



The mining of pearl formation genes in pearl oyster *Pinctada fucata* by cDNA suppression subtractive hybridization

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Source: Marine Biotechnology (article in press, 2011)

Recent researches revealed the regional preference of biomineralization gene transcription in the pearl oyster *Pinctada fucata*: it transcribed mainly the genes responsible for nacre secretion in mantle pallial, whereas the ones regulating calcite shells expressed in mantle edge. This study took use of this character and constructed the forward and reverse suppression subtractive hybridization (SSH) cDNA libraries. A total of 669 cDNA clones were sequenced and 360 expressed sequence tags (ESTs) greater than 100 bp were generated. Functional annotation associated 95 ESTs with specific functions, and 79 among them were identified from *P. fucata* at the first time. In the forward SSH cDNA library, it recognized mass amount of nacre protein genes, biomineralization genes dominantly expressed in the mantle pallial, calcium-ion-binding genes, and other biomineralization-related genes important for pearl formation. Real-time PCR showed that all the examined genes were distributed in oyster mantle tissues with a consistence to the SSH design. The detection of their RNA transcripts in pearl sac confirmed that the identified genes were certainly involved in pearl formation. Therefore, the data from this work will initiate a new round of pearl formation gene study and shed new insights into molluscan biomineralization.

Growth and gonad development of the tropical black-lip pearl oyster, *Pinctada margaritifera* (L.), in the Gambier archipelago (French Polynesia)

Le Moullac G., Tiapari J., Teissier H., Martinez E., Cochard J.-C.

Source: Aquaculture International (article in press, 2011)

The growth and reproductive cycle of cultured black-lipped pearl oysters, *Pinctada margaritifera* (L.), were studied in the Gambier Islands (134°52' W, 23°07' S) from September 2002 to August 2003. Temperatures were recorded throughout the year, revealing seasonal temperature variations between 22.3 and 27.8°C. The mean annual chlorophyll a value, as computed from satellite data, was $0.188 \pm 0.075 \mu\text{g L}^{-1}$. To study growth and reproduction, 720 two-year-old individuals were ear hung on long-lines suspended at a depth of 7 m. Samples were taken twice a month to obtain the following measurements: shell height; wet weight of flesh and total oyster; dry weight of adductor muscle, mantle and visceral mass; and glycogen content. Gonad development was also studied by histology on parallel samples. Growth was relatively fast during the first 6 months of the study: average shell height increased from 89.1 ± 9.1 to 119.7 ± 10.8 mm and total weight from 93.4 ± 24.5 to 155.1 ± 33.6 g, between September and the end of March. Subsequently, from April to August, no significant growth was observed for shell and flesh, while the muscle weight decreased significantly. Condition index (CI), defined as the ratio of wet weight of the visceral mass to shell weight, and histological changes in the gonad revealed 3 significant reproductive events of different intensities. The analysis of correlations revealed a specific effect of the chlorophyll a concentration on the growth of shell and soma, and one of the temperature on tissue glycogen content. This study also showed also that CI could be an efficient indicator of reproductive events in pearl oyster. It thus appears that the development of gonads goes on throughout the year in the Gambier Islands, without any detectable phase of sexual rest.

In vitro effects of noradrenaline on Akoya pearl oyster (*Pinctada imbricata*) haemocytes

Kuchel R.P., Raftos D.A.

Source: Fish and Shellfish Immunology (article in press, 2011)

Exposure to fluctuating environmental conditions in bivalve molluscs can lead to physiological stress and up-regulated production of stress-associated hormones, such as noradrenaline (NA). Since environmental stressors have been found to have an immunosuppressive effect on *Pinctada imbricata*, we investigated the in vitro effects of NA exposure on their defensive haemocytes, focussing specifically on markers of apoptosis. Terminal dUTP nick-end (TUNEL) labelling was used to detect cells displaying DNA fragmentation within tissue exposed to NA. DNA fragmentation was most significant when haemocytes were exposed to 10.0 ng NA/ μ g protein relative to non-treated controls. Similarly, Annexin V-FITC staining, a marker of early apoptotic events, was evident in cells exposed to 5.0 and 10.0 ng NA/ μ g protein after 120 min ($p < 0.05$), and haemocyte adhesion to glass slides declined significantly when cells were exposed to 10.0 ng NA/ μ g protein ($p < 0.05$). A number of morphological and ultrastructural changes in NA-exposed haemocytes were also identified using transmission and scanning electron microscopy. These alterations included chromatin and cytoplasmic condensation, the formation of apoptotic bodies, vacuolisation and blebbing. In NA-treated cells, polymerisation of F-actin was observed around the periphery of the cytoplasm. All of these data suggest that NA induces apoptosis in *P. imbricata* haemocytes.

Diagnostic genetic markers unravel the interplay between host and donor oyster contribution in cultured pearl formation

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Source: Aquaculture 316: 20–24 (2011)

To produce a cultured pearl, a mantle allograft originating from a donor oyster is surgically implanted along with a shell bead nucleus into the gonad of a recipient oyster from the same species (termed the host oyster). Whilst, studies have shown that genomic DNA from a mantle allograft remains present in the pearl sac at the time of pearl harvest, what remains unclear is whether biomineralisation genes from the donor mantle allograft are transcriptionally active and contribute to pearl formation. To help resolve the interplay between host and donor genetic contribution in pearl formation, xenografts were produced, using two *Pinctada* species, *P. maxima* and *P. margaritifera*, to examine which species-specific nacreous genes (N66 and N44) were expressed in the pearl sac. Diagnostic DNA tests revealed that donor oyster cells not only remained present in the pearl sac at the time of pearl harvest, but were found for the first time to be transcriptionally active in the expression of biomineralisation genes, N44 and N66. These results confirm that the donor oyster is an important contributor to the biomineralisation process in pearl culture. Understanding the role the donor and host oyster have in cultured pearl formation provides a solid foundation for elucidating the biological process in general, but it also provides valuable information that can be directly utilised for selective breeding programs in the cultured pearl industry.

Improvement of the production of high-quality pearls by keeping post-operative pearl oysters *Pinctada fucata* in low-salinity seawater

Atsumi T., Ishikawa T., Inoue N., Ishibashi R., Aoki H., Nishikawa H., Kamiya N., Komaru A.

Source: Nippon Suisan Gakkaishi (Japanese Edition), 77(1): 68–74 (2011)

We established a procedure that increased the production of high-quality pearls by keeping post-operative pearl oysters *Pinctada fucata* in low-salinity seawater. Oysters were implanted with a mantle graft and nucleus, and immersed in low (25 psu) or normal salinity (33 psu) seawater in tanks, or suspended from a raft in the sea for 14 days. Then, they were cultured in the sea by the ordinary procedure for several months until pearls were harvested. The harvested pearls were classified as nacreous, prismatic, organic or nucleus. Furthermore, nacreous pearls were classified as high or low quality, based on the proportion of blemishes. From the experiment, oysters immersed in 25 psu seawater showed the highest ratio of high-quality pearls, followed by those in 33 psu seawater. Another practical experiment with ten pearl farmers also showed that the ratio of high-quality pearls was significantly higher in 25 psu seawater than in the sea. These results indicated that the low-salinity treatment of oysters during the post-operative period could be an effective technique to increase the formation of high-quality pearls.

Genetic variation of hatchery and wild stocks of the pearl oyster *Pinctada fucata martensii* (Dunker, 1872), assessed by mitochondrial DNA analysis

Gwak W.S., Nakayama K.

Source: Aquaculture International 19(3):585–591 (2011)

In order to provide baseline information for the genetic resources, genetic variation in wild and cultured *Pinctada fucata martensii* from southern Korea and Japan was studied using nucleotide sequence analysis of 379 base pairs (bp) in the mitochondrial cytochrome oxidase subunit I gene (COI). The study included three hatchery stocks from Korea (Tongyeong) and Japan (Mie and Tsushima) and one wild stock from Korea (Geoje). A total of 3 haplotypes were identified in hatchery stocks of 78 individuals, of which 63 individuals shared 1 haplotype. Overall, nucleotide diversity (π) was low, ranging from 0.000 to 0.002, and haplotype diversity (h) ranged from 0.000 to 0.541. Considerably low haplotype and nucleotide diversities in hatchery stock indicated that low effective population size and consecutive selective breeding of *P. fucata martensii* could be responsible for the reduction in genetic variation. The wild stock exhibited low haplotype diversity (0.507 ± 0.039) with two shared haplotypes. The results of the present study with first record of wild pearl oyster in Korea support the possibility that the transplanted pearl oyster for overwintering experiments could have survived in winter. In order to enhance and/or maintain genetic diversity in the hatchery stock, further research should be directed toward genetic monitoring and evaluation of the hatchery and wild pearl oysters.

