

Removing hemolymph from a bivalve mollusc with a hypodermic needle and syringe requires skill, patience, and concentration. These attributes are revealed with remarkable diversity by the dozens of people I have photographed engaged in this activity. This five-minute presentation will consist of a display of hundreds of these photographs synchronized with Linkin Park's recording of their hit song "Bleed It Out."

Adaptation biochemical mechanisms to cadmium in the far eastern scallop *Mizuhopecten yessoensis*

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Keywords: Cd-binding proteins, metallothioneins, scallop, heavy metals

It s well known that marine mollusks have feature to accumulation heavy metals in tissues. The scallop *Mizuhopecten yessoensis* is the most interesting organism because able to accumulate a toxic cadmium in digestive gland. However in tissue of digestive gland of *Mizuhopecten yessoensis* MT (metallothioneins) have not been found.

The aim of the present study was to establish for individuals *M. yessoensis* (1 year) the accumulation level and subcellular distribution of cadmium. The scallops were obtained from Great Peter's Bay. For metal exposure experiment, specimens *M. yessoensis* were kept for 10 days in aquaria containing aerated seawater. Cadmium was added as $CdCl_2$ at the concentration 300 µg/l. A group of control non-exposed bivalves were kept under similar conditions (except for cadmium treatment).

The results of experiments have shown that Cd-binding ligands have a number of properties similar to MT. They are stable to acetone and temperature treatment and bind some metals, including copper, zinc and cadmium.

The exposure of scallop *M. yessoensis* to cadmium during time experiment results in significant accumulation of cadmium from 0,48 µg Cd / mg of total protein in control group to 14,5 µg Cd / mg of total protein in experiment group, respectively.

Protein chromatographic (FPLC, Superosa 6) from digestive gland of scallop *M. yessoensis* has shown that cadmium is associated with 68-43 kDa Cd-binding protein in control group. In the experimental group cadmium is associated with higher molecular weight Cd-binding protein.

The Zn:Cd:Cu molar rations are 3:1:1 for Cd-binding proteins of control group and 4:13:1 for Cd-binding proteins of experimental scallop, respectively.

The results of this study strongly suggest that far eastern scallop *Mizuhopecten yessoensis* has unique and well-development system of detoxification of heavy metals (high molecular weight proteins). It provides maintenance of relative stability of biochemical systems under heavy metals influence. The study of functioning of mechanisms of cadmium detoxification in marine organisms can improve a more clearly understanding of this adaptation response of marine mollusk and can be used for estimation of pollution by heavy metals.

Poster Abstracts

Abstracts of all posters are listed below (all subject sessions together) in alphabetical order of the first author’s last name.

Seasonal Changes of Gonad Indices of Arabian abalone, Haliotis mariae, in Southern Coast of Oman

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Keywords : Haliotis mariae,spawning season,gonad index ,Oman

The onset, termination and length of the reproductive season, and its factors, of Oman abalone, Haliotis mariae, has been determined through the seasonal change of Gonad Bulk Index (GBI) study. The results demonstrated that the onset of gonad maturation for H. mariae takes place in September, with the rising in seawater temperature and availability of algae.
The peak spawning season was found in December to January when the seawater temperature reaches about 28oC. The spawning will gradually decreases throughout February and March in during which the algae start to be scarce. The annual changes in seawater temperature affect gonad maturation and growth and decay of macro-algae.
Moreover, conditioning and spawning behavior in the natural and culture environment were experienced successfully only during this period of the year.

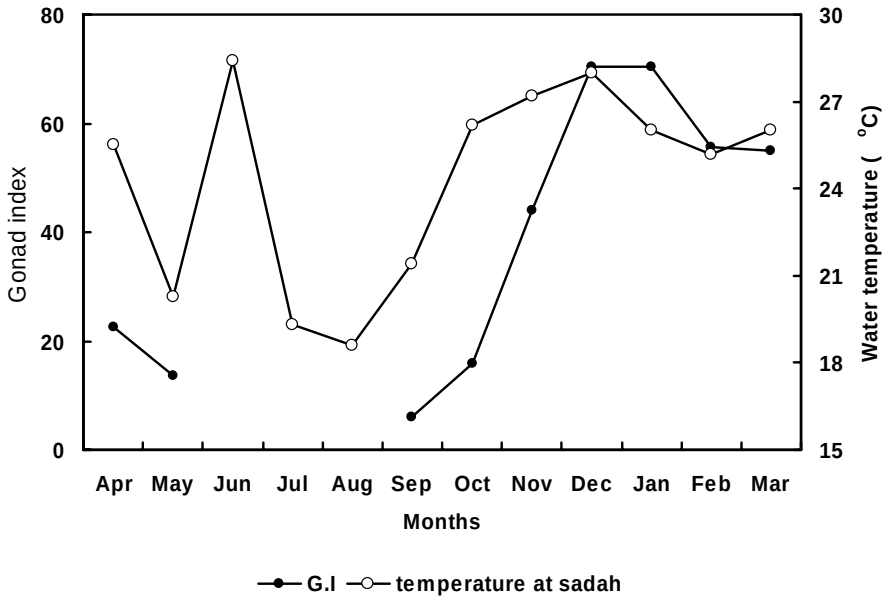

Figure. 1. Seasonal change of gonad index of H. mariae and seawater temperature at sadah area.

Immunocelular localization of CYP1A in different tissues of *Mytilus galloprovincialis*

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Keywords: Mytilus galloprovincialis, CYP1A, immunolocalization

The enzyme cytochrome P4501A (CYP1A), which belongs to the P450 gene superfamily, is a membrane-bound hemoprotein, located in the endoplasmic reticulum of all examined vertebrates and carries out oxidation related to xenobiotics biotransformation. The cellular immunolocalization of CYP1A has been demonstrated in vertebrates, mainly in liver, but also in extrahepatic tissues like lung, skin, kidney and digestive system.

Different species of mollusks are often proposed as sentinel organisms in environmental biomonitorization programs, and the enzyme CYP1A has been used as a pollutant biomarker. However, to date, little is known about the cellular localization of this enzyme and their function in marine invertebrates.

In this study we aimed to localize a putative CYP1A expression in several tissues of mussel Mytilus galloprovincialis by immunohistochemistry in order to elucidate its distribution and function. We have examined a total of 175 specimens of M. galloprovincialis, collected monthly in the Ría of Vigo (NW Spain), from February 2001 to August 2002. Specimens were dissected and the gonads, digestive glands, gills and kidney were remove and fixed formalin's fluids. Histological sections of different tissues were mounted together on gelatin coated slides to process using the peroxidase-antiperoxidase method. The sections were processed for immunohistochemistry using a polyclonal antibody against cytochrome CYP1A. Controls made by omitting the primary antibody produce no immunostaining.

CYP1A immunoreactivity was observed in hemocytes of all the tissues studied and in the adipogranular and Sertoli cells of the mantle. Moreover, immunoreactivity was observed in mioepithelial and endothelial cells as describe other authors. After analysis of these results, we could conclude a wide tissular distribution of CYP1A in Mytilus, but its localization in different cellular types suggests different functions of this enzyme. Further studies are necessary in order to determine their role in each one.

The combined effects of salinity, stocking density and frequency of water exchange on the growth and survival of *Crassostrea rhizophorae* (Guilding, 1828) larvae

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Keywords: mangrove oyster, larval rearing, management, physical factors

The mangrove oyster, *Crassostrea rhizophorae*, is an important species for commercial exploitation along the Brazilian coast, mainly in the Northeast region. Extensive beds of this species, especially around urban areas, are over exploited, and natural production has been decreasing significantly. Oyster culture could help to overcome this problem, but the supply of seeds for commercial purposes, must be assured by hatchery production as the collection of wild seeds will unlikely meet the demands for this kind of venture. However, few studies on the seed production of *C. rhizophorae* are available in the literature and a well-established rearing protocol evaluating the main factors for this species was not yet defined.

This study reports the combined effects of salinity, stocking density and frequency of water exchange on the growth and survival of *Crassostrea rhizophorae* from first to 6th day (trial 1) and 7th to 14th day of development (trial 2). Two salinities (25 and 35 g L^{-1}) and three densities (3, 6 and 12 larvae mL^{-1} in trial 1; 2, 4 and 8 larvae mL^{-1} in trial 2) were tested at three different frequencies of water exchange (24, 48 and 72 h).

Larvae reared in the salinity of 35 g L^{-1} showed the highest survival in trial 1, but it was not significantly different from the salinity of 25 g L^{-1} with water exchange of 72 h. In the second trial, survival was higher in the salinity of 25 g L^{-1}. Growth was higher in both trials in the salinity of 25 g L^{-1}. Overall, water exchanges of 48 and 72 h significantly improved growth and survival. Stocking densities had no significant effect in terms of length, but height was improved in trial 1 at 3 and 6 larvae mL^{-1}. Survival was higher at 6 (32.56%) and 12 (37.1%) larvae mL^{-1} in trial 1 and at 2 (8.54%) larvae mL^{-1} in trial 2.

The results indicate that to maximize growth and survival, *C. rhizophorae* larvae should be reared in the first week at salinity of 25 g L^{-1}, using the stocking density of 12 larvae mL^{-1} and water exchanges of 72 h. In the second week, it should be used the same salinity but the stocking density of 2 larvae mL^{-1} and water exchange of 48 h are recommended.

Morphometry and growth of a Bivalve: the common cockle, *Cerastoderma edule* in the Bay of Somme

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Keywords: Bivalve, Cerastoderma edule, morphometry, growth, estuary, France

Cockle (*Cerastoderma edule*) growth has not been studied a lot yet and the current approach of counting external ridges in order to make age estimation is limited. Nevertheless, growth is a key parameter for the monitoring of bivalve populations, which are heavily exploited in French estuaries. This morphometric study is based on the analysis of cockles shells collected in the Bay of Somme. This site, which has an area of 70 km², is located in the eastern Channel in the north of France, and is one of the most important French cockle fields.

This analysis is part of a study of the dynamic of cockles stocks in the Bay of Somme, which shows sharp inter-annual abundance fluctuations. High-resolution picture analysis was performed with TNPC software (Digital Processing for Calcified Structures) on both valves in order to measure the following parameters: length, width and area, with a 1.10^{-4} millimeter accuracy. Also, the weight of each valve was measured too with a 0.1 mg precision.

Crossed analysis of these four parameters allows quantification of the difference which may exist between left and right valves. In a second time, internal increments census after thin slicing and polishing allow estimating cockles age with a resolution of a day at least.

After age validation through the internal increments, the statistical modeling (GLM), based on external morphometric data, will be calibrated. This will enable to avoid having to study increments in the future.

Furthermore, the coupling of a growth study with environmental parameters acquisition at an adequate frequency could supply some clues to the strong abundance variations of cockles, noticed in the Bay of Somme, and so, will constitute a tool for management in this sector.

Effects of temperature on gonadal occupation index of *Ruditapes decussatus (L.)*

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Keywords: R. decussatus, reproduction, temperature

Mollusc reproduction may be determined through an interaction between endogenous and exogenous factors. Seasonal fluctuations in environmental parameters determine in a great extent the reproductive cycle of bivalves. Temperature has traditionally been assigned a major role to determine gametogenesis. Most of previous studies have evaluated the influence of environmental parameters on gonadal development of bivalves in the field. Only a few studies have considerated the separate effect of temperature.

The aim of this study was evaluate the effects of temperature on gonadal development of *R. decussatus*, mainly determining the effect of temperature rise on gonadal development, estimated as gonadal occupation index . Adult specimens of *R. decussatus*, (37.73±1.57mm) from a beach in the Ría de Arousa (NW Spain), were conditioned at 14±1ºC and 18±1ºC. The clams were fed on a diet of the microalgae *Isochrysis galbana* clone T-ISO. The daily feeding ration, was 0.5% dry weight with regard to clam weight. The gonadal development was evaluated through histological sections, determining the stages of gametogenesis and the gonadal occupation index (GOI).

At the start of the experimental period, 9% of specimens were in the rest period and 90% in the initial stage of gametogenesis. After 60 days under experimental conditions, at 18±1ºC, 100% of specimens were mature while at 14±1ºC, 60% of clams were mature. Statistically significant differences in GOI values appeared between the two conditions, 14ºC and 18ºC (Figure 1), in both males and females (ANOVA, $p<0.05$), confirming the results observed by histological sections. Clams held at 14±1ºC and 18±1ºC matured, furthermore the gonadal development rate is positively related with temperature.

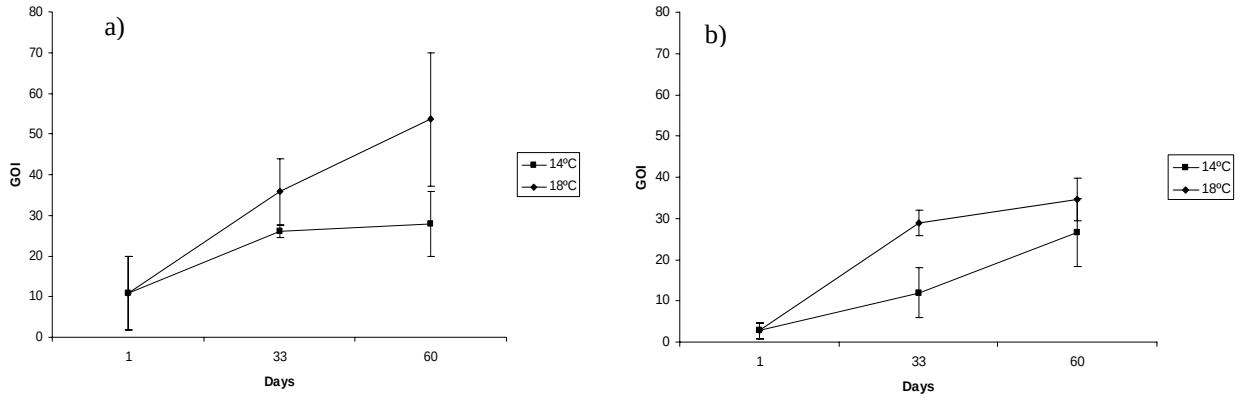

Figure 1: Evolution of Gonadal Occupation Index in males a), and females b), of *R. decussatus.*

Recruitment dynamics of the rainbow lip pearl oyster *Pteria sterna* in Bahía de La Paz, Baja California Sur, México

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Keywords: Spat fall, recruitment variability, pearl oysters.

In the Gulf of California, Mexico, the commercial production of pearls from *P. sterna* relies on the juvenile from wild populations. However the quantities of juvenile seed are erratic and show large interannual fluctuations. For instance, during 2006 juvenile density per collector was in the range of 1,000 to 1,5000 seeds, while in 2007 the density fell to about 30 seeds. This variability has become a critical problem for the commercial production of pearls since it does not guaranties a consistent and sufficient input of seeds for the production of pearls.

The recruitment process is quite relevant and requires a comprehensive study oriented towards gaining understanding on the mechanisms that control the production and survival of seeds in the wild. We believe that this variability in recruitment are dependent on the physiological state of spawners prior to the spawning event, the biotic and abiotic conditions that early life stages encounter during their planktonic life phase, and the intra and inter-specific competition for substrate in the collectors. Here we present our experimental design to approach the problem of the recruitment dynamics of *Pteria sterna* in Bahía de La Paz, B.C.S., México. We also present the results obtained during the first phase of this study.

We are relying on a backward particle tracking analysis using a numerical stochastic simulation of currents in Bay of La Paz, using a hydrodynamics model, to establish the probable location of the banks of spawners inside the Bay. This model is being adjusted using seed density data from collector placed in 10 locations inside the Bay. Once the banks are located we will proceed to determine the relationship between reproductive effort and energy content of spawners prior to the spawning event using calorimetric and stereological techniques. We are also measuring food availability, substrate space competition, temperature, currents and transparency to establish statistical relationships for the recruitment of seeds in the collectors.

Comparison of eastern oyster (*Crassostrea virginica*) and blue mussel (*Mytilus edulis*) feeding rates at low temperatures

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Keywords: Mytilus edulis, Crassostrea virginica, clearance rate, temperature

Eastern oysters (*Crassostrea virginica*) and blue mussels (*Mytilus edulis*) were collected in the Gulf of St. Lawrence, at the northernmost distribution area of *C. virginica*, and maintained in cold water (0, 4 or 9°C) over a 63-day period. Inter-species differences in feeding behaviour were examined using particle clearance rate (*CR*) metrics. For *C. virginica*, feeding activity at low temperatures was clearly an exception; the percentage of the experimental population feeding declined from peak values of 50% at 9°C to null (no animals feeding) at 0°C. For *M. edulis*, the percentage ranged from 100% at 9°C to 17% at 0°C. Only *M. edulis* displayed an adaptation to cold over time (*CR* increased with time). With respect to absolute feeding rates, *C. virginica* cleared significantly ($P < 0.001$) fewer particles than did *M. edulis*. Maximal *CR* was on average 66% lower for *C. virginica* than for *M. edulis*. Results suggest that *C. virginica* is physiologically disadvantaged in terms of grazing on seasonal phytoplankton blooms, including toxic (domoic acid) blooms, which can occur at low temperatures (< 9°C) in eastern Canadian waters.

NMR relaxometry as a non-invasive routine tool for the phenotypic characterisation of *Crassostrea gigas*

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Keywords: *Crassostrea gigas, NMR, growth*

Marine bivalves, especially the Pacific oyster *Crassostrea gigas*, are economically important in French aquaculture. Nevertheless, the ecology and the physiology of this bivalve are not fully understood; consequently, control of its growth and reproduction both in the field and in hatcheries still relies on empirical factors. The investigation of soft tissues in marine molluscs, especially in marine bivalves, classically relies on destructive methods, since a hermetic shell protects the animal. Non invasive characterization of gonad maturation and determination of the sex of Pacific oysters by Magnetic Resonance Imaging MRI was already successfully tested (Davenel et al., 2006; Pouvreau et al., 2006). MR imaging is the most appropriate technique for quantifying the growth of somatic and gonadic tissues and to determine sex. However, this technique is too costly for field studies or to be applied routinely in hatcheries. In this context, we have tested the ability of a low Nuclear Magnetic Resonance relaxometry, a very less expensive technique, to obtain useful phenotypic parameters to control the physiological state of oysters.

NMR measurements were performed with an OXFORD MQA 6005 spectrometer operating to 0,12T. (5MHz) and equipped with a 5 cm probe diameter which allowed to investigate oysters in their first year of maturity (< 45 g total weight). NMR measurements were carried out at three different periods (2007 May 7th, June 4th and June 29th) on 60 oysters which were then dissected to measure oyster-shell internal cavity volume, flesh dry weight and to determine sex and gonad development.

The NMR results showed that it was possible to determine oyster-shell internal cavity volume but also flesh dry weight in less than one minute with a very high determination coefficient R^2 (respectively 0.95 and 0.94). Results showed also that it was possible to identify sex and gonad development, with a rate of success of 83% and 75%, respectively. For the oysters with dry weight higher than 0.7g, the rate of success to identify sex was 100% (fig.1).
Further work is required to design an NMR probe well adapted to bigger oysters and to improve sex discrimination and prediction of gonad development with larger collections.

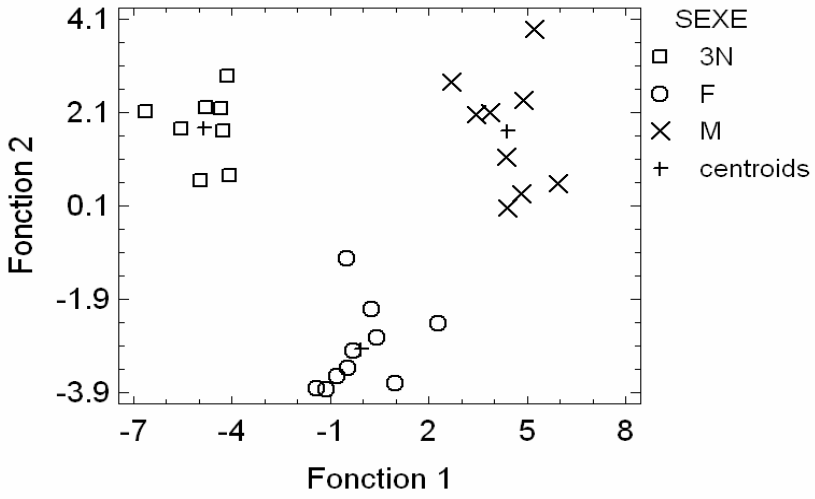

Figure 1. Sex determination by discriminant analysis of NMR signals (dry weight >0.7g)

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The effects of multiple environmental stressors on hard clam survival and physiology

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Keywords: salinity, Karenia brevis, bivalve, hemocytes, flow cytometry

Culture of hard clams (*Mercenaria mercenaria*) generate the third highest revenue of aquaculture species in Florida, USA. In southwest Florida, hard clams experience multiple stressors (freshets, high temperatures and red tide) resulting in substantial losses. Red tides, caused by the dinoflagellate *Karenia brevis,* causes closure of harvesting when concentrations reach 5000 cells L^{-1}. Closure of shellfish beds means clams can be exposed to prolonged environmental stress, particularly during the rainier summer months when low, stressful salinities will coincide with high temperatures. Red tides may also occur during the dry winter months. In 2005 a red tide lasted throughout the year in southwest Florida, spanning both dry and wet seasons. Little is known how hard clams respond to red tide and its interaction with natural environmental stressors such as low salinity and high temperature. Several lab experiments simulating these interactions were conducted to determine the physiological responses of clams to multiple stressors. Clams were exposed to salinities at 10, 20 and 30 ppt for 2 weeks. Clams were then heat shocked at 38°C for one hour (lethal temperature), returned to ambient temperatures and monitored for survival for two weeks. There were significant differences in log-rank survival between treatments ($p<0.05$). Survival was significantly lower at 10 ppt ($p<0.05$). In a subsequent experiment, clams were chronically exposed to *Karenia brevis* (500 cells/ml, 2 times per week) for 2 weeks and transferred to variable salinities (*K. brevis* → Δ salinity; 10, 20 and 30 ppt) for 2 weeks, simulating rainy season effects after red tide. Cellular functions (hemocytes mortality, phagocytosis, and reactive oxygen species (ROS) production) were measured with flow cytometry after 2 weeks in variable salinities to characterize sublethal effects of red tide and lowered salinity. A group of clams were heat-shocked at a sub-lethal temperature (36 °C, 1 hour) to examine interactive effects of red tide, low salinity and thermal stress. Phagocytosis was higher in clams exposed to *K. brevis*, more significantly after heat shock ($p<0.05$). A third converse experiment examined clams' responses to *K. brevis* after prior exposure to variable salinities (Δ salinity → *K. brevis)* which simulated red tide following rainy season. Clams were sampled prior to (t=0) and after exposure to variable salinities (t=1; 10, 20 and 30 ppt for 10 days). After salinity exposure, clams were returned to ambient salinities (30 ppt) and exposed to *K. brevis* (t=2; 500 cells/ml 2 times per week) for 10 days. Heat shock caused a decrease in phagocytosis ($p=.0072$). Phagocytosis decreased from t=0 to t=1 and then increased at t=2 ($p<0.00001$). This trend was apparent despite significant interactive effects of heat shock and salinity ($p<0.05$). Phagocytosis was higher in clams exposed to 10 ppt compared to 20 and 30 ppt ($p=.0366$), even following direct exposure to 10 ppt (t=1) for 10 days. Cellular mortality decreased with heat shock at 20 and 30 ppt, but not at 10 ppt ($p=.0228$). Exposure to *K. brevis* had no significant effect across all treatments, which likely reflects the insensitivity of *M. mercenaria* to red tide when salinity conditions are favorable. This contrasts with the earlier experiment in which *K. brevis* did affect cellular function. Prior exposure to *K. brevis* may have exacerbated the effects of low salinity. A surprising result was that phagocytosis remained elevated in clams exposed to 10 ppt. It was observed that clams fed at all salinities. At sublethal exposures to low salinity (10 ppt), enhanced cellular function may have been necessary to ingest and assimilate nutrients compared to higher salinities (20 and 30 ppt). Chronic exposures, in which mortality is induced, will be performed to see if cellular function remains elevated when clams are near morbidity and mortality. Further experiments will determine the effect of salinity and red tide when *M.mercenaria* is simultaneously exposed to *K. brevis* under the lowest salinities that can support bloom conditions.

