# Is pearl colour produced from *Pinctada margaritifera* predictable through shell phenotypes and rearing environments selections?

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**Abstract :**

The black-lipped pearl oyster, *Pinctada margaritifera* is the most important farmed mollusc species in French Polynesia. Donor oyster selection among wild *P. margaritifera* individuals, chosen according to their inner shell colour, makes it possible to obtain the broadest range of cultured pearl colours of any species. This study demonstrates the relative influence of using black [B] or red [R] outer shell phenotypes, combined with green [G] or yellow [Y] inner shell phenotypes, on pearl darkness level, colour categories and lustre. A large scale grafting experiment was designed and carried out over five grow-out locations, covering three archipelagos: Tuamotus, Society and Gambier. Results revealed that the [B + G] phenotypes may be used as donors to produce dark green pearl, which suit the demands of the Asian market, whereas phenotypes incorporating [R] and/or [Y] phenotypes, may be used to obtain multicolour pearls of medium / light darkness, which suit the demands of the European market. From an environmental point of view the: 1) [B] phenotype showed no significant variation for light and other pearl colour production, and 2) [Y] phenotype produced both the same rate of pearl darkness level and green colour pearls whatever the grow-out location. A classification tree model was built to predict, according to shell phenotype and culture location, the colour and darkness level of harvested pearls. Lustre was shown to be more influenced by the environment than by phenotype. These results should be taken into account in pearl farm production management and in selective breeding programs.

**Keywords** : Pearl oyster, *Pinctada margaritifera*, Shell colour phenotype, Pearl colour, Environment

**Introduction**

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42      In French Polynesia, the marine mollusc *Pinctada margaritifera* (Linnaeus 1758) var.

43  *cumingi* (Reeve) is the top aquaculture species. This mollusc is also widely known as the

44  black-lipped pearl oyster due to the dark marginal coloration of the mantle, although the

45  species is not a true oyster in the biological sense. In this tropical ecosystem, the production

46  of this organic gem (the cultured pearl) and the business that has built up around it is well

47  established. It forms the basis of the most lucrative export industry and the second most

48  important source of income after tourism. The black-lipped "pearl oyster" inhabits subtropical

49  and tropical coral reefs, and is widely distributed in the Indo-Pacific area, down to Northern

50  Australia, throughout the Equator extending up to the southern region of Japan (Le Pennec et

51  al., 2010) and is particularly abundant in the lagoons of French Polynesia. The cultured pearl

52  grafting procedure consists in introducing a nucleus (a bead made from shells of a fresh-water

53  bivalve) and a graft (a piece of mantle tissue from a selected donor "oyster") into the gonad of

54  a recipient "oyster". Pearls need 15 to 24 months of culture to form before they are ready to be

55  harvested. During this time, their successive layers are secreted by the epithelial cells

56  implanted in the recipient "oysters" along with the nucleus (Taylor and Strack 2008).

57      A remarkable specificity of the *P. margaritifera* "pearl oyster" is its ability to produce

58  a very wide range of pearl colours, from the purest white to the deepest black, and that these

59  can have different combinations of main bodycolor and secondary colour (Karampelas et al.

60  2011; Blay et al., 2014; Ky et al., 2014a), passing through every shade of silver, peacock,

61  green, aubergine, purple, golden brown and even rainbow, which can be used to make

62  characteristic multicolour necklaces, in comparison to the two other pearl oyster species *P.*

63  *martenseii* and *P. maxima*. In fact, the "Akoya pearls" produced by *P. martensii*, have the

64  colours pink, white or silver, cream and yellow, followed by white or silver (Tong & Shen

65  2001). In the case of *P. maxima*, cultured pearls in golden or silver-white are regarded as

66  superior to those in yellow or cream (Taylor 2002). The colour of cultured pearls is generally

67  related to their commercial value: darker *P. margaritifera* cultured pearls are more valuable

68  than paler ones, especially on the Asian market. Cultured pearl colour varies according to the

69  original graft made when the nucleus was inserted, and depends mainly on the donor "oyster"