Proximate and fatty acid composition of the gonads of wild versus hatchery-conditioned *Pinctada margaritifera* broodstock

Ehteshami F, Christianus A., Rameshi H., Harmin S.A., Saad C.R.

Source: Aquaculture Nutrition 17(3): 675–682 (2011)

The composition of protein, carbohydrate, lipid and fatty acids of the gonad of wild female broodstock of black-lip pearl oyster, *Pinctada margaritifera*, was compared with oysters fed on a ternary combination of microalgae in hatchery. Artificial feeding was found to be as good as natural feeding in terms of number and size of released eggs. Lipid, protein and carbohydrate reserves of unfed oysters were found to be insufficient to complete oogenesis. The proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of the neutral and polar lipids extracted from female gonads were not influenced by variations in the fatty acid composition of the natural food and ternary combination of microalgae in hatchery. T-Iso, *Chaetoceros calcitrans* and *Chaetoceros muelleri* were able to provide sufficient 22:6n-3 (DHA) and 20:5n-3 (EPA), two of the most important essential fatty acids required for gametogenesis. The n-3/n-6 and 22:5n-3/20:4n-3 ratios were consistently higher in the neutral lipids than in the polar lipids. Conversely, the ratio of 20:4n-3/20:5n-3, 22:6n-3/20:5n-3 and PUFA/SFA was higher in the polar lipids.

Application of a bioenergetic growth model to larvae of the pearl oyster *Pinctada margaritifera* L.

Thomas Y., Garen P., Pouvreau S.

Source: Journal of Sea Research (article in press, 2011)

We applied, for the first time, a dynamic energy budget (DEB) growth model to the larval phase in the pearl oyster *Pinctada margaritifera* var. *cumingii* (Linnaeus, 1758) to evaluate the impact of spatio-temporal variation in the atoll lagoon environment on its capacity for development. The specific parameters of the model, which represent ingestion, temperature effect and the relationship between length and biovolume of the larvae, were determined from experiments or taken from the literature. The interpretation of the values of these parameters allowed us to identify the underlying adaptive character trait: *P. margaritifera* larvae have a good capacity to exploit low food concentrations and a narrow range of thermal tolerance restricted to hydrobiological conditions found in the tropical oligotrophic waters of its distribution zone. Growth simulations show a good fit with the observations made on reared larvae under different conditions: fed on either cultured algae or natural plankton, and with growth data from a natural cohort. Finally, a first application of the model to a pearl-culture lagoon reveals the predominant effect of the vertical structure of trophic resources in determining spatial variation in larval growth.

Integrating optical system designed for multimodal analysis of pearls and its mother oyster to distinguish and appraise cultured pearls

Ju M.J., Lee S.J., Kim Y., Shin J.G., Kim D.H., Kim H.Y., Lee D.S., Lee B.H.

Source: Progress in Biomedical Optics and Imaging — Proceedings of SPIE, 7892, art. no. 78920T (2011)

An optical integrating system composed of optical coherence tomography (OCT) and fluorescence spectroscopy (FS) has been designed and utilized to distinguish pearls by determining their mother oysters used in pearl culturing as well as discriminate and evaluate the pearls, nondestructively. By adopting a wavelength division multiplexing (WDM) and a double clad fiber (DCF) coupler, a FS system could be successfully combined with a fiber-based swept source OCT (SSOCT) system. Applying a common-path configuration, furthermore, the integrating system could be implemented in a simple and effective way with highly minimized group velocity dispersion (GVD) and polarization mismatch problems. The internal structure measurement and the fluorescence spectrum measurement, which were previously performed by two independent apparatus, were concurrently made with the proposed system. From the OCT measurement, we could measure the thickness of the nacre layer, observe the fine sub-structure of the nacre, and inspect the nucleus through the nacre of a pearl. With the fluorescence spectrum measurement, we could categorize the pearls by determining their mother oysters.

Quantitative expression of shell matrix protein genes and their correlations with shell traits in the pearl oyster *Pinctada fucata*

Zhang L., He M.

Source: Aquaculture 314:73–79 (2011)

Shell matrix proteins play an important role in the regulation of shell growth and formation in the pearl oyster, *Pinctada fucata*. This study compared the expression levels of seven shell matrix protein genes (aspein, prismaticin-14, n16, n19, nacrein, msi7, and efcbp) in pearl oysters of various sizes [large (L), medium (M), and small (S)] and analyzed the correlations between expression level and shell size. Except for efcbp and msi7, significant correlations were detected between the expression levels of matrix protein genes and shell size: n19 expression displayed a negative correlation ($P < 0.01$) with increasing shell size, whereas expression levels of aspein, prismaticin-14, n16, and nacrein were greater in oysters with larger shell sizes. Furthermore, stronger correlations were found with shell height for genes related to prismatic layer of shell than shell weight; and with shell weight for genes related to nacreous layer of shell, which makes sense because prismatic proteins are considered to contribute to the extension of the shell length; and nacreous proteins are responsible for the shell thickness. These results contribute to a better understanding of the shell growth mechanisms of the pearl oyster.

The effects of exposure to near-future levels of ocean acidification on activity and byssus production of the Akoya pearl oyster, *Pinctada fucata*

Welladsen H.M., Heimann K., Southgate P.C.

Source: Journal of Shellfish Research 30(1): 85–88 (2011)

Human activities since the beginning of the industrial age have greatly increased the amount of carbon dioxide in the atmosphere. Increased levels of atmospheric carbon dioxide have already caused a 0.1-U decline in global ocean pH, and a continuing decline of 0.3–0.5 U is predicted by the end of 2100. Acidification of the oceans has widespread effects on marine organisms, including reduced rates of calcification and interruptions to normal physiological functions. This study used gaseous carbon dioxide to maintain seawater at 2 treatment levels of pH: 7.8 and 7.6. When compared with controls held at pH 8.1–8.2, pearl oysters (*Pinctada fucata*, Gould) held at pH 7.8 and pH 7.6 showed no significant difference in the number of byssal threads produced or total distance traveled. Byssal threads produced by oysters in the pH 7.6 treatment were significantly thinner than those produced by oysters in the control. However, it is postulated that this was a result of the acute stress of transfer to treatment conditions and not a result of physiological stress caused by near-future levels of ocean acidification. The potential for *P. fucata* to adapt to near-future levels of ocean acidification is discussed.

The effects of egg stocking density and antibiotic treatment on survival and development of winged pearl oyster (*Pteria Penguin*, Röding 1798) embryos

Wassnig M., Southgate P.C.

Source: Journal of Shellfish Research 30(1):103–107 (2011)

Pteria penguin is an important commercial bivalve species that is used in the production of half pearls known as mabè. Expansion of this industry in places with low natural spatfall is reliant on hatchery production of juvenile oysters, which begins with the incubation of fertilized eggs. This study addresses the issue of mortality during egg incubation by examining the effects of egg stocking density and the application of antibiotics. A factorial experimental design was implemented combining 3 egg densities (10, 50, and 100/mL) and 3 antibiotic treatments (control, no antibiotic; 5 mg/mL streptomycin-sulfate; 5 mg/mL tetracycline-erythromycin 2.5:2.5 mg/mL). Antibiotics were added to the culture medium as a single dose and fertilized eggs were incubated for an industry standard period of 24 h. Despite a 23% increase in mean survival during incubation, aquaria treated with tetracycline-erythromycin (1:1) yielded an average of only 9% more veliger larvae than control aquaria as a result of interference with development during the transition of trochophore to shelled larvae (D-stage). Application of the antibiotic streptomycin-sulfate improved mean survival by 16% compared with control aquaria, without significantly compromising development. A high egg density of 100/mL did not significantly reduce survival, but resulted in a 5% reduction in normal development to D-stage. The results of this study show that if tank space is limited during egg incubation, utilizing a high stocking density of 100 eggs/mL will provide the greatest number of D-stage larvae. However, if the supply of eggs is limited, we recommend stocking *P. penguin* eggs at a density ≤ 50 /mL and minimizing mortality by treating the culture medium with the antibiotic streptomycin-sulfate.

Evolutionary patterns in pearl oysters of the genus *Pinctada* (Bivalvia: Pteriidae)

Cunha R.L, Blanc F., Bonhomme F., Arnaud-Haond S.

