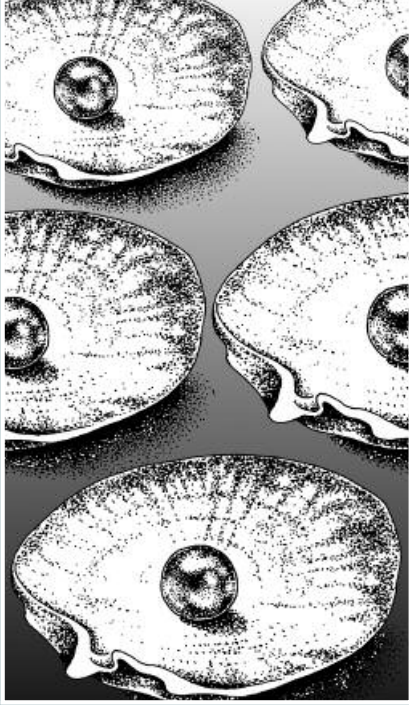

Secretariat of the Pacific Community

Issue 17 - November 2006

# PEARL OYSTER information bulletin

## Inside this issue



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## Editorial

We are back!!! It has been a long time coming, but here is the 17th issue of the *SPC Pearl Oyster Information Bulletin.*

I have assumed the duties of Editor and I would like to thank Neil for the fine job he did as Editor of all previous issues of the Bulletin since 1990. The Bulletin will maintain the same general format that was developed by Neil with a few minor changes. In particular, we encourage submission of longer articles and will be seeking regular updates from major research groups and country statements, where appropriate. The required format for larger articles submitted to the Bulletin can be found on the SPC website at: http://www.spc.int/coastfish/News/POIB/POIB.htm. This website also provides access to previous issues of the Bulletin.

This is a large issue given the two and a half years since the last one. There were pearl oyster sessions at the past two World Aquaculture Society Annual Conferences in Honolulu (2004) and Bali (2005). Abstracts from the Bali meeting are included in this issue together with a large number of abstracts from the scientific literature. Of note in these sections of the Bulletin is the increasing importance of research on pearl oyster genetics and this is further illustrated by abstracts from the IAGA International Symposium on Genetics in Aquaculture, held in Montpelier, France (June 2006).

In the last issue of the Bulletin, Neil commented on the "green" aspects of pearl farming — a theme continued here. As well as abstracts reporting the potential bioremedial benefits of pearl oyster culture, an article in the "Research Notes and Reports" section describes how half-pearl production is contributing to the management plan of a marine reserve in Tanzania. Environmental benefits in the form of increased biodiversity have also been reported at pearl farming sites in the Philippines where an improved environment is thought to result in higher pearl quality. This issue also contains an article outlining innovative technologies adopted at Manihiki as part of a programme to monitor and manage the lagoon environment to ensure the long-term sustainability of the industry. Articles in the "Industry Notes & Reports" section outline trends in production and demand and report increasing global pearl production. This is highlighted by figures suggesting that production of Australian whites in 2006 will be more than double that in 2000! Good sales have been reported in the past year, although demand has not kept pace with production.

This issue also contains a summary of the SPC Regional Pearl Meeting, held in Fiji in December 2005. Given the high level of interest in further development of commercial pearl production within the region, this meeting allowed

a timely overview of production and research activities and identification of bottlenecks and common development goals. The meeting generated a number of recommendations, which were presented to the SPC Heads of Fisheries Meeting in Noumea in April, 2006. They covered issues such as policy, networking and information, marketing, research and development, infrastructure and training.

We are aiming for two issues of the Bulletin per year and I encourage submission of articles that will help us achieve this. We welcome longer research-type articles, reports from researchers and research groups, and articles outlining pearling activities in member countries as well as your comments and any other interesting news. I look forward to hearing from you.

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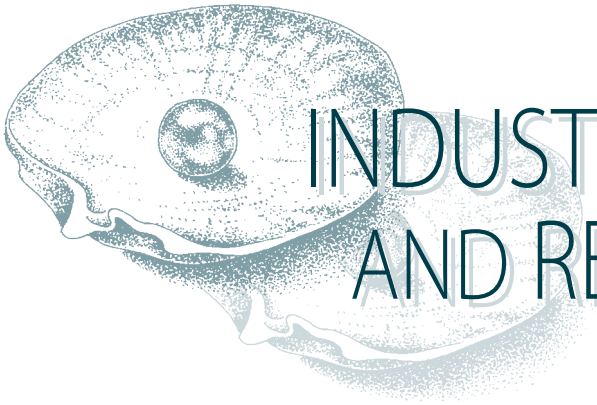
PIMRIS is a joint project of five international organisations concerned with fisheries and marine resource development in the Pacific Islands region. The project is executed by the Secretariat of the Pacific Community (SPC), the South Pacific Forum Fisheries Agency (FFA), the University of the South Pacific (USP), the South Pacific Applied Geoscience Commission (SOPAC), and the South Pacific Regional Environment Programme (SPREP). This bulletin is produced by SPC as part of its commitment to PIMRIS. The aim of PIMRIS is to improve the availability of information on marine resources to users in the region, so as to support their rational development and management. PIMRIS activities include: the active collection, cataloguing and archiving of technical documents, especially ephemera ('grey literature'); evaluation, repackaging and dissemination of information; provision of literature searches, question-and-answer services and bibliographic support; and assistance with the development of in-country reference collections and databases on marine resources.

PIMRIS

Pacific Islands Marine Resources Information System



# INDUSTRY NOTES AND REPORTS

## Scanning the pearl world

**Source:** Pearl World, The International Pearling Journal (April/May/June 2006)

Pearl production is up, and so is demand. Paspaley auctions reportedly enjoyed its best sales in five years this past year in Hong Kong, selling over 150,000 pearls — including all the baroques, indicating a major new trend — with a value of more than US$ 13 million.

But demand, though rising, isn't keeping pace with skyrocketing production. Unsold pearls numbered close to 33,000, leaving growers with a great deal of old inventory to sell at the next auction, even as pearl production continues to rise, driving down prices.

Quality is also rising. According to producers, this is the result of an improving growing environment, especially for South Sea farms in Indonesia, the Philippines and Australia.

In fact, studies in the Philippines show marine diversity increasing 21% in pearl-farm sites compared with unprotected areas. The result is higher-quality pearls and better regeneration of the *Pinctada maxima*.

For reasons that aren't entirely clear, production numbers for South Seas and Tahitians are expected to decrease over the next year or so as a result of fewer oysters being available for grafting.

Martin Coeroli, General Manager of GIE Perles de Tahiti, says that may push prices up 10–20%, but that outcome is likely only for high-lustre, clean, true-peacock-colour 8 mm and 9 mm rounds.

Robert Wan's Tahiti Perles, the largest grower in the French Polynesian islands, sold over 190,000 pearls at its Tahitian pearls auction in Hong Kong last spring. To improve, or at least stabilise, profits without increasing supplies, Wan has chosen to price his goods using the more stable Euro instead of the US dollar. This has raised prices in the United States.

### Expect the best

One indication of the overall high quality of cultured pearls is that more are labelled "metallic lustre" than ever before.

Another sign is that sizes are big and getting bigger. Akoyas are at 9+ mm, and South Sea keshis are at a whopping 15–16+ mm. The wide variety of shapes and natural colours allows retail jewelers to carry hundreds of different items using only one gem material.

### Demand by origin

Australian whites are as popular as they've ever been. According to figures published in Pearl World newsletter, whites from the *Pinctada maxima* are expected to peak at over 9 tons in 2006. By weight, this represents an increase of 260% over production six years ago.

A decade ago, the big round and baroque whites accounted for only 20% of the market. Today, at 50% of total pearl production, they dominate the market. This means that prices for retailers have either become more affordable, as with the large baroques, or have increased relatively painlessly.

Indonesian and Philippine golds are popular, but the recent revelation that golds can be dyed, even

without drilling, has led to more laboratory identifications, adding to costs.

Tahiti deserves credit for its quality-control process, which has helped keep cheap, low-quality pearls out of the market.

While nacre thickness is somewhat disappointing from a purist's point of view, nacre of slightly greater than 0.5 mm is acceptable. To qualify as a Tahitian, a pearl's nacre must be 0.8 mm or thicker, which is more than enough for a bed-nucleated pearl. The Japanese Akoya, after all, with 0.5 mm nacre thickness, was praised for over a century. Chinese rounds and near rounds are plentiful.

### Japanese/Chinese Akoyas

Note that the phrase "Japanese Akoyas" is no longer used, since most strands combine Chinese and Japanese Akoyas. The consensus is that it doesn't matter where they're from as long as the quality is high.

But don't be fooled by tags that say "Made in Japan". The strand is made in Japan, but the pearls on it may be Chinese.

## Golay's global view

**Source:** Pearl World, The International Pearling Journal (January/February/March 2006)

### 21st century pearl renaissance

Pearls have registered a significant revival in recent years. Consumers today are rediscovering the contemporary charm of this aquatic gem. Perceived for generations as classically elegant around the neck of ladies of good families, pearls have taken on a very different allure in the 21st century.

We have always known pearls to be white, round and discreet, but nowadays pearls can be ostentatiously big, in a rich palette of intriguing nuances, taking the form of various fanciful, organic shapes. Celebrities and models on catwalks are lavishly draped with stacks of long sautoirs, heralding a new era of pearl renaissance.

The significant growth in demand for pearl jewellery from 1995 to 2005 can be attributed to four major factors:

1. Full-fledged pearl farming industries in different Pacific regions benefit from accumulated experience and technological breakthroughs, leading to pearl harvests of improved quality and quantity.

2. A profusion of creative pearl jewellery designs in an extensive price range fulfils the demand for *haute joaillerie*, medium to high-end fine jewellery and affordable fashion accessories; and targets all market segments including fine jewellery for mature women, fashion accessories for fashion-conscious clientele and the niche of men's accessories.

3. A fast-growing pearl jewellery retail network comprises independent jewellers, chain stores, department stores, catalogue houses, fashion boutiques and Internet companies.

4. High-profile promotions have been conducted by pearl organisations and companies on a local, national and international scale to reposition pearls as having contemporary chic.

The acquisition rate of pearl jewellery has subsequently increased from the standard 2 to 5 per cent in the past to about 10 per cent, i.e. for every 100 pieces of jewellery sold, about 10 items include pearls as the principal gems. Pearls have become the third major jewellery category after diamond jewellery and plain gold jewellery.

In mature markets where consumers possess a good knowledge and awareness of pearls, such as Japan where the technique of pearl culture originated 100 years ago, the pearl jewellery segment accounts for up to 17 per cent of total jewellery sales. It is estimated that about US$ 1,200 million are spent on pearl jewellery in Japan per year.

Since 1999, the United States has overtaken Japan to become the largest pearl jewellery market, with estimated sales of US$ 1,500 million of pearl jewellery per year.

In fast-growing economies in Southeast Asia, China, Russia and the Middle East, the year-on-year growth rate for pearl jewellery outperforms the rate in the established markets of Japan and Europe.

**Estimated value of pearl production 2004**

| | Market share in value | Production value 2004 |
|---|---|---|
| White South Sea cultured pearls (Australia, Indonesia, the Philippines, Myanmar) | 35% | US$ 220 million |
| Freshwater cultured pearls (China) | 24% | US$ 150 million |
| Akoya cultured pearls (Japan, China) | 22% | US$ 135 million |
| Tahitian cultured pearls (French Polynesia) | 19% | US$ 120 million |
| Total | | US$ 625 million |

Globally, the pearl jewellery market is estimated at US$ 5,000 million, about 10 per cent of the world's total jewellery market.

## Evolution of the pearl jewellery market

Jewellery consumption patterns have shifted in recent years with changing lifestyles and consumer purchasing modes. Self-purchases by women and the rising need for jewellery that can be worn in the day to match outfits and moods have reshaped the jewellery landscape. With a wide spectrum of versatile designs and a highly elastic price range, pearls fit well into the new aspirations of modern consumers.

Between the 1920s and 1970s, most cultured pearls found on the market were Japanese Akoya pearls (white, round, majority 4–8 mm in diameter). Most were sold as classic necklaces for US$ 1,000 to US$ 10,000 per strand targeting the medium to high-end jewellery market segments for a clientele for classic jewellery.

Thirty years later, the mature South Sea pearl industry has brought out large-size South Sea pearls (mostly 9–15 mm), spurring the demand of the top-end prestige clientele for exclusive necklaces over US$ 10,000. Tahitian pearls in large sizes (8–14 mm) and exquisite dark colours are also creating demand for flamboyant, non-conformist pearl jewellery.

On the other hand, a significant quantity of nice-quality freshwater pearls from China at affordable prices is rendering pearls accessible to the mass market in both developed and developing countries. Designers are readily exploiting these affordable gems and creating fanciful accessories, extending the appeal of pearls to the fashion world.

Consumers looking for prestige and status are likely to go for large, higher-valued South Sea pearl jewels. Those with classic taste are more likely to prefer an elegant Akoya pearl necklace of discrete beauty. More audacious and less conventional jewellery lovers may desire the exotic Tahitian pearls. For those who want fashionable accessories to match their seasonal chic, freshwater jewels are the ideal purchase.

In the 21st century, we live in an age when the largest and most beautiful pearls ever produced anytime in history are available and we can enjoy the widest choice of cultured pearls ever offered.

# Risen from the ashes

## A new pearl group attempt: But will it succeed?

**Source:** Pearl World, The International Pearling Journal (January/February/March 2006)

A new umbrella association for the global pearl industry, the International Pearl Organisation (IPO), held its inaugural meeting in September in Hong Kong, calling for genuine, wholehearted support from the worldwide cultured pearl trade for its establishment. About 20 representatives from 14 pearl producing and consuming countries and regions attended this first convocation.

In general, participants supported the formation of the IPO, but were concerned about funding and support from major pearl producers, in particular Paspaley Pearling Co. Pty. Ltd, Australia, which had no representative at the meeting.

Initiated by the President of the CIBJO and also General Manager of GIE Perles de Tahiti, Martin Coeroli, the IPO was seen as a revival of the International Pearl Association, which was formed in 1994 with a view to addressing various topics regarding the pearl sector, from production and trading to promotion and a code of ethics.

People may remember that the IPA, which started up with such enthusiasm, was boycotted by the Japanese and ultimately fell apart due to internal bickering and mismanagement.

Sometime later, Mr Tasaki started a similar group called the WPO (World Pearl Organisation), but this, too, withered and died on the vine.

After both of these failures, sceptics said that there would never be a successful pearl cartel due to conflicting egos and a lack of cooperation between most producing regions and among the major personalities in the producer ranks (who would have to be tapped for the financial resources needed to support coordinated activities).

It is hoped that Mr Coeroli can overcome these historic shortcomings, especially as he is in a perceived neutral leadership position within the industry as a CIBJO executive, and is someone who has been acclaimed as an international pearl marketing expert.

Mr Coeroli said that given Hong Kong's status as the trading centre for all pearl categories, he expected the IPO to be based in Hong Kong and that it would be formed by September 2006.

He explained at the meeting why establishing this organisation was essential: "The strong internal and external competition that our pearl sector is facing, on both production and sales and distribution levels, and the fragmentation of our industry, require more than ever a global body to defend and promote its interests on a worldwide level. IPO is the authoritative body that regroups the main actors and organisations of the pearl industry and that defends and promotes their interests at an international level. As a modern, customer-focused organisation, it also provides key services and support to all of its members and stakeholders".

He stated that the mission was to create a unique platform for the pearl industry; raise awareness of the power of the unique beauty and natural characteristics of pearls; develop knowledge of, and create excitement about pearls among consumers worldwide; and, ultimately, contribute significantly to the growth of the entire pearl industry and its members.

A tentative budget for the first year of operation amounting to US$ 2.55 million was also proposed at the meeting.

Tahiti Perles in French Polynesia and Jewelmer International Corporation in the Philippines were the only major producers attending the meeting, which was held alongside the September Hong Kong Jewellery and Watch Fair.

There were no representatives from Paspaley Pearling Co. Pty. Ltd., the largest pearl producer in Australia, nor from Indonesia or China, important producers of South Sea pearls and freshwater pearls, respectively.

Mr Coeroli said he would maintain efforts in communicating with the Indonesian Pearl Culture Association or ASBUMI and with Robert Sukendy, the largest SSP producer in Indonesia. He said he would also contact the Hong Kong Pearl Association and the Professional Association of Pearls, Zhejiang, to gain support for the organisation from the Chinese freshwater pearl sector.

At the meeting, a steering committee with representatives from different countries was formed to work out the structure and funding of IPO. The members of this committee are Andy Muller of Autore Japan KK; Vidhan Chaudhari of Orient Pearl Co. Pty. Ltd; Yoshihiro Shimizu of Hosei Co. Ltd; Sonny Sethi of Tara and Sons Inc.; David Norman of Aquarian Pearls Pty. Ltd, and Till Schoeffel of Shoeffel GmbH.

# Akoya pearl aquaculture development in Hervey Bay

*Ross Lobegeiger*

**Source:** Queensland Aquaculture News, Issue 28, July 2006

Coral Sea Pearls has established an Akoya oyster (*Pinctada imbricata*) growout operation at Hervey Bay to produce high quality Akoya pearls. This oyster occurs naturally in Queensland. The oysters farmed by Coral Sea Pearls have been the progeny of these local native oysters.

There is a strong demand for Akoya pearls. They are smaller in size (6–10 mm in diameter) than the white South Sea pearls. Their shape is considered perfect. The high quality of the pearls is due to the excellent water quality and unique combination of marine and estuarine waters in the middle part of the Great Sandy Straits, and the water temperature range, which is optimal for producing high quality pearls from Akoya oysters.

## History

The pearl oyster is native to Australian coastal waters. It was first identified more than 100 years ago and is found from Victoria all the way around the northern coastline of the continent to Shark Bay in Western Australia. In the early 1990s, before farming began, the Australian Museum noted the location of several native populations of Akoya pearl oyster in Hervey Bay.

A trial pearl oyster lease has operated in Port Stephens, NSW, since 1999. The trial and other tests indicated that Port Stephens was the best place in eastern Australia to grow high quality Akoya pearls, but Queensland has now proved to be even better.

Coral Sea Pearls has used the experience and expertise from Port Stephens Pearls to develop an Akoya pearl industry in Queensland. The clean waters and good tidal circulation in Queensland provide just the right conditions to produce big, beautiful, lustrous pearls.

## Farm site

Hervey Bay has a long tradition of aquaculture and there are experienced local oyster growers who can contribute their skills to the new industry. Many of the service industries required to support the development of a pearl oyster industry are also in Hervey Bay. It is believed that pearl growing and pearl sales will complement the well-established tourist industry in the Bay.

Akoya pearl oysters are growing on four leases inside Hervey Bay. Pearl oyster will be grown in panel nets at these locations, which provide optimal conditions for pearl production. The land-based site that will be utilised for the pearl operation is located at Urangan boat harbour.

## Environmental considerations

Coral Sea Pearls is aware of how highly the community values the presence of resident and visiting marine wildlife, such as whales, dolphins, turtles and dugong, in Hervey Bay. These animals are an important natural asset for the community and highlight the healthy water quality in the estuary.

Coral Sea Pearls has designed the pearl leases to minimise any potential threat to these species. By following strict company Environmental Codes of Practice on these leases in Hervey Bay, the risk of any entanglement of migrating whales is eliminated. Oyster bags and panels will be secured by taut ropes to minimise the potential for entanglement of any resident fish, dolphins, turtles and dugong.

A detailed, ongoing fauna-monitoring programme is being undertaken for the Department of Environment and Heritage (DEH). Coral Sea Pearls has the only approvals in place given by DEH within Queensland for any aquaculture operation.

# RESEARCH NOTES AND REPORTS

## SPC Regional Pearl Meeting, December 2005, Fiji

*Ben Ponia*[1]

*The Tokatoka Resort in Nadi was the setting for the first regional pearl meeting. Considering the economic significance of this industry and the widespread interest of Pacific Island countries in developing their cultured pearl industry, a regional forum to discuss technical issues has been long overdue. Critical challenges for the sector were identified as seed supply — increasing hatchery capacity; farming — utilising technological advances, such as triploid oysters; pearl quality — improving seeding technician standards; and sales — strengthening marketing.*

### Background

Cultured pearls are among the most alluring products of the Pacific. Although the early days of the gold rush mentality are over, the Pacific Islands remain determined to develop local industries in spite of the challenges involved. This resolve has been evident through past and current efforts.

Pearls are rated as a priority commodity by the Pacific Community. The regional pearl meeting, organised by the Secretariat of the Pacific Community (SPC), was held at the Tokatoka Resort, Nadi, Fiji, from 31 November to 2 December 2005. The meeting's objectives were simple: to provide a technical round-table forum to benchmark the status of pearl production in the Pacific, share information of common interest, and explore areas for technical collaboration.

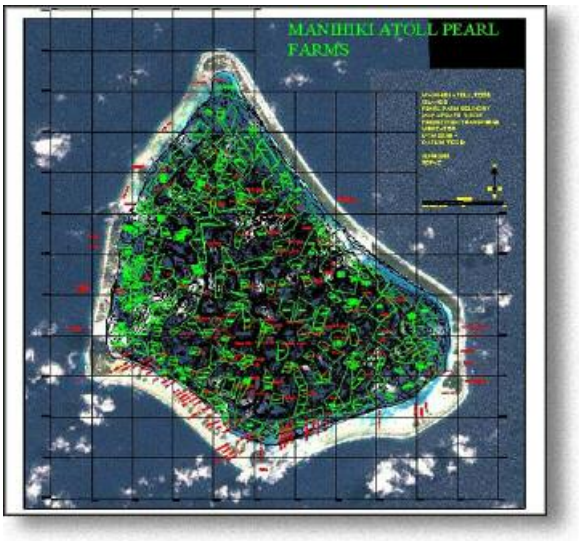
Meeting participants included representatives from governments, the private sector and academia. The meeting was chaired by Mr Maciu Lagibalavu, Director of Aquaculture, Fiji Ministry of Fisheries.

