GEMOLOGICAL CHARACTERISTICS OF SALTWATER CULTURED PEARLS PRODUCED AFTER XENOTRANSPLANTATION

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Experimental saltwater cultured pearls produced after xenotransplantation between *P. margaritifera* and *P. maxima* were studied using UV-Vis-NIR and PL spectroscopy as well as radiography. The results further demonstrate that the graft (*saibo*) largely determines the coloration and nacre thickness of the cultured pearl.

The value of beaded saltwater cultured pearls (SWCPs) depends on five main factors: shape, size (diameter and nacre thickness), color (bodycolor and overtone), luster, and surface condition (Taylor and Strack, 2008; Tayale et al., 2012). Statistics have shown that only 5% of all SWCPs are top quality, yet these account for about 95% of a pearl farm's income (Haws, 2002). To increase the percentage of top-quality SWCPs, several authors have experimented with variables such as environmental factors and the choice of donor and acceptor mollusks (see examples in Lucas, 2008; Southgate, 2008; and Mamangkey, 2009).

Most saltwater pearls are cultivated after transplantation of a piece of mantle tissue. This graft, also known by the Japanese term *saibo*, is cut from a bivalve mollusk donor. A bead, usually from the inner shell of a freshwater mollusk belonging to the Unionidae family, is simultaneously implanted into the gonad of a bivalve mollusk acceptor or host. When the donor and the acceptor bivalves belong to the same species, as is generally the case, the process is known as *allotransplantation*. Allotransplanted mollusks of *Pinctada maxima* typically produce white to light gray, silver, cream, and yellow to golden SWCPs. Allotransplanted mollusks of *Pinctada margaritifera* commonly yield dark gray to black as well as light gray to white SWCPs. Various other natural-color SWCPs can be also found in both bivalves (see Karampelas et al., 2011 and 2012, and references therein).

McGinty et al. (2010 and 2011) presented the results of their genetic studies involving successful xenotransplantation between two different species (*P. margaritifera and P. maxima*) and the influence on the aforementioned SWCP quality factors. This study investigated experimental SWCPs, using methods different from those presented by McGinty et al., to further confirm the effect of the *saibo* from the donor mollusk.

MATERIALS AND METHODS

This study was carried out on 10 successfully cultivated experimental SWCPs (selected from McGinty et al., 2010) with various colors and sizes (see figure 1 and table 1). Seven samples (nos. 1–7) were cultivated in *P. maxima* after transplantation of a *P. margaritifera* tissue graft, while the other three (nos. 8–10) were cultivated in *P. margaritifera* after transplantation of a *P. maxima* graft. All samples were cultivated for 14 months on a farm belonging to Cendanda Indopearls on the Indonesian island of Bali; more on the exact conditions of cultivation can be found in McGinty et al. (2010). None of them had been subjected to any treatment. All but sample 9 were round or near-round, with good to very good surface condition and mostly good luster; see Gübelin Gem Lab (2012) for more information about the grading system used. SWCPs cultivated in *P. maxima* mollusks with *P. margaritifera* grafts had more gray-

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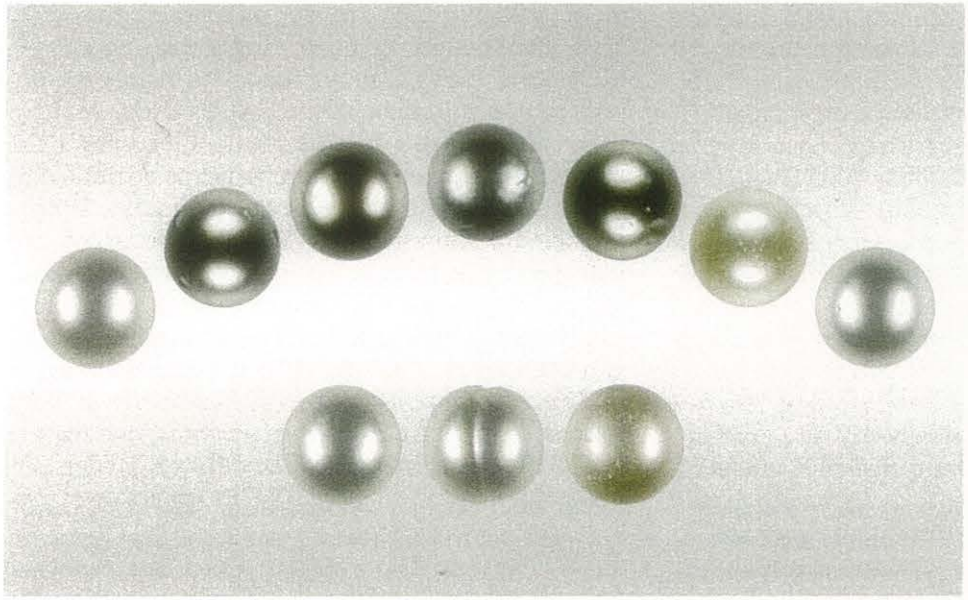


