Sustainability and traceability in marine cultured pearl production

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SUMMARY

For centuries, wild pearl oysters and mussels were fished in the quest for natural pearls and shell material. This eventually led to the drastic overexploitation of oyster stocks in many areas of the globe. Scientific innovation and entrepreneurship eventually unearthed a solution: Researchers discovered a way for humans to farm pearl oysters and induce the formation of a cultured pearl. Pearl cultivation can be one of the most profitable forms of aquaculture and may be carried out in isolated islands where there are otherwise very limited economic opportunities. Pearl farming is also one of the most ecologically sound forms of aquaculture, and if managed accordingly has very little impact on the natural environment. The potential for sustainable development in island economies through pearl farming is large.

There is a positive link between environmental management and the long-term quality of pearls produced, and therefore an incentive for pearl farmers to operate in sustainable ways if they are to profit over time. The interdisciplinary approach of this dissertation has sought to produce research findings and linkages that can support the emergence of sustainable pearl production. Our main objective was to examine select sustainability questions in the context of pearl farming and investigate methods potentially useful in tracing pearls from farm to consumer. One hypothesis is that if sustainability metrics for pearl farms can be established and that these pearls can ultimately be traced through the supply chain, pearl consumers could further support the ecological and social benefits of pearl production in island economies. Emerging demand for responsibly produced raw materials in the jewellery industry suggests that there is indeed considerable potential for this.

The successes of the pearl industries of Australia and French Polynesia have led other Pacific nations to try and set up their own pearl industries, with mixed results. One of the newest examples of this is the Federated States of Micronesia (FSM) (Chapter 3). The challenges and opportunities of setting up a pearl industry in a country such as FSM are reviewed in this thesis: this includes a focus on production techniques, the potential for economic development, improvement of pearl quality and marketing of pearls. Pearl farming may present a great potential for Pacific communities, but being an activity that requires considerable expertise and long-term investment, must be suitably managed to achieve success. In FSM, community pearl farming takes place in a marine protected area (MPA) illustrating the potential of combining marine conseravation and pearl farming. A gemmological study of Micronesian pearls showed that it was not possible to distinguish them from pearl of *Pinctada margartifera* from other producing countries (e.g. French Polynesia), unless they are physically seperated through the supply chain, or traced otherwise.

One of the most important questions relating to pearl farming is its impact on biodiversity. This was tested by studying the influence of pearl farming on reef fish in a pearl producing atoll of French Polynesia. Reef fish are a good relative indicator of biodiversity. Because pearl farming often operates in sensitive environments, it is important to monitor its impact. This study (Chapter 4) showed that pearl farming - in the local context of Ahe- actually has a slight positive influence on fish abundance due to the shelter and food that pearl farming operations can offer reef fish. Importantly, pearl farming did not show to have any impact on reef fish diversity. Multi-factorial mixed model ANOVAs were used to determine the effects of pearl farm activity, position of sites relative to the pass and the distance of studied sites from the shore and pass on fish abundance and fish

diversity. Samples sizes were not sufficient for statistical tests of abundances of individual species, although certain surgeonfishes (e.g. *Acanthurus triostegus*, *Acanthurus xanthopterus*) and butterflyfishes appeared to be more numerous at pearl oyster farming sites. Our results in Ahe show that there were significant effects on fish abundances because of pearl farming, and position relative to and distance from Tiareroa Pass. The position and distance from pass effect can be explained by physical and biological factors that differ markedly both as a whole north and south of the Tiareroa Pass and because of flushing effects with distance from the pass.

The pearl industry has not been spared ecological problems, but it is clear that if it is managed correctly it can greatly contribute to both ecological and social sustainability. Responsible pearl farming must ensure that oysters are stocked in extensive conditions and that biofouling cleaning methods are of low impact on the benthic environment. Research both in French Polynesia and Micronesia does suggest that there is an important potential for pearl farming to operate in synergy with marine protected area (MPA) strategies in a number of countries. There are few other economic activities that can contribute to environmental conservation at the same time.

Innovation is another important aspect in the pearl industry, and rapid developments in technology have incited some pearl farmers to innovate so as to operate more efficiently or harvest pearls of greater quality. One such innovation is the emergence of new pearl nucleus materials, such as the organic-based nuclei that we studied (Chapter 5). Our study highlights how these new nuclei are used in pearl production and investigates the resulting pearl products using gemmological methods. Both generations of these 'new' types of pearls can easily be identified using common gemmological methods.

This research has also focused on finding ways of tracing pearls. This has included developing a novel method in testing pearls: DNA fingerprinting of pearls (Chapter 6). This is the first report of oyster DNA discovered in pearls, and opens up a host of new opportunities in pearl testing. Extracted DNA from pearls was used to identify the source oyster species for the three major pearl-producing oyster species *Pinctada margaritifera*, *P. maxima* and *P. radiata*. Both mitochondrial and nuclear gene fragments could be PCR-amplified and sequenced. This DNA fingerprinting method could be used to document the source of historic pearls and will provide more transparency for traders and consumers within the pearl industry. The final paper of this dissertation (Chapter 7) provides an overview of available and potential methods in tracing pearls from farm to consumer, all the way through the supply chain. Chemical marking, LA-ICP-MS, nucleus branding and other methods are reviewed. It is critical that such solutions can be feasibly integrated in pearl production and are cost efficient. Marking a pearl's nucleus or its surface seem the most promising options.

This study demonstrates that the sustainability potential of pearl farming is important in social and ecological terms. Metrics (e.g. impact on reef fish) can be devised so that sustainability standards for pearl farming can be developed. Other research has showed that there is a case for marine cultured pearls to be marketed as sustainable gems (Nash et al., forthcoming). In order to realise this potential, pearls also need to be adequately traced through the supply chain.

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CHAPTER 1

Introduction: A History of Pearls

Pearls are the oldest gems known to man. Pearls and their shells have been used for human adornment since at least 1500BC (Strack, 2006) and the oldest found and documented pearl has been dated back to 5500BC (Charpentier et al., 2012). Pearls feature in major religious texts such as the Bible and the Koran and pearl shells were already used as decorative objects in 4th millennium BC Egypt (Strack, 2008). Pearls found value and meaning in most ancient cultures, and the symbolism of pearl jewellery has taken on many different facets through the ages (Chadour-Sampson and Bari, 2013). The origin of pearls has often been explained in mythological and cosmological terms.

Pearls were long a symbol of power and purity during the Middle Ages and many royals adorned themselves with pearl jewels. An important period in pearl history were the first voyages of Christopher Columbus and other Spaniards in the 15th and 16th centuries when they discovered pearls and pearl oyster banks in Central and South America. The pearl oyster beds of Mexico, Panama, Colombia and Venezuela were an important source of pearls generating great wealth for the Spanish crown and triggered what is known as the "Pearl Age" amongst European royals and aristocrats (Bari and Lam, 2010). Overfishing of wild oysters in the quest for exquisite natural pearls in the Americas, French Polynesia, the Gulf of Mannar and other areas led to the depletion of many pearl oyster banks (Cariño and Monteforte, 2006). The quest for pearl oyster shell and pearls became truly global over the past few centuries.

Natural pearls form without any form of human intervention in wild molluscs (Strack, 2006). Pearls have been discovered in numerous different freshwater and marine molluscs species and regions around the world. However, the majority of natural pearls have been found by pearl fisheries in select regions. Pearl fishing has been active for millenia in the Arabian/Persian Gulf - a major source of natural pearls-, and was especially active in the 19th and early 20th centuries (Charpentier et al., 2012; Penziner Hightower, 2012). The Gulf of Mannar, located between India and Sri Lanka has also been an important source of natural pearls, up until the middle of the 20th century (Kunz and Stevenson, 1908; Strack, 2006). The Central and South American pearl oyster beds in Mexico, Panama, Colombia and Venezuela were an important source of pearls from the

16th century onwards.

In other regions such as Australia and French Polynesia, wild pearl oysters were primarily fished for their nacreous shell rather than for their pearls (Prokop, 2005). Pearls were a random find, and a welcome additional source of income. The Australian *Pinctada maxima* was especially coveted as a pearl oyster shell due to its large size, it was much better than other oysters as a primary resource in button manufacturing. At the beginning of the 20th century, ca. 75% of pearl shell traded in London was of Australian origin (Mullins, 2005). French Polynesia has been under French control since 1843, and numerous Polynesian lagoons were fished for their *Pinctada margaritifera* pearl oysters during the 19th and 20th centuries (Seurot, 2011). In most of these regions the great value of pearl shell and natural pearls led to a near depletion of wild oyster stocks (Cariño and Monteforte, 2006). The search for pearl shell and natural pearls was also often associated with harrowing working conditions and many pearl divers have fallen seriously ill or lost their lives in the process (Kunz and Stevenson, 1908; Stiles et al., 1943; Bailey, 2001).

The freshwater pearl mussel fisheries were active in China, Scotland, central Europe and USA. Minor pearl fishing took place in the Middle Ages and later in Europe, mostly in the streams of central Europe and Scotland/UK (Strack, 2006).

The trade in natural pearls began to collapse during the Interwar period (1918-1939) and was lastingly hit by news of cultured pearls reaching the market in large quantities in the 1920s (Prokop, 2005). Natural pearls remained a very niche trade up until the beginning of the 21st century, when they re-emerged as star jewels at auctions and in private sales. They have fetched spectacular prices in recent years due to increased demand, rarity and extremely limited supply (Torrey and Sheung, 2008; FT, 2013).

Table 1: Major pearl shell and natural pearl fisheries. Source: Cariño and Monteforte, 2009; Strack, 2006

Region	Period	Main species
Marine		
Arabian/Persian Gulf, Gulf of Mannar, Red Sea	since at least 2000 years ago	*Pinctada radiata, P. fucata, P. margaritifera*
China	2000 years ago	*Pinctada fucata-martensii*
Japan	6th century, intensively in late 19th century	*Pinctada fucata-martensii*
Central and South America	from 16th century onwards	*Pinctada mazatlanica, P. imbricata, Pteria sterna, Pteria colymbus*
Australia, Indonesia, Philippines, French Polynesia	from 19th century onwards	*Pinctada margaritifera, P. maxima*

Freshwater		
China	ca. 8th century	*Unio sp.*
Central Europe, UK	16th century for Bavaria (Kunz and Stevenson, 1908); Roman times (Scotland)	Unio sp., *Pinctada margaritifera margaritifera*
USA	18th century onwards	*Unio sp.*

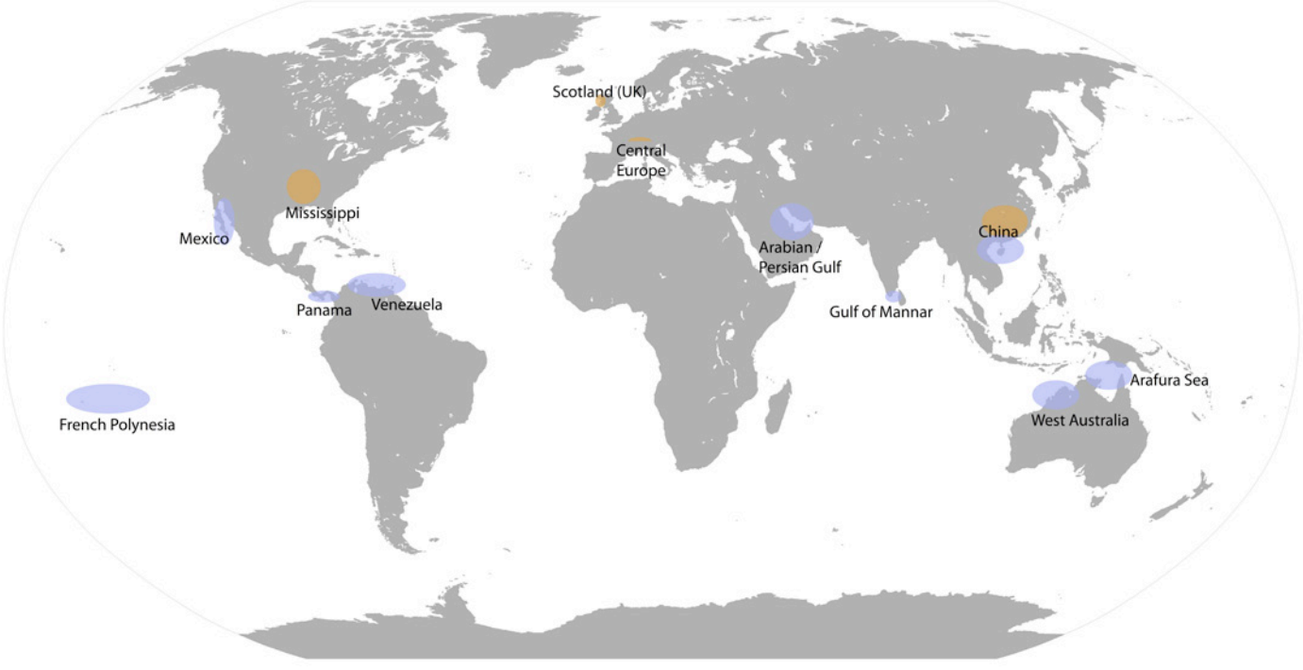

Figure 1: A world map of major historical natural pearl fisheries. Sources of freshwater natural pearls are highlighted in orange, marine natural pearl sources are highlighted in orange. Source: Strack, 2006; Lucas and Southgate, 2008)

The advent of cultured pearls

" The man that solves the problem of pearl oyster cultivation, will not only have the privilege of contributing to scientific and industrial progress: his name will deserve the honor of being included among the founders of empires" Alexander Lyster Jameson (in Cariño and Monteforte, 2009)

Although scientists and entrepreneurs had long sought to discover the exact formation of natural pearls, it was not until the beginning of the 20th century that methods to cultivate loose pearls had been sufficiently refined for products to reach the market. Unlike natural pearls, which form accidentally in wild oysters, cultured pearls form following a human-induced operation (Simkiss and Wada, 1980; Gervis and Sims, 1992; Southgate and Lucas, 2008).

The Chinese had discovered early that mantle tissue was responsible for nacre secretion. 13th century Bud-

dha figurines placed under an oyster's mantle tissue to be subsequently covered in nacre are a good example of this, but these techniques did not produce loose pearls (Kunz and Stevenson, 1908; Simkiss and Wada, 1980). It has long been known that mantle tissue is a key element in unlocking the mystery of pearl production. A number of scientists working on the subject in the 19th century hypothesised that parasites (de Fillipi, 1852) and worms (Kelaart, 1857) could lead to the formation of a pearl sac and possibly a pearl (Simkiss and Wada, 1980). Jameson (1902) confirmed that a pearl sac consisting of epithelial cells was necessary for the formation of a loose natural pearl (Simkiss and Wada, 1980). The end of the 19th century saw a number of individuals attempt the cultivation of pearls. Kokici Mikimoto is largely hailed as being the first to refine these methods using the expertise of Mise and Nishikawa (Strack, 2006). Mikimoto and his team sought to obtain better quality and more round pearls, and were the first to market loose cultured pearls on the international market at the end of the 1910s (Strack, 2006). Since these beginnings of producing loose cultured pearls (with a bead) in marine Akoya oysters, other options have been used to produce cultured pearls. These include cultivating pearls in freshwater mussels and cultured pearls without beads. The different options available in the production of both marine and freshwater cultured pearls are detailed in table 2.

Marine pearl oyster	Gonad-grown	beaded (with nucleus)	Akoya, Rainbow-lipped, South Sea, Tahiti
Marine pearl oyster	Gonad-grown	beadless (without nucleus)	'Keshi' bead rejected (Akoya, South Sea, Tahiti)
Marine pearl oyster	Mantle-grown	beaded	cultured blister pearls (Mabé)
Marine pearl oyster	Mantle-grown	beadless	New type baroque
Freshwater pearl mussel	Gonad-grown	beaded	Ming, Edison, Kasumigaura
Freshwater pearl mussel	Gonad-grown	beadless	'Keshi' bead rejected
Freshwater pearl mussel	Mantle-grown	beaded	Chinese freshwater coin, round etc.
Freshwater pearl mussel	Mantle-grown	beadless	Biwa, Chinese freshwater, USA

Table 2: Options for cultured pearl production. Table modified from Hänni, 2012. By far the most common (<90%) pearl production technique is marine gonad-grown with a bead and freshwater mantle-grown without a bead.

Mikimoto's breakthrough and marketing efforts heralded a new era (Strack, 2006). The affordability of cultured pearls led to their emergence and the gradual demise of the natural pearl trade from the 1920s onwards. Pearls could now be cultured, reducing the pressure on wild oyster populations, providing long-term economic opportunities to remote islands and opening up new consumer markets (Cariño and Monteforte,

2006). Japan was the expert in the production, science and trade of cultured pearls for many decades and remains to this day a major player in pearl production and trade because of its longstanding expertise and tradition (Prokop, 2005). Cultured pearls have become a billion-dollar industry and experienced a tremendous production boom in recent decades in Asia and the Pacific.

The emergence of cultured pearl farming was partially a response by entrepreneurs and scientists to rapidly diminishing and endangered stocks of wild oysters during the 19th and 20th centuries (Cariño and Monteforte, 2009). Cultured pearl farming presented a profitable, renewable and ecologically sensible alternative to unsustainable fishing of wild oysters in the search for exquisite pearls and nacre (mother-of-pearl). The sustainability potential of pearl farming is important. Unlike most other gemstone extraction, pearl farming is inherently dependent on a healthy ecosystem and is renewable. If the coastal reef ecosystems where most marine oysters are bred deteriorate in quality, the livelihood of pearl farmers also diminishes. Yet scant attention has been paid to understanding underlying sustainability issues and how positive synergies could be strengthened (Cartier and Ali, 2012).

The pearl industry today

Cultured pearls dominate the industry, whereas much more valuable natural pearls remain a small niche market (Bari et al., 2010). Pearls are cultured in domesticated saltwater oysters and freshwater mussels in numerous countries worldwide (Figure 3).

The main pearl-producing marine oyster species are *Pinctada* maxima (South Sea pearl oyster), *Pinctada margaritifera* and the Akoya oyster complex (*Pinctada fucata-imbricata-martensii-radiata* complex - see Southagate and Lucas (2008) for further discussion). Akoya cultured pearls have been cultivated in *Pinctada martensii* oysters in Japan since the 1910s, and have been increasingly produced in China and Vietnam in recent decades (Strack, 2006). The culture of *Pinctada maxima* oysters (producing white and golden South Sea pearls) in Australia began in the 1950s and can also be found in Burma/Myanmar, Indonesia and the Philippines (Hänni, 2007; Southgate and Lucas, 2008). French Polynesia has dominated the production of Tahitian cultured pearls (from the *Pinctada margaritifera* oyster) since 1962, but farms in the Cook Islands, Fiji, Micronesia, Marshall Islands have attempted to emulate French Polynesia's success with pearls (Macpherson, 2000; Cartier et al., 2012). Other minor pearl producers include *Pteria sterna* and *Pinctada mazatlanica* production in the Gulf of California (Mexico) since 1993 (Kiefert et al., 2004).

Freshwater cultured pearls have radically transformed the global pearl industry. Japan began producing freshwater cultured pearls in 1925 (Wiesauer, 2012). China began cultivating freshwater cultured pearls in the 1960s (Wiesauer, 2012) and has gone on to produce vast amounts of pearls. In 2010, it is estimated that China

produced 800 and 1000 tons of freshwater cultured pearls of increasingly better qualities (Wiesauer, 2012; Liping and Min, 2013). The global economic crisis in 2008, local political issues and ecological problems faced by pearl farms have greatly reduced production since 2010 (Cartier and Ali, 2013). There is an increasing emphasis on quality and innovation in the Chinese pearl industry (Wiesauer, 2012; Sheperd, 2013).

The market for pearls has grown tremendously in the last 20 years–both in terms of supply and demand. However, both small and large producers are having problems with market access that are associated with supply chain fragmentation in recent years (Müller, 2009; Brodbeck 2010). Over 80% of marine cultured pearls are traded through the pearl centres of Kobe (Japan) and Hong Kong, and both these centres have contributed and accelerated market fragmentation in recent years (Brodbeck, 2010). Global marine pearl production in 2013 is estimated to be worth US$397 million (Müller, 2013). A detailed overview of cultured pearl producing regions and production figures can be found in table 2.

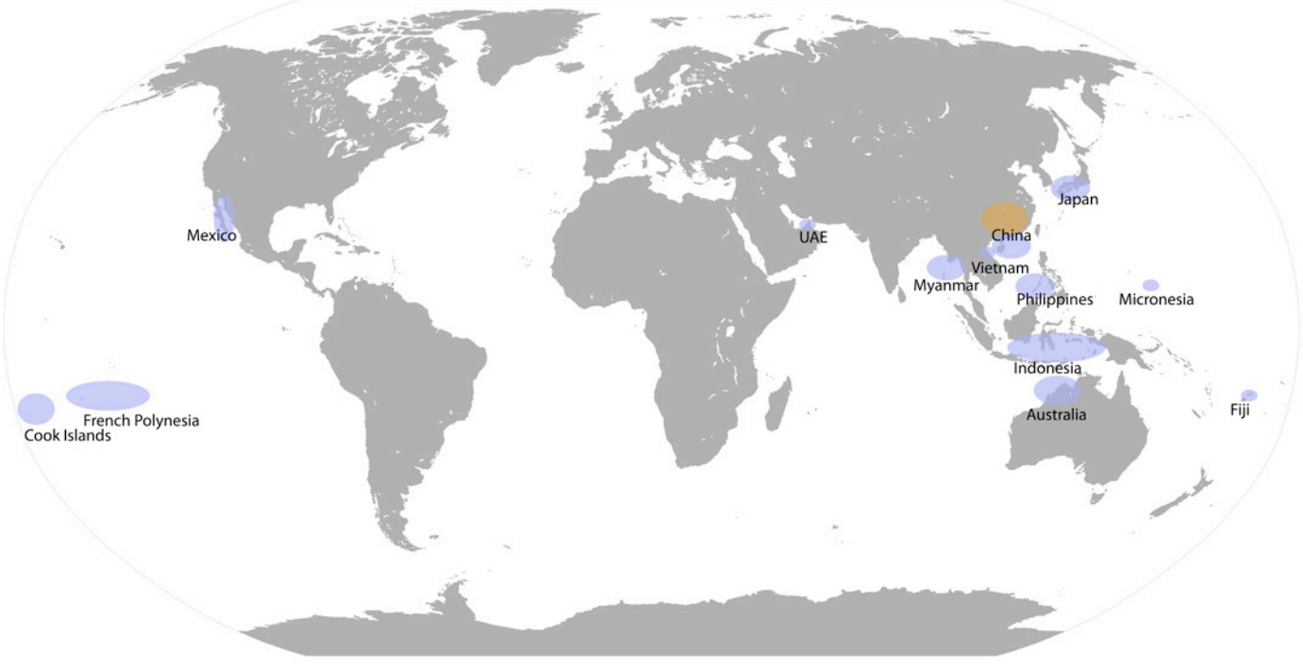

Figure 2: A world map of major cultured pearl farming regions

Country	Freshwater/ marine	Species of pearl oyster	Volume of pearls produced	Value of pearl production	Production since	Source
China	Freshwater and marine	*Hyriopsis cumingii H. schlegelii* and hybrids; *Pinctada fucata-martensi*	800-1000 tons (Freshwater, 2010); 2.6 tons (Akoya, 2013)	n/a (freshwater, 2010); US$ 5 million (Akoya, 2013)	1961 (marine); 1962 (freshwater)	Wiesauer, 2012; Liping and Min, 2013; Sheperd, 2013; Müller, 2013

Japan	Freshwater and marine	*Hyriopsis schlegelii*; Akoya	19 tons (Akoya, 2013)	US$ 100 million (Akoya, 2013)	1916 (marine)	Strack, 2006; Southgate and Lucas, 2008; Müller, 2013
Vietnam	Marine	Akoya	2.8 tons (2013)	US$ 5 million (2013)	1991	Strack, 2011; Müller, 2013
Australia	Marine	*Pinctada maxima,* Akoya	3.75 tons (2013)	US$ 90 million (2013)	1956	Lucas and Southgate, 2008; Müller, 2013
Burma (Myanmar)	Marine	*Pinctada maxima*	1.125 ton (2013)	US$ 24 million (2013)	1960s	Lucas and Southgate, 2008; Müller, 2013
Indonesia	Marine	*Pinctada maxima*	5.45 tons (2013)	US$ 58 million (2013)	1970s	Lucas and Southgate, 2008; Müller, 2013
Philippines	Marine	*Pinctada maxima*	2.1 tons (2013)	US$ 22 million (2013)	1964	Lucas and Southgate, 2008; Müller, 2013
Cook Islands	Marine	*Pinctada margaritifera*	100-150,000 pearls (2010)	US$ 467,000 (2010)	1974	Macpherson, 2000; Strack, 2011; SPC, 2011; Müller, 2013
Fiji	Marine	*Pinctada margaritifera*	9,500 pearls (2011)	n/a	1999	Strack, 2011
French Polynesia	Marine	*Pinctada margaritifera*	14 tonnes (2012)	FCFP 6.9 billion ≈US$76 million (2012)	1963	Domard, 1962; ISPF, 2014
Micronesia	Marine	*Pinctada margaritifera*	2000 pearls (2012)	n/a	1987	Cartier et al., 2012
Mexico	Marine	*Pteria sterna*	3000 pearls (2013)	n/a	1993	Kiefert et al., 2004; D. McLaurin, pers. comm. 2013
U.A.E.	Marine	*Pinctada radiata*	n/a	n/a	2008	M. Al Suwaidi, pers. comm. 2012

Table 3: Cultured pearl production figures

Interactions between pearl farming and the natural environment

The interactions of shellfish aquaculture and the environment have been the subject of considerable research (Prins et al., 1997; Gibbs, 2004; Dowd, 2005; McKindsey et al., 2006; Shumway, 2011). For pearl oysters, past research has investigated the effects of environmental changes on the health of a pearl oyster. This has included hydrobiological parameters such as: temperature fluctuations (Yukihira et al., 2000), salinity changes (Doroudi et al., 2001; Taylor et al., 2004), suspended particulate matter concentrations (Pouvreau et al., 1999; Pouvreau et al., 2000). Other factors include the role of biofouling, predation and potential disease agents (Pit and Southgate, 2003; Bondad-Reantaso et al., 2007; de Nys and Oson, 2008). All these factors can have potentially fatal impacts on an oyster's health (Southgate and Lucas, 2008).

Another research focus has been on the impacts of pearl farming on the natural environment, especially in countries -such as Australia- where environmental regulations are very strict (Jernakoff, 2002, Wells and Jernakoff, 2006). Obviously, the stocking of pearl oysters can have impacts on the surrounding environment (Southgate and Lucas, 2008). Two main issues have been identified. Firstly, the introduction of new species ('invasive species') or modified genotypes (e.g. from hatchery produced spat) of oysters into the natural environment, and associated disease agents (Bondad-Reantaso et al., 2007). In French Polynesia this may also cover the transfer of juvenile Pinctada margaritifera oysters between islands, though more research is required (Arnaud-Haond et al., 2004; Lemer and Planes, 2012). Secondly, the physical impact of pearl oysters in the form of increased biomass, faeces deposition, nutrient consumption and human impacts (Yokoyama, 2002; Gifford et al., 2004; Jelbart et al., 2011). These impacts on the natural environment are increased when pearl oysters are stocked at high densities (Southgate and Lucas, 2008; Jelbart et al., 2011). For example, mass oyster mortalities in Japan, the Cook Islands and French Polynesia in the past three decades are believed to be largely a result of high-stocking densities (Intes 1988; Heffernan, 2006; Kuchel et al., 2011). But under reasonable farming practices, studies have shown that cultured oysters do not limit the growth of wild oyster populations and other organisms (Niquil, 2001). Research in French Polynesia found that the greatest potential impact on the benthic environment were in fact human impacts associated with pearl farming, such as the construction of buildings and marinas (S.N.C. Pae Tai-Pae Uta, 2003). However, the impact, if any, on biodiversity continues to be poorly researched. The main issue with previous research has been the lack of suitable methodology to assess these impacts in complex dynamic marine ecosystems (Gifford et al., 2004; Jelbart et al., 2011).

Impact of oyster stocking to a lagoon environment

Very little research has been done on investigating the effects of having such a great number of oysters 'artificially' hanging in a lagoon. Surveys suggest that up to 8.1 million oysters were continuously cultivated in Ahe lagoon before 2012, whilst the number of wild oysters are not known (Michotey, et al. 2012). Previous research has developed models to determine resource competition and carrying capacity of oysters; these all suggest that carrying capacities have not been reached (Niquil et al., 2001). Prou and colleagues (1999) estimated that approx. 4.3 million wild oysters were in Takapoto lagoon, whereas there were 1.8 million farmed oysters. In Mexico, the farming of pearl oysters has led to the repopulation of wild populations which had previously been overfished (Saucedo and Monteforte, 1997). Zanini and Salvat (2000) found that over 80% of wild Pinctada margaritifera oysters in the lagoon of Takapoto (French Polynesia) were found at depths greater than 20m. This is very different to cultivated oysters, which are found at an average depth of 5-10m (Coeroli and Mizuno, 1985; Buestel et al., 1995). The presence of these farmed oysters and the added biomass could, for example, have an effect on reef fish populations (Jelbart et al., 2011). The impact of pearl oyster farming on reef fish populations is investigated in Chapter 4 (Cartier and Carpenter, 2014) of this thesis.

The sustainability implications of pearl nucleus use

As detailed in table 2 cultured pearls can be produced with or without a nucleus (also called bead). The vast majority of marine cultured pearls are beaded cultured pearls, which means that a nucleus was inserted during the grafting procedure (Strack, 2006). Such a nucleus is generally a round-polished piece of freshwater mussel shell, traditionally from a select number of mussel species found in the American Mississippi delta region (Hänni et al., 2010). Mississippi shell nuclei are still widely used worldwide, because of their superior qualities (e.g. remain stable when the pearl is drilled, hardness). The demand for Mississippi mussel shells from the cultured pearl industry (scarce data available shows that French Polynesia alone imported 39 tons of nuclei in 2005, dropping to 13 tonnes in 2012; Brodbeck 2010, ISPF, 2014) remains strong. The great demand for these wild mussels has had ecological consequences in the US (Strayer et al., 2004), and lower supply from the US has led to sourcing of mussel shell material in other countries such as China. Alternative nucleus materials have been explored in recent decades (e.g. bironite and other artificial materials) but none have benefited from sustained market acceptance. New products used by pearl farmers have surfaced in recent years including silicone-based organic nuclei used in primary stages of pearl production for induction of a larger pearl sac (Chapter 5; Cartier and Krzemnicki, 2013).

Links between ecology and pearl quality

Marine cultured pearl farming does not harm the environment if adequate management practices are implemented, and a healthy ecosystem is a prerequisite to producing beautiful pearls (Kugelmann & Poirine, 2003; Hänni, 2007). 1986, the year in which Tahitian cultured pearls reached their highest per gramme export value was also the year in which the industry was most affected by environmental influences. In 1985, in the atoll of Takapoto 3.5 million pearl oysters (out of 7 million) suddenly died, and this phenomenon was observed in many other pearl-producing islands of the Tuamotu archipelago (Seurot, 2011). Pearl farming is inherently dependent on a healthy ecosystem. Sudden ecological changes can have grave effects both on the oysters and the qualities of produced cultured pearls (Cartier and Ali, 2012). It is empirically shown that good management practises and the economic profitability of a pearl farm are closely linked (Kugelmann & Poirine, 2003). Pearl quality is judged by a variety of parameters that include size, weight, shape, colour, lustre and surface purity. Ecological parameters, for example the age and health of an oyster, play a determining role in the lustre of a cultured pearl (Hänni, 2007; Cartier and Ali, 2011). The environment of a pearl farm and the quality of the pearls it produces is closely tied.

Improving pearl quality

One of the key components to pearl farm profitability is increasing pearl quality and thus pearl value (Fong et al., 2005). Any incremental improvement in the quality of pearls produced (e.g. roundness, size, surface condition) will lead to greater income for a pearl farmer. Pearl oyster husbandry is another important factor, where a pearl farmer must seek to reduce oyster mortalities as much as possible, in order to reduce costs. Techniques vary greatly between different pearl farms and depending on the different environments and latitudes in which they operate. A large amount of research and development work carried out by pearl farmers to improve techniques is not openly available or published. Recent academic research has focused on selection of good broodstock, genetic trait programmes, different grow-out strategies and refinement of surgical techniques. Broodstock is vital in pearl farming. For those farms using hatchery-produced spat (juvenile oysters), they can opt to select broodstock that promotes strong oysters (as host oysters) and desirable nacre production (for donor oysters). The stronger a host oyster, the more likely it is to survive and channel energy into pearl production (Saucedo and Southgate, 2008). Consistently beautiful nacre in a donor oyster (sacrificed for its mantle tissue) is more likely to yield desirable nacre on a subsequently harvested pearl (Acosta-Salmon et al., 2004).

Genetics has become an important topic in pearl production, especially where hatchery-based spat is used. Recent developments in genetics open up huge opportunities for applications in the aquaculture (and thus pearl farming) industry. It is still very much unknown how much and specifically which genetic factors are responsible for different pearl traits (Wada and Jerry, 2008). Current and future efforts focus on the development of selective breeding and targeting commercially important traits of pearl oysters (Southgate et al., 2008; Wada and Jerry, 2008).

Genetic research is also of interest in regions where wild oysters are collected for pearl production (e.g. Australia, French Polynesia) exploring genetic options for more controlled use of mantle donor tissue, with the aim to harvest pearls of more desirable colours, shapes and surface conditions (Acosta-Salmon et al., 2004; Buestel et al., 2009). Genetic programmes have also been set up in order to manage possible natural risks (e.g. typhoons), by having an alternative supply of oysters. All this genetic research takes place by experimenting with pearl oysters, and later examining the produced pearls. Chapter 6 of this dissertation offers a new method of obtaining genetic pearl oyster material, by extracting DNA from pearls rather than from the oyster. This opens up new opportunities in understanding genetic influences on the formation and malformation of pearls.

The healthier an oyster, the more likely it is to produce a high-quality pearl. Good husbandry favours the health of an oyster. This involves finding a suitable farming site, regular removal of biofouling, stocking oysters at favourable depths and exposure to currents, and professional grafting skills (Southgate, 2008). The grafting of mantle donor tissue and introduction of this tissue along with a nucleus into the host oyster's gonad requires specific skills. Hygiene is of utmost importance in the process (Hänni, 2007; Mamangkey, 2009). Subsequent operations (a second or third operation) as are common in South Sea or Tahitian pearl production also require great care. Numerous pearl farmers and operating technicians are continuously aiming to improve operating procedures in order to reduce mortalities, rejections and increase the quality of pearls produced. However, much of the knowledge and developments in pearl oyster operating techniques remain a commercial secret.