Source: Marine Biotechnology 13(2):181–192 (2011)

Pearl oysters belonging to the genus *Pinctada* (Bivalvia: Pteriidae) are widely distributed between the Indo-Pacific and western Atlantic. The existence of both widely distributed and more restricted species makes this group a suitable model to study diversification patterns and prevailing modes of speciation. Phylogenies of eight out of the 11 currently recognised *Pinctada* species using mitochondrial (cox1) and nuclear (18S rRNA) data yielded two monophyletic groups that correspond to shell size and presence/absence of hinge teeth. Character trace of these morphological characters onto the molecular phylogeny revealed a strong correlation. *Pinctada margaritifera* appears polyphyletic with specimens from Mauritius grouping in a different clade from others of the French Polynesia and Japan. Hence, *P. margaritifera* might represent a species complex, and specimens from Mauritius could represent a different species. Regarding the putative species complex *Pinctada fucata*/*Pinctada martensii*/*Pinctada radiata*/*Pinctada imbricata*, our molecular analyses question the taxonomic validity of the morphological characters used to discriminate *P. fucata* and *P. martensii* that exhibited the lowest genetic divergence and are most likely conspecific as they clustered together. *P. radiata* and *P. imbricata* were recovered as monophyletic. The absence of overlapping distributions between sister lineages and the observed isolation by distance suggests that allopatry is the prevailing speciation mode in *Pinctada*. Bayesian dating analysis indicated a Miocene origin for the genus, which is consistent with the fossil record. The northward movement of the Australian plate throughout the Miocene played an important role in the diversification process within *Pinctada*.

Effects of the toxic dinoflagellate *Heterocapsa circularisquama* on larvae of the pearl oyster *Pinctada fucata martensii* (Dunker, 1873)

Basti L., Go J., Higuchi K., Nagai K., Segawa S.

Source: Journal of Shellfish Research 30(1):177–186 (2011)

The effects of the toxic dinoflagellate *Heterocapsa circularisquama* on the activity rate, development rate, prevalence of damage, and survival rate of trochophore and D-shaped larvae of the pearl oyster *Pinctada fucata martensii* were studied in relation to *H. circularisquama* cell densities and exposure duration. In addition, larvae were regularly processed via scanning electron microscopy to investigate morphological damage. The activity rate of both larval stages was significantly decreased after 3–6 h of exposure to *H. circularisquama* at densities ranging from 100 to 2×10^4 cells/mL. The prevalence of damage was significantly high after 3–6 h of exposure to *H. circularisquama* at densities of 100 to 2×10^4 cells/mL and 5×10^3 to 2×10^4 cells/mL for trochophores

and D-shaped larvae, respectively. Cytoplasmic discharge, mass mucus production, irregular shape, delayed or inhibited mineralization of the shell, mantle protrusion, the appearance of abnormal masses in the velum, and the exfoliation of the larvae cilia coupled with epithelial desquamation were frequently observed. The activity rate of D-larvae transformed from trochophores exposed to *H. circularisquama* for 12–48 h at densities ranging from 10 to 2×10^4 cells/mL was significantly reduced. The survival of D-shaped larvae plummeted to less than 0.013 for densities $\geq 5 \times 10^3$ cells/mL. The results indicate that *H. circularisquama* blooms have detrimental impacts on bivalves at early life stages. Blooms of *H. circularisquama* occurring during the spawning periods will influence the natural recruitment in *P. fucata martensii* and will have profound impacts on its population biology. Therefore, shellfish farms should not be built in coastal areas where *H. circularisquama* occurs, or genitors should be relocated during potential blooming periods.

Seasonal changes in recruitment of *Pteria penguin* in north Queensland, Australia

Milione M., Southgate P.C.

Source: Journal of Shellfish Research 30(1):89–94 (2011)

Spat recruitment of the winged pearl oyster *Pteria penguin*, in relation to season, substrate type, and depth was investigated at Orpheus Island in north Queensland for 27 months, from February 2008 to April 2010. Two substrate types (70% shade cloth and open-weave polypropylene mesh bags) were deployed at 2 depths (4 m and 6 m) and checked every 6 wk for 3 spawning seasons to determine any differences in quantity of spatfall between these factors. No significant difference was found in spat recruitment between substrate types ($P = 0.158$) or depth ($P = 0.349$), although there was a significant seasonal effect on spat recruitment ($P < 0.001$), with a peak in the quantity of spatfall in late summer, from February to March, and no spat collected in the winter to spring (July to October). Maximum settlement of spat was 10.2 spat per mesh bag collector in February 2008. Recruitment was significantly reduced ($P < 0.001$) during the 2010 spawning season as a result of disturbance from severe storms generated by tropical cyclone Olga in late January.

Multimodal analysis of pearls and pearl treatments by using optical coherence tomography and fluorescence spectroscopy

Ju M.J., Lee S.J., Kim Y., Shin J.G., Kim H.Y., Lim, Y., Yasuno Y., Lee B.H.

Source: Optics Express 19(7):6420–6432 (2011)

We present an integrated optical system that consists of optical coherence tomography (OCT) and laser-induced fluorescence (LIF) spectroscopy for multimodal analysis of pearls and pearl treatments. The OCT source and the LIF excitation beams were aligned together to illuminate the same spot of a pearl fixed on the sample stage that was under rotation. As a result, both OCT images and LIF spectra of the pearls were detected at the same time and also at the same place. For OCT, a 1310 nm centered swept laser source was used. For LIF, a 405 nm laser diode was used and a lensed multimode fiber was utilized as a fluorescence probe. The tomographic investigation on the internal structure of a pearl allowed us to evaluate and categorize the pearl nondestructively as was previously reported. In addition, the measurements of fluorescence spectrum and its decaying rate helped to determine the species of mother oyster. The proposed multimodal analysis made it possible to classify the pearls and also to disclose the treatments made on the pearls.

Calcein staining of calcified structures in pearl oyster *Pinctada margaritifera* and the effect of food resource level on shell growth

Linard C., Gueguen Y., Moriceau J., Soyeux C., Hui B., Raoux A., Cuif J.P., Cochard J.-C., Le Pennec M., Le Moullac G.

Source: Aquaculture 313(1–4):149–155 (2011)

Marine mollusc shell growth has been widely measured using fluorochrome marking. In order to test the efficiency and reliability of calcein staining on *Pinctada margaritifera* shells and pearls, the present study examined two administration methods, different concentrations and several immersion times. Immersion in a 150 mg L^{-1} calcein solution for 12 h to 24 h appeared to be the best method for marking *P. margaritifera* shells. For pearl marking, injection of a 200 mg L^{-1} calcein solution into the pearl pouch was the optimal method. Calcein marking was then used to measure the influence of food resource levels on the shell growth. Groups of 23-month-old *P. margaritifera* were fed at three trophic levels for two months. The two highest food levels tested ($6000 \text{ cell mL}^{-1}$ and $15000 \text{ cell mL}^{-1}$) induced uniform growth between the dorsal and ventral sides of shell, whereas the lowest food level (800 cell mL^{-1}) induced greater growth on the dorsal side. Shell deposits from the ventral side were observed using a scanning electron microscope, revealing that the difference of

the trophic level over two months had modified the thickness of the aragonite tablets formed. These results showed that the trophic level is a major factor conditioning *P. margaritifera* development.

Environmental influences on stock abundance and fishing power in the silver-lipped pearl oyster fishery

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Source: ICES Journal of Marine Science 68(3): 444–453 (2011)

Stock variability, fishing power, and the contributing environmental factors were examined for the Australian silver-lipped pearl oyster, *Pinctada maxima*. The approach was to compare the longer-term time-series of stock abundance derived from catch per unit effort against the shorter-term 0+ spat-settlement index to seek agreement on causal factors. Indices of stock abundance were established with generalized linear models that simultaneously examined the influence of technological and environmental factors, resulting in a predictive model with a 3-year forecast. The advent of global positioning systems caused a 30% increase in fishing power. A negative relationship between abundance and rainfall, and a positive relationship between abundance and temperature, was detected for both spat settlement and fishery abundance. Northerly winds (negative northings) from December to February significantly enhanced settlement, but easterly winds (negative eastings) in the main fishing month of May influenced fishing power positively. After standardizing for the effects of fishing power, a 150% increase in stock abundance of the exploited component of *P. maxima* stocks was detected between 2004 and 2009. A major contributor to this increase was an exceptionally high settlement of spat in 2005, associated with a rare combination of environmental conditions. Once this year class grows beyond the target size classes, abundance is predicted to decrease to the normal levels.

The effects of dietary supplements of polyunsaturated fatty acid on pearl oyster, *Pinctada margaritifera* L., gonad composition and reproductive output

Ehteshami F., Christianus A., Rameshi H., Harmin S.A., Saad C.R.

Source: Aquaculture Research 42(4):613–622 (2011)

Black-lip pearl oyster, *Pinctada margaritifera* broodstock was collected from the wild. Egg production, hatching rate and larval development were compared between oysters induced to spawn within 2 days after collection in the wild (T1), oysters fed a pure microalgae diet during 24 days before spawning (T2) and oysters fed the same microalgal diet in which 10% of the algae were replaced with 2 µm polyunsaturated fatty acid (PUFA)-rich microspheres (T3). Administration of lipid microspheres resulted in larger sized eggs, a higher percentage of D-larvae and larger sized 48-h-old larvae ($P < 0.05$). The total and neutral lipid contents of the gonad increased after oysters were fed with microalgae only or with supplementary diet. The major neutral and polar fractions of saturated fatty acid (SFA) were 16C and 18C fatty acids, and not influenced by the diet ($P > 0.05$). The gonads of oysters fed supplementary PUFA contained more docosahexaenoic acid (DHA) and less monounsaturated fatty acids. Higher level of DHA in gonads of T3 was associated with oogenesis and embryogenesis success. The n-3/n-6 ratio in the neutral lipid fraction provides a good indication of the spawning condition and predicting egg size and hatching rate.