Figure 1. Ten xenografted saltwater cultured pearls were chosen for this study. Seven samples (nos. 1–7) were cultivated in P. maxima *with transplanted* P. margaritifera *tissue graft, while the other three (nos. 8–10) were cultivated in* P. margaritifera *with transplanted* P. maxima *graft. Composite photo by S. Karampelas (samples not to scale).*

ish color than those cultivated in *P. margaritifera* mollusks with *P. maxima* grafts (again, see table 1).

The samples' UV fluorescence reaction was observed with a 6W long- and short-wave (365 and 254 nm, respectively) UV lamp. Their UV-Vis-NIR spectra were obtained for the 250–1600 nm range using a Cary 5000 spectrometer fitted with a Varian diffuse reflectance accessory. Only the 250–900 nm range, which contains the color-related absorption bands, is presented here. The data sampling interval and spectral bandwidth of each measurement were set at 0.7 nm and the scan rate at 60 nm/minute. Matte black sample holders were used for a more intense signal. Photoluminescence (PL) spectra were acquired using a Renishaw Raman 1000 spectrometer coupled with a Leica DMLM optical microscope at 50× magnification, with an excitation wavelength of 514 nm emitted by an argon-ion laser (Ar^+), a power of 10 mW, a 10-second acquisition time, and a resolution of about 0.1 nm. Digital radiography was performed at the Gübelin Gem Lab with a Comet X-ray unit and a Kodak 6120 digital sensor. Parameters were adjusted to the sample size, with voltage from 60 to 65 kV and current from 5 to 7 mA.

RESULTS AND DISCUSSION

Figures 2–4 show the diffuse reflectance UV-Vis-NIR spectra for six xenotransplanted samples. The spectra

TABLE 1. Characteristics of xenografted SWCP samples.

Sample	Host mollusk	Donor mollusk (*saibo* species)	Size (mm)	Color	Average nacre thickness (mm)
GGL-ATL001	*P. maxima*	*P. margaritifera*	10.65–10.80	Very light gray	1.6
GGL-ATL002	*P. maxima*	*P. margaritifera*	8.10–8.30	Dark gray	0.8
GGL-ATL003	*P. maxima*	*P. margaritifera*	8.60–9.20	Dark gray	0.9
GGL-ATL004	*P. maxima*	*P. margaritifera*	8.80–9.00	Gray	0.7
GGL-ATL005	*P. maxima*	*P. margaritifera*	8.60–8.70	Dark gray	0.6
GGL-ATL006	*P. maxima*	*P. margaritifera*	8.40–8.50	Light gray yellow	0.5
GGL-ATL007	*P. maxima*	*P. margaritifera*	9.50–9.90	Very light gray	1.7
GGL-ATL008	*P. margaritifera*	*P. maxima*	10.10–10.20	Very light gray	1.9
GGL-ATL009	*P. margaritifera*	*P. maxima*	9.10 × 8.90	White	1.6
GGL-ATL010	*P. margaritifera*	*P. maxima*	16.50–16.60	Light yellow	4.4