### Status of pearl farming in the Pacific Islands

French Polynesia remains the powerhouse producer of pearls in the Pacific followed by the Cook Islands with relatively smaller production. Other countries including Fiji Islands, Marshall Islands, Federated States of Micronesia, Tonga, Papua New Guinea, Solomon Islands and Kiribati are in varying stages of commercialisation. In recent years, the average value of pearl exports from the Pacific has been around USD110 million per annum — a decline from a peak of about USD170 million in 2000 mainly caused by an oversupply of pearls and poor quality.

Whilst countries mainly target the cultivation of *Pinctada margaritifera* oyster for its black pearl, there is an opportunity to diversify the range of pearls coming out of the Pacific. For example, Solomon Islands and Papua New Guinea have naturally occurring stocks of *Pinctada maxima*, which produces a luminescent white pearl branded as the South Seas Pearl. A commercial operation in Milne Bay, Papua New Guinea, is presently farming *Pinctada*

1. SPC Aquaculture Adviser. Email: benp@spc.int




**Pearl farm mapping at Manihiki Atoll in the Cook Islands**

*maxima*. The winged oyster (*Pteria penguin*) is found in Fiji and Tonga and can be cultivated for a half pearl with purple undertones similar to those of abalone pearls.

There are diverse development strategies for emerging players in the region. Fiji Islands has made significant progress in commercial production and the 10-year government plan aims for a FJD40 million industry. The private sector is already marketing Fiji Pearls in an exclusive bracket. In Micronesia, the results of hatchery and farm trials are being adapted for small-scale opportunities suited to rural communities, as explained by Mr Masahiro Ito from the College of Micronesia. An example of the feasibility of this approach is provided by Nukuoro atoll where a small pearl farm is being successfully operated under the autonomous management of the local community.

## Constraints and opportunities

In countries where natural stocks of oysters are low, the lack of seed supply is a bottleneck constraining the expansion of pearl farming. Given that the investment time for a pearl enterprise is at least in the order of 10–15 years, it is critical to secure a consistent source of oysters. Fortunately, the technology for breeding pearl oysters is fairly well developed and there are a number of operational hatcheries to learn from. The low-cost, low-technology pearl hatchery on Kiribati continues to sustain an unusually high rate of pearl spat settlement. However, government programmes also often underestimate the level of dedicated effort required to operate hatcheries efficiently and to create the investment climate necessary for commercialisation.

The Pacific region should be supportive of continued pearl research to ensure that it does not lose out on advances in technology that may offer significant benefits. For example, sterile oysters induced through triploidization divert their energy from reproduction into growth. This could significantly reduce the time (and cost) to incubate the pearl, ultimately increasing profitability. Establishing a genetic improvement programme could enable the selection of oysters that generate pearls of specific colour or size.

Environmental management becomes crucial as farming levels intensify. This lesson was learnt the hard way in the Cook Islands, which is still recovering from a severe oyster disease at Manihiki Atoll in 2000, due in part to overstocking. Management measures outlined by Mr Kori Raumea from the Cook Islands Ministry of Marine Resources involve (1) regular environmental monitoring, such as testing of water quality using automated probes deployed on a remote buoy, (2) a digital mapping system to allocate farm leases

**Pearl farm being prepared in Micronesia**

and analyse oyster density, and (3) new legislation and an Atoll Lagoon Management Plan to encourage best farming practices.

The pearl grafting operation ("pearl seeding") is perhaps the most important factor directly affecting pearl quality and it accounts for a significant proportion of production costs. Dr Maria Haws from the University of Hawaii provided some straightforward arguments that clearly show why a poorly skilled seeding technician can drastically reduce revenue to unprofitable levels. According to her analyses, if an "excellent performing" technician and a "poor performing" seeding technician are provided with a thousand oysters, the excellent technician will generate USD19,000 dollars revenue compared to just USD8,600 dollars from the poor technician (Table 1). Even with lower fees, a poor performing technician will still lead to unprofitable levels of revenue.

Clearly, if the Pacific wishes to improve the profitability of the pearl sector, then investing in programmes to raise the standard of pearl-seeding technicians will be essential.

Pearls are jewellery items and maintaining a marketing campaign is essential to entice buyers to these discretionary products. No segment of the industry understands this better than the private sector. Pearl farmers, Mr Temu Okotai from the Cook Islands and Mr Justin Hunter from Fiji shared their experiences of marketing in the business world. In addition, Mr Hunter outlined the integration of their Savusavu-based farm with the aspirations of the local qoliogli, so local communities can share in company profits. Mr Okotai raised the prospect of a Pacific brand for marketing pearls and challenged the region to work together for mutual benefit rather than to compete. The recent stabilising of pearl prices brings some optimism to the marketing environment, although the adage that "high quality pearls will always sell" remains true.

Dr Quentin Fong from the University of Alaska provided economic sensitivity analyses based on a Northern Pacific farm model showing that variation in marketing price is the factor that has the greatest impact on profitability and cost. A one per cent increase (or decrease) causes a five per cent increase (or decrease) in net profits. His findings validated the earlier presentations on the importance of marketing.

## Recommendations for regional collaboration

The following recommendations were made by meeting delegates to synthesise current challenges in the pearl sector and formulate strategies to address them, with an emphasis on the need for regional collaboration.[1]

### *Policy, networking and information*

1. The lack of clear policies in the pearl sector must be addressed.
   a. Existing policies should be revisited to amend or create clear policies where needed;
   b. All stakeholders must be involved in this process.

2. The lack of regulations and legislation — particularly in the area of water rights, leases and tenure for private sector individuals — must be addressed.
   a. Clear regulations and legislation should be put in place.

**Table 1. Performance measure and profitability for varying levels of technician skills (seeding 1000 pearl oysters and charging a fee of USD 3.00 per oyster).**

| Parameter | Technician 1 "Excellent" | Technician 2 "Average" | Technician 3 "Poor" |
|---|---|---|---|
| ***Results at harvest*** | | | |
| Poor quality, unsaleable pearls (%) | 20% | 30% | 40% |
| ***Revenue at harvest*** | | | |
| Revenue from pearls | US$ 19,000 | US$ 13,300 | US$ 8,600 |
| Seeding costs as % of revenues | 37% | 53% | 81% |

1. These recommendations were presented to the SPC Heads of Fisheries Meeting, Noumea, 3–7 April 2006, and subsequently endorsed by country representatives.

3. Policies should incorporate the obligations of a grafting permit required by a seeding technician.
   a. Since many seeding technicians are foreigners, such a permit may need to be incorporated in foreign investment policies.

4. A regional association should be established as a commission for the pearl sector.
   a. Its formation could be endorsed through the Pacific Islands Forum Secretariat (PIFS) Heads of Government meeting;
   b. It should include the main producing countries. Assistance could be provided through regional organisations;
   c. The Pacific regional maritime association (PACMA) could serve as a prototype, with the Secretariat of the Pacific Community (SPC) as the secretariat;
   d. Regional meetings of the pearl sector are urgently needed.

5. The lack of information and resources to share information must be addressed.
   a. SPC and other regional bodies should be encouraged to address this shortfall;
   b. Newsletters, bulletins and websites, etc. should be utilised;
   c. A database may be required;
   d. If necessary, a regional coordinator post should be created and filled;
   e. A network for collaboration should be established.

### *Marketing*

6. SPC should be tasked with gathering and disseminating marketing information to all of its regional member countries in a timely manner.
   a. Information sources such as national marketing reports and international newsletters could be utilised;
   b. *SPC Pearl Oyster Information Bulletin*, the aquaculture portal website, electronic flash message distribution services, etc. could be utilised as information clearing house mechanisms.

7. Countries should be encouraged to develop a national marketing strategy.

8. A regional marketing strategy should be developed.
   a. The regional marketing strategy should take note of the opportunities for, and constraints to, inter-regional "branding" of pearls.

9. A regional association should be formed consisting of national representation for the purpose of marketing (including intelligence, standards, etc.).
   a. PIFS and SPC should be tasked with the formation and administration of this association;
   b. The functions of this group could be carried out through the regional association suggested for policy directives in recommendation 4.

10. The region should adopt the GIA (Gemological Institute of America) standards as a minimum requirement.

### *Research and development*

11. The goal of research and development should be oriented towards increased profitability. This equates to improved pearl quality and retention, more efficient culture methods and stock improvement.
   a. Existing opportunities for research include genetic selection, triploidy and seeding techniques;
   b. James Cook University (JCU) of Australia is well placed to deliver on the research and development programmes identified above.

12. Capacity for pearl disease management must be enhanced.
   a. SPC should collaborate with other key regional agencies such as SOPAC and SPREP in developing guidelines for best farming practices.

13. There should be efficient communication between the various stakeholders involved in the research and development process.
   a. SPC could act as a vehicle for establishing communication, utilising mechanisms such as the *SPC Pearl Oyster Information Bulletin* and aquaculture portal website to achieve this.

14. There should be special emphasis on the monitoring and evaluation of seeding technicians.
   a. Critical control points, such as United States HACCP standards, could be used as a checklist and as minimum standards for technicians and farmers to adhere to;
   b. Where comparisons may be useful, countries within the region should be encouraged to share data on the seeding success/failure rates of their technicians;
   c. A standard reporting sheet could be developed that countries could adopt to develop a common database. There would need to be confidence within countries

and industry on the handling of sensitive seeding information;

d. A regional or national "grafting inspector" may be required within the profession.

### *Infrastructure*

15. The development of hatcheries and spat collection to ensure consistent supply of spat for farming is a critical infrastructure area that needs to be addressed urgently.

### *Training*

16. Training in oyster biology and hatchery culture is a key priority for the region.
    a. JCU and the University of Hawaii at Hilo (UHH) are training institutions within the region. JCU has run courses in this area in the past funded by the Australian Centre for International Agricultural Research (ACIAR);
    a. ACIAR is a potential funding agency within the region;
    a. The concept of an oyster biology/hatchery training programme could include the following:
        - two courses run per year over a three year period;
        - six-week courses covering longline/equipment deployment; microalgae culture spawning induction; hatchery culture; larval, nursery culture and grow out;
        - once the course has been fine-tuned and established, it should be phased into the region by involving the University of the South Pacific (USP) as a regional training centre. This could occur around year 3.

17. Training efforts should be undertaken to increase the number of well-qualified local seeding technicians in the region, particularly in countries with large commercial production.
    a. A source of abundant pearl oysters must be found so that training can take place. This may necessitate a regional training centre where resources can be pooled (e.g. a hatchery);
    a. It is preferable that training be undertaken in-country;
    a. A travelling trainer may be the most effective way of providing training in seeding techniques to remote and distant areas. A "Master Grafting Technician" qualification could be required within the seeding technician profession.

18. A regional workshop on pearl grading should take place to strengthen farmers' knowledge of pearl quality and marketing aspects.
    a. SPC should be tasked with organising and seeking funding for such a workshop;
    a. It may be more effective for a travelling trainer/grader to visit countries.

19. Certification provided by training programmes could be a requirement for those wishing to be granted a permit under a national permit system (e.g. for French Polynesia).
    a. This could ensure a minimum level of technical competence within the industry;
    a. Regional standards could be incorporated into national certification programmes.

**A low cost pearl hatchery in Kiribati**

# Pearls of wisdom

*Dr Olive Heffernan*[1]

**Source:** The Marine Scientist 16:20–23. (2006)

The largest ocean in the world, the Pacific, has a desperate need for effective marine management. Not only is the region home to many unique habitats, it is subject to frequent natural disasters, susceptible to climate variability and change, and has a high reliance on natural resources. PI-GOOS is the Pacific Islands' Global Ocean Observatory System (GOOS), which encourages collection of data imperative for sustainable management of ocean resources.

A case in point is the black pearl industry in Manihiki Lagoon, Cook Islands, which was virtually decimated by a disease outbreak in 2000. With government support and financial aid (see box), a team of scientists and an economist have established the underlying cause of the catastrophe and have helped the islands plan for a sustainable pearl industry based on sound science, good management and capacity building.

## The island of pearls

Manihiki, "the island of pearls", is an idyllic atoll remotely located among the Cook Islands in the Pacific Ocean. Comprising 40 tiny islets encircling a 4 km wide lagoon, this completely enclosed body of water is the source of the island's most valuable asset, the black pearl. Islanders have traditionally derived three products from the black-lipped pearl oyster (*Pinctada margaritifera*) that inhabits the lagoon, namely oyster meat, mother-of-pearl and black pearls.

While locals once dived for wild oysters, this subsistence-based activity transformed into a stalwart aquaculture industry throughout the 1980s and 1990s. From a modest start with a single farm in 1982, the number of farms increased exponentially to 205 in 2003, with an estimated total of one million cultured adult pearl oysters. In the 1990s, tourism was the only sector in the Cook Islands that surpassed the pearl industry in revenue. At its peak in 2000, the pearl industry earned USD18 million in export revenue, accounting for 20 per cent of Cook Islands' GDP.

## Precipitous decline

This same year, however, the shine began to wear off the pearl oyster, as the industry in Manihiki lagoon was decimated by an outbreak of *Vibrio harveyi*, a marine bacterium that commonly infects cultured shellfish. Since the 1990s, there was growing concern that the lagoon would be struck by the disease, which had previously crippled pearl industries in French Polynesia, Indonesia, Myanmar and Australia. Together with low international prices, this led to a dramatic decline in pearl export revenue to NZD 2.8 million in 2005. In the year prior, French Polynesia, the majority supplier of black pearls globally, had rapidly increased the supply to the market, causing a fall in average prices. In Manihiki, the average price received per pearl dropped from USD 200 in the early 1990s to USD 20.00 in 2003.

## Poor practice

Following the slump in export revenue, an investigation by the Island Council and Cook Islands Government found that poor farming practice was weakening and stressing oysters in the lagoon, leaving them susceptible to disease. Overstocking had become common practice under a system with regulations that were limited, unsystematic or not enforced. In the history of Manihiki pearl farming, not a single pearl farmer has been penalised for poor farming practices. In September and October 2000, windless and dry weather conditions reduced the amount of lagoon flushing, causing a rise in water temperature. At the same time, a massive spawning event caused high concentrations of *Vibrio* bacteria in the water. Together, these factors caused the oysters in Manihiki to be badly affected by the *Vibrio* outbreak.

A system of lagoon management regulating farm practices might have prevented the initial factors that led to the demise of the industry. To prevent a catastrophe of this magnitude from reoccurring, the Cook Islands Ministry of Marine Resources (MMR), with the assistance of supporting organisations

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such as NZAID, NIWA, SOPAC and SPC, implemented a series of projects to better understand the system and to achieve sustainable development of pearl oyster aquaculture in Manihiki.

## Mapping Manihiki

The Manihiki lagoon covers an area of 10 by 7 km, with a maximum depth of 70 m. Pearls are farmed between 2 and 30 m. To best utilise this area without overstocking oysters, it was necessary to establish the actual placement of farms within the lagoon and their boundaries in relation to each other.

To achieve this, SOPAC carried out a bathymetric survey in 2002 to define the complex geometry of the lagoon and mapped the position of pearl farms within this area using a Differential Global Positioning System (GPS). Project Leader Robert Smith told The Marine Scientist that a RESON 8010 multibeam system was used for the bathymetric mapping and that IKONOS High-Resolution Satellite Imagery from RadarSat was used as additional data to complement the swath. Map-Info, a Geographical Information System (GIS), was used as a management tool to link the census data on the number and location of pearl farms to the bathymetry. The GIS made it possible to simultaneously view multiple layers of mapping data to get a holistic view of the lagoon and formed the basis of a pearl farm database developed by staff at the MMR. This continues to be maintained for lagoon management and both existing and new pearl farmers have been given a map of their licensed area, showing the farm boundaries and the depths of the lagoon within and around each farm. Mapping the lagoon in this way shows areas at future high-risk of disease outbreak as well as those suitable for farming. It also allows negotiation of boundaries to avoid conflicts.

## Lagoon circulation

In addition to the distribution of farms within the lagoon, information on the carrying capacity and environmental conditions was needed to manage pearl farming in the area sustainably. Collection of baseline data on physical, chemical and biological lagoon parameters began initially in 1996 with the Lagoon Ecology Monitoring and Management Project (LEMMP). The LEMMP established the water quality and made recommendations on advisable oyster stocking densities, stocking rates and management options. This is still used as a reference database on the ecosystem.

To support this information, in 1996 SOPAC completed a lagoon circulation study to work out the properties and movement of water into, out of, and around the lagoon. Manihiki has a small tidal range and is flushed principally by hydraulic pumping of waves. The study found there are slow rates of water exchange between the lagoon and the ocean, suggesting the lagoon takes a long time to "flush" itself with fresh oceanic waters, increasing the likely impacts of disease and pollution. In November 2000, SOPAC completed a further water quality study, using the baseline data from the 1996 lagoon circulation study as a comparison. Immediately following an outbreak, there were notably low levels of dissolved oxygen in the lagoon, indicating that overstocking and slow water exchange were critical factors.

## Long-term monitoring

Following the 2000 study, the need for long-term monitoring of the lagoon was apparent, and yet LEMPP and subsequent studies were labour-intensive and time-consuming. In November 2003, the MMR, with the assistance of SOPAC, installed a Sound Ocean oceanographic monitoring buoy in Manihiki. This has several advantages as a system of data collection. The buoy is fitted with a range of sensors and automatically measures a variety of chemical and physical lagoon parameters every hour, including sea-surface temperature, salinity, air temperature, dissolved oxygen, chlorophyll levels (an indictor of phytoplankton concentration), solar radiation, barometric pressure, pH values, wind direction and speed. To sample areas not covered by the buoy, in 2003 the Ministry purchased a hand-held YSI probe capable of measuring a range of different lagoon parameters, including temperature, pH, salinity, dissolved oxygen and turbidity.

The monitoring system is still in place as part of PIGOOS. Hourly data sampled by the buoy are sent to a base station on a daily basis using a data uplink to an Iridium satellite phone. These data are received, processed and archived online at the SOPAC website (http://www.sopac.org) where they can be downloaded. Currently, a report on the monitoring buoy data is compiled by SOPAC every month and disseminated to pearl farmers, fisheries and the Island Council. According to Smith, the buoys are currently undergoing maintenance and calibration. Due to the isolation of the area, calibrating the instruments and inserting new power sources for the units has been a huge challenge and has required bringing them to a central location for refitting. One will be deployed later this year in Manihiki and the other will be placed in Rakhanga, a lagoon some 30–40 miles NE of Manihiki.

## Net benefits

The disease outbreak continues to have an impact on the pearl industry, with a significant proportion of oysters in Manihiki still affected by the disease.

The local community has responded to the crisis, however and is gradually getting back to where it was through a system of proper management. The information garnered from state-of-the-art monitoring technologies has a number of benefits. The oceanographic data act as an early warning system of environmental conditions, giving the farming community time to respond to events such as the 2000 outbreak. The projects have studied the response of the lagoon to intense mariculture and act as a baseline by which to assess the potential of other lagoons for pearl farming. In addition to bringing state-of-the-art technologies to Manihiki, SOPAC and other supporting organisations have empowered the local community to manage its resources. This has been achieved by capacity building through a series of training initiatives in GIS, pearl technician skills, oyster health surveys and buoy deployment.

## Cost benefit

The Manihiki Island pearl industry has the potential to generate multimillion dollar net benefits for the Cook Islands, but this will only materialise in the presence of an effective management regime. In 2004, a cost-benefit analysis of the Manihiki projects carried out by Emily McKenzie, the then Resource Economist at the SOPAC Secretariat, highlighted the economic benefits of implementing a management plan for the lagoon: the net present value of the industry in Manihiki for 15 years (2004–2019) is NZD 40 million, with additional indirect, secondary benefits when supported by a Pearl Farming Management Plan based on sound oceanographic mapping and monitoring. Without a Pearl Farming Management Plan, incentives remain for resource users to stock the lagoon at unsustainable oyster densities and employ poor farming practices. In this case, the net present value of the projects will be negative over this same time period, falling to NZD 2.8 million.

A Pearl Farming Management Plan has now been developed by the Cook Islands MMR and Manihiki pearl farmers, and according to the Secretary for Marine Resources, was being implemented at the end of March 2006. Based on the practical applications of innovative technologies in hydrography and operational oceanography, the management plan has the potential to change the practice of pearl farming in the region dramatically, helping to ensure its long-term sustainability as an industry.

# Pearls from Africa

*Paul Southgate[1], Jason Rubens[2], Masoud Kipanga[2] and George Msumi[3]*

## Introduction

More often than not, aquaculture is on the wrong side of commentary relating to the environment and conservation. However, collaborative research in Tanzania by the Worldwide Fund for Nature (WWF) and Australia's James Cook University (JCU), in association with Mafia Island Marine Park (MIMP), has shown that not only are some forms of aquaculture compatible with conservation efforts, but aquaculture may provide an important component of the management plan for marine protected areas. Small-scale production of the first cultured pearls from East Africa offers opportunities for income generation for coastal communities in Tanzania and may provide an important part of the management process for sustainable use of coastal ecosystems.