Reproductive cycle and biochemical composition of the American oyster, *Crassostrea virginica*, in Tabasco lagoons, Mexico.

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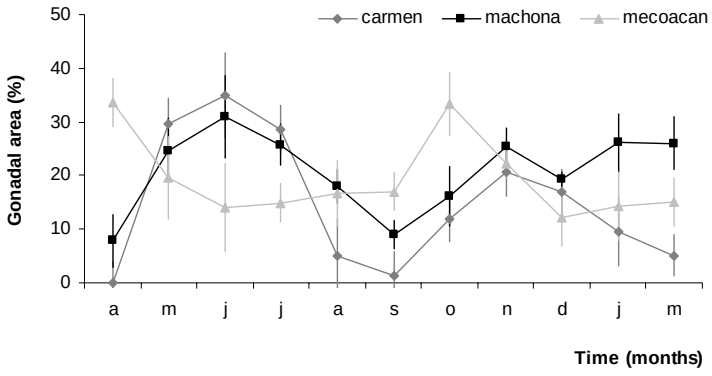
Keywords: Crassostrea virginica, reproductive cycle, biochemical composition,

The reproductive cycle profile and changes in biochemical composition of the American oyster *Crassostrea virginica* reared in Tabasco lagoons (Carmen, Machona and Mecoacan lagoons), were carried out between April 2006 to March 2007 in relation to seasonal changes (temperature, salinity and chlorophyll b & c). Qualitative and quantitative histological analysis of gonad and measurements of protein, carbohydrates and lipids from visceral mass, adductor muscle and mantle tissue were made. Gametic activity was observed over year. The stereological studies (gonadal area, %) showed the existence of two principal spawning periods, spring and autumn for Mecoacan oysters, late summer and late autumn for Carmen and Machona oysters.

As a sexual maturity progressed the glycogen in the visceral mass decreased. The increase in the protein content in the visceral mass correspond with increases in gonad occupation (gamete occupation area %). Lipid levels displayed a clear seasonal pattern linked to the gametogenic cycle. The pattern exhibited by *C. virginica* based on the accumulation de reserves in winter and the subsequent mobilization during gonadal development for the first gametogenic cycle seems to follow a conservative pattern, but for the second one the oysters used the seasonal phytoplanktonic blooms following a opportunistic pattern. These differences in gametogenic cycle and spawning are a reflex of populations' recovery capacities under the different environmental conditions.



Growth and kinetics of lipid and fatty acids of Venerupis pullastra during larval development

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Keywords: clam Venerupis pullastra, growth, larval development, lipids, fatty acids

This study evaluates the larval development, metamorphosis and postlarval stage of *Venerupis pullastra* with respect to growth, kinetic response of lipids and, due to the role of essential fatty acids (i.e. NMI; 20:5n-3 and 22:6n-3), the fatty acid composition. Clam larvae were fed with diets of two species of microalgae supplied individually or mixed - *Isochrysis galbana* clone T-ISO and *Tetraselmis suecica*; species normally used in bivalve hatcheries.

The most salient result was that the largest increase in size and dry weight over the period from when larvae began to feed (larva pediveliger) until metamorphosis was observed for larvae fed with *Isochrysis*. The largest increase in organic weight and lipid content was observed for larvae fed with *Tetraselmis*. During the postlarval stage, all the components studied increased, with the highest increases for the postlarvae fed with the mixed diet. The Larvae fed with *Tetraselmis* did not survive metamorphosis.

In the pediveliger larval stage, the energy supplied by the lipids was similar for the 3 diets (~2.5 Kj/individual/106). In the metamorphosis stage, the larvae fed with *Tetraselmis* presented significantly higher lipid content (5.3 Kj/individual/106), but did not survive metamorphosis. In the postlarval stage, the highest content was found in the larvae fed with the mixed diet (23.8 Kj/individual/106). The energetic data of larval development of *V. pullastra* revealed positive linear anabolism of lipids in the larvae fed with *T.suecica*. The ash adjustment was quadratic (second order polynomial) and an exponential fit was obtained for organic weight and total energy. For the larvae fed with *Isochrysis* and the mixed diet, the best fit in all cases was with a quadratic correction (second order polynomial).
It was shown that the energy necessary to deposit 1 g of shell in *V. pullastra* was approximately 11.53 KJ (2.75Kcal).

The composition of essential fatty acids was analyzed during the initial larval stages (larva veliger and veliger U), observing a decrease in the fatty acids NMID and 20:5n-3 and an increase in 22:6n-3. This demonstrates a structural function of this acid as opposed to the energetic role of 20:5n-3. By comparing the larval and dietary composition, a lack of capacity for elongating and desaturating 20:5n-3 to 22:6n-3 was observed for *V.pullastra* when feeding began (pediveliger stage). The NMID became relatively less important from the start of metamorphosis and were only present in the postlarval stage in residual amounts.

Clearance Rate of *Mytilus galloprovincialis*: On/Off regulation in meagre chlorophyll environments

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Keywords: Mytilus galloprovincialis, mussel, clearance rate, chlorophyll

There is ongoing discussion in the scientific literature concerning the conceptual understanding of the bivalve filtration process. Clearance rate (CR) has been considered to be subject to physiological regulation with the purpose of maximizing energy uptake. Alternatively, filtration may be understood essentially as an "automatized" process, whereby under optimal conditions the filtration system is designed to function at maximum capacity and exposure to adverse conditions results in the cessation of filtration activity. The results of our research group show that the CR of *Mytilus galloprovincialis* is more variable with the presence of diets with a chlorophyll content below 2.08 µg l^{-1} than with diets above this threshold. This variability could indicate the existence of a dichotomic behaviour in the CR response, that is, the mussel filters at its maximum capacity or it ceases filtration. The aim of the present survey is to study the response of the CR of *Mytilus galloprovincialis* under low chlorophyll content exposures, and to determine whether the mussel ceases its filtration, or on the contrary, it shows a physiological regulation, which could be a dichotomic response. This regulation could be addressed to maintain a stable ingestion rate, in terms of total particulate matter (TPM), organic (POM) or chlorophyll (chla). In order to test these hypotheses 4 diets were tested in 23.3 h experiments, measuring the CR of 9 individuals every 40 min, by means of Flow-through chamber method. Besides an experimental diet that simulates the seston characteristics of the Ría de Arousa (standard diet), 3 diets with a chlorophyll content below 2.08 µg l^{-1} and different content of TPM, POM and chla (Table) were tested.

The evolution of the CR for each mussel with diets containing less than 2.08 µg chla l^{-1} showed periods of maximum filtration (Maximum CR, Table) and periods in which the CR decreased sharply, reaching the cessation in some cases. This resulted in a population average CR below the maximum values (CR, Table) and a Pearson's Variation Coefficient significantly higher in these diets (CR's CV, Table). The diet factor exerted a significant effect (ANOVA, $p<0.001$) on the accumulated ingestion rates (total ingestion measured in mg per individual during the 23.3 h period). Statistical analyses *a posteriori* (Tukey) showed an ingestion rate of the standard diet higher than observed in the other diets, which showed the same ingestion rate in terms of organic content (A. POM, Table) and chlorophyll (A. chla, Table). Therefore, results of this study suggested that the CR response of *Mytilus galloprovincialis* after 23.3 h of exposure presents both periods of maximum filtration and cessation, a regulation that could be addressed to obtain a stable ingestion rate in terms of organic matter or chlorophyll.

Diet	TPM mg l^{-1}	POM mg l^{-1}	chla µg l^{-1}	CR l h^{-1}	Maximum CR (l h^{-1})	CR's CV (%)	A. POM (mg)	A. chla (µg)
Standard	1.06	0.69	13.13	4.07	5.98	24	67.2	1280
1	1.35	1.10	0.898	0.94	5.04	102	26.5	21.7
2	0.89	0.72	0.627	1.63	5.56	81	27.3	23.8
3	1.39	0.43	0.480	2.64	6.39	68	24.2	27.0

Feeding and digestive response of *Mytilus galloprovincialis* to long-term variation in food regime

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Keywords: Mytilus galloprovincialis, differential absorption, enzymatic digestive activity

The aim of the present study is focused on the physiological response of *Mytilus galloprovincialis*, with special emphasis on its digestive response: differential absorption of biochemical components and enzymatic activity (protease, amylase, cellulase and laminarinase) of the digestive organs under a reduction in diet concentration. The mussels, 80 individuals, were acclimated for 7 days in an open-flow tank with a mixed diet of *Isochrysis* aff. *galbana* and pulverized sediment in a proportion that results in 4.77 ±0.19 µg chlorophyll l^{-1} diet, equivalent to the average values observed in the Ría de Vigo, seston content of 0.62±0.04 mg l^{-1}, organic content of 0.47±0.04 mg l^{-1} and protein, lipid and carbohydrate percentage of 53.2%, 31.7% and 15.1%, respectively. The next 14 days food concentration was reduced 2.7 times (1.75±0.19 µg chla l^{-1}, 0.23 mg l^{-1}, 0.17 mg organic l^{-1}, 55.6% protein, 30.8% lipids and 13.6% carbohydrates), resulting in a diet that fits within the 25th percentil of the observed chlorophyll values in the Ría de Vigo (NW Spain). The feeding and digestive behaviour of *M. galloprovincialis* was determined at the end of acclimatizing period (t = 0) and in the 14th day of exposure to the meagre diet (t =14).

No differences were observed in the CR values (Table) and no pseudofaeces production was observed, which means the mussels did not carry out a preingestive regulation depending on the diet. This response resulted in a low organic ingestion rate (OIR) when the mussels were exposed to the low concentration diet (Table). With regard to the postingestive regulation, a significant decrease of the AE was observed (Table). Attending to the differential absorption of biochemical components, a significant decrease the protein and carbohydrate AE observed in the low concentration diet, whereas the lipid AE remained constant (Table). Concerning to the absorption rate (AR) a significant decrease was observed for all the biochemical components in the low concentration diet (Table), which involved a significant change in the assimilatory balance. With regard to the specific enzymatic activity, and independently of the diet, the protease activity was higher in the digestive gland, amylase activity was similar in both organs and cellulase and laminarinase were higher in the crystalline style. On the other side, the reduction in diet concentration caused a significant decrease in the weight of digestive organs. In the case of the digestive gland, this decrease did not result in a decrease of total or specific activity, whereas in the crystalline style resulted in a significant decrease of total activity and specific activity of amylase, cellulase and laminarinase, but the specific activity of protease remained constant. The physiological response under the low concentration diet, which simulates the meagre food periods of the Rías Gallegas, is related changes in the ingested food, whereas the digestive response (i.e. AE) can not compensate the lower ingestion rate. Therefore, the exposure of *Mytilus galloprovincialis* to low concentration diets could imply a decrease in the net energy uptake per unit of organic ingested, which could be caused by the lack of coupling between enzymatic activity and AE, and consequently with the AR, in the specific case of protein.

Time days	CR l h^{-1}	OIR mg h^{-1}	AE	AE Prot	AE Lip	AE Carb	AR-Prot mg h^{-1}	AR-Lip mg h^{-1}	AR-Carb mg h^{-1}
t = 0	3.8	1.77	84.9	0.88	0.80	0.84	0.79	0.43	0.22
t = 14	4.0	0.68	79.6	0.80	0.79	0.79	0.30	0.17	0.07
p	0.143	<0.001	<0.05	<0.001	0.557	<0.001	<0.001	<0.001	<0.001

Characterization of oyster summer mortalities according to the French Ifremer/REMORA monitoring network; with complements from phytoplanktonic (Ifremer /REPHY databank) and meteorological data (Météo-France databank)

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The wide range of data from the French Ifremer/REMORA network, collected since 1993 allow an overall characterization of mortalities of 1-year old and 2-year old cupped oysters *Crassostrea gigas*, cultivated in the main French oyster areas (Fleury *et al*, 2003), and thus a better understanding of the causes of these mortalities (Ifremer /MOREST project).

The average annual mortalities on intertidal sites range from 10 to 20%. Most of the mortalities take place in Spring and Summer. Mortalities of 1-year-old oysters occur in Marennes and several sites in Brittany, whilst 2-year-old oyster mortalities occur in the same sites and in baie des Veys (Normandy). In addition, mortalities of 2-year-old oysters appear more variable according to different annual surveys.

Internal (physiological) factors and external (environmental) factors have been considered successively.
A Principal Component Analysis (PCA) on seasonal mortalities, growths and gonad maturations showed that, at the time scale of the REMORA monitoring (seasonal data), no strong correlation can be set up between mortalities and growth or maturation.

2-factor ANOVAs (Sites and Years) on the Spring+Summer mortalities differentiated the variance weights of these factors according to the year-class of animals: the site effect was slightly higher (51%) for the 1-year class, whereas the year effect was higher (74%) for the 2-year class. This was reinforced by the result of another PCA that included meteorological and environmental data such as rain, sun, air and water temperature and chlorophyll a, which displayed that the 1-year oyster mortalities were the most correlated (even if not significantly) with chlorophyll a and temperature (which are site dependant), whereas the 2-years oyster mortalities had a closer correlation to the rain (year dependent).

In conclusion, this survey reveals more clearly the effect of external factors than internal ones on oysters mortalities. It also reveals that the causes of mortality may be somewhat different for 1-year-old oysters (preponderance of geographical factors) than 2-year-old oysters (meteorological factors). (Soletchnik *et al*, 2007).

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Effect of temperature during air exposure on metabolic demand and the incidence of gaping in suspension-cultured *Mytilus spp.*

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Keywords: Mytilus spp, air exposure, temperature, metabolic demand, valve gaping, stress

Intertidal mussels have developed mechanisms to cope with periodic air exposure. Their strategies combine depression of metabolic rates, enhancement of anaerobic capacity and intermittent use of air breathing. Therefore, valve gaping may occur during air exposure to sustain oxygen requirements of aerobic metabolism and/or to eliminate volatile products of the anaerobic metabolism. However mussels cultured in suspension do never experience air exposure. They have a lower tolerance of air exposure than intertidal mussels and have a higher reliance on air breathing. Then gaping is frequently observed with suspension-cultured mussel during the post-harvest processing activities. However, gaping compromises the water content of mussels and hence their viability that is why its incidence should be better managed by mussel growers during their post-harvest processing.

Our study was designed with suspension-cultured mussel to observe the effect of temperature during valve closure on metabolic rates and the related incidence of valve gaping. We especially investigate whether the ambient temperature during the air exposure or the magnitude of the difference between the water temperature during the collection of mussels and the temperature of the air would increase the metabolic demand and hence the incidence of gaping. Fist experiments were conducted in December (temperature of the water = 0.5° C) with cultured mussels (*Mytilus spp*.) from the Baie des Chaleurs (Quebec, Canada). Three groups of mussels were tightly shut to prevent air breathing or water loss and placed respectively at 4, 10 or 24 °C for 14h. At the end of the treatment, oxygen uptake was measured with mussels of each group (in addition to control groups placed in sea water at 4, 10 or 24 °C for 14h) as well as their mantle cavity fluid pH. To examine the switch from aerobic to anaerobic pathways required to sustain the metabolic demand, we determined the anaerobic (lactate, alanopine, octopine and strombine dehydrogenase) and aerobic (citrate synthase and cytochrome c oxidase) capacity of the posterior adductor muscle. We also determined the adenylate energy charge (AEC) and the heat shock protein concentration (HSP) in the posterior adductor muscle and gills respectively as biochemical indicators of the stress induced by the experimental condition and partly responsible for the metabolic demand. To determine the incidence of gaping after the period of valve closure, mussels from each group were allowed to gape for 5 minutes. Then the incidence was observed and we measured the muscle tone of gapers and non-gapers with a dynamometer. First results with mussel sampled last December indicate no difference in muscle strength between gapers and non-gapers and demonstrate that gaping is a controlled movement potentially associated with metabolic requirements. Although the period of valve closure increased the incidence of gaping in all groups in comparison to the controls we did not observe any direct correlation between gaping and the temperature. However, the mantle cavity fluid pH decreased with increasing temperature. It may reflect a decreasing oxygenation of tissues due to a higher metabolic demand which may also explain the highest oxygen uptake observed in mussel from the same group at the end of the experiment. These preliminary results show that oxygen requirements related to a period of valve closure of 14h increase with an increasing temperature. Others results (aerobic/anaerobic capacity, HSP, AEC) and the next experiment in May should better explain the relation between the temperature, the stress, the metabolic demand and the incidence of gaping. We will also consider the effect of the nutritive status and the reproductive cycle of mussels on the metabolic demand.

Cellular and molecular characterization of early stages of spermatogenesis in the pacific oyster *Crassostrea gigas.*

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Keywords: Crassostrea gigas, spermatogenesis, reproductive cycle, germ cell characteristics

The Pacific oyster, *Crassostrea gigas*, is a successive and irregular protandrous hermaphrodite mollusc. Reproduction in this species is seasonal and timing of each step of germ lineage development is broadly affected by environmental conditions. The gonad is consists in gonadal tubules invaginated in a connective storage tissue where haemolymphatic vessels are numerous. The volume of the gonadal tubules is directly related to the developmental stage of gametogenesis from autumn (reproduction arrest followed by spermatogonial mitosis) to summer (ripe gonad). During early gmetogenetic stages, the sex of animals is undetectable and no molecular specific markers of spermatogonia and/or spermatocyte are actually disposable. That is why we have undertaken a cellular and molecular characterization of early spermatogenetic stages using different complementary approaches.

First, the ultrastructural characterisitics of the gonad were explored and a specific interest was devoted to the main cellular features of spermatogonia and spermatocytes. The gonadal tubules are surrounded by myoepithelial cells associated with an acellular matrix delimiting the outer part of the tubule; the inner part is composed by intragonadal somatic cells and germinal cells. Two types of spermatogonia were identified, where type I spermatogonia are large, scarce and pale cells leaned against the base of the tubule (nuclear diameter 5.5 ± 0.5µm). Type II spermatogonia are clustered and dark cell which appear smaller than type I (nuclear diameter 4.3 ± 0.3µm). The aspect of nuage-like material in cytoplasm is described from pale spermatogonia to primary spermatocytes (nuclear diameter 3.6 ± 0.3µm), while no structure related to chromatoid body could be observed in oyster spermatocytes and spermatids. Suspension of dissociated germ cells were also prepared and fractionated over density gradient and/or FACS (Fluorescence-activated cell sorting) in order to isolate spermatogonia and primary spermatocytes. Cells suspensions were validated according to structural/ultrastructural properties to develop a differential proteomic approach leading to characterize new molecular markers of spermatogonia. Laser microdissection (LMD) was also considered as an alternative technique to isolate islets of gonadal tubules at different stages of gametogenesis. The expression of genes known to be potentially involved in the mitotic activity of spermatogonia was measured by real time PCR in gonad and connective cells in order to identify pertinent markers of early spermatogenesis. The fine identification of the cells expressing these genes of interest could then be investigated by *in situ* hybridization. The characterization of specific molecular markers of the early stages of spermatogenesis is a first step in the study of gametogenesis regulation in a Lophotrochozoan.

Lipid requirements of the scallop *Pecten maximus* (L.) during larval and post-larval development in relation to addition of *Rhodomonas salina* in diet

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Keywords: nutrition, biochemical characteristics, fatty acids, sterol profils

The main objective of this study was to evaluate the effect of the addition of *Rhodomonas salina* in the diet of *Pecten maximus* on growth, survival, metamorphosis success in relation to biochemical content. Two different microalgae mixtures were used as nutritional treatments for *P. maximus* larvae. The first was a standard diet namely PTC (*Pavlova lutheri* (P), *Isochrysis galbana* (T), *Chaetoceros calcitrans* (C)) and the second was an experimental diet namely PTCR (*Pavlova lutheri*, *Isochrysis galbana*, *Chaetoceros calcitrans* and *Rhodomonas salina* (R)). Food quality is an essential factor in the success of the larval, post-larval and juvenile development. A diet rich in polyunsatured fatty acids present an advantage for growth, survival and metamorphosis success. Larvae and post-larvae fed with the diet containing *R. salina* showed a higher accumulation of AA (20:4n-6), but a lower concentration of DHA (22:6n-3) and EPA (20:5n-3). Addition of *R. salina* seems to be advantageous by an earlier appearance of metamorphosis, but no difference was identified on growth and survival between diets. A preferential incorporation of DHA and AA in polar lipids observed in both diets, indicated the importance of these fatty acids on metabolism of *P. maximus*. The composition of sterol observed in larvae fed with an addition of *R. salina* show a high level of brassicasterol. Advantage of the preferential accumulation of brassicasterol with the addition of *R. salina* is not clear but other studies suggest that brassicasterol can replace cholesterol in some functions.

Phytoplankton selection by the mussel *Mytilus galloprovincialis* in a natural environment (Alfacs bay, N.W. Mediterranean Sea)

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Keywords: filter feeding, Mediterranean sea, Mytilus galloprovincialis, phytoplankton

Filter feeding in bivalves have been a topic of interest for a long time. Despite the amount of studies performed, little attempts have been made within natural resources. Most of the knowledge comes from laboratory experiments using known phytoplankton species to determine filter feeding selection. In order to get a more realistic idea of phytoplankton consumption by mussels (*Mytilus galloprivincialis*), an experimental approach was designed in Alfacs bay (N.W. Mediterranean sea). The area of study, located in the Ebro delta, has peculiar geographical and environmental conditions and high phytoplankton production, which makes it an ideal site to develop mussel aquaculture.