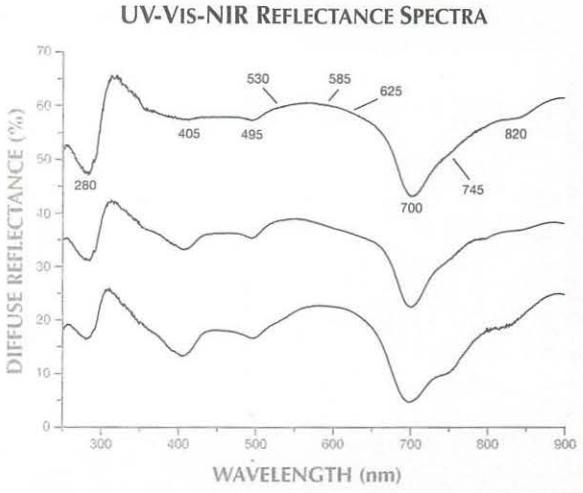
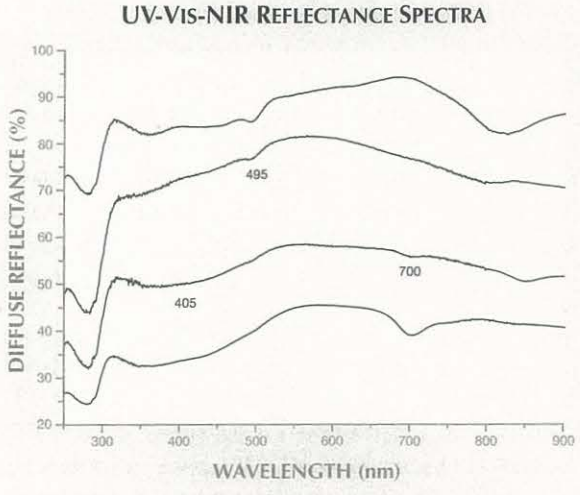


Figure 2. These diffuse-reflectance spectra of a black allografted P. margaritifera *SWCP (bottom) and two gray xenografted samples from a* P. maxima *host and* P. margaritifera *donor (GGL-ATL002 and GGL-ATL004) show absorptions at 280 nm, from 330 to 460 nm (with apparent maxima at 330–385 nm and 385–460 nm), and at 405, 495, and 700 nm. Also observed is a weak continuous absorption with a maximum at 820 nm, plus some less-intense features at 530, 585, 625, and 745 nm. For clarity, the spectrum of GGL-ATL004 is shifted up 5% and that of the black natural-color SWCP is shifted down 5%.*

present an absorption (a decrease in diffuse reflectance) at around 280 nm. Figure 2 shows two natural-color samples cultivated after xenotransplantation into *P. maxima* mollusks with *P. margaritifera* grafts, GGL-ATL002 (dark gray) and GGL-ATL004 (gray), as well as one black natural-color SWCP from *P. margaritifera* after allotransplantation (bottom spectrum). All three spectra contain six main absorption bands: from 330 to 460 nm, with maxima at 330–385 nm and 385–460 nm, and at 405, 495, 700, and 745 nm (plus a continuous band extending through the visible range with a maximum in the near infrared at around 820 nm). Also observed are three less-intense bands at around 530, 585, and 625 nm, which are common in allotransplanted *P. margaritifera* SWCPs (Elen 2002; Karampelas et al., 2011). Differences in the spectra patterns are due to the different relative intensities of these bands. The 700 nm band is currently known only from allotransplanted *P. margaritifera* SWCPs (Elen, 2002). Moreover, the 405 nm band has not been observed in natural-color allotransplanted *P. maxima* SWCPs (Karampelas, 2012). These results are in accordance with those found experimentally by McGinty et al. (2010 and 2011), as well as other authors (e.g., Wada and Komaru, 1996). In other words, the *saibo*—in this case, *P. margaritifera* tissue—is mainly responsible for the coloration of the SWCPs. None of these bands is linked to a specific pigment, except for the one at approximately 405 nm, which is attributed to a kind of porphyrin (Iwahashi and Akamatsu, 1994).

Figure 3 shows the UV-Vis-NIR spectra of two light yellow samples, cultivated after xenotransplantation. Sample GGL-ATL006, cultivated in a *P. maxima* mollusk with a *P. margaritifera* graft, is a bit grayish. Sample GGL-ATL010 is cultivated in a *P. margaritifera* mollusk with a *P. maxima* graft. Both spectra contain the characteristic absorption feature from 330 to 460 nm observed in yellow to golden natural-color allotransplanted SWCPs from *P. margari-*

Figure 3. The light yellow xenografted sample GGL-ATL010 (second spectrum from the top; P. margaritifera *host and* P. maxima *donor) shows a weak absorption feature from 330 to 460 nm (with weak bands at 330–385 nm and 385–460 nm), as well as other bands at 495 nm and in the near-infrared region. Similar bands are observed to analogous natural-color (light brownish yellow) allografted sample from* P. maxima *(top spectrum), with different relative intensities of the same bands. The light gray yellow xenografted sample GGL-ATL006 (second spectrum from the bottom;* P. maxima *host and* P. margaritifera *donor) presents similar absorptions, as well as two additional bands at around 405 and 700 nm. Similar bands are observed in the natural-color allografted sample from* P. margaritifera *(bottom spectrum). The spectrum for GGL-ATL006 is shifted down 10% for clarity.*