Working in partnership with the Tanzanian Government, WWF has supported the development of MIMP in central Tanzania since the park's establishment in 1995. The park supports a local population of 18,000 people, who are poor and rely heavily on limited natural resources. Fishing and coconut production are the traditional sources of income on the island. WWF and MIMP are exploring, with local communities, sustainable livelihoods that will facilitate conservation measures and address the high levels of poverty on the island. Given the physical geography of the island, aquaculture is probably the most promising area to develop new income-generating activities. Over the past four years, MIMP and WWF have jointly undertaken preliminary research to investigate the feasibility of cage culture of rabbit fish (*Siganus* spp.) and pearl culture.

As demonstrated in the Pacific, cultured pearl production can provide considerable opportunity for income generation for coastal communities. Pearl production may occur on small family-based pearl farms and individuals may enter the industry at a number of levels to produce oyster shells (mother-of-pearl), half-pearls or round pearls, or they may simply collect spat from the wild for sale to pearl farms. Furthermore, the pearl industry provides opportunity for the involvement of women and provides the raw materials for local handicraft manufacture, which may include lower grade pearls or pearl shell.

Preliminary research at Mafia Island showed that the blacklip pearl oyster was abundant within MIMP. Furthermore, Mafia Island oysters reached a large size, indicating good growth rates and the potential to produce pearls of a large size. The mother-of-pearl of Mafia Island oysters was also of high quality showing very good colouration. These findings provided the basis for subsequent research to determine the quality and market acceptance of half pearls produced at Mafia Island. A trial batch of 50 *Pinctada margaritifera* was "seeded" for half pearl production (Fig. 1). Each oyster was seeded with 4–5 hemi-spherical nuclei following anaesthesia with benzocaine, which allows accurate positioning of pearl nuclei on the inner surface of the shell while minimising stress to the oyster. Seeded oysters were then placed into clean seawater to recover before being placed into panel (pocket) nets. Nets containing seeded oysters were suspended from a bamboo raft within the MIMP for a further six months. Local fishermen and MIMP/WWF staff were also trained in basic husbandry methods for *P. margaritifera* as

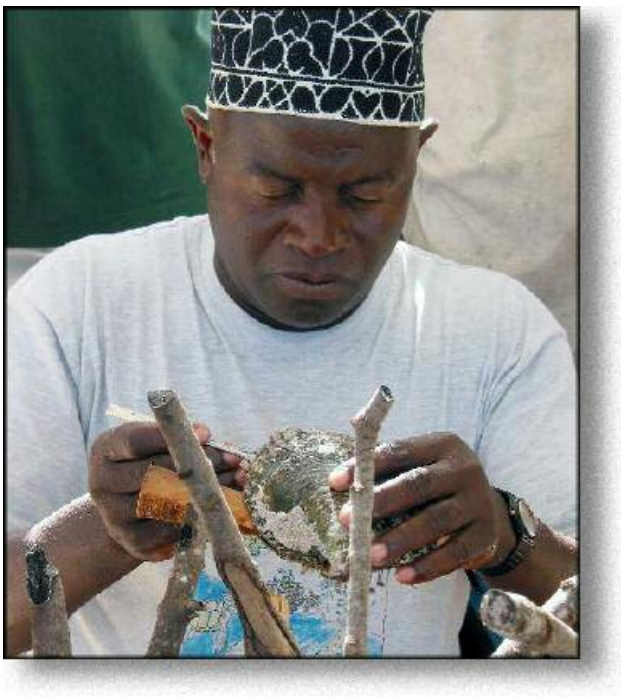

**Figure 1. "Seeding" anaesthetised pearl oysters for half pearl production**

1. Pearl Oyster Research Group, School of Marine Biology & Aquaculture, James Cook University, Australia.
2. World Wide Fund for Nature (WWF) Tanzania Programme Office, Dar es Salaam, Tanzania.
3. Mafia Island Marine Park, Tanzania.

well as half-pearl production techniques that are not technically demanding.

Trial seeding for half pearls at MIMP proved extremely successful with negligible oyster mortality and production of high quality half pearls (Fig. 2). Some of the pearls have been made up into sterling silver jewellery settings (Fig. 3) and have been used to assess market acceptance of pearl jewellery at resorts on Mafia Island and retail outlets in Dar-es-Salaam and Zanzibar.

In many countries, pearls must be exported to be sold, and must compete with pearl products from other countries in the international market place. Tanzania, however, is fortunate as it is visited by large numbers of tourists who travel to both coastal resorts and inland game reserves. It is anticipated that tourism will provide the main market for pearl products from Tanzania over the short term. Over a longer term, expansion of pearl production in Tanzania may benefit from the experience and existing distribution networks of Tanzania's well-established gemstone industry.

## Future development of pearl culture in Tanzania

The long-term sustainability of this project will depend on reliable sources of culture stock. Expansion of current spat collection activities and development of local hatchery production are immediate goals for the project. Ongoing research will also investigate the potential for round pearl production within the MIMP and development of local jewellery making skills.

Preliminary research has shown very clearly that high quality half pearls can be produced within the MIMP. Assuming appropriate demand, pearl jewellery provides a promising opportunity for income generation for communities within the MIMP and may play a key role in the management plan for the park. This project provides a model for similar developments in other parts of Tanzania and the East African coast.

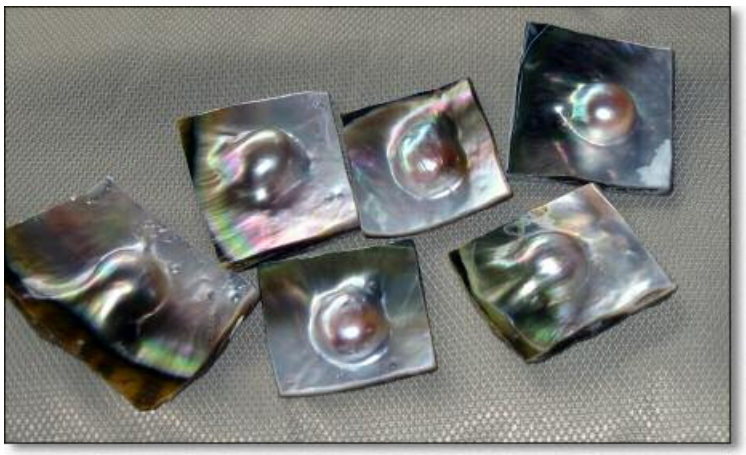

**Figure 2.**
**Half pearls produced within the Mafia Island Marine Park**

**Figure 3.**
**Finished products: half pearl jewellery**

# Pohnpei Pearl Project enters commercial phase

*Kathryn Dennis*

**Source:** Center for Tropical and Subtropical Aquaculture Regional Notes 17(1):1–7 (March 2006)

The College of Micronesia (COM) Land Grant Program plans to help launch at least four locally owned private pearl oyster farms this summer as it ends the first year of Phase III, or the commercialisation phase, of its "Pearl Project." Two farms in Pohnpei and two to three community-based farms in the outer islands will mark the beginning of what stakeholders hope will become a thriving industry in Pohnpei.

"Once we show that pearl farms can be successful, then others will feel more comfortable and get into the action," says Singeru Singeo, executive director of the Land Grant Program at the College of Micronesia, which has its central office in Pohnpei, Federated States of Micronesia. The commercial phase began last summer with funding from USDA/CSREES and the US Department of the Interior's Technical Assistance Office.

The goal is to harvest at least 10,000 (and up to 30,000) pearls as early as 2010 or as late as 2011 in order to go to auction in Guam and/or countries such as Japan, says Singeo. By 2008 (or 2009), then, the industry's private farms must reach an annual production level of 100,000 pearl oysters of a size suitable for seeding. Once an oyster is seeded, it takes almost two years for it to produce a pearl of appropriate market size and quality. Also in 2008, the first private farms — launched this summer with seeded oysters from the COM's project — will conduct a test harvest with the goal of 3,000 to 5,000 pearls.

Sounds like an impossible dream? Not so, suggests Masahiro Ito. The project's chief scientist, Ito claims local staff regularly produce at or above the level necessary to reach these industry goals: 20,000 to 30,000 three-month-olds from a single hatchery run. They are on schedule to complete seven runs this year, four of them by mid-June, he says. Although not all the spat produced at the hatchery (to the size of 1 to 2 mm) eventually reaches optimum size for seeding, experience has shown that 80 per cent of the spat that grow out in an ocean nursery for three months become two-year-old oysters ready for seeding.

The COM Land Grant employs six local technicians at its hatchery and other facilities — all of whom have proven they can successfully and regularly spawn spat and grow out seeded oysters without assistance. Training in grafting and seeding by a master technician began last year, and two local people will continue with this training.

From the beginning of the Pearl Project, the plan has focused on eventual commercialisation and the creation of an export market. The heart of the project, however, has been skills training for Micronesians. "Training local people is integral to the sustainability of the project and ultimately the industry," says Singeo. The project has invested a great deal in training opportunities.

"One expert cannot handle it all. It was clear from the start of the project that we needed to train local technicians," Ito says.

Over the last four years, the project has trained more than 70 people from local communities in ocean grow-out techniques and farm maintenance, and more than 10 people in hatchery techniques, including microalgae culture, broodstock and farm site selection, farm set-up and ocean nursery culture, says Ito. Training began the first year in 2001, as the project team and trainees turned a rundown warehouse into a low-tech, efficient hatchery at Nett Point in Pohnpei and had their first successful spat run in the project's first nine months.

The target was unemployed people. "Usually, you pay tuition to learn. They can't do that here and don't have the money," Ito says. Instead, the project gave trainees a stipend for lunch and transportation. The project has also employed local people on a casual hire basis, since the workload is not consistent throughout the year. The best and most dedicated trainees became the six technicians that now run the project's Nett Point hatchery and nursery as well as pilot farm sites on Pakin Atoll. "By 2005, they had complete confidence in themselves. They could do spawning anytime; they could achieve larval settlement," says Ito. He speaks matter-of-factly about project success in modest terms, yet exudes pride when he talks about "our boys".

To build that confidence, Ito explains, he took a step back from these core trainees in 2003, a difficult position as he watched them struggle on their own. He says 2003 was tough, but "they had to learn and make their own improvements".

Developing talent is critical to creating a productive industry — and so was building a hatchery. A

young pearl industry needs a regular source of spat to survive. Built in 2001, the hatchery system includes six 1000 L tanks. For grow out, the project's Nett Point training facility includes 12 sets of longline, each 100 m long, in an area of 1 ha. Two subfarms in Pakin Atoll have 11 sets of longlines.

Hatchery broodstock began with 25 oysters from the wild. The project team carefully selected the best oysters from the 1000 wild specimens collected in the first year. Technicians have learned to check shell color as they check the gonad condition before spawning, says Ito. Today, the hatchery and pilot farms have 25,000 broodstock ready for spawning, plus 15,000 young adults, all of which were produced in the hatchery.

The idea is for the Pearl Project to lease its oysters, ready for seeding, to private farms at the suggested rate of 5 cents per year per juvenile or one-year-old oyster, and 10 cents per year per two year-old oyster. This year the project will have available for lease a total of 10,000 oysters: 5000 seeded and 5000 ready for seeding. In 2007, up to 35,000 could be ready for lease.

Demand for the project's oysters will play a role in determining how many farms will launch this summer, along with factors such as how many business people are willing to take on the risk, says Singeo. Priority will go to those farmers with the best chance for success, e.g. people with previous business management experience and resources such as boats and staff.

More than a dozen, potential farm sites have been identified by project technicians on Pakin Atoll and other areas such as Kitti, Mwoakilloa and Pingelap. "When people see our less than ideal location with strong trade winds, which create rough conditions and muddy water, we hope they say *If they can do it at Nett Point, then maybe we can do it at our place*," Ito says.

Clearly, the new farms will need assistance. COM does not want to be their sole support, so in 2004 it held the first of many stakeholder meetings to discuss pearl industry development. "The college's role is one of research, extension, and training. Another institution or government agency should take responsibility for the promotion of commercial activity," Singeo says.

So far, two members of the cabinet of the Pohnpei State Governor, Johnny David, have agreed to help. In early February, Kikuo Apis, director of the Economic Affairs Office, decided to take the lead in organising a public sector advisory group, and Yosuo Phillip, director of the semi-autonomous Economic Development Authority, decided to lead the formation of a private sector advisory group, says Singeo. "The two gentlemen certainly are in a position to provide the necessary links between the Pearl Project and the government's actions as may be required," he says. (Apis and Phillip could not be reached for comment by deadline.)

The Pohnpei government needs to develop policies to support the development of an industry: permits for leasing ocean and lagoon areas, standards for pearl quality, tax incentives, and more. Also, a government agency could set up a small loan program to help farmers cover prohibitive costs, such as the services of seeding technicians, Singeo says. At the same time, he emphasises that the government must know its place, facilitating but not operating businesses.

A lot of people, resources, and institutions must come together by this summer, not to mention by 2010, when Singeo and Ito hope the industry will harvest at least 10,000 pearls, the minimum number it takes to attract serious buyers.

Almost two years ago, Ito conducted the first test harvest with oysters that had been seeded only 10 months before. Despite the early timing (harvesting usually occurs 20 to 22 months after seeding), the July 2004 harvest resulted in a high percentage of high quality (11–31% A-grade luster flaw, 64–81% green rate, and 16–26% blue rate) and roundness (13–31% round rate), Ito reports. The project has completed a harvest every summer since then and follow-up experiments this year may show more detailed results, he says.

"We have proven that we can make quality pearls from local oysters," says Singeo. Determined and hopeful that past success predicts an impressive future for the project in its commercialisation phase, he appraises the achievements of the project in establishing a hatchery, spawning, training and demonstration, seeding and quality pearl production, scientific research, and jumpstarting stakeholder meetings and support activities.

As Ito puts it, "We are just doing what we planned."

Even so, the plan means a great deal to a great many people, even if they do not know it yet. "Micronesia really needs economic development projects," Singeo says, noting that the Compact of Free Association between the United States and the Federated States of Micronesia will not last forever. "The only way for the islands to survive in the future, to have the money to support a government and infrastructure, is to have money coming from exports," he says. "Pearls are ideal because shelf life is long, weight and shipping costs are low, and selling prices are not cheap."

# ABSTRACTS, REVIEWS AND CURRENT CONTENTS

## World Aquaculture Society Conference, World Aquaculture 2005 May 9–13 2005, Bali, Indonesia

### South Sea pearling in Indonesia: past, present and future

*Braden Panji Poernomo*

Indonesia produced its South Sea Pearls (SSP) in 1928 when Japanese pearling pioneer Dr. Sukeo Fujita successfully cultured round pearls in South East Sulawesi (Celebes) after 8 years of experimenting with wild *Pinctada maxima*. Production peaked in 1936 when it exceeded 18.75 kg. The outbreak of World War II saw the Dutch Colonial Government take control of the farm and the cessation of pearling activities. Japanese investors recommenced SSP cultivation in the 1970s. At that time only wild oysters were available for cultivation and the natural oyster beds were rapidly depleted. By the 1980s, investment in hatchery technology began. By 1990 the Cogent Trading Company harvested a commercial crop of pearls from hatchery bred oysters. This initial success led to rapid development of *Pinctada maxima* hatcheries and pearl farms. By 1994 there were 87 pearl companies of which 43 had produced commercial crops of pearls. By the mid-1990s, the industry was almost solely reliant on hatchery technology as a means of sustaining oyster supplies. Production rapidly escalated and by the end of the decade, Indonesia was fast becoming the largest volume producer of SSPs in the world. In 1998, this exciting period of development was massively disrupted by economic and political upheaval. The deterioration in general law and order in the late 1990s and early into the new century saw pearl farmers suffer at the hands of criminals. This combined with general economic malaise and a decline in the pearl market led to companies (especially Japanese) ceasing operation and in 2001 there were only 26 pearl companies that remained viable. The improving political climate since 2001 has had a stabilising effect on pearl farm investment in Indonesia leading to steady improvements in production and pearl quality. There are fewer farmers producing larger and better crops. Pearl markets that were dramatically affected by a number of major world events (terrorism, the outbreak of SARS and a weak Japanese economy) have been steadily improving. The outlook for pearling in Indonesia is now once again positive. Current annual production is in excess of 3,500 kg with an estimated value of more than US$ 60M. Pearling is an ideal activity for Indonesia and other developing nations. It employs a large unskilled, semi-skilled and skilled labour force and can have a very positive effect on regional development in some of the world's most remote areas. The new style pearl farms that are successful have learnt to work with nature and local communities to ensure continued viable production and security. Technology that was once considered secret and only available through contracting foreign experts has been transferred to the local people. The challenge of the future lies in marrying stable production with developing markets and ensuring a balance of supply and demand. Indonesia needs to now demonstrate to the world that it is a mature and conscientious producer of a much valued product.

## Prospects for pearl culture in Indonesia and some aspects of the industry

*Amatun Nur*

Throughout history, pearls have held a unique place within wealthy and powerful circles. The development of a pearl requires many months of patience and investment on the part of the producer before it is of any value. There are essentially three types of pearls: natural, cultured and imitation. A natural pearl forms when an irritant, such as a piece of sand, works its way into a particular species of oyster, mussel, or clam. A cultured pearl undergoes the same process. The only difference is that the irritant is a surgically implanted bead or piece of shell called mother-of-pearl. Imitation pearls are a different story altogether. The art of culturing pearls was invented in Japan in 1893 by a man named Kokichi Mikimoto. He discovered that when a tiny bead of mother-of-pearl was introduced into an oyster, the oyster would begin to cover the irritant with nacre. There is no foolproof method of ensuring that an oyster will produce a quality pearl. The weather is extremely important for the quality of the pearl. If the water becomes too cold, the oysters may die or fail to grow. Before they are ready to be harvested, pearls take a long time to form in successive layers around the epithelial cells implanted in the host oysters along with the artificial nucleus. When the pearls are ready to be marketed, all these characteristics are assessed and classified. Commonly, in Indonesia, pearls are produced by *Pinctada maxima* culture. In the global market, these are known as the expensive *South Sea pearls*. The spread of *Pinctada maxima* in Indonesia extends along the coasts of Java, Madura, Lampung, Nusa Tenggara and up to Papua. Since 1980, the number of pearl culture farms has grown significantly. Statistics show there were approximately 87 pearl culture farms in 1988, although there are only 49 farms now because of plundering, which has resulted in foreign enterprises moving out of Indonesia, and also because of a depression in the export value of pearls. Plundering, which has affected almost every farm, is still a big problem. According to the leader of the Indonesia Pearl Culture Association from 1998 to 2003, Indonesia needs government laws and regulations specifically for pearl production. These rules should not be merged with those for other commodities. Pearl producers in Indonesia still believe that the prospect for pearls is outstanding. The economic sector in Indonesia has grown significantly and this is a parameter of pearl value. Pearls predominately come from Japan, Australia, Indonesia, Myanmar, China, India, the Philippines and Tahiti. Japan, however, controls roughly 80 per cent of the world pearl market, with Australia and China coming in second and third, respectively. The South Seas around Australia, Indonesia, and Myanmar are renowned for their large, white pearls, while Japan's pearls are highly valued for their lustrous character. The pearl's image of beauty, uniqueness and rarity will ensure a lustrous future for the industry. Whatever the outcome may be, it is clear that beautiful, high quality pearls will remain in use and will be cherished for as long as humans walk this precious earth.

## Pearl cultivation with Indian *Pinctada margaritifera*

*Ajai Kumar Sonkar*

A research project was initiated in January 2004 at North Bay near Port Blair, Andaman and Nicobar Islands. In the experiments, which involved operating on several thousand oysters from a collection of natural stocks, it was recorded that the shape of the oysters was deformed and their growth was obstructed because most of them were found in congested crevices and splits in the coral rocks. This caused inadequate growth of the body, resulting in a substantially smaller region that restricted nuclei implantation to sizes bigger than 8 mm. The size of the shell also reduced the cavity between the valves. Uncommonly, the mantle tissue of the Indian black lip oysters was found to be extra active. It used to shrink when opening the valve for surgery, causing a high rate of rejection of graft tissue and implanted nuclei. To overcome these problems, experiments were performed using variations and amendments of the basic surgical technique. The results were exceptionally encouraging: more than 90 per cent of the oysters produced pearls with a mortality rate of zero. The new technique enables the implantation of bigger nuclei. Detailed facts are discussed in the paper. Indian *P. margaritifera* produces quite different pearl colours, ranging from silvery copper to silvery gold, silvery green to light lavender, etc. In other experiments, abalones and conchs were operated on with astonishing results, which are also discussed in the paper.

## Overview of French Polynesia research contributions on pearl oyster *Pinctada margaritifera* farming

*Dominique Buestel*

Research on pearl oyster was initiated by the French Polynesian government following the massive mortalities that happened in the 1980s. In view of the complexity of the problem, a general research program called PGRN (Programme Général de Recherche sur la Nacre - General Research Program on Pearl Oyster)

was conducted from 1990 to 1999. The research program had two areas of focus. The first was on the pathology, physiology and biology of the pearl oyster. The aim of the second one was to study the lagoon ecosystem and develop rational management of pearl culture. Subsequent research was oriented towards improving pearl quality and developing popular communication for pearl farmers. New propositions to develop a program to organise the pearl industry and make it sustainable are currently being discussed at the European Commission. Following the PGRN, the acknowledged current crisis highlights the need for applied research initiated by the Service de la perliculture (Pearl Culture Agency), with pearl farmers agreement, on the following areas: reinforcing farm cost-effectiveness by improving graft yields, pearl quality and rearing practices; controlling reproduction in the hatchery to ensure the selection of high-performance farmed oysters; making pearl farming sustainable by optimising spat collection in the wild and mastering production in the hatchery; and making pearl farming safe through health and environmental surveillance and through preserving the genetic variability of oyster populations. At present, this research is mainly conducted in collaboration between the Service de la perliculture and the Ifremer Center of Tahiti. New collaborations are needed to improve the effectiveness of these research programs.