Distribution, abundance and population structure of *Pinctada radiata* (Mollusca: Bivalvia) in southern Tunisian waters (Central Mediterranean)

Derbali A., Jarbouli O., Ghorbel M.

Source: Cahiers de Biologie Marine 52(1):23–31 (2011)

The pearl oyster *Pinctada radiata* is an alien species introduced to the Mediterranean Sea and recorded in Tunisia several years ago. However, since its record in the Gulf of Gabes, no studies have been carried out about the spread of this invasive mollusc. Thus, the status of this species is still poorly known and there is a knowledge gap about its distribution and abundance. The present work is a contribution to the knowledge of the pearl oyster distribution and densities in southern Tunisian waters, at depths between 0 and 100 metres. The results showed a scattered distribution pattern of the species according to location and depth ranging between 0 and 145 ind.m⁻². *Pinctada radiata* was encountered from the intertidal zone to 40 m depth, with a highest population densities recorded at depth range of 2–20 m. The total stock was estimated to be 27584.9 ± 11504.5 million individuals. Oyster distribution seems influenced by the substrate type. This high population of pearl oysters was associated with large cover of seagrass *Posidonia oceanica* which provides an excellent substratum for attachment. The littoral zone seems not to be the preferred habitat for the prolifera-

tion of this immigrant species. Oysters' size increased steadily with depth, ranging from a mean of 37.98 ± 0.40 mm SH at 0–1 m to 60.98 ± 0.68 mm SH at 20–100 m depth range. Size structure analysis showed that deep water population was dominated by large individuals reaching 96 mm SH. Findings of this study suggested that *P. radiata* is well proliferate in the Gulf of Gabes.

Can the quality of pearls from the Japanese pearl oyster (*Pinctada fucata*) be explained by the gene expression patterns of the major shell matrix proteins in the pearl sac?

Inoue N., Ishibashi R., Ishikawa T., Atsumi T., Aoki H., Komaru A.

Source: Marine Biotechnology 13(1):48–55 (2011)

For pearl culture, the pearl oyster is forced open and a nucleus is implanted into the gonad with a mantle graft. The outer mantle epithelial cells of the implanted mantle graft elongate and surrounding the nucleus a pearl sac is formed. Shell matrix proteins secreted by the pearl sac play an important role in the regulation of pearl formation. Recently, seven shell matrix proteins were identified from the pearl oyster *Pinctada fucata*. However, there is a paucity of information on the function of these proteins and their gene expression patterns. Our study aims to elucidate the relationship between pearl type, quality, and gene expression patterns of six shell matrix proteins (msi60, n16, nacrein, msi31, prismaticin-14, and aspein) in the pearl sac based on real-time PCR analysis. After culturing for about 2 months, the pearl sac tissues were collected from 22 individuals: 12 with high quality (HP), nine with low quality (LP), and one with organic (ORG) pearl formation. The surface of each of the 12 HP pearls was composed only of a nacreous layer; in contrast, that of the nine LP pearls was composed of nacreous and prismatic layers. The six target gene expressions were detected in all individuals. However, delta threshold cycle (CT) for msi31 was significantly higher in the HP than in the LP individuals (Mann-Whitney's U test, $p = 0.02$). This means that the relative expression level of msi31, which constitutes the framework of the prismatic layer, was higher in the LP than in the HP individuals.

Comparison of expression patterns of shell matrix protein genes in the mantle tissues between high- and low-quality pearl-producing recipients of the pearl oyster, *Pinctada fucata*

Inoue N., Ishibashi R., Ishikawa T., Atsumi T., Aoki H., Komaru A.

Source: Zoological Science 28(1):32–36 (2011)

The production of a cultured pearl is the result of a complex interplay between the donor and recipient oysters. However, there is a paucity of information on the relationship between donor and recipient oyster gene expression patterns and pearl quality. Shell matrix proteins affect not only the formation of the shell, but also that of the pearls. We compared the gene expression patterns of five shell matrix proteins (msi60, nacrein, msi31, prismaticin-14, and aspein) in the mantle edge (ME), which forms the prismatic layer, and the mantle center (MC), which forms the nacreous layer, between high- (HP) and low quality pearl- (LP) producing recipient oysters. After culturing for about two months, ME and MC tissues were collected from nine recipient oysters: four with HP, five with LP. In the ME, the average threshold cycle (CT) for aspein was higher in HP than in LP (t-test, $p = 0.03$). Additionally, in the MC, the average CT for msi60 was lower in HP than in LP ($p = 0.06$). This means the relative expression level of msi60 in the mantle of HP was higher than that of LP, and expression level of aspein in the mantle of HP was lower than that of LP. Pearl quality was closely related to the expression patterns of shell matrix protein genes of recipient oysters.

Macrofauna associated with an introduced oyster, *Pinctada radiata*: Spatial scale implications of community differences

Tlig-Zouari S., Rabaoui L., Cosentino A., Irathni I., Ghrairi H., Hassine O.K.B.

Source: Journal of Sea Research, 65 (1), pp. 161–169 (2011).

The macrozoobenthos associated with the introduced pearl oyster *Pinctada radiata* has been sampled at two different spatial scales of three sectors (order of hundreds of kilometres) and of eight localities (order of tens of kilometres). Moreover, the NW sector was selected to compare three localities with the presence of *P. radiata* (low density) and one locality where it was totally absent. The first design was hierarchical, with random localities nested within sectors; the second one was an asymmetrical factorial design, in which the presence/absence of *Pinctada* and hydrodynamism were considered. Similarity relationships were investigated by means of multivariate clustering, similarity percentage analysis and nm-MDS ordination; the two experimental designs have been tested by permutational MANOVA and analysis of dispersion (PERM-DISP). Most of the variability of the associated zoobenthic community appeared to be mainly captured by

local environmental factors; the meso-scale variability was more discriminating than differences at larger spatial scale. Large scale NW-SE biogeographic gradient may also have some effects in the assemblage composition. Although the whole arrangement of samples in the MDS plane showed a clear Bray-Curtis distance between the locality without *Pinctada* and all the remaining sites, pair-wise contrasts were not all significant. The factor “presence/absence” was not significant in this design, whereas the exposure was more indicative of differences in the local assemblage composition. These results may not confirm that the community structure variability is due to the impact of *Pinctada* invasion because the potential and subtle community shift may be masked by the overwhelming influence of just the local environmental gradients. In spite of this, the introduced oyster may play the role of an engineer species at high densities, contributing to the complexity of the benthic habitat and influencing the trophic pattern of its fauna.

Growth and survival of the Atlantic pearl oyster *Pinctada imbricata* (Röding 1798), under suspended culture conditions using hanging ropes and pearl nets

Semidey D., Marquez A., Lodeiros C.

Source: *Zootecnia Tropical* 28(4): 521–533 (2010)

Growth and survival of juvenile pearl mother *Pinctada imbricata* was evaluated through six months of culture in suspended hanging ropes and pearl nets using a longline in Turpialito Bay, Cariaco Gulf, Venezuela. Pearl oyster seeds 15 mm long were cultured, evaluating survival, shell length, muscle, rest of the body and byssus mass monthly. Variations of salinity, temperature, chlorophyll a, seston, dissolved oxygen and shell fouling, were determined every two weeks. After six months, the oyster reached dorso-ventral length close to 50 mm. Shell growth pattern was similar for both types of culture, although byssus mass was higher in hanging ropes, while shell length was greater in pearl nets. No significant differences were found in body mass. Survival showed similar patterns in both culture systems, although a decrease in numbers occurred in January for the oysters in pearl nets, associated to the presence of the gastropod *Cymatium* sp. The phytoplanktonic biomass was positively correlated to growth in *P. imbricata*, showing to be an important factor in growth modulation. The results suggest the use of the rope system for the culture of *P. imbricata*.

The molluscan bio-fouling community on the Red Sea pearl oyster beds

Wronski T.