Figure 3: Different shapes of Tahitian cultured pearls. From left to right: round, circled, oval, drop, round.

Gemmology - the science of gemstone testing- is concerned with testing of pearls. This field is important in differentiating between cultured and natural pearls, pearls of different types and possible treatments to pearls (Farn, 1986). Although the improvement of pearl quality is largely seen as something that needs to take place at a production level (i.e. at a pearl farm), it is clear that better understanding of pearl formation (and how to produce a higher quality of pearls) can be gained through collaboration with gemmological scientists. A good example of this is the phenomenon of circled pearls, which is still poorly understood (Caseiro, 1993; Cartier et al., 2012). Many pearl farmers would like to reduce the share of circled pearls in their harvests because these are of low value but remain a significant part of harvests of many Pinctada pearl producers (Figure 3, Table 4). The formation of a pearl can be much better understood by examining its internal structure of a pearl, rather than just its surface. Gemmological techniques such as X-ray shadow imaging and X-ray computer microtomography (Krzemnicki et al., 2010), scanning electron microscopy (Ji et al., 2013) offer good opportunities to study such formation mechanisms and understand how a circled pearl form pearls and how this could potentially be avoided (Caseiro, 1993). Chapter 7 of this dissertation also details how different gemmological methods could be used to trace cultured pearls through the supply chain, from farm to consumer. If rewards for environmental and social commitment at a production and trading level are to be disbursed then traceability mechanisms may be necessary in assuring supply chain accountability and credibility (Conroy, 2005).

Table 4: Maison de la Perle Auction November 2012: 345,352 cultured Tahiti pearls on sale. 22.8% of the pearls offered at this auction were graded as circled pearls. Source: Maison de la Perle, 2012

Size	Share (in number of pearls)
7-9mm	46%
10-11mm	40%
12-14mm	13%
15-17mm	0.6%
18mm and up	0.001%

Marine pearls as a sustainability case study

Cultured pearl farming presents a profitable, renewable and ecologically sensible alternative to unsustainable fishing of wild oysters in the search for exquisite pearls and nacre (mother-of-pearl). It is the most profitable form of aquaculture and can be carried out lucratively in isolated islands where there are otherwise very

limited economic opportunities (Sims, 2003). The positive socio-economic benefits of cultured pearl farming are well reported (Tisdell & Poirine, 1998, Macpherson, 2000; Southgate & Lucas, 2008). Because of its potentially low environmental impact, there is clearly a potential synergy between pearl farms and marine conservation (Cartier and Ali, 2012; Cartier and Carpenter, 2014).

Cultured pearls have become important economic pillars of French Polynesia and the Cook Islands, as main sources of export revenue. In French Polynesia alone, in 2010, there were 397 individuals/companies who collected spat (juvenile oysters) and 429 licensed pearl farms (Service de la perliculture, 2010), producing pearls with an export value of $130 million (Müller, 2009). In French Polynesia 7000 people depended on this industry at its peak in 2000 (Murzyniec-Laurendeau, 2002). In the Cook Islands, black-lip pearl production can be done within existing forms of (indigenous) social and economic organization (Macpherson, 2000). Producing a beautiful pearl is not reserved to large-scale entrepreneurs, a great number of small-scale and artisanal stakeholders also benefit from the pearl oyster resource in different ways (Rapaport, 1994; Poirine, 2003). Within cultured pearl farming there are considerable economies of scale between the wide range of small-scale and large-scale actors (Tisdell & Poirine, 1998). The attractiveness of cultured pearl farming led a great number of actors to enter pearl farming, creating problems of overproduction and a fragmentation of the sector (Brodbeck, 2010). However, this overproduction concerns low qualities of pearls, whereas production of high-quality pearls does not meet world market demand (Brodbeck, 2010). If appropriate management measures are implemented, these production and marketing issues can be addressed and resolved (Müller, 2009; Brodbeck, 2010). The decline of the industry has had an effect on many actors in the industry at all levels of the supply chain. The prices that pearl farmers receive for their harvests have significantly dropped in recent years, creating a demand for more sustainable trading relationships (Kugelmann & Poirine, 2003; Brodbeck, 2010). The cultured pearl industry is currently undergoing huge transformations due to globally induced economic (value chain fragmentation) and environmental changes (climate change, pollution), and must revert to a high-quality production of pearls in order to prosper sustainably in future (Southgate & Lucas, 2008).

Traceability and supply chain certification as a new model

The positive synergies of marine pearl farming can prosper further if the resource continues to be managed responsibly and value chains are designed to support these positive environmental and socio-economic impacts. “Ultimately, traders and consumers of pearls could further strengthen the livelihoods of pearl farmers and the positive ecosystem services they provide. Consumers of jewellery should be made aware of the positive synergies that lie in the process of cultivating marine pearls. Indeed, if the farmers who operate in

the waters of the Pacific do not prosper, the ecosystem services provided by these waters will cease to exist." (Cartier and Ali, 2012). One way of promoting the positive business, ecological and social benefits of pearl farming is by highlighting its sustainability credentials. This is an emerging trend in supply chain management. There are two main approaches to this in the context of marine cultured pearls: product-based transparency or certification mechanisms (Nash, 2013).

Aims and outline of thesis

This thesis' research covers a number of fields related to sustainability and traceability questions in marine cultured pearl production. This dissertation is part of a larger project on the sustainability of pearl farming, and how responsible pearl production can be promoted throughout the supply chain. Fieldwork for this thesis has been carried out in Australia, China, Fiji, French Polynesia, Indonesia, Japan, Mexico, Micronesia and UAE.

There is a nascent trend in the jewellery industry for so-called ethical or sustainable jewellery. Pearls are an ideal raw material to study for this, given their renewable and low-impact nature (Cartier and Ali, 2012). An overview of sustainability issues and opportunities in the production and trade of marine cultured pearls is offered in Chapter 2. Importantly, pearl farms not only have a potential of operating with low ecological impact, but can also have a restorative impact on the natural environment (e.g. wild oyster population restoration in Mexico; Saucedo and Monteforte, 1997). Furthermore, pearl farms can potentially seize an opportunity by exploring synergies with marine conservation activities. The sustainability of pearl farming is not limited to ecological factors; it also encompasses social and business aspects of a pearl farm.

Pearl farming has been heralded as a viable option for developing Marine Protected Area (MPA) and promoting local economic development in the Pacific, as for example in Micronesia (Chapter 3). Chapter 3 details the processes required to produce a pearl from spat production to marketing of pearls, and highlights the challenges and opportunities of setting up a pearl industry in a new region.

The impacts of pearl farming on biodiversity remain poorly understood, and one chapter of this thesis has investigated the influence of pearl farming on reef fish populations in French Polynesia (Chapter 4). Reef fish are a good indicator of relative biodiversity. Research in the Philippines and in French Polynesia (Chapter 4) suggest that pearl farms can actually have a positive impact on reef fish populations, and have no significant impact on fish diversity. Understanding the impacts of pearl farming on biodiversity is important in determining what a 'sustainable' pearl farm is, and devising appropriate tools to measure impacts and outcomes.

Pearl farming has benefited from numerous technological advancements in different fields. Numerous pearl

farmers have sought to innovate in order to produce higher qualities of cultured pearls and operate more efficiently. Chapter 5 investigates the use of a totally new type of nucleus used in marine cultured pearl production that can shorten the period of pearl production and opens up some new opportunities for pearl farmers. Concurrently, documenting and investigating such innovations is important in order to maintain consumer confidence in the pearl trade.

One hypothesis of this dissertation is that a pearl farm can be sustainable, and pearls stemming from such a farm could be marketed as such thereby creating additional value for pearl producers and the pearl supply chain. For example, Fiji-, Mexican-, and Micronesian cultured pearls have been traded at higher than average prices for cultured pearls because of their market differentiation strategies by marketing their pearls as unique, responsibly produced and traceable to source.

This observation has led to the final phase of this research in investigating how responsibly produced marine cultured pearls could be traced from farm to market, so that such pearls can be correctly marketed whilst hindering fraud and ensuring consumer confidence. The reviewed methods could form the basis of a future certification and traceability system that can ultimately be used to reward pearl farmers and traders for environmental and social commitments. In the case of marine cultured pearls, there are several feasible options that have been explored of how a pearl could be traced from a remote Pacific atoll to the final consumer. This has included developing a novel method to document pearls by extracting DNA from a pearl and using this genetic material to identify its mother oyster species (Chapter 6). The final chapter of this dissertation (Chapter 7) offers an overview of methods that could potentially be used to trace a pearl from where it is produced all the way to the end consumer.

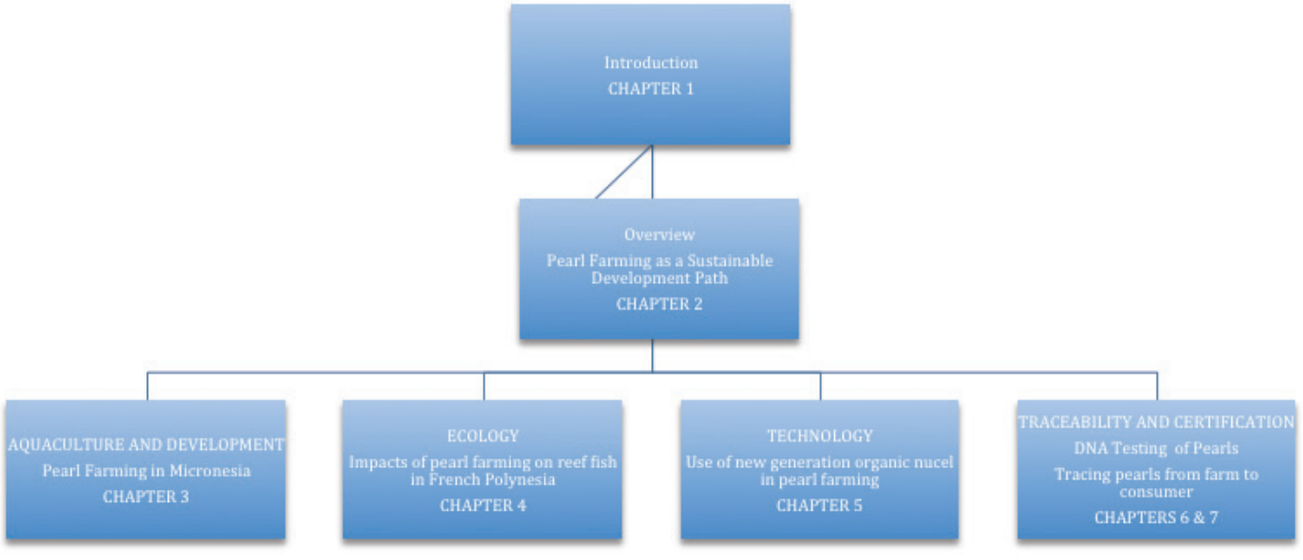

Figure 4: Structure of dissertation

CHAPTER 2

Pearl Farming as a Sustainable Development Path

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Authors: Cartier, L.E., Ali, S.H.

Pearl Farming as a Sustainable Development Path

by Laurent E. Cartier and Saleem H. Ali

Laurent Cartier

Buoys off the coast of Bali support the longlines from which oyster nets hang. Pearl farming, particularly in the Pacific, has proven a strong pillar of local economic development and environmental preservation.

For centuries, wild pearl oysters and mussels were fished in the quest for natural pearls and shell material. This eventually led to the drastic overexploitation of oyster stocks in many areas of the globe.[1] Scientific innovation and entrepreneurship eventually unearthed a solution: Researchers discovered a way for humans to farm pearl oysters and induce the formation of a cultured pearl. A century after this discovery, many pearl farming regions are vulnerable to climate change and coral and coastal habitat destruction. Pearl farming might provide a win-win opportunity for such communities.

Overcoming Biodiversity Loss

At present, marine biodiversity is facing huge threats in the Pacific region as a result of climate change, overfishing, and unregulated coastal development.[2] The preservation and conservation of marine resources have become a priority in many areas and regions. Corals and fisheries are the basis for functioning marine ecosystems on which humans rely for food and well-being, and these ecosystems must thus be preserved and rebuilt.[3,4] Conservation biologist Joe Roman and colleagues have argued that "in the long run, the most effective forms of conservation will be those that engage local stakeholders; the cultivation of sustainable ecosystems and their services must be promoted along with conservation of endangered species and populations."[5] There is ample evidence that for conservation to work it needs to also provide tangible benefits for local communities.[6]

Clearly, great opportunities lie in combining the conservation of marine biodiversity with viable economic activities for local people in order to preserve highly sensitive ecosystems.[7] Given that cultured pearl farming is one of the few economic activities

in which sound environmental management and conservation are a prerequisite to economic success, the sector offers some interesting insights with regard to sustainability in the marine realm. In Mexico, pearl oysters of the species *Pinctada mazatlanica* and *Pteria sterna* were declared in danger of extinction and put under protection in 1940. This was the first marine conservation measure in Latin America.[8] Could pearl farming today promote environmental conservation by providing financial incentives that are frequently lacking in environmental conservation projects?[9]

An Introduction to Pearl Farming

The production of a cultured pearl is a complex process that requires a thriving marine ecosystem, important knowledge and skill, and several years of patience. In Australia, pearl farmers can collect wild adult oysters under a strict quota system. Otherwise, young oysters can either be collected as spat from the wild during natural spawning seasons, using artificial collectors, or be artificially spawned in hatcheries. Two to three years after the birth of an oyster, it can be seeded, an operation during which a nucleus and mantle tissue are implanted to induce the formation of a cultured pearl. After this, the nucleus is gradually covered with nacre (mother-of-pearl) by the oyster. This period of growth of a cultured pearl takes a further one to two years, depending on a range of factors. From birth of the oyster to the harvest of the pearl usually takes at least three to four years.

Not all oysters will survive, and not all oysters will produce a beautiful pearl. Estimates suggest that 95 percent of a pearl farm's income comes from 2 percent of its pearls.[10] The skills of the seeding technician play one important role. Environmental deterioration or sudden ecological changes will also affect the oyster and hamper its potential for producing a high-quality pearl, as pearl oysters are remarkably sensitive organisms. Consequently, financial and ecological sustainability are intimately and inextricably linked.[11] The more pristine an environment, the healthier the oysters are and the higher the likelihood of harvesting valuable, high-quality pearls. Ultimately, for a pearl farmer, it pays to maintain a thriving ecosystem.

There is ample evidence that for conservation to work it needs to also provide tangible benefits for local communities.

Using Local Resources and Promoting Natural Capital

Despite procedural challenges, pearl farming can be one of the most profitable forms of aquaculture and may be carried out in isolated islands where there are otherwise very limited economic opportunities.[12] Cultured pearls have become important economic pillars in French Polynesia and the Cook Islands as main sources of export revenue. In French Polynesia, pearl farming has reduced pressure on fish stocks, stemmed outer-island emigration, and provided economic alternatives for an economy otherwise heavily reliant on French financial assistance and tourism. At its peak in 2000, the pearl sector provided employment to 7,000 people in French Polynesia.[13] In the Cook Islands, black pearl production can be carried out within existing forms of indigenous socioeconomic organization.[14] Other small island developing states in the Pacific have attempted to emulate these successes and tried to establish pearl farming operations, but most have failed to sustain the production of commercially viable pearls at a community level.[15]

A recent and tentative success story is in the Federated States of Micronesia where a community pearl farming project has been envisaged as a model for combining marine conservation and community development, thereby providing economic opportunities using local resources. There, a new integrated marine plan is being implemented in which pearl farming complements lost income that artisanal reef fishing communities have to incur due to the introduction of no-fishing zones and marine protected areas. This new source of income has created an incentive for conservation by reducing pressure on reef fish stocks, and it is increasing the resilience of these communities in the face of climate change. This reflects findings of a recently published United Nations Environment Programme report on the green economy that revealed that "there is a clear link between poverty eradication and better protection and restoration of habitat, marine fishery resources and biodiversity."[16,17]

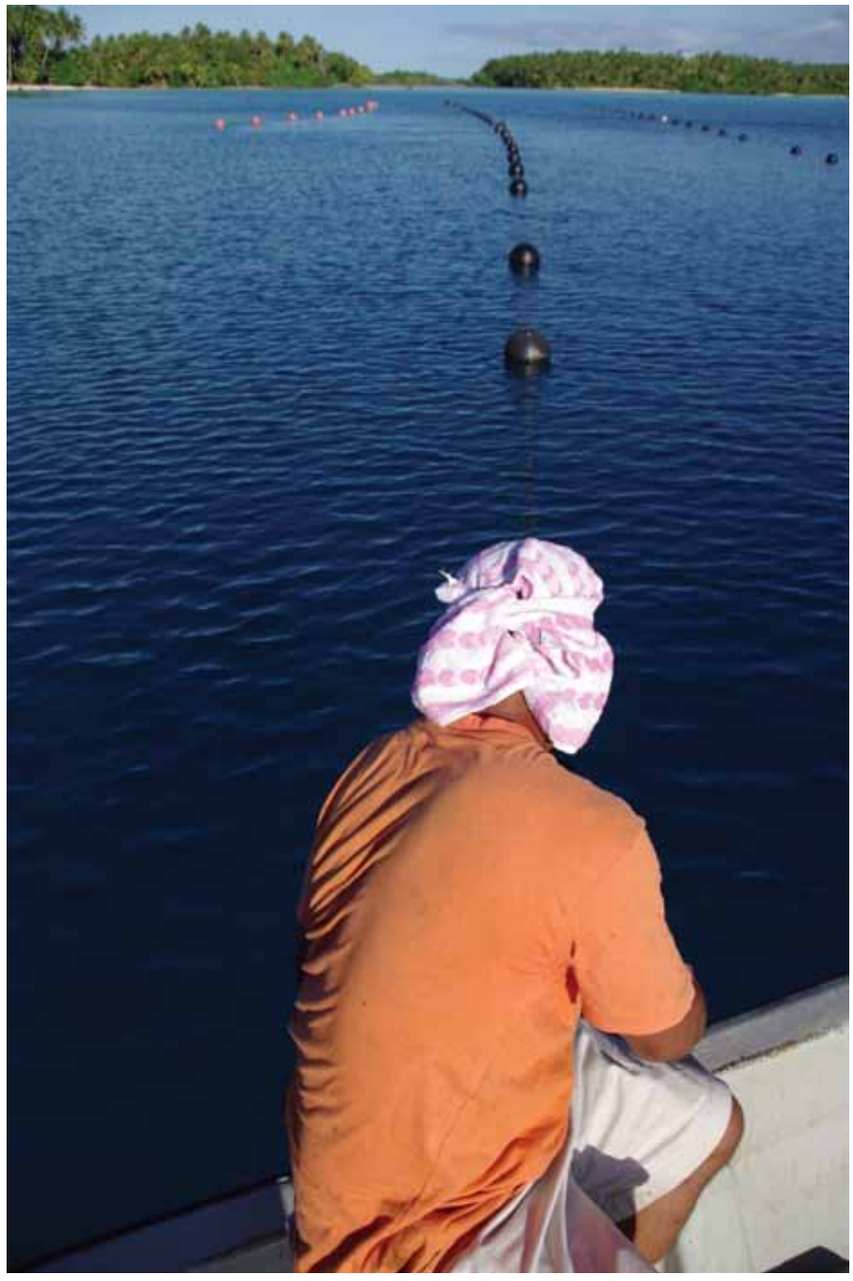
Maximizing Revenue: Emphasis on Quality and Innovation

The business of selling cultured pearls has become challenging for many pearl farmers. With the effects of the 2008 global economic crisis, issues of high production and changing demand, fragmentation both at a supply and

distribution level, and rising competition from Chinese freshwater cultured pearls, the marine cultured pearl industry finds itself in a difficult business environment.[18] In French Polynesia, a number of pearl farmers who worked farms of varying size have gone out of business in recent years. The average price of exported pearls from French Polynesia has been divided by four in the past decade. This price decline can be explained by overproduction, a reduction in the average quality of pearls produced, and a host of other market factors.[19] However, research has shown that prices for large, high-quality cultured pearls have not dropped.[20] A pearl farm with a focus on quality pearls produced through responsible farming practices still very much has its place in the international market. Demand for cultured pearls should be strengthened by creating further incentives for luxury consumers. Pearls could be marketed as a sustainable alternative in an increasingly ecologically conscious jewelry market.

Nevertheless, the revenue streams of pearl farmers are changing and diversifying beyond the simple sale of pearls.[21] Ecotourism that allows for direct purchase of raw pearl, jewelry, and culinary products is one option, although the remoteness of some destinations like Micronesia and Polynesia limit this avenue.

Another option is to explore the additional uses of oysters beyond the pearls they produce. Historically, oyster shells were used in button manufacturing. Today, shells are again in demand for furniture and ornamental purposes. A pearl oyster shell consists of 95 percent calcium carbonate. Ground and purified, it can be used for medicinal purposes in relieving osteoporosis,[22] in bone replacement therapy,[23,24] as a source of calcium for dietary supplements,[25,26] and in beauty products. The properties of the oyster shell's periostracum (the outer, organic-rich layer of the shell) have even been investigated for military and maritime uses.[27] Adductor muscle meat of pearl oysters is frequently sold for human consumption and the dried meat of the oyster is being used to enrich soils in certain countries, such as the United Arab Emirates. Clearly, these many different uses provide opportunities for pearl farmers to diversify their sources of revenue and allow them to minimize resource waste.



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Small-scale pearl farming off Pakin in the Federated States of Micronesia contributes so effectively to ecosystem health that it is permitted inside of marine protected areas.

Pearl oysters have also been investigated for environmental remediation based on their filtering abilities. They are among the filter-feeding marine organisms with the highest clearance rates. A single *Pinctada margaritifera* oyster in French Polynesia can filter between 11.5 and 25.9 liters per hour per gram of tissue dry weight, thereby serving as an important buffer and regulator of water purity and quality.[28] Pearl oysters have been investigated as a tool for heavy metal remediation in coastal ecosystems in Australia, with a study showing that pearl oysters could assist in the removal of pollutants from coastal waters while producing commercially viable cultured pearls.[29] Thus the oysters could also provide an ecosystem service that should be accounted for in cost-benefit analyses of different possible development paths. Given that pearls are a saleable good themselves, their price could be calibrated to account for the services they provide. Such an approach provides a means of internalizing a market for ecosystem services through a particular product (thus circumventing the usual "market absence" critique of payment for ecosystem services approaches).

Furthermore, the ocean is the world's largest carbon sink and is an important regulator in global carbon storage, sequestration, and release. It is estimated that "the most crucial, climate-combating coastal ecosystems cover less than 0.5 percent of the sea bed. These areas, covering features such as mangroves, salt marshes, and seagrasses, are responsible for capturing and storing up to some 70 percent of the carbon permanently stored within the marine domain."[30] The ecosystem services provided by pearl farmers are further reaching than the conservation of sensitive corals and fisheries. They could play a growing role in the management of "blue carbon." Pearl farming in the Federated States of Micronesia and the United Arab Emirates is carried out in coastal mangrove ecosystems, which are protected by the pearl farmers because their oysters are dependent on the nutrients provided by the mangroves. Such pearl farms would be ideal candidates to qualify for funding from a future blue-carbon credit-trading scheme.

Conclusion

At present, within pearl farming, the difficult business environment presents an obstacle to promoting sustainability. But consumer demand could increase the sustainability benefits the sector provides to coastal communities and ecosystems. Responsible consumer choice for pearls as a jewelry product rather than other gemstones should be further encouraged. Scientists need to conduct more research into understanding how marine pearl farming's potential in marine conservation and restoration can be extended and to find better ways of cohabiting and engaging with nearby fishing communities.

Ultimately, traders and consumers of pearls could further strengthen the livelihoods of pearl farmers and the positive ecosystem services they provide. Consumers of jewelry should be made aware of the positive synergies that lie in the process of cultivating marine pearls. Indeed, if the farmers who operate in the waters of the Pacific do not prosper, the ecosystem services provided by these waters will cease to exist.

As a model of private entrepreneurship in small island developing states (e.g., Fiji, the Federated States of Micronesia, and French Polynesia), pearl farming has modestly emerged as an economic activity that can offer alternatives in a time of climate change. It can offer many valuable lessons for development opportunities in remote coastal communities. It also provides evidence that marine conservation can be integrated within a viable economic activity leading to sustainable long-term growth in vulnerable Pacific environments. S

Acknowledgments

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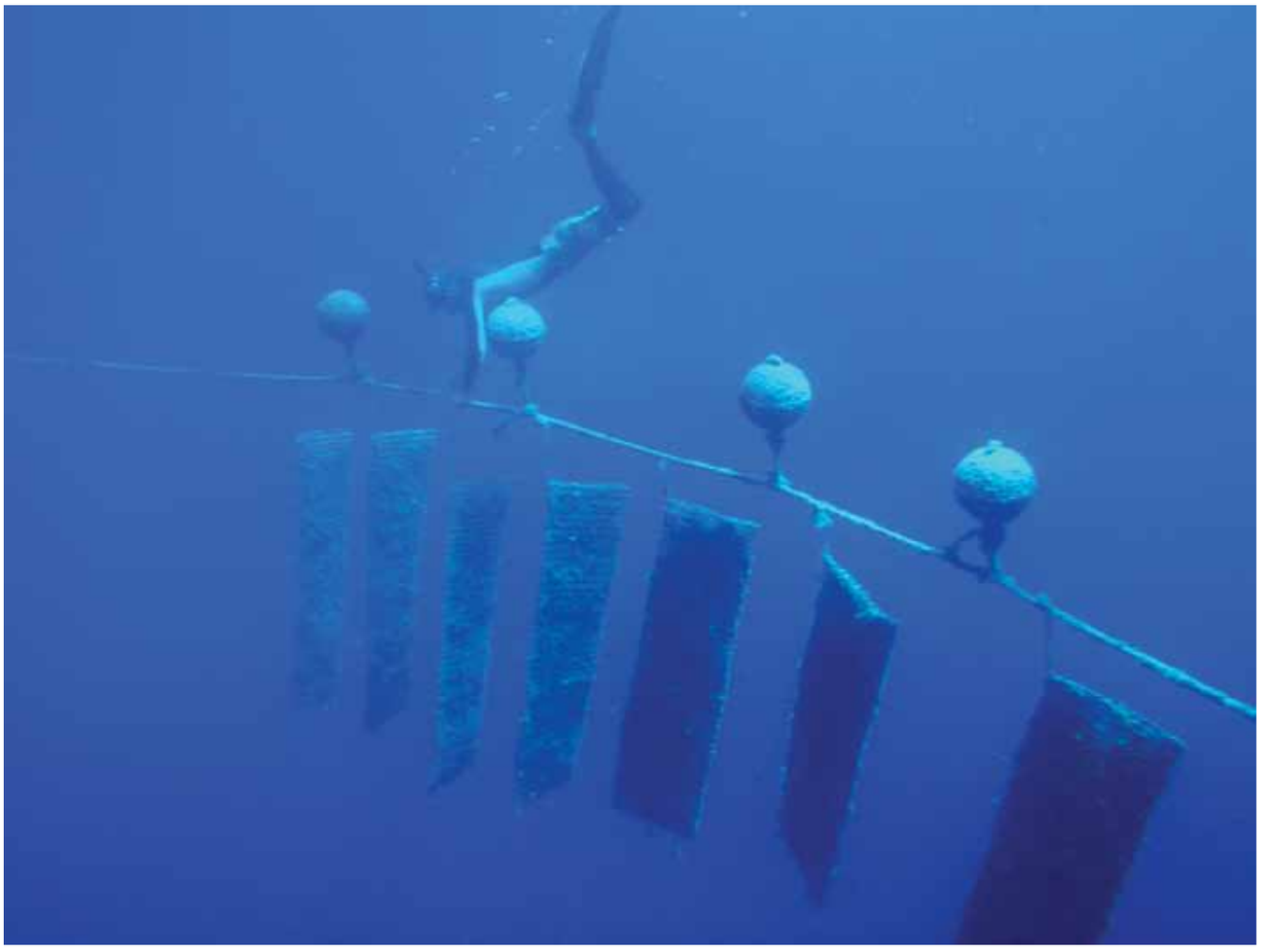

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Cultivated oysters are protected by nets like these, submerged in the lagoon of Ahe, French Polynesia. Both the nets and oysters require regular cleaning to maintain healthy growth, and this labor-intensive work provides opportunity for a range of local jobs.

from the blacklip pearl oyster *Pinctada margaritifera* in Pohnpei (the Federated States of Micronesia): Years 1 and 2. *CTSA Regional e-Notes* 9 (September 2011).

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CHAPTER 3

Pearl farming and production in the Federated States of Micronesia

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Authors: Cartier, L.E., Krzemnicki, M.S., Ito, M.

CULTURED PEARL FARMING AND PRODUCTION IN THE FEDERATED STATES OF MICRONESIA

Laurent E. Cartier, Michael S. Krzemnicki, and Masahiro Ito

The current production of cultured pearls from the black-lipped pearl oyster (*Pinctada margaritifera*) in the Federated States of Micronesia (FSM) includes mostly beaded as well as blister and nonbead-cultured pearls in a wide array of colors. Pearl farming is carried out on four islands, with plans for commercial production in the near future. The sector is envisaged as a model for economic development and marine conservation. To successfully compete in the marketplace, pearl farmers in the FSM should focus on producing high-quality cultured pearls and explore market differentiation strategies such as the "Micronesian Blue" product. Gemologically, the FSM cultured pearls are indistinguishable from those of French Polynesia that are produced using the same mollusk species.

In Micronesia, a group of more than 2,000 small islands in the western tropical Pacific Ocean, *P. margaritifera* oyster shells have been used by local populations and sold to itinerant traders since the 18th century (Clarke et al., 1996). Martin (1996) noted that in the 1800s, German divers gathered 50 tonnes of oysters from Chuuk Lagoon. The Japanese occupation of Micronesia (1914–1944) prompted further interest in pearl oyster resources, and shells were fished and a trial cultured pearl farm established in nearby Palau. In 1986, the FSM gained sovereignty after nearly 40 years as a U.S.-administered trusteeship. That year, 8,595 kg of black-lipped oysters were harvested in Chuuk Lagoon (Smith, 1992). Until 1987, however, there were no serious efforts to develop a cultured pearl farming industry in the area (Clarke et al., 1996). In the past 25 years there have been numerous attempts to establish commercial and community-based pearling operations. Current efforts are promising, and a variety of cultured pearl colors, including "Micronesian Blue," are beginning to reach the international market (figures 1 and 2).

Black cultured pearl production from the *P. margaritifera* mollusk was valued at more than US$100

Figure 1. These earrings contain "Micronesian Blue" cultured pearls (~10.5 mm in diameter). Photo courtesy of Natsuko Shiraki, © Hasuna Co. Ltd., Tokyo.

See end of article for About the Authors and Acknowledgments.
GEMS & GEMOLOGY, Vol. 48, No. 2, pp. 108–122,
http://dx.doi.org/10.5741/GEMS.48.2.108.

Figure 2. These bracelets are made with baroque-shaped cultured pearls (~7.3–9.0 mm) from the FSM. Photo courtesy of Natsuko Shiraki, © Hasuna Co. Ltd., Tokyo.

million in 2009 (Müller, 2009). This mollusk has a wide geographic distribution, including the Pacific Ocean, Indian Ocean, Red Sea, and off the coast of Mexico (Strack, 2006). However, commercial cultivation of this mollusk only takes place in French Polynesia, the Cook Islands, and Fiji, and is just beginning to emerge in the FSM. The industry as a whole is only 50 years old; the first successes in French Polynesia were reported in 1961 (Domard, 1962).

Pearl farming and associated economic activity has brought considerable development to remote regions of French Polynesia and the Cook Islands (Southgate and Lucas, 2008). At its peak in 2000, the French Polynesian cultured pearl sector employed 7,000 people (Murzyniec-Laurendeau, 2002). In recent decades, a number of other developing Pacific countries—through government and donor-funded projects—have attempted to emulate these successes in culturing black pearls from *P. margaritifera*. These include Kiribati, the Marshall Islands, Papua New Guinea, the Solomon Islands, and Tonga (Strack, 2006; Southgate and Lucas, 2008). The FSM is an ideal candidate for pearl farming projects because of its ecological similarity to the islands of French Polynesia. The country is highly dependent on foreign aid through the U.S. Compact of Free Association agreement, receiving a projected US$92.2 million in 2011 ("The Federated States of Micronesia…," 2010). Clearly, the production of high-value cultured pearls could foster indigenous economic development.

This article reviews various initiatives since 1987 to establish a Micronesian cultured pearl industry and evaluates the viability of community-based farming projects and marketing opportunities for "development pearls." It examines the implications of recent developments in the global black cultured pearl industry for the nascent FSM industry. The hatchery production of juvenile oysters is highlighted, as are a number of pearl oyster husbandry techniques and factors that influence the quality of the resulting cultured pearls. Finally, gemological characteristics of the bead-cultured pearls are presented. One of the authors (LC) visited the FSM pearl farms in October 2011, whereas another author (MI) has been working in the FSM on developing pearl farming and other aquaculture activities since 2001.

HISTORY AND INDUSTRY STRUCTURE

In 1987, the Pacific Fisheries Development Foundation and Pohnpei Research Division began evaluating the feasibility of a domestic cultured pearl industry. Since then a number of pilot projects and initiatives in the FSM have been started by local government, donors, and private citizens. Survey work and a feasibility study were briefly carried out on Ahnt Atoll but ceased in 1991 (Clarke et al., 1996). The primary focus of subsequent efforts was on Nukuoro Atoll, the only island in the FSM known to have a sufficient population of wild spat, thus eliminating the need for costly hatchery production of juvenile oysters. In 1994, Australia and the Pohnpei state government began funding a local project, and by 1995 there were 3,000 oysters seeded with round nuclei and 100 shells implanted with blister nuclei (Clarke et al., 1996). Low retention rates were attributed to the "poor condition of the oysters, the rudimentary working conditions and the relative inexperience of the local staff" (Clarke et al., 1996; p. 4). These factors, along with others detailed later in this article, have posed serious challenges to donor-funded community pearl farms in the FSM.

The Nukuoro farm was eventually incorporated in 2009 as Nukuoro Black Pearl Inc. (Leopold, 2011). The first significant harvest was sold locally in 2002, with 800 cultured pearls bringing US$10,000 (Sehpin, 2002). Three years later, financial irregularities were reported at Nukuoro (Sehpin, 2005). That same year saw the development of a bioeconomic model for small-scale pearl farms that was based on production and financial data from the Nukuoro farm, along with another farm in the Marshall Islands (Fong et al., 2005). However, pearl cultivation ceased in 2009. According to the Nukuoro municipal government, the oysters were left in the lagoon, and 10,000–20,000 have now reached an operable size but cannot be implanted due to lack of funding.