70  (Tayale et al, 2012; Ky et al., 2013). The predominant bodycolors of *P. margaritifera* cultured

71  pearls are grey, yellow and white. *P. margaritifera* cultured pearls also have attractive

72  overtones (secondary colours): green, blue, aubergine (reddish purple) and peacock. These

73  overtones may be present in a variety of combinations and are considered a bonus factor for

74  the value of the pearl. Tayale et al. (2012) and Ky et al. (2013) demonstrated significant donor

75  and family effects, respectively, on pearl darkness level, using individual wild donors and

76  hatchery-bred families. Although dark pearls are generally more valuable, a completely black

77  pearl with no overtone is considered less desirable and may be worth 50% less than one of

78  similar quality with green overtones. Lustre is another important quality trait, which

79  constitutes one of the components of the Tahitian cultured pearl official grade. The reflection

80  of light from the edges of the cultured pearl, defines its shininess / glossiness or the "lustre" of

81  its surface.

82      The factors that contribute to the colour of a pearl include the oyster species (genetic),

83  the culture zone where the recipient oyster is reared during pearl development

84  (environmental) and the interactions of the organism with the environment (Snow et al. 2004).

85  It is important to understand the influences of both the phenotypic criteria used for donor

86  selection and the environment in the formation of cultured pearl colour, as numerous farms

87  and grow-out sites are very widely geographically separated in French Polynesia and

88  therefore subject to disparate environmental regimes. For example, in the context of a

89  hatchery-based genetic selection program focused on colour determination, an understanding

90  of the interactions between animals and their environment would be essential for ensuring

91      maximum genetic gains when multiple grow-out locations are used or when a particular end

92      product is targeted (Wada and Komaru, 1996). To date, few studies have been made on

93      relations between donor phenotype and pearl colour in *P. margaritifera*, but Tayale et al.

94      (2012), found evidence that individual wild donors influenced cultured pearl colour through a

95      duplicated grafting experiment on *P. margaritifera*. This study also revealed correlations

96      between the inner shell colour of the donor and the colour proportions observed in the

97      harvested pearls. More recently, Ky et al. (2013) showed the existence of a family effect (i.e.,

98      oysters produced by hatchery system) on pearl colour demonstrating the possibility of setting

99      up a sib genetic selection plan for *P. margaritifera*.

100         Whereas shell morphology is a species-specific characteristic, shell colour varies very

101     widely between *P. margaritifera* individuals used as donors, permitting production of an

102     equally broad range of pearl colours. In the present study, we focused on the relationships

103     between the donor phenotype criteria and their influence on pearl colour parameters with

104     regards to recipient-environment interactions. We conducted an experimental graft using

105     specific selected donor oyster phenotypes chosen on the basis of both outer and inner shell

106     colour, and analysed the colour parameters (pearl darkness level, pearl visual colour

107     categories and lustre) of the corresponding pearls harvested, under five contrasting rearing

108     environments. The results could help the French Polynesian pearl industry in the selection of

109     appropriate donor oysters for future colour broodstock establishment and responding to

110     market demands for particular pearl colours.

## Materials and methods

### Animals

Wild *Pinctada margaritifera* were collected as spat in the lagoon of Mangareva Island (Gambier archipelago, French Polynesia) to serve as donors and recipients. Passive techniques were employed for catching spat using commercial spat collectors made from modern synthetic materials, to which planktonic mollusc larvae become attached fifteen to twenty days after their release. The technique consists of immersing a rope at a depth of 3m, which is stretched out between buoys and moored to pinnacles or dead weights placed on the lagoon floor. Surface buoys keep the 100- to 200-metre rope suspended off the bottom. Collectors were attached at roughly one metre intervals.

After nearly one year of subsurface rearing (3–5 m below the surface), the young pearl oysters (4–5 cm in diameter) were then removed from the collectors on which they had developed. These juveniles were pierced and tied together onto a CTN (Cord Technical Nakasai) rearing system, where they were left until grafting. This rearing method involves drilling a small hole through the base of the shell in the dorsal-posterior region. This process doesn't affect living tissue. The CTN were protected using plastic mesh to prevent predation in the lagoon. At six months post transfer, the pearl oysters were removed from the water and washed with a high pressure spray, thus removing any parasites (mainly epibionts). This process causes no injury or mortality and maximizes growth rate. Mature oysters aged near 20 months, measuring at least 7 cm in length, were taken from the rearing station, detached and stored ready to be used in the grafting procedure.