Source: *Zoology in the Middle East* 51: 67–73 (2010)

In the Red Sea, pearl oyster banks occur most extensively around the Dahlak and the Farasan Islands. Pearl oysters (*Pinctada*, Pteriidae) form extended beds by attaching themselves to hard substrates. Such beds attract a diverse bio-fouling fauna. Most dominant are the molluscs, but little is known about the associated biota of pearl oyster beds, their distributional abundance, and the structure of this community. In this study, the macro-molluscan fauna living on pearl oyster beds in the Red Sea around the Farasan Islands was studied using a quantitative survey of the by-catch left by pearl oyster divers. Bivalvia represented 99.6% of the malaco-fauna on pearl oyster beds around the Farasan Islands, while gastropods and chitons represented only 0.4%. In total, 33 mollusc species were identified (24 bivalves, 7 prosobranch gastropods, one basomatophore gastropod and one chiton), with *Brachidontes variabilis*, a species which is not found on Arabian Gulf pearl oyster beds, the most common bivalve (71% of all molluscs), and *Diodora ruppellii* the most common gastropod (0.12% of all molluscs). The results are discussed and compared with the pearl oyster beds from the Arabian Gulf.

Estimating the heritability for growth-related traits in the pearl oyster, *Pinctada fucata martensii* (Dunker)

Wang H., Du X., Lü W., Liu Z.

Source: *Aquaculture Research* 42(1): 57–64 (2010)

Twelve paternal half-sib families (or 36 full-sib families) of the pearl oyster, *Pinctada fucata martensii* (Dunker), were produced according to the requirements of hierarchical genetic mating design. A total of 4320 individuals, aged 15 months, were measured for seven growth-related traits. Predicated upon the additive-dominance genetic analysis model, varying genetic variance components and then heritabilities of the growth-related traits of interest were estimated using analysis of variance. Results showed that seven growth-related traits had larger additive genetic variances ($P < 0.05$); the dominance genetic variance of shell weight (SW) was smaller ($P > 0.05$), the dominance genetic variances of other six traits were all larger ($P < 0.05$). Narrow- and broad-sense heritabilities for the seven traits were, respectively, 0.64 ± 0.10 and 0.78 ± 0.12 for shell length, 0.49 ± 0.06 and 0.63 ± 0.09 for shell height, 0.38 ± 0.14 and 0.54 ± 0.16 for shell breadth, 0.41 ± 0.17 and 0.56 ± 0.11 for hinge

length, 0.53 ± 0.11 and 0.68 ± 0.08 for body weight, 0.35 ± 0.07 and 0.55 ± 0.08 for tissue weight and 0.67 ± 0.10 and 0.75 ± 0.16 for SW. All heritability estimates were statistically significant ($P < 0.05$). According to these results, the mass selection procedure is suggested for the breeding of *P. martensii*.

Molecular phylogeny of pearl oysters and their relatives (Mollusca, Bivalvia, Pterioidea)

Tëmkin I.

Source: BMC Evolutionary Biology, 10(1), art. no. 342 (2010)

Background: The superfamily Pterioidea is a morphologically and ecologically diverse lineage of epifaunal marine bivalves distributed throughout the tropical and subtropical continental shelf regions. This group includes commercially important pearl culture species and model organisms used for medical studies of biomineralization. Recent morphological treatment of selected pterioideans and molecular phylogenetic analyses of higher-level relationships in Bivalvia have challenged the traditional view that pterioidean families are monophyletic. This issue is examined here in light of molecular data sets composed of DNA sequences for nuclear and mitochondrial loci, and a published character data set of anatomical and shell morphological characters.

Results: The present study is the first comprehensive species-level analysis of the Pterioidea to produce a well-resolved, robust phylogenetic hypothesis for nearly all extant taxa. The data were analyzed for potential biases due to taxon and character sampling, and idiosyncracies of different molecular evolutionary processes. The congruence and contribution of different partitions were quantified, and the sensitivity of clade stability to alignment parameters was explored.

Conclusions: Four primary conclusions were reached: (1) the results strongly supported the monophyly of the Pterioidea; (2) none of the previously defined families (except for the monotypic Pulvinitidae) were monophyletic; (3) the arrangement of the genera was novel and unanticipated, however strongly supported and robust to changes in alignment parameters; and (4) optimizing key morphological characters onto topologies derived from the analysis of molecular data revealed many instances of homoplasy and uncovered synapomorphies for major nodes. Additionally, a complete species-level sampling of the genus *Pinctada* provided further insights into the on-going controversy regarding the taxonomic identity of major pearl culture species.

Histopathology of oedema in pearl oysters *Pinctada maxima*

Jones J.B., Crockford M., Creeper J., Stephens F.

Source: Diseases of Aquatic Organisms 91(1):67–73 (2010)

In October 2006, severe mortalities (80 to 100%) were reported in pearl oyster *Pinctada maxima* production farms from Exmouth Gulf, Western Australia. Only *P. maxima* were affected; other bivalves including black pearl oysters *P. margaritifera* remained healthy. Initial investigations indicated that the mortality was due to an infectious process, although no disease agent has yet been identified. Gross appearance of affected oysters showed mild oedema, retraction of the mantle, weakness and death. Histology revealed no inflammatory response, but we did observe a subtle lesion involving tissue oedema and oedematous separation of epithelial tissues from underlying stroma. Oedema or a watery appearance is commonly reported in published descriptions of diseased molluscs, yet in many cases the terminology has been poorly characterised. The potential causes of oedema are reviewed; however, the question remains as to what might be the cause of oedema in molluscs that are normally iso-osmotic with seawater and have no power of anisomotic extracellular osmotic regulation.

Effects of glycopeptides on development, growth and non-specific immunity of pearl oyster *Pinctada fucata* (Gould)

Zhang S., Long L.J., Zhou Y.C., Yin H., Xiao Z., Chen Y.F.

Source: Aquaculture Nutrition 16(5):520–527 (2010)

The effects of glycopeptides, prepared from pearl oyster *Pinctada fucata*, on embryonic development, larval and juvenile growth and adult non-specific immunity of *P. fucata* were investigated in this study. Glycopeptides had a pronounced stimulatory effect on embryonic development and larval and juvenile growth of *P. fucata*, enhancing with increased glycopeptide concentrations. All of haemocytes, phagocytosis, aggregation, serum microbiostatic activity and bacteriocidal activity all showed significant increase after 60-day feeding, relative to unfed controls. The major conclusion is that glycopeptides had a pronounced stimulatory effect on the non-specific immunity of pearl oysters.

Java's forgotten pearls: The history and disappearance of pearl fishing in the Segara Anakan lagoon, South Java, Indonesia

Schwerdtner Máñez K.

Source: Journal of Historical Geography 36(4):367–376 (2010)

Pearls have been a valued resource in most cultures that had access to them. A number of historically important pearling grounds were situated in the waters around today's Indonesia. One of these areas, now largely forgotten, was the Segara Anakan lagoon in South Java. In the seventeenth century, Dutch colonists exploited the lagoon's pearls. Afterwards, the lagoon's oysters were locally exploited as a food item until the late 1970s. While the pearl fishery attracted considerable attention in the colonial literature, its disappearance, by contrast, went largely undocumented. Nowadays, the oysters no longer are found in the lagoon as a result of extensive sedimentation processes. Their former existence is only preserved in the memory of local people. This article examines the history and fate of the pearls of Segara Anakan, providing an example of a formerly valued species whose existence simply became forgotten outside the area.

Characteristics of biogenic calcite in the prismatic layer of a pearl oyster, *Pinctada fucata*

Okumura T., Suzuki M., Nagasawa H., Kogure T.

Source: Micron 41(7):821–826 (2010)

The fine structure of the calcite prism in the outer layer of a pearl oyster, *Pinctada fucata*, has been investigated using various electron beam techniques, in order to understand its characteristics and growth mechanism including the role of intracrystalline organic substances. As the calcite prismatic layer grows thicker, sinuous boundaries develop to divide the prism into a number of domains. The crystal misorientation between the adjacent domains is several to more than ten degrees. The component of the misorientation is mainly the rotation about the c-axis. There is no continuous organic membrane at the boundaries. Furthermore, the crystal orientation inside the domains changes gradually, as indicated by the electron back-scattered diffraction (EBSD) in a scanning electron microscope (SEM). Transmission electron microscopy (TEM) examination revealed that the domain consists of sub-grains of a few hundred nanometers divided by small-angle grain boundaries, which are probably the origin of the gradual change of the crystal orientation inside the domains. Spherular Fresnel contrasts were often observed at the small-angle grain boundaries, in defocused TEM images. Electron energy-loss spectroscopy (EELS) indicated the spherules are organic macromolecules, suggesting that incorporation of organic macromolecules during the crystal growth forms the sub-grain structure of the calcite prism.

Gene expression patterns in the outer mantle epithelial cells associated with pearl sac formation

Inoue N., Ishibashi R., Ishikawa T., Atsumi T., Aoki H., Komaru A.