At present, pearl culturing takes place on four of the FSM's 607 islands, all within the state of Pohnpei: Pakin, Pohnpei (Nett Point), Pingelap, and Pweniou (a tiny islet off Pohnpei Island; figure 3). The first two farms each have 10,000 oysters, whereas the latter ones each have 3,000 oysters. All of these farms are in preparation for commercial pearl cultivation. Municipal government recently discontinued cultivation on a fifth island (Mwoakilloa) pending additional investment.

The waters in the FSM region, especially near Pohnpei, are rich in nutrients from nearby coastal mangrove forests. Water temperatures near Pohnpei's Nett Point farm vary between 27°C and 30°C, and salinity ranges from 35.0 to 35.5 parts per thousand. Testing at various sites within the Pohnpei lagoon has revealed that water currents, nutrient availability, and shelter vary greatly from site to site. Appropriate sites for pearl farming have been chosen taking these factors into account. The healthier the oyster, the lower the probability of disease, complications, or mortality and the higher the likelihood of harvesting high-quality cultured pearls.

The most encouraging efforts in support of pearl culturing in the FSM involve a project at the College of Micronesia (COM) Land Grant Program, which supplies hatchery-grown spat and technical assistance to the four operations mentioned above. In 2001, work began on a demonstration and training hatchery at the program's facilities at Nett Point on Pohnpei. The aim of the hatchery was to supply high-quality spat to islands that have insufficient natural oyster populations (Ito et al., 2004). This project has received funding from the U.S. Department of Agriculture (USDA), the U.S. Department of the Interior's Office of Insular Affairs, and the COM program. The ultimate goal is to "develop a self-sustaining pearl industry, integrating both community-based and commercial pearl farming operations" by 2016 (Ito, 2006). Investors have visited the FSM to explore the possibility of a large-scale commercial pearl farm, and such an enterprise would ensure the long-term viability of the hatchery, which is still being subsidized.

Figure 3. This map shows the location of past and present cultured pearl operations in the FSM. The hatchery that produces oyster spat is located at Nett Point on Pohnpei. The brood stock for this hatchery was initially collected from Ahnt, Pohnpei, and Pakin Islands. Pearl farms are presently in operation on Pakin, Pohnpei, Pingelap, and Pweniou (just off Pohnpei) Islands. Former farms on Mwoakilloa and Nukuoro are no longer producing any cultured pearls. Illustration by Augustin Hiebel.

Another project has received two rounds of funding from the Center for Tropical and Subtropical Aquaculture (CTSA) to investigate the development of pearl farming in the FSM (Haws, 2004), as well as to make hatchery production more efficient and to determine the spawning seasons of black-lipped pearl oysters (Haws et al., 2004). Most of the hatchery-based work was attempted in the Marshall Islands. This project has been discontinued due to a lack of funding. There was no overlap with the COM-based project, and the activities described in this article all stem from work at COM designed to produce cultured pearls marketed under the "Micronesian Blue" label.

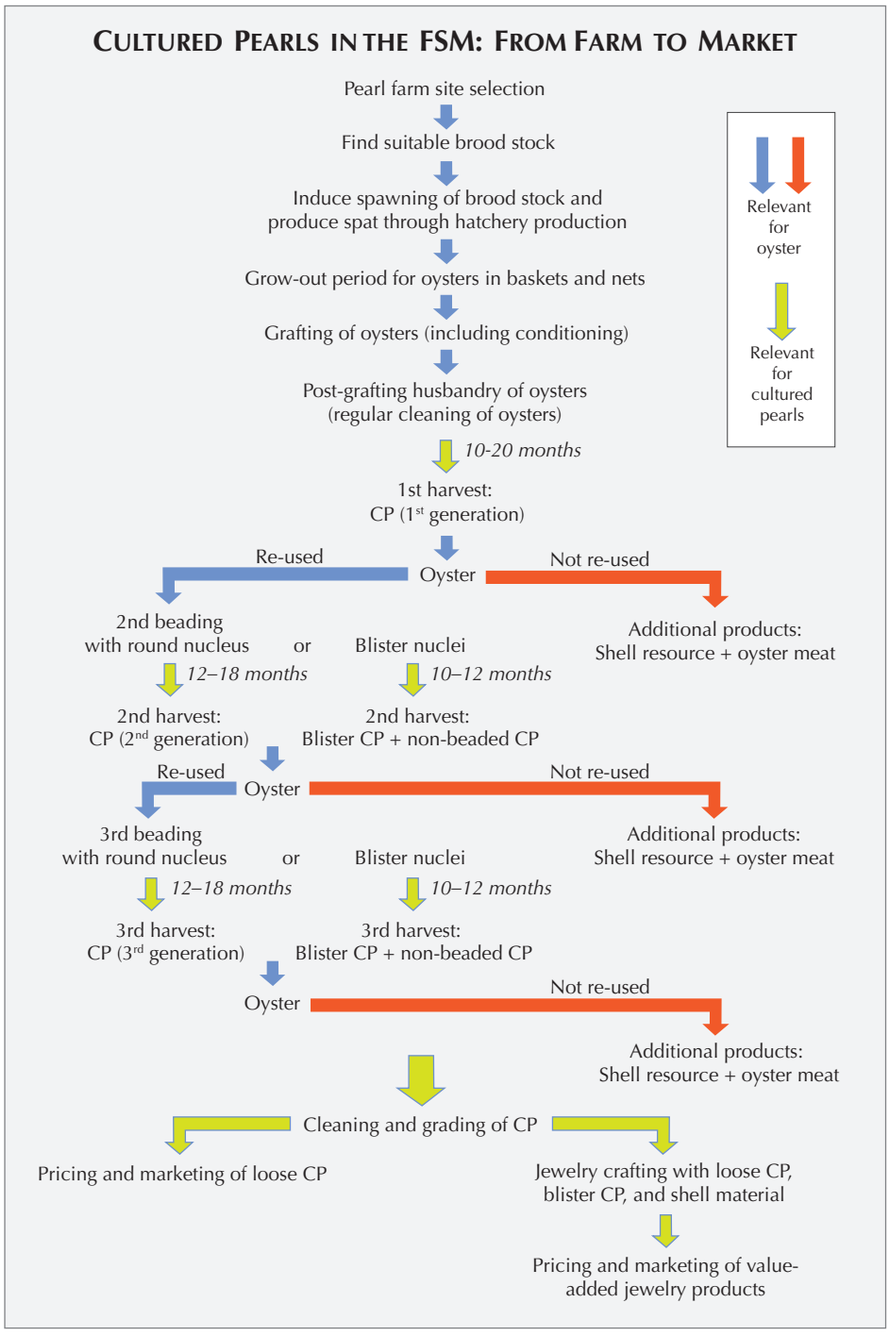


Figure 4. This diagram illustrates the different steps in setting up a pearl farm and obtaining cultured pearls (CP) in the FSM. It shows the potential of using the same oyster several times in the production of cultured pearl products and what resources can be obtained from this process. The periods indicated are from the time of seeding to the time of harvest. Modified after Haws (2002).

PEARL FARMING

The entire FSM pearling procedure, from farm site selection to marketing of the cultured pearls, is presented in figure 4.

Spat Production. Whereas the French Polynesian industry has relied on the collection of wild spat, the emerging FSM cultured pearl sector—apart from Nukuoro—relies on hatchery production using mature oysters (i.e., "brood stock"). Many Pacific islands have seen overfishing and a significant depletion of wild oyster stocks. Winds, currents, hydrology, and the placement of spat collectors and substrates also play major roles in determining the number of spat

that can be collected in the wild. Surveys have been conducted around the islands of Ahnt, Pakin, and Pohnpei to determine the feasibility of wild spat collection, but the populations were far too low. To address the shortage of wild spat in Micronesia, two hatcheries were set up in 2001: at Nett Point operated by COM (mentioned above) and on the southern part of Pohnpei Island run by the Marine and Environmental Research Institute of Pohnpei (Haws, 2004).

The key to high-quality hatchery-based spat production is careful selection of mature brood stock oysters collected in the wild. The brood stock strongly influences the color and quality of the cultured pearls. Brood stock for the Nett Point hatchery were collected by one of the authors (MI) and collaborators during multiple transect dives on the islands of Ahnt, Pohnpei, and Pakin from 2001 through 2004.

Whether spat is collected in the wild or produced in a hatchery, oyster reproduction follows very specific cycles that must be taken into account. Interestingly, the FSM seems to have no distinct spawning seasons. However, there are roughly two periods, March–June and September–December, when oysters release eggs and sperm and fertilization can take place. As in French Polynesia, this corresponds to seasonal changes in ocean water temperature and nutrient content (Southgate and Lucas, 2008). Full moon is usually a very good time to induce spawning in the hatchery setting, and this is done by stressing the oysters, such as by a rapid change in water temperature. Spawning in the wild is also induced by a change in environmental factors, though much less rapidly. One episode of spawning in a hatchery can yield 1–2 million oyster larvae per 1,000 liter tank. These larvae are fed various types of algae (figure 5), and they eventually develop into spat. Meanwhile, the water conditions are closely monitored. The combination of algal feed and water conditions is critical to producing strong, high-quality spat. Around day 17–19, spat collectors (e.g., 30 × 50 cm pieces of shade cloth attached to ½ in. PVC pipe frames, known as "Christmas tree" collectors) are placed in the tanks. Approximately 500–2,000 spat accumulate on the 60–70 collectors deployed in each tank. The spat are left there for 42–46 days, until they reach a size of 2–5 mm in antero-posterior shell length. Following this stage, they are transferred from the hatchery tanks into oceanic spat collectors or pearl oyster nets for nursery grow-out.

Figure 5. At the Nett Point hatchery, four species of algae are typically used to feed oyster larvae: Cheatoceros *(yellow),* Pavlova *(yellow-brown),* Rhodomonas *(orange), and* Tetraselmis *(green). Photo by L. Cartier.*

In Brief

- Efforts to produce black cultured pearls in the Federated States of Micronesia (FSM) date back to 1987.
- Farms on four islands in the state of Pohnpei (Pakin, Pohnpei, Pingelap, and Pweniou) are preparing for commercial pearl cultivation, with a total of 26,000 hatchery-reared oysters.
- These farms are projected to yield 6,500 cultured blister pearls and 2,000 loose bead-cultured pearls in 2012, with increasing production in the future.
- The cultured pearls show a range of colors; those with particularly distinct blue overtones are most prized, and branded "Micronesian Blue."

Nursery and Husbandry. Baskets with juvenile oysters are taken to the pearl farm (e.g., figure 6), and left on the seabed in shallow waters to reduce predation. Spat mortality is initially assessed by onsite counting approximately four months after fertilization, and the baskets are examined every six weeks for predators. Carnivorous snails and crabs are major causes of spat mortality. The young oysters are later transferred to lantern baskets (figure 7). When they are between 1.5 and 2.5 years in age they are removed from the baskets, drilled, and hung on chaplet lines (see figure 8). In most areas of the FSM, netting is not required at this stage because predation is less of a threat. Biofouling, the settling and growth of animals and plants

Figure 6. This photo shows the farming operation near Pweniou Island off Pohnpei. Photo by L. Cartier.

on the oysters, must be removed in 1–2 month intervals to ensure the proper health and growth of the pearl oysters (figure 9). Once the shell is deemed sufficiently large (10–12 cm in diameter) and healthy, the oyster can be grafted to induce the formation of a cultured pearl.

Grafting. The grafting operation requires a host and a donor oyster, and a skillful technician (e.g., Hänni, 2007). Whereas the donor oyster (which is sacrificed) is selected for the quality of its mantle, the host oyster is chosen for its vigor (Haws, 2002). An international grafting technician regularly visits the FSM to train locals in grafting techniques for both round and blister cultured pearls, with the aim that by 2013 they can meet the requirements of a nascent cultured pearl industry. The nuclei consist of Mississippi mussel shell material and range from 5.5 to 13.0 mm in diameter.

Figure 7. Two-year-old oysters in lantern baskets are examined at the Pweniou pearl farm. Inside the basket, technicians found two predatory snails. Photo by L. Cartier.

Typically, the first-generation operation is carried out to produce a loose cultured pearl. Cultured blister pearls are sought in older generations of pearl oysters, which can be regrafted two or three times. For the production of bead-cultured pearls, the seeded oysters are kept in the water between 10 and 20 months. An oyster deemed unsuitable for regrafting may then be seeded to produce several cultured blister pearls (figure 10). In this case, the oyster is left in the water 10–12 months. Because a pearl sac is already present, such oysters are very likely to bear "keshi" nonbead-cultured pearls as well. This strategy maximizes the resource: Rather than sacrificing the oyster, it is reused to produce cultured blister pearls that can be manufactured into simple jewelry.

PRODUCTION, PROCESSING, AND MARKETING

Loose cultured pearls and blister products are harvested several times a year, but the output remains small. Production from the COM project in the FSM during the past decade was around 15,000 round cultured pearls and 3,000 cultured blister pearls. The majority of them came from the Nett Point farm on

Figure 8. Grafted oysters are attached to ropes using the "ear-hanging" method, forming chaplets. Photo by L. Cartier.

Pohnpei. They were sold as samples from the COM project to selected Japanese jewelry designers and shops for promotional purposes.

The four farms linked to the COM program are projected to yield 6,500 cultured blister pearls and 2,000 loose bead-cultured pearls in 2012, with a steady expansion in the coming years. The cultured blister pearls are expected to come from Pohnpei (3,000 pieces), Pakin (2,000 pieces), and Pweniou (1,500 pieces), and they will be sold on the local and international markets. As pearl farming moves toward commercial operation in the near future, round cultured pearls will also enter the international market.

The FSM produces far fewer dark cultured pearls than French Polynesia, because it uses lighter-colored brood stock. They are cleaned and processed with nothing more than sea salt and a polishing cloth. Most cultured blister products are crafted into jewelry and sold locally. Two charity sales in Pohnpei in 2010 led to revenues of US$6,000 and $13,500. The entire local market in the FSM is estimated at only US$100,000 per year, and the country drew just 20,000 tourists in 2010. If the pearl sector is to grow, it must expand beyond the local market. Nearby Guam, for instance, is an important tourist destination.

The FSM pearl industry must also find suitable niches worldwide and generate greater income through marketing differentiation (Fong et al., 2005). Although not yet commercially available on the international market, "Micronesian Blue" cultured pearls are being sold at charity sales and were used in two Japanese jewelry collections. The FSM products are also being marketed as "development pearls" because of their contributions to the local economy and marine conservation. Additional marketing strategies are being examined to avoid the failures of numerous donor-funded projects to promote community-based pearl farming over the past three decades (Ito, 2011a).

QUALITY: THE KEY TO PEARL FARM VIABILITY

The greater the proportion of high-quality cultured pearls in a harvest and the lower the oyster mortality rates, the more likely a farm will be profitable. Haws (2002) calculated that 95% of a farm's earnings come from just 2% of the cultured pearls. Le Pennec et al. (2010) estimated that for 2,000 grafted oysters, only 3% yield "beautiful" cultured pearls; improving this rate to 4% would considerably increase farmers' incomes. Conversely, Fong et al. (2005) projected that for a farm with 25,000 seeded oysters, a 5% increase in mortality would raise production costs per cultured pearl by nearly 21%.

Figure 9. Regular cleaning of oysters, as shown here on Pakin Island, is vital to maintaining their health. This step also creates jobs for local villagers. Photo by L. Cartier.

Figure 10. An oyster that yielded a first-generation cultured pearl was re-grafted to produce four cultured blister pearls. The remaining pearl sac produced a nonbead-cultured pearl. Photo by L. Cartier.

Le Pennec et al. (2010) noted that out of 1,000 oysters grafted in French Polynesia, 250–300 saleable cultured pearls (25–30%) are typically produced in the first generation. In a study of the Nukuoro farm and another farm in the Marshall Islands, Fong et al. (2005) found that 10,725 marketable cultured pearls (42.9%) were produced from a harvest of 25,000 first-seeded oysters. This success rate is surprisingly high given that mortality rates should be similar to those in other areas of Micronesia (see below) and that the two farms were not commercially successful. The lack of an industrywide grading system for cultured pearls also makes such comparisons difficult.

Improving Cultured Pearl Quality. Murzyniec-Laurendeau (2002) showed that in a sample harvest of 271,000 *P. margaritifera* cultured pearls from French Polynesia, circled goods (cultured pearls with concentric rings or grooves visible on the surface) accounted for 23% of the volume but only 6% of the value. If formation mechanisms of circled cultured pearls can be better understood, practices can be adapted to minimize their production in favor of more valuable cultured pearls. There is a surprising lack of collaboration between gemologists and scientists researching biomineralization, aquaculture, and oyster genetics. Greater synergy across disciplines would advance cultured pearl production and quality.

A three-year research project was initiated by COM in 2007 to understand how grafting techniques could be optimized to improve quality (Ito, 2009). The study also investigated formation mechanisms of circled cultured pearls and disproved the widely held idea that they result from nucleus rotation in the pearl sac (see also Caseiro, 1993). Ito (2009, 2011b) argued that if this were the case, nonlinear patterns should be found on circled cultured pearls. However, Ito's (2011b) study of 4,011 samples found no evidence for this, and proposed a mantle cell proliferation mechanism of circled cultured pearl formation.

A great deal of experimentation has gone into understanding the optimal conditions for oysters and how the quality of harvested cultured pearls can be improved through certain pearling practices. A trial project was initiated by COM in 2005 to investigate the circling phenomenon in cultured pearls, and this study also offered an overview of mortality and rejection rates (figure 11). These rates were higher than in a normal pearl farming context, because the aim was scientific experimentation rather than commercial success; the total success rate was only 28%. Nucleus rejection rates for second-generation grafting of these trial oysters decreased to 10–15%, which is good by international comparison.

The harvesting success rates and qualities are highly dependent on farm site, nursery expertise, skills of the grafting technicians, and whether pearl farming was carried out for experimental or commercial purposes. The following practices are recommended in the FSM: Waiting until the oysters reach a good size (10–12 cm in shell diameter) before grafting, maintaining low stocking densities of oysters, extending the period between grafting and harvest, and regularly (every 6–8 weeks) removing any biofouling from the oysters.

ECONOMIC CONSIDERATIONS AND DEVELOPMENT STRATEGIES

The average price (at export) of black cultured pearls in French Polynesia has fallen by a factor of four in

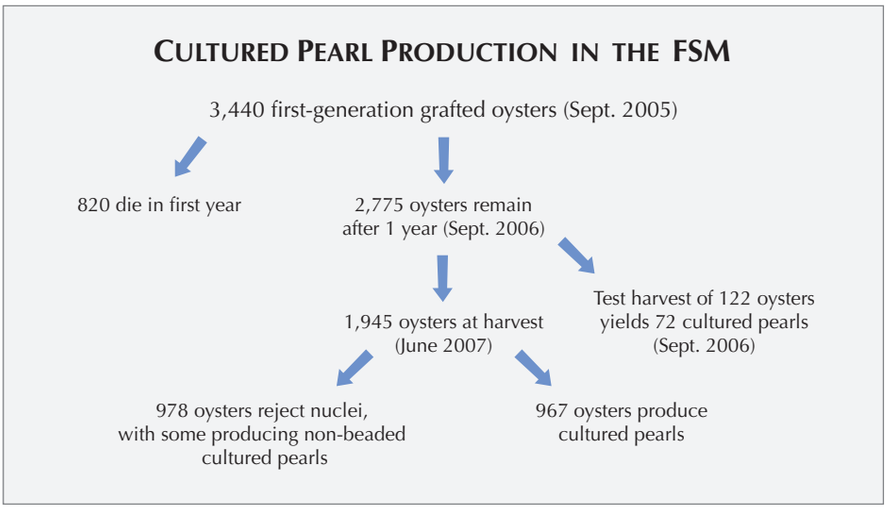


Figure 11. This chart shows the oyster mortality and rejection rates for a 2005–2007 trial project in the FSM. These figures are higher than those in other pearl farming regions, but do not reflect current rates in the FSM, which are much lower.

the past decade, from 1,800 CFP francs (US$19.68) to 460 CFP francs (US$5.03; Talvard, 2011). However, this depreciation is also the result of diminishing quality in the output of many pearl farms. Government authorities continue to carry out quality control of exported cultured pearls, and those of very low quality are destroyed. However, both the average size and average quality of these cultured pearls are lower than a decade ago. Such developments in the French Polynesian industry—which accounts for more than 95% of the world's black cultured pearls—are bound to also affect minor producers such as the Cook Islands, Fiji, Mexico, and the FSM.

A number of reports have noted the lack of large (>13 mm) high-quality black cultured pearls in the international market (Shor, 2007; Torrey and Sheung, 2008; Italtrend, 2010) and the fact that the average price of these larger goods has not decreased. Some reports suggest an overproduction of small black cultured pearls of low to medium quality, but obviously this cannot be generalized to include all types and qualities of these goods at present.

For two farms in the FSM and the Marshall Islands, both with 25,000 seeded oysters, Fong et al. (2005) calculated the average cost of producing a cultured pearl to be US$19.15. This was over a 20-year period, and both farms examined for that study have since ceased operation. In French Polynesia, as elsewhere, large pearl farms (>200,000 oysters) benefit from economies of scale (Poirine, 2003). Poirine and Kugelmann (2003) calculated with data from 2000 that the average cost per cultured pearl in French Polynesia for a large-scale farm was 902 CFP francs (US$9.93), compared to 1,889 CFP francs (US$20.79) for a small-scale farm of <25,000 oysters. Although pearl farming still has the potential to bring economic development to remote coastal communities, the long-term viability of these farms may be at risk due to challenging market factors, not to mention environmental and climate considerations.

Do small-scale farms have a future? The revenue models presented by Johnston and Ponia (2003) and Fong et al. (2005) do not reflect the economically unfavorable evolution of the black cultured pearl market in the past decade. The assumptions of their models render all small-scale pearl farms unprofitable if the recent global slump in black cultured pearl prices is taken into account. Yet other research in French Polynesia and the FSM suggests that there is a future for small-scale pearl farms that adopt alternative strategies, including:

- Maximizing revenue by marketing oyster meat and oyster shell resources (as jewelry or as raw material for medicinal purposes)
- Reducing spat costs through innovation in hatchery production
- Reducing oyster mortality
- Emphasizing cultured pearl quality over quantity
- Strategizing market differentiation through branding (e.g., Fiji)
- Adopting value-added activities such as jewelry crafting and developing synergies with tourism
- Emphasizing technology so that dependence on costly international assistance is minimized
- Making pearling a seasonal activity for local people, complemented by income from fishing, farming, or tourism

Technology Transfer. Even with these strategies, the transfer of technology to local inhabitants is essential. In several countries, the production of cultured blister pearls has been envisioned as an economic development strategy, and donors have funded such projects using *P. margaritifera* in Kiribati (Teitelbaum, 2007), Tanzania (Southgate et al., 2006), and Tonga (Teitelbaum and Fale, 2008). Yet none of these has achieved sustained commercial success, domestically or abroad. Typically, these types of internationally funded projects emphasized farming methods and handicraft-making techniques without training locals in sales and marketing (Ito, 2011a).

In contrast, current efforts in the FSM focus on training locals in all aspects of cultured pearl production and marketing. This ensures that the skills necessary for a pearl farming sector can be sustained locally without long-term foreign aid. Micronesians, not foreigners, are training local workers as technicians at the COM project's Nett Point hatchery on Pohnpei. This is widely regarded as a positive step in the development of aquaculture because it fosters local expertise and community collaboration, making the sector more likely to succeed. Overall, the project has four aims:

1. Standardizing hatchery and ocean grow-out protocols to realize mass spat and seedable oyster production
2. Training local technicians in hatchery-subsequent husbandry practices and grafting techniques
3. Training locals in basic jewelry manufacturing methods
4. Incorporating pearl farming into an integrated aquaculture and marine protected area development project and an ecosystem-based community fisheries management plan, with the goal of promoting alternative livelihood opportunities and local marine conservation

This project in the FSM is unique in the sense that the local grafting technicians being trained also have pearl farming and cultured pearl grading skills, and are themselves capable of training others. Indigenous youths who have learned basic jewelry design and manufacturing techniques (figure 12) then process the cultured blister pearls for sale locally and regionally (in Guam, for instance). Cultured blister pearl jewelry has recently sold in the local market for an average of US$20 per piece, an encouraging development (figure 13).

Figure 12. In a workshop on Pakin Island, local youths are taught how to drill shells containing cultured blister pearls so that they can be processed into jewelry. Photo by L. Cartier.

Management: The Key to a Successful Industry. After five decades of black cultured pearl farming and trading in French Polynesia, it has become clear that the management of both production and marketing is critical to ensuring long-term success. The striking differences in the industry development and

Figure 13. These pieces of cultured blister pearl and shell-derived jewelry, manufactured by indigenous youths, are sold in the local market. The diameter of the shell is ~10 cm. Photo by M. Ito.

government regulation between Australia (the main producer of white South Sea cultured pearls by value) and French Polynesia (the dominant source of black cultured pearls) have been examined by several authors (Tisdell and Poirine, 1998; Poirine, 2003; Müller, 2009). While French Polynesia, in Müller's words, adopted a "laissez-faire" approach to marine concessions, production, and trade, Australia chose to enforce strict quotas on output. Although the FSM pearl industry is unlikely to attain such international importance, questions regarding how the sector should be managed will need to be addressed as the sector develops.

While Poirine (2003) advocated economic regulation of the (Polynesian) cultured pearl sector through an auction system of limited marine concessions, another model has emerged in the FSM. Because most indigenous spat must be grown in a hatchery (Nukuoro notwithstanding), scientists control the oyster supply. Any pearl farm involved in the COM project that does not adhere to strict environmental and other guidelines must return its oysters to the Nett Point hatchery. The oysters remain the property of the hatchery, ensuring scientific oversight of the sector. Additional management models are currently under development.

Marine Conservation. Sound pearling practices have a positive impact on local fish stocks, since fry thrive around oyster farms and commercial fishing within these areas is prohibited (Pae Tai – Pae Uta, 2003). Unlike the extraction of many other gem resources, the cultivation of pearls depends directly on responsible environmental management. Low stocking densities have a positive influence on the health of oysters and are more likely to lead to high-quality harvests (Southgate and Lucas, 2008). Very high stocking densities can lead to mass mortality of oysters, as demonstrated on the island of Manihiki and the subsequent demise of the Cook Islands cultured pearl industry (Macpherson, 2000; Southgate and Lucas, 2008).

Pearl farming is one of the most profitable forms of aquaculture. With limited environmental impact and a high-value resource that can be produced in remote atolls, it has often been described as an ideal business model for developing Pacific coastal communities (Sims, 2003). In regions such as the FSM, which depend on artisanal fishing and subsistence farming and enjoy few if any alternative opportunities, pearl farming may reduce human pressures on the environment and generate cash income for local communities. Through alternative economic opportunities, such as pearl farming, pressures on rapidly diminishing fish stocks can be reduced. The income lost by abstaining from fishing in certain areas—Pakin or Pweniou islands, for instance—can be recouped by income from pearl farming. Marine protected areas (MPA) with no-fishing zones have been established in some parts of Pakin and Pweniou. In Pakin, for example, the model has been extended to become an integrated MPA in which pearl farming is carried out but fishing is not allowed. This innovative approach ensures that fish stocks can recover and gives locals access to alternative sources of income.

GEMOLOGY OF MICRONESIAN CULTURED PEARLS

Materials and Methods. For this study we examined 18 *P. margaritifera* cultured pearls obtained from Pohnpei's Nett Point farm by author LC (figure 14). The samples ranged from 3.86 to 13.00 ct, and measured approximately 8.1–12.1 mm in diameter. The selection was chosen to best represent the range of possible colors and qualities from the FSM's current cultured pearl production; three samples were of the "Micronesian Blue" variety.

In addition to visual examination and close microscopic inspection, all samples were analyzed by X-radiography using a Faxitron instrument (90 kV and 100 mA excitation) and Fuji film. On three samples (FSM_15, FSM_16, and FSM_17), we also measured

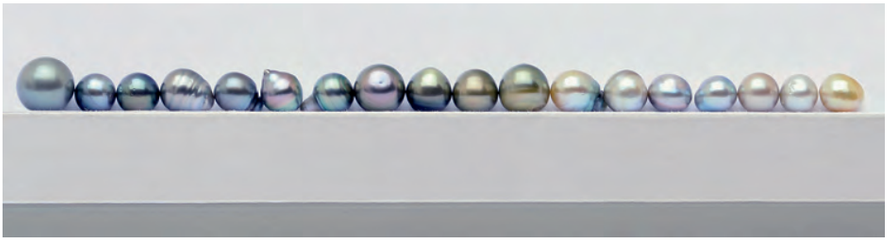


Figure 14. A range of colors and overtones were observed in the cultured pearl samples from the FSM (8.1–12.1 mm in diameter). Photo by M. S. Krzemnicki, © SSEF.

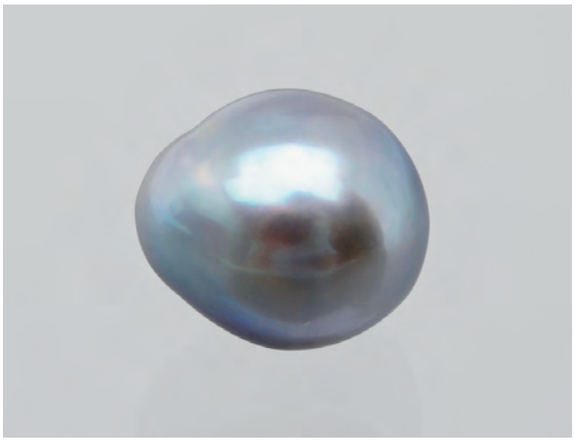


Figure 15. This light gray sample (8.4 mm in diameter) displays distinct blue and purple overtones characteristic of "Micronesian Blue" cultured pearls. Photo by M. S. Krzemnicki, © SSEF.

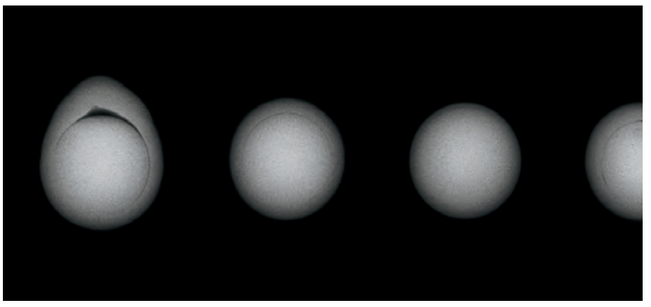


Figure 16. These X-radiographs of four bead-cultured pearls from Micronesia show varying nacre thicknesses, described here from left to right. Sample FSM_4 shows a small triangular cavity at the interface between the bead and nacre. FSM_10 has a medium nacre overgrowth (~1 mm), while FSM_14 shows a rather thin nacre layer (~0.5 mm), and FSM_16 has a thicker nacre overgrowth (~1.5 mm). Images by M. S. Krzemnicki, © SSEF.

UV-Vis reflectance spectra using a Varian Cary 500 spectrophotometer with a diffuse reflectance accessory. Furthermore, all 18 pearls were examined with a long- and short-wave UV lamp. Luminescence spectra of three cultured pearls (FSM_15, FSM_16, and FSM_18) were collected with an SSEF-developed UV-Vis spectrometer (based on an Avantes spectrometer) coupled with a luminescence accessory consisting of a mounting with three 365 nm LED lamps.

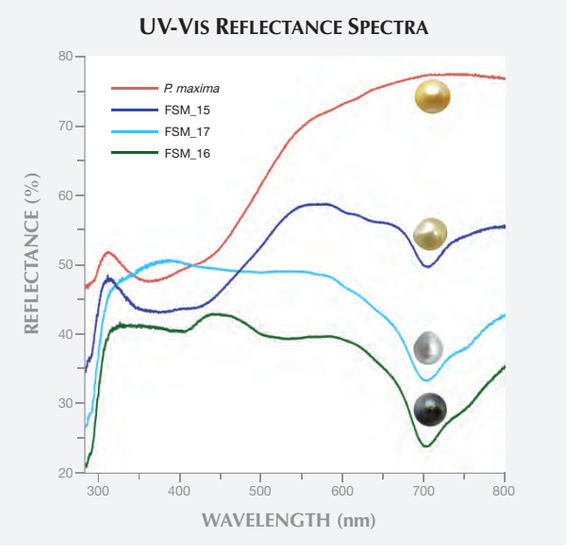
Results and Discussion. The cultured pearls' shape varied greatly from perfectly round to semi-round, button, drop, baroque, and circled. The color range included white, yellow, light gray to dark gray and brownish gray, and black (again, see figure 14). Most showed moderate to distinct overtones, with interference and diffraction colors dominated by green, purple, and particularly distinct blue hues (e.g., figure 15). The color distribution was partially uneven, especially in those showing circled features and surface imperfections such as dots, indentations, and bumps.

As the cultured pearls were taken directly from the production site prior to processing, the moderate to high luster represents their original state rather than their polished appearance. This was especially obvious under high magnification, which revealed fine fingerprint-like structures caused by the regular stacking of the aragonite platelets of the nacre.

X-radiographs (e.g., figure 16) revealed a distinct bead nucleus in the center of each sample, surrounded by nacre with a thickness of 0.5–3.9 mm. The off-shaped cultured pearls in particular showed distinct variations in nacre thickness, whereas the round to semi-round samples had typical (for *P. margaritifera* cultured pearls) nacre thickness of 0.8–1.4 mm.

UV-Vis spectra revealed a trough in reflectance at about 700 nm (figure 17), which is characteristic

Figure 17. The UV-Vis reflectance spectra of three P. margaritifera *cultured pearls from the FSM are compared to the spectrum of a yellow cultured pearl from* P. maxima*. The* P. margaritifera *samples show a distinct trough in reflectance at 700 nm that is characteristic for this species, but not seen in the* P. maxima *sample. The spectra are shifted vertically for clarity.*



of the color pigments (porphyrins) in the shell and cultured pearls of *P. margaritifera* (Miyoshi et al., 1987; Karampelas et al., 2011). Interestingly, even the reflectance spectrum of the yellow cultured pearl (FSM_15) showed this feature. This is in contrast to yellow cultured pearls from the gold-lipped pearl oyster (*P. maxima*), which look very similar but do not show this trough. This supports the use of UV-Vis spectroscopy for separating yellow to "golden" cultured pearls from these two species (see also Elen, 2002).