Preliminary results of an extensive field study are presented in this abstract. Bay water was collected in order to identify the phytoplankton community and compared with the species found in mussel stomach contents, feces and pseudofeces. Among all the phytoplankton species identified, we present the results of 3 abundant species, which represented the majority of the phytoplankton community during a period of time. Results showed a different feeding selection depending on the species ingested. Mussels showed a positive selection towards the dinoflagellate *Prorocentrum minimum* as it was more abundant in stomach content and feces than in the bay water, and very little was discarded as pseudofeces. The diatom *Cyclotella meneghiniana* did not present any type of selection .There were no significant differences between the percentage of the species found in the bay water, stomach content, feces or pseudofeces. But the diatom *Pseudo-nitzschia* spp., a potential HAB species although never proved to be toxic in the area of study, was selected negatively by the mussels. This phytoplankton species was much more abundant in the bay water and pseudofeces and very little was found in stomach content and feces. Thus, mussels seem to have a preference towards some phytoplankton species, being capable of filter feeding selection.

Table 1: percentage values (± se) of the 3 phytoplankton species studied in the bay water, and stomach content, feces and pseudofeces of mussels.

	Prorocentrum minimum	*Cyclotella meneghiniana*	*Pseudo-nitzschia spp.*
Bay water	9.55 ± 7.30	29.09 ± 9.19	24.73 ± 3.19
Stomach content	26.43 ± 5.16	28.97 ± 6.50	1.36 ± 2.26
Feces	39.34 ± 5.16	52.08 ± 6.50	0.13 ± 2.26
Pseudofeces	3.74 ± 5.16	35.80 ± 6.50	28.25 ± 2.26

Genetic diversity among four different world-wide lines of Pacific oyster *Crassostrea gigas* by microsatellites.

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Keywords: Crassostrea gigas, microsatellite, genetic diversity

Recently in Mexico, oyster producers have joined with scientists to explore alternatives for the culture of *Crassostrea gigas*, especially in coastal areas of the Baja California Peninsula, at the Pacific Northwest, where temperatures are not as extreme as for example in the Gulf of California. Our goal is the conformation of a broodstock of *C. gigas*. For this, the fact that *C. gigas* has been disseminated throughout the world would be exploited, looking for genetic differentiation among stocks, and evaluating the combined and specific effects of crossing them on performance (growth and survival). In the present study, five microsatellite loci were used to characterize genetic variability and differentiation among four lines of hatchery produced stocks: Australia (Au), Chile (Cl), Tasmania (Ts) and United States (US). All loci showed moderate to high polymorphism in all four *C. gigas* lines, as observed in the average number of alleles per locus (6.0-22.0), and in the average expected heterozygosity (0.488-0.894). Significant departures from Hardy-Weinberg equilibrium were observed in sixteen out of twenty comparisons. F_{ST} values (0.041-0.134) and Nei´s genetic distances (0.207-0.607) were statistically significant in all six pair wise comparisons, supporting the existence of four lines genetically differentiated. The ranking in the level of genetic variability was as follow: US (N_A=17.4; H_E= 0.820), Cl (N_A=14.4; H_E= 0.755), Au (N_A=13.2; H_E =0.722), Ts (N_A=8; H_E = 0.696). Beside the lowest values of variability, the Tasmanian line showed the higher pair wise genetic distances (0.475-0.607) and F_{ST} (0.134-0.109) values with the rest of the lines.

Optimisation of larval culture of the blue mussel, *Mytilus edulis*

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Keywords: Mytilus edulis, mussel, culture, veliger larvae, algae

The blue mussel *Mytilus edulis* is one of the most important aquatic animals cultivated in the world. In Europe, *M. edulis* culture relies on seed collected from the wild. However, securing a reliable supply of seed is a major challenge to the majority of European blue mussel farmers. The availability of hatchery-produced seed can provide an alternative source of seed. Here we report on optimisation trials that investigate various aspects of *M. edulis* veliger larval culture including rearing temperature, feeding regularity and alternative microalgal diets. *M. edulis* larvae were reared at 14, 17 and 21 °C. Growth rate was significantly higher in larvae reared at 17 and 21°C in comparison with larvae reared at 14 °C, but no differences were observed between the former temperature regimes. Two different feeding regularity strategies were tested, namely (1) feeding daily and (2) every 2-3 days. No differences in larval growth rate were observed between the two feeding strategies. The effect of 5 different microalgal diets on larval growth and proportion of eyed larvae 26 days after fertilization (DAF) was evaluated. The diets used were (1) *Isochrysis* aff. *galbana* clone T-ISO, (2) *Chaetoceros calcitrans* fo. *pumilus*, (3) *C. muelleri*, (4) *I.* aff. *galbana* clone T-ISO and *C. calcitrans* fo. *pumilus*, and (5) *I.* aff. *galbana* clone T-ISO and *C. muelleri*. Growth rate and the proportion of eyed larvae 26 DAF was significantly higher in larvae fed with the combination diets in comparison with the mono-diets. Among the mono-diets, larvae fed with *I.* aff. *galbana* clone T-ISO grew significantly faster than larvae fed with either of the *Chaetoceros* diets.

Reproductive Cycle of *Chione fluctifraga* (Sowerby, 1853) (Bivalvia: Veneridae) during La Niña 1998-1999 at Ojo de Liebre Lagoon, Baja California Sur, Mexico.

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Key words: Chione fluctifraga, La Niña, Reproduction

The clam *Chione fructifraga* (Sowerby, 1853) stretches from San Pedro, California, U.S.A to Bahía Magdalena, Mexico, and from the northern Gulf of California to Guaymas, Sonora, Mexico. It is commercially exploited only in the Gulf of California, whereas it remains an unexploited resource at Ojo de Liebre lagoon despite of the existence of clam banks with densities of at least 50 clams per square meter. As reported for other bivalves, this species may display changes in its reproductive cycle as a result of El Niño and La Niña phenomena, which at the Pacific coast result mostly in either temperature increase or decrease; these climatic phenomena occur every 3 or 7 years with variable intensity.

The reproductive cycle of the clam *C. fluctifraga* was investigated, as well as its relationship with temperature at Ojo de Liebre lagoon, Baja California Sur, Mexico, from June 1998 to November 1999. The La Niña period was identified through those months with negative deviations from the monthly temperature average of a 25-year series. It was found that the period influenced by La Niña comprised from October 1998 to November 1999, while the period from June to September 1998 corresponded to the end of an El Niño period. Twenty five clams were collected from a sandy intertidal zone in Ojo de Liebre lagoon and fixed in 10% formalin. Gonads were embedded in paraffin, 7-µm sections were obtained, hematoxylin-eosin stained and mounted on synthetic resin. The clam's reproductive cycle was divided into five stages for gonad phase the characterization: undifferentiation, development, ripening, spawning and post-spawning. Temperature was recorded at the sampling site. The relationship between temperature and spawning was investigated through a non-parametric Spearman's rank correlation.

Undifferentiation was observed from September 1998 to November 1999, with the peak (93%) in November 1999. Development took place from June to September 1998 and from December 1998 to September 1999, with the peak (86.5%) in June 1998. Ripening was observed from June to August 1998 and from July to September 1999, with the highest frequency (39%) in July 1999. Spawning occurred from July to November 1998 and from August to September 1999, with the peak (73%) in August 1998. Post-spawning was detected from September to November 1998 and from August to November 1999, with the highest percentage (45%) in September 1998. As for spawning, it is worth noting that the difference in frequency was higher in 1998 relative to 1999. In 1998, spawning took place over three months, reaching a frequency of up to 73%, while in 1999 it occurred only during two months and reached a mere 47%, also in August. However, this decline in spawning frequency failed to reach statistical significance, likely because of the low number of data. Temperatures between September and November 1998 were notoriously higher (2ºC higher approx.) than those in the same months of 1999. When correlated with spawning frequency through a Spearman's rank test a significant correlation was evident ($r = 0.6$, $P < 0.01$), indicating a direct relationship between temperature and spawning frequency. It is concluded that the spawning intensity of *Chione fluctrifaga* declined during La Niña 1998-1999 compared to 1997-1998, at the end of El Niño, likely as a result of temperature decrease.

Biomineralization markers during early stages of shell formation in the abalone *Haliotis tuberculata*

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Keywords: Haliotis tuberculata, larvae, shell formation, carbonic anhydrase, CGRP

The shell of marine Molluscs is a composite biomaterial made of calcium carbonate intimately associated with organic matrix components that are secreted by mantle epithelial cells. Shell formation begins in early larval development stage with the secretion of the periostracal lamina acting as a substrate for further mineralized layers. In the adult shell of the abalone (*Haliotis tuberculata*), a succession of an organic external coating (periostracum), a prismatic calcitic layer, and an internal aragonitic coating (nacre) is typically observed. It is now established that the selection of each $CaCO_3$ crystal-morph builds on nucleating or inhibiting matrix proteins that are deposited at the calcification site, and on specific enzymes controlling the precipitation process. Although the structure of the adult shell has been well studied, the early steps of shell formation and the mechanisms of its regulation are still unclear. In a previous work on abalone shell formation, we demonstrated that mineralization occurred at an early pre-torsional veliger stage, and that the mineral phase initially deposited was essentially composed of aragonite (Jardillier et al., 2008). To better understand the biochemical and molecular mechanisms involved in the regulation of shell formation, we have evaluated the activity of a well known biomineralization marker, the carbonic anhydrase (CA), and the presence of a molecule presumed to regulate shell formation, the CGRP-like molecule.

Carbonic anhydrase is present in all organisms, ranging from bacteria to vertebrates. It catalyses the hydration of CO_2 to form HCO_3^- and participates in several important physiological functions such as acid–base regulation, respiration and mineralization. Using the pH decrease method, CA activity has been evaluated in seven stages of the abalone life cycle from the early trochophore to the pre-metamorphic stage. A significant increase of CA activity was found during larval development of the abalone. A first CA peak was measured at the early trochophore stage, that corresponds to the onset of shell mineralization and a second activity peak appeared just before metamorphosis. These results confirm the role of CA enzyme in mollusc shell biomineralization.

In another hand, the calcitonin gene related peptide (CGRP), at high concentrations, acts as calcitonin in the control of vertebrates' calcium metabolism. Related CGRP molecules (CGRP-like) were recently evidenced in marine invertebrates, namely in molluscs, and their implication in the control of $CaCO_3$ formation was previously suggested (Duvail & Fouchereau-Peron, 2001). To gain a further sense of understanding of the role of these molecules in the developmental cycle of *H. tuberculata*, the quantity of CGRP-like molecules were measured in whole larval extracts by Radio-Immuno Assay (RIA). Our results evidenced significant variations over the larval development that may correspond to particular stages in shell mineralization.

These preliminary results confirm the role of CA in mollusc shell formation and suggest that CGRP-like molecules may control the early stages of abalone shell biomineralization. Experiments are underway to confirm the role of both CA and CGRP-like molecules in early shell growth and their interactions with other components such as matrix proteins.

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Effects of *Karenia brevis* on the defense responses of the hard clam *Mercenaria mercenaria,* the oyster *Crassostrea virginica,* and the green mussel *Perna viridis*

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Keywords: immune system, Karenia brevis, bivalve, hemocytes, flow cytometry

Harmful algal blooms can occur and persist along the west coast of Florida. The dinoflagellate *Karenia brevis* has been shown to be the main algae responsible for those blooms, commonly called "red tides". Previous studies have demonstrated that *K. brevis* can cause massive death among fishes, birds and marine mammal, and induce serious health problems among humans such as severe respiratory problems, but also neurotoxic shellfish poisoning (NSP) by consumption of contaminated shellfishes. Besides brevetoxins production, *K. brevis* has been demonstrated to possess a hemolytic activity. Because harmful algae like dinoflagellate *Prorocentrum sp.* have a deleterious impact in mollusks gastrointestinal epithelial cells, the asumption that *K.brevis* could have the same interaction and be in direct contact with bivalves' immune cells (i.e. hemocytes) during blooms episodes was made. In an attempt to determine if *K.brevis* blooms affect South Florida mollusks immune system, this study focused on three bivalves of interest: the hard clam *Mercenaria mercenaria*, the eastern oyster *Crassostrea virginica*, and the invasive green mussel *Perna viridis.* In a preliminary experiment, *K. brevis* hemolytic activity was verified using horse red blood cells (*i.e.* erythrocytes). The assay was performed with algae cultures 15-17 days after initial inoculation in L1-Si medium. The results showed that 60 % of horse erythrocytes were lysed after 24 hours of incubation. In another experiment, defense responses for each the three mollusks species were studied, such as hemocytes mortality, phagocytic capacity and reactive oxygen species (ROS) production. After direct exposure to *K.brevis* (1000 cells/mL + L1-Si medium; L1-Si medium alone; seawater control) all treatments and immune responses were examined using appropriate fluorescent dyes and flow cytometry techniques. Significant differences (ANOVA; $p<0.05$) between controls and *K.brevis* exposed hemocytes were observed at three incubation times (2 hours, 4 hours and 24 hours). Results showed an increase in hemocyte mortality for all three species when exposed to *K. brevis*. In addition, a decrease in the phagocytic capacity of hemocytes from *M. mercenaria* (ANOVA; $p<0.05$), and an increase in the ROS production of hemocytes from *C. virginica* and *P. viridis* (ANOVA; $p<0.05$) were observed.

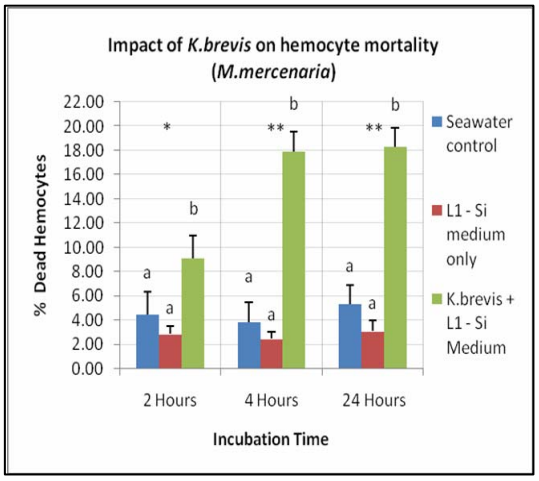

Figure: Effect of *K.brevis* upon hemocytes mortality at T=2h, 4h, 24h. (Mean ±standard error, n=4). Letters indicate statistical differences between incubation treatments; *: indicate statistical differences between incubation times

Ingestion rate, assimilation and absorption efficiency of four microalgae species in *Ostrea edulis* (L.) broodstock.

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Keywords: Ostrea edulis, Conditioning, Feeding, Ingestion rate, Assimilation, Absorption efficiency.

Because of their commercial value, research has been devoted to the feeding requirements of some marine bivalves. Despite this particular attention, the incidence of food quality and quantity from larvae to adults remains poorly known. Suspension feeding bivalves collect their food from the water by retaining suspended organic particles when transported through the gills by means of the ciliary system on gill filaments. Because particles retention intensity depends on bivalves and microalgae species, ingestion rate, assimilation and absorption efficiency of four microalgae have been studied in *Ostrea edulis* broodstock (L.) to determine the ideal diet for its conditioning. Three hundred and sixty 1.5 year old *O. edulis* oysters (40-50 mm of total length) originated from the Bay of Cancale (Normandy, France) have been transfered to the "Station Expérimentale d'Argenton" (Brittany, France) where such experiments were carried out from February to May 2008. They were progressively adapted to experimental conditions by increasing temperature by 1°C during 10 days until 19°C and daily fed a bi-specific diet (*Isochrysis affinis galbana* and *Chaetoceros gracilis*) adjusted to 1600 μm^3 μl^{-1}. In each triplicate tanks per nutritional condition 30 European flat oysters were homogeneously distributed. Continuous 1 µm filtered-seawater was delivered at 40 l h^{-1} flow and constant temperature (19°C). Four different microalgae species were used as nutritional mono-specific regime: *I. aff. galbana* (T: volumetric size ≈ 40 μm^3, dry weight, 20 pg $cell^{-1}$), *C. gracilis* (Cg: ≈ 80 μm^3, 25 pg $cell^{-1}$), *Skeletonema costatum* (Sc: ≈ 80 μm^3, 40 pg $cell^{-1}$) and *Tetraselmis suecica* (Ts: ≈ 300 μm^3, 30 pg $cell^{-1}$). Seawater was sampled twice a day at inlet and outlet of each experimental tank to determine phytoplankton concentration using an electronic particle counter type Multisizer 3. The number of cells per oyster and its phytoplankton volume equivalent (μm^3) were daily determined. Faeces and pseudofaeces were collected, centrifuged, dried and combusted twice according to a previous described protocol while ingestion rate assimilation and absorption efficiency based on formulas established by Wilson and Connover were applied.

Active feeding as a way to improve detoxification patterns in phycotoxin-contaminated bivalve molluscs.

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Keywords: bivalve molluscs, phycotoxins, detoxification, food

Since the eighties, world coasts have been undergoing recurrent blooms of toxic microalgae. In temperate areas at least three main syndroma are affecting the consumers: paralytic shellfish poisoning (PSP) amnesic shellfish poisoning (ASP) and diarrheic shellfish poisoning (DSP);. Okadaïc acid and its derivates are the main diarrheic toxins but they belong to a broader spectrum of toxins, i.e "lipophilic toxins'. These later also include pectenotoxins, yessotoxins, azaspiracids and the fast acting toxins (FAT) like gymnodimines and spirolides. Toxic microalgae occurrences in coastal waters may lead to further toxin bioaccumation in filter-feeders, which is a real thread for human consumption. As a consequence, a ban of shellfish harvesting and marketing is decided by Administration as soon as toxin content in shellfish meat is exceeding the regulatory threshold. Such closures generally induce important economic losses and development of semi-industrial detoxification plants might represent a valuable countermeasure in the future.

This study focused on three experiments involving different bivalve / toxin associations: Pacific oyster *Crassostrea gigas* and PSP, blue mussel *Mytilus edulis* and DSP, European clam *Ruditapes decussates* and gymnodimine. All three studies are based upon a comparison between detoxification kinetics of either starved (sea water alone) or fodder algae-fed bivalves. Oysters were contaminated in controlled tank with 200 cell.mL^{-1} of *Alexandrium minutum* during 10 days and afterwards detoxified with either sea water alone or with *Skeletonema costatum* at concentration: 2000 cells.mL^{-1}. Mussels were collected in a DSP contaminated area of Vilaine Bay, in June 2007, and then detoxified in two different tanks. The first tank contained only sea water and the second tank was supplied with *Skeletonema costatum*. Clams were contaminated with *Karenia selliformis* at concentration 200 cells mL^{-1} and then detoxified with sea water or fed *Isochrisis galbana* (12 000 cells mL^{-1}).

The major part of any of these three types of algal toxins was detected in the digestive tract: and the detoxification pattern was mainly that of the digestive gland. Digestive gland (DG) detoxification kinetics could be described by a linear Ordinary Differential Equation (ODE) of the first order (except for Clams first day detoxification). For steady conditions, the ODE can be solved explicitly by an exponential function of time. For the first couple (oysters / PSP), the depuration coefficient was 0.09 d^{-1} in sea water and 0.27 d^{-1} (ratio: 3) with *S.costatum* diet. For an initial toxicity of 200 µg eq. saxitoxine (STX) for 100g of oyster wet meat, it would require almost 8 days in sea water and less than 4 days with *S.costatum* diet to drop toxin content below the regulatory threshold (80µg eq.STX for 100 g of wet meat). For the second couple (mussels / DSP) , the depuration coefficient was 0.05 d^{-1} in sea water and 0.13 d^{-1} with *S.costatum* diet (ratio: 2.6). For the last couple (clams / gymnodimine), the ratio between the two coefficients was 2.6, with a coefficient of 0.72 d^{-1} in seawater and 1.85 d^{-1} with *I.galbana* diet.

The input of fodder algae significantly increased detoxification kinetics for all types of shellfish / toxin couples. Finally, the effects of environmental data (temperature and carbon concentration) upon DSP-contaminated Norwegian mussel were investigated. It is important to underline that temperature was far colder in Norway than in all experiments carried out in France. ODE models of the first order taking into account or not the environmental conditions were tried. Although it was difficult to assess any significant effects of environmental parameters, the 'food effect' was, there again, observed.

The short lived *Argopecten ventricosus* – How do intrinsic or extrinsic factors shape the lifespan of a species?

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Keywords: Argopecten ventricosus, growth, aging, ROS

The catarina scallop *Argopecten ventricosus*,,is among the shortest-lived scallop species. It reaches market size within a year and is of high commercial value, (Maeda-Martínez et al. 2000). Due to its very short lifespan of only 2-3 y, this scallop is an ideal model to study how internal and environmentally factors modulate growth and aging. In our study we are testing the effect of one abiotic physical factor: elevated temperature and of the biotic stressor predation on growth and physiological aging of the catarina clam of the pacific coast of Baja California Sur, Mexico.

Animals were obtained from a hatchery (genetically similar animals) and grown in the laboratory for 1 ½ Months to a size of 5 mm until the young animals were exposed to different experimental conditions in the laboratory and in the field. One part of the animals is being raised in the laboratory under different temperature regimes: *in situ* field temperatures and *in situ* field temperatures but elevated max. 5°C. Another part of the animals is reared directly in the field inside the sea grass as well as nestier trays (predator protected area) and outside sea grass (predator exposed area).
The growth, metabolic rate and biochemical parameters are being analyzed in all different experimental groups over a time frame of two years to evaluate the physiological adaptations to the different environments conditions and experimental stressors.

Scallops in the field grew more rapidly than animals maintained in the laboratory. The mean growth was 0.5 mm/day for the laboratory animals, compared to 2.3 mm/day for field animals. Animals grown at 5°C higher temperature in the Lab grew 4% slower than animals at *in situ* temperatures. Currently the measurements of metabolic rate and of scope of growth are carried out to determine the energetic balance and expenditure in the different treatments. Tissue antioxidant capacities and the activity of antioxidant enzymes are being measured. First results of the field animals before and after release to different environments, and the laboratory animals exposed to two different temperatures will be shown in the Symposium in September.