Source: Marine Biotechnology 13:474–483 (2011)

For pearl culture, nucleus and mantle grafts are implanted into the gonad of the host oyster. The epithelial cells of the implanted mantle graft elongate and surround the nucleus, and a pearl sac is formed. Shell matrix proteins secreted by the pearl sac play an important role in pearl formation. We studied the gene expression patterns of six shell matrix proteins (msi60, n16, nacrein, msi31, prismalin-14, and aspein) in the epithelial cells associated with pearl sac formation. There were differences in the expression patterns of the six genes in the epithelial cells, and the relative expression levels for msi60 and aspein differed between the mantle graft and pearl sac (48 days after implantation). Therefore, the gene expression patterns of the epithelial cells were genetically undetermined, and changed between before and after pearl sac formation. The gene expression patterns of the epithelial cells of the pearl sac may be regulated by the host oysters.

Utility of shell-closing strength as the indicator of good health in breeding and culture management of Japanese pearl oyster *Pinctada fucata*

Aoki H., Ishikawa T., Fujiwara T., Atsumi T., Nishikawa H., Okamoto C., Komaru A.

Source: Aquaculture 308:S115–S118 (2010)

The aim of this study was to evaluate the suitability of shell-closing strength (SCS), a newly developed trait, as a physiological indicator in the Japanese pearl oyster *Pinctada fucata*. SCS is the load value necessary to open the shell of a pearl oyster to 10 mm using a shell opener. We developed a new instrument for the measurement of SCS consisting of a force gauge and a shell opener, and examined the relationship between SCS and physiological and nutritive indexes in pearl oysters. The results of the experiments showed that (1)

oysters exhibiting good physiological conditions can be selected by using SCS as the indicator; oysters with higher SCS showed lower mortality, higher condition factor, and glycogen content in the adductor muscles than those with lower SCS; (2) SCS varies remarkably among genetic oyster lineages through the culturing period, indicative of the involvement of genetic factors in the determination of SCS; (3) the SCS of pearl oyster tended to increase with the body and the adductor muscle weights, although the protein and glycogen contents of the soft tissue exhibited typical changes during the period in which the water temperature is high (spring to summer). These results suggested that SCS is an efficient indicator of health of pearl oysters, and is useful for breeding and culture management of this species.

Assessing pearl quality using reflectance UV-Vis spectroscopy: Does the same donor produce consistent pearl quality?

Mamangkey N.G.F., Agatonovic S., Southgate P.C.

Source: Marine Drugs 8:2517–2525 (2010)

Two groups of commercial quality (“acceptable”) pearls produced using two donors, and a group of “acceptable” pearls from other donors were analyzed using reflectance UV-Vis spectrophotometry. Three pearls with different colors produced by the same donor showed different absorption spectra. Cream and gold colored pearls showed a wide absorption from 320 to about 460 nm, while there was just slight reflectance around 400 nm by the white pearl with a pink overtone. Cream and gold pearls reached a reflectance peak at 560 to 590 nm, while the white pearl with pink overtone showed slightly wider absorption in this region. Both cream and gold pearls showed an absorption peak after the reflectance peak, at about 700 nm for the cream pearl and 750 nm for the gold pearl. Two other pearls produced by the same donor (white with cream overtone and cream with various overtones) showed similar spectra, which differed in their intensity. One of these pearls had very high lustre and its spectrum showed a much higher percentage reflectance than the second pearl with inferior lustre. This result may indicate that reflectance is a useful quantitative indicator of pearl lustre. The spectra of two white pearls resulting from different donors with the same color nacre (silver) showed a reflectance at 260 nm, followed by absorption at 280 nm and another reflectance peak at 340 nm. After this peak the spectra for these pearls remained flat until a slight absorption peak around 700 nm. Throughout the visible region, all white pearls used in this study showed similar reflectance spectra although there were differences in reflectance intensity. Unlike the spectral results from white pearls, the results from yellow and gold pearls varied according to color saturation of the pearl. The results of this study show that similarities between absorption and reflectance spectra of cultured pearls resulting from the same saibo donor are negligible and could not be detected with UV-Vis spectrophotometry. Nevertheless, this technique could have a role to play in developing less subjective methods of assessing pearl quality and in further studies of the relationships between pearl quality and that of the donor and recipient oysters.

Haemocyte morphology and function in the Akoya pearl oyster, *Pinctada imbricata*

Kuchel R.P., Raftos D.A., Birch D., Vella N.

Source: Journal of Invertebrate Pathology 105(1): 36–48 (2010)

The morphology and cytochemistry of *Pinctada imbricata* haemocytes were studied in vitro. Three distinct blood cell types were identified; hyalinocytes, granulocytes, and serous cells. Haemocytes were classified based on the presence/absence of granules, and nucleus to cytoplasm ratio. Granulocytes were the most common cell type ($62 \pm 2.81\%$), followed by hyalinocytes ($36 \pm 2.35\%$), and serous cells ($2 \pm 0.90\%$). Granulocytes, and hyalinocytes were found to be immunologically active, with the ability to phagocytose Congo red stained yeast. Of the cells involved in phagocytosis, granulocytes were the most active with $88.8 \pm 3.9\%$ of these haemocytes engulfing yeast. Cytochemical stains (phenoloxidase, peroxidase, superoxide, melanin, neutral red) showed that enzymes associated with phagocytic activity were localised in granules within granulocytes. Based on their affinities for Giemsa/May-Grünwald stain, haemocytes were also defined as either acidic, basic or neutral. Hyalinocytes and serous cells were found to be eosinophilic, whilst granulocytes were either basophilic (large granulocytes), eosinophilic (small granulocytes) or a combination of the two (combination granulocytes). Light, differential interference contrast and epi-fluorescence microscopy identified three sub-populations of granulocytes based on size and granularity; small ($4.00\text{--}5.00\ \mu\text{m}$ in diameter, with small granules ($0.05\text{--}0.5\ \mu\text{m}$ in diameter), large ($5.00\text{--}9.00\ \mu\text{m}$ in diameter, with large granules ($0.50\text{--}2.50\ \mu\text{m}$ in diameter) and combination ($5.00\text{--}9.00\ \mu\text{m}$ in diameter, with both large and small granules). These observations demonstrate that *P. imbricata* have a variety of morphologically and functionally specialized haemocytes, many of which may be associated with immunological functions.

Gene expression patterns and pearl formation in the Japanese pearl oyster (*Pinctada fucata*): A comparison of gene expression patterns between the pearl sac and mantle tissues

Inoue N., Ishibashi R., Ishikawa T., Atsumi T., Aoki H., Komaru A.

Source: Aquaculture 308:68–74 (2010)

Shell matrix proteins play an important role in the regulation of shell and pearl formation in the Japanese pearl oyster (*Pinctada fucata*). There is a paucity of information on the function of these proteins and their gene expression patterns. The purpose of this study was to compare the gene expression patterns of six shell matrix proteins (nacrein, msi60, msi31, n16, prismaticin-14 and aspein) in the mantle center (MC) (which forms the nacreous layer) and mantle edge (ME) (which forms the prismatic layer), with those in the pearl sac (PS) (which forms the nacreous layer on the pearl surface). There was a significant correlation between the PS and MC ($P < 0.05$) for the six gene expression patterns. In addition, cluster analysis showed that the expression patterns of the six genes were grouped into three clusters (nacreous cluster: msi60 and n16, prismatic cluster: msi31, prismaticin-14 and aspein, and nacrein). These results indicate that formation of the nacreous and prismatic layers of the shell and pearl is affected by the gene expression patterns of the shell matrix proteins, and that the two genes of the nacreous cluster (msi60 and n16) and the three genes of the prismatic cluster (msi31, prismaticin-14 and aspein) are regulated by unknown factors.

Quantifying sponge erosions in Western Australian pearl oyster shells

Daume S., Fromont J., Parker F., Davidson M., Murphy D., Hart A

Source: Aquaculture Research 41(9):e260–e267 (2010)

This paper describes several methods to estimate the amount of sponge erosion in pearl oyster shells (*Pinctada maxima* Jameson, 1901). Internal erosion can be calculated from non-destructive surface area estimates because a clear relationship was found between the eroded area on the surface of the shells and internal erosions. Grading shells into three different categories, according to the severity of erosion, also proved to be a suitable method to quickly assess the amount of sponge erosions. All methods were more accurate in shells with larger amounts of erosions. Using the described methods, the Lacepedes fishing ground was more diverse in bioeroding sponge species compared with sites at the 80-Mile fishing ground locations. *Pione velans* Hentschel, 1909 was the dominant bioeroding sponge species but *Cliona dissimilis* Ridley and Dendy, 1886 eroded more shell material than *P. velans*. Observed patterns suggest that *P. velans* had a more recent recruitment than *C. dissimilis*. We suggest that visual grading together with estimates from the shell surface will be sufficient to determine the general long-term trends in bioeroding sponge severity. However, results of monitoring programmes need to be supplemented by histological examination to confirm sponge activity and species identity.