The samples showed inert to distinct yellow reactions to long-wave UV radiation, and distinctly weaker fluorescence to short-wave UV. Often the reaction was not uniformly distributed, but correlated to the lighter gray surface regions of the cultured pearls. The luminescence spectra of three cultured pearls characterized by distinct yellow fluorescence (FSM_18), moderate yellow fluorescence (FSM_15), and essentially no reaction (FSM_16) to the long-wave UV lamp all revealed two broad luminescence bands that correlated in intensity with the visual strength of their fluorescence (figure 18). By comparison, gray to dark cultured pearls from *Pteria sterna* from the Sea of Cortez in Mexico show additional spectral features above 600 nm that correspond to the red luminescence commonly observed in them (Kiefert et al., 2004; Sturman, 2009).

Figure 18. The luminescence spectra of three cultured pearls from P. margaritifera *with distinct yellow (FSM_18), moderate yellow (FSM_15), and nearly no fluorescence (FSM_16) to long-wave UV radiation are compared to the spectrum of a brown* Pteria sterna *cultured pearl from Mexico, which fluoresced strong red to long-wave UV radiation. The strong luminescence intensity below 400 nm for all samples is due to the excitation wavelength of the LED light source.*

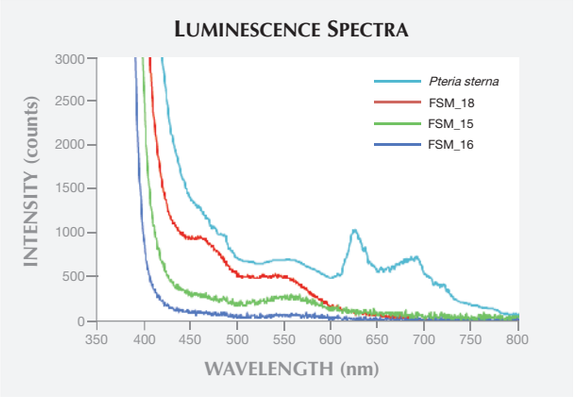


Figure 19. Blue overtones in "Micronesian Blue" cultured pearls (here, 12 mm in diameter) may be diagnostic of these products in the marketplace. Photo courtesy of Yuhei Hosono, © Le Collier, Tokyo.

Based on their observed and measured characteristics, our Micronesian samples were similar in many respects to cultured pearls produced in French Polynesia using the same species. The blue overtones, in some cases quite distinct, may serve to distinguish the "Micronesian Blue" cultured pearls in the international market (e.g., figure 19).

CONCLUSION

Pearl oyster farming is still in its infancy in the FSM, yielding small quantities of cultured pearls compared to the massive production in French Polynesia. Pearling activities and production are expected to expand in the FSM in the near future. Technical assistance through the COM program should ensure the supply of high-quality *P. margaritifera* oysters to support the nascent industry, as well as the adoption of responsible production practices.

Demand for the FSM's cultured pearls appears to be growing as they reach the international market, especially in Japan, where samples from initial harvests have been sold to selected jewelry designers who are marketing them as Micronesian cultured pearls. For the industry to succeed, a market differentiation strategy must be adopted. The decision to brand a portion of the production as "Micronesian Blue" cultured pearls is an important step in that direction.

Figure 20. This necklace features Micronesian cultured pearls (8.5–13.3 mm) of various colors. Photo courtesy of Yuhei Hosono, © Le Collier, Tokyo.

The FSM's cultured pearls come in a wide spectrum of colors and overtones (e.g., figures 14 and 20). Gemological and analytical instrumentation cannot conclusively separate these cultured pearls from those produced by *P. margaritifera* in French Polynesia and other areas. However, they are easily separated from *Pteria sterna* cultured pearls through UV-Vis reflectance spectroscopy. In addition, yellow cultured pearls from the FSM can be separated from yellow South Sea samples cultivated in the *P. maxima* oyster.

Through the careful selection of suitable brood stock, "Micronesian Blue" cultured pearls may become a high-value niche product on the international market in the near future. With an emphasis on quality and limited production, the FSM pearl sector has a realistic chance of economic success without foreign aid.

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CHAPTER 4

The influence of pearl oyster farming on reef fish abundance and diversity in Ahe, French Polynesia

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The influence of pearl oyster farming on reef fish abundance and diversity in Ahe, French Polynesia

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ABSTRACT

Many cultured pearl farms are located in areas of the Pacific that have thriving, highly diverse fish communities but the impacts of farming on these communities are poorly understood. We studied the effects of pearl oyster farming on shore fish abundance and diversity in the lagoon of Ahe, French Polynesia by adapting roving diver census methods to the coral reef bommies of the lagoon and compared 16 sites with high pearl farming impact to others with no direct impact. Pearl farming has a slightly positive effect on reef fish abundance (N) and no significant impact on fish diversity (H) or community composition. This is important when considering the ecological sustainability of pearl farming in French Polynesia and suggests that a potential synergy between pearl farms and marine conservation should be further explored.

1. Introduction

Aquaculture – the farming of aquatic organisms – is widely heralded as a promise in taking over from where extractive fisheries are failing (Folke and Kautsky, 1992; Shumway et al., 2003). However, a lot of questions remain about the ecological impacts of fish and shellfish aquaculture (Stewart, 1997; Naylor et al., 2000; Diana, 2009). The world's marine aquaculture production totaled 18.3 million tonnes in 2011, with 75.5% attributed to marine molluscs (FAO, 2012). Bivalve cultivation is often carried out in extensive aquaculture systems not requiring fertilizers, feed or closed water systems; and is often regarded as more ecologically sound than intensive aquaculture (Crawford et al., 2003; Gibbs, 2004). Pearl oyster farming involves the cultivation of low trophic level organisms – pearl oysters – which thrive under low stocking densities in bays and lagoons, where the right mix of required nutrients and sheltered conditions are met (Southgate and Lucas, 2008). Unlike many other forms of aquaculture, it does not focus on food production but on the production of valuable cultured pearls and it is considered to have one of the lowest potentials for environmental impact, although the extent of their impacts are not well understood (Wells and Jernakoff, 2006; O'Connor and Gifford, 2008).

Cultured pearls have become a billion-dollar industry and experienced a tremendous boom in recent decades in Asia and the Pacific. French Polynesia has dominated the production of Tahitian cultured pearls (from the *Pinctada margaritifera* oyster) since 1962, with a production share of over 98% at present (Cartier et al., 2012). What began as an experiment has turned into an industry, which at its peak in 2000 exported ~$200 million worth of raw pearls (Southgate and Lucas, 2008). In 2011, pearl farming in French Polynesia was carried out on 26 atolls and 4 high-islands, with a total of 10,000 ha authorised for pearl farming (Andréfouët et al., 2012). Given the rapid development of the industry and the relative paucity of data on the impacts of the sector on the natural environment and biodiversity, research is needed so that the costs and benefits can be weighed (Klinger and Naylor, 2012).

The impacts of shellfish aquaculture on biodiversity continue to be poorly understood (Diana, 2009). Marine areas in Asia and the Pacific boast some of the greatest marine biodiversity on the planet (Carpenter and Springer, 2005). But much of this is at risk as the rate of global marine biodiversity loss continues to grow (Carpenter et al., 2008; Butchart et al., 2010). Research has shown that exploitation, habitat loss, invasive species and pollution are responsible for most organism extinctions (Wilcove et al., 1998; Diana, 2009). Aquaculture has the potential to preserve biodiversity by reducing exploitation and pressures on wild stocks. However, aquaculture can also lead to the deterioration of biodiversity and natural ecosystems through habitat destruction, competition for resources (e.g. food) and the introduction of invasive species (Naylor et al., 2000; Diana, 2009). These impacts can be at odds with the ultimate success of aquaculture as an appropriate environment is needed for profitable grow-out of farmed organisms.

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Oysters are vulnerable to even subtle environmental changes and the quality of a produced pearl is intimately associated to the quality of water in which the oyster grows (Gervis and Sims, 1992; Hänni, 2007). This includes effects from biofouling, predation and potential disease agents (Pit and Southgate, 2003; Bondad-Reantaso et al., 2007; de Nys and Ison, 2008) that can have potentially fatal impacts on an oyster's health (Southgate and Lucas, 2008). Therefore, environmental stewardship, livelihoods and profitability are inextricably linked. This is why responsibly managed pearl farms have been advocated as agents of biological conservation and restoration of wild oyster populations (Saucedo and Monteforte, 1997; Sims, 2003; Cartier and Ali, 2012). Although the impacts of pearl farming on the natural environment are difficult to quantify, largely due to the large number of factors and the large spatial variation in farming sites around the world, two main types of potential impacts have been identified (Southgate and Lucas, 2008; The WorldFish Center, 2008). Firstly, the introduction of potentially invasive species or modified genotypes (e.g. from hatchery produced spat or wild spat from other areas) of oysters into the natural environment, and associated disease agents (Arnaud-Haond et al., 2004; Bondad-Reantaso et al., 2007; Lemer and Planes, 2012). Secondly, the physical impact of pearl oysters in the form of increased biomass, faeces deposition, nutrient consumption and human impacts (Yokoyama, 2002; Gifford et al., 2004; Jelbart et al., 2011). Mass oyster mortalities in Japan, the Cook Islands and French Polynesia in the past three decades are believed to be largely a result of high-stocking densities, potentially augmented by adverse weather conditions (Intes, 1988; Heffernan, 2006; Kuchel et al., 2011; Andrefouet et al., 2013). But under reasonable farming practices, studies have shown that cultured oysters do not limit the growth of wild oyster populations and other organisms (Niquil et al., 2001). However, the impact, if any, on biodiversity continues to be poorly researched. The main issue with previous research has been the lack of suitable methodology to assess these impacts in complex dynamic marine ecosystems (Gifford et al., 2004; Jelbart et al., 2011).

Reef fish are a good indicator of relative biodiversity, and thus provide a useful tool to determine the impacts of pearl farming through quantification of shore fish abundance and diversity. Previous research in the Philippines (Palawan) has suggested that the effects of pearl farming on reef fish populations may be positive in comparison to control sites (Carpenter, unpubl. data). Most pearl farms in French Polynesia are found in lagoon environments of the Tuamotu Archipelago (Le Pennec et al., 2010). The interaction between reef fish and the pearl farms is high, as many pearl farms working stations are built on coral reefs (Pae Tai-Pae Uta, 2003). Pearl oysters are frequently stocked in nets to protect them from predators. These nets also offer shelter and substratum to fish larvae and juveniles. Biofouling – the settlement and growth of plants and animals (de Nys and Ison, 2008) – attached to nets and oysters provide additional biomass and food to fish, potentially promoting fish abundance.

More research is required to understand the effects of having such a great number of oysters 'artificially' hanging in a lagoon. The atoll of Ahe had 77 cultured pearl farms working on 1188 ha of marine concessions: nearly 11% of the lagoon area was used for commercial pearl oyster cultivation in May 2012 (Andréfouët et al., 2012; Fournier et al., 2012a). The relatively high concentration of pearl farms provides a suitable environment in which to study reef fish populations and how they are affected by pearl farms. Recent research associated to pearl farming on Ahe, has focused on picoplankton (Bouvy et al., 2012), lagoon hydrodynamics (Dumas et al., 2012), plankton concentrations (Fournier et al., 2012a,b), bivalve larvae dynamics (Thomas et al., 2012a,b) and prokaryotes (Michotey et al., 2012). These studies clearly indicate that the western portion of the lagoon that we include in our study, should be partitioned a priori relative to position to the one major connection of the lagoon to the open ocean. To our knowledge, no research has been carried out on what impact this and pearl farming may have on coral reefs and reef fish populations. The presence of these farmed oysters and the added biomass could have an effect on reef fish populations. Although a historical comparison is not possible, we can compare pearl farming sites to sites without any direct impacts from pearl farming. The aim of this paper is to determine what effects pearl farming has on reef fish populations in a French Polynesian atoll environment, thereby elucidating the relative impacts of the activity on local biodiversity and how farming is influencing fish habitats and possibly modifying fish feeding habits.

2. Materials and methods

2.1. Study locations

The study locations are found in the atoll of Ahe (14°29′S; 146°18′W) in the Tuamotu Archipelago of French Polynesia. The lagoon of Ahe lagoon has a surface of 142 km^2 and a mean depth of nearly 42 m (Fournier et al., 2012b). It is considered a semi-enclosed atoll, with one pass (Tiareroa Pass) that is located in the western part of the lagoon (Fig. 1). Ahe was chosen for this study because it is one of the best studied atolls in French Polynesia and has a long history of pearl farming activity (Thomas, 2009; Andréfouët et al., 2012). Other research has shown the difficulty of comparing different lagoons in French Polynesia (Pouvreau et al., 2000). It was thus important to develop a suitable method adapted to the conditions of the Ahe lagoon.

The lagoon of Ahe has an abundance of bommies (*karena* in Tahitian), which are isolated patch reefs. Bommies are very good study sites because they present important structurally complex habitats for reef fish (Lewis, 1997; Planes et al., 2012). Ault and Johnson (1998) concluded that the spatial and temporal variation of reef fish is strongly affected by species' vagility and reef connectivity. So, the reduced vagility of reef fish within bommie habitats may provide clearer patterns than when comparing this method to contiguous fringing reefs (Lewis, 1997). A large portion of pearl oyster farming activity is done over deep water in the lagoon where reef fish monitoring is not practical because of limited bottom time on SCUBA gear. However, a number of pearl farms and oyster lines are found in the immediate vicinity of bommies.The bommies in the immediate vicinity of farming activity were designated as Impacted areas for reef fish censuses (Table 1). All reefs in the lagoon of Ahe may have some indirect effects due to potential eutrophication from pearl oyster farming activities (Bouvy et al., 2012), but recent research on sediments under pearl farms in Ahe found no significant organic enrichment (Gaertner-Mazouni et al., 2012). NoDirect impact reefs were away from the general vicinity of recent farming activity. The classification of a site as Impacted or NoDirect impact was done by consulting local pearl farmers on the past and present influence of pearling at that specific site, taking into account spat collection activities at the site and studying marine consession maps of the past few years. Subsistence fishing occurs throughout the lagoon at low levels and we make the assumption that this was not significantly different between Impacted and NoDirect impact reefs. The oceanographic features of the Ahe Lagoon vary according to distance from the main pass and aspect regarding prevailing winds, with considerable circulation and flushing differences related to position north or south of the pass (Dumas et al., 2012) and were coded accordingly for analyses. We reconnoitered other parts of the lagoon, but chose to focus on the western part of the lagoon in order to reduce variability due to hydrodynamic differences (Dumas et al.,

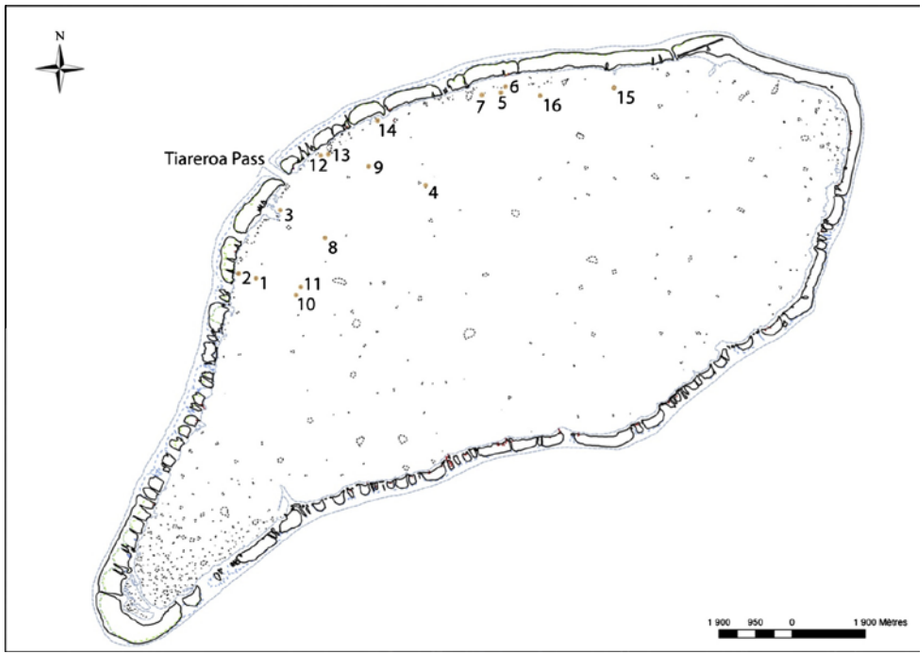

Fig. 1. Map of censused coral bommies in Ahe lagoon, French Polynesia. Studied sites are marked in orange, along with the number of the studied site. Ahe has only one main pass, which is located between stations 3 and 12. Modified map courtesy of Marine Ministry of French Polynesia.

Table 1
Censused stations with GPS coordinates of latitude and longtitude reef designation and census results. The table includes whether the site is impacted through pearl farming (Impacted or NoDirect impact), the type of reef, the position of the site relative to the pass (North or South), the distance of the site from the shore, number of species identified (S), abundances (N_{1-1000}, $\sqrt{N}$, N_{1-4}), Shannon diversity for each of the sample sites ($H_{(N1-1000)}$, $H_{(\sqrt{N})}$ and $H_{(N1-4)}$), farming impact and position of the censused site relative to the pass. N_{1-1000}, $\sqrt{N}$, $H_{(N1-1000)}$ and $H_{(\sqrt{N})}$ correspond to coding of abundance category as 1–1000 and later transformed as a square root. N_{1-4} and $H_{(N_{1-4})}$ correspond to abundance coded as 1–4.

Station no.	Lat	Long	Impact	ReefType	Position	Distance from shore (m)	Distance from pass (m)	S	N_{1-1000}	$\sqrt{N}$	N_{1-4}	$H_{(N1-1000)}$	$H_{(\sqrt{N})}$	$H_{(N_{1-4})}$
St1	14°28′13.9″S	146°22′01.3″W	Impacted	Bommie	SPass	590	2741	66	11,388	591	163	2.975	3.729	4.12482
St2	14°28′52.9″S	146°22′05.9″W	Impacted	Bommie	SPass	128	2788	67	9607	553	164	2.963	3.779	4.14737
St3	14°28′00.0″S	146°21′29.3″W	Impacted	Bommie	SPass	412	857	69	8718	536	166	2.969	3.818	4.17617
St4	14°27′39.2″S	146°19′23.9″W	NoDirect	Bommie	NPass	2139	3634	58	6457	433	139	2.835	3.678	4.00392
St5	14°26′20.0″S	146°18′18.3′W	Impacted	Bommie	NPass	444	6004	72	11,889	642	179	3.093	3.843	4.21300
St6	14°26′15.2″S	146°18′15.3″W	Impacted	Bommie	NPass	313	6150	70	13012	671	176	3.139	3.814	4.17355
St7	14°26′21.6″S	146°18′33.5″W	Impacted	Bommie	NPass	409	5557	68	10,805	650	181	3.236	3.911	4.17534
St8	14°28′22.3″S	146°20′51.3″W	NoDirect	Bommie	NPass	1717	1841	75	6609	529	180	3.257	4.007	4.26525
St9	14°27′23.0″S	146°20′14.0″W	NoDirect	Bommie	NPass	1144	2150	74	8057	546	178	3.064	3.929	4.25350
St10	14°29′11.4″S	146°21′16.3″W	NoDirect	Bommie	SPass	1669	3038	57	4584	386	137	2.965	3.752	4.00238
St11	14°29′04.7″S	146°21′12.4″W	NoDirect	Bommie	SPass	1754	2845	63	6723	456	146	2.919	3.734	4.06793
St12	14°27′13.0″S	146°20′54.3″W	NoDirect	Bommie	NPass	198	1088	72	9144	566	171	3.070	3.856	4.20169
St13	14°27′11.0″S	146°20′47.5″W	NoDirect	Bommie	NPass	250	1282	71	7370	529	171	3.138	3.915	4.20368
St14	14°26′43.6″S	146°20′05.6″W	NoDirect	Bommie	NPass	188	2794	69	8871	543	165	3.001	3.813	4.16651
St15	14°26′14.5″S	146°16′40.9″W	Impacted	Bommie	NPass	480	8851	70	14,857	772	192	3.322	3.920	4.19636
St16	14°26′22.5″S	146°17′45.2″W	NoDirect	Bommie	NPass	655	6933	70	10,510	625	177	3.170	3.880	4.18573

2012). Our field time at this remote atoll was limited and we did not have resources to complete sufficient replication in all different parts of the lagoon. The studied stations were classified as north or south of the pass to take into account the influence of the pass as described by other studies (Dumas et al., 2012; Pagano et al., 2012). We hypothesized that distance from shore and distance from the pass may also have an effect on fish community composition and this distance was measured by mapping collected GPS coordinates on Google Earth. The relative size of bommies censused was kept as close as possible to minimize effects of bommie size on reef fish community composition.

2.2. Fish sampling

Fish density and diversity data was collected at both Impacted and NoDirect impact bommies (Table 1 and Fig. 1) using the Roving Diver Census (RDC) method (Schmitt et al., 2002), modified for use on bommies. This method is typically more comprehensive for censusing diversity of fishes but is less accurate for estimating abundance of species than more widely used belt transect methods (Schmitt et al., 2002). The RDC is particularly suited to use on bommies where belt transects are difficult to position because of the typical vertical drops from the surface. Controlling for depth effects

on fish communities using a transect belt or point-count methods (Bohnsack and Bannerot, 1986) is difficult on bommies because of uneven topography and variable depth that the vertical drop of the bommie meets the less acute lagoon bottom. The starting point for the fish census was chosen haphazardly according to optimal small boat mooring sites near the bommie and the diver descended to 20 m and started recording all species observed and their relative abundance (single = 1 individual, few = 2–10, many = 11–100, abundant = over 100) encountered throughout the 60 min duration of the census. The census diver swam steadily in a counterclockwise direction around the bommie decreasing depth after each circumnavigation so that the last circumnavigation at the end of the 60 min census took place in shallow water at the top of bommie. Relative abundance (N) throughout the census for each species (Bacchet et al., 2006), and the number of species observed (S) was enumerated on a list of species pre-recorded on waterproof paper secured to a plastic slate.

2.3. Data analysis

Coding of abundance of reef fishes using the RDC method (Schmitt et al., 2002) can vary because of the logarithmic scale used in estimation of numbers of individuals. One method is to code abundance (N_{1-4}) single as 1, few as 2, many as 3 and abundant as 4 (Schmitt et al., 2002). This de-emphasizes the importance of numerous species. Two alternative methods are to code each abundance category as the maximum number of individuals (N_{1-1000}: single = 1, few = 10, many = 100, abundant = 1000) and to transform these as a square root ($\sqrt{N}$) to avoid departures from normality. These codings gives more weight to the abundant individuals in the community. We employed all coding methods to give a potential range of results from the inherently inaccurate RDC method of estimating abundances that was employed for use on bommies. Abundances (N_{1-1000}, $\sqrt{N}$ and N_{1-4}) and the Shannon diversity index (H) for each estimate of abundance ($H_{N1-1000}$, $H_{(\sqrt{N})}$, $H_{(N1-4)}$) were calculated using Primer V6 (version 6.1.15 from Primer-E Ltd, 2012; Clarke and Warwick, 2001).

A four-factor analysis of variance (ANOVA) with unbalanced data was used to test if pearl farming, position within the Ahe lagoon, distance from the pass or distance from shore has an impact on the abundance and diversity of reef fish communities using the SAS statistical package (version 9.3, 2012). The first factor was pearl farming on either Impacted or NoDirect impact bommies. The second factor was position in the lagoon either north (NPass) or south (SPass) of the main pass in the western part of the lagoon (Fig. 1). The third and fourth factors were distance from the pass and distance from shore in meters, respectively (Table 1) since this varied considerably among stations (Fig. 1). The interaction term was tested between the main factor hypothesized (pearl farming impact) and each of the secondary factors (position in lagoon, distance from the pass and distance from shore).

After examination of the univariate results, we hypothesized that the beta diversity of the fish communities on bommies would be influenced by both pearl oyster farming and position in the lagoon and tested this using the ANOSIM feature of the Primer software (Clarke and Warwick, 2001). We tested this using two factors coded separately for pearl farming and position and using a composite single factor as follows: Impacted north of the pass, No Direct impact north of the pass, Impacted south of the pass, and NoDirect impact south of the pass. Resemblance similarity was calculated using a presence and absence matrix of species and the Sorenson similarity measure (Clarke and Warwick, 2001).

3. Results

We collected complete reef fish data at 16 sites in the lagoon of Ahe in October 2012 (Fig. 1, Table 1, Table S1). The observed fish species were consistent with the literature of reef fishes in the Tuamotus (Bacchet et al., 2006). In both cases Station 10 (NoDirect impact, SPass) was found to have the lowest censused fish abundance of all sites (N_{1-1000}: 4584, $\sqrt{N}$: 586, N_{1-4}: 137). Station 15 (Impacted, NPass) showed the highest abundance of reef fish of all studied sites (N_{1-1000}: 14857, $\sqrt{N}$: 772, N_{1-4}: 192). The number of observed species was lowest at Station 10 (S = 57, NoDirect, SPass) and highest at Station 8 (S = 75, NoDirect, SPass). The total number of different species observed across all 16 stations was 151 (Table S1). The difference between stations 8 and 10 in terms of number of species (i.e. diversity) observed is a good example of natural variability of fish abundance in the lagoon. All censused stations with number of species identified (S), abundances (N_{1-1000}, $\sqrt{N}$, N_{1-4}), Shannon diversity for each of the sample sites ($H_{(N1-1000)}$, $H_{(\sqrt{N})}$, $H_{(N1-4)}$), farming impact and position of the sites relative to the pass are listed in Table 1. Mean abundances of fishes ($\sqrt{N}$ and N_{1-4}) where slightly higher at Impacted sites than NoDirect impact sites and at sites North of the pass versus South of the pass (Table 2). Diversity of fishes ($H_{\sqrt{N}}$ and $H_{(N1-4)}$) were nearly equal or slightly higher at Impacted sites than NoDirect impact sites and at sites North of the pass versus South of the pass.

3.1. Effects on fish abundance

Results from multi-factorial mixed model ANOVAs, to determine the effects of pearl farm activity, position of sites relative to the pass and the distance of studied sites from the shore and pass on fish abundance and fish diversity are listed in Tables 3 and 4. There was a significant overall model effect on fish abundance regardless of coding method (N_{1-1000}, $\sqrt{N}$, N_{1-4}) and both pearl farming impact and position north or south of the pass were significant independent factors (Table 3). There was no significant distance from shore effect in any of the tests. Distance from pass effect was significant using abundance coding N_{1-1000} and $\sqrt{N}$, but not N_{1-4}. There were significantly higher abundances for all coding methods (N_{1-1000}, $\sqrt{N}$, N_{1-4}) north of the pass than south of the pass (Tables 3 and 4). There were also significantly higher abundances at heavily impacted sites than sites not directly impacted by pearl oyster farms (Tables 3 and

Table 2
Means of fish abundance (N_{1-1000}, $\sqrt{N}$, N_{1-4}) and species diversity ($H_{(N1-1000)}$, $H_{(\sqrt{N})}$, $H_{(N1-4)}$) at all censused stations (n = 16). N_{1-1000} and $H_{N1-1000}$ correspond to coding of abundance category as 1–1000 before square root transformation. $\sqrt{N}$ and $H_{(\sqrt{N})}$ correspond to coding of abundance category as 1–1000 and transformed as a square root. N_{1-4} and $H_{(N1-4)}$ correspond to abundance coded as 1–4. The numbers in parentheses after the mean represent the number of stations sampled in the category followed by the standard deviation.

	Impacted	NoDirect Impact	NPass	SPass
N_{1-1000}	11,468 (7, 2062.29)	7591.67 (9, 1763.61)	9780.09 (11, 2708.47)	8204 (5, 2630.03)
$\sqrt{N}$	630.71 (7, 80.282)	512.56 (9, 73.850)	591.45 (11, 91.653)	504.40 (5, 82.494)
N_{1-4}	174.43 (7, 10.690)	162.67 (9, 17.255)	173.55 (11, 13.359)	155.20 (5, 12.950)
$H_{(N1-1000)}$	3.100 (7, 0.141)	3.047 (9, 0.131)	3.120 (11, 0.133)	2.958 (5, 0.022)
$H_{(\sqrt{N})}$	3.831 (7, 0.068)	3.840 (9, 0.106)	3.870 (11, 0.085)	3.762 (5, 0.037)
$H_{(N1-4)}$	4.172 (7, 0.029)	4.150 (9, 0.101)	4.185 (11, 0.068)	4.104 (5, 0.069)

Table 3
Summary two-way ANOVA table with fish abundance (N_{1-1000}, $\sqrt{N}$, N_{1-4}) as a dependent variable. N_{1-1000} corresponds to coding of abundance category as 1–1000 before square root transformation. $\sqrt{N}$ corresponds to coding of abundance category as 1–1000 and transformed as a square root. N_{1-4} corresponds to abundance coded as 1–4.The three factors in the ANOVA were: Impact, Position and Distance Shore. Df was 4 for the general model and 1 for all individual factors. The number of stations sampled is 16 (n = 16).

Source	F	Pr > F
N_{1-1000}		
General model	21.38	<0.0001**
N_{1-1000} **(Type III SS)**		
Impact	6.79	0.0244*
Position	1.55	0.2391
Distance Shore	7.07	0.0222*
Distance Pass	10.53	0.0078*
Distance Shore × Impact	1.75	0.2101
Position × Impact	0.02	0.8873
Distance Pass × Impact	0.83	0.3805
N_{1-1000} **(Type I SS)**		
Impact	52.12	<0.0001**
Position	19.77	0.0010**
Distance Shore	3.09	0.1064
Distance Pass	10.53	0.0078*
$\sqrt{N}$		
General model	18.30	<0.0001**
$\sqrt{N}$ **(Type III SS)**		
Impact	4.73	0.0524
Position	5.48	0.0391*
Distance Shore	6.32	0.0288*
Distance Pass	6.93	0.0233*
Distance Shore × Impact	1.4	0.2594
Position × Impact	0.01	0.9207
Distance Pass × Impact	1.73	0.2134
$\sqrt{N}$ **(Type I SS)**		
Impact	33.71	0.0001**
Position	29.30	0.0002**
Distance Shore	3.24	0.0994
Distance Pass	6.93	0.0233*
N_{1-4}		
General model	5.46	0.0114*
N_{1-4} **(Type III SS)**		
Impact	1.58	0.2348
Position	6.73	0.0249*
Distance Shore	2.10	0.1751
Distance Pass	0.01	0.9238
Distance Shore × Impact	0.58	0.4619
Position × Impact	0.65	0.4371
Distance Pass × Impact	1.57	0.2342
N_{1-4} **(Type I SS)**		
Impact	4.95	0.0479*
Position	14.66	0.0028*
Distance Shore	2.22	0.1644
Distance Pass	0.01	0.9238

* Significant.
** Highly significant.

4) in all comparisons except for the Type III sums of squares when abundance is coded to de-emphasize the importance of numerous species (N). However, there was no significant interaction between the main abundance factor (Impact) and the three secondary factors (Distance Shore, Position, Distance Pass) and therefore Type I sums of squares can be considered representative for this comparison. Our samples sizes were not sufficient for statistical tests of abundances of individual species, although certain surgeonfishes (e.g. *Acanthurus triostegus*, *Acanthurus xanthopterus*) and butterflyfishes appeared to be more numerous at pearl oyster farming sites (Table S1).

Table 4
Summary two-way ANOVA table with fish diversity ($H_{(N1-1000)}$, $H_{(\sqrt{N})}$,$H_{(N1-4)}$) as a dependent variable. $H_{(N1-1000)}$ corresponds to coding of abundance category as 1-1000 before square root transformation. $H_{(\sqrt{N})}$ corresponds to coding of abundance category as 1–1000 and transformed as a square root. $H_{(N1-4)}$ corresponds to abundance coded as 1–4. The three factors in the ANOVA were: Impact, Position and Distance Shore. Df was 4 for the general model and 1 for all individual factors. The number of stations sampled is 16 (n = 16).

Source	F	Pr > F
$H_{(N1-1000)}$		
General model	2.70	0.0867
$H_{(N1-1000)}$ **(Type III SS)**		
Impact	0.11	0.7444
Position	3.02	0.1103
Distance Shore	0.38	0.5481
Distance Pass	0.88	0.3682
Distance Shore × Impact	0.52	0.4833
Position × Impact	0.61	0.4496
Distance Pass × Impact	3.08	0.1049
$H_{(N1-1000)}$ **(Type I SS)**		
Impact	0.89	0.3653
Position	8.89	0.0125*
Distance Shore	0.12	0.7316
Distance Pass	0.88	0.3682
$H_{(\sqrt{N})}$		
General model	1.70	0.2199
$H_{(\sqrt{N})}$ **(Type III SS)**		
Impact	0.04	0.8425
Position	4.26	0.0636
Distance Shore	0.23	0.6418
Distance Pass	0.28	0.6101
Distance Shore × Impact	0.11	0.7456
Position × Impact	0.10	0.7523
Distance Pass × Impact	1.99	0.1840
$H_{(\sqrt{N})}$ **(Type I SS)**		
Impact	0.06	0.8140
Position	6.02	0.0321*
Distance Shore	0.45	0.5174
Distance Pass	0.28	0.6101
$H_{(N1-4)}$		
General model	3.12	0.0605
$H_{(N1-4)}$ **(Type III SS)**		
Impact	0.66	0.4337
Position	5.83	0.0344*
Distance Shore	1.62	0.2290
Distance Pass	1.65	0.2257
Distance Shore × Impact	0.16	0.6977
Position × Impact	2.30	0.1556
Distance Pass × Impact	1.08	0.3202
$H_{(N1-4)}$ **(Type I SS)**		
Impact	0.52	0.4849
Position	7.27	0.0208*
Distance Shore	3.04	0.1089
Distance Pass	1.65	0.2257

* Significant.

3.2. Effects on fish diversity

There was no overall model effect and therefore none of the independent factors influence fish diversity (Table 4) coded to emphasize numerous species ($H_{(\sqrt{N})}$). Only position North or South of the pass had a marginally significant overall model effect on fish diversity. There was a slightly higher mean number of species in Impacted areas (68.9 species, standard deviation = 2.04) versus No Direct impact areas (67.7 species, standard deviation of 6.71) but this was not significant.

3.3. Effects on community composition

One-way ANOSIM analysis shows that community composition is significantly different in three out of four comparisons involving stations north versus south of the pass (Table 5). Only one of four

comparisons involving Impacted and NoDirect impact stations was signficant and the one that was significant also involved a comparison between North and South of the Pass.