Donor pearl oysters were selected from a group of healthy animals by an expert grafter, following a set of criteria including colour of the visceral mass and gills, shell size and appearance, and muscle resistance when shells were opened. The grafter used a speculum

135    to open the pearl's valves and a spatula mirror to observe the inner shell colour. Using this

136    procedure, two donor phenotypes were selected according to inner shell colour: predominance

137    of "green" [G] (n = 70) or of "yellow" [yellow] (N = 56) (Figure 1). Among these 126 donor

138    oysters, a second selection was made on the basis of outer shell colour phenotypes; which had

139    either a "black" [B] (N = 71) or a red colour [R] (N = 55) (Figure 1). Combinations of inner

140    and outer shell colours gave the following phenotypes: [B + G] (N = 42), [B + Y] (N = 28),

141    [R + G] (N = 28) and [R + Y] (N = 28) (Figure 1).

142    **Grafting procedure and experimental design**

143        The grafting operation (Figure 2) was made by two experts from Regahiga pearl farm

144    (Mangareva Island - Gambier archipelago) (Ky et al., 2015a). The nuclei used for this purpose

145    are Japanese 1.8 BU nuclei, which are beads made from the shells of freshwater bivalves,

146    (5.45 mm diameter, 0.26 g weight - Imai Seikaku Co. Ltd., Japan). Freshwater mussels are

147    used to make pearl nuclei because the shells of these bivalves have thick nacreous layers with

148    a hardness, specific gravity and thermal conductivity that make them particularly suitable

149    (Gervis and Sims, 1992). The epithelial cells required for grafting were excised from the

150    mantle of the selected donor pearl oysters (N = 126). In the method used, small strips of

151    epithelium are prepared before being transplanted into the recipient oysters; the grafts are

152    pieces measuring approximately 4 mm$^2$. Each receiving mollusc is selected based on its

153    visible health status. The grafter uses a speculum to gently pry open the recipient oyster's

154    valves. The grafter first incises the recipient oyster gonad in which they then place the nucleus

155    and graft. The whole operation takes 30 seconds to 1 minute. A total of 2520 grafts were

156    performed (20 grafts per donor) over 5 days. The grafted oysters were placed in separate

157    subdivisions in transparent retention bags (10 grafted oysters per retention bag) with their

158    hinges facing upwards, so that the nuclei could not slip out of place due to the pull of gravity.

159    Traceability and correspondence between grafted oysters and each donor oyster was

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160    maintained using plastic numbered labels attached to the retention bags. All the grafted

161    oysters were checked for nucleus retention / rejection and mortality at 45 days after the graft

162    experiments, as described in Ky et al. (2014b).

163         Grafted pearl oysters that had retained their nuclei were sorted from the others by

164    checking visually for the absence of nuclei in their sections of the retention bag: when no

165    bead was detected inside the bag it was inferred that the pearl oyster had retained its nucleus.

166    These oysters were counted within each retention bag and were drilled (by making a hole

167    through the base of the shell in the dorsal-posterior region, without affecting living tissue) and

168    fixed onto chaplets (one chaplet was made up per originated donor oyster after removing the

169    net retention bags). In contrast, oysters that had rejected their nuclei were identified by the

170    presence of the rejected nucleus in their retention bags, i.e., outside these receiver molluscs,

171    because, if a nucleus is expulsed from the shell, it will be found in the bag. These oysters were

172    counted and were not reused in production, but sacrificed. Mortality in the post-grafting

173    period was assessed visually and by counting the number of oysters with open shells or the

174    presence of shell fragments inside the bags at 45 days post grafting.