Larval settlement and metamorphosis of the pearl oyster *Pinctada fucata* in response to biofilms

Yu X., He W., Li H., Yan Y., Lin C.

Source: Aquaculture 306:334–337 (2010)

Biofilms is an important mediator of larval settlement and metamorphosis for the pearl oyster *Pinctada fucata*, which is an important aquaculture species in South China. However, the role of biofilms in larval settlement and metamorphosis in this species remains unclear. This paper presents the first laboratory study investigating larval settlement and metamorphosis of *P. fucata* in response to natural biofilms of different ages and biofilm-conditioned seawater. Natural biofilms from 1 to 9 day-old except 6 day-old significantly induced larval settlement, while there was no obvious positive correlation between microbial abundance of the biofilms and percentages of larval settlement and metamorphosis. These results indicate that the inductive effect may be closely associated with biofilms community structure and extracellular products in the biofilms rather than microbial abundance. No inductive effects were observed when larvae were exposed to biofilm-conditioned seawater. Thus, the inductive cues from the natural biofilms seem to result from its surface-associated characteristic rather than waterborne. In addition, this study also provides valuable information for studies of mechanism of larval settlement and metamorphosis in this species, which is useful information for the hatchery industry of this pearl oyster.

The consequences of differential family survival rates and equalizing maternal contributions on the effective population size (Ne) of cultured silver-lipped pearl oysters, *Pinctada maxima*

Lind C.E., Evans B.S., Taylor J.J.U., Jerry D.R.

Source: Aquaculture Research 41(8): 1229–1242 (2010)

The effective population size (Ne) is a critical gauge of how efficiently an aquaculture operation is capturing or maintaining genetic diversity and can govern the long-term success of genetic selection programmes. In communally reared pearl oysters (*Pinctada maxima*), high variance in family sizes is a significant contributor towards low Ne and its severity may be compounded by differential survival rates of individual families. To determine the effect of variable survival on Ne in cultured *P. maxima*, families from two commercial populations were analysed using DNA parentage analyses to monitor survival and changes in relative contributions. Significant shifts in relative contributions were observed between 72 days and 18 months of age in both commercial cohorts ($P < 0.001$). Survival rates were found to be highly variable among families (ranging from 2.5% to 49.5%) when reared in a common environment. Additionally, we investigated whether equalizing maternal family sizes before communal rearing will reduce family size variance, and increase Ne, compared with stocking at naturally produced proportions. Family equalization (E) significantly improved Ne ($P = 0.013$) compared with rearing at natural (N) proportions (E: $Ne = 7.18 \pm 0.34$; N: $Ne = 5.60 \pm 0.15$); however, this practice may unintentionally magnify negative influences of poor performing families if survival is correlated with other commercially important traits. It is concluded that highly variable family survival will affect Ne in communally reared *P. maxima*, and the practice of equalizing family sizes in order to maximize Ne may only become consistently beneficial once further progress is made towards understanding, and then reducing variation in family survival rates.

Population and family growth response to different rearing location, heritability estimates and genotype \times environment interaction in the silver-lip pearl oyster (*Pinctada maxima*)

Kvingeda R., Evans B.S., Lind C.E., Taylor J.J.U., Dupont-Nivet M., Jerry D.R.

Source: Aquaculture 304(1–4): (2010)

Genetic parameters and genotype by environment interactions were estimated from the growth responses of silver-lip pearl oyster (*Pinctada maxima*) families originating from three populations (Aru, Bali, West Papua) reared at two Indonesian commercial sites (Bali and Lombok). Microsatellite-based DNA parentage analyses were used to assign oysters to their family and population of origin and four shell growth traits (anterior-posterior measurement (APM), dorsal-ventral measurement (DVM), shell width (SW) and wet weight (WW)) recorded at 14 and 18 months of age. Significant size differences were observed in all shell growth traits between oyster populations, and between oysters cultured at the different locations. Oysters from Bali and West Papua grew faster than those sourced from Aru at both culture sites, whilst the Lombok site produced the fastest growing oysters overall. Significant size differences were also present in shell traits among families, indicating a large amount of genetic variability present for potential breeding programs. Although there were significant familial size differences for shell traits, genetic correlation analyses showed little evidence for re-ranking of family performance among the two culture sites ($rg = 0.89-0.99$), suggesting low genotype by environment deviations among sites. Heritability of the shell traits DVM, APM and WW was moderate (0.15 ± 0.003 (DVM), 0.23 ± 0.030 (APM)) and as a consequence should respond to targeted selection.

Spat collection and experimental culture of the Atlantic pearl oyster, *Pinctada imbricata* (Bivalvia: Pteriidae), under suspended conditions in the Caribbean

Velasco L.A., Barros J.

Source: Journal of the World Aquaculture Society 41(3): 281–297 (2010)

Several suspended culture experiments of the Atlantic pearl oyster, *Pinctada imbricata*, were carried out in the Colombian Caribbean. Seed was collected at two seasons of the year (rainy and dry) using onion bags collectors. Shell growth and survival of the oysters, as well as frequency and size of predators, were evaluated under different stocking densities (10, 20, 30, and 40% coverage of the bottom of the net), culture systems (pearl nets and pocket nets), seasons (rainy and dry), and depths (4 and 9 m). A greater amount of *P. imbricata* spat was collected during the dry season (297 ind./m²) than in the rainy season (72 ind./m²). Density influenced significantly the growth of *P. imbricata* but not its survival. The highest antero-posterior measure (APM) values appeared at lower densities (10 and 20% bottom coverage). Growth and survival values of the pearl oysters were greater in pocket nets than in pearl nets. Culture season did not affect the survival of the oysters but their growth was greater in the dry season than in the rainy season. Culture depth influenced oyster growth but not their survival, with recorded shell APM being greater at 9 m than at 4 m. The follow-

ing predators: Cymatiidae, Portunidae, Majidae, and Xanthidae were found inside the culture nets. Highest mortality values of the pearl oysters coincided with high frequency and/or size of predators. Frequency of cymatids was higher during the rainy season and in pearl nets with high oyster density. Xantids appeared mostly in pocket nets and majids during the dry season.

Evaluation of natural and commercial probiotics for improving growth and survival of the pearl oyster, *Pinctada mazatlanica*, during late hatchery and early field culturing

Aguilar-Macías O.L., Ojeda-Ramírez J.J., Campa-Córdova A.I., Saucedo P.E.

Source: Journal of the World Aquaculture Society 41(3):447–454 (2010)

Survival and growth of pearl oyster, *Pinctada mazatlanica*, juveniles fed microalgae supplemented with natural and commercial probiotics were measured for 21 d at the hatchery. Probiotics tested were (1) a *Lactobacillus* sp., (2) a mix of two bacilli, *Burkholderia cepacia* and *Pseudomonas aeruginosa*, (3) a marine yeast, *Yarrowia lipolytica*, (4) Epicin-hatchery® as commercial probiotic, (5) an antibiotic oxytetracycline, and (6) the control group fed *Isochrysis galbana*, *Pavlova salina*, and *Chaetoceros muelleri* only. When the hatchery phase ended, the effects of probiotics were followed during the early stages of field cultivation (90 d). Different from the control group, natural probiotics significantly improved performance of juveniles during both phases, particularly in the field. The treatment with *Lactobacillus* sp. significantly increased survival by 72%, growth in shell height by 63%, and growth in wet weight by 83% over the control. The marine yeast and mix of bacilli provided intermediate results, enhancing survival by 55–65%, shell height by 55–58%, and wet weight by 70–76% compared with the control. Conversely, growth and survival in the treatments with Epicin and oxytetracycline were significantly lower than in the control. These results show the potential of natural probiotics for improving hatchery rearing of this pearl oyster.

Xenografts and pearl production in two pearl oyster species, *P. maxima* and *P. margaritifera*: Effect on pearl quality and a key to understanding genetic contribution

McGinty E.L., Evans B.S., Taylor J.U.U., Jerry D.R.

Source: Aquaculture 302(3–4):175–181 (2010)

Mantle xenografts between two *Pinctada* pearl oyster species, *P. maxima* (silver-lip pearl oyster) and *P. margaritifera* (black-lip pearl oyster), were used to examine their influence on pearl quality and to further our understanding of the respective contribution host and donor oysters have in pearl formation. Here, host oysters were implanted with mantle tissue originating from either the same species (allograft) or the other species (xenograft) and pearl formation allowed to proceed for 14 months, where after pearls were harvested and nuclei retention and pearl quality traits (nacre deposition/weight, shape, colour, complexion and lustre) recorded. Results showed that xenografts did not significantly affect pearl sac formation and subsequent nuclei retention ($F = 5.64$, $df = 3$, $P < 0.05$), but did influence pearl colour, complexion, shape, nacre deposition and nacre weight ($P < 0.05$). Nacre deposition and weight were found to be higher in xenografts comprising of a *P. maxima* donor and *P. margaritifera* host, than in the reciprocal xenograft and allografts. Pearl colour and complexion were also strongly influenced by the donor oyster species used as xenografts, with *P. maxima* host oysters implanted with mantle from *P. margaritifera* producing generally black colour based pearls. Conversely, *P. margaritifera* hosts implanted with *P. maxima* mantle produced silver colour based pearls. This study demonstrates the potential of xenografts as a means to improve pearl quality traits such as pearl size, and highlights the role that donor oysters have in the realisation of pearl growth, colour and surface complexion.