## Pearl culture farming in French Polynesia: overview of activities and development

*Sandra Langy*

Pearl farming was initiated in French Polynesia in the 1960s by some passionate pioneers with technology provided by Japanese specialists. *Pinctada margaritifera* grafted to obtain Tahitian cultured pearls were cultivated at Hikueru and Bora Bora in 1963. Following these experiments, and because of the quantities of pearl oysters available from the natural stock, pearl production increased exponentially. From 100 kg in the 1980s, it reached a maximum of 11 tons in 2000. Today, production has stabilised at around 10 tons annually. Pearl farming is the second biggest income earner following tourism with a value of CFP5 billion (USD150 million). Thus, it has been an extraordinary economic and social tool, encouraging islanders to return to their remote islands with 7000 jobs offered. Pearl farming is established in 30 atolls and islands of the Tuamotu and Gambier archipelagos and some volcanic islands of the Société archipelago. The majority of pearls (80 per cent) are produced by 68 big pearl farms (more than 30 ha) out of the thousand farms listed. Tahitian black pearls have achieved the status of a luxury product and since 1983 have been French Polynesia's leading export product. However, since 2001, the international economic situation, in association with mass production leading to lower quality, has resulted in a crisis situation. Large and small farms have had to face and overcome a dramatic fall in production. The value of pearl production fell by 30.7 per cent from 2002 to 2003 (USD10.00/g), but an improvement was noticed in 2004 with prices per gram reaching USD20.00.

## Developing Akoya pearl culture on the Australian east coast; challenges and constraints

*Wayne O'Connor*

Developing aquaculture in increasingly urbanised societies poses significant challenges. An example has been the recent attempt to establish a pearl industry in the subtropical waters of New South Wales (NSW) on the Australian east coast. NSW is Australia's most populous state with the vast majority of population and development occurring along its coast in a phenomenon known as the sea change. This development has led to concern over land and water use. In 1993, interest began in developing a marine pearl industry using the native pearl oyster *Pinctada imbricata*. The NSW government was approached and research was undertaken to establish the status of the *P. imbricata* population in NSW, its amenity to culture, its capacity to produce high quality pearls and the likely impacts that a pearl industry might have. In 2001, an application to establish a 92 ha commercial farm was made, but met with considerable local public opposition. Concerns regarding the social, economic and environmental impact were raised and the government responded by establishing an independent Commission of Inquiry. Among the chief community concerns regarding the farm were: pollution of the environment through the accumulation of sediment in the immediate vicinity of the farm; the impact that sedimentation might have on fauna beneath the farm and on nearby seagrass beds; the extent of sediment plumes that arise from cleaning activities; the impact of pearl farms on estuarine carrying capacity; the potential for additional pearl oyster spat settlement to foul commercial edible oyster infrastructure; and the impact that pearl culture might have on large marine fauna, such as turtles, whales and dolphins. While the Commission recommended that, with certain constraints, the farm could proceed, the government ultimately overturned the decision, citing environmental concerns, and refused the development application. A subsequent, significantly altered application was lodged in late 2003, but this application also met with local opposition and was similarly refused in mid 2004. Despite research on the environmentally benign nature of the proposal and the question of economic

viability, the failure to initially effectively allay public concerns ultimately prevented the acceptance of these two applications. This case highlighted the need for effective community engagement on aquaculture issues to allay many unfounded concerns. The future of a pearl industry in NSW is unclear.

## Atoll community-based satellite blacklip pearl oyster *Pinctada margaritifera* farming model for alternate or supplementary income generation in the Republic of the Marshall Islands

*Manoj Nair*

US-affiliated Pacific Island countries such as the Republic of the Marshall Islands (RMI) and Federated States of Micronesia (FSM) have developed pearl farming as an income generating local industry. Blacklip pearl farming is one of the very few commercial activities that can be undertaken by outer island communities in RMI, which have been traditionally almost solely dependent on copra for income generation. To provide an alternative income source, pearl farming is advocated as pearl oysters are cultured using simple low cost technology that is suitable for small-scale operations/community-based production. The ultimate end product, a cultured pearl, is high in value, non-perishable and easy to transport to well-established markets. RMI has two existing commercial pearling companies. However, these two pearling companies completely depend on hatcheries to provide spat (small settled pearl oysters) for their farming operations. The concept of satellite farming involves technology transfer by providing hatchery-produced spat to community-based grow-out farmers on the outer islands. These groups can thereby supplement their incomes by growing out spat to sizes requested and paid for by the larger commercial pearl companies. The confidence and skills gained by the grow-out farmers should empower them to start their own pearl farms at these outer islands in the future. This is the very concept of a recent project jointly funded by the College of the Marshall Islands Land Grant and United Nations Development Program (UNDP) on spat grow-out by outer island communities for alternate/supplementary income generation. The project is part of the overall UNDP project, Sustainable Livelihoods Development Project in the Republic of the Marshall Islands. In addition, the research component of this project concurrently addresses some of the remaining constraints to the expansion of the existing commercial pearl farms by improving site-specific pearl hatchery propagation and farm grow-out husbandry methodologies.

## The hatchery as a research tool

*Mereani Bellais*

The culture of blacklip pearl oyster, *Pinctada margaritifera*, is the second main economic activity in French Polynesia. This activity started commercially in the sixties by grafting mother-of-pearl from wild brood stock. Anarchic and excessive development of this unsustainable method of production has considerably reduced the number of natural wild pearl oysters. In the late 1980s, in anticipation of an eventual scarcity of natural resources, the French Polynesian government created a hatchery specialising in the production of *P. margaritifera* at the atoll of Rangiroa, an hour north of Tahiti. The initial aim was to reassure pearl producers about the permanent availability of *P. margaritifera* spat. Life cycle development and the main factors involved in the reproduction process of *P. margaritifera* have been studied in this artificially controlled environment. The Rangiroa Territorial Hatchery has developed successful larval rearing techniques that produce millions of spat. These techniques are described in the paper. Since then, other research, for example, on performance, quality, growing rate and selection of phenotypic characters such as colour, has been undertaken in this hatchery.

## Lineage and pearl quality from silver- or gold-lip pearl oysters *Pinctada maxima*

*Joseph J.U. Taylor*

Pearl value increases exponentially with quality. Shape is one of the major determining factors in pearl quality and hence value, with round pearls being of far greater value than any other shape. As a result, the ultimate aim of any pearl producer is to maximise the percentage of round shaped pearls. In order to determine whether or not lineage has a role to play in determining pearl quality, two cohorts of *Pinctada maxima* were monitored throughout the cultivation period from the larval stage until pearl harvest (a period of four years). Cohort A was the result of spawning selected silver-lip oysters that originated from the Aru Islands of south-east Indonesia. Cohort B was the offspring of oysters from the Raja Ampat Islands of north-east Indonesia. Broodstock used to produce cohort B all had gold/yellow-lipped nacre. Both cohorts received identical treatment throughout the cultivation period and were grown at a commercial pearl farm at Alyui Bay, West Papua, in Indonesia. The results following harvest demonstrated significant differences in the quality

of pearls produced, particularly in terms of shape. Cohort A produced rounder pearls than cohort B. This preliminary study demonstrates that significant differences in pearl quality can arise due to lineage. Clearly, improvements in pearl quality and value could result through a process of selective breeding.

## A reliable DNA pedigree system for *Pinctada maxima* breeding studies

*Brad S. Evans*

The silver-lip pearl oyster industry in Australia is worth approximately AUD175 million and has traditionally been based on the harvest of wild stock. More recently, pearling companies have moved away from sole reliance on wild stocks into hatchery propagation of spat as a buffer against poor recruitment (Rose and Baker, 1994). Hatchery propagation has the added benefit that genetic selection can be practised to improve the growth rate and disease tolerance of oysters, as well as uniformity and quality of pearls. Since pearls are generally sold in allotments characterised by weight, shape, lustre and surface complexion, reduced variance in these traits as a consequence of selection will dramatically increase prices received (Rose and Baker, 1994). The downside of hatchery production can be a rapid increase in inbreeding if hatchery genetics are not understood and controlled. We have developed a reliable DNA pedigree system for *Pinctada maxima*. The system comprises a suite of eight highly polymorphic tri- and tetra-nucleotide microsatellite markers. The use of tri- and tetra-nucleotide microsatellites removes the problem of stutter peaks and subsequent automatic binning, which are often associated with the more common di-nucleotide microsatellites. The results of examining the expected Mendelian inheritance of alleles from 40 full sib families where the true parents were known indicate that the likelihood of null alleles within this suite is very low. The eight polymorphic markers that comprise the DNA pedigree system were chosen for reliability and ease of scoring from an initial set of 40 tri- and tetra-nucleotide markers developed from an enriched microsatellite library created by the Molecular Genetics for Biology group at James Cook University. Our DNA pedigree system has been used on a commercial scale to genotype parental broodstock, to con-Mendelian inheritance of the loci in the progeny from known single pair matings, to assess the differential contribution of broodstock in commercial batch spawnings and to maintain pedigree information for selective breeding in *P. maxima*. We have also shown the utility of this method for the familial assignment of individual D-larvae, allowing for analysis of broodstock contributions as early as two days after fertilisation. This system will be used to support ongoing collaborative *P. maxima* aquaculture research by Atlas Pacific Limited and James Cook University, to increase our understanding of hatchery genetics, and to help elucidate the genetic basis of commercially important pearl quality traits in this species.

## Towards a selective breeding program for silver- or gold-lip pearl oysters *Pinctada maxima* in Indonesia

*Jens Knauer*

Atlas Pacific Limited and James Cook University recently embarked on a collaborative research project aimed at establishing a selective breeding program for the silver- or gold-lip pearl oyster, *Pinctada maxima*, in Indonesia. The initial phase of the project, estimated to last four years, will focus on developing the molecular tools and acquiring the fundamental genetic information essential to instigation of a future selective breeding program for this species. Special objectives of the current project will be to determine the contribution of additive genetic variation to the expression of pearl growth and quality traits, to examine whether genotype by environment interactions significantly influence pearl quality when oysters are farmed at different locations, and to develop a DNA marker suite to enable retention of pedigree information during selective breeding. The scope of the project will be outlined and an overview of the milestones achieved thus far presented.

## Producing silver- or gold-lip pearl oysters *Pinctada maxima* in a commercial hatchery; mass spawnings versus family lines

*Jens Knauer*

Traditionally, in commercial hatcheries silver- or gold-lip pearl oysters, *Pinctada maxima*, are produced in mass spawnings involving a large number of broodstock. This method is very simple but does not have a measure of control over mating pairs. In contrast to these mass spawnings, it is also possible to generate family lines using a single mating pair. Data on the effects and success of both methods are presented, and the implications for commercial farms in terms of broodstock and hatchery management are discussed.

## Evaluation of growth and economic traits of Chinese-Indian F1 hybrids of the pearl oyster *Pinctada martensi*

*Aimin Wang*

Pearl oyster culture is a very important industry in Southern China and mostly occurs in Guangdong, Guangxi and Hainan Provinces. Although China is among the world's largest producers of marine pearls, quality can still be improved. To try to improve pearl quality we have investigated growth and pearl quality in F1 hybrids of Chinese (SW- Sanya wild stock) and Indian (IC - India cultured) pearl oysters. Hybrids (SI) of SW females x IC males showed faster growth than SW stocks, LC (Liusa cultured) and XC (Xinchun cultured) pearl oysters. LC and XC pearl oysters are common cultured populations in China. Hybrids of SW females x IC males also showed greater shell depth than IC. Nacre colour in SW males x IC female hybrids (IS) was better than that of SW, LC, and XC. The hybrids (IS) were thought to be suitable as saibo donors.

## The nutritional value of tropical microalgae for blacklip pearl oyster *Pinctada margaritifera* larvae

*Erika Martinez-Fernandez*

Over recent years, the pearling industry has seen growing interest in hatchery production of pearl oysters. Hatchery methods for pearl oyster larvae are generally based on those developed for temperate bivalves and until recently have included the use of temperate species of microalgae as larval food. Larvae of tropical species of pearl oysters (e.g. *Pinctada margaritifera*, *P. maxima* and *P. mazatlanica*) are generally reared at water temperatures between 27 and 30°C. During the last few years, there have been major developments in the culture and availability of tropical microalgae as food for tropical bivalve species. This study investigated the nutritional value, for *P. margaritifera* larvae, of seven small (<10 tropical microalgae species: *Isochrysis* sp. (T-ISO, CS-177), *Pavlova salina* (CS-49), *Pavlova* sp. (CS-50), *Chaetoceros muelleri* (CS-176), *Chaetoceros* sp. (CS-256), *Micromonas pusilla* (CS-170) and an unidentified Prasinophyta (CS-126) (Codes are CSIRO catalogue codes). Mono species binary and ternary algal diets were assessed for their nutritional value for early (D-stage veliger) and late (umbo stage veliger) stage larvae. Unfed control treatments were run for all experiments. Significant differences ($P<0.05$) were observed in overall survival of *P. margaritifera* D-stage larvae fed monoalgal diets after 10 days of culture. Lowest survival was recorded for larvae fed the diatom CS-256, while survival was highest for larvae fed the *Pavlova* sp. (CS-50). Overall survival of umbo stage larvae did not show significant differences ($P<0.05$) for all treatments including the unfed control after eight days of culture. Growth of D-stage veliger larvae fed monoalgal diets showed significant differences ($P<0.05$). Best growth was obtained by larvae fed the brown *Pavlova salina* and *Pavlova* sp. (CS-50), followed closely by *Isochrysis* sp. (T-ISO). Poorest growth was obtained for larvae fed the diatom CS-256 and the Prasynophyta CS-126. Growth of umbo stage larvae was significantly ($P<0.05$) higher for larvae fed the brown *Pavlova* sp. (CS-50) and *Pavlova salina*, followed closely by the diatom *Chaetoceros muelleri*. Poorest growth was obtained for larvae fed the diatom CS-256, and the Prasynophyta CS-126. Results of the binary and ternary microalgae diet combinations and overall conclusions will be discussed.

## Successful remote settlement of eyed silver- or gold-lip pearl oyster *Pinctada maxima* larvae

*Joseph J.U. Taylor*

Remote settlement and cold storage transport of eyed larvae is successful with a number of commercially important temperate bivalve species. Similar techniques could lead to increased efficiency and cost savings for the South Sea Pearl industry if proven viable for the silver- or gold-lip pearl oyster *Pinctada maxima*. Six attempts to transport eyed larvae under cold storage conditions were made involving three sites in Indonesia (Table 1). In preparation for transport, larvae were packaged in 45-micron mesh, wrapped in damp toweling and sealed in oxygen plastic bags. For transport, larvae were stored in polystyrene boxes. The temperature was maintained at 5 to 8°C during transport and the total time for transport was between 28 and 32 hours. On arrival at destination, larvae were re-immersed in seawater and health was inspected. It was noted that the larvae required 15 to 20 minutes re-immersion in seawater before any activity was observed. Successful settlement was achieved in every attempt (Table 1). Survival through metamorphosis to age 45 days was variable (Table 1). Larvae transported for batch 4 were of poor quality as evidenced by the failure of those left at Alyui Bay to survive metamorphosis. All batches successfully survived beyond six months of age and batches 1 and 2 were seeded for pearls in the second half of 2004. The advantages of this technique to *P. maxima* producers are many and include the ability of several hatcheries to work together in order to improve and expand production at reduced cost. Hatcheries with different peak times for production could work together to extend their respective seasons and better target the best times for natural spawning and quality egg production.

**Table 1.** **Survival results for 45 day old *P. maxima* following cold storage transportation of eyed larvae. A = West Papua; B = North Bali; C = North Maluku.**

| Batch | Transfer date | Route | Eyed larvae x $10^6$ | Spat x $10^6$ | Survival |
|---|---|---|---|---|---|
| 1 | Sep. 02 | A–B | 5.75 | 0.61 | 10.6% |
| 2 | Oct. 02 | A–B | 10.00 | 1.03 | 13.0% |
| 3 | Sep. 03 | A–B | 8.00 | 1.04 | 13.0% |
| 4 | Oct. 03 | C–B | 12.00 | 0.01 | 0.1% |
| 5 | Feb. 04 | C–B | 7.80 | 1.48 | 19.0% |
| 6 | Mar. 04 | A–B | 17.80 | 1.46 | 8.2% |

## Production of triploids of the pearl oyster *Pinctada margaritifera* in French Polynesia

*Jean-Claude Cochard*

Triploids are commonly used in aquaculture and their sterility and superior growth could be of some interest to the pearl culture industry. Rearing time could thus be shortened and the grafting process facilitated. Attempts to produce triploids have been conducted using cytochalasin B in the Service de la Perliculture hatchery in Rangiroa atoll and in the Ifremer-COP experimental facilities at Vairao (Tahiti). Zygotes and embryos were stained with Hoechst 33258 and examined under epimicroscopy for evaluation of ploidy. The larvae were reared using techniques developed at Rangiroa Hatchery. At settling stage, artificial collectors were immersed in the tanks containing pearl oyster larvae. After 10 days, each collector with oyster spat was suspended on a breeding line in the lagoon for on-growing (three months to one year). This step enabled analysis of the chronology of embryo development. At day 28–29, the expulsion of the polar body (PB) occurred on average (n = 17) 12.2 min after fertilisation. The second PB was expelled after 27.3 min. The two-cell stage was observed after 55.5 min. In 2003, experiments on the retention of the second PB resulted in 95% triploid embryos before cleavage. Survival to straight hinge stage was 65% of diploid controls. However, up to 40% of the larvae were abnormal in shape. Larval growth rate and survival were not significantly affected in most cases. After one year, ploidy was checked on gill samples of spat. Only two out of 130 treated juvenile pearl oysters were identified as diploids; the remaining 98% were triploids. Mean diameter differed significantly (59.9 mm and 63.9 mm for triploids and diploid controls, respectively), probably due to the higher density of triploid spat on collectors. Growth and development of the gonad after a two-month conditioning period in the hatchery will be described.

## Taxonomic status of the Indian pearl oyster *Pinctada fucata* (Gould)

*Tharammal Sankaran Velayudhan*

Pearl oysters that produce pearls come under the genus *Pinctada*. The most important species under this genus are *Pinctada maxima, P. margaritifera, P. m. galtsofei, P. mazatlanica, P. fucata* and *P. radiata*. There are several other species, such as *P. chemnitzii, P. sugillata, P. atropurpurea* and *P. anomioides*, which are not available in quantity and have less commercial value in respect of pearls and pearl production. Winged pearl oysters, *Pteria penguin, P. formosa, P. sterna* and *P. colymbus*, produce pearls very rarely, while the windowpane oyster *Placuna placenta* produces seed pearls of very small size (Shirai, 1994). Since pearl oysters produce pearls of different colour and shape, study of the taxonomy, distribution, abundance and dimensional variation among species is most important. Out of the 28 species of pearl oysters that are known from different parts of the world, seven species are known from the Indian coasts, *viz. Pinctada fucata* (Gould), *Pinctada margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve), *P. anomioides* (Reeve), *P. atropurpurea* (Dunker), and *Pteria penguin* (Jameson ). The most important works on the taxonomy of Indian pearl oysters are those of Prashad (1932), Hynd (1955), Rao (1970), Rao and Rao (1974) and Velayudhan and Gandhi (1987). Foreign works have been reviewed by Gervis and Sims (1992). Pearl oysters enjoy a worldwide distribution. Their distribution has been described by Hynd (1955), Alagarswami (1991), Gervis and Sims (1992) and Shirai (1994).

### Site dependent growth and nacre quality in Akoya pearl oysters in south eastern Australia

*Wayne A. O'Connor*

Evaluations of the potential for farming the pearl oyster, *Pinctada imbricata*, on the central New South Wales (NSW) coast saw pearl oysters deployed to a number of other sites in Victoria and NSW. In a series of trials extending over three years, the impacts of site on growth, survival and nacre quality were assessed. When groups of sibling oysters were deployed at locations extending from as far south as Port Phillip Bay, Victoria, to the central coast of NSW, growth, survival, nacre thickness and quality differed significantly between sites. In general, reductions in oyster growth were observed as latitude increased. These were putatively ascribed to declining mean water temperature, although, significant differences in growth were also found among sites at which temperature was unlikely to have been the predominant factor. The shells of oysters deployed at the various locations were sectioned and the thickness of the nacre layer was strongly correlated with growth. The quality of the nacre (colour and lustre) produced at each site was then assessed by an independent panel and scored according to commercial desirability. The scores for colour and lustre varied significantly between sites, but neither was correlated with growth. Further, those sites that scored highly for colour did not necessarily score well for nacre lustre. In those sites that were monitored over successive years, the relative performance with respect to colour and lustre varied over time.

## World Aquaculture Society Conference, World Aquaculture 2006 9–13 May 2006, Florence, Italy

### Quantitative expressions of growth in the silver-lip pearl oyster *Pinctada maxima* cultured at three sites and two depths in West Papua, Indonesia

*Anne M. Lee*

A means of quantifying growth in bivalves is to fit mathematical growth models (e.g. Special von Bertalanffy Growth Function (VBGF), General VBGF, Gompertz, Richards, linear and logistic models) to length-at-age data. In cultured bivalves, where the absolute age of an animal is known, parameters like the asymptotic length ($L_\infty$), growth constant (K) and theoretical time where length is zero ($t_0$) may be estimated with greater accuracy.