4. Discussion

Our results in Ahe show that there were significant effects on fish abundances because of pearl farming, and position relative to and distance from Tiareroa Pass. The position and distance from pass effect can potentially be explained by physical and biological factors that differ markedly both as a whole north and south of the Tiareroa Pass and because of flushing effects with distance from the pass (Dumas et al., 2012; Lefebvre et al., 2012; Thomas et al., 2012a,b). Dumas et al. (2012) found that water temperature is generally higher south of the pass, circulation of water is typically separate north and south of the pass, and that potential retention time (e-flushing) for oyster larvae is generally longer north of the pass. Generally, residence time is positively related to biomass and production in Ahe Lagoon (Lefebvre et al., 2012) and this could possibly promote greater fish abundance. In addition, hydrodynamic factors influence a generally greater bivalve larvae abundance and size in the northern part of Ahe Lagoon (Thomas et al., 2012b) and these factors may also influence higher recruitment of fishes in this part of the lagoon.

Flushing time relative to distance from the pass (Dumas et al., 2012) may also influence productivity and food availability to fishes. This may have a greater effect on the schooling, more abundant fishes since distance from the pass only had a significant effect when abundance was coded to emphasize the more abundant fishes (N_{1-1000} and $\sqrt{N}$, but not N_{1-4}).

Pagano et al. (2012) also showed that zooplankton abundance and biomass were found to be higher in the northern part of the lagoon than in the south, during the windy season (the season that our censuses occurred). Higher zooplankton concentration might promote the presence of planktivorous fish (Alldredge and King, 2009). Given that wind-driven circulation is influential in oyster spawning, bivalve larval dispersal and plankton availability (Fournier et al., 2012b) these wind regimes are likely to also influence the abundance of fish populations. Studies on other Polynesian lagoons have shown the influence the amount of ocean-lagoon water exchange and wind-driven circulation can have on spatial abundance and diversity of fish through control of nutrient and fish larvae circulation (Morize et al., 1990; Caillart et al., 1994; Planes et al., 2012).

While position relative to and distance from Tiareroa Pass was found to have a potential confounding effect in our study, the main factor of proximity to pearl farms also had a slightly significant positive effect on fish abundance. There are two primary ways that presence of pearl farms could positively influence reef fish abundance: increasing structure of the reef and increasing proximal food availability. The nature of different reef fish habitats has a strong effect on fish abundance and diversity in the Tuamotu Archipelago (Caillart et al., 1994) and although the bommies we studied were similar in structure, the presence of pearl oyster farms influences structure in proximity to these bommies. Reefs with greater rugosity and shelter sites typically support higher density and diversity of reef fishes (e.g. Ménard et al., 2012; Planes et al., 2012; Dustan et al., 2013). The many baskets and nets used to protect oysters increase rugosity in proximity to reefs and offer shelter to small fish from predators and substratum to fish larvae and juveniles.

In addition to increasing available structure, pearl farms and spat collection sites may also act as types of Fish Aggregating Devices with the additional food they offer (Achari et al., 2001), although spat collection sites are concentrated more to the southwest of Ahe lagoon, mostly outside our study area (Thomas, 2009). Biofouling attached to nets and oysters provides additional biomass and food to fish, potentially promoting fish abundance. A closer examination of abundances and diversity specifically of fish grazers on these biofouling organisms could further test this hypothesis. Nets and oysters need to be cleaned at regular intervals to remove biofouling (de Nys and Ison, 2008). Different cleaning methods could have different effects on fish populations, as the disposal of this biofouling material in the water may give preferential treatment to certain species (Le Pennec et al., 2010). One innovative biofouling removal technique on Ahe involves grazing reef fish to remove biofouling (Pae Tai-Pae Uta, 2003). This is used at stations 1 and 2 (Impacted), which show higher fish abundance than stations 10 and 11 (NoDirect control sites). Further research should also test if different cleaning methods such as biological control, freshwater or hypersaline baths, mechanical cleaning and high-pressure water cleaning (de Nys and Ison, 2008), have significant positive or negative effects of fish abundance and community composition. This is especially important if aquaculture and pearl farming is to increase its ecological and social license to operate by demonstrating its low impacts (Stewart, 1997).

Our results indicate that pearl oyster farming in the western part of Ahe lagoon does not influence fish diversity or community composition which indicates that this activity does not negatively impact fish communities. This reaffirms the potential of pearl farms as eco-friendly economic activities (Sims, 2003; Cartier and Ali, 2012). However, this result is surprising given that many pearl farmers report an overrepresentation of oyster predating species such as triggerfishes and pufferfishes (Balistidae and Tetraodontidae) (Le Pennec et al., 2010). Oyster predation and protection from predators is a large cost for pearl farmers in French Polynesia (Le Pennec et al., 2010) and has also been reported in Australia (Pit and Southgate, 2003), India (Dharmaraj et al., 1987), Mexico (Saucedo and Monteforte, 1997), the Caribbean (Urban, 2000) and the Solomon Islands (Friedman et al., 1998). However, it remains unclear if pearl oyster predators are overrepresented in zones of high pearl oyster densities because they are attracted to the presence of numerous pearl oysters or if they naturally occur in these areas in high numbers (Coeroli et al., 1984; Le Pennec et al., 2010). Further research is needed that specifically tests the abundances of oyster predators on control versus high impact sites may elucidate this question.

Previous research has identified the lack of suitable methodology to assess the impacts of pearl farming (Jelbart et al., 2011). A

Table 5
Results of one-way analysis of similarities (ANOSIM) for all censused sites. Only comparisons with the R statistic higher than the Global R statistic and have a significance level lower than 0.05 are considered significant (*).

Groups	*R* statistic (Global *R* = 0.404)	Significance level (%)	Possible & actual permutations	Number ⩾ observed
Impacted SPass, NoDirect SPass	0.519	0.10	10	1
Impacted SPass, Impacted NPass	0.426	0.029*	35	1
Impacted SPass, NoDirect NPass	0.287	0.071	56	4
NoDirect SPass, Impacted NPass	0.778	0.029*	35	1
NoDirect SPass, NoDirect NPass	0.462	0.036*	56	2

sufficient number of control sites is required in order to take into account the complex dynamic marine ecosystem (Gifford et al., 2004; Jelbart et al., 2011). Our methodology was adapted to the topography of Ahe's lagoon and the coral bommies for replication of roving censuses at all 16 sites. Given the difference in geography and topography of French Polynesia's islands particularly with regard to abundance of bommies, it may be difficult to compare different lagoons in French Polynesia using this same methodology (Pouvreau et al., 2000).

We have shown that pearl farming can have a slightly positive effect on reef fish abundance in the western area of Ahe lagoon, French Polynesia. Most importantly, it does not appear to negatively alter fish abundance and community structure. This confirms preliminary (unpublished Carpenter 2005) findings from the pearl farming areas of the Philippines which showed similar results. Hoffmann et al. (2010) have argued that conservation is an important factor in reducing marine biodiversity loss. With overexploitation as the greatest threat to biodiversity in the oceans it is advantageous to have an industry that provides jobs but that is environmentally sustainable. Pearl farming appears to offer viable conservation and development solutions. For example, in the Federated States of Micronesia (FSM), community pearl farming has been tested as a way of reducing fishing pressure on reefs (Cartier et al., 2012). However, the emerging consensus is that MPAs and conservation can only work if there is a tangible benefit for local populations (Klein et al., 2008). Alternative sources of income must be provided for an MPA system to have credibility and achieve long-term success. The income lost by abstaining from reef fishing in certain areas can be compensated by income generated from pearl farming. Pearl farming could be integrated into an MPA business model, even more so because of its potential low impact and their service as ecosystem indicators (Sarver et al., 2003; Gifford et al., 2004). This fits well into the vision advocated by Sala et al. (2013) for business models for marine reserves. However, further studies are clearly needed to test the generality of our results in other pearl farming situations.

French Polynesia coral reefs are considered some of the least degraded in the world (Salvat et al., 2008), and could be further protected by extending no-fishing grounds, shark sanctuaries and fish nurseries. Our preliminary evidence suggests that pearl farms could serve as de facto MPAs for these purposes or at least as minimum impact economic zones and this result deserves further testing. Cultured pearls represent the largest source of foreign currency for French Polynesia (70% of exports in 2010 were pearls – Talvard, 2011), offer valuable employment opportunities and this conservation-pearl farming synergy may offer interesting opportunities. However, it should be emphasized that not all pearl farming activities are ecologically friendly and that in order to be sustainable, guidelines must be followed. French Polynesia's pearl sector has not been spared ecological problems in its five decades of existense, especially with mass mortalities in 1985 whose origins were poorly understood (Cabral, 1989). Responsible pearl farming must ensure that oysters are stocked in extensive conditions and that biofouling cleaning methods are of low impact on the benthic environment (de Nys and Ison, 2008; O'Connor and Gifford, 2008). Because pearl farming operates in sensitive environments, it is important to monitor its impact. Reef fish are a good relative indicator of biodiversity and reef degradation (Lecchini et al., 2012). Reef fish could further be used to monitor the relative impacts of pearl farming on biodiversity, in order to ensure and manage the long-term ecological sustainability of the sector.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2013.11.027.

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CHAPTER 5

New developments in cultured pearl production: use of organic and baroque shell nuclei

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New developments in cultured pearl production: use of organic and baroque shell nuclei

Laurent E. Cartier and Michael S. Krzemnicki

Cultured pearls can be produced both with and without a nucleus. Marine pearl oysters that produce Akoya, South Sea and Tahitian cultured pearls typically use nuclei for their pearl products. The nucleus material used for these beaded cultured pearls is traditionally from freshwater Mississippi mussels. In recent years, there have been a number of attempts to use alternative pearl and shell materials as nuclei. This includes different types of shells, Bironite, laminated/powdered shell, freshwater cultured pearls and even natural pearls. The most recent development, detailed in this article, is the use of organic nuclei for the production of 2nd generation beaded baroque cultured pearls. Pearls cultured in this way first appeared on the market at the 2012 BaselWorld show. This paper examines how these pearls are linked to this new type of nucleus, how it is used in the pearl farming process, and details a gemmological study of the different generations of final pearl products.

Introduction

The process of culturing round pearls was discovered and refined at the beginning of the 20th century. The initial Mise-Nishikawa method was further developed by Kokichi Mikimoto and his company who brought round cultured pearls to the international market from 1919 onwards (Simkiss and Wada, 1980; Strack, 2006). Producing such cultured pearls required three things: a host oyster, a donor oyster's saibo (mantle tissue) and a nucleus (Taylor and Strack, 2008; Hänni, 2012). The grafted mantle cells slowly form a pearl sac around a spherical nucleus (pearl sac is complete after about 30 days, Cochennec-Laureau et al., 2010), which is responsible for secreting and depositing regular layers of nacre onto the nucleus and eventually leading to a cultured pearl. The basic method of forming such a beaded cultured pearl has not changed much since its beginnings.

The authors were presented with samples of a new type of pearl product from French Polynesia by a pearl trader during the 2012 BaselWorld show. These pearls had unusual shapes, came in large sizes (up to 23mm) and were characterised by a high visually appealing lustre (Figure 1). These pearls were called "Keshi baroque" cultured pearls. However after we carried out radiographic analysis, it was already clear that these were baroque-shaped beaded cultured pearls, making the use of the term "Keshi" wrong (Hänni, 2006; Segura and Fritsch, 2012). Similar baroque-shaped beaded cultured pearls were later encountered at the September Hong Kong Jewellery show, in French Polynesia and Switzerland. Samples were donated to the Swiss Gemmological Institute SSEF and we were able to carry out closer examination of these cultured pearls to understand their formation mechanisms.

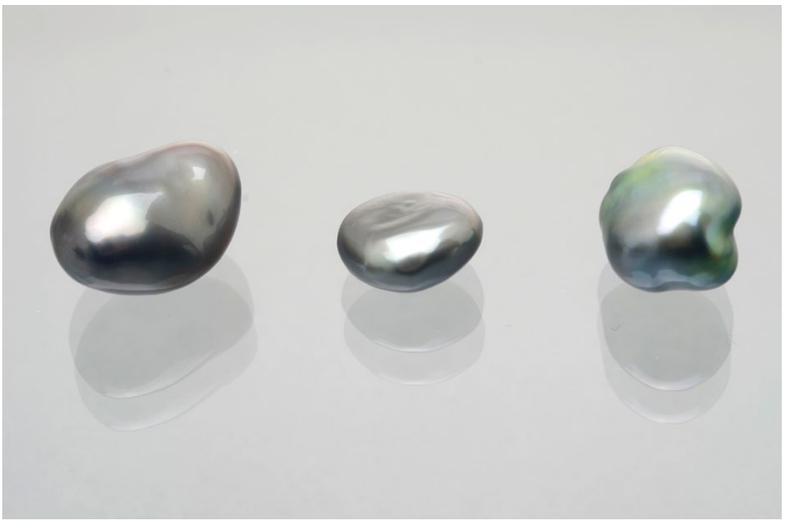

Figure 1. Baroque-shaped beaded cultured pearls examined during the BaselWorld 2012 show. The sample on the left has a diameter of 23mm © L.E. Cartier

Figure 2. Different products from the Pinctada margaritifera *oyster. From left to right: baroque-shaped beaded cultured pearls, round beaded cultured pearls, beadless ("keshi") cultured pearls and "Tokki" cultured pearls. © L.E. Cartier*

Figure 3. Different types of nuclei material commonly used in South Sea / Tahitian pearl farming. Mississippi mussel shell (left), Pinctada maxima shell (middle) and 'US White' Mississippi shell material (right). The sample on the far left is 7.5mm in diameter. © L.E. Cartier

Figure 4. Organic nuclei that are inserted into the oyster. The sample on the left illustratively shows the absorbing capacity of these nuclei. The result of this expansion will be a larger pearl sac, compared to regular nuclei. The first generation pearl (harvested after 9-12 months- see Group A in Figure 6) is not sold. © L.E. Cartier

Recent developments in nucleus materials

The traditional source of nuclei for cultured pearls comes from the Mississippi and Tennessee watershed regions (Alagarswami, 1968; Gervis and Sims, 1992; Strack, 2006, Superchai et al., 2008). These areas had a long tradition of "musseling" because of the importance of different mussels to the US button manufacturing industry. However, the button industry experienced stiff competition from Japan button manufacturers and later from plastic buttons and by 1919 was struggling (Claassen,1994). "Musseling" activity recovered when demand for nuclei from the pearl industry grew: first from the Japanese Akoya industry, and from the 1960's onwards from Australia and French Polynesia.

Mississippi shells are especially sought after in pearling for their size, specific gravity, drilling properties, thermal properties and purity (Kanjanachatree et al., 2007). The colour and purity of a nucleus is important for Akoya cultured pearls as they are frequently characterised by smaller nacre thickness, and the nucleus must not become visible for a pearl to remain commercial (Ward, 1995). There is a general consensus in the industry that Mississippi shell material is the best option in the quest to produce high-quality cultured pearls (Figure 3). Research has shown that the type of nucleus material has a significant influence on the quality, shape and surface perfection of a resulting cultured pearl (Te Reko Parau, 2010: 37-38). For example, investigation shows the use of *Pinctada maxima* shell as nuclei material for Tahitian cultured pearls to be just as good as Mississippi shell material (Scoones, 1990; Bertaux, 2006).

Mississippi mussels used in nucleus production are all from the Unionidae family. It is estimated that 80% of US shell production comes from Tennessee at present (TWRA, n.d.). There are currently 10 species of freshwater mussels that can be harvested commercially in Tennessee. These include: Pink heelsplitter (*Potamilus alatus*), Washboard (*Megalonaias nervosa*), River pigtoe (*Pleurobema cordatum*), Lake pigtoe (*Fusconaia flava*), Mapleleaf (*Quadrula quadrula*), Southern (Snoot nose), Mapleleaf (*Quadrula apiculata*), Three ridge (*Amblema plicata*), Elephant Ear (*Elliptio crassidens*), Ebony (*Fusconaia ebena*) and Monkeyface (*Quadrula metanevra*) (TWRA, 2012). The last available export statistics of US mussel shell production value was $821,000 in 2010 (Olson, 2012). There are concerns with the health of mussel populations in different areas of the Mississippi, due to overfishing, pollution and ecological change (Strayer et al., 2004). The long-term supply of Mississippi shell material is uncertain, which has led to an increase in the cost of Mississippi nuclei, especially for larger sizes. Manufacturers of nuclei material have been increasingly sourcing mussel shell material from other countries in recent years, especially China.

Due to the high cost of Mississippi nuclei and the dependence on the resource there have been numerous experiments to use alternative materials (Roberts and Rose, 1989; Ventouras, 1999; Superchi et al., 2008). These include Tridacna spp. (Gervis and Sims, 1992; Ju et al., 2011), Chinese freshwater cultured pearls (Hänni et al., 2010), nuclei composed of powdered and compressed shell material (MRM, 2012) and natural pearls of low quality (Hänni et al., 2010). Bironite, a processed form of natural dolomite, has also been tested but did not find wider acceptance in the market (Snow, 1999). This article reports on a new innovation in the choice of nucleus material - the use of organic rather than inorganic shell nuclei (Figure 4). The authors in 2010 had been informed of new types of organic nuclei before the appearance of the above described baroque-shaped beaded cultured pearls. It was clear to us that this new pearl product was linked to the new type of organic nucleus.

Organic nuclei: concept and applications in the pearl culturing process

The studied organic nuclei were produced by Imai Seikaku Co. Ltd. (Awaji Island, Japan). They have similar properties to super absorbent polymer (SAP) spheres: they absorb surrounding liquid and grow. Initially compact, the nuclei become soft and gelatinous (see Figure 4). The nuclei are coated with a thin film, which makes them compatible with the oyster's tissue. As with regular nuclei, they also include a bio-coating that consists of fibronectins (FNC- α, Patent No. 62309272). Fibronectins found in the bio-coating favour the healing process in the oyster after the surgical operation of saibo and nucleus insertion.

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Figure 5. A pearl oyster operating technician inserting an organic nucleus into a Pinctada margaritifera *oyster. © L.E. Cartier*

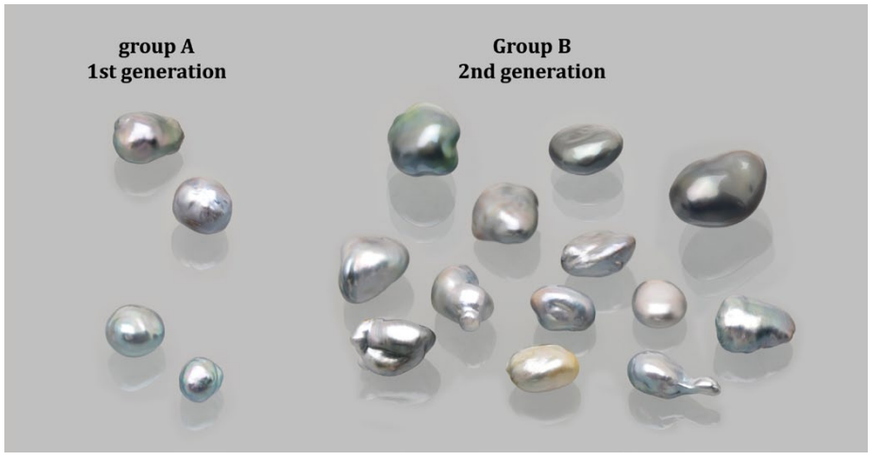

Figure 6. The cultured pearl samples investigated in this study. The pearls from group A were formed as a 1st generation product with an organic gelatinous nucleus. These cultured pearls are not introduced into the pearl trade but are only created to produce an inflated pearl sac. The upper two pearls are from Pinctada margaritifera *(Micronesia), the lower two from* Pinctada fucata *(Japan). The cultured pearls of group B are the 2nd generation product and all come from French Polynesian* Pinctada margaritifera *production. The 2nd generation pearls contain a baroque shaped bead made from a freshwater shell. © M.S. Krzemnicki*

Once a pearl oyster is deemed of sufficient size, it can be grafted/seeded. However, the age an oyster is grafted varies ranging from 1.5-2.5 years to 3-4 years for *Pinctada margaritifera* (Cartier et al., 2012; pers. comm, John Rere, 2012). The organic nucleus can be inserted into the gonad with a piece of donor mantle tissue very similar to the normal production of beaded cultured pearls. In salt-water and in the enclosed environment of the oyster's gonad the growth of the nucleus is distinctly slower than seen in Figure 4 but still considerable. The saibo will remain close to the nucleus because the organic nucleus is expanding. The majority of nucleus growth occurs before the pearl sac is completely formed (i.e. in first hours/days after operation). This nucleus is covered with nacre and a first generation pearl can be harvested several months later (generally 9-12 months). These pearls are, to our knowledge, not marketed because of their small nacre thickness and light weight; the aim is to sacrifice these in order to have a large and still young pearl sac with a good potential to produce nacre of high quality (lustre, overtones).

The pearl sac is baroque (due to the nature of the nucleus - see shape of bloated nucleus in Figure 4), and much more flexible than a pearl sac that had hosted a regular nucleus because of the continuous pressure and irregular expansion of the organic nucleus. A large baroque shell nucleus can now be inserted and is left in the oyster for the regular 12 months required for a cultured pearl to deposit good nacre thickness. The end product is a large baroque beaded cultured pearl as seen in Figure 1. It has to be added that this is a niche product and that all the cultured pearls produced in this manner come in baroque shapes. To our knowledge so far, no round cultured pearls have been cultured using this specific type of organic nucleus.

Gemmology

Materials and methods

For the gemmological investigation, we analysed in total 17 cultured pearl products, which were loaned or donated to the SSEF (see acknowledgements). All pearls show a more or less baroque shape, combined with a light grey to dark grey colour, partially with high lustre and distinct overtones (Figure 6). The size and weight of these pearls range from 2.2 ct to 41.2 ct.

Based on information from the suppliers and radiographies, the studied pearls can be divided into two groups. Cultured pearls of group A are 1st generation products containing an organic gelatinous nucleus (as seen in Figures 4 and 5). They are produced solely to create a large pearl sac in a short time of about 9-12 months. The samples of group B are large 2nd generation cultured pearls with a baroque-shaped shell piece as nucleus. They are the result of grafting a large bead in a young but large pearl sac produced by the 1st generation pearl product. In our study, the cultured pearls are from *Pinctada margaritifera* from French Polynesia and Micronesia (two samples of Group A); and two samples from *Pinctada fucata* (group A, samples 65913-O and -P) from Japan.

Apart from a visual examination and a close microscopic inspection, all samples have been analysed by radiography (on Agfa X-ray films) and X-ray luminescence (Hänni et al. 2005) using a Faxitron instrument (90 kV and 4 mA excitation). On two samples (65913-P, and 65913-B) we additionally collected UV-Vis reflectance spectra (Varian Cary 500 with a diffuse reflectance accessory) and specific gravity (Mettler Toledo hydrostatic balance). For a better understanding of internal structures and nuclei, eight samples (62860-B, 65913-A, -F, -H, -L, -M, -N, -O) were selected based on radiographies and further analysed by X-ray microtomography (CT-scan), using a Scanco µ-CT 40 scanner (70kv). Subsequently, these specimens together with samples 65913-C, -J (in total 10 samples) were cut and polished to better study their internal structures by microscopy. On one cut sample (65913-A) we did a ED-XRF chemical analysis (Thermofisher Quant'X) to identify its freshwater nature.

Analytical results

The visual examination revealed that most of the pearls are characterised by a high lustre and well developed colour overtones. Especially for the large baroque-shaped pearls of the 2nd generation (group B), this surface quality is in some cases obvious and outstanding (Figure 1). Apart from irregular streaks, there are practically no dots and blemishes, and neither so-called circling features which are so common in cultured pearls especially from *Pinctada margaritifera* (Ito, 2011). This indicates a rather juvenile pearl sac from which the nacre for these large pearls precipitated; and especially that an expanding organic nucleus may avoid rotation and certain blemish formation on the pearl.

Some of the pearls from group B however show small roundish surface bumps due to small grown-on additional cultured pearls. This feature is quite commonly observed in beaded cultured pearls and is well known in the trade by the Japanese term "Tokki" (Krzemnicki et al. 2010, Krzemnicki et al. 2011).

The specific gravity was determined on a sample containing an organic gelatinous nucleus (group A: 65913-P) and a cultured pearl with a shell bead as nucleus (group B: 65913-B). The low SG of 1.36 for the group A specimen is strongly indicative of the pearl's quasi-hollow nature. Most of its weight is actually due to water incorporated in the gelatine. As a consequence, these pearls (65913-L to -P) were nearly floating on the immersion liquid (methylene-iodide) used for the radiographies. The SG of 2.74 for the beaded sample (group B) is characteristic for most pearls and actually reflects the density of calcium carbonate.

To identify the mollusc species and to detect a possible colour treatment, we chose from each group a sample of light grey colour (group A: 65913-P; group B: 65913-B) to analyse them with UV-Vis reflectometry. The resulting spectra (Figure 7) are characteristic for the natural colour pigments in *Pinctada fucata* (65913-P) and *Pinctada margaritifera* (65913-B) (Komatsu & Akamatsu 1978; Miyoshi et al., 1987; Iwahashi & Akamatsu 1994; Karampelas et al., 2011; Cartier et al., 2012), thus confirming the provided information about their origin.

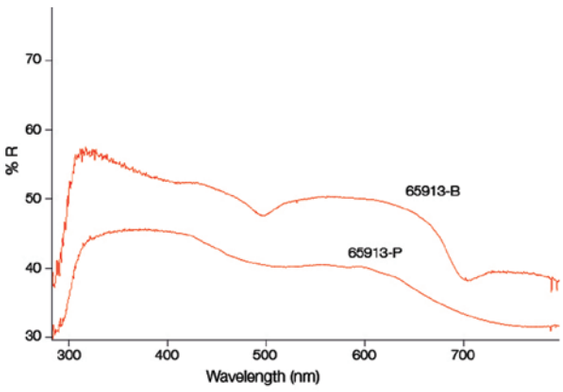

Figure 7. UV-Vis reflectance (R%) spectra of a light grey cultured pearl from Pinctada fucata *(sample 65913-P, group A, 1st generation) and* Pinctada margaritifera *(sample 65913-B, group B, 2nd generation). The dip at 700nm is a characteristic feature for* Pinctada margaritifera *and separates these pearls easily from other grey pearl species. © W. Zhou, SSEF*

Radiography

Comparing the radiographies of the pearls from group A (organic nucleus) and group B (shell piece) reveals very characteristic features, which makes a separation into these two groups straight-forward (Figure 8). All samples from group A show a large dark and featureless internal cavity (low X-ray absorbance) covered by a thin nacreous overgrowth (0.3 – 0.5 mm thick), which is the result of a short growth period (6-12 months) in both the *Pinctada fucata* and *Pinctada margaritifera* recipient oysters (pers. comm., Takuya Imai, 2012). All samples from group B except pearl No. 65913-H show a more or less baroque-shaped nucleus (shell piece), covered by a rather thick nacreous layer (0.5 – 4.0 mm). The baroque-shaped nuclei partially show weak linear to slightly curved lines, that are a result of layering in the shell material. The shell piece for this production was cut from freshwater shells (e.g. from Mississippi or Chinese freshwater mussels). This was confirmed by the distinct X-ray luminescence reaction of the cut samples of group B and by the high trace amount of manganese found in one of these cut pearls (sample 65913-A) when analysed by EDXRF. Thus, we can state that this new cultured pearl product is very similar to normal cultured pearls using spherical nacre beads cut from freshwater shells (e.g. Akoya-, Tahiti- and South Sea cultured pearls). As a consequence, the studied beaded cultured pearls (group B) show on radiographies a slightly brighter bead surrounded by a darker grey nacre layer. This observation is well known from any saltwater cultured pearl using a freshwater bead as the freshwater bead is absorbing X-rays slightly more than the saltwater nacre (Hänni et al., 2005). Due to the rather baroque shape of the freshwater bead this difference in grey between nucleus and nacre layer is however much less marked on the radiographies than when using a round bead. Thus, the identification of our studied beaded cultured pearls (group B) may sometimes be more challenging.

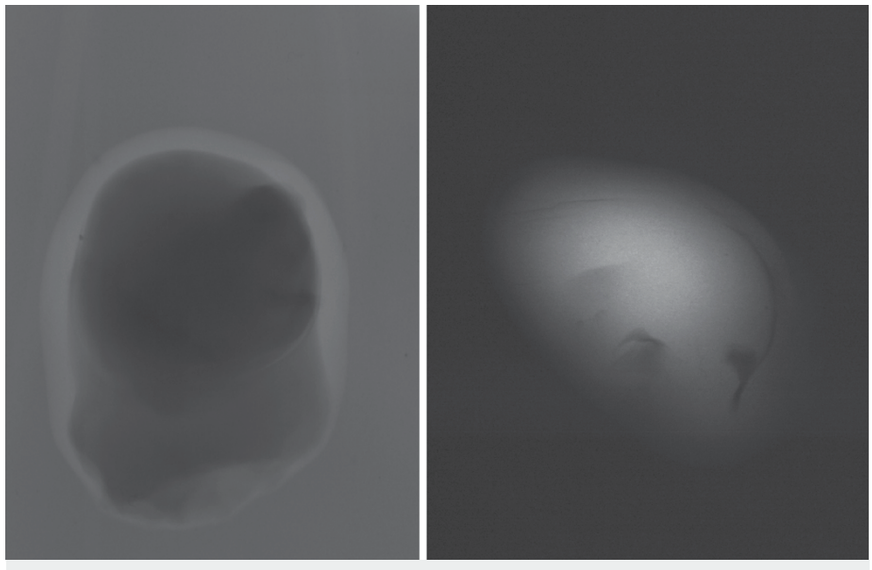

Figure 8. Radiography showing a specimen of 1st generation (group A) on the left containing an organic gelatinous bead (sample 65913-L) and a sample from the 2nd generation (group B) with a freshwater shell piece (sample 65913-A). The organic nucleus is nearly transparent to X-rays, therefore resulting in a dark centre, whereas the freshwater shell piece is slightly more absorbing (more bright) than the surrounding nacreous layer. A fine curved layering is visible in the shell piece together with some organic matter (dark) at the bead/nacre interface. © M.S. Krzemnicki

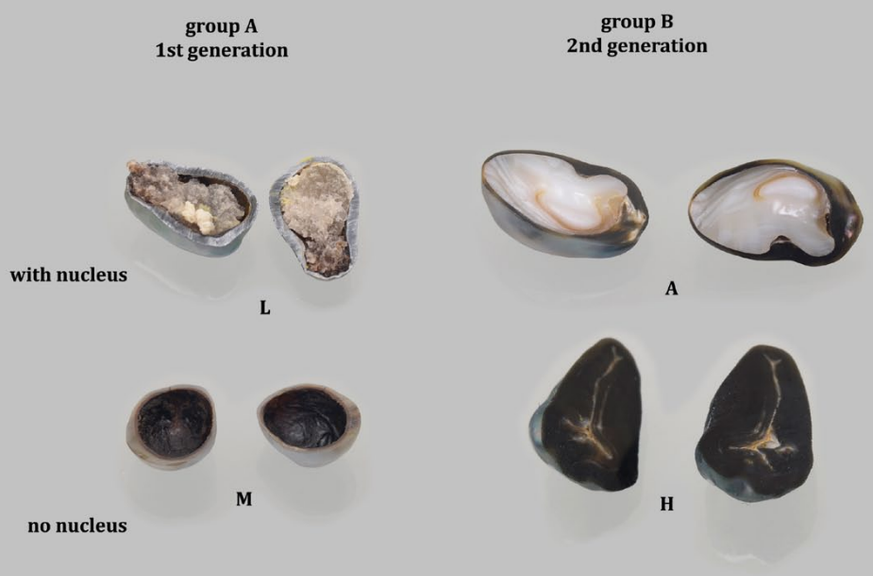

Figure 9. Four samples cut in half, showing different structures. Left are the two pearls from group A (1st generation): The upper left still contains a decomposed version of the gelatinous organic nucleus (sample 65913-L), whereas the lower left is without the organic nucleus (sample 65913-M). On the right side are two samples from group B (2nd generation): One containing a characteristic baroque-shaped shell piece as a bead (sample 65913-A), the lower sample only with an irregular cavity structure due to the rejection of the shell piece (65913-H). The upper row thus shows the normal products of the 1st and 2nd growth generation - both still with nucleus - whereas the lower row shows beadless cultured pearl products from both generations. © M.S. Krzemnicki

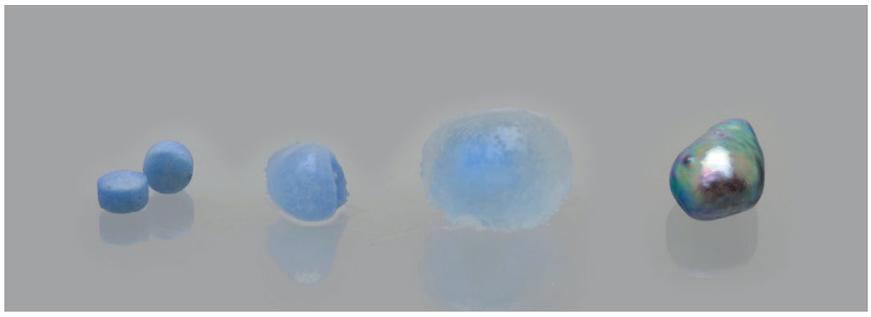

Figure 10. Organic nuclei used for the 1st generation cultured pearls such as sample 65913-O. The three stages of swelling show how these organic nuclei would inflate when soaked in water for five hours. When inserted into the gonads of an oyster, they expand less rapidly. © L.E. Cartier

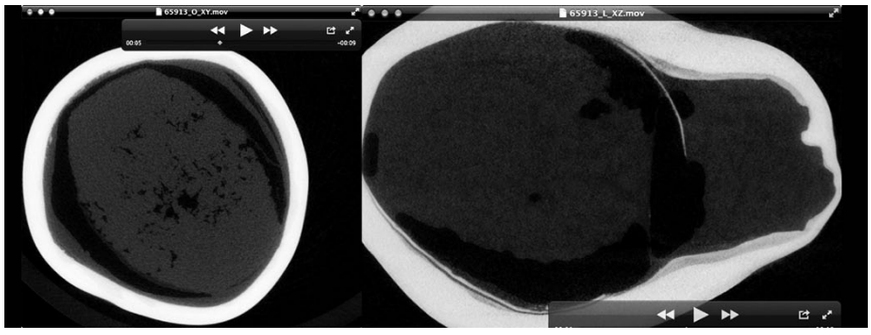

Figure 11. X-ray tomographic sections of two pearls with organic nucleus: Left a pearl (sample 65913-O) where the button-shaped organic nucleus has just slightly expanded revealing a somewhat granular appearance (grey). Right a pearl (sample 65913-L) where the organic nucleus burst outwards after a first expansion (already covered with a thin lining of nacre), thus resulting in a distinct baroque shaped pearl. The black parts in the tomographic slices are cavities, whereas the white and light grey inner lining of the sections represent the nacreous layer and the inner layers of organic matter deposited first by the young pearl sac. © M.S. Krzemnicki

After cutting of the samples

The close visual observation of the cut samples shows again very different features in the pearls of group A (1st generation with organic gelatinous nucleus) and group B (2nd generation with baroque shaped shell beads). Interestingly, we encounter in both groups/generations "normal" products containing a nucleus - organic (in specimen 65913-L, -O, -P of group A) or inorganic (in all samples of group B except 65913-H) - but also "beadless" products, which might be the result of bead rejection (Figure 9). When cutting sample 65913-M (group A), we found that it contained water with a distinct foul odour. We assume that its organic nucleus was either rejected at some point or consumed/transformed. When cutting the other samples of group A, the organic gelatinous nuclei fast began to swell due to the cooling of the cutting wheel with water.