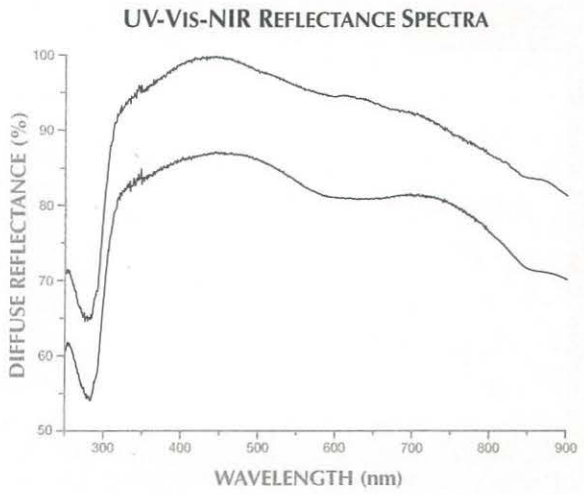
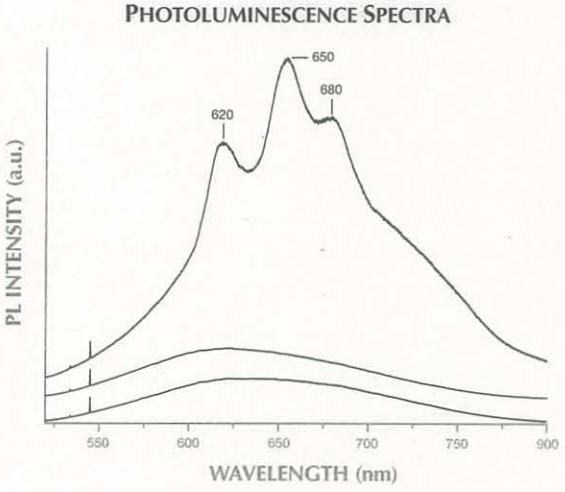


*Figure 4. Two very light gray ("white-silver") SWCPs were cultivated after xenografting: GGL-ATL007 (*P. maxima *"host" and* P. margaritifera *donor) and GGL-008 (*P. margaritifera *"host" and* P. maxima *donor). These display only weak absorptions in the visible region that are not characteristic of either bivalve species. The samples' very light gray coloration is due to a weak continuous absorption through the visible region, with a maximum in the near-infrared region. The spectrum for GGL-ATL007 is shifted up 10% for clarity.*

tifera and *P. maxima* (Elen, 2002). Both spectra also have a weak band at around 495 nm, similar to yellowish allotransplanted SWCPs of both mollusks (Karampelas, 2012). A weak band at around 700 nm and a shoulder at about 405 nm are also observed in the spectrum of sample GGL-ATL006. These absorption bands, present in allotransplanted SWCPs in *P. margaritifera* and absent from those cultivated in *P. maxima* (again, see figure 3), spectroscopically confirm the genetic results from McGinty et al. (2010 and 2011).

Figure 4 presents two samples of very light gray or "white-silver" color from the xenotransplantation of GGL-ATL007 and GGL-ATL008. The two spectra look similar; virtually the entire visible region is transmitted. A weak continuous absorption through the visible range with a maximum in the near-infrared region was responsible for the samples' light gray color. Very similar spectra can be observed in some white as well as other light-colored (white-silver and light yellow) allografted samples from *P. maxima* and *P. margaritifera*. The absorption band at around 700 nm is present in all of the colored samples (allografted or xenografted) cultivated using *saibo* from *P. margaritifera*, but was absent from the two light-colored samples (GGL-AUT001 and 007). The 700 nm absorption was absent, or sometimes present as a shoulder, in white to light-colored allotranplanted samples from *P. margaritifera* (Elen, 2002; Karampelas et al., 2012). Thus, the absence of the 700 nm band from a light-colored SWCP does not preclude the possibility that it was cultivated using *saibo* from *P. margaritifera*.