Cultured pearl oysters *Pinctada maxima* of known ages (0.58 to 4.83 years) were grown on suspended longlines at three sites (Ganan, Manselo, Batu Terio) and two depths (5 and 15 m) in West Papua, Indonesia. Length (antero-posterior measurement) data for 450 animals were collected over 18 months and fitted into mathematical growth models using the Levenberg-Marquardt non-linear regression algorithm. The criteria for estimating best fit was a low mean residual sum of squares (MRSS), high coefficient of determination ($r^2$) and low deviation of the asymptotic length ($L_\infty$) from the maximum length ($L_{max}$) as calculated by $\sqrt{(L_\infty - L_{max})^2}$. The results are shown in the table below:

**Table 1.** **Growth parameters of different growth models with length data of P. maxima**

| Model | Formula | K | $L_\infty$ | $t_0$ | b | $r^2$ | MRSS | Deviation of $L_\infty$ from $L_{max}$ |
|---|---|---|---|---|---|---|---|---|
| Special VBGF | $L_\infty[1-e^{k(t-to)}]$ | 0.927 | 168.38 | 0.117 | | 0.985 | 14.921 | 1.93 |
| General VBGF | $L_\infty[1-e^{k(t-to)}]^b$ | 0.343 | 184.06 | 0.580 | 0.309 | 0.987 | 13.259 | 13.75 |
| Gompertz | $L_\infty e[-e^{(-k(t-to))}]$ | 1.213 | 166.03 | 0.554 | | 0.979 | 19.876 | 4.28 |
| Richards | $L_\infty[1-be^{(-k(t-to))}]^{1/b}$ | 0.001 | 166.03 | 1.213 | 0.001 | 0.979 | 20.375 | 4.28 |
| Logistic | $L_\infty[1+e^{-k(t-to)}]^{-1}$ | 0.200 | 172.44 | 0.768 | 3 | 0.950 | 50.285 | 2.13 |

The model with the best fit using MRSS and $r^2$ as criteria appeared to be the General VBGF. However, this model tended to overestimate $L_\infty$. The Special VBGF had very similar MRSS and $r^2$ to the General VBGF, but without a large deviation from $L_\infty$. This model was deemed the best to describe the growth of *P. maxima* aged 0.58 to 4.83 years cultured in West Papua. While the other models produced a high correlation be-

tween predicted and actual lengths, they had a tendency to either overestimate (General VBGF and logistic) or underestimate (Gompertz and Richards) $L_\infty$.

Estimates of $L_\infty$, $t_0$ and K for *P. maxima* cultured at three different sites and two depths were obtained using the Special VBGF as the best-fit model. The results showed that the site with the highest growth was Manselo (K = 0.991) while Ganan had the slowest growth (K = 0.863). While oysters cultured at 5 m grew faster than oysters cultured at 15 m, it appeared that at 15 m depth, oysters attained a larger size.

**Table 2. Growth parameters of P. maxima cultured at three sites and two depths and fitted with the Special VBGF.**

| | Site | | | Depth | |
|---|---|---|---|---|---|
| | Ganan | Manselo | Batu Terio | 5 m | 15 m |
| $L_\infty$ | 167.49 | 169.22 | 168.82 | 164.54 | 170.85 |
| $t_0$ | 0.087 | 0.131 | 0.116 | 0.187 | -0.025 |
| K | 0.863 | 0.991 | 0.909 | 0.974 | 0.762 |

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## Progress towards a selective breeding program for silver or gold-lip pearl oyster *Pinctada maxima* in Indonesia

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James Cook University and Atlas Pacific Limited recently embarked on a collaborative research project aimed at establishing a selective breeding program for the silver-or-gold-lip pearl oyster, *Pinctada maxima*, in Indonesia. The initial phase of the project, estimated to last four years, will focus on developing the molecular tools and acquiring the fundamental genetic information essential to instigation of a future selective breeding program for this species. Specific objectives of the project are to 1) screen the genetic diversity and performance of available wild populations to identify superior founder stocks as a base for future selection, 2) estimate heritability of pearl growth and quality traits, 3) determine if genotype by environment interactions significantly influence pearl quality, and 4) develop a microsatellite DNA pedigree market suite to enable retention of pedigree information during early developmental stages prior to the feasible application of physical tags. The scope of the project will be outlined and results to date presented.

## Towards selective breeding of the silver-lip pearl oyster (*Pinctada maxima*) – understanding genotype by environment (GxE) interactions

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Before commencing a breeding programme involving a new aquaculture species it is important to identify populations or strains that can be used as a founder genetic base and that possess "super" characteristics for commercial production. Also of interest is an understanding of how the phenotypic expression of important traits is influenced by disparate local environmental conditions (so called genotype by environment interactions). Currently, there is interest in the selective breeding of the silver-lip pearl oyster *Pinctada maxima* in Indonesia. Before a breeding programme commences for this species, however, we want to identify the best population on which to base the founder stock and because improved oysters will be grown at multiple sites throughout Indonesia, determine the influence local environmental conditions have on the

expression of oyster growth and pearl quality. In this experiment we produced spat from 27 full-sib families derived from three wild Indonesian *P. maxima* populations (Bali, Raja Empat, Aru) and communally stocked them at three different grow-out sites in Indonesia. Microsatellite-based DNA parentage analyses were used to assign oysters to their family and population of origin, and growth and morphometric data was obtained at 6 and 12 months of age. Results from this experiment will be presented and the implications of possible genotype by environment interactions on a future selective breeding programme for *P. maxima* discussed.

## Genotype by environment (GxE) interactions in silver pearl oysters (*Pinctada maxima*) and their effects on spat survival and growth

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The benefits of selective breeding as a way to improve productivity have been amply demonstrated in livestock and several important aquaculture species; however, selective breeding practices as a means to improve productivity have rarely been applied to pearl oysters. Applying modern breeding methodologies to the pearling industry could have dramatic impacts on productivity and profitability through improvements in growth characteristics of oysters, as well as increases in the uniformity and quality of pearls. Before selective breeding begins, however, we need to understand the fundamental genetic mechanisms determining the expression of commercially important traits. In particular, we need to determine if the trait under selection has a strong heritable genetic basis and what influence the environment has on the overall realisation of trait expression. Also of interest is whether the genetic potential of an individual will be realised under disparate local environmental conditions (so called genotype by environment interactions). In an effort to understand the importance genotype by environment effects might have in a future selective breeding program for the silver-lipped pearl oyster, *Pinctada maxima*, we assessed the relative performance (i.e. in survival and growth traits) of spat from six families when communally reared at different densities, salinities and food availability. The results from this trial will be presented and the implications for selective breeding programs for the silver-lipped pearl oyster discussed.

## Genetic structure and diversity of a high dispersal mollusc, *Pinctada maxima*, throughout northern Australia and the Indo-West Pacific region

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The silver-lipped pearl oyster, *Pinctada maxima*, is the most important pearl producing species throughout northern Australia and southeast Asia and due to its long planktonic larval phase has a large capacity to widely disperse. Currently very little information is known on the genetic structure and diversity of this species within the biogeographically complex Indo-West Pacific and northern Australian regions. We analysed genetic variation from over 400 individuals from the Solomon Islands, Indonesia, Australia, Vietnam and Papua New Guinea at six polymorphic microsatellite loci and one mitochondrial gene region (cytochrome oxidase 1). Analyses based on these genes indicate that significant genetic structure exists within and between Indonesian, Australian and eastern Pacific populations and therefore that *P. maxima* is not genetically homogenous throughout its broader distribution. Knowledge on the population structure of this species will be used to identify genetically differentiated sub-populations that may have desirable traits for aquaculture and future breeding programs.

## Factors affecting the maintenance of genetic variation in hatchery cultured silver-lipped pearl oyster (*Pinctada maxima*)

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Commercial hatchery processes were examined to highlight critical factors leading to the loss of genetic variation in artificially propagated silver-lipped pearl oyster (*Pinctada maxima*) populations. Microsatellite based DNA parentage analyses were employed to assess the practice of mass spawning as a contributor to

significant genetic diversity loss. Here a communal spawning comprising 14 male and 9 female broodstock was performed in Bali, Indonesia, with the resulting cohort of larvae reared by commercial methods in 4 x 5,000 L tanks. Initial broodstock contributions and subsequent relative family survival were estimated throughout larval development by sampling and genotyping at critical stages of morphological development (2, 8, 21 and 28 days post-fertilisation), and as settled spat (35 days old). The results from this study and the implications of varying broodstock contributions and differential family survival on the genetic diversity of *P. maxima* will be discussed.

### Allelic variation within the N66 (Nacerin) gene of the silver- or gold-lipped pearl oyster, *Pinctada maxima*

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The silver- or gold-lipped pearl oyster (*Pinctada maxima*) is renowned for producing large pearls of excellent quality and this species is valued for pearl production and shell products which as sold as mother-of-pearl. The inner nacreous layer of *P. maxima* displays considerable variation in both colour and lustre and such qualities are likely to be linked to organic components within the molluscan shell. The N66 gene of *P. maxima* is a known homologue of the nacerin gene first isolated from *Pinctada fucata*. This gene is expressed in both the dorsal region of the mantle and the mantle edge and may be involved in the crystallisation of the nacreous layer. The N66 protein contains a repetitive region consisting largely of Asn and Gly residues. We developed specific primers to amplify a ~ 700 base pair fragment spanning the repetitive region of the N66 gene in *P. maxima*. We have used these primers and the polymerase chain reaction (PCR) to look for allelic variation within this portion of the N66 gene. At least four alleles ranging from 708 to 784 base pairs were readily resolved by size separation. DNA sequencing of several individuals homozygous for the most common 708 base pair allele suggests that size homoplasy exists within this allele size class. The functional significance of this allelic variation is unknown, but future work will explore correlations between N66 alleles varying in repeat region size and sequence and phenotypic characteristics of the nacreous layer of *P. maxima*.

## Other abstracts

### Reproduction, growth and culture

### Effect of stocking density on growth and survival of the rainbow pearl oyster, *Pteria sterna* (Gould 1852) during nursery culture and late culture in Bahía de La Paz, Baja California Sur, México

*Monteforte-Sánchez M., Bervera H., Ramìrez J.J., Saucedo P. and López C.O.*

**Source:** Aquaculture International 13(5):391–407 (2005).

Growth and survival of the rainbow pearl oyster, *Pteria sterna* (Gould 1852), was evaluated in field culture at Bahìa de La Paz, México. Mexican-made Nestier™ trays were used in nursery culture from March to July 1999 at four different stocking densities (25, 50, 75 and 100 individuals/tray). Late culture proceeded from July 1999 to March 2000 in sandwich nets and rail cages. Each artifact received 70 to 75 individuals. We studied the long-term effect of nursery culture stocking treatments. Growth patterns were examined using shell volume (height x width x depth, in $cm^3$). Survival was estimated monthly. Growth and survival were acceptable regarding routine operations, but variations in this experiment depended on stocking density and type of late culture device. The interaction of density and culture device was significant for shell volume at the end of the experiment ($F = 3614.14$; $P < 0.0001$). Final shell volume depended on stocking density in nursery culture ($F = 8.09$, $P < 0.001$), but culture device had no influence ($F = 0.76$; $P = 0.3$). The results indicated that growth and survival in nursery culture were not proportionally related to stocking density. The change to late culture improved overall response. Advantages in growth were favorable for D50 C only. Rail cages promoted better survival than sandwich nets. Based on the natural behavior of *P. sterna*, the optimal stocking density may be higher than the ranges tested in the present study. We recommend new strategies to improve the actual culture technology for *P. sterna*. A 3-dimensional culture unit might be an important advantage for this species regarding territorial exploitation and efficiency of spatial management in the production cycle.

## The commercial oysters of the Gulf of Mexico and the Caribbean Sea: ecology, biology, and fisheries

*Mackenzie C.L.*

**Source:** Proceedings of the Gulf and Caribbean Fisheries Institute 56:521–535 (2005).

The species of commercial oysters present in the Gulf of Mexico and the Caribbean Sea are the eastern oyster, *Crassostrea virginica*, the mangrove oyster, *Crassostrea rhizophorae*, and the Atlantic pearl oyster, *Pinctada imbricata*. *C. virginica* inhabits estuaries along the Gulf of Mexico coast and supports fisheries from western Florida to southern Mexico. The mangrove oyster grows in estuarine lagoons on various islands, including Cuba, Jamaica, Hispanola, Puerto Rico and Isla Margarita, and on mainland shores in the Caribbean Sea. The pearl oyster ranges from North Carolina to Brazil, but the only current fishery for it exists near Isla Margarita Island off the coast of Venezuela. The eastern and mangrove oysters inhabit turbid shallow waters, while the pearl oyster inhabits clear deeper (3–11 m) waters. The oysters have similar life histories: synchronous spawning by males and females, external fertilisation, a planktonic larval period of one to two weeks, and settlement onto hard surfaces. The principal predators of all the oyster species are boring gastropods and crabs. In the US Gulf of Mexico, most eastern oysters currently are harvested by about 1200 dredging and tonging boats and 3,300 men. Recent annual harvests, about 4.5 million bushels, are sold in the southern and eastern US. Mexico's eastern oyster fishery is comprised of about 350 outboard motor boats; most harvesting is by tonging. Annual landings in Mexico are about 1.0 million bushels. In the Caribbean Sea, an estimated 200 fishermen gather mangrove oysters in various estuaries. Annual landings total 50,000–60,000 bushels with the most being landed in Cuba. The pearl oyster is harvested near Isla Margarita, Venezuela, by dredging with outboard motor boats. About 40 boats and 120 fishermen comprise the fishery; annual landings are about 20,000 bushels.

## Histological changes in the gonad of the blacklip pearl oyster (*Pinctada margaritifera* Linnaeus, 1758) during the reproductive season in north Queensland, Australia

*Acosta-Salmon H. and Southgate P.C.*

**Source:** Molluscan Research 25(2): 71–74 (2005).

Histological changes in the gonad of cultured blacklip pearl oysters (*Pinctada margaritifera*) were studied during the main reproductive season. Collection of oysters from culture stock held at Magnetic Island and Orpheus Island, north Queensland, was conducted between August 2003 and February 2004. Oysters from each site were collected every month. Samples of gonad tissue from the oysters were processed for histological analysis. The sex ratio of oysters at both sites was skewed towards males. Active gonad development was recorded in all months during the study. Results of this study indicate that reproduction of *P. margaritifera* at Orpheus Island and Magnetic Island is a continuous process between August and February, as shown by the presence of ripe and partially spawned oysters during the majority of this period.

## Factors influencing recruitment of hatchery reared pearl oyster (*Pinctada mazatlanica*; Hanley 1856) spat

*Saucedo P.E., Bervera-Leon H., Monteforte M., Southgate P.C. and Monsalvo-Spencer P.*

**Source:** Journal of Shellfish Research 24(1):215–219 (2005).

We experimentally evaluated collection of *Pinctada mazatlanica* spat using substrates of different textures and colours placed at different depths within settlement tanks. When larvae reached the pediveliger stage (day 25), black-colored spat collectors ("envelope" type) composed of the following materials were offered as settlement substrates: onion bag, mosquito net, fishing net, and 63% shade-cloth. Spat collectors measured 30 x 30 cm and were composed of an outer bag and inner substrate made of the same material. The influence of the colour of spat collector material on recruitment was investigated using a second type of spat collector composed of onion bags as the outer bag and inner substrate ("bag" type). Bags were prepared in three different outer/inner (O/I) colour combinations of green/green, green/black, and red/black (O/I). Both types of collectors were deployed at different depths within settlement tanks. Approximately 2.7% of the initial larval population survived, resulting in 35,583 spat. Of these, 71.3% recruited to bag type collectors, 21.9% to envelope type collectors, and 6.8% to the surfaces of the culture tanks. Spat recruitment was significantly affected by collector material ($P < 0.01$), with fishing net and shade-cloth yielding the highest and lowest density of spat, respectively. Similarly, spat collector colour combination significantly influenced spat collection ($P < 0.01$), with higher recruitment to red and green substrates. For both spat collector types, there was significantly greater ($P < 0.01$) recruitment of spat to collectors in the middle of the

water column (60–90-cm depth). This study showed that both the type and colour of material used for spat collectors, as well as depth of deployment, influenced recruitment of *P. mazatlanica* spat. These results will help fine-tune current hatchery techniques for this species.

### Reproductive condition of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Linnaeus 1758) from Chuuk Lagoon, Federated States of Micronesia, during the summer months in 2003

*Kang Do-Hyung, Park Heung-Sik, Yi,Soon-Kil and Choi Kwang-Sik*

**Source:** Ocean and Polar Research 27(3):351–358 (2005).

The reproductive condition of tropical blacklip pearl oyster, *Pinctada margaritifera*, collected during the period July through September 2003 from Chuuk Lagoon, Federated States of Micronesia, was investigated using histology. The level of gonad development for each pearl oyster was determined using the average score of five microscopic fields; the average score was used as the maturity index (MI). All wild pearl oysters collected in July exhibited fully ripe eggs in their ovaries (40–50 µm in diameter), indicating that they were ready for spawning. In mid-August, most wild pearl oysters were in spawning and the MI dropped dramatically from mid to late September, suggesting that the wild pearl oyster completed spawning during this period. In contrast, the cultivated pearl oysters collected in mid-September held ripe eggs in the ovaries and only a few of them spawned, indicating that gonad maturation of the cultivated pearl oyster was somewhat slower than that of the wild pearl oyster in Chuuk Lagoon during the summer period. Histological analysis also indicated that spawning of the pearl oyster is rather incomplete and they may spawn continuously during summer.

### Growth and mortality of *Pinctada imbricata* (Mollusca: Pteridae) in Guamachito, Araya Peninsula, Sucre State, Venezuela

*Marcano J.S., Prieto A., Larez A., Alio J.J. and Sanabria H.*

**Source:** Ciencias Marinas 31(2):387–397 (2005).

Growth, size distribution and mortality of *Pinctada imbricata* from Guamachito, Araya Peninsula, Venezuela, were analysed between January and December 2000. Monthly samples were taken on perpendicular transepts to the coastline with an artisanal dredge, and data on sea surface temperature, dissolved oxygen and salinity were registered. The size distribution was variable, with a size predominance between 50 and 70 mm in length, which represented 75% of the population. The greatest quantity of juveniles was observed in March, when the mean length of the population was 55.8 mm. The variation in mean wet and dry weights presented a similar trend, with minimum values in June, July, September and October. The length/wet weight and length/dry weight relationships were significant ($P<0.05$). The parameters of the seasonal von Bertalanfy growth equation were $L_\infty = 85.15$ mm, $K = 1.42$ yr$^{-1}$, ts = 0.2 and C = 0.20, indicating slow growth in the period from July to November (WP = 0.42) that could be associated with the environmental conditions of the area. Estimated longevity was three years. Total mortality rate (Z) was 3.45 per year for small individuals (10–36 mm) and 14.36 per year for larger individuals (36–84 mm). The high growth rate of *P. imbricata* supports its use for aquacultural purposes in the region. The Guamachito oyster bank shows good conditions to support sustained fishery exploitation, considering fishery parameters such as registered catch, optimal effort, minimum extraction size, fishing mortality and natural mortality.

### Cryopreservation of black-lip pearl oyster (*Pinctada margaritifera*, L.) spermatozoa: effects of cryoprotectants on spermatozoa motility

*Lyons L., Jerry D.R. and Southgate P.C.*

**Source:** Journal of Shellfish Research, 24(4):1187–1190 (2005).

Cryopreservation of sperm is seen as an important step in developing effective hatchery culture techniques for the black-lip pearl oyster, *Pinctada margaritifera*. As a preliminary investigation into cryopreservation of the gametes of this species, we tested five cryoprotectant agent combinations for their ability to retain sperm motility: (1) M trehalose and 5, 10 and 15% dimethyl sulphoxide (DMSO); (2) Hanks calcium-free balanced salt solution (C-F HBSS) and 5%, 10% and 15% DMSO; (3) C-F HBSS and 5, 10 and 15% propylene glycol (PG); (4) 1 M trehalose and an equal combination of DMSO and PG making up 5, 10, 15% total volume; and (5) C-F HBSS and an equal combination of DMSO and PG making up 5%, 10% and 15% total volume. Total rapid and progressive sperm motilities were estimated through computer assisted sperm

analysis (CASA). Sperm cryopreserved in 1 M trehalose and 5% DMSO retained the highest total (86.0 ± 1.2% SE), progressive (46.0 ± 1.2% SE) and rapid (25.1 ± 0.6% E) motilities of all cryoprotectant solutions, whereas those cryopreserved with PG generally retained poor motility. Although the 1 M trehalose and 5% DMSO treatment compared favourably with that of fresh sperm for total motility ($P$ <0.01), all cryoprotectant treatments were poor at retaining the proportion of original rapid and progressively moving sperm. This study highlights the potential for cryopreservation of gametes from *P. margaritifera*, which will benefit selective breeding and conservation programs with this important commercial species.

### Growth patterns of the pearl oyster *Pinctada margaritifera* L. in Gazi Bay, Kenya

*Mavuti K.M., Kimani E.N. and Mukiama T.*

**Source:** African Journal of Marine Science 27(3):567–575 (2005).

Culture of pearl oysters is rapidly increasing worldwide, including the western Indian Ocean. The oyster *Pinctada margaritifera* L., which produces the most highly valued black pearls, occurs in East Africa, and has been exploited there for the shell for many decades. The growth patterns of *P. margaritifera* from a natural population in a sheltered back-reef, and from oysters translocated to a tidal current-swept site, are described. Both sites are within Gazi Bay, Kenya. The growth rate in the natural population ranged from 31.3 mm year$^{-1}$ (60–65 mm size-class) to 7.6 mm year$^{-1}$ (105–110 mm size-class). The von Bertalanffy growth coefficient (K), calculated with a fixed L. of 127.2 mm, was 0.30 for the natural population and 0.38 for the translocated oysters. The mean growth rate during the north-east monsoon season was approximately double that during the south-east monsoon season. The daily rate of nacre deposition ranged from 1.3 pm to 5.9 pm (mean 3.45 pm); it declined with the size of oysters and was marginally higher at the high-energy current site. At that rate, it would take approximately two years to produce a marketable cultured half pearl with a 2.5 mm layer of nacre. The results of the study are relevant to the understanding of the influence of the environment on growth, and are applicable to the optimisation of the growth rate of pearl oysters in the inshore region along the east coast of Africa.