Growth of diploid & triploid *C. gigas* at different sites/years is explained by an index of monthly productivity/temperature, but differential survival is explained by accumulated thermal degrees and productivity

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Keywords: Crassostrea gigas, diploid-triploid, Index, environmental-conditions, El Niño-La Niña

The Pacific oyster, *C. gigas*, a species whose origin is from temperate climates, is also cultured in tropical northwest Mexico since the 1970's. However, large collapses have been recorded from the 1990's on, especially when this oyster is cultured within sites of the Gulf of California (Fig. 1a). Around the world summer mortalities of this oyster have been recorded, but extreme environmental conditions have not been tested for their effects. Because triploid oysters have been found to respond better than diploids to abnormal environmental conditions, and to establish if culture of triploid oysters rather than diploids is an alternative for avoiding production collapses within the Gulf of California culture sites, two studies were conducted comparing diploids and triploids at multiple sites simultaneously, the first during 2006 and the second in 2007. For the 2006 study, significant differences between culture sites (all within the Gulf of California: Ceuta, Chicura & Soldado) and ploidy were observed for growth and survival. For the 2007 experiments (Ceuta, Bacorehuis & Rancho Bueno) significant differences in growth were also evident between diploid and triploid oysters, but not for survival.

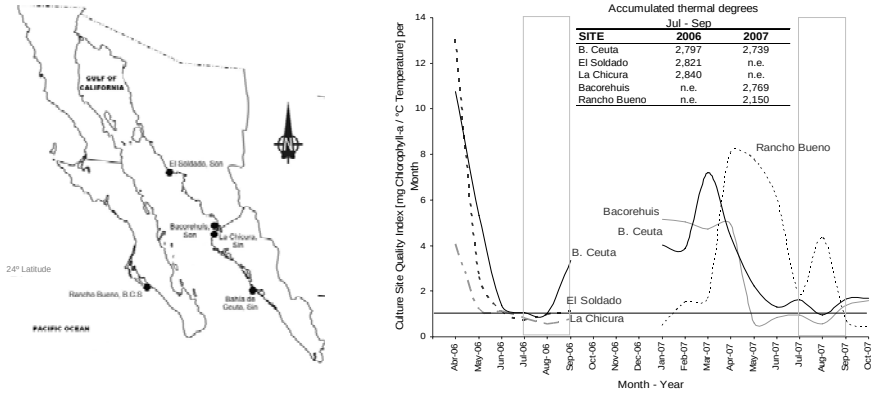

Fig. 1: (a) Culture sites at the Northwest of Mexico; (b) Culture site quality index and accumulated thermal degrees during 2006 & 2007

These two years were characterized for presenting contrasting environmental conditions, caused by an El Niño event during 2006, and a La Niña event in 2007. This prompted us to search for associations of temperature and productivity with growth and survival. We estimated a 'Culture Site Quality Index' as the relationship between monthly chlorophyll-a concentration and temperature at each site (Fig. 1b), and found that it explained the differences in growth between sites and within year, but not the differences on survival. We also estimated the total thermal degrees that accumulate at each 3-mo intervals, finding that for both years, the largest accumulation of thermal degrees in a single 3-mo period occurred from July to September (Fig. 1b-table). Accumulation of thermal degrees of $\geq$2,800^{o}C over a 3-mo period combined with a monthly average availability of $\leq$1 mg chlorophyll-*a* / m^3 per culture site resulted in low survival, and this associated with low carbohydrates and glycogen reserves regardless of ploidy.

Tropical vs. subtemperate environments: growth, survival & biochemical composition of *Crassostrea gigas* diploids and triploids

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Keywords: C. gigas, diploid-triploid, environmental-conditions, growth, survival

The Pacific oyster, *Crassostrea gigas*, a species originating from temperate waters, is cultured in Mexico at both environmental conditions, subtemperate (Pacific side of the Baja California Peninsula) and tropical (Gulf of California). Much research has been done internationally in temperate areas on what is defined as 'summer mortalities', but not on understanding if this species is adapted to perform in a wide range of environmental conditions. We evaluated growth, survival & biochemical composition of diploid and triploid oysters by growing them simultaneously for 9-mo at three oyster grow-out sites: Bacorehuis Lagoon (tropical continental Mexico, within the Gulf of California), Ceuta Bay (tropical continental Mexico, south of the mouth of the Gulf of California), and Rancho Bueno Lagoon (subtemperate west coast of the Baja California Peninsula). The best site for growth was found for oysters grown in the subtemperate site, but differences in growth between the two tropical sites were also found (Fig. 1). Shell length, total weight and tissue weight of triploid oysters was greater than diploids regardless of culture site, but whole oyster shell width (thickness) was not different between diploids and triploids only when grown at the temperate site. Survival was site dependent, with the largest monthly survivals seen at the subtemperate site. Ploidy was not a significant effect on survival, but an interaction between ploidy and site was observed for survival, resulting from a lower survival of triploids than diploids at the subtemperate site, whereas the same survival was seen at both tropical sites for diploids and triploids. Biochemical composition was associated to the patterns of growth and survival, with the largest concentrations of glycogen and triacylglycerides for oysters grown at the subtemperate site.

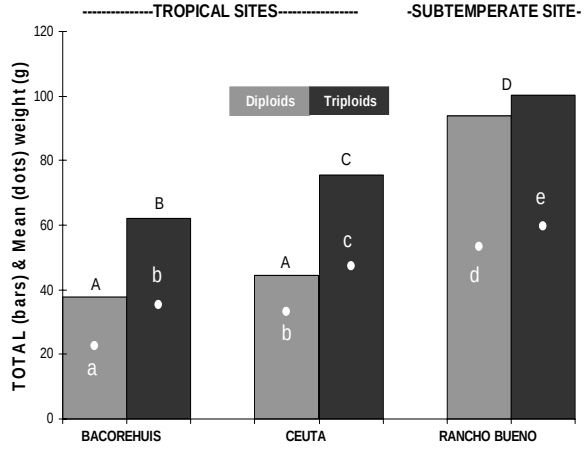

Fig. 1. *C. gigas* diploids and triploids growth between culture sites (two tropical, one subtemperate).

These results provide with information that allows prediction of what might be expected for Pacific oyster culture globally under an increasing warming climate change.

Furthermore, when considering the development of a selective breeding programme, they point to the need to understand if a comprehensive breeding programme for the northwest of Mexico, with such contrasting environmental conditions, will suffice for all cultured sites, or if specific breeding programmes will have to be developed for tropical vs. temperate sites because a significant genotype by environment interaction might exists for growth or survival.

How does reproduction and recruitment patterns match? The case of *Chlamys varia* in Ría de Betanzos-Sada, NW Spain

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Keywords: Chlamys varia, reproduction, recruitment

Although it is not abundant, the black scallop *Chlamys varia*, is considered as a potential species for aquaculture in Galicia. Before starting aquaculture operations it is advisable study the reproductive pattern, in order to optimize the production of spat both in hatchery or by means of collectors. Regarding to employment of collectors, in Galicia spat settlement of *C.varia* has been recorded only in the Ría de Betanzos-Sada. Settlement of black scallop in other Rías has been disappointing. Obviously, the recruitment takes place after the spawning but the relationship between both processes is not always clear. It was intended to increase the knowledge on this subject by studying the pattern of spawning and recruitment of *C.varia* in Ría Betanzos-Sada, a small Galician Ría where this pectinid settles in reasonable high numbers. To perform this work the reproductive cycle was studied employing the evolution of a gonad index and the recruitment by the control of settlement of spat on collectors deployed in the area.

Individuals of *C.varia* mean size 36.5 ± 5.2 mm attached to mussel ropes in Ría Betanzos-Sada, supposedly settled in summer 2005 were collected in November 2006 and placed in cages at 6 m depth in Ría Betanzos-Sada. Roughly fortnightly 20 animals were sampled and the organs dissected. A gonad condition index (GI) was calculated as GI =100 (gonad DW/shell DW), were DW is dry weight. From May 17th to September 19th collector bags were deployed every 3 weeks. Also every 3 weeks, from June 28th to September 19th two collectors bags of each deployment date were sampled and the number and size of the spat of *C.varia* recorded. In February the spat settled on the collectors deployed at the different dates were detached and measured.

C. varia starts gametogenesis in winter, reaching maximum gonad weight by May. >From this date begins a protracted spawning season ending by November. There is a period of maximum reproductive activity taking place between early May and end July; afterwards there is a period of lowered activity ending by the beginning of November. Apparently, there is a continuous main spawning season between May and July, all the population being involved, followed by a minor one between August and October when only part of the animals participate in the reproduction. Settlement starts in June and goes on in a continuous way until July. In August 7th a new cohort starts to settle and continues until end September.

Two recruitment episodes were recorded, both taking place for a period of roughly two months, the first one of higher intensity, peaking at ≈ 300 spat bag-1, the second at ≈ 100 spat bag-1, differences apparently related to the different spawning intensity. At detachment in February 2008, the population shows a very scattered distribution partially related to the two recruitment events, but affected by higher mortality on the first cohort and ranges from 3 to 40 mm. Spreading recruitment over a long period can be a good strategy in order to increase the probability of success. Even, as *C.varia* is a protandric species with sex change related to age/size, this strategy can allow part of the population reaching the adequate size for sex reversal the next year.

The role of suspended mussel culture (*M. Edulis*) in nutrient dynamics of oligothrophic systems: the study set-up

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Keywords: Mussel, carrying capacity, nutrient dynamics, remineralization, oligothorophic

In the context of mussel culture, it is appropriate to define sustainable production in terms of carrying capacity (often divided into four categories: physical, production, ecological and social). In the current study we will focus on production and ecological carrying capacity of suspended mussel culture. There are various ways in which mussel culture influences their surrounding ecosystem and which subsequently determine the carrying capacity. Mussel populations can influence the ecosystem by filtering particulate matter from the water-column, and excrete the remainder either in a dissolved form or as feces and pseudofeces. Thereby it may increase the transport velocity of organic matter to the seabed, causing either increased nutrient turnover or retention in the sediment. The directly excreted inorganic nutrients and the inorganic nutrients originating from remineralization of biodeposits can in turn stimulate phytoplankton growth. The current study will deliver more insight in specific nutrient pathways resulting in better predictive generic modelling of carrying capacity in mussel culture.

The overall objective of this study is to determine changes in nutrient and food dynamics induced by suspended mussel culture under low nutrient and low seston conditions. To achieve this, five sub-objectives have been conducted:

1. To quantify rope-scale changes in particle/food dynamics due to preferential filtering.
2. To quantify rope-scale changes in dissolved nutrient dynamics due to direct excretion and remineralization of biodeposits.
3. To quantify the fraction of biodeposits that is trapped within the longlines to provide information on nutrient sinks in the system.
4. To quantify rates of nutrient regeneration from mussel mediated biodeposits.
5. To establish a nutrient budget and carrying capacity estimates for suspended mussel farms in an oligotrophic environment.

The proposed objectives will be achieved by three successive research blocks. The first block (I) contains a seasonal *in situ* study carried out in pelagic chambers to evaluate changes in particle/food dynamics and dissolved nutrient release rates induced by mussel culture. The in- and out flowing water will be analyzed for phytoplankton shifts and nutrient fluxes and those dynamics will be evaluated as a function of environmental conditions and culture characteristics. On the basis of stoichiometric data the nutrient retention in mussel biomass will be included. The second block (II) focuses on biodeposition. Firstly the egestion and allocation of biodeposits will be determined by correlating total egestion rates to total amount of biodeposits trapped within the ropes. Secondly we will focus on remineralization of mussel biodeposits. By incubating freshly collected biodeposits we will quantify specific remineralization rates as a function of temperature, initial food availability, bacterial activity and associated fauna. The third block (III) focuses on modelling the nutrient fluxes induced by mussel culture. By integration of rope scale fluxes (block I and II) to farm scale fluxes and using predictive models we will be able to determine the feedback of nutrient regeneration on ecosystem processes.

Gametogenic cycle and biochemical composition of the pullet carpet shell *Venerupis senegalensis* on the Ria de Aveiro Lagoon (northwestern coast of Portugal)

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Keywords: Pullet carpet shell, Venerupis senegalensis, biochemical composition, condition index, reproductive cycle

The pullet carpet shell *Venerupis senegalensis* is an Atlantic–Mediterranean warm-temperate species that inhabits sandy to muddy bottoms, usually from the low tide mark to a depth of 40 m. This species is commercially exploited in Portugal, Spain, France and Italy. Presently, in mainland Portugal *V. senegalensis* is more abundant on the western coast, namely in the Ria de Aveiro coastal lagoon. Within this lagoon, fishermen harvest clams by walking the intertidal areas and using artisanal hand-tools. This species can also be harvested using hand-dredges, which are operated from boats with the help of a long handle. Within the last five years the population of this species declined due to overfishing and recruitment failure, resulting in a substantial threat to the sustainability of the fishery. Recruitment failure may be related to the physiological viability of larvae that depends on environmental factors and/or on reproductive broodstock condition. In the present poster we present results of a work that aimed to study the dynamics of the pullet carpet shell reproductive cycle along with its nutrient storage and exploitation strategy in the Ria de Aveiro throughout two years (2006-2007). The gametogenic cycle of *V. senegalensis* was analyzed through histological preparation using qualitative criteria. Condition index and biochemical composition were determined in order to provide information on energy storage and utilization. Knowledge of the reproductive cycle of *V. senegalensis* provides a valuable insight into the biology of this species and it is important for futures stock enhancement programs which will contribute to the sustainability of the fishery. The information obtained in this study is also important to provide the optimal reproductive time for artificial spawning induction in aquaculture. From a management point of view, this information is important to obtain a good quality of larvae and consequently to promote successful restocking actions based on aquaculture production. This study was partially funded by the PROMAR (Interreg IIIA) and Tecnologias de Produção Aquícola (MARE) projects.

Genetic diversity of two Portuguese populations of the pullet carpet shell *Venerupis senegalensis*, based on RAPD markers

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Keywords: Pullet carpet shell, Venerupis senegalensis, RAPDs, genetic diversity

In Portugal, the pullet carpet shell *Venerupis senegalensis* is a commercially important species that occurs in estuaries and coastal lagoons. In subtidal areas this species is harvested using dredges or by divers in apneia, whereas in intertidal areas several rudimental hand-tools are used to collect this species. The pullet carpet shell was once abundant in the Ria Formosa lagoon (southern Portugal). However, in the early 1980's the abundance of this species declined dramatically due to over-fishing. Despite the high commercial value of this species, the lack of interest by local fishermen in harvesting this species lead us to suppose that the population density in Ria Formosa has decreased below a threshold level, such that natural recovery is not possible. Therefore, an active intervention would be necessary to restore stocks to reproductive viability. In order to reverse this negative trend, a project to produce larvae and juveniles of *V. senegalensis* was implemented aiming to test different technical approaches to restock this species in Ria Formosa. To minimize the deleterious effects of hatchery practices on the genetic make-up of the released stock, the broodstock should be obtained from the natural population to be restocked or, if polymorphism of this population is proven to be low and the population to be in genetic decline, from the genetically closest population. Since besides the Ria Formosa population no other information is currently available on the genetic variation within Portuguese populations of *V. senegalensis*, the genetic structure of another representative natural population (Ria de Aveiro) was analysed by random amplified polymorphic DNA (RAPD) techniques. Twenty individuals from each population were analysed by RAPD profiles. Of the 10 primers screened, six amplified clear and reproducible bands. In this poster we present the results of the molecular variance analysis that described the genetic variation within and among populations, and the correlation with their geographic origin. This study was funded by the Promopesca (Interreg IIIA) project.

Involvement of Insulin-like pathway in the regulation of reproduction and storage metabolism of the Pacific oyster *Crassostrea gigas.*

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Keywords: Crassostrea gigas, Insulin-like pathway, seasonal cycle, storage cells

The reproductive strategy of the pacific oyster *Crassostrea gigas* is based on annual massive production of gametes. The high energetic cost of this reproductive effort is supported by glycogen reserves stored in specialised cells called vesicular cells. The seasonality of glycogen metabolism linked to the reproductive cycle as well as the annual restructuration of gonadal storage tissue suppose the occurrence of regulatory events depending on environmental parameters (temperature, stress, food consumption…) and internal neuroendocrine factors. Insulin-like molecules are known to be involved in multiple regulatory pathways including growth, reproduction, glycogen metabolism and longevity in vertebrate and invertebrate species. In *C. gigas*, a ligand oILP (Hamano *et al.*, 2005) and a receptor CIR (Gricourt *et al.*, 2003) have been characterised. The availability of the receptor sequence led to envisage the localisation of IL target cells and to precise the potential functions of ILs.

The expression of CIR was first measured using real time PCR in various tissues of standard animals (palps, gills, mantle, gonadal area). CIR expression was mainly detected in mantle edge in october and in gills and palps in april. In the gonadal area, a mixed tissue containing storage and reproductive cells with a ratio varying according to the seasonal cycle, the expression of CIR could not be easily associated with one specific cell type. We also used a laser microdissection approach to isolate both cell types.

Animals exposed to different trophic levels in controlled conditions during one month in october were also tested. Food restrictions appeared to result in a significative increase of CIR expression in palps.

Insulin-like effects were known to be mediated by two major signalling pathways whom effectors seems to be well conserved throughout evolution. Designation of degenerated oligonucleotidic primers in conserved regions led us to identify by PCR approach some of the effectors of the signalisation pathways like Ras end Pten. Other members of the cascade were now investigated and more particularly final effectors of the signal. The choice of pertinent antibodies addressed to the active/inactive forms of the effectors would be very informative in order to understand this regulatory pathway in *C. gigas* and how environmental parameters could be integrated.

Involvement of lectins in Manila Clam *Ruditapes philippinarum* defense

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Keywords: manila clam, galectin, c-type lectin, Perkinsus olseni, vibrio, innate immunity

The lectins play crucial roles in the innate immunity systems of vertebrates and invertebrates. In this study, we studied about noble galectin (MCGal) and c-type lectin (MCL3) from the Manila clam *Ruditapes philippinarum.* The lectins were cloned from Manila clams infected with *Perkinsus olseni* and characterized. Galectin protein has 309 amino acid residues, and a predicted molecular weight of 33.9 kDa and C-type lectin was 17.38 kDa. Carbohydrate recognition by the recombinant Manila clam lectins, as determined by hapten inhibition of hemagglutination, revealed that rMCGal has features common to the galectin family, i.e., significant affinity for galactose and N-acetylgalactosamine. rMCL3 agglutinated rabbit erythrocytes in presence of Ca^{2+} and it was partially inhibited by GalNAc, Man, lactose and raffinose, whereas the polysaccharides bovine mucin type II and candida mannan inhibited the agglutination completely. Galectin and C-type lectin mRNA expression were detected mainly in the heart, mantle, foot, adductor muscle, palp and siphon tissues of *Perkinsus* infected clam. Also, immunohistochemistry using an each antibody confirmed protein expression in these tissues and in the hemocytes. The mRNA temporal expression of these lectins in Manila clams challenged with *Perkinsus* or *Vibrio* species was up-regulated as compared with non-challenged healthy clams. Especially, rMCGal agglutinated *Vibrio tapetis,* and agglutination was inhibited by incubation with α-lactose. rMCGal also bound to the surface of *P. olseni*. As a result, these lectins seem to play a crucial role in Manila clam defense, particularly with respect to pathogen recognition through opsoninzation or agglutination.

The identification of a new member of the neuropeptide Y receptor family from the oyster *Crassostrea gigas*, that is differentially expressed in progeny exhibiting opposed susceptibility to summer mortality

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Keywords: Crassostrea gigas, Gene expression, Neuropetide Y, reproduction, nutrition

Summer mortality has been reported in the Pacific oyster *Crassostrea gigas* for many years in different parts of the world. The causes of this phenomenon are complex, resulting in the interaction between the oyster, the environment and opportunistic pathogens. The multidisciplinary program Morest coordinated by Ifremer was initiated to go further in the understanding of the causes of summer mortality in France and to reduce its impact on oyster production. Among factors responsible for survival, it has been demonstrated that the genetic variability contribute largely (45%) to survival. Families with high and low survival rates were identified, and a high heritability for this character has allowed the production of selected lines “R” for Resistant and “S” for Susceptible to summer mortality (Samain et al., 2007).

In order to characterize summer mortality resistant markers and identify the biological mechanisms involved in summer survival, a cDNA microarray has been produced by the Max Planck Institute for Molecular Genetics (Berlin), resulting from the collaborative work of the consortium Marine Genomics Europe and the European Aquafrst program. The slide contains 9059 unigenes spotted in duplicates, previously assembled in a unique database (http://www.sigenae.org/aquafirst/).

This cDNA microarray has been hybridised using samples originating from gonad tissue of R and S oysters before, during and after a summer mortality event observed in the field. Differentially expressed genes between R and S were identified using a SAM analysis. Among the 423 non-redundant genes found differentially expressed between the two progenies, one belongs to the neuropeptide Y (NPY) receptor family. It has been considered as a very important candidate because NPY is known to be involved in the central regulation of appetite, sexual behaviour and reproductive function. As a matter of fact, it has been shown that physiological mechanisms that control energy balance are reciprocally linked to those that control reproduction, and together, these mechanisms optimise reproductive success under fluctuating metabolic conditions. NPY function as physiological integrator in two different neuro-endocrine systems: one governing feeding and the other controlling reproduction. All these considerations are very relevant in regard to R and S behaviour. Indeed, comparison between the two selected progenies showed significant differences in term of gonad investment and spawning, especially in eutrophic environment. These have results suggested that R families can survive summer mortality because they are not as reproductively active as S families (Fleury et al., 2008).

In the present study, we report the characterization of a member of the Neuropeptide Y receptor family in the cupped oyster *C. gigas*. The spatio-temporal expression of this gene is presented according to the tissues, the sex and developmental and reproductive adult stages. Its expression is also compared under different food availability conditions. The putative role of this Neuropeptide Y receptor in reproduction and in nutrition is then discussed as well as these pathways might be significant to the higher rate of summer mortality of the sensitive selected progeny.

Fleury et al 2008. Gene 410, 187-196.
Samain et al. 2007. Aquaculture 268, 227-243

Bonamia-ostreae induced mortalities in one-year old European flat oysters *Ostrea edulis*: experimental infection by cohabitation challenge

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Keywords: Ostrea edulis, Bonamia ostreae, cohabitation experiment, transmission, heart smear

Bonamiosis is a parasitic disease (causative agent: *Bonamia ostreae*) affecting the European flat oyster *Ostrea edulis,* responsible for a drastic decline in the aquaculture production of this oyster species. Therefore a selective breeding program for resistance to bonamiosis has been undertaken since 1985 by Ifremer, leading to the production of several selected oyster families.

In the present study, a 6-month cohabitation challenge experiment was performed in order to transmit the disease from wild oysters (injected with the parasite) to two tested families of oysters originating from the selective breeding program. Mortalities were checked daily, and ventricular heart smears were performed on dying or moribund oysters to detect the level of infection to *B. ostreae*.