The extent of the swelling of the organic nucleus is rather reduced within the oyster's gonad over the growth period of several months, compared with the swelling ability when the organic nucleus is soaked in water for a few hours (Figure 10). The organic nucleus may either just swell rather uniformly (see sample 65913-O in Figure 10) or may expand after grafting into the gonads by bursting open the original shape as can be seen in sample 65913-L using three-dimensional analysis by X-ray microtomography (Figure 11). Hence the 1st generation pearl will be of distinctly baroque shape if the organic nucleus bursts.

Figure 12. The cut pearls of group B (2nd generation) with baroque shaped shell pieces cut from freshwater shells. The pearls (65913-A, -C, -F, and -J) all show curved layers in the shell bead, as can be expected in the thick hinge of the shell. © L.E. Cartier

Typically for this product, the beaded samples (group B, 2nd generation) contain baroque-shaped shell pieces, often with layered structures. Due to these structures, we assume that the beads were cut from the hinge of freshwater shells, where nacre thickness is at a maximum (Figure 12).
One sample (65913-H), although collected as part of the 2nd generation beaded cultured pearls (group B) does not contain any bead, but actually reveals a long and irregular-shaped cavity-structure (Figure 9: lower right pearl), typical and characteristic for beadless cultured pearls. This pearl formed in an already existing pearl sac which collapsed after rejection of the baroque shaped shell bead. Similar beadless cultured pearls are well known in the trade and often sold as "Keshi" cultured pearls, although this term is not well defined (Hänni, 2006). In fact, this pearl is the only pearl of this study, which could be given that trade name, whereas all others (of group B) contain a bead and thus have to be named as beaded cultured pearls. See also the next section which discusses these two new cultured pearl products.

Discussion

For this investigation, we studied and analysed two new pearl products: a first generation product using an organic nucleus, so far not described in the gemmological literature (group A pearls in this study) and a second generation product using freshwater shell pieces as beads for large baroque-shaped cultured pearls.

The innovation (by Imai Seikaku Co. Ltd., Japan) to use organic nuclei in the first generation has two main reasons. First, to increase the growth rate and size of a pearl sac in a 1st generation and thus to be able to produce large sized cultured pearls faster than by the traditional method of grafting beads of increasing size from one generation to the next. Inserting a small nucleus also means that a smaller incision into the gonad is necessary, thereby reducing the risk of rejection and oyster mortality. A second reason for using this type of inflating bead is that a relatively young pearl sac has a better capacity to secrete nacre and produce a pearl with good colour and lustre (Figure 13), as statistical studies of pearl harvests have shown (Caseiro, 1995). When comparing a third generation pearl harvest to that of a first generation harvest, it is obvious that the average lustre of pearls is higher in first generation pearls (pers. comm., John Rere, 2012). The rationale behind this innovation in nucleus technology is simple: reduced pearl growth time lowers costs, and a potentially larger high quality pearl brings more income to a pearl farmer.

The freshwater pearl industry, which traditionally produces cultured pearls without a nucleus, has also experimented with different materials (Scarratt et al., 2000). A recent product is so-called "soufflé" freshwater cultured pearls (Sturman and Strack, 2010; Wiesauer, 2012). These are pearls that were filled with mud that is later removed after drilling. The aim is also to produce large cultured pearls in a relatively short time, using the mud to expand the pearl sac and promote larger pearl size. This is similar to the process described in this article, with the difference that the organic nucleus leads to greater expansion and the first generation pearls of this study are not commercialised.

Figure 13. Pinctada margaritifera *oysters selected and sacrificed for mantle tissue ('saibo'). The beautiful orient and lustre of the shell is the primary criterion in selecting suitable donor oysters. This is more likely to be found in a young healthy oyster © L.E. Cartier*

All the pearls examined during this study were of more or less baroque shape. Bead rejection is far less than average both with the 1st generation organic nuclei and 2nd generation baroque shell nuclei. For the organic nuclei the reasons are: 1) the organic nuclei is relatively small (average 6.5mm), 2) this requires a relatively small incision in the gonad, and 3) the expanding nucleus strongly reduces the risk of the saibo becoming detached from the nucleus (pers. comm., John Rere, 2013). However, due to the nature of the nucleus' and pearl sac's expansion (see Figure 10), it is difficult to produce round cultured pearls using this technique. Furthermore, there have been no reports of circled pearl formation; we assume that this is because the pearl sac is constantly under pressure from an expanding nucleus.

As the average price of cultured pearls, for example in French Polynesia, has decreased in recent years, cost issues have become increasingly important for pearl farmers (ISPF, 2011). Nuclei are a huge cost point for farmers (Fong et al., 2005). The price of a large nuclei suitable for a third-generation pearl (e.g. 16 mm) is proportionally much higher than a regular first generation nucleus (e.g. 7 mm). A pearl farmer must thus make a careful calculation of costs and risks, and this explains why many farmers in French Polynesia produce far less third-generation cultured pearls (Cartier, 2012).

Although the pearls seen in Figure 1 were first described as "Keshi" baroque cultured pearls, the use of this term is wrong. These pearls contain a baroque-shaped shell nucleus and are therefore beaded cultured pearls. This innovation in nucleus material and the resulting pearls are also interesting samples to study in order to better understand formation of the pearl sac and of pearls. The lack of circled pearls when using the approach described in this article may also shed more light on the formation mechanism of circled pearls and how to avoid these in order for a pearl farmer to have a higher average quality of pearl production.

Although the cultured pearl samples (of 2nd generation) studied in this article come from *Pinctada margartifera*, these nuclei are also reportedly being used in *Pinctada maxima* production in Indonesia (pers. comm., Takuya Imai, 2012). The baroque-shaped beaded cultured pearls described in this article are a niche product on the market at present. They have been produced to also meet demand for large baroque cultured pearls. It will be interesting to follow what developments new types of nuclei, such as the organic nuclei described in this article, will lead to in the production of cultured pearls. Both generations of these new types of pearl product can be clearly identified as beaded cultured pearls using the techniques available in a gemmological laboratory.

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CHAPTER 6

DNA Fingerprinting of Pearls to Determine Their Origins

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PLOS ONE

DNA Fingerprinting of Pearls to Determine Their Origins

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Abstract

We report the first successful extraction of oyster DNA from a pearl and use it to identify the source oyster species for the three major pearl-producing oyster species *Pinctada margaritifera*, *P. maxima* and *P. radiata*. Both mitochondrial and nuclear gene fragments could be PCR-amplified and sequenced. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in the internal transcribed spacer (ITS) region was developed and used to identify 18 pearls of unknown origin. A micro-drilling technique was developed to obtain small amounts of DNA while maintaining the commercial value of the pearls. This DNA fingerprinting method could be used to document the source of historic pearls and will provide more transparency for traders and consumers within the pearl industry.

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Introduction

Pearls produced by oysters of the Pteriidae family are among the most valuable and oldest gems. Oyster shells and pearls have been used for human adornment since antiquity [1], [2], [3], [4], [5], [6]. Today pearls are cultured in domesticated saltwater oysters and freshwater mussels and have become a billion dollar industry [7]. Whereas a natural pearl forms without any human intervention in a wild oyster, a cultured pearl is the result of a human-induced injury. The value assigned to a pearl depends largely on its quality, rarity, and whether it originated naturally or through culture [8]. Thus there is significant interest in being able to scientifically document the provenance of both historic natural pearls [8], [9] and modern cultured pearls. This is rarely possible for the most valuable white to slightly cream-colored pearls using current methods such as UV-visible photospectrometry and micro-Raman spectroscopy [10], [11], [12], [13]. The higher value of natural pearls has led to many fraudulent attempts to pass off cultured pearls as natural ones [14], [15], [16]. To date, the distinction between natural and cultured pearls has been based on X-ray shadow images (Fig. 1A, Fig. 1B and Fig. 1C) and more recently X-ray computer microtomography [15]. Other acts of fraud involve using cultured pearls from *Pinctada maxima* and *P. margaritifera* to resemble natural pearls from *P. radiata* [17]. Although all three types of oysters have been fished for centuries in the quest for natural pearls, those from *P. radiata* from the Arabian/Persian Gulf (“Basra Pearls”) have traditionally been the most coveted [6].

Marine cultured pearls are produced mainly in three species of oysters: *P. margaritifera*, *P. maxima* and the Akoya pearl oyster (*P. fucata-imbricata-martensii-radiata* complex) (Fig. 1D). The *P. maxima* oysters that produce white and golden South Sea cultured pearls are found in Australia, Burma, Indonesia and the Philippines [6], [7], [18]. Pearls from *P. margaritifera* are called black cultured pearls (or Tahitian cultured pearls) and are now produced mainly in French Polynesia, Fiji, Cook Islands and Micronesia [7], [19], [20], [21]. Akoya cultured pearls are produced mainly in China, Japan and Vietnam [6], [7]. Pearls from *P. radiata* are cultured exclusively in the Arabian/Persian Gulf. The majority of natural pearls come from *P. radiata* oysters, due to a long history of pearl fisheries in the Arabian/Persian Gulf [22]. Although they play a smaller role in the natural pearl trade, *P. maxima* and *P. margaritifera* oysters have produced many natural pearls of considerable size over the last centuries [4], [23], [24]. Natural pearls have a very small niche market and remain very rare because of extremely limited production in recent decades [8].

A cultured pearl consists of nacreous aragonite (calcium carbonate, $CaCO_3$) tablets (Fig. 1E) bound by an organic matrix that covers a nucleus typically made from freshwater mussel shell material (Fig. 1C and Fig. 1D) [25], [26]. A cultured pearl results from a surgical operation that subjects the oyster to a human-induced injury. After a marine pearl oyster has reached a suitable size, a small piece of external mantle tissue from a donor oyster is inserted along with a nucleus (a spherical piece of mussel shell, also called bead) (Fig. 1C) into a host oyster’s gonad. The grafted mantle cells form a pearl sac that is responsible for secreting and enveloping the implanted material with aragonite, ultimately resulting in a pearl [27], [28]. The growth of a cultured pearl usually takes 6–24 months during which the cultured pearl obtains a nacreous overgrowth between 0.5 mm and 2 mm [7].

The nacreous part of a pearl consists of approximately 92% $CaCO_3$, 4% organic matter (OM), 4% water and minute amounts of residual substances [29]. The OM (consisting mostly of conchioline and porphyrines), which is also secreted by the pearl

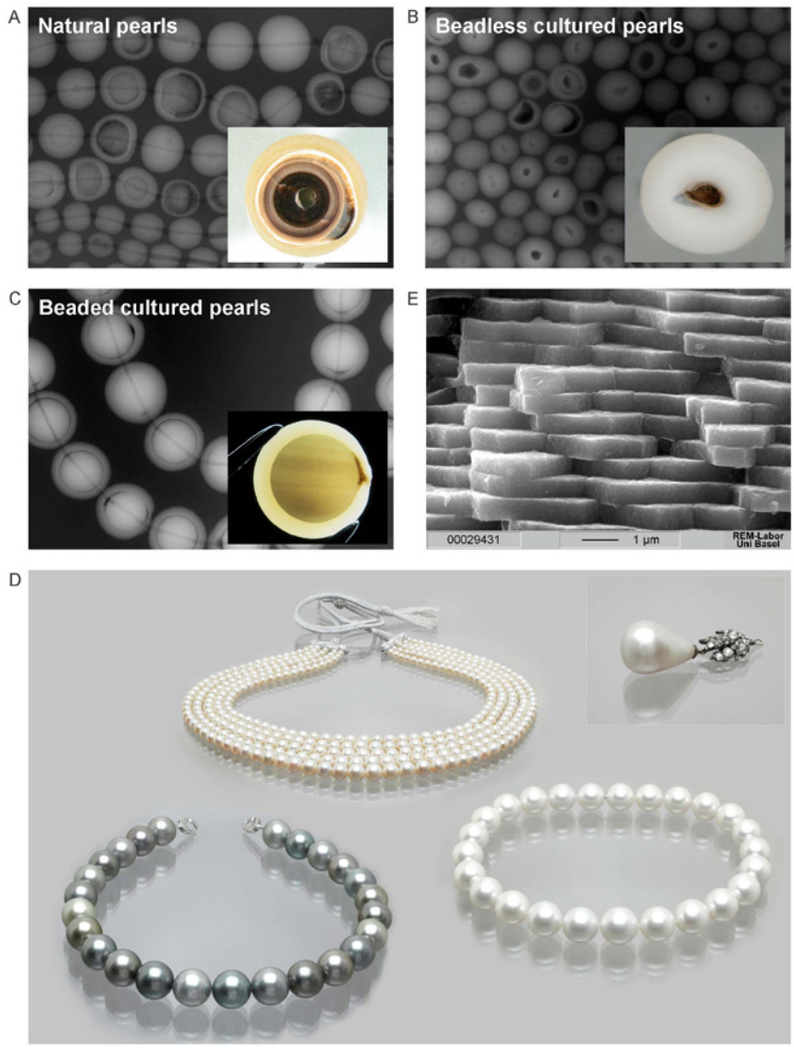

Figure 1. Pearls of *Pinctada margaritifera, P. maxima* and *P. radiata*. A) Natural pearls (*P. radiata*): radiography of a necklace and a cross-section of a pearl showing the three layers: the periostracum rich in organic material (OM) (inner layer), the prismatic layer (middle layer), and the aragonitic nacre or mother of pearl layer (outer layer). B) Beadless (without a nucleus) cultured pearls also called 'Keshi' (*P. maxima*): radiography of a necklace and a cross-section showing the nacreous layer around an inner cavity filled with OM. C) Beaded cultured pearls: radiography of a necklace with *P. margaritifera* pearls and cross section of an Akoya pearl showing the nacreous layer around an internal nucleus and an OM "pocket" on the right (Photos and radiographies A–C: H.A. Hänni). D) Necklaces with *P. margaritifera* pearls (lower row left), *P. radiata* pearls (upper row) and *P. maxima* pearls (lower row right). The inset shows the historic natural pearl "the Peregrina" which was found in the 16th century. This pearl and its necklace were sold for $11.8 million at a Christie's auction in December 2011 in New York. The PCR-RFLP method described here could provide scientific validation of the provenance of historic pearls (Photos: Swiss Gemmological Institute SSEF). E) Scanning electron microscope side-view image of aragonite tablets of the nacreous layer of a *P. margaritifera* pearl (Photo: Marcel Düggelin, ZMB, Basel University).
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sac, serves as a framework for the $CaCO_3$ matrix (Fig. 1E) during the biomineralization process [30]. OM can also be found in concentrated pockets (Fig. 1C). Up until now, DNA has not been extracted from a pearl's OM, but proteins have been extracted and analyzed [31], [32], [33]. Earlier reports of DNA recovery were from calcified mussel shells [34] and the ligament that holds the valves together [35]. DNA has also been extracted from other organic gems and $CaCO_3$ material (e.g. bones and teeth, corals, eggshells, ivory) [36], [37], [38], [39], [40], [41].

The aim of this research was to develop a DNA-based method to determine the oyster species that produced a pearl as a first step towards providing more precise information regarding its likely geographical origin. The DNA fingerprinting technique described here can be used to differentiate pearls from different oysters that were deliberately or accidentally mixed and may eventually differentiate cultured pearls that have been mixed in with natural pearls. DNA fingerprints could also establish the provenance of historic pearls such as the "Peregrina" pearl shown in Fig. 1D. Here we demonstrate that DNA can be extracted from a pearl's OM and used to determine the oyster species that produced the pearl. We developed a micro-drilling technique to extract the DNA that will not affect the commercial value of a pearl. These new methods will provide many advantages to the international pearl industry.

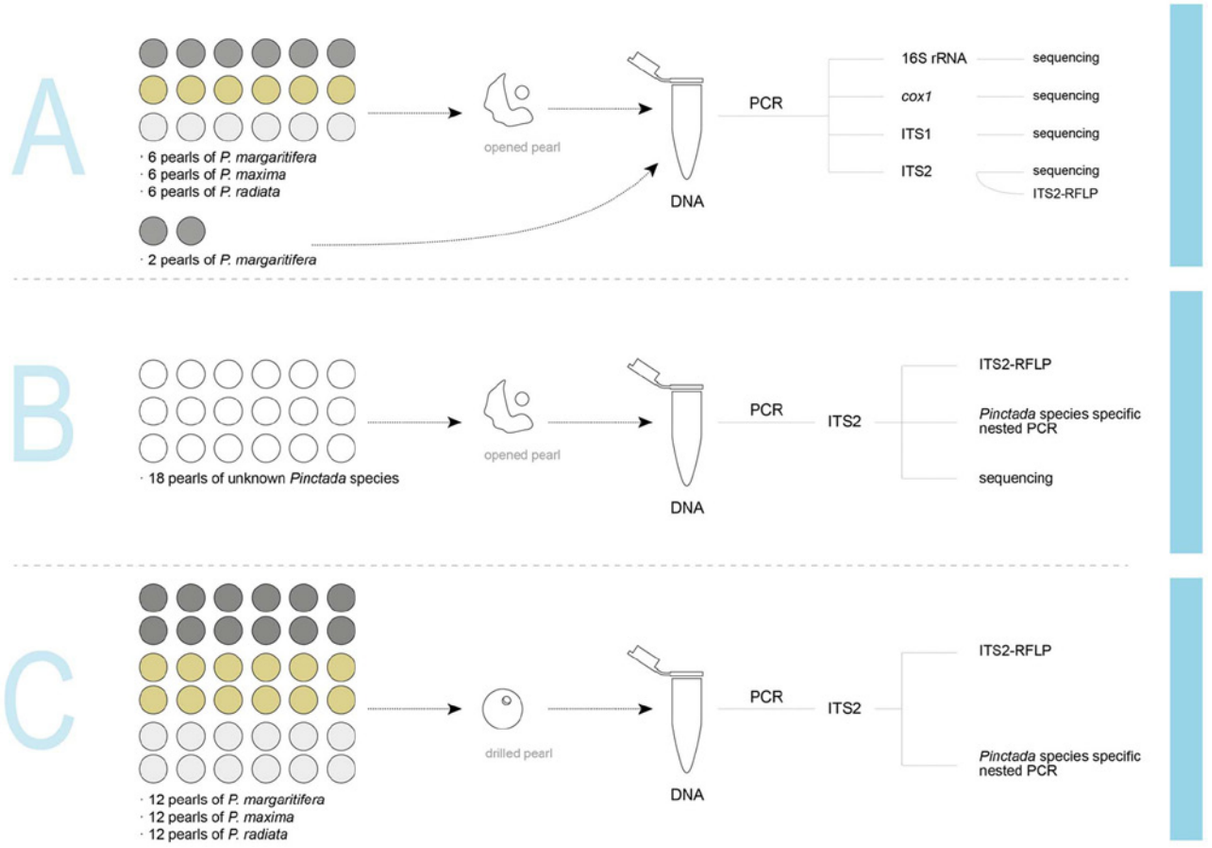

Figure 2. Schematic representation of the experimental procedures used for DNA extraction and PCR amplicon analysis. In methods A and B pearls were broken open using forceps to expose the internal organic material and nacre (mother of pearl). In method C samples were obtained by drilling a 1-mm diameter hole through the pearls and the hole was enlarged internally using a 0.9 mm drill head.
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Results and Discussion

Pearls contain DNA that allows assignment of source *Pinctada* species

We developed a DNA extraction method from pearls to allow us to identify the *Pinctada* species that produced the pearl. We considered a DNA extraction to be successful when at least one of the four target loci was amplified by PCR and correctly identified the source *Pinctada* species. The target loci included the two mitochondrial, 16S ribosomal (rRNA) and cytochrome oxidase subunit I (*cox1*), and the two nuclear internal transcribed spacers ITS1 and ITS2. These genes were chosen because they are commonly used in oyster phylogenetic studies and are known to be variable among *Pinctada* species [42], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52].

The *Pinctada* species were successfully identified for 100% of tested pearls from *P. margaritifera* (7/7 pearl samples) and *P. radiata* (6/6) and 60% of pearls from *P. maxima* (3/5) (Table 1 and Table 2) using method A (Fig. 2A). One pearl (PMX4) that was predicted to be *P. maxima* based on morphological criteria was instead associated to *P. margaritifera* by ITS2 and 16S rRNA sequences. The reason for this mismatch is explained below. The recovery of sequences up to 675 bp in length (Table 1) indicates that DNA is well preserved in pearls even when pearls were harvested years earlier and stored for several years at normal atmospheric conditions in a drawer or safe. The OM present in the $CaCO_3$ matrix in a pearl might be a source of DNA (Fig. 1C and Fig. 1E) [53], [54]. The negatively charged DNA molecule is known to have a high affinity for the Ca^{2+} ion of $CaCO_3$ [55], [56], [57], which might enhance its conservation in organic gems such as pearls. DNA recovery has been reported for several ancient $CaCO_3$ materials, including eggshells from the Holocene, horse bones from the Pleistocene and other ancient bones and teeth [38], [39], [40], [58].

Mitochondrial genes are present at a higher copy number per cell than nuclear genes and are thought to degrade more slowly due to their organellar location [59]. Thus they are often preferentially targeted in degraded, ancient and diluted samples [58], [59]. Nevertheless, we had greater success amplifying and sequencing the nuclear ITS2 gene than the mitochondrial 16S rRNA or *cox1* genes. These results suggest that the DNA is well preserved in the interior of the pearl.

Complete ITS2 sequences were obtained for *P. margaritifera* and *P. maxima* (Table 1), but two of the *P. radiata* samples (PR2 and PR4) had ~30 bp of internal sequence characterized by double peaks consistent with heterozygosity in this small region (Table 1). Intra-individual ITS polymorphism is common in oyster species [47], [49], [51]. Moreover, because cultured pearls are formed by grafting nacre-secreting mantle tissue from a donor oyster into the gonad of a recipient oyster (host), the two organisms might have

Table 1. DNA profiles of pearl samples from *Pinctada margaritifera* (PMR), *P. maxima* (PMX) and *P. radiata* (PR) based on four different molecular markers.

Pearl sample	Pearl weight (carats/mg)	Sample weight (mg)	16S rRNA	*cox1*	ITS1 rRNA	ITS2 rRNA
PMR positive control			PMR (AB214436.1)[a]	PMR (AB259166.1)	PMR (AY877501.1)	PMR (AY877506.1)
			511 bp (99%)[b]	575 bp (99%)	675 bp (99%)	575 bp (100%)
PMX positive control			PMX (AB214435.1)	PMX (GQ452847.1)	PMX (AY172345.1)	PMX (AY877505.1)
			509 bp (100%)	476 bp (99%)	593 bp (99%)	571 bp (100%)
PR positive control			PR (AB214442.1)	PR (GQ355875.1)	*P. martensii*[c] (AY172344.1)	*P. fucata*[c] (AY877582.1)
			524 bp (100%)	575 bp (99%)	580 bp (99%)	591 bp (99%)
PMR1	11.1/2228	426	PMR (AB214436.1)	PMR (AF374329.1)	PMR (AY877501.1)	PMR (AY877506.1)
			511 bp (99%)	425 bp (99%)	675 bp (99%)	575 bp (100%)
PMR2	8.1/1610	19	PMR (AB214436.1)	PMR (AF374329.1)	PMR (AY877501.1)	PMR (AY877506.1)
			455 bp (99%)	425 bp (99%)	378 bp (100%)	575 bp (100%)
PMR3	7.4/1480	24	n.d.[d]	n.d.	n.d.	PMR (AY877506.1)
						575 bp (100%)
PMR4	7.4/1480	124	PMR (AB214436.1)	PMR (AF374329.1)	PMR (AY877501.1)	PMR (AY877506.1)
			455 bp (99%)	425 bp (99%)	378 bp (100%)	575 bp (100%)
PMR5	13.1/2618	318	PMR (AB214436.1)	PMR (AF374329.1)	n.d.	PMR (AY877506.1)
			455 bp (100%)	425 bp (99%)		575 bp (100%)
PMR6	9.8/1964	23	PMR (AB214436.1)	PMR (AF374326.1)	n.d.	PMR (AY877506.1)
			454 bp (99%)	425 bp (100%)		575 bp (100%)
PMX1	33.0/6598	78	PMX (AB214435.1)	n.d.	n.d.	PMX[e]
			451 bp (100%)			
PMX2	29.5/5898	135	PMX (AB214435.1)	n.d.	n.d.	PMX (AY883851.1)
			451 bp (100%)			571 bp (100%)
PMX3	20.9/4180	34	PMX (AB214435.1)	PMX (GQ452847.1)	n.d.	PMX (AY282737.1)
			451 bp (100%)	204 bp (100%)		571 bp (100%)
PMX4	25.3/5070	105	PMR (AB214436.1)	n.d	n.d.	PMR (AY877506.1)
			454 bp (99%)			575 bp (99%)
PMX5	13.5/2694	38	n.d.	n.d.	n.d.	n.d.
PMX6	8.4/1672	59	n.d.	n.d.	n.d.	n.d.
PR1	6.2/1234	108	PR (AB214442.1)	n.d.	*P. martensii* (AY144602.1)	*P. fucata* (AY877582.1)
			444 bp (100%)		226 bp (99%)	590 bp (99%)
PR2	5.4/1090	79	PR (AB214442.1)	PR (GQ355875.1)	*P. martensii* (AY144602.1)	*P. fucata* (AY877588.1/AY877600.1)[f]
			444 bp (100%)	543 bp (99%)	226 bp (99%)	221 bp/239 bp (100%)
PR3	5.1/1030	296	PR (AB214442.1)	n.d.	n.d.	*P. fucata* (AY877582.1)
			523 bp (100%)			491 bp (99%)
PR4	4.5/908	224	PR (AB214442.1)	PR (GQ355875.1)	*P. martensii*[e]	*P. fucata* (AY877588.1/AY877600.1)[f]
			523 bp (100%)	543 bp (99%)		221 bp/239 bp (100%)
PR5	4.5/904	151	n.d.	*P. fucata* (DQ299941.1)	n.d.	*P. fucata* (AY877582.1)
				149 bp (91%)		491 bp (99%)
PR6	4.2/842	83	PR (AB214442.1)	PR (GQ355875.1)	n.d.	*P. fucata* (AY877605.1)
			523 bp (100%)	543 bp (99%)		242 bp (99%)

[a]*Pinctada* species assignment was based on the highest BLAST score (highest query coverage and maximal base pair identity). GenBank accession number shown in brackets.
[b]amplicon size (base pair) and maximal identity (%) of the sequence to the BLAST query.
[c]*P. fucata* and *P. martensii* are conspecific to *P. radiata* on the basis of their ITS sequences [50], [51].
[d]not determined.
[e]sample had lower sequence quality, but the BLAST query in GenBank indicated the correct *Pinctada* species. The ITS2 sequences could be amplified and successfully analyzed using PCR-RFLP.
[f]these two accession numbers correspond to ITS2 sequences which flanked an internal sequence of ~30 bp characterized by double peaks consistent with heterozygosity.
doi:10.1371/journal.pone.0075606.t001

Table 2. Sequencing success rate associated with different molecular markers from pearl DNA extracts of *Pinctada margaritifera*, *P. maxima* and *P. radiata* using methods A, B and C (Fig. 2).

Method A[a]	16S rRNA	*cox1*	ITS1	ITS2	Total % of successfully identified pearls
P. margaritifera	86% (6/7)[b, c]	71% (5/7)	43% (3/7)	100% (7/7)	100% (7/7)[c]
P. maxima	60% (3/5)[c]	20% (1/5)	0% (0/5)	60% (3/5)	60% (3/5)[c]
P. radiata	83% (5/6)	67% (4/6)	50% (3/6)	100% (6/6)	100% (6/6)
Total % of successfully sequenced markers	78% (14/18)	56% (10/18)	33% (6/18)	89% (16/18)	89% (16/18)

Methods A, B and C[a]	Method A[a]	Method B[a]	Methods A+B[a]	Method C[a] practically ''non-destructive''
	ITS2	ITS2	ITS2	ITS2
P. margaritifera	100% (7/7)[b, c]	100% (7/7)[c]	100% (14/14)[c]	92% (11/12)
P. maxima	60% (3/5)[c]	80% (4/5)[c]	70% (7/10)[c]	58% (7/12)
P. radiata	100% (6/6)	100% (6/6)	100% (12/12)	92% (11/12)
Total % of successfully sequenced markers	89% (16/18)	94% (17/18)	92% (33/36)	81% (29/36)

[a]in methods A and B the pearls were broken open using forceps to expose the inner material used to extract DNA. In method C the powder used for DNA extraction was obtained by drilling a 1-mm diameter hole in the pearls and the hole was enlarged internally using a 0.9 mm drill head.
[b]percentage (%) of successfully identified pearls (identified pearls/total pearls tested).
[c]from a total of twelve *P. maxima* and *P. margaritifera* samples analyzed in method A or in method B, one pearl that was predicted to belong to *P. maxima* based morphological criteria was identified as *P. margaritifera* according to the DNA fingerprint.
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different ITS sequences that will be mixed in the pearl [60]. Sequence polymorphisms were found among *P. margaritifera* pearls in mitochondrial 16S rRNA and *cox1* sequences as well as in the ITS2 sequence of PMX4. No polymorphisms were detected among *P. maxima* pearls. DNA sequences were deposited in GenBank under accession numbers KF283999–KF284026 (ITS1 and ITS2), KF284042–KF284058 (16S rRNA) and KF284059–KF284070 (*cox1*).

None of the four loci could be amplified from the *P. maxima* pearls PMX5 and PMX6 (Table 1). Pearl PMX5 contained a malodorous brown liquid consistent with degradation of the OM and possibly degradation of the corresponding DNA. Other *P. maxima* pearls generally contained little visible OM and had thinner and more resistant outer nacreous layers around the internal nucleus. *P. margaritifera* and *P. radiata* pearls were characterized by a relatively higher visible OM content, which was correlated with higher PCR amplification success. We had successful amplification from samples composed only of white powder, indicating that DNA can be obtained through demineralization from the $CaCO_3$ structure (Fig. 1) of the nacre and/or from small samples (e.g.: PMR2 = 19 mg, Table 1).

We failed to amplify any DNA from the two intact pearls of *P. margaritifera* (pearls PMRA and PMRB, Fig. 2A) that were not broken open before adding them to the ethylenediaminetetraacetic acid (EDTA) buffer. Pearls are often washed with freshwater and cleaned using salt or ground up walnut shells to remove surface impurities, and some pearls can be treated using, for example, the maeshori method that involves the use of solvents such as methyl alcohol [61]. Moreover, we sterilized the pearls for 20 min in a sodium hypochlorite solution prior to DNA extraction. These treatments may explain why we could not extract DNA from the outer layer. The minimal surface area exposed to the EDTA might also have hampered DNA extraction. Other studies showed that recovery of DNA from freshwater shell material of *Margaritifera margaritifera* was strongly affected by exposure time and grinding intensity [34]. We did not further develop testing procedures for entire pearls because this totally destructive method would not be acceptable in the pearl trade. We therefore focused our efforts on developing the less destructive micro-drilling method described later in this paper.

A PCR-RFLP test to determine pearl origins

Sequences of ITS regions have been widely used to differentiate *Pinctada* species [47], [49], [51], [52] and an RFLP method has already been developed on the intergenic spacer (IGS) of nuclear ribosomal RNA to distinguish the closely related *P. fucata*, *P. imbricata* and *P. martensii* [49]. We developed a PCR-RFLP method based on the ITS2 region to differentiate among the three examined *Pinctada* species (Fig. 3).

To validate the PCR ITS2-RFLP method, 18 pearls of unknown identity were included in a blinded analysis (Fig. 2B). ITS2 was successfully amplified from 17 out of 18 pearls (Fig. 4A, Table 2). PCR with *P. margaritifera* specific primers amplified only the corresponding *P. margarifiera* pearl samples (Fig. 4B) and the PCR ITS2-RFLP analysis allowed us to correctly identify each pearl (Fig. 4C) except for BL4 that we identified as *P. margaritifera* instead of *P. maxima*. As explained below, we consider the PCR ITS2-RFLP assay to be more accurate than the conventional assay based on morphological criteria. The results of the PCR ITS2-RFLP assay were confirmed by sequencing the ITS2 region amplified in each pearl (GenBank accession numbers KF284027–KF284041; Table S1). The method was successful across a variety of pearls of different sizes, shapes and composition of the extracted material (weight range from 38 mg to 672 mg) (Table S1).