### Growth and biometric relationships of the pearl oyster *Pinctada fucata* (Gould) on transplanting from the Gulf of Mannar to the Arabian Sea

*Mohamed K.S., Kripa V., Velayudhan T.S. and Appukuttan K.K.*

**Source:** Aquaculture Research 37:725–741 (2006).

Comparative studies were made on the growth and biometric relationships of the pearl oyster *Pinctada fucata* (Gould) Tuticorin stock at (Tuticorin (TST) - parent stock) transplanted from Tuticorin Bay (8.7°N; 78.2°E) in the Gulf of Mannar along the Indian southeast coast to Kollam Bay (8.8°N; 76.5°E) in the Arabian Sea along the Indian southwest coast (Tuticorin stock at Kollam (TSK) - transplanted stock). At the time of transplantation, Kollam Bay did not have a native stock. However, within a year, the transplanted stock spawned and oyster spat was collected from within the farm (Kollam stock (KS) - progeny stock). The growth in dorso-ventral measurement and total weight in Kollam Bay was 1.4–1.6 times and 3.1 to 6.8 times respectively greater than that observed at Tuticorin. Furthermore, at Kollam Bay, the thickness observed at the end of the first year was similar to that obtained at the end of the second year in Tuticorin. Both the TSK and KS had significantly higher instantaneous growth rates (IGR) than TST. All the stocks displayed significantly different biometric relationships. The increased growth in Kollam Bay is attributed to the almost two-times greater productivity in the Arabian Sea compared with the Bay of Bengal. It is concluded that in the case of *P. fucata*, site and interaction with the environment are important determinants of growth and shell dimension. The present study clearly indicates that the environmental conditions prevailing along the southeast Arabian Sea are congenial for the growth, gametogenesis, spawning and settlement of *P. fucata* larvae. In spite of strong monsoonal influences in the hydrology of Kollam Bay, the growth and reproduction of *P. fucata* stocks indicate its relative hardiness and ability to adapt to a changed environment.

### The pearl oysters, *Pinctada maxima* and *P. margaritifera*, respond in different ways to culture in dissimilar environments

*Yukihira H., Lucas J.S. and Klumpp D.W.*

**Source:** Aquaculture 252: 208–224 (2006).

Growth, condition index (CI) and survival of the pearl oysters, *Pinctada maxima* and *P. margaritifera*, were measured in three size groups of oysters over 14 months at two dissimilar environments in the Great Barrier Reef lagoon. These were the Australian Institute of Marine Science (AIMS) in a mainland bay and Orpheus Island

Research Station (OIRS) in coral reef waters. Temperature, suspended particulate matter (SPM) and particulate organic matter (POM) were monitored during the study. Temperature at AIMS fluctuated more widely than at OIRS both daily and seasonally, with annual ranges of 20–31°C and 22–30°C, respectively. Mean SPM concentration at AIMS (11.1 mg $L^{-1}$) was much higher than at OIRS (1.4 mg $L^{-1}$) and fluctuated widely (2–60 mg $L^{-1}$). Mean POM level was also substantially higher at AIMS, being 2.1 mg $L^{-1}$ compared with 0.56 mg $L^{-1}$ at OIRS. Von Bertalanffy growth curve analyses showed that *P. maxima* grew more rapidly and to larger sizes than *P. margaritifera* at both sites. For the shell height (SH) of *P. maxima*, growth index $\varphi' = 4.31$ and 4.24, asymptotic size $SH_\infty = 229$ and 205 mm, and time to reach 120 mm SH ($T_{(120)}$) = 1.9 and 2.1 years at AIMS and OIRS, respectively. For *P. margaritifera*, $\varphi' = 4.00$ and 4.15, $SH_\infty = 136$ and 157 mm, and $T_{(120)} = 2.5$ and 3.9 years at AIMS and OIRS, respectively. *P. maxima* had significantly lower growth rates and lower survival of small oysters during winter compared with summer. There were, however, no significant differences between the two sites in growth rates of *P. maxima* and final CI values. In contrast, *P. margaritifera* showed significant differences between sites and not seasons, with lower growth rates, survival of small oysters, final CI values and asymptotic sizes at AIMS. The low winter temperatures, but not high SPM at AIMS, adversely affected *P. maxima*. Conversely, the high SPM levels at AIMS, but not temperature, adversely affected *P. margaritifera*. This was in accordance with earlier laboratory-based energetics studies of the effects of temperature and SPM on these two species. *P. maxima* has potential to be commercially cultured in ca. > 25°C waters with a wide range of SPM levels, including oligotrophic coral reef waters with appropriate particle sizes. It is possible to culture *P. margaritifera* in turbid conditions, but its poor performance in these conditions makes commercial culture unlikely.

## Growth, mortality, recruitment and sex-ratio in wild stocks of silver-lipped pearl oyster, Pinctada maxima (Jameson) (Mollusca: Pteriidae), in Western Australia

*Hart A.M. and Joll L.M.*

**Source:** Journal of Shellfish Research 25(1): 201–210 (2006).

Growth, mortality, recruitment and sex-ratio of wild stocks of the silver-lipped pearl oyster *Pinctada maxima* were studied at sites spanning the geographic extent of the commercial fishery using mark-recapture experiments, recruitment cohort analysis and research surveys of stock abundance and reproductive status. Growth parameters ($L_\infty$, K) from the von Bertalanffy growth equation were estimated at 210 mm dorsoventral measurement (DVM) (±16 mm SD) and 0.74 at the Lacepede Islands, $L_\infty$ of 199 mm DVM (± 6 mm SD) and K of 0.79 on 80 Mile Beach, and $L_\infty$ of 194 mm (± 6 mm SD), and K of 0.72 at Exmouth Gulf respectively. Estimates of natural mortality (M) by tagging were very low (0.02–0.03), compared with catch-curve analysis, which estimated M to be between 0.1 in deeper (30–34 m) populations and 0.18 in shallow (9–12 m) populations. Settled *P. maxima* spat (0+ and 1+ age classes) on adult shell were quantified (e.g., 1,317 spat found on 119,000 shells in 2003) to obtain an annual recruitment index, which showed clear temporal trends in abundance. Over 7 y (1992 to 1995; 2001 to 2003) the annual recruitment index varied from 5.1–8.0 spat per 1,000 shell for the 0 + age class, and 3.5–6.2 spat per 1,000 shell for the 1+ age class. Preliminary predictions of future abundance showed promise; however, more work is required on spatial and habitat effects on spat settlement before the potential of the 0+ and 1+ recruitment indices can be realised. We also confirm that Western Australian populations of *P. maxima* are protandrous hermaphrodites, with a 50:50 sex ratio not achieved until females are 170 mm DVM, which is above the maximum size fished.

## The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae

*Martínez-Fernández E., Acosta-Salmón H. and Southgate P.C.*

**Source:** Aquaculture, 257:491–503 (2006).

Recent years have seen major developments in the culture and availability of tropical microalgae as a food source for tropical bivalve species. The nutritional value of seven small (< 9 µm) tropical microalgae species (two diatoms (*Chaetoceros muelleri* and *Chaetoceros* sp.); three golden-brown flagellates (*Isochrysis* sp., *Pavlova salina* and *Pavlova* sp.) and two green-flagellates (*Micromonas pusilla* and an unidentified coccoid CS-126)) were analysed for carbohydrate, lipid and protein contents as well as fatty acid composition. Each species of microalgae was fed singly to early (D-stage veliger) and later (umbo-stage veliger) stage larvae of the black-lip pearl oyster, *Pinctada margaritifera*. Highest survival of D-stage larvae over the 10 day experiment was recorded for those fed *Pavlova* sp. (CS-50). Greatest shell growth was shown by D-stage larvae fed the golden-flagellates *Pavlova* sp. (CS-50) and *Pav. salina*. Based on growth of D-stage larvae, the microalgae could be divided into three groups: (1) larvae fed *Pav. salina* and *Pavlova* sp. showed significantly

greater growth than those fed other microalgae; (2) those fed *Isochrysis* sp., *C. muelleri* and *M. pusilla* showed significantly greater growth than unfed larvae; and (3) larvae fed *Chaetoceros* sp. and CS-126 did not grow at a rate greater than unfed larvae. Growth of D-stage veliger larvae was significantly correlated with carbohydrate, lipid and protein content of microalgae and with levels of dietary polyunsaturated fatty acid, specifically DHA ($r = 0.829$, $P = 0.021$). In a second experiment, survival of umbo-stage larvae (including the unfed control) did not differ significantly between treatments ($P < 0.05$) after eight days of culture. Larvae fed *Pavlova* sp. and *Pav. salina* showed the greatest incremental growth increases, but these were not significantly greater than those of larvae fed TISO and *C. muelleri* ($P>0.05$). Growth of umbo-stage larvae fed *M. pusilla*, *Chaetoceros* sp. and the Prasinophyta sp. (CS-126) did not differ significantly from that of unfed larvae ($P < 0.05$). This study is the first comprehensive assessment of the nutritional value of tropical microalgae species for pearl oyster larvae. The results provide a basis for development of more effective larval culture techniques by identifying microalgae supporting good growth of *P. margaritifera* larvae of different ages.

## Effect of temperature and body size on food utilisation in the marine pearl oyster *Pinctada fucata* (Bivalvia: Pteridae)

*Mondal S.K.*

**Source:** Indian Journal of Marine Sciences 35: 43–49 (2006).

Physiological parameters such as clearance rates, absorption efficiency, oxygen consumption and ammonia excretion were estimated for four size groups ranging from 16 to 60 mm in Dorso-Ventral Measurements (DVM) of the marine pearl oyster *Pinctada fucata* at different water temperatures. The results were integrated by means of two physiological indices, namely scope for growth (SFG) and net growth efficiency ($K_2$). The rates of clearance, oxygen consumption and ammonia excretion were found to be strongly correlated ($P$ less than or equal to 0.01) with size groups (as tissue dry weight) at water temperatures from 18 to 31°C. Absorption efficiency ranged from 43.2 to 56.9% and was not related to body size in the tested temperature range. Oxygen consumption and ammonia excretion increased with temperature within the same size group from 18 to 31°C. Clearance rate increased with temperature from 18 to 28°C, but declined with further increases in temperature to 31°C. Excreted energy contributed 2.4 to 4.0% to the total absorbed energy for different size groups and water temperatures. The SFG and $K_2$ were higher at 26 and 28°C and were lowest at 18°C for all size groups. The results showed that the optimum physiological conditions for survival and growth of *P. fucata* were in the temperature range of 26 to 28°C.

## Transport and recruitment of silver-lip pearl oyster larvae on Australia's North West Shelf

*Condie S.A., Mansbridge J.V., Hart A.M. and Andrewartha J.R.*

**Source:** Journal of Shellfish Research 25(1):179–185 (2006).

Silver-lip pearl oyster (*Pinctada maxima*) spat surveyed in the Eighty Mile Beach section of the North West Shelf have been used in conjunction with outputs from a particle dispersion model to identify likely spawning grounds. The dispersion model consisted of a 3-dimensional regional circulation model in which large numbers of individual particles were tracked over the period 1994 to 1999. From the settlement areas defined by the spat data, larvae were tracked back in time over their estimated pelagic phase of 24–31 days within the main spawning period of mid October to late December. The reverse calculation was also undertaken, looking at larval dispersion from known broodstock populations. Results demonstrate that large tidal currents in the region move larvae back and forth across the shelf, whereas lower frequency currents influence their net transport. Whereas some model larvae travelled more than 60 km, most were transported less than 30 km. The model results suggest that spawning in the Eighty Mile Beach region is concentrated around the recently surveyed broodstock distribution between 8 and 15 m depth, with potential smaller contributions from the northeast. These spawning events are likely to lead to successful recruitment locally and alongshore to the southwest. They also feed larvae into neighbouring shallow coastal environments (through tidal oscillations) and deeper waters to the west (–20 m). However, spat abundances seem to be low in these areas, suggesting that recruitment is strongly limited by habitat availability and possibly high mortality rates in shallow water. High local abundances of broodstock and spat observed occasionally in deeper water (–30 m) seem to be supported by intermittent larval transport from inshore populations. However, spawning in this area seems to contribute little to recruitment in the inshore populations.

### Economic feasibility of small-scale black-lipped pearl oyster (*Pinctada margaritifera*) pearl farming in the Central Pacific

*Fong Q.S.W., Ellis S. and Haws M.*

**Source:** Aquaculture Economics & Management 9(3):347–368 (2005).

This work provides an analysis of the economic feasibility of one of many small-scale aquaculture operations being considered, black pearl oyster farms, as one type of supplemental economic activity for outer island communities in the Central Pacific. Specifically, projections of financial performance of a small-scale 25,000 seeded pearl oyster farm using the Tahitian longline method are being conducted. Estimates of initial capital investment and annual operating costs are being formulated, and an annual cash flow and enterprise budget are being developed. Results show that initial capital investment is US$ 202,076. Annual operating expenses are $ 293,726 during full operation. The largest costs contributing to annual operating expenses are seeding (46%), labour including farm owner's opportunity cost (24%), and depreciation (9%). The base model presented in this work suggests profitability over a 20-year horizon. Net returns over a 20-year farm horizon based on an 8% discount rate indicate a positive NPV of $102,945. Sensitivity analyses on profit due to the variability of market price, survival, and cost of seed and other inputs are conducted and results presented.

### The effect of age and shell size on accumulation of fouling organisms on the Akoya pearl oyster *Pinctada fucata* (Gould)

*Guenther J., Southgate P.C. and de Nys R.*

**Source:** Aquaculture 253: 366–373 (2006)

Biofouling is a significant operational cost for pearl aquaculture. Understanding the factors that affect the settlement and development of fouling organisms contributes to improved management practices to control biofouling. Field experiments were carried out to document the accumulation of fouling organisms, measured as percentage cover, with respect to age (2-year-old and 3-year-old, independent of shell size) and shell size (45–55 mm and 65–75 mm dorso-ventral measurement, DVM, independent of age) of the Akoya pearl oyster *Pinctada fucata*. After 16 weeks, 3-year-old *P. fucata* were significantly more fouled than 2-year-old oysters. In contrast, there was no significant difference in fouling cover between 3-year-old *P. fucata* with DVM of 45–55 mm and 65–75 mm. Fouling communities were dominated by the hydroid *Obelia* sp., the bryozoan *Parasmittina* sp. 1, the bivalve *Saccostrea* sp. 1 and the ascidian *Didemnum* sp. To determine a potential relationship between the accumulation of fouling organisms and periostracum cover, differences in periostracum cover with age (2-year-old and 3-year-old) and shell size (45–55 mm and 65–75 mm DVM) of *R. fucata* were examined. Periostracum, measured as both area covered and area without periostracum, decreased significantly with age, but not with shell size. The high percentage cover of fouling on all oysters and the large area where periostracum was undefined make any conclusion as to the role of the periostracum in preventing fouling unclear. These findings, however, support age as a key factor for the development of husbandry protocols, with older *P. fucata* shells being cleaned more frequently than younger shells to counteract the increased settlement of fouling organisms.

## Shell structure and biomineralisation

### Chemical modification studies on alkaline phosphatase from pearl oyster (*Pinctada fucata*): a substrate reaction course analysis and involvement of essential arginine and lysine residues at the active site

*Chen H.-T, Xie L.-P. Yu Z.-Y, Xu G.-R. and Zhang R.-Q.*

**Source:** International Journal of Biochemistry and Cell Biology 37(7): 1446–1457 (2005).

Alkaline phosphatases (ALP, EC 3.1.3.1) are ubiquitous enzymes found in most species. ALP from a pearl oyster, *Pinctada fucata* (PALP), is presumably involved in nacreous biomineralisation processes. Here, chemical modification was used to investigate the involvement of basic residues in the catalytic activity of PALP. The Tsou's plot analysis indicated that the inactivation of PALP by 2,4,6-trinitrobenzenesulfonic acid (TNBS) and phenylglyoxal (PG) is dependent upon modification of one essential lysine and one essential arginine residue, respectively. Substrate reaction course analysis showed that the TNBS and PG inactivation of PALP followed pseudo-first-order kinetics, and the second-order inactivation constants for

the enzyme with or without substrate binding were determined. It was found that the binding substrate slowed PG inactivation, whereas it had little effect on TNBS inactivation. Protection experiments showed that substrates and competitive inhibitors provided significant protection against PG inactivation, and the modified enzyme lost its ability to bind the specific affinity column. However, the TNBS-induced inactivation could not be prevented in the presence of substrates or competitive inhibitors, and the modified enzyme retained the ability to bind the affinity column. In conclusion, an arginine residue involved in substrate binding and a lysine residue involved in catalysis were present at the active site of PALP. This study will facilitate description of the role that ALP plays in pearl formation and the mechanism involved.

### cDNA cloning and characterisation of a novel calmodulin-like protein from pearl oyster *Pinctada fucata*

*Li S., Xie L., Ma Z., Zhang R.*

**Source:** FEBS Journal 272 (19):4899–4910 (2005).

Calcium metabolism in oysters is a very complicated and highly controlled physiological and biochemical process. However, the regulation of calcium metabolism in oysters is poorly understood. Our previous study showed that calmodulin (CaM) seemed to play a regulatory role in the process of oyster calcium metabolism. In this study, a full-length cDNA encoding a novel calmodulin-like protein (CaLP) with a long C-terminal sequence was identified from pearl oyster, *Pinctada fucata*, expressed in *Escherichia coli* and characterised in vitro. The oyster CaLP mRNA was expressed in all tissues tested, with the highest levels in the mantle, which is a key organ in calcium secretion. In situ hybridisation analysis reveals that CaLP mRNA is expressed strongly in the outer and inner epithelial cells of the inner fold, the outer epithelial cells of the middle fold, and the dorsal region of the mantle. Oyster CaLP protein, with four putative $Ca^{2+}$-binding domains, is highly heat-stable and has a potentially high affinity for calcium. CaLP also displays typical $Ca^{2+}$-dependent electrophoretic shift, $Ca^{2+}$-binding activity and significant $Ca^{2+}$-induced conformational changes. $Ca^{2+}$-dependent affinity chromatography analysis demonstrated that oyster CaLP was able to interact with some different target proteins from those of oyster CaM in the mantle and the gill. In summary, our results have demonstrated that oyster CaLP is a novel member of the CaM superfamily, and suggest that oyster CaLP protein might play a different role from CaM in the regulation of oyster calcium metabolism.

### Extraction and purification of matrix protein from the nacre of pearl oyster *Pinctada fucata*

*Ma C., Zhang C., Nie Y., Xie L., Zhang R.*

**Source:** Tsinghua Science and Technology, 10(4): 499–503 (2005).

A soluble matrix protein P14 with an apparent molecular mass of 14.5 kDa was isolated from fragmented nacre of pearl oysters (*Pinctada fucata*) treated with 10% NaOH solution to investigate nacre matrix proteins and their effect on $CaCO_3$ crystal. The protein was characterized by gel exclusion chromatography and reversed-phase high performance liquid chromatography after demineralisation by 10% acetic acid. The X-ray diffraction pattern of P14 crystals indicates that P14 plays an important role in nacre biomineralisation. P14 can induce aragonite formation, stimulate $CaCO_3$ crystal formation, and accelerate aragonite precipitation. Heating of the acid insoluble nacre residue, which was named conchiolin, in 10% sodium dodecyl sulfate solution supplemented with 10% p-mercaptoethanol solution for 10–20 min at about 100°C gave two other soluble proteins with molecular masses of 19.4 kDa and 25.0 kDa. The present study suggests that these two proteins are linked to the insoluble organic matrix by disulfide bridges because the extraction yield increases when p-mercaptoethanol is added to the medium.

### Cloning and characterisation of a homologous $Ca^{2+}$/calmodulin-dependent protein kinase PSKH1 from pearl oyster *Pinctada fucata*

*Dai Y., Xie L., Xiong X., Chen L., Fan W., Zhang R.*

**Source:** Tsinghua Science and Technology 10(4): 504–511 (2005).

Many of the effects of $Ca^{2+}$ signaling are mediated through the $C^{2+}$/calmodulin complex and its acceptors, the $Ca^{2+}$/calmodulin-dependent protein kinases, including PSKH1. Studies of the proteins involved in calcium metabolism in oysters will help elucidate the pearl formation mechanism. This paper describes a full-length PSKH1 cDNA isolated from pearl oyster *Pinctada fucata*. Oyster PSKH1 shares 65% homology with human PSKH1 and 48% similarity with rat CaM kinase I in the amino acid sequence, and contains a

calmodulin-binding domain. The results of semi-quantitative reverse transcription-polymerase chain reaction and in situ hybridisation revealed that oyster PSKH1 mRNA is highly expressed in the outer epithelial cells of the mantle pallial and in the gill epithelial cells. These studies provide important information describing the complex $Ca^{2+}$ signalling mechanism in oyster calcium metabolism.