Figure 5. The photoluminescence spectrum of xenografted sample GGL-ATL005 (with saibo *from* P. margaritifera*) shows bands at around 620, 650, and 680 nm. The sharp bands in the 520–550 nm region are due to the Raman effect. The light colored samples (GGL-ATL007 and GGL-ATL008) present less intense bands with a broad apparent maximum around 630 nm. All spectra intensities are adjusted to the main Raman band and shifted for clarity.*



In Brief

- Ten saltwater cultured pearls (SWCPs) cultivated after xenotransplantation between *P. maxima* and *P. margaritifera* mollusks were studied using UV-Vis-NIR and PL spectroscopy as well as X-ray microradiography.
- In xenotransplantation, the graft (*saibo*) from the donor mollusk largely determines the coloration and nacre thickness of the cultured pearl.
- Through spectroscopy studies, gemological laboratories can identify (with the exception of some light-colored SWCPs) the species of the donor (e.g., the 700 nm absorption band characteristic of graft from *P. margaritifera*) but not that of the host.

PL spectra of the dark-colored xenografted samples using *saibo* from *P. margaritifera* displayed bands in the orange to red region at about 620, 650, and 680 nm with green excitation (figure 5), similar to those in allografted *P. margaritifera* samples (Miyoshi et al., 1987). The light-colored xenografted samples—cultivated with both grafts—showed less-intense bands (again, see figure 5); similar results were found in allografted samples from both mollusks. Moreover, like allotransplanted SWCPs from the same mollusks, the light-colored samples were inert to short- and long-wave UV radiation (GGL-AUT001 and GGL-AUT006–010), while the others showed a weak greenish yellow and weak yellow reaction, respectively.

From the X-radiographs, the samples cultivated with a *P. maxima* donor and a *P. margaritifera* host generally contained thicker nacre (approximately 1.6–4.4 mm) than those cultivated using a *P. margaritifera* donor and a *P. maxima* host (0.5–1.8 mm; see also table 1). Allografted SWCPs from *P. maxima* had thicker nacre (as well as nacre weight) than allografted *P. margaritifera* SWCPs after cultivation for the same period of time in the same farm and under similar conditions; see examples in McGinty et al. (2010). This was probably due to the different growth rate (directly related to the nacre deposition rate) of *P. maxima* and *P. margaritifera* bivalves; *P. maxima* have a higher growth rate than their *P. margaritifera* counterparts (Yukihira et al., 2006; Saucedo and Southgate, 2008). Nevertheless, the growth rate of *P. maxima* and *P. margaritifera* can vary with environmental conditions such as salinity and water temperature (Gervis and Sims, 1992; Yukihira et al., 2006; Saucedo and Southgate, 2008). The radiography results here do confirm that the *saibo* plays an important role in nacre deposition (McGinty et al., 2010 and 2011).

CONCLUSION

Xenotransplantation between *P. margaritifera* and *P. maxima* can yield gem-quality SWCPs, as documented by McGinty et al. (2010 and 2011). This study using UV-Vis-NIR spectroscopy as well as radiography confirmed the histological and genetic findings by various researchers (e.g., Arnaud-Haond et al., 2007; McGinty et al., 2010) that the *saibo* from the donor mollusk is mainly responsible for the color as well as the nacre thickness. Using spectroscopic means, gemological laboratories can identify (with the exception of some light-colored SWCPs) the mollusk species of the donor (e.g., the 700 nm absorption band characteristic of *saibo* from *P. margaritifera*) but not the host. The host mollusk probably plays some role in the nacre deposition. For instance, xenotransplanted SWCPs with a *P. margaritifera* host and *saibo* from *P. maxima* have slightly thicker nacre than the allotransplanted SWCPs from *P. maxima* (McGinty et al., 2010). Additional research is needed to shed light on this.

Moreover, several studies have shown that selecting the best-secreting *saibo* for transplantation into a healthy host mollusk is the key to SWCP quality (e.g., Acosta-Salmón et al., 2004; Southgate, 2008). Further research is also needed on all five quality factors in xenografted SWCPs, including comparison with allografted SWCPs from the same mollusk species under identical conditions, after careful selection of donor and host mollusks. These investigations would clearly show if quality can be improved through xenografting. Another meaningful experiment, suggested by various authors, would be to see if xenografting between other Pinctada species (e.g., *P. fucata*) or even related species (e.g., *Pteria sp.*) can yield high-quality SWCPs.

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