Potential applications in the pearl industry

To minimize the potential loss in pearl value that would result from damaging the pearl to obtain sufficient material for a DNA test, we developed a micro-drilling methodology (Fig. 5) that could be especially useful for determining the origin of historic natural

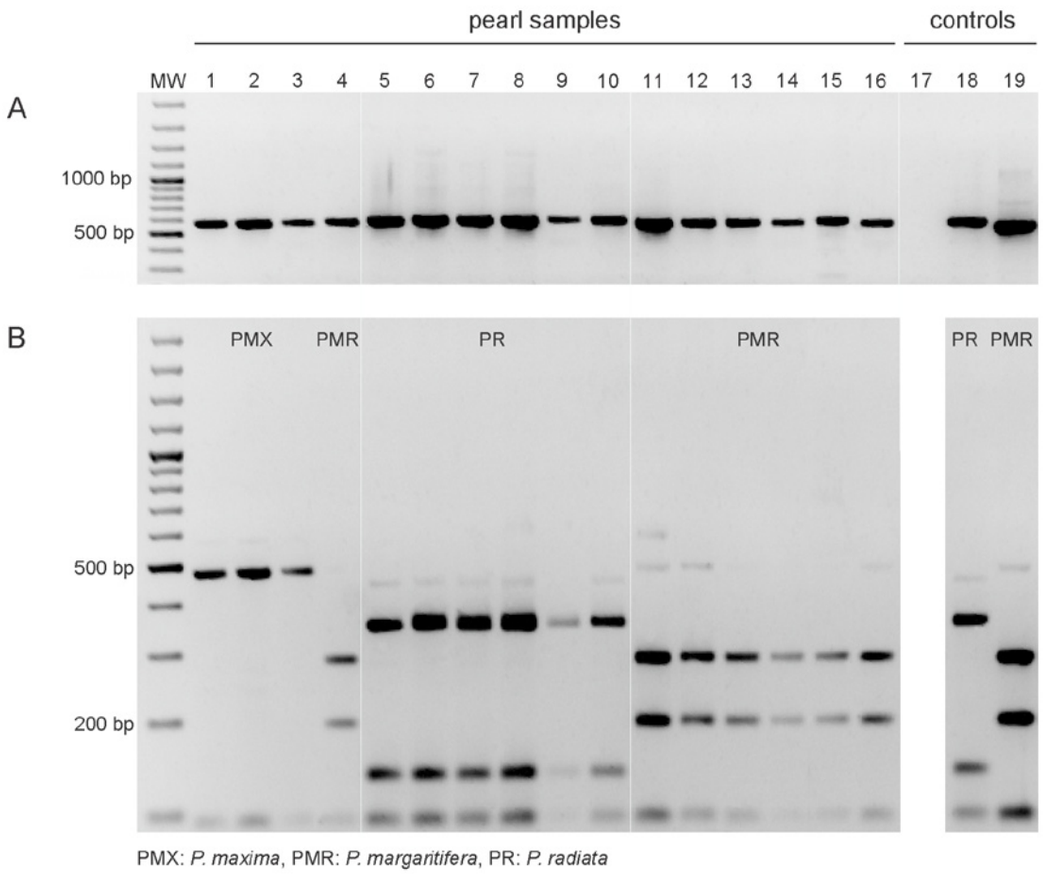

Figure 3. A PCR-RFLP assay of the ITS2 region applied to pearls from *Pinctada margaritifera*, *P. maxima* and *P. radiata*. (A) PCR products of 575 bp (*P. margaritifera*), 571 bp (*P. maxima*) and 590–91 bp (*P. radiata*) obtained with ITS2 universal primers (5.8S-F and 28S-R) and (B) RFLP patterns of ITS2 amplicons (from A) obtained after digestion with *Rsa*I. MW: molecular weight size marker, 100-bp DNA ladder; lanes 1–3: *P. maxima* (PMX) pearls; lane 4: *P. margaritifera* (PMR) pearl; lanes 5–10: *P. radiata* (PR) pearls; lanes 11–16: *P. margaritifera* pearls; lane 17: PCR negative control; lanes 18 and 19: *P. radiata* and *P. margaritifera* positive controls. Note: The *P. maxima* positive control is shown in Figure 4.
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pearls of high value (see for example Fig. 1D). We tested this method on twelve pearls for each *Pinctada* species (Fig. S1 and Fig. 2C). For both *P. margaritifera* and *P. radiata*, 11 out of 12 pearls could be successfully identified using as little as 10 mg of recovered drill powder (Table 2 and Table 3). For *P. margaritifera* it was possible to amplify the ITS2 with a direct PCR, but in *P. radiata* and *P. maxima* a nested PCR approach using an additional specific primer internal to the ITS2 region was needed. All of our experiments indicate that DNA recovery is more difficult from *P. maxima* than the other species.

P. margaritifera or *P. maxima*, which method is more accurate?

An unexpected outcome was the mixed identity assigned to the cultured pearls PMX4 and BL4 (Table 1 and Table S1, Fig. 3 and Fig. 4). These pearls were assigned to the *P. maxima* species by pearl experts at the Swiss Gemmological Institute SSEF through visual observation, mainly because of their cream color. However, their DNA fingerprints (PCR ITS2-RFLP and sequences of 16S rRNA and ITS2) clearly indicated that these pearls originated from *P. margaritifera*. The ITS2 sequence of PMX4 differed from *P. margaritifera* by only two single nucleotide polymorphisms (Table 1). Based on our overall results, we believe that the visual assignment of species origin was incorrect, as it is well known that *P. margaritifera* not only produces grey to black pearls, but also yellowish to white ones, which are very similar in color to pearls from *P. maxima* [10], [19]. A recent study [45] found a Japanese *P. maxima* oyster, identified based on its morphology clustering with *P. margaritifera*, on the basis of its *cox1* sequence and concluded that the mismatch was due to inaccuracy of the morphological measurement. Similarly, a specimen identified as *P. radiata* on the basis of morphology had an ITS1 sequence matching *P. chemnitzi* [51]. These mistaken identifications based on morphology illustrate well the need for an accurate method to determine the origins of pearls produced by *Pinctada* oysters.

Conclusions

We were able to extract DNA from individual pearls and develop a PCR-RFLP assay to determine which oyster species produced the pearl. This method can potentially be used to document the provenance of historic pearls and determine which oyster species produced either natural or cultured pearls. The ability to extract relatively large DNA molecules from pearls opens the possibility of applying next generation DNA sequencing (NGS) technologies [38]

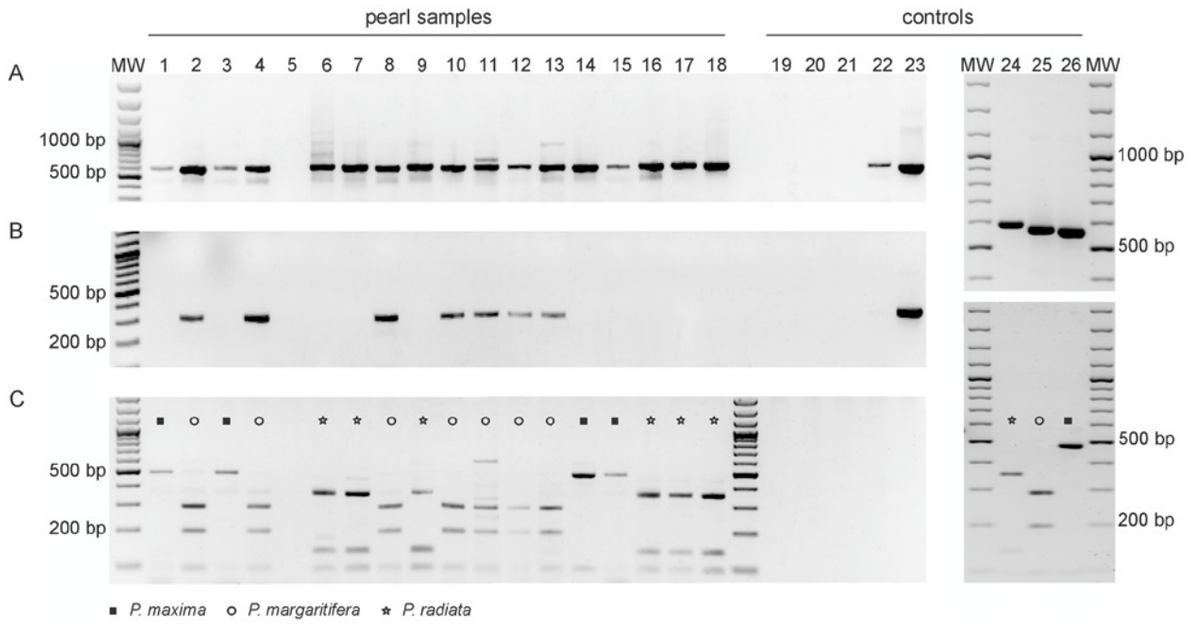

Figure 4. Blind PCR-RFLP assay with eighteen pearls of unknown identity. (A) PCR products of 575 bp (*Pinctada margaritifera*), 571 bp (*P. maxima*) and 590–91 bp (*P. radiata*) obtained with ITS2 universal primers (5.8S-F and 28S-R) and (B) of 335 bp obtained with 28S-R and the *P. margaritifera* specific primer ITS2-Marg-F. (C) RFLP patterns of ITS2 gene fragments (from A) obtained after digestion with *Rsa*I. MW: molecular weight size marker, 100 bp DNA ladder; lanes 1–18: pearl isolates; lanes 19–20: DNA extraction negative controls; lane 21: PCR negative control; lanes 22–23: *P. radiata* and *P. margaritifera* positive controls; lanes 24–26: *P. radiata*, *P. margaritifera* and *P. maxima* positive controls showing ITS2 PCR products (upper gel) and ITS2-RFLP patterns (lower gel).
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to provide more extensive sequence data that would provide even more precise information on pearl origins. We anticipate that NGS technologies coupled with detailed population genetic analyses of reference oyster populations could enable individual pearls to be assigned to specific oyster populations, allowing a scientific assignment of a pearl's origin and providing more transparency for traders and consumers within the pearl industry.

Materials and Methods

Animal sample preparation and DNA extraction

Three oyster specimens each of *P. margaritifera*, *P. maxima* and *P. radiata* were collected at pearl farms in Pohnpei (Federated States of Micronesia) in December 2011, Bali (Indonesia) in May 2013 and Ras Al Khaimah (United Arab Emirates) in January 2012 and stored at −20°C. A 0.5–1.0 g piece of adductor muscle was ground in liquid nitrogen and total genomic DNA was extracted according to the manufacturer's recommendations using the QIAGEN DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany). DNA was diluted to 10 ng/µl and stored at −20°C until further use. These DNA samples were used as positive controls for the PCR-RFLP and sequencing analyses.

Pearl material

All samples were non-drilled marine cultured pearls of known origin. All pearls contained a nucleus (a spherical bead of freshwater mussel shell) typically used in pearl production. Natural pearls were not used because they are much more valuable and their geographic and species provenance is rarely well documented. In total, 74 pearls were studied using three different methodologies (A, B and C: see Fig. 2). For method A six pearls of each *Pinctada* species were analyzed using destructive DNA extraction methods (PMR1–6 for *P. margaritifera*, PMX1–6 for *P. maxima* and PR1–6 for *P. radiata*) and two additional *P. margaritifera* pearls, PMRA and PMRB, were analyzed non-destructively. For method B a blind test based on destructive DNA extraction was carried out using 18 pearls from an unknown source (BL1–18) that was later revealed. For method C, the DNA of 12 pearls of each *Pinctada* species (PR7–18, PMX7–18 and PMR7–18) were analyzed using micro-drill sampling (pearls are shown in Fig. S1). *P. margaritifera* pearls were collected in French Polynesia between 2007 and 2010, except nine pearls harvested in Fiji in 2010–2011 (PMRB in method A, and PMR9 to 16 in method C). *P. maxima* pearls were grown either in Australia or Indonesia and harvested between 2005–2009, except for two pearls from the Philippines, PMX16 and PMX17 (method C) harvested in 2003 and 2010, respectively. *P. radiata* pearls were harvested at pearl farms in Ras Al Khaimah (United Arab Emirates) in 2009 and 2010. Pearls were provided by RAK Pearls (United Arab Emirates) and Dr. Masahiro Ito (Pohnpei, Micronesia), Andy Müller (Kobe, Japan), Frieden AG (Thun, Switzerland) and Jörg Gellner (Zürich, Switzerland). Pearl weights ranged from 1154–3190 mg (5.8–15.9 carats) for *P. margaritifera*, from 856–6598 mg (4.3–32.9 carats) for *P. maxima* and from 504–1754 mg (2.5–8.8 carats) for *P. radiata*.

Preparing pearls for DNA extraction

The three different DNA extraction and analysis methodologies (A, B and C) are illustrated in Fig. 2. To minimize the possibility of DNA cross contamination, DNA extraction from pearls was performed in a different laboratory room and sterile hood than DNA extraction from the adductor muscle. All pearls were surface

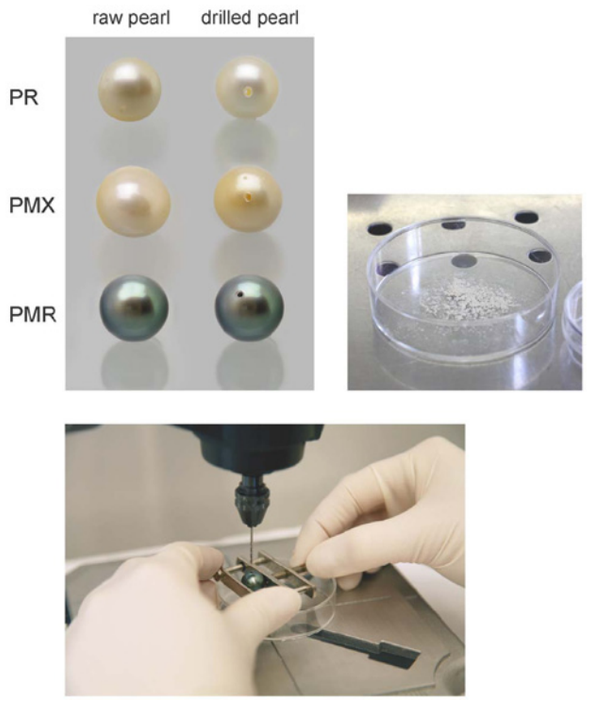

Figure 5. Examples of pearls of *Pinctada margaritifera*, *P. maxima* and *P. radiata* used in this study before and after micro-drilling. We used a drill head attached to a Dremel Workstation to produce pearl powder used for DNA extraction. Recovered pearl powder (nacre and organic material) can be seen in the Petri dish. *P. margaritifera* (PMR), *P. maxima* (PMX) and *P. radiata* (PR).
doi:10.1371/journal.pone.0075606.g005

sterilized by stirring in a 4% sodium hypochlorite solution for 20 min. For methods A and B (Fig. 2), the pearls were broken open using sterile forceps in a sterile hood, except PMRA and PMRB which were tested in their original state (i.e. as intact pearls). The inner nucleus was discarded and the remaining material was pulverized in a mortar, added to a 2 ml microfuge tube and weighed. The two intact pearls were added to 2 ml microfuge tubes and weighed. 500 µl of 0.5 M EDTA at pH 8.0, was added to each sample to dissolve the $CaCO_3$. For method C (Fig. 2) the material used for DNA extraction was removed by drilling a hole using a Dremel® (Model 8000, Dremel Europe, Breda, Netherlands) with a 1 mm drill head fixed on a Dremel® Workstation (Fig. 5). The pearl was held in a vise over a sterile Petri dish that collected the resulting drill powder. A second non-fixed 0.9 mm drill head was used to enlarge the interior part of the drill hole without damaging the surface around the drill hole. The drill powder was suspended in 1000 to 2000 µl 0.5 M EDTA (pH 8.0). All pearl samples in the EDTA solution were vigorously vortexed for two min and incubated overnight at 56°C in a water bath.

DNA extraction

Total DNA was extracted directly from the pearl-EDTA solution using a Fast DNA Spin Kit for soil (MP Biomedicals, Irvine, CA, USA). The extraction procedure was done according to the manufacturer's recommendations except that in the first step 1000 or 700 µl of sodium phosphate buffer included in the kit was directly added to the microfuge tube when it contained 500 µl or 1000 µl EDTA, respectively. When samples were incubated in 2000 µl EDTA, the sample was divided evenly into two 2 ml microfuge tubes and each tube received 700 µl of sodium phosphate buffer. The Lysing Matrix E tubes provided in the kit were not used. Homogenization with the FastPrep instrument was not performed; instead the samples were vortexed vigorously for two minutes. The resulting DNA samples were used directly, diluted ten times, or concentrated in a vacuum centrifuge prior to PCR.

PCR amplification

DNA samples were screened for the presence of the mitochondrial-encoded 16S rRNA and the *cox1* genes and the nuclear-encoded ITS1 and ITS2 of the rRNA gene cluster. *Pinctada* ITS2 gene sequences were retrieved from GenBank and aligned using the multiple sequence alignment program ClustalW 1.8 [62]. Sequences that were polymorphic between *P. margaritifera*, *P. maxima* and *P. radiata* were used to design species-specific forward primers ITS2-Marg-F, ITS2-Max-F and ITS2-Rad-F. All primers, annealing temperatures and PCR conditions used in this study and the expected lengths of the PCR amplicons are listed in Table S2.

PCR was carried out in 20 µl reactions containing 1 µl of DNA template, 2 µl of PCR buffer (Fermentas GmbH, St. Leon-Rot, Germany), 5% bovine serum albumin (New England Biolabs, Inc., Beverly, MA), 5% dimethylsulfoxide (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), 200 µM of each dATP, dCTP, dGTP, and dTTP (New England Biolabs, Inc.), 0.50 µM of each primer and 1.4 U of Dream DNA polymerase (Fermentas GmbH). The initial denaturation (5 min at 94°C) was followed by 40 cycles of 94°C for 30 s, as annealing temperature of 45–55°C for 30 s and 72°C for 60 s with a final extension at 72°C for 7 mins.

Sequencing of 16S rRNA, *cox1*, ITS1 and ITS2

All PCR amplicons were purified on a MultiScreen PCR plate (Millipore, Molsheim, France) and resuspended in 30 µl of sterile double-distilled water. Sequencing reactions were performed with 3–10 ng of purified PCR product and primers at a final concentration of 0.10 µM using an ABI PRISM BigDye Terminator v3.0 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. PCR products were sequenced in both directions using the same primer pairs as in the amplification reaction (Table S2). The obtained products were cleaned by gel-filtration through Sephadex G-50 columns (Amersham Biosciences, Uppsala, Sweden) on MultiScreen HV plates (Millipore). Purified products were sequenced using an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) at the Genetic Diversity Centre of the ETH Zürich. DNA sequences were edited using the Sequencher package (Gene Codes, Ann Arbor, MI, USA). Only the unambiguous parts of the sequence were used to define the species through homology with the NCBI Databank.

PCR-RFLP analyses

To discriminate between *Pinctada* species, a PCR-RFLP analysis was performed on the PCR-amplified ITS2 gene fragment. Candidate restriction endonucleases were identified using the software Nebcutter 2.0 [63]. Restriction analysis was done in 12 µl reaction mixtures with 5 µl of amplified product, 100 µg/ml bovine serum albumin (New England Biolabs, Inc.), 1.2 µl enzyme buffer (New England Biolabs, Inc.) and 0.5 units of *Rsa*I (Fermentas GmbH). Reactions were incubated for 90 min at 37°C and then stored at −20°C. Restriction fragments were separated by electrophoresis in ethidium bromide-stained 2%

Table 3. ITS2 profiles of pearls from *Pinctada margaritifera* (PMR), *P. maxima* (PMX) and *P. radiata* (PR) using a practically non-destructive method (Fig. 2C).

Pearl label	Pearl weight (carats/mg)	Sample weight (mg)	ITS2 direct PCR[a]	ITS2 nested PCR[a]	ITS2-RFLP	PMR, PMX or PR ITS2 nested PCR[a]
PMR7	6.7/1335	43	no	no	no	no
PMR8	7.5/1511	45	*yes*	*yes*	*yes*	*yes*
PMR9	7.9/1588	60	*yes*	*yes*	*yes*	*yes*
PMR10	12.2/2441	61	*yes*	*yes*	*yes*	*yes*
PMR11	11.5/2307	59	*yes*	*yes*	*yes*	*yes*
PMR12	9.7/1934	59	*yes*	*yes*	*yes*	*yes*
PMR13	10.2/2048	74	*yes*	*yes*	*yes*	*yes*
PMR14	6.5/1310	75	*yes*	*yes*	*yes*	*yes*
PMR15	15.9/3190	50	*yes*	*yes*	*yes*	*yes*
PMR16	12.3/2464	39	*yes*	*yes*	*yes*	*yes*
PMR17	6.7/1335	71	*yes*	*yes*	*yes*	*yes*
PMR18	7.5/1511	100	*yes*	*yes*	*yes*	*yes*
			92% (11/12)[b]	92% (11/12)	92% (11/12)	92% (11/12)
PMX7	11.6/2320	90	no	no	no	no
PMX8	15.6/3120	50	no	no	no	*yes*
PMX9	6.4/1290	20	no	no	no	*yes*
PMX10	7.2/1450	60	no	*yes*	*yes*	*yes*
PMX11	18.6/3720	110	no	*yes*	*yes*	*yes*
PMX12	20.2/4030	90	no	no	no	no
PMX13	12.4/2470	100	no	no	no	no
PMX14	17.4/3480	70	no	no	no	no
PMX15	12.0/2400	60	no	no	no	no
PMX16	12.1/2420	100	no	*yes*	*yes*	*yes*
PMX17	10.4/2080	70	no	*yes*	*yes*	*yes*
PMX18	9.3/1860	40	no	*yes*	*yes*	*yes*
			0% (0/12)[b]	42% (5/12)	42% (5/12)	58% (7/12)
PR7	6.9/1380	40	no	*yes*	*yes*	*yes*
PR8	4.9/970	20	no	*yes*	*yes*	*yes*
PR9	4.7/940	10	no	*yes*	*yes*	*yes*
PR10	6.0/1210	13	no	*yes*	*yes*	*yes*
PR11	6.1/1220	40	no	no	no	*yes*
PR12	5.4/1080	33	no	*yes*	*yes*	*yes*
PR13	6.5/1310	40	no	*yes*	*yes*	*yes*
PR14	6.2/1240	20	no	no	no	*yes*
PR15	7.0/1400	20	no	no	no	*yes*
PR16	5.2/1050	20	no	*yes*	*yes*	*yes*
PR17	4.2/850	20	no	*yes*	*yes*	*yes*
PR18	5.1/1020	20	no	no	no	no
			0% (0/12)[b]	67% (8/12)	67% (8/12)	92% (11/12)

[a]direct PCR was conducted using ITS2 universal primers (5.8S-F and 28S-R). Nested PCR was conducted with the universal ITS2 primers or primer pair 28S-R and *Pinctada*-specific forward primers internal to the ITS2 fragment (ITS2-Marg-F, ITS2-Max-F or ITS2-Rad-F).
[b]percentage of successfully identified pearls (identified pearls/total pearls tested).
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agarose gels. A 100 bp ladder (GIBCO-BRL Life Technologies Inc., Gaithersburg, MD, USA) was used as a size marker. The digested PCR products were compared with equivalent RFLP profiles obtained from the reference positive control *P. margaritifera*, *P. maxima* and *P. radiata* adductor muscle DNA extracts.

Supporting Information

Figure S1 Pearls from *Pinctada margaritifera* (PMR), *P. maxima* (PMX) and *P. radiata* (PR) used in method C (Fig. 2).
(PDF)

Table S1 Blind test: PCR-RFLP and analysis of the ITS2 sequences from eighteen pearls of unknown identity.
(PDF)

Table S2 PCR primers, amplicon lengths and references.
(PDF)

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Author Contributions

Conceived and designed the experiments: JBM LEC EPF MSK BAM. Performed the experiments: JBM. Analyzed the data: JBM LEC MSK BAM. Contributed reagents/materials/analysis tools: JBM LEC EPF MSK HAH BAM. Wrote the paper: JBM LEC MSK BAM.

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CHAPTER 7

Tracing cultured pearls from farm to consumer: A review of potential methods and solutions

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Tracing cultured pearls from farm to consumer: A review of potential methods and solutions

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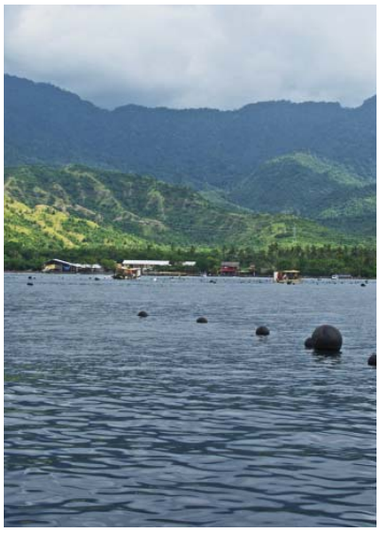
Abstract: This article reviews various methods that could be used to determine the geographic origin of cultured pearls, potentially allowing a consumer to trace them back to the farm. Chemical marking using different substances is possible due to the porosity of the nucleus and nacre. It is also possible to affix a logo marker to the nucleus that can later be imaged using X-radiography. In addition, radio-frequency identification chips are today so small that they can be housed within the nucleus of a cultured pearl. Also discussed is the potential of using trace-element chemistry to differentiate mollusc species and pearling regions. Carbon and oxygen isotopes could also be useful given that they reflect the waters in which a cultured pearl grew, and DNA testing may offer options in the future.

Keywords: cultured pearl branding, cultured pearl traceability, LA-ICP-MS, RFID chips, shell and cultured pearl DNA



Introduction

Branded jewellery products are more successful than non-branded goods (Kapferer and Bastien, 2009). There is continued demand from jewellery consumers for branded goods and increasing desire for traceability of products (Conroy, 2007; Ganesan *et al.,* 2009). Cultured pearls are an interesting case study where some products are branded (e.g., *Figure 1*), but traceability to source is something that is difficult to verify independently at present. A cultured pearl strand with a branded tag does not provide a clear guarantee of origin for the end consumer, given that individual cultured pearls can easily be exchanged or strands re-strung. At the same time, there is a growing interest in tracing cultured pearls through the supply chain, so that an end consumer knows which farm their cultured pearls came from. Producers who operate responsibly are investigating ways of marking their cultured pearls so that provenance can be guaranteed to the end consumer.

Any method used to trace cultured pearls must largely be invisible so as to maintain the commercial value of the end products. Cultured pearls are produced both with a nucleus (e.g., Akoya, South Sea and Tahitian) and without a nucleus (e.g., Chinese freshwater beadless products); for general reviews, see for example Gervis and Sims (1992) and Southgate and Lucas (2008). Different labelling/traceability approaches may be required for these two types of cultured pearls, based on their internal structure. This article reviews a wide range of methods — chemical, physical and biological — that potentially could be used in tracing cultured pearls through the supply chain.

Chemical marking

Pearls consist of fine polycrystalline calcium carbonate ($CaCO_3$) crystals and traces of organic matter. The mother-of-

Figure 1: A branded necklace of South Sea cultured pearls (12 mm in diameter) produced by Atlas Pearls in northern Bali and West Papua (Indonesia). Photo courtesy of Atlas Pearls, Claremont, Western Australia.

Figure 2: Cross-section of a 'chocolate' beaded cultured pearl. The light-coloured bead (i.e., nucleus) and the darker overgrowth are clearly visible. It is evident in the enlarged image at the bottom right that the brown colour has been artificially added. This demonstrates the porosity of a cultured pearl and its potential for absorbing chemically doped or colour-doped solutions. The colour has penetrated approximately 0.5 mm. Photo by H. A. Hänni.

pearl (also called nacre) surface of pearls is made up of aragonite tablets. A pearl's porous structure means that it has a good potential for absorbing chemically doped or colour-doped solutions. A good example of this are dyed cultured pearls (e.g., *Figure 2*), which can be found in many different colours (Hänni, 2006; Strack, 2006). In a similar way, cultured pearls from selected producers could be marked using a colourless doped solution — that is unique to a pearl producer — after harvest. If chemically doped, these pearls could later be identified in a gemmological laboratory using EDXRF spectroscopy (Hänni, 1981). However, the applicability of this approach is limited given that EDXRF spectroscopy is not in widespread use in the jewellery industry.

Alternatively, rather than marking the cultured pearl after harvest, one could mark the nucleus before insertion using a specific solution. However, if the nacreous overgrowth is too thick, it may not be possible to identify the chemical signal from the nucleus. Another approach would be to remove a tiny amount of nucleus material from a drilled cultured pearl for chemical analysis.

The authors have experimented with the diffusion of fluoroamine (NH_2F) into a cultured pearl, something a pearl farmer could easily do. The subsequent detection of fluorine could then be linked back to that farm. Fluorine is a relatively light element that is not detectable by EDXRF spectroscopy, but is best analysed by nuclear magnetic resonance (NMR). However, NMR is cost-intensive and the instrument's sample chamber is typically smaller than the diameter of a cultured pearl.

If only a limited number of pearl farms are involved in such chemical marking of their cultured pearls, it could be viable to supply each of them with different cost-effective and nontoxic chemicals that could be detected in a gemmological laboratory.

Labelling the nucleus or the surface of a cultured pearl

Initial experiments using physical labels affixed to a cultured pearl nucleus were carried out in 2010 by author HAH. Thin (0.05 mm) rings consisting of gold wire were affixed to several Mississippi shell nuclei (the nucleus material commonly used in the pearl industry) and used to produce cultured pearls. The aim was to investigate the possible rejection of labelled nuclei by the molluscs and to see whether this gold label (or the associated adhesive) would influence cultured pearl growth. Results after six months showed that the labelling materials (gold and glue) had no influence on cultured pearl production and this spurred further efforts to investigate the production of nucleus logos.

Any such logo marker must be extremely thin, be composed of noble metal (and therefore be resistant to corrosion) and have the same convex shape as the nucleus to ensure that the resulting cultured pearl is also round. However, the production of such round metal labels, generally 3–4 mm wide and 0.05 mm thick, is relatively expensive. Different label production techniques were tested, such as galvanic production, pressing, etching and cutting with a

Figure 3: Silver logo labels (3 mm in diameter) for a pearl farm. These can be affixed onto the bead prior to insertion and later be used to trace a beaded cultured pearl back to its farm. Photo by H. A. Hänni.

laser or water jets; these are widely used techniques in manufacturing (Schultze and Bressel, 2001). The water jet technique was most precise for cutting the contours of the logo, but still considered too expensive.

Several dozen logo tags (e.g., *Figure 3*) were affixed to shell nuclei and sent to different marine farms to be tested in cultured pearl production. After the usual 12–18 month growth period, these 'tagged' cultured pearls were harvested and successfully examined with X-radiography *(Figure 4)*. Due to the position of the logo in the peripheral part of a cultured pearl, there is only a statistically small chance of the logo being damaged during drilling.

The production of such logo markers is relatively expensive, even if produced in large quantities. In addition, these cultured pearls need to be tested using X-rays, which is relatively unfeasible for a jeweller. (X-rays used for medical purposes, such as in dentistry, are not strong enough to visualize all required details within a cultured pearl of, e.g., 10 mm.) Nevertheless, for beaded cultured pearls that use a nucleus (e.g., Akoya, South Sea and Tahitian), this method is an option. For beadless cultured pearls (e.g., Chinese freshwater cultured pearls), the introduction of a label together with the saibo (donor mantle tissue) would have the disadvantage of positioning the logo in the centre of the cultured pearl, resulting in a high likelihood of damage during the drilling process.

Another approach is to mark the surface of the cultured pearl rather than the nucleus. This could involve either laser engraving with a unique number (similar to laser inscriptions on diamonds) that can later be used to identify its source or embossing a hologram onto the surface of the cultured pearl that can be read with a suitable reader. Both of these methods are currently being investigated in French Polynesia ('Redonner ses Lettres…', 2013; 'Le Tahiti Pearl Consortium Disparaît', 2013). These methods are slightly destructive to a cultured pearl's surface and it remains to be seen if they are acceptable to the pearl trade.

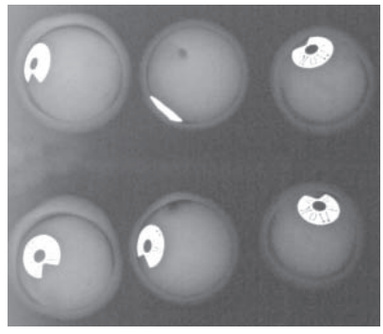


Figure 4: X-radiographs of three Tahitian cultured pearls with a branded nucleus. The farm-specific logos are in silver, which has a high density making it quite visible with X-rays. Three cultured pearls are shown in two slightly different orientations in this composite image. The diameter of the cultured pearls is approximately 8 mm and the width of the logos is 3 mm. Image by H. A. Hänni.

RFID – radio frequency identification

Radio frequency identification (RFID) technology has undergone rapid development in the past decade and is now a widely used method in many technology applications (Want, 2006). It is increasingly being employed in jewellery management solutions (Wyld, 2010). Through the miniaturization of RFID chips (transponders in millimetre sizes), the use of electromagnetic frequencies is a feasible option for the tagging/traceability of cultured pearls. Transponders are chips that contain relevant data which can be accessed with an RFID reader. These devices are inexpensive and they could be easily used in jewellery retail stores ('June HK Fair Special…', 2013). Information stored on the chips could include the production location, harvest date and details about the pearl farm. Additional information can be added to the RFID chip after a cultured pearl has been harvested, including its quality grade, inventory data and unique identification information that could be useful for theft recovery.

RFID chips have been introduced into commonly used Mississippi shell nuclei, which are currently being piloted by pearl farmers in the Pacific Ocean.

Figure 5: A composite shell bead that has been sliced and polished to show a small RFID chip (3 mm long) embedded within it. The information on such a chip can be accessed using an RFID reader. Photo by H. A. Hänni.

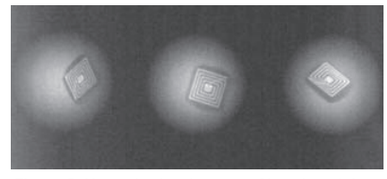


Figure 6: X-ray shadow images of bead nuclei (7.5 mm diameter) consisting of pieces of shell with embedded RFID chips. These are being marketed by Fukui Shell Nucleus Factory. Image by H. A. Hänni.

One nucleus manufacturer (Fukui Shell Nucleus Factory, Hong Kong) has already brought to market nuclei that contain RFID chips (see 'June HK Fair Special…', 2013). *Figure 5* shows such a 'micro-chip embedded nucleus' which, depending on its size, costs US$2–3 per piece. According to the manufacturer, these nuclei consist of two layers of shell material (i.e., laminated nuclei) and a 3 mm RFID chip that is located 1 mm below the surface of the nucleus *(Figure 5)*. *Figure 6* shows an X-ray shadow image of such chip-embedded nuclei.