### Characterisation of two new genes implicated in mineralisation process of *Pinctada margaritifera*

*Cochennec-Laureau N., Fleury E., Belliard C. and Levy P.*

**Source:** Journal of Shellfish Research 24(2):646 (2005).

A transplant is a complex surgical procedure performed to obtain a pearl after about 12 to 18 months. One of the key steps in this procedure is the transfer of the graft (piece of mantle) from the donor oyster. A functional relationship exists between the coating nature of the pearl and the cellular structure of the pearl bag portion of the graft. We have identified two genetic markers of biomineralisation in *Pinctada margaritifera*, the pearl oyster: Perline and Calcine, responsible for secretion of aragonite and calcite, respectively. After isolating and sequencing a portion of each of these genes, we verified the specificity of these sequences using molecular methods (RT-PCR, in situ hybridisation) and biocomputing methods (alignments, phylogenetic trees, amino-acid composition). We have attempted to make a graft cartography to distinguish zones coding for expression of Perline and Calcine mRNA. The goal of this research is to define precisely the zone for selection of a graft (in a non empiric way) to achieve a desired balance between Perline and Calcine.

### The carbonic anhydrase domain protein, nacrein, is expressed in the epithelial cells of the mantle and acts as a negative regulator in calcification in the mollusc *Pinctada fucata*

*Miyamoto Hiroshi, Miyoshi Fumiko and Kohno Jun*

**Source:** Zoological Science (Tokyo) 22(3):311–315 (2005)

Signals and organic matrix proteins secreted from the mantle are critical for the development of shells in molluscs. Nacrein, which is composed of a carbonic anhydrase domain and a Gly-X-Asn repeat domain, is one of the organic matrix proteins that accumulates in shells. In situ hybridisation revealed that nacrein was expressed in the outer epithelial cells of the mantle of the pearl oyster *Pinctada fucata*. The recombinant nacrein protein inhibited the precipitation of calcium carbonate from a saturated solution containing $CaCl_2$ and $NaHCO_3$, indicating that it can act as a negative regulator for calcification in the shells of molluscs. Because deletion of the Gly-X-Asn repeat domain of nacrein had a significant effect on the ability of nacrein to inhibit the precipitation of calcium carbonate, it is conceivable that the repeat domain has a primary role in the inhibitory function of nacrein in shell formation. Together these studies suggest that nacrein functions as a negative regulator in calcification in the extrapallial space between the shell and the mantle by inhibiting the precipitation of $CaCO_3$.

### A novel carbonic anhydrase from the mantle of the pearl oyster (*Pinctada fucata*)

*Yu Z., Xie L., Lee S. and Zhang R.*

**Source:** Comparative Biochemistry and Physiology B 143(2): 190–194 (2006).

A novel carbonic anhydrase (CA) has been purified from the mantle of the pearl oyster, *Pinctada fucata*, by ammonium sulfate precipitation and affinity chromatography. Its molecular mass was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to be approximately 38 kDa. Native-PAGE shows that the novel CA can bind a fluorescent probe, 5-dimethylamino-1-naphthalenesulfonamide (DNSA), known to specifically bind carbonic anhydrase. Compared to carbonic anhydrase I (CAI) from human erythrocytes, the novel CA migrates faster, indicating that it is more acidic. The effect of an inhibitor on the enzyme activity was also examined. The CA from the mantle showed a weak resistance to acetazolamide (AZ), a specific inhibitor of CA. When DNSA was bound to CA, it caused the wavelength of emission maximum intensity to blue shift to 454 nm upon excitation at 326 nm. Histochemical data indicates that the enzyme is distributed widely throughout the mantle tissue, being concentrated at the edge of the mantle. The evidence presented indicates a function for CA in the process of pearl formation and biomineralisation.

### Biphasic and dually coordinated expression of the genes encoding major shell matrix proteins in the pearl oyster *Pinctada fucata*

*Takeuchi T. and Endo K.*

**Source:** Marine Biotechnology 8(1): 52–61 (2006).

Regional expression patterns of shell matrix protein genes of *Pinctada fucata* were investigated using real-time quantitative polymerase chain reaction (PCR) and in situ hybridisation. Six shell matrix proteins examined in this study indicated a distinct biphasic pattern of expression, falling into one of the following three groups: (1) expressed only in the more dorsal region of the mantle (MSI60 and N16); (2) expressed only in the more ventral region (MSI31, Prismalin-14, and Aspein); and (3) expressed in both regions (nacrein). The ubiquity of the last protein probably reflects its general role as a carbonate-producing enzyme, while the other groups are interpreted as corresponding to the distinction between the two varieties of shell layers, the aragonitic nacreous layer and the calcitic prismatic layer. In addition, the constituent genes of each of these two groups indicated similar levels of relative expression among different sites, even among different individuals, suggesting that the genes of each group share a single upstream regulatory factor, respectively, and that these genes are expressed in a dually coordinated fashion.

### A novel matrix protein family participating in the prismatic layer framework formation of pearl oyster, *Pinctada fucata*

*Zhang C., Xie L., Huang J. Liu X., Zhang R.*

**Source:** Biochemical and Biophysical Research Communications 344(3):735–740 (2006).

Understanding the molecular composition and the formation mechanism of the shell matrix framework is of great interest for biomineralisation in mollusk shell. The cDNAs encoding a novel matrix protein family (KRMP) were cloned from the mantle of pearl oyster, *Pinctada fucata*. Analysis of the deduced amino acid sequences revealed that KRMP have a high proportion of lysine, glycine, and tyrosine, and their predicted isoelectric points are higher than those of any other identified shell matrix protein to our knowledge. The deduced amino acid sequences of KRMP can be divided into three regions, including an N-terminal signal peptide, a lysine-rich basic region interacting with acidic proteins or $CO_3^{2-}$, and a Gly/Tyr-rich region involved in the protein cross-link via a quinone-tanning process. RT-PCR and in situ hybridisation demonstrated that KRMP mRNA was specifically expressed in the mantle edge, involved in the prismatic layer formation. Taken together, it seems that KRMP is a matrix protein family participating in the framework formation of the prismatic layer.

### A novel putative tyrosinase involved in periostracum formation from the pearl oyster (*Pinctada fucata*)

Zhang C., Xie L., Huang J., Chen L. and Zhang R.

**Source:** Biochemical and Biophysical Research Communications 342(2):632–639 (2006).

Tyrosinase (monophenol, L-DOPA: oxygen oxidoreductase, EC 1.14.18.1), a kind of copper-containing phenoloxidase, has great interest for scientists in relation to its important role in periostracum formation. A cDNA clone encoding a putative tyrosinase, termed OT47 because of its estimated molecular mass of 47 kDa, was isolated from the pearl oyster, *Pinctada fucata*. This novel tyrosinase shares similarities with the cephalopod tyrosinases and other type 3 copper proteins within two conserved copper-binding sites. RT-PCR analysis showed that OT47 mRNA was expressed only in the mantle edge. Further in situ hybridisation analysis and tyrosinase activity staining revealed that OT47 was expressed at the outer epithelial cells of the middle fold, differing from early histological results in *Mercenaria mercenaria*, suggesting a different model of periostracum secretion in *P. fucata*. Taken together, these results suggest that OT47 is most likely involved in periostracum formation. The identification and characterisation of oyster tyrosinase also help to further understand the structural and functional properties of molluscan tyrosinase.

### Cloning and characterization of a novel G protein ß-subunit of pearl oyster (*Pinctada fucata*), and its interaction sites with calmodulin

*Chen Lei, Xie Liping, Xiong Xunhao, Dai Yiping, and Fan Weimin*

**Source:** Comparative Biochemistry and Physiology B 142(2), 142–152 (2005)

A cDNA clone encoding a novel G protein ß subunit of ß1 subclass, pfGß1, was isolated from the pearl oyster (*Pinctada fucata*). The deduced amino acid sequence of pfGß1 (341 amino acids) shares high homology

with the northern European squid (*Loligo pealei*) and great pond snail (*Lymnaea stagnalis*) Gß1, while it has diverged from bovine (*Bos taurus*) and human. Well-conserved amino acid domains in the G protein ß subunit, seven WD repeats, were found in the deduced amino acid sequence. Alignment analysis showed that the beginning amino acid residues in variable fragments of the seventh WD motif are different from those of any other Gß. Prediction of the 3D structure of pfGß showed that pfGß belongs to ß-propeller family proteins, whose members contain 4–8 antiparallel P-sheets resembling the blades of a propeller. In situ hybridisation and Northern blotting analysis revealed that the pfCi mRNA hybridisation signals were widely expressed in various tissues except muscle, and were abundant in the epithelia of the gill, gonad and outer fold of the mantle. We also investigated the interactions between Gßγ and calmodulin (CaM), and specific amino acid residues that may be critical for the binding of Gßγ to CaM were also identified. Furthermore, functional studies of the interaction showed that the binding of CaM and Gßγ increases alkaline phosphatase (ALP) activity, an indicator for mineralisation in MC3T3-E1 cells. The ALP activity of the mutants of pfGßγ that impaired the interactions of Gßγ with CaM was higher than for the control group; however, it was lower than for the WTC group. Together, these results suggest that Gßγ might interact with CaM and point to an important physiological function in modulating cellular functions.

### Shematrin: a family of glycine-rich structural proteins in the shell of the pearl oyster *Pinctada fucata*

*Yano M., Nagai K., Morimoto K. and Miyamoto H.*

**Source:** Comparative Biochemistry and Physiology B 144(2): 254–262 (2006).

Random sequencing of molecules from a cDNA library constructed from mantle mRNA of the pearl oyster *Pinctada fucata* was used to obtain information on organic matrix proteins in the shell. In the determined sequences, we identified seven distinct cDNAs encoding similar glycine-rich domains. Complete sequence analysis of these cDNAs showed that the predicted sequences of the proteins, which we named shematrins, possessed similar domains comprising repeat sequences of two or more glycines, followed by a hydrophobic amino acid. In addition, in shematrin-1, -2 and -3, a repeat domain designated as $XG_nX$ (where X is a hydrophobic amino acid) was conserved. It is of further note that all the shematrin proteins have RKKKY, RRKKY or RRRKY as their C-terminal sequence. According to Northern blot analysis, all shematrins are exclusively expressed in the mantle, particularly in the edge region of the mantle. Furthermore, peptide fragments similar to shematrin-1 and -2 were detected in the prismatic layer of the shells by MALDI-TOF/TOF MS analysis. These findings suggest that many shematrins are synthesised in the mantle edge and secreted into the prismatic layer of the shell, where the protein family is thought to provide a framework for calcification.

## Pearl oyster health

### Effects of exogenous lipid peroxides on mortality and tissue alterations in Japanese pearl oysters *Pinctada fucata martensii*

*Sugishita Y., Hirano M., Tsutsumi K., Mobin S.M.A., Kanai K. and Yoshikoshi K.*

**Source:** Journal of Aquatic Animal Health: 233–243 (2005)

The effects of exogenous lipid peroxides, suspected to be a cellular injury factor that causes mass mortalities of cultured Japanese pearl oysters *Pinctada fucata martensii*, were investigated in vivo and in vitro. Cumulative mortalities of experimental oysters exposed to oxidized oils in suspension (fish feed oil) or emulsion (methyl linoleate) were approximately 40% after eight or nine weeks, whereas mortalities were 0.0% (suspension) and 5.6% (emulsion) in control oysters exposed to unoxidized oils. Pathological changes observed in experimental oysters were characterised by blebbing and necrosis of cells in various organs that had spread from the digestive organ and were identical to those observed in diseased oysters from natural mass mortalities. The thiobarbituric acid values of experimental oysters were consistently and significantly higher than those of control oysters. An in vitro exposure examination also demonstrated that oxidized oil caused conspicuous blebbing and necrosis in the epithelial cells of the digestive organ. These results suggest that organic pollution caused by suspended solids containing lipid peroxides, emulsion of oxidized oils, or both, is a major environmental factor that chronically damages tissues of cultured oysters and can cause mass mortalities.

## An investigation of the pathology associated with mass mortality events in the cultured Japanese pearl oyster *Pinctada fucata martensii* at four farms in Western Japan

*Hirano Mizuki, Sugishita Yoshiyuki, Mobin SMA, Kanai Kinya and Yoshikoshi Kazuma*

**Source:** Journal of Aquatic Animal Health 17(4): 323–337 (2005).

The epidemiological and histopathological characteristics of the mass mortality of cultured Japanese pearl oysters *Pinctada fucata martensii* were investigated. Rearing experiments with Japanese pearl oysters in farms revealed that mass mortality occurs as a regular annual event in particular farms in western Japan. Diseased oysters had marked atrophy and red-brown discoloration of the soft parts of the body. Light microscopy revealed that the epithelia of the stomach, the ducts of the digestive diverticula (DD), and the DD themselves showed marked blebbing and necrosis to varying degrees during earlier stages of the disease. At advanced stages, muscle fibres of the adductor muscle, heart, mantle, and other parts of the body and the connective tissues of various organs involving the vascular system also exhibited considerable atrophy and necrosis. There were no remarkable changes in the branchial and pallial epithelia. No viral, bacterial, mycotic, or parasitic causative organisms were found in diseased oysters. The results of a case study of mass mortality at one farm suggested that there is some causal relationship between outbreaks of this disease and the existence of neighboring fish farms. These findings suggest that mass mortality is not due to an infectious disease. We discuss pathological features and possible causes of this disease.

## Excavating sponges that are destructive to farmed pearl oysters in Western and Northern Australia

*Fromont J., Craig R., Rawlinson L. and Alder J.*

**Source:** Aquaculture Research 36(2):150–162 (2005).

Species of the family Clionaidae (Porifera: Demospongiae) that excavate shell of the silver-lip pearl oyster, *Pinctada maxima*, in north Western and Northern Australia are described. Two species belong to the genus *Cliona* and one species to the closely related genus *Pione*. *Cliona orientalis* has only recently been reported from Australia in living and dead coral on the Great Barrier Reef, and this is the first report of this species from north Western and Northern Australia. *Cliona dissimilis* is reported from Australia for the first time. *Pione velans* was first described from Shark Bay, Western Australia, and this study extends its distribution from Albany, south Western Australia, to Port Bremer in the Northern Territory. The most common species found was *C. dissimilis*. *Pione velans* and *C. orientalis* were also present, although the latter species was rare. *Cliona dissimilis* and *P. velans* were found to be sexually reproductive in some shells with specimens of *C. dissimilis* with oocytes in May and *P. velans* in September 1999. Egg development was synchronous, indicating that the sponges were oviparous and would broadcast gametes in a spawning event.

## Effect on bivalve molluscs of a harmful dinoflagellate *Heterocapsa circularisquama* isolated from Omura Bay, Japan, and its growth characteristics

*Yamatogi T. Sakaguchi M., Matsuda M., Iwanaga S., Iwataki M. and Matsuoka K.*

**Source:** Nippon Suisan Gakkaishi 71(5): 746–754 (2005).

*Heterocapsa circularisquama* is a red-tide forming dinoflagellate causing mass mortality of bivalve molluscs in Western Japan. Bioassays on bivalve molluscs were conducted using two strains of *H. circularisquama* isolated from Omura Bay, Nagasaki Prefecture, in 1999 and 2002. Pearl oyster *Pinctada fucata* died within 2 to 3 days of being exposed to 5,000 cells $mL^{-1}$ of *H. circularisquama*. Short-neck clam *Ruditapes philippinarum* died within 4 to 6 days of being exposed to 5,000 cells $mL^{-1}$ of *H. circularisquama*. The growth characteristics of *H. circularisquama* isolated from Omura Bay in 1999 were examined in 54 different combinations of temperature (10–30°C) and salinity (16–36) under a light intensity of 80 µmol $m^{-2}$ $s^{-1}$. *H. circularisquama* reproduced at 12.5–30°C and 16–36 in salinity. The highest growth rate was obtained at 30°C and 32 in salinity. Under the conditions, the maximum specific growth rate was 0.91 $day^{-1}$. The effect of light intensity on the growth of *H. circularisquama* was examined for seven different conditions ranging from 10 to 140 µmol $m^{-2}$ $s^{-1}$ under a 14 h light and 10 h dark cycle and 25°C. *H. circularisquama* grew well at a light intensity of 20 µmol $m^{-2}$ $s^{-1}$ and higher, then growth became saturated at 80 µmol $m^{-2}$ $s^{-1}$. Under the conditions of this experiment, for growth, the half-saturation constant of light intensity (Ks) and threshold value (Io) were 24.0 µmol $m^{-2}$ $s^{-1}$ and 15.5 µmol $m^{-2}$ $s^{-1}$, respectively. The occurrence of *H. circularisquama* in Omura Bay, Imari Bay and Tachibana Bay was confirmed in the present study by observation of body scale structures.

### Diseases of pearl oysters and other molluscs: a Western Australian perspective

*Jones J.B. and Creeper J.*

**Source:** Journal of Shellfish Research 25(1): 233–238 (2006).

Mollusc culture, particularly the cultivation of pearl oysters, is an important component of the aquaculture industry in Western Australia. As a result, there has been a long-term investment in surveys of commercial mollusc species for potential diseases of concern. A number of pathogens, particularly haplosporidans, identified within wild-stock shellfish have the potential to adversely affect mollusc populations. Others pose risks for translocations associated with aquaculture. The microsporidan *Steinhausia mytilovum* (Field), found in ova of the blue mussel *Mytilus galloprovincialis* (Lamarck), poses intriguing questions about the origin and dispersal of its host.

## Taxonomy and genetics

### Natural hybridisation between *Pinctada fucata* and *Pinctada maculata* inferred from internal transcribed spacer regions of nuclear ribosomal RNA genes

*Masaoka T. and Kobayashi T.*

**Source:** Fisheries Science 71(4):829–836 (2005).

Genetic evidence was obtained of the occurrence of natural hybridisation between female *Pinctada fucata* and male *Pinctada maculata* among wild pearl oysters (n = 20) collected for use as the mother shell for private pearl farming in the Oshima Strait at Amami-o-shima, Kagoshima Prefecture, Japan. A polymerase chain reaction-based species identification method for *Pinctada* was developed using polymorphisms in the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA (rRNA) gene. This method enabled the amplification of the ITS regions using a primer set specific for *P. maculata* and *P. fucata*. However, 10 of 20 individuals morphologically identified as *P. fucata* had sequences specific to both *P. maculata* and *P. fucata* in the ITS region. These putative hybrids showed sequences of a maternally inherited mitochondrial 16S rRNA gene, identical to that of *P. fucata*. Shells of the putative hybrids were difficult to discriminate from those of *P. fucata* exhibiting similar taxonomic traits. Moreover, the hybrids exhibited slower growth than *P. fucata* but faster growth than *P. maculata*.

### Species identification of *Pinctada imbricata* using intergenic spacer of nuclear ribosomal RNA genes and mitochondrial 16S ribosomal RNA gene regions

*Masaoka Tetsuji and Kobayashi Takanori*

**Source:** Fisheries Science (Tokyo) 71(4):837–846 (2005).

For pearl production, pearl oyster seeds from foreign pearl oysters, as well as hybrids between native and foreign pearl oysters, are produced in Japanese hatcheries. However, it is very difficult to identify these pearl oysters and hybrids based on morphological measurements. Thus, a molecular identification method for distinguishing Atlantic pearl oysters *Pinctada imbricata* from the Indian-Pacific pearl oyster group including *P. martensii* and *P. fucata*, was developed. The polymerase chain reaction (PCR) products of the partial intergenic spacer (IGS) of nuclear ribosomal RNA (rRNA) genes exhibited length polymorphism between *P. imbricata* (590 bp) and the other two species (427 bp). Restriction fragment length polymorphism analysis of the PCR products (PCR-RFLP) cleaved with Mse I observed in the IGS of nuclear rRNA genes also gave different profiles between *P. imbricata* and the other two species. The difference in PCR-RFLP using AN I was also detected in the mitochondrial 16S rRNA gene regions between *P. imbricata* and the other two species. Thus, the method developed enables the distinction of *P. imbricata* from *P. martensii* and *P. fucata*.

### Recent foundation of Mexican populations of pearl oysters (*Pteria sterna*) revealed by lack of genetic variation on two mitochondrial genes

*Arnaud-Haond S., Blanc F., Bonhomme E. and Monteforte M.*

**Source:** Journal of the Marine Biological Association of the United Kingdom 85(2): 363–366 (2005).

The rainbow pearl oyster *Pteria sterna* is distributed along the Mexican Pacific coasts and Gulf of Baja California, where it represents a great economic potential. Five sampled populations collected in Baja California

were used to screen two mitochondrial DNA genes that have proven useful in population genetics studies of several other bivalve species. The lack of polymorphism detected in these samples suggests a recent event of very low population size, most likely a founder event, which is congruent with the absence of *P. sterna* in the fossil records in Baja California.

### Variability of ribosomal DNA ITS-2 and its utility in detecting genetic relatedness of pearl oyster

*He M., Huang L., Shi J. and Jiang Y.*

**Source:** Marine Biotechnology 7(1):40–45 (2005).

The objective of this study was to detect interspecific and intraspecific genetic variations of the second internal transcribed spacer of ribosomal DNA (ITS-2), and explore the feasibility of using it as a molecular marker for phylogenetic analyses and species identification among pearl oysters. ITS-2 sequences of six pearl oysters were amplified via polymerase chain reaction. The amplified DNA fragments were about 500 bp, spanning the partial sequences of 5.8S and 28S rRNA genes. The GC contents of all species used in this study were higher than the AT contents. The variations of sequences involved substitutions as well as insertions/deletions and were mainly concentrated in spacer regions. Sequences of about 30-bp in spacer regions showed no variations among five *Pinctada* species. Intraindividual and intraspecific polymorphisms of ITS-2 sequences were detected in some species; the interspecific variability was significantly larger than the variability within species, and the variability at the genus level was higher than that at the species level. Both neighbour-joining and parsimony analyses of ITS-2 sequences revealed the distinguishable species boundary of six pearl oysters, and indicated that *P. chemnitzi* and *P. nigra* were closely related species, as were *P. maxima* and *P. margaritifera*. The findings revealed that ITS-2 sequences could be an appropriate tool for phylogenetic study of pearl oysters.