The first infections occurred after 4 months of challenge in the tested oysters (Family 1 and Family 2). The cumulative mortalities after 6 months were 58% for the wild oysters, 9% for Family 1 (20-month old at the beginning of the experiment) and 20% for Family 2 (8-month old). The parasite could be detected in 66.8% of the dying wild oysters, 67.5% of the dying oysters of Family 1, 89% of the dying oysters of Family 2 and only 11% of the surviving oysters of Family 2. The mortality was significantly higher in Family 2 than in Family 1 (χ^2=20.87, $p<0.001$, 1 d.f.) as well as the level of infection by the parasite found in heart smear (χ^2=24.34, $p<0.001$, 4 d.f.). This result demonstrates that prespawning oysters as young as 1 year-old can become infected with the parasite and die from bonamiosis. This result is inconsistent with the commonly accepted critical age of 2 years-old for the disease development. The most probable cause of the discrepancy in the development of bonamiosis between the 2 tested families is a difference in genetic background.

Microsatellite development in *Ostrea edulis* and *Mytilus edulis*

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Keywords: microsatellite, European flat oyster, blue mussel, M13 tail

The European flat oyster *Ostrea edulis* and the blue mussel *Mytilus edulis* are both very valuable commercial species across Europe. Despite their economical importance, only a few microsatellite markers have so far been developed in those species. The aim of this study was to develop new microsatellites for *O. edulis* and *M. edulis* that could be used for population genetics studies, genetic variability assessment of stocks, parentage analysis or genetic and QTL mapping studies.

For *Ostrea edulis*, an enriched library was made by ecogenics GmbH (Zurich, Switzerland) from size selected genomic DNA ligated into SAULA/SAULB-linker and enriched by magnetic bead selection with biotin-labelled $(GT)_{13}$ and $(CT)_{13}$ oligonucleotide repeats (Gautschi et al. 2000a,b). Of 758 recombinant colonies screened, 179 gave a positive signal after hybridization. Plasmids from 133 positive clones were sequenced and primers were designed for 94 microsatellite inserts. Optimisation of PCR conditions was first performed on agarose gel by changing the annealing temperature, the $MgCl_2$ concentration and the primers concentration. Successful amplification was achieved on 2% agarose gel for 76 primer pairs.

For *Mytilus edulis*, an enriched library was made by ecogenics GmbH (Zurich, Switzerland) from size selected genomic DNA ligated into SNX forward/SNX reverselinker and enriched by magnetic bead selection with biotin-labelled $(GT)_{13}$ and $(CT)_{13}$ oligonucleotide repeats. Of 750 recombinant colonies screened, 157 gave a positive signal after hybridization. Plasmids from 157 positive clones were sequenced and primers were designed for 62 microsatellite inserts. Out of 31 primer pairs tested, successful amplification was achieved on 2% agarose gel for 22 of them after optimisation of the PCR conditions.

Further optimisation was performed on a 3130*xl* Genetic Analyzer (Applied Biosystems), using an economic method for the fluorescent labeling of PCR fragments. This M13-tail protocol consists of using 3 primers during the PCR: a sequence-specific forward primer with M13 tail at the 5' end, a sequence-specific reverse primer and the universal fluorescent-labeled M13 tail. Four different M13 tails were used, each labeled with a different fluorescent dye (FAM, VIC, PET, NED).

Test of polymorphism for the optimised microsatellites of *O. edulis* and *M. edulis* was performed by genotyping 16 individuals per population, in two wild populations.

Functional characterization of several Transforming Growth Factor-β (TGF-β) signalling components in the oyster *Crassostrea gigas*

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Keywords: Crassostrea.gigas, growth factors, TGF-beta, Activin

Members of the Transforming Growth factor beta (TGF-β) superfamily are known to have important roles in cell proliferation, cell differentiation, reproduction and development. To understand the molecular processes underlying the physiological control of proliferation and differentiation in Lophotrochozoans, genes encoding TGF-β pathway components have been characterized in the Pacific oyster *Crassostrea gigas*.

During this work, we improved our knowledge on the TGF-β signalling components by the identification of a new type II receptor, named Cg-ActR2. This receptor shows highest identity with human activin type 2 receptor (ActR2). Expression pattern of Cg-ActR2 transcripts was examined in adult tissues and during embryonic and larval stages of development. Though being ubiquitously but weakly expressed in adult tissues, Cg-ActR2 appears highly expressed in early embryonic stages with a maternal expression in oocytes and an increased expression in blastula and gastrula stages. To gain insight into the early larval development implication of Cg-ActR2, we have microinjected capped mRNAs encoding both full length and truncated proteins in zygotes of zebrafish and medaka used as reporter organisms. First results show Cg-ActR2 interferes with medaka endogenous activin pathway components as observed by specific expression of relevant molecular markers.

The TGF-β signalling process appears to be widely conserved in the animal kingdom. As previously observed in zebrafish used as a reporter animal, oyster TGF-β pathway components (Ligands and Receptors) seem to be able to interact with vertebrate components. Though structural homologies between oyster factors and their orthologous factors in other species are obvious, the way oyster components interact to each other remains unknown. To address this question, plasmids encoding *Crassostrea gigas* TGF-β ligands and TGF-β receptors were co-transfected in mammalian cell lines with reporter vectors encoding Firefly Luciferase under the control of either BMP or TGF-β/Activin responsive elements. The results show that Cg-TGF-β, a TGF-β/activin-related ligand, interacts with Cg-ActR2 together with Cg-TβRI or to a lesser extent with Cg-ALR1 but not with Cg-BMP-R1 and induce the TGFβ/activin pathway as observed by a nine fold increase of luciferase activity with respect to basal level. In this specific context Cg-TβsfR2 receptor, an exotic BMPR2 with two ligand binding domains does not seem to induce the TGF-β/Activin pathway alone but inhibits this pathway in combination with any other tested oyster TGF-β pathway component.

Altogether this study suggests a preservation of the functionality of the TGFβ/activin pathway in Lophotrochozoans and demonstrates a conservation of the hierarchy of the interactions between the various components of the pathway (orthologous ligands apparently bind orthologous receptors).

Peculiarities of pumping activity of some invertebrates in the fouling communities of the White Sea

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Keywords: pumping rate, filter feeding, competition for food, fouling communities

The principal components of fouling communities developing in the White Sea are bivalves, solitary ascidians and sponges. Interactions between these organisms are strongly competitive, one of the relevant resources is food. As a rule competitive capability of filter feeding organism is estimated through filtering rate. In this study we consider both pumping rate and configuration of feeding currents.

The study was carried out in 2005–2006 on the White Sea Biological Station of Zoological Institute RAS. Pumping rate was determined by means of thermister flowmeter (LaBarbera, Vogel, 1976). Current's configuration was reconstructed using computer processing of video records (Lezin et al., 2007).

The greatest pumping rate was registered for sponge *Halichondria panicea* and blue mussel (*Mytilus edulis*). Solitary ascidians (*Styela rustica*, *Molgula citrina*) and bivalve *Hiatella arctica* demonstrated greatly lower pumping activity.

The differences in pumping rate correlate with configuration of feeding currents. So, sponge and mussel create wide zone of water currents almost completely surrounding animal's body. In contrary, solitary ascidians and *H. arctica* form comparatively narrow currents. However having mobile siphons these organisms are able to select the most suitable direction of currents and thereby to optimize own feeding efforts.

Thus, two type of feeding activity can be distinguished. The first of them consist in non regulated wide filtering zone and high pumping rate. The second one is characterized by low pumping rate and ability to efficiently exploit surrounding space owing to mobility of siphon.

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Measure black pearl oyster shell growth: a comparison of two fluorochromes

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Keywords: Pinctada margaritifera, growth, tetracycline, calcein

Cultured pearls are obtained by the introduction of a pearl nucleus and a piece of mantle tissue from a donor oyster ("saibo") into a number of recipient oysters. The pearl is composed of many nacre layers made of aragonite crystals. The thickness of the nacre layer is an important criterium for evaluating the quality of Tahitian pearl

In order to understand the influence of environmental factors (temperature, food,…) on the growth of black pearl oyster (*Pinctada margaritifera*), pearl growth and recipient oyster shell growth must be determined. To measure pearl and shell growth, a visible mark of the beginning and the end of experiment must be detectable on calcified structures. Stains which are successfully incorporated into the growth calcified structure, can be used as internal growth markers. Because of the non traumatic nature of this method, the chemical markers, calcein and tetracycline, were chosen for this experimentation. Those substances have already been tested successfully on others molluscs. They are also inexpensive and easy to use. We conducted a series of experiments to test the suitability and the reliability of these two growth marker on black pearl oyster.

The treated animals were juveniles and showed a length average of 4 cm and a width average of 3 cm. Two different way of marker administration were used in these experiments. Calcein was delivered by immersion and injection, tetracycline by immersion only. For each growth marker, different concentrations were used : 50mg/L, 100mg/L and 150mg/L for calcein ; 600mg/L, 800mg/L and 1000mg/L for tetracycline. A series of immersion times were examined for each concentration : : 6h, 12h and 24h for calcein ; 24h, 32h and 48h and for tetracycline. After the immersion/injection period, molluscs were reared in the laboratory for one and two months. Information about the retention time and food influence on markers incorporation will be presented.

Pre-ingestive behavior of *Crassostrea corteziensis, Nodipecten subnodosus,* and *Atrina maura*

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Keywords: Crassotrea corteziensis, Nodipecten subnodosus, Atrina maura, selective pre-ingestion, filtration rate

Feeding behavior in juveniles of three species of mollusks, lions paw *Nodipecten subnodosus*, pleasure oyster *Crassostrea corteziensis*, and pen shell *Atrina maura* were recorded. These species are commercially important to the aquaculture industry in the northwestern part of Mexico. However, seed production has not been completely developed. It was demonstrated that *Chaetoceros calcitrans* and *Chaetoceros muelleri* is a very good food for these species, with the preference of these species for this microalgae considered the same. In this work, we tried to determine if there was selective pre-ingestive behavior by these species that could partially explain the good results of the diets.

The juvenile mollusks were conditioned in the laboratory in a continuous-flow system. Short experiments were done to determine pre-ingestive selection by comparing the filtration rates of different microalgae. The system consisted of a chamber adapted to a continuous flow rate of 80 or 100 ml min^{-1}, temperature of 22±0.5 °C, and salinity of 37.5 PSU. The organisms were acclimated for an hour before starting the filtration-rate measurements. With *N. subnodosus* and *C. corteziensis*, the microalgae were *Isochrysis galbana*, *Pavlova lutheri*, *Nannochloropsis oculta*, *Chaetoceros muelleri*, *Chaetoceros calcitrans*, *Nannochloropsis oculata*, *Tetraselmis suecica*, and *Phaeodactylum tricornutum*. The measurements were taken at 30-min intervals for 3h; two experiments with three repetitions were done. The concentrations were equivalent to 40,000 cells ml^{-1} of *Isochrysis galbana* in ash free, dry weight. With *A. maura*, the microalgae were three diatoms known for good nutrition for this mollusk species, *C. muelleri, Thalassiosira pseudonana, Thalassiosira fluviatilis* and the chlorophyceae *Dunaliella tertiolecta* that is known as a poor food. The microalgae were also tested at five flow rates: 40, 60, 80, 100, and 120 ml min^{-1}.

The results showed that the scallop *N. subnodosus* did not have a preference for any of the microalgae species; the oyster *C. corteziensis* selected for microalgae size, of which the least selected was *N. oculata* and the most ingested were the diatoms and *T. suecica*; the penshell *A. maura* showed no pre-ingestion preference at low flow rates, but at medium and high flow rates there was a preference, with the least preferred food (*D. tertiolecta* at the two highest flow rates. This result indicated that *A. maura* has a distinct pre-ingestion behavior related to flow rate.

Calcium permeability of egg capsules and variations in ionic and osmotic concentrations in the egg capsule fluid of *Crepidula fornicata* during embryonic development.

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Keywords: Gastropod, egg capsule fluid, ions, osmotic pressure

Calcium permeability of the capsule wall was studied at oviposition, by dipping freshly-laid egg capsules in ^{45}Ca as a tracer. This study also determined total calcium content of the embryos at different developmental stages. Osmolarity and major ion concentrations in egg capsule fluid were higher than seawater at uncleaved and trochophore stages, and then dropped to the same level as sea water at veliger stage. Concentrations of Cl^- and Na^+ were relatively high at oviposition, peaked at trochophore stage, and finally dropped close to concentrations of seawater at hatching. In contrast, concentrations of Mg^{++} and Ca^{++} decreased steadily during capsular development. Radiotracer permeability experiments in recently laid egg capsules confirmed that the capsule wall is impermeable to this ion at that stage. However, because of the dissolution of the inner layer of the wall during the final part of capsular development, the wall becomes permeable to calcium and probably to the rest of the major ions studied.

Autumn conditioning of the flat oyster *Ostrea edulis* L.

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Keywords: Ostrea edulis, conditioning, temperature, photoperiod, gametogenesis

The main objective of broodstock conditioning is to induce gonad maturation several months prior to that observed in wild stocks. Broodstock conditioning is essential in the provision of larvae for culture. The present study was designed to investigate the effect of temperature and photoperiod on *O. edulis* conditioning during the autumn season.

Oysters were conditioned (12 tanks, 30 animals per tank) in a continuous flow-through system of filtered (10 μm) seawater at a flow rate of 1L per hour per oyster. Oysters were fed with a mixed diet of micro-algae equal to 6% dry weight algae/dry weight oyster per day and per oyster. The temperature regimes utilized were: 15ºC (**T15**) and temperature gradually raised from 14ºC to 18ºC in a month (**TG**). Three different photoperiod regimes were applied: 8 h daylight (**P8**), 16 h daylight (**P16**) and photoperiod gradually increased from 8 h daylight to 16 h daylight in a month (**PG**). Six experimental conditionings were assayed by combining the two different temperatures and the three photoperiods: T15-P8; T15-P16; T15-PG; TG-P8; TG-P16; TG-PG. About ten oysters per condition were sampled at approximately monthly intervals (initial 18/10/2005; S1 21/11/2005; S2 19/12/2005; S3 30/01/2006). Histology, image analysis (SigmaScan Pro 5) and stereology methods were employed to determine the percentage of the gonad volume occupied by germinal cells.

The examination of histological sections and the stereology results (Fig. 1) showed that higher occupation of the gonad by germinal cells was obtained at TG in relation to T15 and at PG in relation to P8. Partial spawnings between December and January were obtained principally in the tanks with the TG-PG conditions, but also in TG-P16 and T15-PG conditions. In conclusion the conditions of gradually increased temperature and photoperiod accelerated the sexual maturation of the oysters in relation to a temperature of 15ºC and a photoperiod of 8 h daylight.

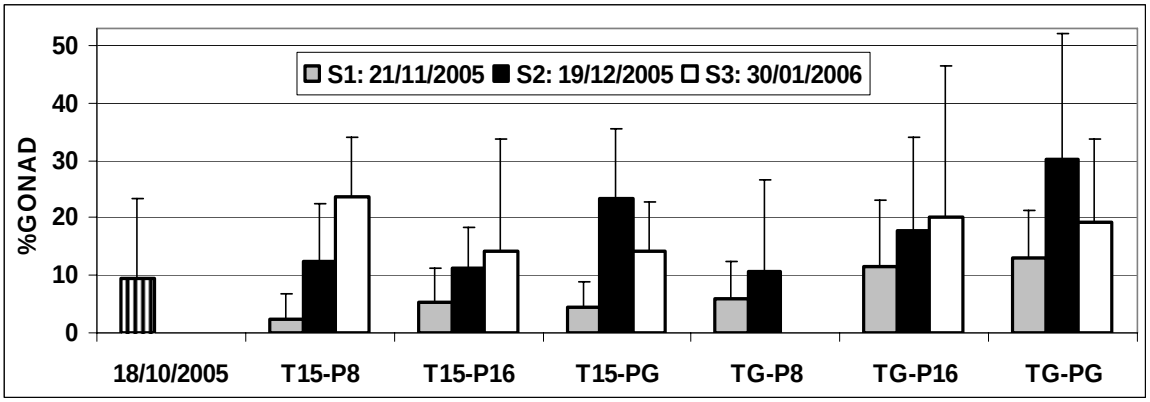

Figure 1. Evolution of the percentage of gonad volume occupied by germinal cells (mean+SD).

Development and implementation of a photobioreactor for continuous culture of microalgae in commercial hatcheries of shellfish

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Keywords: Photobioreactor, mollusc hatchery, Isochrysis affinis galbana, Crassostrea gigas

Over the last five years, French mollusc hatcheries have extended because of an increasing demand for *Crassostrea gigas* spat supply, mainly triploids. Despite various substitute diets tested, live microalgae are still essential food for the production of bivalve mollusc larvae. Providing live food, which represents up to 30% of total production costs, is regarded as a major constraint in mollusc hatcheries. Continuous culture could be an attractive alternative to batch production as it allows automation of the production process and provides stable quantity and quality of microalgal biomass.

As a first step a photobioreactor was developed in order to match the technical and economical limitations inherent in hatcheries. This work led to a closed tubular, artificially illuminated airlift photobioreactor based on a succession of elementary modules connected by flanges. Air injections ensured culture circulation with a swirling motion due to special tangential inlets. Study focused on the influence of various parameters (*i.e.* gas flow rate, air sparger, velocity factor of the flange and column radius and height) on radiative transfer, hydrodynamic and biological productivity. Coupled with technical-economical aspects, these experimental approaches and modelling converged to a 120-L unit equipped with 30 mm radius and 1 m length columns.

Once developed the photobioreactor was implemented in a French mollusc hatchery in order to check its productivity in an industrial environment, and continuous cultures of *Isochrysis affinis galbana* were run.

Development of peripheral tools was then after undertaken to achieve full automation of the process. In order to prevent external contamination of long lasting cultures an "in-line nutrient mixer" was designed to allow either automated initial sterilisation of the photobioreactor and mixing of nutrients with sea-water according to dilution rate. Finally larval feeding is automatically controlled by means of a loop based on a special light-attenuation sensor developed in our laboratory. Thus continuous cultures of microalgae should be coupled in term to open flow through larval rearings. The completion of the project will ensure automation of the whole microalgae-larvae production process and should contribute to lowering hatchery production costs.

Evolution and formation of *Nautilus macromphalus* nacre: biochemistry and proteomic of the shell organic matrix

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Keywords: Biomineralization, organic matrix, evolution, glycosylation, mass spectrometry

In mollusks, the shell formation is a genetically controlled process handled by the calcifying mantle cells. One of the most studied shell texture is the nacre, also called mother-of-pearl, the lustrous aragonitic layer that constitutes the internal part of the shell of several bivalves, a few gastropods and one cephalopod, the nautilus. Like other shell texture, nacre contains a minor organic fraction, which displays a wide range of functions in relation with the biomineralization process. Here, we have characterized biochemically the nacre matrix of the cephalopod *Nautilus macromphalus*.

The acido-soluble matrix contains a mixture of polydisperse and discrete proteins, glycoproteins and proteoglycans, which inhibits the *in vitro* precipitation of $CaCO_3$, interacts with the formation of calcite crystals, and binds Ca^{2+} ions in solution. Although the saccharidic moieties are not involved in binding calcium, they participate to the modulation of the shape of calcite crystals grown *in vitro*. On 2-DE, the different components of the nacre matrix migrate either at very acidic or at very basic p*I*. In addition, we have used a 'shellomic' approach (proteomics applied to the shell matrix) on the acetic soluble and acetic insoluble matrices, as well as on spots obtained after 2-D electrophoresis. Our data demonstrate that the so-called insoluble and soluble matrices contain numerous shared peptides. Furthermore, while most of the obtained partial sequences do not fit with known molluscan shell proteins, few of them partly match with shell proteins of bivalvian origin.. These findings have implications in the knowledge of the macro-evolution of molluscan shell matrices.

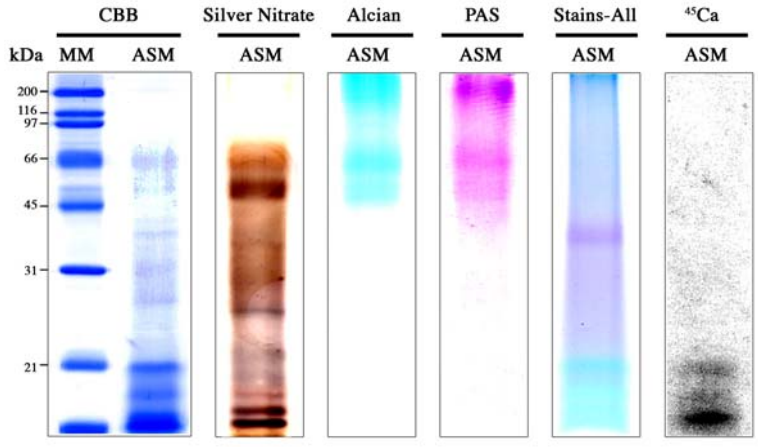

Figure 1: SDS-PAGE analysis of the ASM of *Nautilus macromphalus* nacre. 12% acrylamide gels were stained with CBB, silver nitrate, Alcian blue, PAS, Stains-All, from left to right. The last right lane corresponds to the result of the calcium overlay test (^{45}Ca). MM, molecular mass markers.

Effect of self-fertilization elimination on physiological responses of juveniles of the simultaneous hermaphrodite scallop *Argopecten purpuratus*

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Argopecten purpuratus, a scallop of commercial importance is a functional hermaphrodite. The usual methods of breeding cause an unavoidable degree of self-fertilization with the subsequent deleterious effects on survival and growth rates. Applying knowledge of the cellular mechanisms of spawning in this species we developed a method to eliminate self-fertilization in this species. By this *in vitro*-fertilization method, oocytes obtained by scraping the female portion of the gonad, were induced to reinitiate meiosis by serotonin, and then they were fertilized with sperm from different individuals. The progeny obtained by this method was compared with others obtained by cross and self-fertilization. The larvae obtained by any of the fertilization methods were cultured in hatchery with periodical sampling for survival and growth rates. After settling and metamorphosing they were transferred to the sea. Every 60 days, juveniles were counted and measured. When juveniles attained about 5 cm in length (eight months at sea), physiological rates were measured and scopes for growth were calculated. Metabolic rates (M) were obtained from oxygen consumption, ingestion rates (I) were obtained as the product of clearance rate and the organic mass of food in suspension, excretion rates (E) were determined by the increase of ammonia in the water. Each rate was converted to the corresponding energy units. Absorption efficiencies (%) were determined using the ratio method of Conover. The absorbed energy (A) was calculated from ingestion rate and absorption efficiency. From these values, the scopes for growth for juveniles coming from the different groups of larvae were calculated using the following equation:

$$SFG = A - (M + E)$$

The survival of early juveniles coming from larvae obtained by *in vitro* fertilization was higher than those from individuals coming from larvae obtained by cross or self-fertilization. After 8 months of culturing in the sea, those juveniles coming from larvae obtained by *in vitro* fertilization procedure presented the highest sizes and those coming from self-fertilization resulted in the smallest sizes. No differences were detected either in metabolic, ingestion or excretion rates between the juveniles coming from the different groups of larvae. However, absorption efficiency showed to be worse for those juveniles coming from self-fertilization treatment. This lower absorption efficiency determined that the scope for growth for these individuals was the smallest one.