175         At this time, recipient oysters grafted from the same donor oyster (chaplets) were

176    randomly split into five groups, of which one was kept at Regahiga pearl farm and reared in

177    Atiaoa bay (site nomenclature: GMR-Atiaoa), a second group was transferred by boat to

178    another bay of Mangareva Island called Taku (GMR-Taku), a third was transferred by plane

179    to Ahe atoll, a fourth to Rangiroa atoll and the last to Tahaa Island. After 20 months of

180    culture, the cultured pearls were harvested. Some *keshi* (small non-nucleated pearls formed

181    when an oyster rejects and expulses the implanted nucleus) were harvested, but not graded.

182    **Measurement of cultured pearl colour**

183         Cultured pearls were cleaned by ultrasonication in soapy water (hand washing) with a

184    LEO 801 laboratory cleaner (2-L capacity, 80 W, 46 kHz) and were then rinsed in distilled

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185   water (Ky et al., 2014a). Colour evaluations were made (without a jeweller's loupe) on the

186   cultured pearls by two professional graders from GIE Poe O Rikitea working together.  They

187   classed the pearls according to:

188   - Darkness of overall coloration, with three categories depending on its level: high, medium

189   and light (Figure 3a);

190   - Visually-perceived colour category (due to pigments: bodycolor; and secondary colour:

191   overtone). Four "colour categories" were made into which all the harvested pearls could be

192   classified from the highest harvest rate to the lowest: 1) grey, 2) peacock, 3) green and 4)

193   "other" colours, which included blue, white and aubergine (Figure 3b).

194   **Statistical analysis**

195        Univariate analysis was performed for each variable. Categorical variables were

196   expressed as numbers and percentages. To compare donor phenotypes that led to each

197   level/category of the "colour" variables (darkness level, colour categories and presence /

198   absence of lustre), $\chi^2$ tests and Fisher's exact tests for count data were performed to check if

199   there was a difference between distributions. If the overall test was significant, pairwise

200   comparisons between all pairs of proportions were calculated to identify which groups

201   differed (represented by letters in each table).

202        A discriminant model was built based on pearl darkness data, using the classification

203   and regression tree (or CART) algorithm with the *rpart* function in R software. The optimal

204   tree size was determined by cross validation. The discriminative capacities of the model were

205   next estimated. Sensitivity and specificity help measure the performance of the classifier. In

206   our model, the sensitivity estimates if the tree correctly predicts that a dark pearl will be

207   produced and the specificity evaluates if it correctly predicts when a pearl will not be dark (or,

208   equivalently, predicts a light or medium pearl). These two statistics measure true positive and

209   true negative performance.

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210 Statistical tests were performed using R® version 3.2.1 software (R Foundation for

211 Statistical Computing). The significance threshold was set at $p \leq 0.05$.

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213    **Results**

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215    **Nucleus retention, pearl harvest and oyster mortality / predation.**

216          Among the 2519 grafts, the nucleus retention, rejection and oyster mortality rates were

217    86.1% (N = 2168), 11.6% (N = 293) and 2.3% (N = 58), respectively, at 45 days post grafting.

218    After 18 months of culture, a total of 1910 cultured pearls and 97 *keshi* were harvested (with

219    20.3% mortality / predation).

220          The cultured pearl harvest rates at each site were: GMR-Atiaoa: 94%, GMR-Taku:

221    87%, Ahe: 90%, Rangiroa: 88%, and Tahaa: 86%. Location was found to have a highly

222    significant effect ($p = 0.003$) on pearl harvest rate, with the GMR-Atiaoa site presenting a

223    higher rate than the group made up of Rangiroa, Taku and Tahaa, but no significant difference

224    with Ahe.

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226    **Shell colour phenotype effects on pearl darkness, colour and lustre.**

227          According to the external colour shell variant of the donor oyster, comparison between

228    the [B] (black) and [R] (red) phenotypes revealed a significant impact on pearl darkness level.

229    Using [B] phenotype as the donor led to a very significantly higher rate ($p < 0.001$) of dark

230    pearls (+ 9%) in comparison with results from [R] phenotype donors (Table 1). By contrast,

231    using [R] phenotypes as donors showed a significantly higher rate ($p = 0.005$) of medium dark

232    pearls (+ 6%) in comparison with [B] phenotype donors (Table 1). No significant differences

233    were observed in the percentage of pearls in the light darkness level class ($p = 0.11$) between

234    [B] and [R] phenotype donors (Table 1).