### Development and characterisation of novel tetra- and dinucleotide microsatellite markers for the French Polynesia black-lipped pearl oyster, *Pinctada margaritifera*

*Herbinger C.M., Smith C.A. and Langy S.*

**Source:** Molecular Ecology Notes 6(1):107–109 (2006).

Ten microsatellite loci were developed for the black-lipped pearl oyster *Pinctada margaritifera* with a magnetic bead enrichment protocol. These tetra- and dinucleotide markers were polymorphic, with 10 to 43 alleles observed in 97 individuals from two Tuamotu atoll populations. Most loci revealed significant genic differentiation between the two populations and also exhibited some degree of heterozygote deficiencies, probably due to the presence of null alleles. These loci should be very useful for describing genetic structure, genetic variability and reproductive success in the various aquaculture and wild populations of pearl oyster in French Polynesia.

### Species identity and phylogenetic relationship of the pearl oysters in *Pinctada* Roeding, 1798 based on ITS sequence analysis

*Yu D.H. and Chu K.H.*

**Source:** Biochemical Systematics and Ecology 34(3):240–250 (2006).

Analysis of ITS 1 and ITS 2 sequences in the pearl oysters *Pinctada albina*, *Pinctada chemnitzi*, *Pinctada fucata*, *Pinctada fucata martensii*, *Pinctada imbricata*, *Pinctada margaritifera*, *Pinctada maxima*, *Pinctada nigra* and *Pinctada radiata* was carried out. A homogeneity test of substitution patterns suggests that GC contents are highest in *P. margaritifera* and *P. maxima* and chromosomal rearrangements occurred in *P. chemnitzi*. These observations indicate that *P. margaritifera* and *P. maxima* are primitive species and *P. chemnitzi* is a recent species. Phylogenetic analysis shows that the pearl oysters studied constitute three clades with *P. margaritifera* and *P. maxima* forming the basal clade, congruent with results revealed by the substitution pattern test. The second clade consists of *P. fucata*, *P. fucata martensii* and *P. imbricata*. Low genetic distances among these taxa indicate that they may be conspecific. The remaining species make up the third clade and low genetic divergence between *P. albina* and *P. nigra* suggests that they may represent the same species. The ITS 1 sequence of *P. radiata* in GenBank is almost identical to that of *P. chemnitzi* determined in the present study and we suspect that the specimen used for the *P. radiata* sequence was misidentified.

### Low genetic differentiation among widely separated populations of the pearl oyster *Pinctada fucata* as revealed by AFLP

*Yu D.H. and Chu K.H.*

**Source:** Journal of Experimental Marine Biology and Ecology 333(1): 140–146 (2006).

Genetic variation within and among five populations of the pearl oyster *Pinctada fucata*, from China (Daya Bay, Sanya Bay and Beibu Bay), Japan (Mie Prefecture) and Australia (Port Stephens) was studied using AFLP. Three primer pairs generated 184 loci, of which 91.8–97.3% were polymorphic. An overall genetic diversity of 0.38 among populations and an average of 0.37 within populations (ranging from 0.35 in the Japanese population to 0.39 in the Beibu Bay population) were observed. Genetic differentiation among the five populations is low but significant as indicated by pairwise $G_{ST}$ (0.0079–0.0404). AMOVA further shows that differentiation is significant among the five populations but is not significant at a broader geographical scale, among the three groups of Chinese, Japanese and Australian populations or among the two groups of Australian and north Pacific populations. The low level of genetic differentiation indicated that *P. fucata* populations in the west Pacific are genetically linked. Among the five populations, the Australian one is more differentiated from the others, based on both pairwise AMOVA and $G_{ST}$ analyses, and is genetically isolated by distance as indicated by the Mantel test. However, genetic differences among the three Chinese populations are not correlated with geographic distances, suggesting that Hainan Island and Leizhou Peninsula may act as barriers blocking gene flow.

### Theory and methods of genetic improvement in mariculture mollusks: a review

*Zhang G.-F. and Liu X.*

**Source:** Journal of Fisheries of China 30(1):130–137 (2006).

A strategy for genetic improvement in aquaculture mollusks, which involves theory, method, materials and application system, is very important for the sustainable development of marine molluscan breeding and the industry. Selection and hybridisation in the traditional way have proved effective in the genetic improvement of mariculture mollusks. Growth was increased significantly by selective breeding of oysters as well as through resistance to MSX and QX disease. A framework for genetic improvement of mollusks has been derived from breeding applications for abalone and scallop. Systematic breeding with selection and hybridisation has made substantial progress based on establishment of families in *Haliotis discus hannai*, *H. diversicolor*, *Argopecten irradians*, *Chlamys farreri* and *Pinctada martensii*. The first self-fertilised family of bay scallop was established in 1999 followed by a series of family lines with different traits, which formed the basic elements for genetic breeding. The hybridisation of Pacific abalone between different populations with a large genetic distance has showed remarkable traits. Hybrid Pacific abalone have been used to improve the traits of growth and resistance in aquaculture and 98% of the seed used in farming is hybrid. Hybridisation between populations is also used in other mariculture molluscs, such as scallop, bay scallop, pearl oyster and small abalone. Hybridisation between different populations and systematic and massive selection are the dominant approaches in the genetic improvement of mollusks. So far, in China there has been considerable work on material systems for molluscan genetics and breeding as well as on the basic theory of heritability, heterosis, inbreeding depression, and genetic-environment interactions for genetic improvement. Different lines have been produced by self-fertilisation, inbreeding and crossing and some self-fertilised lines of bay scallop have been bred to F4. The heritability of colour traits is useful as a marker for selective breeding. It is necessary to deal with molecular marker-assisted selection and design breeding. The molluscan mortality that occurs in aquaculture has mainly resulted from physiological depression under the interaction of genetics and environment. So the phenomena of minimisation and abnormal mortality in mariculture mollusks should be described as "trait depression".

## Environmental issues

### Oil pollution and tropical littoral communities: Biological effects of the 1975 Florida Keys oil spill

*Chan E.I.*

**Source:** 2005 International Oil Spill Conference, IOSC 2005:5382 (2005).

This study reports on the biological effects of the July 1975 oil spill in the Florida Keys for a one-year period. Floating seagrass served as a natural sorbent for oil and stranded in the intertidal zone. A soluble component of oil, or possibly an organic cleaning solvent, leaching from this debris, was probably

responsible for mass mortality of subtidal echinoderms on the rocky platform. Several crab species were eliminated from the rocky shores, mangrove fringes, and Batis marsh communities for several months. Subtidal pearl oysters (*Pinctada radiata*) from the grass flat community suffered extensive mortalities, also attributable to a soluble component of oil. Red mangrove (*Rhizophora mangle*) seedlings on the fringe and in the mangrove swamp, sustaining greater than 50% oiling of their leaves, were killed. Dwarf black mangroves (*Avicennia nitida*) with greater than 50% oiling of pneumatophores also died, as did some where the substrate remained oiled one year later. Elevated temperatures, exceeding lethal limits for many intertidal organisms, were observed in oil-covered substrates. Oil persisted in the substrate of rocky shores and mangrove-marsh areas for at least one year after the spill.

### The effects of aquaculture on bottlenose dolphin (*Tursiops* sp.) ranging in Shark Bay, Western Australia

*Watson-Capps J.J. and Mann J.*

**Source:** Biological Conservation 124(4):519–526 (2005).

The increasing presence of aquaculture in coastal waters calls for a better understanding of its environmental effects. Currently little information is available on the impact of shellfish farms on cetaceans. Here we compare long-term ranging patterns of adult female bottlenose dolphins (*Tursiops* sp. in Shark Bay, Western Australia) before and during full-scale pearl oyster farming operations, to determine if they were displaced. When the exact location of the oyster farm was determined, the dolphins decreased their use of that area after the farm was in place. Tracks of adult female dolphin movement near the oyster farm were compared to tracks of dolphin movement near an ecologically similar area where no oyster farm existed. Tracks near the oyster farm were less likely to enter the oyster farm itself than tracks near an ecologically similar location. This suggests that shellfish aquaculture could have a large impact on small cetaceans. The analytical techniques discussed apply broadly to aquatic and terrestrial animals.

### Quantification of in situ nutrient and heavy metal remediation by a small pearl oyster (*Pinctada imbricata*) farm at Port Stephens, Australia

Gifford S., Dunstan H., O'Connor W. and Macfarlane G.R.
**Source:** Marine Pollution Bulletin 50(4):417–422 (2005).

The use of pearl oysters has recently been proposed as an environmental remediation tool in coastal ecosystems. This study quantified the nitrogen, phosphorus and heavy metal content of the tissue and shell of pearl oysters harvested from a small pearl oyster farm at Port Stephens, Australia. Each tonne of pearl oyster material harvested resulted in approximately 703 g metals, 7452 g nitrogen, and 545 g phosphorus being removed from the waters of Port Stephens. Increasing current farm production of 9.8 t $yr^{-1}$ to 499 t $yr^{-1}$ would balance current nitrogen loads entering Port Stephens from a small sewage treatment plant located on its southern shores. Furthermore, manipulation of harvest dates to coincide with oyster condition would likely remove substantially greater quantities of nutrients. This study demonstrates that pearl aquaculture could be used to assist in the removal of pollutants from coastal waters, while producing a commercially profitable commodity.

### Effect of the pollutants lead, zinc, hexadecane and octocosane on total growth and shell growth in the Akoya pearl oyster, *Pinctada imbricata*

*Gifford S.P., MacFarlane G.R., O'Connor W.A. and Dunstan R.H.*

**Source:** Journal of Shellfish Research 25(1): 159–165 (2006).

Pearl oysters (*Pinctada imbricata*) were held in the laboratory and exposed to various levels of the heavy metals, lead and zinc, and the aliphatic hydrocarbons, hexadecane and octacosane, for two months. Individual oysters were followed over the course of the experiment, allowing specific calculation of total oyster growth (wet weight) and shell growth. Significant reductions in total oyster growth were observed when oysters were exposed to high concentrations (270 µg $L^{-1}$) of either zinc or lead. Exposure to the aliphatic hydrocarbons had no effect on total oyster growth. High concentrations of lead completely halted shell growth, the first demonstration of pollutant induced cessation of biomineralisation in pearl oysters. Conversely, exposure to moderate levels of lead and the long-chain hydrocarbon octacosane resulted in significant increases in shell width growth.

## The value of evidence about past abundance: Marine fauna of the Gulf of California through the eyes of 16th to 19th century travellers

*Sàenz-Arroyo A., Roberts C.M., Torre J., Carinõ-Olvera M. and Hawkins J.P.*

**Source:** Fish and Fisheries 7(2):128–146 (2006).

Eyewitness accounts written by early travellers to 'the new world' provide valuable insights into how seascapes once looked. Although this kind of information has been widely used to chart human impacts on terrestrial ecosystems, it has been greatly overlooked in the marine realm. Here we present a synthesis of 16th to 19th century travellers' descriptions of the Gulf of California and its marine wildlife. The diaries written by conquerors, pirates, missionaries and naturalists described a place in which whales were 'innumerable,' turtles were 'covering the sea' and large fish were so abundant that they could be taken by hand. Beds of pearl oysters that are described had disappeared by 1940 and only historical documents reveal the existence of large, widespread, deep pearl oyster reefs, whose ecology and past functions we know little about. Disqualifying the testimonies of early visitors to a region as 'anecdotal' is dangerous; it may lead to setting inappropriate management targets that could lead to the extinction of species that are rare today but were once much more abundant. Moreover, it represents unfair historical judgement on the work of early natural historians, scholars and scientists. We suggest that the review and analytical synthesis of reports made by early travellers should become part of the prerequisites for deciding how to manage marine ecosystems today.

## An assessment of the environmental impact of wild harvest pearl aquaculture (*Pinctada maxima*) in Western Australia.

*Wells F.E and Jernakoff P.*

**Source:** Journal of Shellfish Research 25(1):141–151 (2006).

Typical operating procedures used in the wild harvest pearl aquaculture (*Pinctada maxima*) industry in Western Australia are described as a basis for examining the potential environmental impact of the industry. A risk analysis workshop was held, which included industry representatives, marine scientists, regulatory agencies and conservation interests. The goal of the workshop was to document the main potential environmental and ecological risks that arise from the various activities carried out by the *P. maxima* industry. Thirteen environmental and ecological issues were identified across the *P. maxima* fishery. None were considered to be high risks; all were ranked as either moderate (23%) or low (77%). Moderate risk rankings included: introduction of disease from seeding; attraction of other fauna; and introduction of exotic organisms. Low risks were: spread of disease; introduction of disease from hatchery; introduction of disease from translocation; impact on protected and endangered species resulting from entanglement; impact of habitat; impact on protected and endangered species resulting from farm lighting; nutrient impacts in sediment; perceived change in water quality; potential for litter; and reduction of primary productivity. The low ratings given to disease risks took into account current strict regulatory controls for minimising disease risks. The industry is considered to be environmentally benign. However, recommendations are made on how to further minimise risk.

## The Akoya pearl oyster shell as an archival monitor of lead exposure

*MacFarlane G.R., Markich S.J., Linz K., Gifford S., Dunstan R.H., O'Connor W. and Russell R.A.*

**Source:** Environmental Pollution 143(1): 166–173 (2006).

The Akoya pearl oyster (*Pinctada imbricata*) was experimentally exposed to (a) constant levels of lead (Pb) at 180 µg $L^{-1}$ for nine weeks, or (b) two short-term (pulse) exposures of Pb at 180 µg $L^{-1}$ (three weeks each) with an intervening depuration period (three weeks), to assess its utility as (i) an accumulative monitor of Pb contamination, and (ii) an archival monitor for discriminating constant versus pulsed Pb exposure events. *P. imbricata* showed similar reductions in growth (based on shell morphology and wet weight) and Pb accumulation patterns for whole tissue and shell in response to both Pb exposure regimes. Thus the whole oyster was deemed an inappropriate accumulative monitor for assessing short-term temporal variation of Pb exposure and effect. However, using secondary ion mass spectrometry, Pb was shown to accumulate in the successively deposited nacreous layers of the shell of *P. imbricata*, documenting the exposure history of constant versus pulsed Pb events. Patterns of Pb deposition not only reflected the frequency of Pb exposure events but also their relative durations. Thus, the shell of *P. imbricata* may be employed as a suitable biological archive of Pb exposure.

## PEARL PRODUCTION

### Mantle regeneration in the pearl oysters *Pinctada fucata* and *Pinctada margaritifera*

*Acosta-Salmon H. and Southgate P.C.*

**Source:** Aquaculture 246: 447–453 (2005).

Mantle tissue in pearl oysters (Pteriidae) is responsible for secreting the mother-of-pearl or nacre lining the shell. When grafted into another oyster, excised mantle tissue, commonly called 'saibo', is responsible for cultured pearl production. This study was undertaken to assess the process of mantle regeneration in the Akoya pearl oyster *Pinctada fucata* and the blacklip pearl oyster *Pinctada margaritifera* following mantle excision. Prior to saibo excision, oysters were anaesthetised with 2 mL $L^{-1}$ propylene phenoxetol. Saibo tissue was excised from 50 *P. fucata* and 10 *P. margaritifera*. After excision, all oysters were returned to culture conditions. All oysters were maintained in panel (pocket) nets on a longline at Magnetic Island, north Queensland, Australia. Oysters were anaesthetised and sacrificed 3, 6, 9, 12, 15, 20, 30, 45, 60 and 90 days after saibo excision to assess mantle regeneration using histological and histochemical techniques. Survival over the 90-day study was 100% and 70% for *P. fucata* and *P. margaritifera*, respectively. After excision, the mantle tissue healed within the first three days and began growing as connective tissue. Muscular development was seen between days 60 and 90 after excision. Shell formation abilities were recovered by day 15 when secretory cells and conchiolin secretions were first observed. Both *P. fucata* and *P. margaritifera* regenerated mantle to its original extent within the 90-day study. This is the first description of in vivo mantle regeneration in *pearl* oysters and the results have major implications for the lucrative cultured pearl industry. Our results indicate that mantle tissue donors need not be killed for pearl production. Those producing saibo that results in good quality pearls could be used as future parent-stock in breeding programs or for subsequent saibo donation and pearl production.

### Use of relaxants to obtain saibo tissue from the blacklip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*)

*Acosta-Salmon H., Martinez-Fernandez E. and Southgate P.C.*

**Source:** Aquaculture 246:167–172 (2005).

Cultured pearl production requires mantle tissue ('saibo') from donor oysters to be grafted into a number of recipient oysters. Use of anaesthetics is essential to keep donor pearl oysters alive for further utilisation and to minimise damage to them while excising saibo tissue. Propylene phenoxetol and benzocaine were chosen as relaxants as they have proven effective with pearl oysters over a short period of time and allow rapid recovery without mortality. *Pinctada margaritifera* and *Pinctada fucata* were exposed to propylene phenoxetol at a concentration of 2.5 mL $L^{-1}$ and benzocaine at concentrations of 250, 500 and 1200 mg $L^{-1}$. Once relaxed, oysters were observed every 5 min to evaluate the condition of the tissues. Oysters were classified as either 'suitable saibo donor' or 'non-suitable saibo donor' depending on their suitability for use as saibo donors for pearl production. Survival of oysters in all treatments was 100%. With the exception of oysters exposed to 250 mg $L^{-1}$ of benzocaine, where no relaxation was recorded, oysters in all other treatments became relaxed and showed good condition and acceptable characteristics for use as saibo donors.

### Wound healing after excision of mantle tissue from the Akoya pearl oyster, *Pinctada fucata*

*Acosta-Salmon H. and Southgate P.C.*

**Source:** Comparative Biochemistry and Physiology A 143(2):264–268 (2006).

Pearl oysters are usually sacrificed to donate mantle tissue for pearl production. However, if oysters are anaesthetised, they are able to survive mantle excision and regenerate this tissue. Mantle excision causes a large wound and severs the pallial artery, which necessitates rapid wound repair to avoid death by bleeding. This study was undertaken to assess the wound healing process in the mantle of the Akoya pearl oyster, *Pinctada fucata*, following mantle excision. Forty-seven *P. fucata* were relaxed with 2.5 mL $L^{-1}$ propylene phenoxetol before mantle tissue was excised. Oysters were relaxed and sacrificed 1, 3, 6, 12, 25, 36, 48, 66, 80 and 105 h after excision to assess mantle healing using histological techniques. Muscular contraction that effectively reduced the size of the wound was observed within 1 h after mantle excision. Accumulation of haemocytes and connective tissue occurred 3–6 h after excision and wound plugging was achieved within 6 h of excision. Proliferation of epithelial cells to cover the wound site was observed within the first 25 h after mantle excision and growth of connective tissue and formation of the pallial artery were observed within 105 h after mantle excision.

## The influence of culture method and culture period on quality of half-pearls ('mabé') from the winged pearl oyster *Pteria sterna*, Gould, 1851

*Ruiz-Rubio H., Acosta-Salmõn, H., Olivera A., Southgate P.C. and Rangel-Dàvalos C.*

**Source:** Aquaculture 254: 269–274 (2006).

Two groups of 96 winged pearl oysters, *Pteria sterna*, with mean (± S.D.) dorso-ventral shell height (DVM) of 75.0 ± 6.0 mm, were used to evaluate the effect of two culture methods (pocket nets and plastic cages) on the quality of half-pearls (mabé) produced from them over a period of 9 months. Prior to implantation, oysters were anaesthetised using benzocaine and two plastic nuclei were implanted on the left valve and one on the right valve of each pearl oyster. Oysters were returned to culture conditions and were sampled to determine nacre secretion and pearl quality 5, 7, 9 and 11 months after nucleus implantation. There was no significant difference ($P > 0.05$) between growth rates of oysters cultured in plastic cages and those held in pocket nets. However, a greater yield of commercial quality pearls was produced by oysters grown in plastic cages. Nacre thickness at the top of the resulting half-pearl blisters showed no significant difference between oysters held in the different culture apparatus ($P > 0.05$) or between different months ($P > 0.05$). Highest mabé quality was recorded 9 months after nucleus implantation when water temperatures were low. Growth of pearl oysters during this study resulted in enlargement of adductor muscle, which in some cases grew to cover the mabé. This result indicates that *P. sterna* with a dorso-ventral shell height greater than 75 mm should be used for the production of mabé. Furthermore, the results suggest that a culture period of 9 months is required to optimise the quality of mabé produced from *P. sterna* cultured in plastic cages.

## Effects of relaxants before embedding nucleus on survival rate and pearl qualities in *Pinctada fucata*

*Kanjanachatree K., Piyathamrongrut K. and Kaewteen P.*

**Source:** Songklanakarin Journal of Science and Technology 28(1): 87–97 (2006).

Nucleation in spherical pearl production from Akoya pearl oysters *Pinctada fucata* (Gould, 1850) showed a low survival rate, and the nuclei were frequently excluded. Using relaxants before nucleation could lower the oysters' metabolism, thus improving nucleation. This experiment showed that appropriate concentrations of propylene phenoxetol, 30% magnesium sulfate and 10% MS 222 were 2.5, 15 and 2 mL/L treated for 6, 11 and 7 minutes, respectively. At the beginning of anesthetisation, the oysters had high oxygen consumption, but this decreased continuously until constant, while those without the relaxant treatment had higher oxygen consumption. Oysters reared in the sea as soon as nucleation was finished had a higher rate of survival after eight months than those that remained in cement tanks for four weeks before being reared in the sea, and bead rejection also decreased. However, pearl formation in the oysters without the relaxant treatment was significantly better than in those with the treatments: the average pearl diameter obtained from the former was 6.62 mm, while those from the latter were 6.52, 6.48 and 6.46 mm when treated with 30% magnesium sulfate, propylene phenoxetol and 10% MS 222, respectively.



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