Our results show that *in vitro* fertilization is a method that let obtain a higher percentage of surviving juvenile scallops which will grow faster than individuals coming from cross fertilization, a method that can't avoid a degree of inbreeding. If we consider the higher mortality of juveniles coming from cross and self fertilization procedures, we may explain the lack of differences detected in some of the physiological processes. We suggest that those individuals with a higher degree of inbreeding died much before we made the physiological measurements.

Effect of geographic origin, season of the year and temperature on broodstock conditioning, spawning success and larval viability of *Ruditapes decussatus* (Linné, 1758)

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Keywords: clams, Ruditapes decussatus, geographic origin, season, temperature, broodstock conditioning

Culture of *Ruditapes decussatus* is clearly limited by the availability of seed once this production proceeds almost exclusively from natural recruitment. Artificial spawning and larval rearing programs could provide an alternative source of spat.

This study was designed to evaluate the effect of different conditioning temperatures on the broodstock maturation, spawning success and larval viability of two geographically (North and South of the Iberian Peninsula) distinct populations of the European clam (*R. decussatus*) in different seasons of the year in order to create an "optimal" artificial spawning and larval rearing programs.

Two batches of clams from each population were collected in winter and autumn, and conditioned at 18±1, 20±1 and 22±1ºC. Of the three variables analysed the season of the year was the most determining factor on gametogenic development, spawning and larval rearing. Geographic origin and conditioning temperature also greatly affected the spawning. The results also showed that the winter conditioning was more effective than the autumn one and the best conditioning temperatures was 20±1ºC and 22±1ºC for North and South populations, respectively.

Theses results suggest that the efficiently conditioning temperature for each population of the same species is related with the seasonal temperature regime from the geographic origin. Larval viability and growth performance seemed to be independent on the broodstock conditioning.

Ovarian gene expressions during vitellogenesis in the Pacific oyster, *Crassostrea gigas*

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Keywords: Crassostrea gigas, vitellogenin, follicle cells, estrogen receptor

Vitellogenins are the precursors of the major yolk protein (vitellin) s in oviparous vertebrates and invertebrates. In invertebrates, many studies on cDNAs encoding vitellogenin are reported in crustaceans. In molluscs, a full-length cDNA encoding vitellogenin was cloned from the Pacific oyster *Crassostrea gigas*, and its amino acid sequence was deduced. The deduced primary structure of vitellogenin in *C. gigas* was shown to be similar to vitellogenins of fish, crustacean and nematode species, especially in the N-terminal region.

The levels of vitellogenin mRNA in various tissues from female oyster and stage-specific expression were measured by reverse transcription-mediated PCR. Vitellogenin mRNA expression was detected only in the ovary, and indicated maximum level in March (the early stage of maturation). To determine the distribution of oyster vitellogenin mRNA expression in ovary, we performed *in situ* hybridization using DIG-labeled RNA probes. A strong signal was detected in the follicle cells. It is concluded that the follicle cells are the site of vitellogenin synthesis.

The synthesis, secretion and processing of vitellogenins differ among phyla. Vitellogenins are synthesized by extraovarian tissues such as the liver in vertebrates and the fat body in insects, secreted into the circulatory system, and transported into the ovary. In teleosts, as in other oviparous vertebrates, it is clearly established that the vitellogenin gene expression is regulated by estradiol-17ß (E_2) via estrogen receptor (ER). In the Pacific oyster, E_2 is detected in the ovary, and its content shows a synchronous profile with gonadal maturity.

To investigate the estrogen signaling in the vitellogenesis, a cDNA encoding the Pacific oyster, *Crassostrea gigas*, estrogen receptor (cgER) was cloned. Comparisons of the amino acid sequence of cgER with other mollusc ERs show high similarities of the C domain (95-97%), and the E domain (56-66%). The phylogenetic analysis indicated that the cgER is an ortholog of the other mollusc ERs. Reporter gene assay revealed that cgER is unresponsive to estrogen. This result is similar to those of other mollusc ERs.

We examined the localization of cgER in the oyster ovary at the vitellogenic stage using anti cgER peptide antiserum. The immunohistochemical study indicated that cgER was mainly localized in the nuclei of follicle cells, the site of vitellogenin synthesis, in the oyster ovary. This result suggests that cgER could work as a nuclear receptor. Our results will facilitate further research to understand the vitellogenesis in the oyster.

Molecular cloning of a cDNA fragment encoding putative vitellogenin from the scallop *Pecten maximus* L.

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Keywords: Pecten maximus, scallop, vitellogenin, oogenesis

The vitellogenin is a precursor of a lipid-binding product named as vitellin, which is involved in lipid and metal storage. Vitellin is accumulated in oocytes of most oviparous animals. Vitellogenins are present in almost all species of oviparous animals from nematodes to vertebates, including marine bivalves. Results of the homology and motif searches argue for emergence of vitellogenin before the cnidiarian-bilaterian divergence.

Total RNA was extracted from *P. maximus* ovary. Several clones containing a partial cDNA sequence of vitellogenin were obtained by reverse transcription-PCR (RT-PCR) and cloning. The primer pairs used were ACTGGTGTATTGCTGTCTGC (forward) and ACTGGTGTATTGCTGTCTGC (reverse). The *P. maximus* vitellogenin partial sequence (EMBL accession number AM943022) contained 1290 nucleotides coding to an ORF of 430 amino acids, corresponding to positions 16 to 442 in *Patinopecten yessoensis* vitellogenin.

A homology search in the UNIPROT database (WU-blastp, matrix Blosum62) showed considerable similarity with marine bivalve homologues: 80 % identity and 90 % positives with *Patinopecten yessoensis* vitellogenin; 37% identity and 58% positives with *Crassostrea gigas* vitellogenin; 42% identity and 65% positives with *Mytilus edulis* vitellogenin. About 20-25% identity was found with N-terminal regions of other vertebrate and invertebrate vitellogenins. A search against specific databases containing protein conserved domains and functional sites by means of InterProScan (EMBL-EBI) and Conserved Domains Search (NCBI) programs indicates that the protein contains a conserved region (pfam01347) found in several lipid transport proteins including the amino terminal region of vitellogenin, microsomal triglyceride transfer protein and apolipoprotein B-100.

Molecular characterization of vitellogenin is important to study its synthesis and transfer to oocytes during vitellogenesis. The site synthesizing vitellogenin differs among phyla. *In* situ hybridization experiments showed that in bivalves the synthesis takes place in the ovary, in the follicle cells (*C. gigas*; Matsumoto *et al*., 2003) or in the auxiliary cells (*P. yessoensis*, Osada *et al*, 2004). Auxiliary and follicle cells are in contact with growing oocytes and they are suspected of playing a role in oocyte nutrition. Furthermore, vitellogenin levels are being used as a biomarker of endocrine disruption in fish and one such application could be envisaged in marine molluscs.

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Influence of sex and gametogenesis stage on Heat shock Proteins and Metallothionein levels in the Pacific oyster *Crassostrea gigas*

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Keywords: Crassostrea gigas, reproduction, Heat Shock Proteins, metallothioneins, sex, gonad, maturation stage

The Pacific oyster *Crassostrea gigas* is the most commercially-important marine mollusk and has been successfully introduced into many parts of the world as an aquaculture species. Sex determinism of C. gigas is predominantly under genetic rather than environmental factors. A dominant male allele in a single locus model determines male (M/M or M/F) or protandric females (F/F). Females are capable of changing sex after a first year being male. However, once gametogenesis has begun, oysters are unable to change sex until after spawning and the gonad is undifferentiated. The gonad of oysters is not permanent and constitutes a network of tubules interspersed in connective tissue and surrounding the digestive gland. Different germ cell developmental stages can occur simultaneously in the gonad: undifferentiated cells, growing germ cells, and mature gametes provided by multiple cohorts of germ cells, each with synchronous development. Nevertheless, a main developmental stage can be defined at some periods of the year. Reproductive development is temperature dependent in *C. gigas* and this intertidal species routinely encounters large fluctuations in temperature.

One physiological adaptation to living in a stressful habitat like the intertidal area is the synthesis of stress proteins, as Metallothioneins and Heat-Shock Proteins (HSPs). Metallothioneins play a role in the metabolism of the relatively non-toxic essential metals (zinc and copper) as well as in the detoxification of toxic metals such as cadmium. Molecular chaperones, HSPs, act to rescue damaged proteins and prevent them from aggregating. Among HSP families, the most studied proteins are members of the HSP70 family.

We demonstrate that the baseline levels of HSPs stayed almost similar in gills, mantle and digestive gland between oysters at low and high tidal levels. On the contrary, endogenous HSPs and MTs levels in gonads changed strongly during gametogenesis. In female gonads, the constitutive form of HSP70 and the MTs increased between rest and ripe stages and decreased after spawning. In male gonads, HSP70 and MTs levels increased between rest and growing stages and decreased immediately at the ripe stage. Females presented higher concentrations of HSP70 and MTs than males during the spawning period. No significant difference was found among oysters sampled at low and high tidal levels. We hypothesize that the high levels of stress proteins in eggs may increase survival of oyster progeny.

Hox genes in the black scallop *Chlamys varia* (L.)

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Keywords: homeobox genes, caudal, Gbx, Lox5, Posterior Hox genes

Homeobox genes (Hox genes) encode a family of transcription factors that have essential roles in the regulation of development and differentiation in eukaryotes. Although they have been extensively studied in different phyla, relatively little is known about homeobox-containing genes and their function in molluscs. These transcription factors present the homeodomain, a highly conserved DNA-binding motif of 60 amino acids and encoded by a short 180 bp DNA fragment, the homeobox. The conservation of this motif and its proliferation in metazoans emphasizes the importance of the role of homeobox genes in development and differentiation. Hox genes are clustered in most genomes that have been analyzed: in non-vertebrates there is one Hox cluster whereas in mammals there are four Hox clusters.

In previous studies we analyzed the homeobox-containing genes of the bivalve mollusc *Pecten maximus* (L.) and we demonstrated the presence of *proboscipedia* and *caudal* gene homologues. We also analyzed the homeobox-containing genes of the bivalve mollusc *Mytilus galloprovincialis* (Lmk.) and have demonstrated the presence of *Hox* genes belonging to the anterior, PG3, and central classes. Recently we have identified two *cdx* and a *Gbx* gene fragments in the oyster, *Ostrea edulis* (L.) and a *lab* gene from the clamp, *Venerupis pullastra* (Montagu).

We have designed a pair of degenerate oligonucleotide primers that correspond to conserved homeodomain sequences. Genomic DNA was isolated from adult black scallops. DNA was amplified using 0.5µM of each primer and Hot Master Taq DNA Polymerase. PCR was performed at 95ºC (5 min) and then at 95ºC (1 min), 40ºC (1 min), 72ºC (30 s) for 39 cycles, and finally 10 min at 72ºC. Positive PCR products were reamplified and later cloned in the pGem-T Easy Vector System II (Promega). DNA of individual clones, obtained using GenElute Plasmid Miniprep Kit (Sigma), was double strand sequenced by using ABI Prism dRhodamine Terminator Cycle Sequencing kit.

In this study we have identified the first homeobox genes in a bivalve mollusc, the black scallop *Chlamys varia* (L.). Following PCR amplification of *Chlamys varia* DNA, we were able to recover two *Gbx* gene fragments from the non-Hox/ParaHox class; the derived amino acid sequence was designated Cvox Gbx (23 aa) and Cvox Gbx1 (72 aa) from *Chlamys varia* homeobox. We also isolated and analyzed two ParaHox genes, *caudal* gene homologues. The derived amino acid sequences (23 aa) were designated Cvox Cad and Cvox Cad1. In this study we also report the isolation of a Lox5 gene fragment (Cvox Lox5), ftz gene orthologous. Finally, we have identified two posterior Hox genes (Cvox Post-1 and Cvox Post-2).

Comparison of the Cvox homeodomain with those of several homeodomain proteins reveals a high level of conservation. Molecular alignments and phylogenetic analysis indicate that these scallop genes are homologues of the *caudal* ParaHox, GBX-class, Lox5 and Posterior Hox genes.

A comparative study of the feeding behaviour of *Crassostrea virginica* living in bottom and surface waters

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Keywords: Crassostrea virginica, alimentation, depth variations, digestive enzymes activity, bivalve larvae

Modelling the carrying capacity of an oyster farming site necessitates a thorough understanding of bivalve physiology. Whether the physiological data collected from oysters living on the bottom can be extrapolated to suspension-cultured oysters and vice-versa is questioned. The aim of this research was to compare the feeding behaviour of American oysters (*Crassostrea virginica*) living in bottom and surface waters in a shallow bay exploited by oyster farmers, in New-Brunswick, Canada. Three sampling stations were set up, one in 2006 and two in 2007. Water depth was 3.5 m at high tide at each station. Three floating and three non-floating Vexar® bags containing 250 oysters (~ 50 mm shell length) were anchored at each sampling station. The activity of α-amylase, cellulase, and laminarinase was measured in the digestive gland of bottom and surface oysters during spring, summer and fall, as an indicator of algae and vegetal detritical matter consumption. The number of bivalve larvae in the oyster's stomachs was used as an indicator of micro-zooplankton consumption. The dry mass of soft tissues per mL of internal shell volume was used to describe the physiological condition of the animals. Water temperature as well as the concentration of chlorophyll *a* and seston were similar in bottom and surface waters. In addition, no significant difference in condition index and cellulase activity was detected between the oysters held at the bottom and at the surface. However, the activity of α-amylase and laminarinase was higher in bottom than in surface oysters (two-way ANOVA, $F = 8.63$ and 9.66 respectively, $P < 0.002$). Mean activity of α-amylase and laminarinase was 2.69 ± 1.68 and 1.44 ± 0.75 μmol/ g wet tissue/ min in surface oysters and 3.62 ± 1.68 and 1.86 ± 0.94 μmol/ g wet tissue/ min in bottom oysters. The number of bivalve larvae in the stomach (mainly *Mya arenaria* and *Ensis directus)* was highly variable and no significant difference could be detected between surface and bottom oysters. The mean content was 3.64 ± 6.7 and 1.4 ± 2.2 larvae/ stomach in surface and bottom oysters respectively. A significant effect of season was also noted on the activity of cellulase and α-amylase ($F = 21.58$ and 6.89 respectively, $P < 0.001$). Our results suggest that even in shallow waters, feeding behaviour may differ between oysters living at different depths. Therefore, physiological data obtained from bottom oysters may not be applicable to surface oysters.

Aspects of reproduction and biochemical metabolism in *Chamelea gallina* from the North Adriatic Sea

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Keywords: Chamelea gallina, North Adriatic Sea, Gametogenetic cycle, Biochemical composition

The Venus clam *Chamelea gallina* is an infaunal filter-feeder, widely distributed throughout the Adriatic Sea, where it has great economic importance. In the present study, some aspects of the biological cycle of *C. gallina* have been investigated by evaluating the time-course of gonad maturation and variation in gross biochemical composition and energy values. Specimens with homogeneous size (length range 25-35 mm) were collected monthly from January to December 2001 for biochemical measurements (protein, lipid, total carbohydrate and glycogen contents and energy values) and from November 2001 to November 2002 for the study of the gametogenetic cycle, performed by microscopic observations of histological sections of gonadic tissue.

Protein content varied narrowly and irregularly, whereas lipids remarkably increased from April to June 2001. Total carbohydrates and glycogen exhibited a similar erratic trend, and various relative maxima and minima were recorded. Total energy content, evaluated by converting carbohydrate, lipid and protein values into calorie contents, was especially stable all through the year, ranging from the maximum of 13.2 kJ/g in September to the minimum of 10.7 kJ/g in December. Histological observations indicated that gametogenesis started in September and kept on throughout winter until late spring. Ripe gonads were first observed in February, and the highest percentage of ripe individuals was detected in May, when also spawned clams were found. Spawning appeared to persist during warm months up to July, and the maximum number of spawned clams was recorded in June. The gonadic index was pretty suitable as a proxy for the reproductive status of the natural population: in particular, it showed an increasing trend from January, peaked in May, decreased sharply to the minimum value of August, (when most animals resulted sexually inactive from the histological observations) and started rising from September-October, highlighting the gradual onset of gametogenesis (as observed in the histological sections).

An inverse relationship was established between the gonadic index and glycogen content, particularly between the lowest levels of glycogen and the time-span of sexual maturity and gamete emission. The good correlation between gametogenesis and depletion of energetic reserves have been already documented in various bivalve species *(Mytilus edulis, M. galloprovincialis, Ruditapes philippinarum, R. decussatus*). However, our results did not evidence the conversion from glycogen to lipids, already observed in the clam *R. philippinarum* as well as in other bivalve species, as only a slight increase of lipid content was detected during the period of gamete maturation. As a consequence, glycogen is indicated as the most important component in the metabolic energy storage for *C. gallina*.

Identification and expression of *Cg-DMl*, a factor of the DM family, in the pacific oyster *Crassostrea gigas*

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Keywords: Crassostrea gigas, Dmrt1, Dmrt4, Dmrt5, sex determination

The pacific oyster *Crassostrea gigas* is a successive not systematic protandric hermaphrodite. To be able to predict and therefore to control its gonad differentiation (with different environmental conditions), we need to know when (during the development and the gametogenetic cycle) and by which factor does its sex determinism occur. In order to answer this question we first looked for a molecular factor of the DM family, ortholog to Dmrt1 (a conserved sex determinism factor in Invertebrate and Vertebrate). In this study a molecular factor of the DM family called *Cg-DMl* (*Crassostrea gigas* DM like) has been identified for the first time in Molluscs. It is 359 aa long, with three conserved domains (two common to all the family factors and one specific to members such as Dmrt4 and Dmrt5) and its gene presents one intron of 598 bp. *Cg-DMl* mRNA expression, investigated by qPCR during the development and along a complete adult gametogenetic cycle, was reported ubiquitous in different adult tissues and significantly higher during the development after the metamorphosis, compared to the adults. This *Cg-DMl* sequence, which might not be the factor of sex determinism in *C. gigas*, was also used to look for (i) different potential splicing forms of the gene between males and females as in *Drosophila*, (ii) another potential member of this family whose expression would better agree with a role in sex determinism. Another potential sex determinism factor, not belonging to the DM family, was also investigated in parallel and has been first characterized. Our results suggest that *Cg-DMl* may control a broad range of biological processes and may contribute to a better understanding of the DM family member evolution.

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On-site nursery techniques for Manila clam (*Tapes philippinarum*) in Venice lagoon

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Keywords: Tapes philippinarum, condition index, spat culture, growth, air-survival test

The first attempts of introduction of Manila clam in Italy date back to early 80's, when a first stock of spat coming from a British hatchery was introduced in Venice Lagoon. Rapidly, this clam has proven to be perfectly adapted to this environment and, due to the favourable conditions found and to further introductions in the surrounding areas, it has been spread all over the Northern Adriatic lagoons, locally replacing in the shallow areas the native clam specie *T. decussatus.* Consequently, harvesting Manila clam in the wild has become a very remunerative activity in the area. Current productions include both fishing and farming activities but the importance of this latter is raising in relevance.
After a maximal production raised in 1999 (approx. 40 000t), a significant decrease in clam production for the Venice lagoon has been observed in the recent years (approx. 18 000t in 2002), as a consequence of different main concerns (overexploitation of the natural beds, illegal harvest, use of high-impacting fishing gear, deterioration of environmental conditions). To face this situation in 2000, local government and water authorities proposed to the clam growers unitary consortium (COVEALLA) a Master Fishing Plan for the Venice Lagoon; in 2006 similar guidelines were reprised in a plan redacted by GRAL, a consortium of public authorities locally involved on activities related with clam farming and fishing.

Among the different directives promoted by both plans and aimed to give sustainability to clam farming procedures, particular relevance has been given to the rational management of natural spat. Clams culture in Italy relies almost exclusively upon the collection of natural spat and its management in controlled areas; for those reasons hatchery and nursery activities for the clam culture are still poorly developed locally. Local farmers are used to harvest large-size spat (> 10mm) in order to avoid important losses due to predation during the growing phases, whilst a large amount of small or medium-size spat undergoes to high predation rate or is damaged during the harvest of seed of bigger size by the use of high-impacting fishing gears.

The aim of this work is the identification of the most suitable systems for the clam spat culture to be used in Venice lagoon. In the first part of the research the potential of five different techniques used elsewhere from similar purposes (FLUPSY, lanterns, plastic bags, nets, poches) have been assessed, taking into account the peculiar feature of the Venice lagoon and using the large datasets available for this area (morphology, hydrology, sediments, chemical and physical features, phytoplankton, pollutants). In this way the most suitable areas for each technique have been identified and plotted by means of GIS oriented maps.
Afterwards, the first set of experiments (the research is still on going) has evaluated the effectiveness of "poche" technique; a similar culture system (suspended bags) has been tested using the same wild spat, in two different areas (sites A and B) and with different culture densities (500g, 1000g, 1500g per poche). Different biometrical and gravimetrical measurements [length (L), height (H), thickness (T), wet weight (WW), dry weight (DW), shell weight (SW)] have been taken in order to evaluate growth, whilst condition indexes (DW/SW, DW/LHT, WW/SW, WW/LHT) and air-survival tests were used to check the physiological status of the reared animals.
During the first three months of rearing the factor "area" affected growth in a much more significant way than the factor "density", indicating that local conditions play a major role also on a local scale (the distance between the two sites is 4 km). The condition index analysis confirmed this pattern, whilst air survival tests outlined that clam kept at lower density were affected by lower stress.

High frequency variability of clam, *Ruditapes philippinarum,* fluids and shell composition in the Auray River

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Keywords: proxy, calibration, Ruditapes philippinarum, estuary, salinity

Calcareous organisms such as corals, foraminifera and bivalves record information about environmental conditions (i.e. temperature, salinity, primary production...) into their skeleton during their growth. Chemical compositions of their skeleton are thus useful for paleoclimatic studies. As an example, carbonate $\delta^{18}O$ has been widely used as a proxy for temperature reconstruction.