235    When cultured pearl colour categories were considered, the proportions of grey (+ 5%) and

236    green (+ 4%) pearls were slightly higher when [B] phenotype was used as donor than when

237    [R] phenotypes were used ($p < 0.05$) (Table 1). By contrast, using [R] phenotypes as donors

238    produced a significantly higher rate ($p = 0.003$) of peacock pearls (+ 6%) in comparison with

239    [B] phenotype (Table 1). No significant differences ($p = 0.10$) were observed between [B] and

240    [R] donor phenotypes for the "other" colour category of cultured pearls (composed of blue,

241    aubergine and white) were observed ($p = 0.11$) (Table 1). For the lustre parameter of pearls,

242    no significant difference was observed ($p = 0.99$) between pearls from [B] or [R] donor

243    phenotypes (Table 1).

244        For inner shell colour donor phenotypes, comparison between the [G] (green) and [Y]

245    (yellow) revealed a significant impact on pearl darkness level. Using [G] phenotype donors

246    produced a very significantly higher rate ($p < 0.001$) of dark pearls (+ 12%) in comparison

247    with the [Y] phenotype (Table 2). By contrast, using [Y] phenotype donors produced a

248    significantly higher rate of pearls with medium ($p = 0.005$; + 7%) and light (p = 0.002; + 5%)

249    darkness levels in comparison with [G] phenotype (Table 2). Considering cultured pearl

250    colour categories, the proportions of peacock (+ 4%) and green (+ 4%) pearls were slightly

251    higher when the [G] phenotype donors were rather than [Y] phenotypes ($p < 0.05$) (Table 2).

252    By contrast, using [Y] phenotypes as donors led to a significantly higher rate ($p = 0.003$) of

253    "other" pearl colours (+ 5%) than using [G] phenotype donors (Table 2). No significant

254    differences were observed between [G] and [Y] donor phenotypes for proportions of grey

255    colour pearls ($p = 0.12$) (Table 2). For the lustre parameter, a significantly higher number of

256    pearls with lustre was observed ($p = 0.01$) when the donors had the [Y] phenotype (+ 5%)

257    rather than the [G] phenotype (Table 2).

258        When the outer and inner shell colours were considered in combination, comparison

259    between the four phenotypes [B+G], [B+Y], [R+G] and [R+Y] revealed a significant impact

260    on pearl darkness level. Using [B+G] phenotypes as donors led to a very significantly higher

261    rate ($p < 0.001$) of dark pearls (+ 14%) in comparison to the means produced from the other

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262    phenotypes [B+Y], [R+G] and [R+Y] (Table 3). By contrast, using these latter three

263    phenotypes as donors led to a significantly higher rate of medium ($p < 0.001$; + 10%) and

264    light ($p = 0.009$; + 5%) darkness level pearls on average compared with the [B+G] phenotype

265    (Table 3). For colour categories of pearls, each phenotype combination showed a specificity:

266    1) the [B+G] phenotype showed a significantly higher rate ($p = 0.01$) of green pearl colour (+

267    5% on average) in comparison with the other combinations; 2) the [B+Y] phenotype led to

268    more grey ($p = 0.03$; + 7% on average); 3) the [R+G] phenotype for more peacock colour ($p <$

269    $0.001$; + 10%), and 4) the [R+Y] phenotype for the "other" colour group ($p < 0.001$; + 8%)

270    (Table 3). For the lustre parameter, no significant difference was observed ($p = 0.99$) between

271    the different donor combination phenotypes (Table 3).