Comparative morphometric study of the invasive pearl oyster *Pinctada radiata* along the Tunisian coastline

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Source: Biologia 65(2):294–300 (2010)

In order to study the relative growth of the pearl oyster *Pinctada radiata* in Tunisia, a total of 330 individuals of this species were collected from six sites along the Tunisian coastline. Quantitative measurements of collected oysters were conducted for shell height, shell length, shell width, hinge length, height and width of the nacreous part and wet weight. The size structure of the sampled populations was described and the relative growth between different morphometric characteristics was estimated as allometric growth lines for the six *P. radiata* samples. It appeared that the majority of examined samples were dominated by large individuals that exceeded a shell height of 42 mm. The maximum size (100.5 mm), recorded in Bizerta lagoon, is bigger than that recorded elsewhere in particular in the Red Sea. Size distribution analysis also showed that the majority of *P. radiata* samples were dominated by two or more size groups. Differences of allometric regression were found between

the examined samples for the tested relationships. Moreover, the Factorial Discriminant Analysis, coupled with Ascending Hierarchic Classification, classified the sub-populations according to geographic locations.

A histological examination of grafting success in pearl oyster *Pinctada margaritifera* in French Polynesia

Cochennec-Laureau N., Montagnani C., Saulnier D., Fougerouse A., Levy P., Lo C.

Source: Aquatic Living Resources 23(1):131–140 (2010)

Pearl oyster grafting is a complex surgical operation that should lead to pearl formation after approximately eighteen months. Although this technique has been used for many years in French Polynesia, the grafting process is still not standardised. While studies have been carried out in order to improve graft performance and yield, these remain highly variable due to post-grafting mortality, nucleus rejection and unreliable pearl quality, all of which constrain pearl farm profitability. The present study uses histological analysis to monitor oysters that either rejected or retained their nuclei. Both groups of oysters are compared in terms of evolution of the graft, which could influence retention, and the development of a pearl sac in cases where grafting was successful. Data show that rejection phenomena are linked to a number of causes, notably an inflammatory reaction in the “receiving” oyster, the presence of numerous tissue lesions and the quality of the grafted tissue. These results suggest that study is needed on the different concomitant elements of the grafting process: the graft “donor” oysters, the nucleus and the “receiving” oyster and their interactions.

Occurrence of the protozoan parasite, *Perkinsus olseni* in the wild and farmed pearl oyster, *Pinctada fucata* (Gould) from the Southeast coast of India

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Source: Aquaculture 299(1–4):8–14 (2010)

The pearl oyster, *Pinctada fucata* (Gould), is a commercially important bivalve distributed in the Gulf of Mannar along the southeast coast of India and had supported a healthy, traditional pearl fishery until the 1950s. But, during the past few decades, the natural pearl oyster beds in the Gulf of Mannar have showed a sharp decline leading to the closure of the traditional pearl fishery and was presumed to be due to over-exploitation and pollution. Except for a preliminary report on *Perkinsus marinus* infection in *Crassostrea madrasensis* in 1988, no incidence of perkinsosis or other Office International des Epizooties (OIE) notified protozoan infections in mollusks has been reported from the Indian subcontinent and the pathogen profile of *P. fucata* from the region has not been studied. Since *Perkinsus* spp. is known to have destroyed many oyster beds worldwide, the present study was taken up to screen the pearl oyster population along the Gulf of Mannar coast for the presence of *Perkinsus* spp. and examine the probable role of *Perkinsus* in the decline of the natural pearl oyster beds. Thirty individuals of adult *P. fucata* were collected from wild populations at three different locations in the Gulf of Mannar and 10 individuals from a pearl farm containing hatchery reared stock at Tuticorin. The tissue samples were subjected to Ray's fluid thioglycollate medium (RFTM) culture, histology and polymerase chain reaction (PCR). All the samples showed enlarged blue-black hypnospores in RFTM, indicating the presence of *Perkinsus* spp. *Perkinsus*-like organisms were also observed in the histological preparations. Screening of the tissues using the *Perkinsus* genus specific internal transcribed spacer (ITS) 85 and ITS 750 primers, amplified the product specific to the genus *Perkinsus* (ca. 700 base pairs) and further, the specific identity of the parasite was determined by sequencing the amplified PCR products which showed 99% identity to *Perkinsus olseni*. The pairwise genetic distance values and phylogenetic analysis also confirm that the present isolate from *P. fucata* is a member of the *P. olseni* clade. This preliminary investigation suggests a possibility that perkinsosis could be one of the major reasons for the decline of the *P. fucata* beds in the Gulf of Mannar over a period of time. This forms the first report on the existence of *P. olseni*, an OIE listed pathogen in the wild and cultured *P. fucata* populations from the Indian subcontinent.

A layered structure in the organic envelopes of the prismatic layer of the shell of the pearl oyster *Pinctada margaritifera* (Mollusca, Bivalvia)

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Source: Microscopy and Microanalysis 16(1):91–98 (2010)

The organic interprismatic layers of the mollusc *Pinctada margaritifera* are studied using a variety of highly spatially-resolved techniques to establish their composition and structure. Our results show that both the interlamellar sheets of the nacre and interprismatic envelopes form layered structures. Additionally, these organic layers are neither homogeneous in composition, nor continuous in their structure. Both structures play a major role in the biomineralization process and act as a boundary between mineral units.

Comparative regeneration of excised mantle tissue in one year and seven year old Indian pearl oyster, *Pinctada fucata* (Gould) grown under land-based culture system

Rao G.S., Pattnaik P., Dash B.

Source: Indian Journal of Fisheries 57(1):39–43 (2010)

Excised mantle tissue (saibo) from the donor oyster is one of the important factors determining quality of cultured pearls. The present study was conducted to compare the process of regeneration of excised mantle tissue in one year and seven year old donor oysters, *Pinctada fucata* grown under land-based culture system. Menthol was used as relaxant prior to excision of mantle tissue, which was found to be effective at a concentration of 500 mg L⁻¹. The mantle tissue was found to regenerate within 3 months post-excision in both one year as well as seven year old *P. fucata*, with 100% survival. On gross examination, the regenerated mantle tissue of the 1 year as well as the 7 year old oysters appeared similar to that of the normal mantle tissue of the control group. Histological analysis demonstrated complete regeneration of the mantle tissue and its associated structures in both the groups. This is the first description of in vivo mantle regeneration in 7 year old pearl oyster, *P. fucata*. The findings revealed that even aged donor oysters yielding good quality saibo can be recovered after mantle excision and could be further used as saibo donors for quality pearl production.

Conference presentations

World Aquaculture Society Annual Conference: World Aquaculture 2011, Natal, Brazil

Breeding for perfection – A journey towards understanding the genetics behind production of gem quality South Sea Pearls

Jerry D.R., Evans B.S., Zenger K., Taylor J., Lind C.E., Kvingedal R., McGinty E., Safari A.

The production of a cultured pearl is the result of a complex interplay between two pearl oysters, whereby a mantle tissue graft originating from a donor oyster is implanted along with a calcite seed nucleus into the gonad of a second host oyster. The donor tissue degenerates to a single epithelia cell layer which grows around the implanted nucleus to form a pearl sac and subsequently a pearl. Despite pearl aquaculture being worth ~USD 625 million dollars annually, there currently are few genetic improvement programs for pearl oysters. One of the major reasons for the paucity of improvement programs is a lack of understanding of the genetic basis of how a pearl is produced, and in particular whether traits such as pearl colour, lustre, shape and surface complexion are genetically determined and can be improved through selection. Additionally, given the potential role of the two oysters needed to produce a cultured pearl, it is not known if selection needs to be more targeted towards the donor or the host oyster.

In 2005, researchers at James Cook University were approached by a commercial pearling company to help them commence a selective breeding program for *P. maxima*, which produces the much sought after large “South Sea” pearl and we began for the first time to gather information on the genetic basis of pearl formation. In this presentation I will outline the journey we have taken to unravel the complex genetic basis of pearl formation starting from manipulating the phenotype of a pearl, determining the quantitative genetic basis of pearl quality traits, to ultimately sequencing the pearl oyster transcriptome in the search for quantitative trait locus (QTL) linked to quality traits.

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