However, as element incorporation is widely influenced by animal metabolism, calibration between recorded messages in biogenic carbonates and encountered environmental parameters is often difficult. These biological influences are generally called "vital effect". A mechanistic understanding of the transfer processes and the incorporation of elements from seawater into biogenic carbonate is necessary to improve the use of most proxies.

For this purpose, bivalves are ideal model-organisms: their high growth rates and large size allow high spatial and temporal resolution approaches. The present study focuses on the euryhalin Manila clam, *Ruditapes philippinarum,* which lives buried a few centimeters into sandy and muddy sediments of intertidal and subtidal areas. Bivalve biomineralization takes place in extra-pallial fluids located into the space delimited by the shell and the mantle. In order to better understand how elements are transferred to the different compartments of the clam (i.e. haemolymph, tissues, extra-pallial fluids and shell carbonate), the elemental composition of these compartments and of the surrounding water were simultaneously monitored in a tidal environment submitted to cyclic changes.

A monitoring study was performed at a high temporal resolution in the Auray River estuary (Gulf of Morbihan, France). Seawater and Manila clams were sampled in the subtidal zone by scuba diving every two hours during a 48 hours experiment. At this location, tidally driven variations of environmental parameters (i.e. salinity, turbidity, dissolved oxygen, $ä^{18}O_{water}$, $ä^{13}C_{DIC}$ ...) were observed. Salinity varied between 20 (low tide) and 32 (high tide). The first results indicate that the osmolarity in seawater equals that in the haemolymph and extra-pallial fluids, except at low tide when osmolarity is higher in the fluids. These results show that *Ruditapes philippinarum* is an osmoconformer. However, we can hypothesize that at low tide, when salinity decreases beneath a threshold, our clams could close their valves to protect themselves from low salinity. Trace elements analyses of the shells by electronic microprobe and of the fluids by ICP-MS are also investigated. Variations in the commonly used strontium and magnesium proxies in the different compartments allow us to test if, at high temporal resolution, their incorporation is regulated by the organism or only by the environment.

Temporal variability in the Pacific Oyster spatfall along the French Coast: a new monitoring project.

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Keywords: Crassostrea.gigas, spatfall, recruitment, reproduction, larvae

In France, spatfall of Pacific Oysters occurs traditionally in two shellfish area of the south west of France: the Marennes-Oléron bay and the Arcachon basin. The spat collected in both sites ensures approx. 70 % of the needs of French oyster production (this other part is produced in hatcheries). But, over the last ten years, recruitment has become highly variable especially in Arcachon basin. For instance, in this site, spatfall (assessed at the end of the reproductive season) can vary between 50 oysters per collector to more than 20 000 oysters per collector (Figure 1). This huge variation has prompted questions from oysters producers and the French National Committee for Shellfish Culture who want explanations for the phenomenon.

It is well known that recruitment of bivalves shows large variability in time and space, depending directly on environmental factors such as temperature, phytoplankton or salinity or more indirectly on climatic conditions. For Pacific oysters, previous regional studies are available in Marennes oléron and Arcachon bays and showed clearly that the reproduction of this species is highly dependent on temperature, phytoplankton (quality, quantity) especially in spring for gametogenesis and in summer for spawning and larvae survival. But these studies were only regional and this idea is now to approach the phenomenon on a larger scale. In the other side, there is now evidence of the ecological impacts of recent climate change in many environments and for many species, and more precisely for recruitment of some European bivalves (e.g. *Macoma balthica*, Philippart et al., 2003) and a similar hypothesis can also be formulated for the Pacific oyster in France, since the reproduction ability of this species seems to "move" to the north of France. New sites are now investigated for spat collecting by oyster producers (e.g. Rade de Brest).

In that context, a national monitoring project is currently starting at Ifremer in 2008. More precisely, the major aim of this new project, named Velyger, is to monitor (1) gametogenesis, (2) larval abundance and (3) spatfall intensity for Pacific Oysters in several shellfish area in France (Arcachon, Marennes Oleron, Bourgneuf, Quiberon and Rade de Brest) and in relation with environmental and climatic factors. We expect from this project to give deeper information on the reproduction of this specie through a supra-regional approach and to confirm previous conclusion drawn in Arcachon or Marennes Oléron. Another aim of that project is to re-analyse the long historical time series of larval abundance (more than 30 years available at Arcachon and Marennes Oleron) in relation with environmental and climatic factors in order to test if the climatic changes that occur now are partly responsible of this new phenomenon. This project will also give 'on line' data to oysters producers to help them to anticipate as better as possible the variability of the reproductive cycle of the year.
The aim of the presentation is to give an overview and the first results of this project.

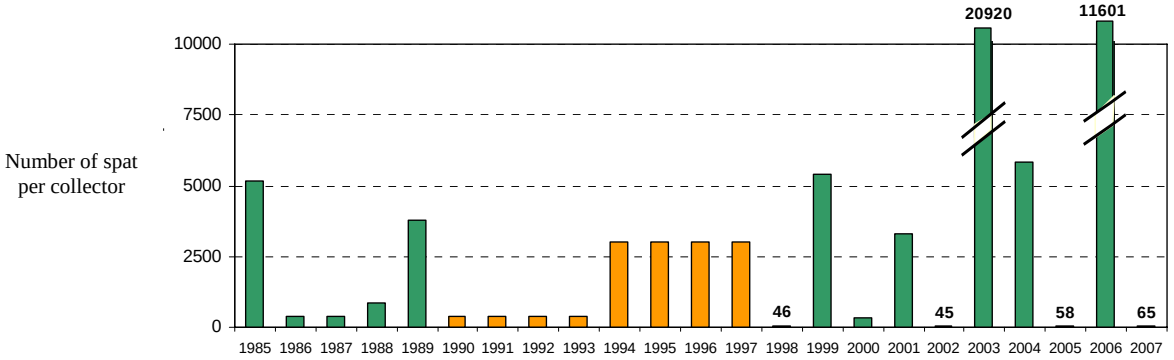

Figure 1 : Interannual variablity of spatfall for Pacific Oyster in the Arcachon Basin fron 1985 to 2007 (sources : Ifremer LER/AR). From 1990 to 1997, data were assessed only through investigation to oysters producers.

Biometry of *Octopus vulgaris*

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Keywords: Octopus vulgaris, biometry, morphometry, relative growth

The common octopus *Octopus vulgaris* (Cuvier, 1797) is a cephalopod of great commercial interest in Spain, and in Andalusia it represents a high percentage of the total mollusc captures (Rodríguez-Rúa *et al.*, 2005). Octopus's fishery has been over exploited in Spain for the last decades, which has prompted administration to take fishery regulation measurements, and to search for alternatives such as aquaculture. Although paralarvae high mortality rates make culture unfeasible to date, the common octopus is a good candidate for aquaculture due to some of its biological characteristics such as direct embryologic development, short lifetime cycle, and rapid growth. Within this framework, the knowledge of biological characteristics such as biometry and morphometry becomes of primary importance.

A total of 675 individuals of *O. vulgaris* (355 males and 320 females) were captured from the Western coasts of Andalusia (Southern Spain) by means of trawling and octopus pots. Individuals were anaesthetized on ice and the following morphometric characteristics were measured. W: Wet Weight (g), TL: Total Length (cm), MDL: Mantle Dorsal Length (cm), MVL: Mantle Ventral Length (cm), and MW: Mantle Width (cm). Sex was determined microscopically. Sexual development was established, by means of histological techniques, and four gametogenic stages were identified: I Immature, II Maturing, III Mature, IV Spawning.

The common octopus relative growth was fitted to the allometric equation $Y = a X^b$, where Y is weight in grams, a is a constant, b is the allometric coefficient, and X any chosen length in cm. The results show different values of the allometric coefficient for each of the linear magnitudes measured (Figure 1).

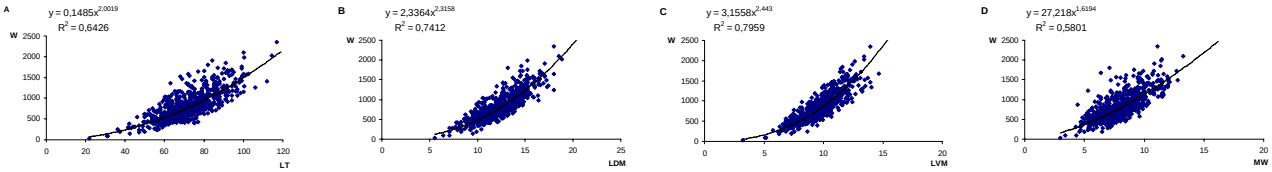

Figure 1. Relative growth in *O. vulgaris*. Length-Weight regressions for different length measurements: Total Length (A), Mantle Dorsal Length (B), Mantle Ventral Length (C), and Mantle Width (C).

Despite data dispersion, all b values recorded are quite different form 3, which implies allometric growth in every case. Guerra (1980) reported different b values for W *vs* MDL (b=2.917) and W *vs* MW (b=1.0788) regressions in the Mediterranean (NE Spain). The best regression curve fitted here was W *vs* MVL (R^2= 0.7959), so we used W-MVL relationship to study differences between sexes, sexual development and annual seasons.

We tested statistical significant differences in the W-MVL relationship with ANCOVA test (α=0.05). We found differences between sexes (F=9.021; p=0.003). We did not find differences in sexual development among females (F=2.061; p=0.105), but there were differences between autumn and the other seasons: autumn-winter (F=24.178; p≤0.0001); autumn-spring (F=7.511; p=0.007); autumn-summer (F=14.451; p≤0.0001). In males, we found differences between stage II and stages III (F=17.673; p≤0.0001) and IV (F= 21.496; p≤0.0001); and between autumn-spring (F=9.192; p=0.003) and autumn-summer (F=10.639; p=0.001). Autumn is the post-spawning season, were most immature (females) or maturing (males) specimens concentrate (Rodríguez-Rúa *et al.*, 2005). The W-MVL relationship in this season differs from the rest, which points out that, in maturing males and immature females, physiological condition of specimens modify *O. vulgaris*. relative growth of these two morphometric variables.

Sterol composition during the reproductive cycle of the lions-paw scallop (*Nodipecten subnodosus)*

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Keywords: Pectinidae, reproduction, lipids

The lions-paw scallop *Nodipecten subnodosus* is in great demand in the seafood marketplace because of its large adductor muscle and has been exploited through fisheries for decades in the Baja California Peninsula. Gonad development and spawning occurs mainly during summer and the relative reliance on tissue reserves or recently ingested food for maturation depends on the availability of food. This report provides new information based on analyses of tissue sterols in a natural population at a site with low productivity. Samples of seston and five scallops were collected during four seasons of the annual reproductive cycle: August, November, February, and May. Levels of sterols in seston, muscle, mantle, digestive gland, and female and male gonad portions were analyzed. Histological analysis and determination of the gonadosomatic index confirms previous studies in the reproductive cycle: ripeness in August, spawned in November, resting in February, and developing in May. In all tissues, cholesterol was the principal sterol, followed by brassicasterol. However, in seston samples, dihydrocholesterol and campesterol were more abundant than brassicasterol in February and May, although their levels were always lower than cholesterol. In contrast, the proportion of dehydrocholesterol and 24-methylenecholesterol to total sterols were higher in some tissues than in seston. These differences in the proportions of some sterols between lions-paw scallop tissues and available food confirm the selective retention of specific sterols or even possible bioconversion between different sterol classes. A general pattern of accumulation of practically all sterols was observed in female gonads, and to a lesser extent, in male gonads, in relation to gametogenesis. In contrast, levels of all sterols in the digestive gland were lower in August and November compared to February and May, suggesting a possible transfer of sterols from this organ to gonads during gonadal development, as previously observed for total lipids in the same species at this site. Only small variations of cholesterol and brassicasterol in somatic tissues, such as mantle and adductor muscle, were observed throughout the reproductive cycle.

The challenge of optimising larval stocking density across a range of culture environments

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Keywords: Perna canaliculus larvae, stocking density, stress, water quality

Inevitably, the culture of shellfish larvae requires them to be held in unnaturally crowded conditions. Elevated population densities may contribute to potentially deleterious factors in the culture environment, including reduced water quality, competition for resources, pathogen transmission and physical disturbance (e.g. collision). Optimum culture densities are usually determined by a compromise between the above considerations and the need to produce commercially viable quantities. However, before such optima can be identified, an understanding of the interactions between stocking density and exogenous environmental factors is required. Here we use performance criteria (survival, growth, ingestion and appearance) to determine the interactions of varying water exchange rates or physiological challenge with Greenshell™ mussel (*Perna canaliculus*) larval stocking density. Larvae were reared in 2.5L polycarbonate bullet-shaped tanks supplied with 1µm-filtered 18ºC seawater containing microalgae (*Chaetoceros calcitrans* + *Isochrysis galbana*, 40 cells.µL^{-1}). Veligers were stocked to tanks 10 days post-hatching at either 200 or 400 larvae.mL^{-1}. Water exchange treatments were designed to create minute-volume densities of 20 larvae.mL.min^{-1} (20 mL.min^{-1} @ 400 larvae.mL^{-1} or 10 mL.min^{-1} @ 200 larvae.mL^{-1}), 10 larvae.mL.min^{-1} (40 mL.min^{-1} @ 400 larvae.mL^{-1} or 20 mL.min^{-1} @ 200 larvae.mL^{-1}), 5 larvae.mL.min^{-1} (40 mL.min^{-1} @ 200 larvae.mL^{-1}) or 2.5 larvae.mL.min^{-1} (80 mL.min^{-1} @ 200 larvae.mL^{-1}). Six replicates of each treatment were monitored until the majority of larvae had reached pediveliger stage, 21 days after hatching. No biomass effect was measured on culture water quality (Total ammonia-N <0.02 mg.L^{-1}; dissolved oxygen >98%, pH 8.1 ±0.1), any effects of varying water exchange rates were therefore more likely to be mechanical or due to maintenance of the food environment. Performance data revealed a complex interaction between density and water flow. Survival was inversely correlated to water exchange, led by the 200 larvae.mL^{-1} @10 mL.min^{-1} treatment (60±6% survival between days 10 and 21), followed by the two 20 mL.min^{-1} exchange treatments (42-44% survival) and the 40 or 80 mL.min^{-1} treatments (23-26%). Although ingestion and lipid body accumulation followed a similar pattern, net shell growth was strongly influenced by resident larval density; 200 larvae.mL^{-1} treatments supported faster growth, notably at the 20 mL.min^{-1} exchange, which produced the highest overall pediveliger yield.

A hypothesis that the degree of stress experienced by young veligers was influenced by population density was tested using a similar design. Two-day old veligers were reared at 200, 400 or 800 larvae.mL^{-1} receiving 40 or 80mL.min^{-1} water exchange. Treatments were exposed to either chemical (water sourced from a eutrophic pond, pH 8.6) or physical perturbation (fine particulate matter sloughed from pipe surfaces after mechanical cleaning) and survival assessed after 6 days. Chemical challenge caused mortality in direct proportion to stocking density, with highest survival at 200 larvae.mL^{-1} (45-49%), followed by 400 larvae.mL^{-1} (30-36%) and 800 larvae.mL^{-1} (26-27%). Water exchange rate exerted no significant influence ($p>0.05$). The effects of particulate challenge were less severe; treatments were therefore monitored to pediveliger stage. Particles acted as nuclei for diatom chains which attached to shells and obstructed the velum; they were largely retained within the tank, resulting in heavier accumulations and greater impacts in the faster water exchange treatments. Higher density larval populations appeared to 'self-clean', binding particulates in mucus or dense pseudofaeces, thus reducing impact. Optimum culture density is therefore dependent upon the tank environment and the acceptable degree of risk exposure; these potentially conflicting requirements will be further discussed in conjunction with considerations of food washout.

Oxygen consumption estimation of mussel culture in Alfacs Bay

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Keywords: Mytilus galloprovincialis, respiration, Mediterranean estuaries, shellfish culture

Mussel culture on the Spanish Mediterranean coast is carried out in the two Ebro Delta bays, Fangar and Alfacs, with an annual production of 3000 t y^{-1}. Both areas are characterized by their high environmental variability in terms of temperature, salinity and oxygen concentration.. *Mytilus galloprovincialis* culture cycle begins in September-October, when farmers prepare the growth ropes using the seed previously collected in January. At that time, the seed has an average size of 3-4 cm. Main commercial harvest is during summer, when the mussel reaches 6-7 cm length. Growth and mortality of mussels in both areas are strongly affected by the high seawater temperatures of July and August, which can cause important mortality episodes.

The role of low dissolved oxygen in the water on the mortality events is nowadays under discussion, and no previous studies on oxygen consumption have been made. Therefore, the goal of the present study is to estimate oxygen consumption of mussels along an annual culture cycle. In accordance, animal size and temperature were variable along the experiments in order to find out a more realistic approach of mussels physiological response throughout the cycle.

Mussels were collected in the field and transferred to a laboratory tank, where the seawater temperature was adjusted to the environmental temperature. Water temperature ranged from 9 to 29º C throughout the experiments. Mussels were place in individual closed chambers between 1 or 2 days after mussel collection for oxygen consumption measurements. These measurements were recorded in continuous using an 8 channel respirometer connected to oxygen microoptodes. The duration of the experiments lasted from 12 to 24 hours, depending on the respirometer chamber volume and animal size.

Oxygen consumption showed a strong weight dependence, which was described as a power function: $Y = 0.3525\ X^{-1.0241}$; $R^2 = 0.7064$, where Y is the weight specific oxygen consumption rate (µmol O2 g^{-1} dry wt h^{-1}) and X is tissue dry weight. Mussel oxygen consumption increased with increasing temperatures up to 20 ºC, when the metabolism appears to reach a plateau, followed by a decline until 29 ºC. At this elevated temperature, some animals died before oxygen was fully uptake from the respirometer chamber. These results will be use to estimate oxygen uptake by the cultured mussels in Alfacs Bay.

Prostaglandin levels in oysters during acute stress and spawning

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Keywords: Crassostrea gigas, Crassostrea corteziensis, reproduction, endocrine control

To obtain a successful reproduction in laboratory of any marine organism it is necessary to know all the factors involved in the gonadic maturation and spawning. It is known that in some molluscs, gonadic development is directly affected by temperature and feeding, which also affect the synthesis and secretion of hormones that control reproduction, such as prostaglandins (PG). PG can have different functions in relation to the species, the physiological state of the organism, and their concentration. In vertebrates, they are known to modulate reproduction and the immune system. In theory, it is possible to increase ovogenesis, ovulation, and spawning in fish and molluscs when they are injected with PG. However, the administration of PG in pharmacologic doses may promote maturation, but it can also increase the susceptibility to stress conditions and pathogens, and in the long term, mortality. We undertook 1) to analyze how stress can affect PG levels in oysters in a temporal manner and 2) to determine the expected levels of PG during spawning.

For the first objective, we used *Crassostrea corteziensis* oysters, to which a sampling stress was applied, that consisted in taking out the whole organism from its shell and making a transversal cut with a bisturi approx. 1 cm from the antero-posterior axe. Oysters were sampled 3, 5, 10, 30, and 60 minutes after the initial stress, and the levels of total proteins, lipids, carbohydrates were analyzed in gonad, digestive gland and mantle, and PGE_2 was analyzed in gonad. We found that levels of PGE_2 were affected by the sampling stress, with significantly higher levels 30 min after applying the stress.

To determine the PGE2 levels during spawning, we used mature *C. gigas* that were induced to spawn. Oysters were sampled 1 hr. before, during and 18 hrs. after they had spawned. A sample of gonad was analyzed by histology and in the rest, the PGE_2 levels were analyzed. We found that PGE_2 levels were highest during the spawning activity, but decrease after that being still higher than levels found 1 hr. before spawn, in not stimulated oysters.

The Ifremer Station of Argenton, an experimental tool dedicated for molluscs study from larvae to adult, from hatchery to the surroundings

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Keywords: bivalve molluscs, hatchery, conditioning, larvae, postlarvae, phytoplancton

The Ifremer Mollusc Experimental Station is located 25 kms North of Brest (Brittany). Its location takes advantage of its relative isolation, far from the city. Land farming wastes are limited, the main nearby river mouth, Aber Ildut, being situated 3 miles far away. Moreover waste waters are highly diluted in the famous “Four” Channel, particularly well known for its seawater mixture. Research carried out in Argenton since 20 years has shown the interest of this site for a hatchery location. Thus seawater quality remains constant, and its low turbidity presents undoubtedly a great advantage for filtration efficiency equipment. Studies on mollusc feeding requirements are accordingly easy to set up and lead to non biased results. Moreover it is closed to the Brest Ifremer Centre, the European Sea Marine Institute and the University of Western Brittany. This new experimental facility has been effective since 2002 and grew progressively so as to be currently operated with a permanent staff of 9 persons, 4 researchers, 1 engineer and 4 technicians. The buildings cover 800 m^2 with half this surface being allocated to animal experimentation. The station of Argenton is integrated to Ifremer support device to French shellfish farming and is involved in the control of hatchery juveniles supply (project OJUVE) as well as the sustainability of natural spatfall production (project VELYGER). Both projects rely on the knowledge of the biology of the reproduction of molluscs, larval development and metamorphosis. Complementary ecophysiological approaches allow the integration of environmental parameters on mollusc growth at different stages of life, from larva to adult. The objectives of VELYGER which started in 2008, are detailed in a poster in the present symposium and we accordingly focus here on the main results achieved in the last 4 years through OJUVE: development of non invasive methods (RMI and NMR) for oyster reproduction study, development of new approaches for the qualification of gametes, development of continuous microalgae cultures in dedicated tubular photobioreactor, development of flow through larval rearing techniques in large and small volumes for ecophysiological studies and genetic selection, as well as prophylaxis method improvement.

Reproductive cycle of *Spisula solida* (Linnaeus, 1767) (Mollusc: Bivalve) in the littoral of Huelva (SW Spain)

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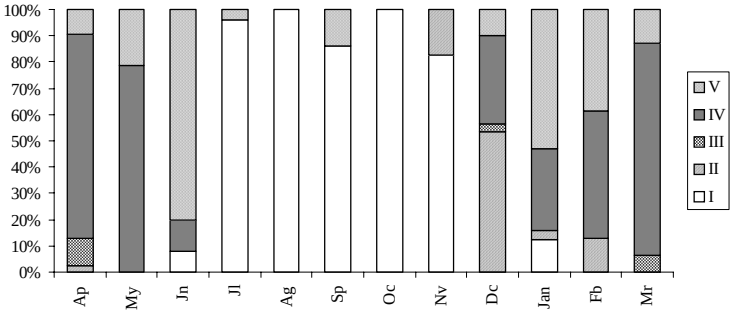
Keywords: *Spisula solida, gametogenic cycle, histology, spawning, sexual maturity*

Spisula solida has a wide geographical distribution. It has been reported from South Iceland and Norway to the Atlantic coast of the Iberian Peninsula, Morocco and Madeira, but is not found in the Mediterranean. This species is of commercial importance in Spain, where it is the target of a specialized artisanal fishery. Studies of the reproductive cycle of *S. solida* in Andalusia (South of Spain) have not been carried out to date. The aim of this paper is to establish the gametogenic cycle and sexual maturity size of a population of *S. solida* in Punta Umbria (Huelva, SW Spain). This will provide a detailed and reliable patttern of the reproductive process.