One disadvantage of these nuclei is the relatively high cost of the chips, which would be wasted in cultured pearls of low quality. Also, the 3 mm size of the straight-edged chips is rather large when taking into account that the nucleus has a spherical shape. The size and position

*Table I: LA-ICP-MS analyses of cultured pearls and shells from various species and locations.**

Habitat		Saltwater														
Sample		*Pinctada maxima* (silver) shell			*Pinctada maxima* (gold) shell			*Pinctada radiata* shell			*Pinctada margaritifera* cultured pearl			*Pinctada margaritifera* cultured pearl		
Source		Indonesia			Philippines			United Arab Emirates (RAK)			Rangiroa, French Polynesia			Fiji		
CaO	wt.%	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03
Na_2O	wt.%	0.91	0.91	0.88	0.84	0.84	0.86	0.97	0.92	0.95	0.75	0.75	0.74	0.90	0.86	0.93
Li	ppm	0.53	0.40	0.40	0.40	0.33	0.23	0.56	0.50	0.56	0.38	0.44	0.40	0.57	0.64	0.69
B	ppm	18.2	20.5	21.2	15.1	15.1	15.6	1.8	1.9	1.7	10.7	10.8	10.6	16.3	14.6	14.0
Mg	ppm	169	180	199	115	115	121	476	392	463	206	198	226	120	98	98
P	ppm	34.0	33.0	35.0	6.6	6.5	6.6	73.2	71.5	71.7	13.8	13.2	12.9	13.3	13.5	12.7
K	ppm	71.1	63.6	51.1	131	157	180	104	118	106	60.0	51.2	42.7	82.7	87.2	83.7
Cr	ppm	2.3	1.9	2.1	2.0	1.9	1.8	2.0	1.8	2.0	2.5	2.2	2.2	2.3	2.4	2.2
Mn	ppm	3.4	3.3	3.4	6.2	6.6	6.2	0.19	0.18	0.18	1.3	1.4	1.4	88.5	92.9	56.9
Fe	ppm	18.4	19.6	17.3	18.3	19.1	22.8	15.6	18.1	18.1	26.9	26.7	29.0	25.4	26.8	28.3
Cu	ppm	0.14	0.08	<0.06	0.09	0.11	0.10	0.26	0.35	0.29	0.11	0.10	0.06	0.04	0.08	0.07
Zn	ppm	<0.08	0.34	0.41	0.38	0.44	0.55	0.59	0.73	0.59	0.28	0.21	0.45	<0.05	0.31	0.12
Sr	ppm	1030	1070	1130	1040	1040	1080	802	775	795	166	169	163	964	138	113
Ba	ppm	0.35	0.49	0.43	0.28	0.26	0.30	0.17	0.15	0.23	0.28	0.37	0.30	0.19	0.39	0.23
Pb	ppm	0.12	0.10	0.10	3.0	3.6	14.9	0.13	0.13	0.12	0.06	0.05	0.05	0.05	0.05	0.04
Ag	ppm	<0.003	<0.004	<0.006	<0.005	0.01	0.01	<0.009	<0.009	<0.004	<0.004	<0.004	<0.004	<0.005	<0.005	<0.005

Habitat		Saltwater						Freshwater								
Sample		*Pteria sterna* shell			*Pteria penguin* shell			*Hyriopsis* shell			*Unio* shell			Ming cultured pearl		
Source		Mexico			Irian Jaya, Indonesia			China			Scotland			China		
CaO	wt.%	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03	56.03
Na_2O	wt.%	0.97	0.95	0.88	0.67	0.66	0.70	0.28	0.28	0.28	0.25	0.25	0.25	0.34	0.34	0.35
Li	ppm	0.64	0.57	0.47	0.47	0.56	1.14	<0.07	0.12	0.09	<0.07	<0.09	<0.08	<0.03	0.07	<0.03
B	ppm	5.0	5.5	7.3	8.5	8.5	9.7	4.0	4.0	3.5	3.7	4.4	4.4	0.20	<0.13	<0.14
Mg	ppm	132	73	57	165	147	112	23.9	23.5	15.4	18.3	18.7	56.4	14.0	20.0	18.0
P	ppm	123	122	135	158	150	121	192	183	188	100	113	127	183	191	160
K	ppm	65.7	66.0	65.0	41.8	40.8	50.5	19.6	8.9	5.6	34.6	66.4	95.7	19.0	28.0	6.0
Cr	ppm	2.2	2.0	2.0	2.1	2.2	2.2	2.1	1.7	1.6	2.3	1.4	1.8	2.3	2.6	2.3
Mn	ppm	1.7	1.5	0.8	1.6	1.5	2.2	661	1580	861	519	501	477	133	268	137
Fe	ppm	11.6	13.4	12.8	12.4	12.4	13.7	13.3	12.3	15.1	14.9	16.3	16.3	28.0	29.8	32.4
Cu	ppm	0.10	0.10	0.07	0.06	0.05	0.05	0.17	0.20	0.22	0.33	0.25	0.38	0.25	0.41	0.38
Zn	ppm	0.41	0.40	0.56	0.43	0.33	0.30	0.29	<0.11	0.18	0.67	0.26	0.53	2.3	2.6	1.8
Sr	ppm	1000	908	923	1280	1320	1170	761	877	360	250	260	267	305	446	409
Ba	ppm	0.20	0.17	0.90	1.11	0.96	1.03	247	288	225	469	516	543	57.6	117.3	76.2
Pb	ppm	0.36	0.45	0.40	0.27	0.24	0.29	0.02	0.02	0.02	0.12	0.07	0.14	0.05	0.06	0.13
Ag	ppm	<0.003	0.005	<0.003	<0.002	<0.003	<0.003	<0.006	<0.003	<0.005	<0.004	<0.004	<0.005	<0.02	<0.02	<0.02

* CaO = 56.03 wt.% was used as an internal standard on the basis of the $CaCO_3$ formula for aragonite and calcite. Ag was measured to identify cultured pearls dyed black using silver nitrate. Iodine was analysed in all samples but could not be quantified for lack of an iodine standard. Be, Al, Sc, Ti, V, Co, Ni, As, Rb, Y, Cd, REEs (La, Ce, Nd, Tb, Yb and Lu), and Bi were measured at or just below the detection limit (sub-ppm). Each sample was analysed in three different spots, corresponding to the three columns for each sample.

of these chips within the nucleus means they may often be damaged during the cultured pearl drilling process. Rapid developments in RFID technology are promising, but we may need to await the further miniaturization of the chips before they become a feasible option for the cultured pearl industry.

Advanced fingerprinting of pearl and shell materials

Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) has become more widely used in the last few years in geosciences, even in gemmology (e.g., Saminpanya *et al.,* 2003; Abduriyim and Kitawaki, 2006). Many laboratories and researchers now employ it for the chemical characterization of gems because it has a low detection limit and can also detect light elements. With this method it is possible to carry out high-resolution spot analyses, which allows us to take into account possible chemical zoning in gem materials, including cultured pearls. The technique has been used for characterization of cultured freshwater pearls (Jacob *et al.,* 2006) and natural saltwater pearls from Australian *Pinctada maxima* molluscs (Scarratt *et al.,* 2012). To our knowledge, there are no published LA-ICP-MS data on a wider range of cultured pearls or shell samples from various mollusc species.

For this study, a preliminary LA-ICP-MS investigation of cultured pearls and shell material was undertaken at the University of Bern. The instrumentation used a 193 nm ArF laser, and synthetic glass (SRM612) was used as a standard for calibration before and after each round of measurements. This was also done to ensure the reproducibility of measurements and detect possible impurities in the chamber that might affect subsequent data. The pits produced on the surface of the samples during ablation had a diameter of 160 µm. As such, the technique is quasi-nondestructive.

Table I lists the results for the seven shell samples and three cultured pearls from different locations that were analysed. It is clear that further research is required to compile a useful LA-ICP-MS database that might permit origin determination of cultured pearls from different species.

Figure 7: The Atlas Pearl farms that produced the necklace shown in Figure 1 *are located in Bali (shown here) and West Papua, Indonesia. Giving consumers access to the origin of their cultured pearls may create additional value for pearl farmers. Photo by L. Cartier.*

Another possible (and nondestructive) method for chemically fingerprinting gem materials is particle-induced X-ray emission (PIXE), which has been applied to ruby and emerald (Calligaro *et al.,* 1999; Yu *et al.,* 2000). More recently, PIXE was used on cultured pearls (Murao *et al.,* 2013). Other studies have measured oxygen and carbon isotopic values of nacre and cultured pearls in an attempt to identify geographic origin (Yoshimura *et al.,* 2010). However, all these techniques remain academic and expensive, and they presently do not fulfil the requirements for a rapid and cost-effective tracing method for cultured pearls.

A final method that is very new but merits description is DNA fingerprinting of cultured pearls. Oyster shells and pearls have a biological origin and contain small amounts of organic matter between aragonite layers and in the form of organic pockets. A recently published study described how DNA can be extracted from this organic material in cultured pearls in a practically nondestructive manner (Meyer *et al.,* 2013). The DNA can be used to identify the oyster species of the cultured pearl and the authors also proposed that geographic origin determination might also be possible using next generation sequencing (NGS) techniques in the near future. A similar approach has been used for geographic origin and species determination of ivory (Wasser *et al.,* 2004).

Conclusion

The aim of this review is to show the range of currently available methods that potentially could be used to trace cultured pearls through the supply chain. Supply chain accountability and product traceability are becoming increasingly important issues in the jewellery industry. The branding strategies of various producers, wholesalers and jewellery companies would benefit from additional support through an efficient traceability method. Furthermore, there is a potential for responsible pearl farmers (e.g., *Figure 7*) to capture greater value for their products if they can be traced all

the way to the consumer, but the supply chain accountability and provenance need to be guaranteed (Conroy, 2005; Cartier 2012; Cartier and Ali, 2012). As technology continues to evolve, the search for methods to trace cultured pearls through the supply chain should be addressed in collaboration with the gemmological community and the focus should be on developing cost-effective solutions that are feasible for those at all levels of the supply chain (producer, wholesaler, retailer and consumer).

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CHAPTER 8

Final remarks and outlook

Marine cultured pearl farms are found in the Pacific and Pacific Rim region. Cultured pearls have become a billion-dollar industry and experienced a tremendous boom in recent decades. Pearl oysters are often farmed in remote areas that offer pristine ecological conditions. They are farmed in both bays and coral-rich lagoons, where the right mix of required nutrients and sheltered conditions can be found for them to thrive and produce beautiful cultured pearls. High-quality cultured pearls can only be produced under optimal ecological- and labour conditions.

Cultured pearl farming and surrounding services have become a vital source of income and significantly contributed to economic development in a large number of remote coastal communities in the Pacific region. Appropriate management and marketing strategies need to be adopted so that it remains successful and profitable in the long-term. Thereby also providing economic opportunities in remote islands and beautiful cultured pearls for the trade and end consumers.

Whilst average prices of different qualities of marine cultured pearls have significantly dropped in recent years, this has also created demand for more sustainable trading relationships (Kugelmann & Poirine, 2003; Brodbeck, 2010; Müller, 2013). Sustainability branding and certification of responsibly produced cultured pearls offers a promising opportunity for certain producers in the marine cultured pearl industry (Cartier et al., 2012; Nash, 2013).

Though the global pearl industry presents a fragmented supply chain, the luxury consumer is fundamentally linked to the livelihood of producers and the environment in which the pearls are grown. Pearl oyster farming has -due to the requirements it needs for it to prosper and it's renewable nature as a resource- a good potential to meet sustainability criteria and foster responsible luxury.

Developing pearl farming in the Pacific

A number of Asian and Pacific nations have attempted to emulate successes in Australia and French Polynesia by setting up 'South Sea' and 'Tahitian' pearl oyster farming operations of their own, either through private or government investment. The motive for this -for example in Kiribati, Tonga, Solomon Islands, Cook Islands, Micronesia, and Marshall Islands is the potential of pearl farming as a potentially high-value activity that can be carried out in remote coastal areas and has low environmental impact. There are numerous chal-

lenges associated with developing a pearl industry and introducing community pearl farms as aid projects, as experiences in Micronesia and elsewhere have shown (Cartier et al., 2012). Small producers have problems with market access, unlike some of the large-scale pearl farms in the region. Pearl farming can be viable development path for certain coastal communities but it must be planned and managed with understanding of the obstacles and opportunities of pearl farmers in a social, environmental and economic context.

Pearl farms as business models for marine protected areas (MPA)

Marine pearl oysters are farmed in both bays and coral-rich lagoons, where the right mix of required nutrients and sheltered conditions can be found for them to thrive and produce beautiful cultured pearls. The interaction between the pearl oysters and the marine environment is intimate and these oysters are vulnerable to even subtle environmental changes. A prime example of this fragile equilibrium is Lake Biwa and Ago Bay in Japan, which have been a victim of its own success (Yoshimura et al., 2010). It was partially in Ago Bay that the process of culturing pearls was developed in the early 20th century. Economic activity and pearl overproduction led to pollution, which eventually resulted in recent years to a drastic reduction in pearl quality and the eventual death of great numbers of oysters (Prokop, 2005). The economic viability and long-term future of pearl farming ventures is directly dependent on a healthy marine environment; there is a clear economic incentive for long-term marine conservation in ecosystems that are very vulnerable to environmental degradation. Marine Protection Areas (MPA) have been widely heralded as a solution for marine conservation, but MPAs often fail because there are few tangible benefits for local communities to conserve the waters (Kareiva, 2006). There are few economic activities that can be as ecologically sustainable and offer valuable employment opportunities in remote coastal areas of the Pacific. Cartier and Carpenter (2014) have shown that fish abundance is slightly higher around pearl farming areas in a study of Ahe (French Polynesia) and that pearling shows no significant impact on reef fish diversity (see Chapter 4). Based on this empirical evidence, pearl farming could be integrated into hybrid MPAs and thereby offer a viable business models for MPAs in select areas, as advocated by Sala et al. (2013).

Tracing pearls from farm to consumer

Traceability and supply chain accountability are becoming increasingly important themes in the jewellery industry (Kapferer, 2009). A number of pearl farmers are branding their pearls in order to capture a greater share of the pearl's value. Developing methods to support this trend is important. A wide variety of presently available and new methods (e.g. DNA testing of pearls) are being tested to determine how best a pearl could

be traced from farm to consumer (Chapter 7; Hänni and Cartier, 2013).

Outlook

This dissertation is part of a larger project that is investigating how the positive ecological and socio-economic benefits of pearl farming could be extended and supported, both through institutional and market-based mechanisms. This dissertation has formed the basis for sustainability principles for pearl farming that have been developed and will be presented in 2014. This research thus forms the beginning of a larger journey in fostering the emergence of marine cultured pearls as sustainable gemstones. There are several aspects that need to be pursued. The industry is currently undergoing huge transformations due to globally induced economic (e.g. lower demand and overproduction) and environmental changes (e.g. pollution, climate change), and must revert to a high-quality production of pearls in order to prosper sustainably.

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Table S1: List of all observed different fish species at 16 studied sites in Ahe, French Polynesia. Coding was following: single=1, few=10, many=100, abundant=1000.

	St1	St2	St3	St4	St5	St6	St7	St8	St9	St10	St11	St12	St13	St14	St15	St16
	Impacted, SPass	Impacted, SPass	Impacted, SPass	NoDirect, NPass	Impacted, NPass	Impacted, NPass	Impacted, NPass	NoDirect, NPass	NoDirect, NPass	NoDirect, SPass	NoDirect, SPass	NoDirect, NPass	NoDirect, NPass	NoDirect, NPass	Impacted, NPass	NoDirect, NPass
Triaenodon obesus						1								1		1
Aetobatus narinari					1											
Chanos chanos			1													
Gymnothorax javanicus												1		1		
Synodus variegatus		1			1	1		10			10	10	10			
Crenimugil crenilabis		10													10	
Tylosurus crocodilus								1								
Neoniphon opercularis	100	10			100	10	10	10							100	1
Neoniphon sammara			10													
Sargocentron spiniferum	10	10	100	100	10	10	10		10	10	1	100			100	
Myripristis berndti				100												
Myripristis kuntee	100	1000	100		100	100	100	100	100	10	100	100	100		100	100
Myripristis violacea	100	10	100		10			100	10			10				10
Fistularia commersonii	1		1			1								1		
Aulostomus chinensis		10	10		1	1			1			10	1	1	1	
Heteropriacanthus cruentatus				100												
Priacanthus hamrur									10	10						
Cheilodipterus quenquelineatus		10				10										
Cephalopholis	10	100	100	100	1	10	10	100	100	100	10	100		10	100	10

argus																
Epinephelus fasciatus																
Epinephelus merra	10	1		1	10	1						1	10	10		1
Epineph. polyphekadion	10	100	100	10	100	100	10	10	10	10	100		10	10	100	10
Plectropomus laevis									1							
Plectropomus leopardus	1			1	10						1		1	10	1	1
Caranx melampygus		100	10				10				10		10	10		
Decapterus macrosoma										100	100					
Aphareus furca			10		1	1	1	1					1	1	1	
Aprion virescens					10			10		10	10					10
Lutjanus bohar		10														
Lutjanus fulvus	1000	10	10				100	100				100				
Lutjanus gibbus	1000	100		100	1000	10	100	100	100	100	100	100	10	10	1000	1000
Lutjanus kasmira												10				
Lutjanus monostigma	100	10	10	10	100	100	100	100	10	10	10	100	10	10	100	100
Lethrinus atkinsoni																
Lethrinus olivaceus	1		10		10	1	10	1				10	1	10	10	
Monotaxis grandoculis	1000	1000	10	100	1000	100	1000	100	100	100	100	1000	100	100	1000	1000
Mulloidich. flavolineatus	1000	1000		1000		100	100	100	100	100	100		1		1000	1000
Mulloidich. vanicolensis								10			1000					
Parupeneus barberinus	10	1	10		10	1			1		1	1	1		1	1
Parupeneus cyclostomus												1				10
Parupeneus insularis				10						1	1					
Parupeneus multifasciatus	100	1000	100	100	100	10	100	100	100	100		100	100	100	100	100
Chaetodon auriga	10	100	100	100	100	100	100	100	100		10	100	100	100	100	100
Chaetodon bennetti	1		1	10	10	100	100	1	10	10	10		10	1	10	10
Chaetodon			100	10	10	10	10	10	10	10	10	100	100		100	10

citrinellus																
Chaetodon ephippium	100	100	10	10	100	1000	100	100	10	10	10	100	10	100	100	100
Chaetodon lineolatus			10													
Chaetodon lunula	100	100	100	10	1000	1000	1000	100	100	100	100	100	100	100	100	100
Chaetodon lunulatus	100	10	100	10	10	100	100	100	100	10	10	100		100	100	100
Chaetodon ornatissumus		1	10									1	10			
Chaetodon reticulatus			10									10	10			
Chaetodon semeion	1	10														
Chaetodon trifascialus									10			1		10		
Chaetodon ulietensis	1000	1000	1000	100	100	100	100	100	100	100			100	100	100	100
Chaetodon unimaculatus												100				
Chaetodon vagabundus	100															
Forcipiger flavissimus			10					10	10			100	10			
Heniochus chrystostomus	10		100	10				10	10		10	10	10	1		
Heniochus monoceros	10	10	10	10	10	10	10	10	10	10		10	10	10		10
Centropyge flavissima	10	10	100	10	100	100	100	100	10	10	10	100	100	100	100	100
Centropyge loricula																
Pygoplites diacanthus	1		1	10			1	1	10	10	10	10	10		1	10
Kyphosus cinerascens		10														
Sectator ocyurus																10
Abudefduf septemfasciatus		100			10		10									
Abudefduf sexfasciatus	1000			1000	100	1000	1000	1000	1000		1000	10	100	1000	1000	1000
Amphiprion chrysopterus				10												

Chromis atripectoralis	10													10		
Chromis iomelas				1				10	10			1				
Chromis margaritifer	10	100	1000	100	100	100	100	100	1000	10	10	1000	1000	1000	100	100
Chromis viridis	100				1000	1000	1000		100	100	100	1000	1000	1000	1000	1000
Dascyllus aruanus	100	100	1000	100	1000	1000	1000	100	100	100	100	1000	1000	1000	1000	1000
Dascyllus flavicaudus	1		100	100	100	100	100	100	100	10	100	1000	1000	1000	10	100
Dascyllus trimaculatus				100												
Plectroglyphidodon dickii												1				
Pomacentrus coelestis		100	100	100	10	1000	1000		100	100	10	10	10		100	100
Pomacentrus pavo	100				1000	1000	1000							100	100	100
Stegastes fasciolatus		10	100				100	10		100		10	100	100	100	100
Anampses caeruleopunctatus					1	1	1				1		1	1		1
Bodianus anthioides												1				
Bodiaus axillaris			1	10		1		10	10		1		1			
Cheilinus chlorurus	10	10		10	10	100	100	100	10	100	100		10	10	100	100
Cheilinus trilobatus		10			10	10	10	10		10	10			10	10	
Cheilinus undulatus		1						1			1			1		10
Cirrhilabrus exquisitus									10			1			10	
Coris aygula		1		1											1	1
Epibulus insidiator	10		10	1			10	10	10	10	10			1	10	10
Gomphosus varius	10	10			10	10	10	10	1	10	10	10	10	10	10	10
Halichoeres hortulanus	10		10	10		10		10	100	10		100	100		10	10
Halichoeres ornatissimus									10			10				
Halichoeres trimaculatus					10	10		10	10		1		10	10	100	100
Labroides bicolor	1	10	10	10	10	1	10	10	10	10	1	10	10	10	10	10
Labroides dimidiatus	10	10		100	100	100	100	100	100	100	100	100	100	100	100	100
Oxycheilinus bimaculatus					1											

Pseudocheilinus evanidus		10	10	1	100	10	10	10				10	10	10		
Pseudocheilinus hexataenia						10	10					10				
Stethojulis bandanensis					1	1			10		1					
Thalassoma amblycephalum	100	100	100	100	100	100	100	10	1000	100	100	100	100	1000	1000	100
Thalassoma hardwickii	100	1000	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Thalassoma quinquevittatum								1	10			10	10	10		
Cetoscarus ocellatus	10		10	10	10		100	10	10	10		10		10	10	10
Chlorurus frontalis	10		100	10		10	100	100	100	100	100	100	100	100		100
Chlorurus microrhinos	100	100	100		100	1000			100	100	100		100	100	1000	100
Chlorurus sordidus	100	100	100	10	100	100	100	100	100	100	100	100	100	100	100	100
Hipposcarus longiceps	100	10			10	10	10	10	10	10		10	100	10	100	100
Scarus altipinnis		100											100	100	10	
Scarus ghobban	100	100	10	100	100	100	100	100	100	10	100	100		100	100	100
Scarus niger	10	100	100	10		100	100	10	10	10	100	100	100	10	100	100
Scarus psittacus																
Scarus schlegeli	100	10	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Amblygobius phalaena			1											1		
Ctenogobiops feroculus									1							
Eviota afelei							1									
Gnatholepis anjerensis															1	
Nemateleotris magnifica									10				10			
Ptereleotris evides		10	10		100	100	100	100	10				100	100	100	100
Ptereleotris heteroptera																
Ptereleotris monoptera		10	10		1000	1000	100	100			100				1000	100
Acanthurus achilles			10													
Acanthurus mata	1000	100	100	1000	1000	100	100	1000	1000	1000	1000	1000	100	100	1000	1000
Acanthurus	10	100	100	100	100	100	100	100	100	10	10	100	100	100	100	100

nigricaudus																
Acanthurus nigrofuscus	10															
Acanthurus olivaceus					10					1					100	
Acanthurus pyroferus								10								1
Acanthurus thompsoni								100	100		1	100	100			
Acanthurus triostegus		1000	1000	10		100	100	10	10	1			100	100	100	10
Acanthurus xanthopterus	1000	100	10		100		100	10			10				10	
Ctenochaetus binotatus													10			
Ctenochaetus flavicauda					10	100	100		100	1		1	1		100	100
Ctenochaetus striatus	1000	100	1000	1000	100	100	100	100	100	100	100	100	100	100	100	
Naso annulatus																
Naso brevirostris	100	100		100	1000	1000		1000	1000	1000	1000		100	100	1000	100
Naso hexacanthus							1									
Naso lituratus	10	10	1000	10	10	10	10	10	10	10		10	10	10		10
Naso vlamingii	10								10		1					
Zebrasoma scopas	10	10	10	10	100	10	10	100	100	10	100	100	100	100	100	100
Zebrasoma veliferum	10	10	10	10	10	100	100	100	10	100	100	10	100		100	10
Siganus spinus																
Zanclus cornutus	100	100	10	100	100	100	100	100		100	100	100	100	100	100	100
Sphyraena qenie																
Balistapus undulatus	10	1	10					1				10	10	10		1
Balistoides viridescens	10	10	10	1	10		10	10	10	10	10	1	1	10	100	
Melichthys vidua									1							
Pseudobalistes flavimarginatus	10	10			10		10	1	1			1				1
Pseudobalistes fuscus																
Rhinecanthus aculeatus			1		10	10	100							10	10	
Sufflamen bursa											1					
Aluterus scriptus			1													
Arothron meleagris					1							1				
Canthigaster solandri					10			10								
Canthigaster valentini			10			10		10	10				10	10	10	10
Total number of observed species	66	67	69	58	72	70	68	75	74	57	63	72	71	69	70	70

Figure S1. Pearls from *P. margaritifera* (PMR), *P. maxima* (PMX) and *P. radiata* (PR) used in method C (Fig. 2).

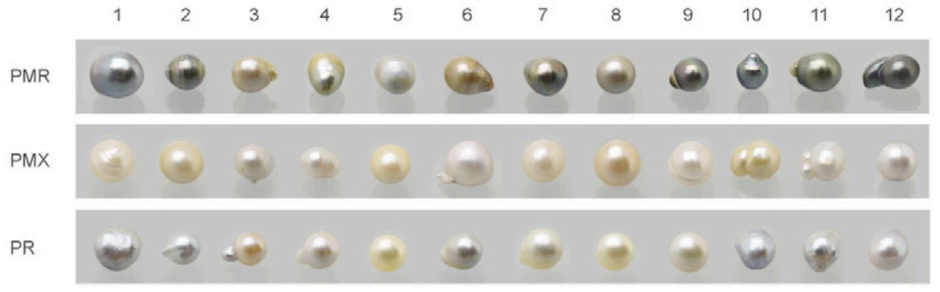

Table S1. Blind test: PCR-RFLP and analysis of the ITS2 sequences from eighteen pearls of unknown identity.

Pearl sample	Pearl weight (carats / mg)	Sample weight (mg) [a]	ITS2-RFLP [b] results	ITS2 [b] rRNA sequence match	GenBank accession number	Correctly identified?
BL1	16.8 / 3358	103	*P. maxima*	*P. maxima* (AY883851.1) [c] 461 bp (100 %) [d]	KF284027	*yes*
BL2	10.5 / 2092	158	*P. margaritifera*	*P. margaritifera* (AY877506.1) 292 bp (100 %)	KF284028	*yes*
BL3	10.1 / 2012	61	*P. maxima*	*P. maxima* (AY877504.1) 492 bp (99 %)	KF284029	*yes*
BL4	9.2 / 1834	115	*P. margaritifera*	*P. margaritifera* (AY877506.1) 437 bp (100 %)	KF284030	***no***
BL5	9.1 / 1816	165	Not determined	Not determined		***no***
BL6	8.8 / 1754	146	*P. radiata*	*P. fucata* [e] (AB214478.1) 207 bp (100 %)	KF284031	*yes* *yes*
BL7	8.3 / 1658	417	*P. radiata*	*P. fucata* (AY877582.1) 538 bp (99 %)	KF284032	*yes*
BL8	7.8 / 1550	672	*P. margaritifera*	*P. margaritifera* [f]		*yes*
BL9	7.3 / 1456	178	*P. radiata*	*P. fucata* (AY877582.1) 511 bp (99 %)	KF284033	*yes*
BL10	6.8 / 1354	38	*P. margaritifera*	*P. margaritifera* [f]		*yes*
BL11	6.6 / 1324	547	*P. margaritifera*	*P. margaritifera* (AY877506.1) 437 bp (100 %)	KF284034	*yes*
BL12	6.0 / 1198	154	*P. margaritifera*	*P. margaritifera* (AY877506.1) 435 bp (100 %)	KF284035	*yes*
BL13	5.8 / 1154	615	*P. margaritifera*	*P. margaritifera* (AY877506.1) 437 bp (100 %)	KF284036	*yes*
BL14	4.3 / 868	142	*P. maxima*	*P. maxima* (AY877505.1) 494 bp (100 %)	KF284037	*yes*
BL15	4.3 / 856	84	*P. maxima*	*P. maxima* (AY877505.1) 494 bp (100 %)	KF284038	*yes*
BL16	4.1 / 818	184	*P. radiata*	*P. fucata* (AY877582.1) 489 bp (99 %)	KF284039	*yes*
BL17	3.3 / 652	170	*P. radiata*	*P. fucata* (AY877582.1) 538 bp (99 %)	KF284040	*yes*
BL18	2.5 / 504	88	*P. radiata*	*P. fucata* (AY877582.1) 489 bp (99 %)	KF284041	*yes*

[a] the sample used for DNA extraction consisted of material extracted from the interior of the pearl, including ground nacre and OM.

[b] ITS2: nuclear internal transcribed spacer 2 located between the 5.8S and 28S ribosomal RNA genes. RFLP: restriction fragment length polymorphism.

[c] *Pinctada* species assignment was based on the highest BLAST score (highest query coverage and maximal base pair identity). GenBank accession number shown in brackets.

[d] amplicon size (base pair) and maximal identity (%) of the sequence to the BLAST query.

[e] *P. fucata* is conspecific to *P. radiata* according to their ITS sequences [50].

[f] sample had low sequence quality, but the BLAST query in GenBank recovered the correct *Pinctada* species.

Table S2. PCR primers, amplicon lengths and references.

Gene product	Organellar localization	Primer sequence (5'-3')	Primer name	Tann (°C)	Fragment length (bp)	Reference
16S rRNA [a]	Mitochondrial	CGCCTGGTTGATTAAAAACATTGCTGC	16S pinctada for	55	PMR: 511	[49], [50]
		CCGGTTTGAACTCAGATCACGTA	16S pinctada rev		PMX: 509	
					PR: 524	
cox1 [a]	Mitochondrial	TCGTATAGAGCTCCGTCGACCTG	LCX	45	PMR: 576	[45]
		TGGAACAAAACTGGATCGCC	HCY		PMX: 576	
					PR: 576	
ITS1 [a]	Nuclear	CACACCGCCCGTCGCTACTA	sp-1-5	52	PMR: 675	[44], [51]
		ATTTAGCTGCGGTCTTCATC	sp-1-3		PMX: 701	
					PR: 627	
ITS2 [a]	Nuclear	GCAGGACACATTGAACATCG	5.8S-F	52	PMR: 575	[51]
		CCAAGGACGTTCTTAGCAGAAG	28S-R		PMX: 571	
					PR: 590-591	
P. margaritifera ITS2 [a]	Nuclear	CTGTTCTGTCATGACGACGG	ITS2-Marg-F	52	PMR: 335	This study
P. maxima ITS2 [a]	Nuclear	GGGCCTATTTCCGTGTTGAG	ITS2-Max-F	52	PMX: 332	This study
P. radiata ITS2 [a]	Nuclear	CTGTCGATGGATGACTTACACG	ITS2-Rad-F	52	PR: 336-337	This study

[a] 16S rRNA: mitochondrial 16S ribosomal RNA gene; *cox1*: mitochondrial cytochrome oxidase subunit I gene; ITS1: nuclear internal transcribed spacer 1 located between the 18S and 5.8S ribosomal RNA gene; ITS2: nuclear internal transcribed spacer 2 located between the 5.8S and 28S ribosomal RNA genes.

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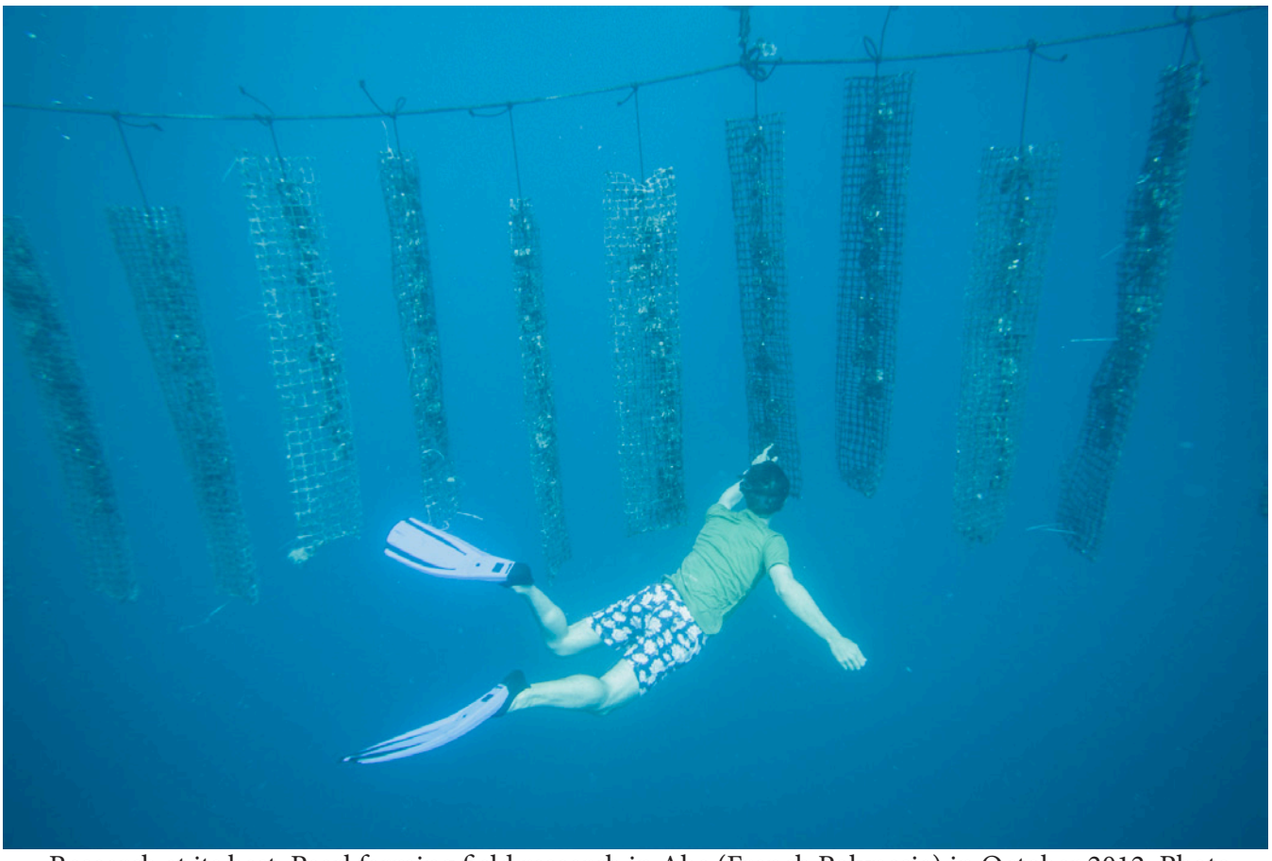

Research at its best. Pearl farming field research in Ahe (French Polynesia) in October 2012. Photo courtesy of Andy Bardon.

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