A total of 4601 specimens of shell length ranging from 9.1 to 38.4 mm were captured between April 2001 and May 2002. The gonad development was determined by means of histological methods and variations in condition index. For histological study, individuals were fixed in Gendre fluid (Paraformaldehyde, Picric Acid, 70% Ethanol and Acetic Acid) and transferred into 70% Ethanol for preservation. Clearing and paraffin embedding were performed using standard procedures. Sections were cut 5 µm thick and stained with Hematoxylin/eosin and Hematoxylin/VOF. Five gametogenic stages were identified in males and females (I immature, II maturing, III mature, IV spawning, V post-spawning). The index used was: FDW (mg)/L^3 (mm^3), where FDW: flesh dry weight, L: length. FDW/L^{-3} was calculated using monthly mean values of the different parameters.

Although *S. solida* is a gonochoric species, two hermaphrodite individuals were found. The sex-ratio obtained was 3:2, males being more abundant than females ($p>0.5$). Development of the gonad began in November, with a 17% of the individuals in maturing stage and finished in July, with the spawning period taking place from December to June. The highest percentage of spawning individuals was detected in March (81%). Resting took place from July to October, with maximum peaks in August and October (100% of specimens in immature stage) (Figure 1). Gametogenesis is related to decreasing temperature, and spawning occurs with low temperatures values that increases progressively. Size at first sexual maturity was 12 mm of shell length. Monthly variation of the index FDW/L^3 did not match satisfactorily with the gametogenic cycle characterized, so it was not proved valid as a reliable method for gametogenic cycle studying in this population of *S. solida*.

Figure 1. Relative frequency of gonad development stages in *S. solida.*



Immunolocalization of cholesterol side chain cleavage enzyme (P450scc) in *Mytilus galloprovincialis*

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Keywords: Mytilus galloprovincialis, P450scc, steroidogenic pathways, immunolocalization

The biosynthesis of steroid hormones has been demonstrated in gonadal tissues, as well as some non gonadal tissues such as the cortical adrenal gland and the kidney. All steroid hormones are synthesized from a common precursor, cholesterol. In vertebrates, the initial limitant step in all steroidogenic pathways is the cleavage of the cholesterol side-chain to form pregnenolone, which occurs in the inner mitochondrial membrane. This reaction is catalyzed by cholesterol side-chain cleavage cytochrome P450 enzyme (P450scc), which is the expression of the CYP11A1 gene. In bivalves, in spite of their unability or very low ability to synthesize cholesterol, has been refered the presence and biosynthesis of steroid hormones, as well as the involvement of the P450 cytochrome system. However, few studies have focused on the localization of steroidogenic pathways in these organisms.

We have examined the localization tissular, cellular and subcellular of P450scc in mussel *Mytilus galloprovincialis* by immunohistochemical and immunochemical analysis. We have found no evidence of this enzyme in any gills, kidney and gonadal cell throughout year. Converselly, specific immunoreactivity was only present in apical cytoplasm of the basophilic cells of the digestive gland. SDS-gel electrophoresis and western blot analysis of different subcellular fractions from digestive gland reveal this enzyme as one single band of protein with a molecular weight of 51.4 KDa. Moreover, cytochrome P450scc seems mainly located in microsomes, where its proportion, relative to μg of proteín, was 6 times higher than in mitochondria fraction.

Histologically, the digestive gland of molluscs is formed by branched blind tubules termed digestive diverticula. The epithelium of these diverticula is formed by at least two types of cells: digestive and basophilic cells, which have a role in the absorption-digestion of nutrients and in the enzyme production-secretion, respectively. In mammals, cholesterol is internalised by endocytosis processes where the formation of coated pits are involved. In *Mytilus galloprovincialis* has been recently documented the presence of coated pits in the apical membrane of basophilic cells showing evidence of endocytotic processes. Moreover, a well developed endoplasmic reticulum and Golgi complex as well as scarce mitochondrias have been ultrastructurally described in the apical region of these cells. Therefore, in contrast to mammals, where P450scc carries out its function mainly in the inner mitochondrial membrane, the subcellular distribution of this enzyme in *Mytilus galloprovincialis* appears to coincide with the ultrastructural characteristics of the basophilic cells described by other authors.

In summary, our immunochemical results suggest that in mussels part of the cholesterol ingested in the diet could be endocyted and subsequently converted into pregnenolone by P450scc enzyme, mainly in the endoplasmic reticulum of the basophilic cells of the digestive gland. Therefore, these should represent the first cell type where the steroidogenic pathways begins.

Energy storage and allocation during reproduction of Pacific winged pearl oyster *Pteria sterna* at Bahía de La Paz, Baja California Sur, Mexico

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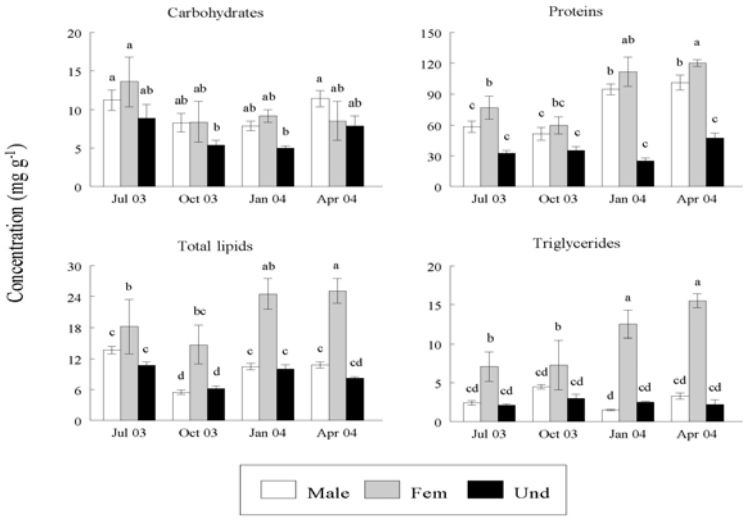
Keywords: Pteria sterna, reproductive physiology, biochemical composition, bioenergetics

Seasonal variations in storage, partitioning, and allocation of energy reserves (proteins, carbohydrates, lipids, and triglycerides) between the gonad and somatic tissues (gonad digestive gland, mantle tissue, adductor muscle) related to reproduction of Pacific winged pearl oyster *Pteria sterna* were investigated. Tissue samples were collected every three months and analyzed with histological and biochemical techniques. Energy coefficients were also calculated with data from chemical composition of tissues. The main reproductive season occurred from January through April, when water temperature reached annual minimums of 21-22 °C and there was a higher frequency of ripe gonads, more and larger postvitellogenic oocytes, and higher protein, lipid, and triglyceride levels in gonad tissue. During the study period, there were two spawning peaks detected: October 2003 and January 2004. However, the evidence indicates that gonad samples in all developmental stages occurred throughout the year. This result, together with data from previous studies, places *P. sterna* as a multi-spawning species. Gametogenesis was sustained from energy mainly obtained from the digestive gland (~35%) and secondly from the adductor muscle (~28%). Both tissues contributed two-thirds of the total energy needed to meet the demands of the entire reproductive cycle. In energy units, ~17 kJ/g were provided by the digestive gland and ~13 kJ g/g by the adductor muscle. Only proteins from these two tissues were mobilized to the gonad for maturation of sex organs, since carbohydrates were rather stored despite the progress of gametogenesis. The role of mantle tissue was negligible. Given the location of Bahía de La Paz in a transition ecotone between the cooler temperate and warmer tropical provinces, *P. sterna* may displays several periods of gonad growth as a strategy to regulate reproductive success. These periods are sustained, in minor proportion, from reserves stored in somatic tissues during summer when primary productivity is low (conservative strategy), and more intensely, from high food supply in winter and spring when stored reserves diminish (opportunistic strategy). This combined conservative vs. opportunistic strategy appears to be typical of species inhabiting temperate and subtropical areas.



Invasion of the Pearl Oyster *Pinctada radiata* (Leach 1814) in Maliakos Gulf, Central East Greece (Aegean Sea): First Reporting

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Keywords: pearl oyster, Pinctada radiata, invasion, Maliakos Gulf, Aegean Sea, Greece

The subtropical pearl oyster *Pinctada radiata* (Leach 1814), of Indo-Pacific origin, has a long record of appearance as a non-indigenous species (NIS) in the East Mediterranean. Since the first reporting in Suez at 1883 it seems that the species is well established in certain regions of the Levantine Sea (Egypt, Libya, Tunis, Israel, Cyprus) with significant occurrence in Sicily, Malta and islands nearby. In the Aegean Sea the first reporting of occurrence was in 1963 in Saronicos Bay where it was introduced for aquaculture purposes. Since then, there were reports of its occurrence in the SE Aegean (Karpathos, Rhodes), in the NE Aegean (Lesvos, Turkey's coast) and Central Aegean round the island of Euboea (1992-1994). Recently, reports for its occurrence in Lakonikos Bay (South Peloponese, 2002), Cyclades (Serifos, South Aegean, 2006) and Crete (facing Levantine, 2003) indicate that *P. radiata* has a migratory potential compliant with the Lessepsian migration pattern.

The present work documents for the first time the distribution of the pearl oyster in the environmentally protected area (Natura 2000) of Maliakos gulf in the Central East Greece (Aegean Sea). It has been found initially as bivalve empty shell at Agios Ioannis beach (Fig. 1) during 2005. From hydrological data this part of the bay receives first the inflow from the Aegean probably explaining the invasion by spat drifted in by the current. During the summer of 2007 spat of the animal has been collected as attached biofoulant on pergolari of a long-line mussel farm (*Mytilus galloprovincialis*) along the southern part of the Gulf at Molos area. The spat was covering the whole surface of the culture system at a depth defined by the length of the pergolari (3- 3.5 m) hanging about 1-1.5 m below the sea surface from the mother rope. Later in December, live animals could be found along the coast line of Molos. *Pinctada radiata* inhabits the beach by sticking on the gravel and the shore stones, competing for space with the declining native flat-oyster *Ostrea edulis*. It has also been found to attach on the small "oyster reefs" that are created by the conjunction of more than 3 empty dead native oyster shell aggregation. Mean biometric values and standard deviation, of animals collected during the low tide period were 50.09±5.49mm for width, 48.13±2.56mm for length, 16.12±1.70mm for height and 10.03±2.99g for wet weight.

The introduction of a NIS in an environmentally protected area where it competes with the native species and interferes with bivalve aquaculture practises raises concerns regarding the ecological balance and the proper management of the ecosystem and therefore has to be investigated further. At any rate, the species is more or less established in the bay now, and the authorities have to reconsider its NIS status for future fisheries and aquaculture guidelines.

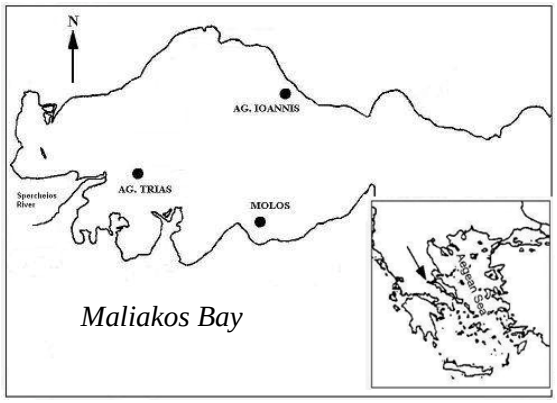

Figure 1: Outline of Maliakos semi-closed embayment in Central East Greece (Central West Aegean Sea). Bullets indicate sites of mussel farming.

Ionic mapping of *Ruditapes phillipinarum* shells affected by the Brown Ring Disease

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Keywords: Ruditapes phillipinarum, Vibrio tapetis, WDS mapping, trace elements

In 1987, mass mortalities of cultured manila clams were noticed in Landeda (Brittany) north Finistere, which was the first production site in France (500t in 1987). In 1989, Paillard and Maes showed that mortalities were associated with the presence of a brown deposit on the inner surface of the valves. This disease, named Brown Ring Disease (BRD) is caused by a bacterium *Vibrio tapetis*.
Vibrio tapetis colonizes the periostracum and inhibits the normal process of shell biomineralization. The clam's response to the bacterium attack consists in production and accumulation of a brown organic matrix on the inner face of the shell, which cause its death. But in some cases, clams recover by deposing an aragonitic white layer on the brown organic matrix.

We explore the use of trace elements to explain this phenomenon. Indeed, carbonate minerals accept a wide range of trace elements in their structure, which are used as tracers of environmental conditions like Sr, or tracers of organic matrix as S. For this purpose, we test Ionic imaging and quantitative analysis transects on 3 shells using a WDS microprobe. Mapping by WDS microprobe is currently used in geology to study location of trace elements in rocks minerals, but was not yet applied in biology because element concentrations are very low, near the detection limits.
We adapt the methodology to characterize the recovery of manila clams face to BRD using ionic mapping of C, Ca, Sr, S, K, Mg, Na, or Fe, on clams' slices. For each individual, a repaired zone (we also chose different stages of shell repair) and a safe zone of the same valve are compared.
Trace elements mapping seems to be a powerful tool to characterize the shell repair and growth after a BRD attack.

Effect of phytoplankton abundance and size structure on recruitment of mussel (*Mytilus edulis*) in Oosterschelde Estuary, The Netherlands

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Keywords: Mytilus edulis, larvae, spat, abundance, algal size classes, Oosterschelde

Shellfish culture in Dutch waters, especially mussel bottom culture in Oosterschelde, relies on natural recruitment of spat. However, there is limited evidence of natural recruitment in Oosterschelde. We hypothesise that recruitment of mussels may be affected by food limitation during the larval life stages. In this study, number of mussel larvae, and subsequent numbers of spat, and the abundance of phytoplankton size classes were determined. Biweekly time-series data of mussel larvae, settlement rate, and 5 algal size classes (<5, 5-10, 10-15, 15-20 and >20 µm), including algal biovolume from 4 different locations in Oosterschelde were analyzed to determine whether settlement rate can be predicted from phytoplankton availability. The study may lead to better understanding of recruitment success of these young animals in Oosterschelde. Although variation in settlement rate was found between years and locations, there was no apparent correlation with phytoplankton abundance and size structure. The reasons for recruitment success or failure of mussels in the Oosterschelde remain largely elusive.

Genetic variability of pearl oyster *Pinctada maxima* from wild and farmed population in Lombok Island - Indonesia: A Preliminary Study

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Keywords: allozyme electrophoresis, genetic variability, pearl oyster, Pinctada maxima

Pearl production is an essential economic activity in Eastern Indonesia, especially in Lombok Island. The production relies almost on the collection of wild brood stocks, which it makes dependent on natural resources. A good knowledge of brood stocks is essential for the management of wild and farmed population, especially for breeding and seed production. The aim of this study was to analyse the structure of the genetic variation of pearl oyster from the Sumbawa Island as a wild population and in Lombok Island as a farmed population by using an allozyme electrophoresis. The result will be a useful reference for the comparison of the genetic variation of the wild and farmed population.

Samples were taken from the wild and farmed population. The result of the investigation from the wild population showed, from the 9 enzymes analyzed, 15 loci were detected and 5 polymorphic loci have been identified and labelled as Mdh*1, Mdh*2, Pep*1, Pep*2, and one loci Gpi *1 (with the polymorphism loci proportion equal to 0.333) and the ratio of heterozygosity (Ho/He) was 0.064. While from the farmed populatio, four (4) polymorphic loci were identified as Mdh*1, Mdh*2, Pep*1 and Pep*2.

Table 1. Genetic variability in Pearl oyster populations from Lombok Island - Indonesia based on electrophoresis analysis of 15 loci

Parameter	Population		Reduction
	Wild	Farmed	
Number of sample examined	30	32	-
Number of loci examined	15	15	-
Number of polymorphic loci	5	4	0.222
Proportion of polymorphic loci	0.333	0.266	0.201
Number of allele per locus	1.600	1.533	0.041
Heterozigosity	0.064	0.038	0.406

The reduction of 40% genetic variation may be caused by bottleneck effect, due the ratio of brood stock too low and number of individual brood stock which spawn were different.

The gametogenic cycle of Venerupis rhomboides *(Pennant, 1777) in the Andalusian coast (S Spain)*

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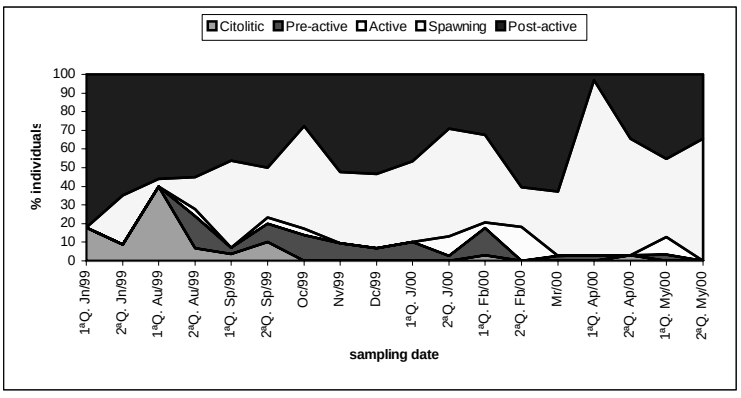
Keywords: Venerupis rhomboides, gametogenic cycle, ripening, spawning, histology

Venerupis rhomboides (Pennant, 1777) is a bivalve mollusk of commercial interest which is exploited all along the Spanish coast. In Andalusia, it is exploited by the shellfish fleet, and there are culture experiences already running. The main objective of this study is the determination of the gametogenic cycle of *V. rhomboides*, in the Andalusian coast, by means of histological techniques, in order to contribute to the knowledge of this species which may set some biological basic for its aquaculture developments. There are not many references available regarding the histology of this species. The studies published are focused on the following subjects: its genetic identification, its mortality due to processes of contamination, its fertility (studied by means of stereologicals methods), and its culture.

A total of 548 specimens were captured in the coast of Malaga (N 36º28' W 4º44') at 15 m depth, during a year (from June 1999 to May 2000). The individuals were histologicaly processed, embedded in paraffin and they were cut to 7 µm. The sections were stained with Hematoxilin of Carazzi / Eosin and Hematoxilin of Carazzi / V. O. F. (Light Green, Orange G and Acid Fucsine) (Gutiérrez, 1967). The different stages of gonad development were scored in accordance with the scale proposed by De Villiers (1975) for *Donax serra* (Röding, 1798).

According to the histological results we concluded that spawning ocurred between the second two-weeks of August and the second two-weeks of May, both inclusive, with a maximum peak in the first two-weeks of April (94% of the population in spawning) (Fig.1). In summer and winter the percentages of spawning individuals decreased, being lower than in spring. Therefore, in summer months seemed to be a period of relative sexual rest, with the highest number of individuals whose gonad had undergone regression in the first two-weeks of August. The results obtained were mostly similar to those observed by others authors, with a small disphase in the gametogenic cycle that may be due to latitudinal and thermal differences.

Fig.1. Proportion of the states of gonad development of *V. rhomboids.*



Spatio-temporal variation of the reproductive effort of female Manila clam *Ruditapes philippinarum* in Korean waters measured using enzyme-linked immunosorbent assay (ELISA)

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Keywords: Ruditapes philippinarum, reproductive effort, ELISA, GSI

Manila clam *Ruditapes philippinarum* is one of the most important natural resources and commonly occurring along the western and southern coast of Korean peninsula. To manage the clam populations in natural habits as well as in commercial clam beds, understanding reproductive biology of clam is crucial. In an attempt to measure reproductive effort of the female clam, *R philippiarum* egg-specific polyclonal antibody has been developed and applied in quantification of the clam eggs in Korea. The present study reports reproductive effort of the clam measured in spring and fall 2007. Forty clams were collected from 24 different locations during late April and May and November. A thin cross-section was made in the middle of the body for histology and the remaining tissues were lyophilized for the immunoassay. Reproductive condition and sex was determined from histology and accordingly female clams exhibiting eggs in the slide were selected for the measurement of the reproductive effort. Twenty mg of the homogenized tissues containing eggs were sonicated in PBS and diluted up to 1,000 fold. To assess the reproductive effort, an indirect sandwich enzyme-linked immunosorbent assay (ELISA) was used as the rabbit anti-oyster egg specific antibody as a primary antibody and the goat anti-rabbit IgG alkaline phosphatase-conjugated as a secondary antibody. After adding ρNPP as a substrate, optical density of the final antibody-antigen complex was then measured at 405 nm. Quantity of the egg in an unknown sample was then estimated from a titration curve constructed from reference materials included in the plate. The reference materials were prepared by adding known quantity of the clam eggs into known quantity of egg-free somatic tissue powder. The reference materials were then homogenized and serially diluted up to 1,000 folds. The reproductive effort was then expressed as a weight-based gonad somatic index (GSI), a ratio of eggs to the total tissue weight. Histology indicated that clams collected during April and May were in early developing to ripe, while most clams collected in November were in spent or sexually indifferent stage. The weight-based GSI ranged <1 to 38.6 during spring and <1 to 17.5 in fall. A positive correlation was found between the size of clam and the reproductive effort.

Transcriptomic response of *Argopecten purpuratus* post-larvae to copper exposure under experimental conditions

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Keywords: Argopecten purpuratus, copper, supression subtractive hybridization libraries, gene expression.

Few studies have described the molecular response of larvae in mollusc species to heavy metal exposure. In this study, we investigated the response of the *Argopecten purpuratus* post-larva to copper under experimental conditions and focused on the analysis of the differential expression patterns of specific genes associated with response to copper. A suppression subtractive hybridization method was used to identify specific copper-regulated genes in post-larvae after 4 days and 8 days of exposure to 2.5 and 10 µg x L^{-1} Cu^{+2}. This method revealed 175 different sequences corresponding to 9 major physiological functions. The expression of 12 potentially regulated genes was analyzed by real-time PCR in post-larvae at different sampling times over the time course of copper stress.
This study contributes to the characterization of potential genetic markers that could be used in future environmental monitoring, and could lead to explore new mechanisms of stress tolerance in marine mollusc species especially in first stages of development.
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