272    **Environment effect on pearl darkness, colour and lustre according to shell colour**

273    **phenotypes.**

274    *[B] phenotype*

275        When [B] phenotype oysters were used as donors, a significant environment  effect

276    was detected on darkness level for: 1) Tahaa location, which showed the significantly highest

277    rate of dark pearls ($p = 0.015$; +11% on average) in comparison with the four other sites; 2)

278    GMR-Taku location, which showed the significantly highest number of medium darkness

279    level pearls ($p = 0.004$; +11% on average); and 3) no significant difference ($p = 0.134$)

280    between site location was observed for the proportion of lightest colour pearls (Table 4). With

281    [B] phenotype donors, the significantly lower rates of grey, peacock and green pearls were

282    observed at Tahaa ($p < 0.001$; -20%), Ahe ($p < 0.001$; -23%) and GMR-Atiaoa ($p = 0.002$; -

283    7%) locations, respectively (Table 4). No significant difference ($p = 0.38$) was observed

284    between sites for the percentage of "other" pearl colours produced (Table 4).

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285    For the lustre trait, a very highly significant site effect was seen when [B] phenotypes were

286    used as donors, with $p < 0.001$ for GMR-Taku location, which showed the lowest rate of

287    pearls with lustre in compared with the four other sites (-18% on average) (Table 4).

288    *[R] Phenotype*

289    When the [R] phenotype was used as the donor, a significant environment effect on

290    darkness level was detected for: 1) GMR-Atiaoa location, which showed both the highest

291    level of dark pearls ($p < 0.001$; +18% on average) and the lowest level of light pearls (p <

292    0.001; -10%) in comparison with the four other sites; and 2) Ahe location, which showed the

293    highest amount of medium darkness level pearls ($p = 0.005$; +20% on average) (Table 4).

294    With [R] donors, a significant environment effect on colour was detected for: 1) Ahe location,

295    which showed both the highest amount of grey pearls ($p < 0.001$; +34% on average)

296    compared with the four other sites; 2) Tahaa location, which showed the highest amount of

297    peacock pearls ($p < 0.001$; +15% on average) compared with GMR-Atiaoa, GMR-Taku and

298    Rangiroa sites (whereas Ahe showed the lowest rate of peacock pearls); and 3) both Rangiroa

299    and Tahaa locations, which showed similar amounts of the "other" colours of pearls,

300    significantly higher than the three other locations ($p < 0.001$; +12%). By contrast, no

301    significant site effect was detected for green colour ($p = 0.91$) (Table 4).

302    With [R] phenotype donors, a very high significant site effect was observed for lustre (p <

303    0.001) at GMR-Taku location, which showed the lowest rate of pearls with lustre in

304    comparison to the four other sites (-25% on average) (Table 4).

305    *[G] phenotype*

306    When the [G] phenotype donors were used, a significant environment effect was

307    detected for darkness level at: 1) Rangiroa, which showed the significantly lowest rate of dark

308    pearls ($p < 0.001$; -13% on average) in comparison to the four other sites; 2) both GMR-Taku

309    and Rangiroa locations, which showed the same highest level of medium darkness level

310    pearls, in comparison to the three other sites ($p < 0.001$; -13% on average); and 3) no

311    significant difference ($p = 0.250$) between site location was observed for the production of

312    light pearl rate (Table 5). For [G] phenotype used as donor, and for the colour categories, a

313    significant environment effect was detected for: 1) Tahaa location, which showed the lowest

314    level of grey pearls ($p < 0.001$; -14% on average) in comparison to the four other sites; 2)

315    GMR-Atiaoa, GMR-Taku, and Tahaa locations, which together showed a significantly higher

316    rate of peacock pearls ($p < 0.001$; +26% on average) in comparison with the two other sites;

317    3) GMR-Atiaoa location, which showed a significantly lower level of green pearls than the

318    other sites ($p = 0.04$; -6% on average); and 4) Ahe and Rangiroa locations, which had a

319    significantly higher amount of pearls of "other" colours ($p = 0.005$; +8%). For [G] phenotype

320    used as donor, and for the lustre trait, a very high significant site effect existed ($p < 0.001$) for

321    GMR-Taku location, which showed the lowest rate of pearls with lustre in comparison to the

322    four other sites (-24% on average) (Table 5).

### *[Y] phenotype*

324            When [Y] phenotypes were used as donors, no significant environment effect was

325    detected on the darkness level of pearls (Table 5). For the colour categories, a significant

326    environment effect was detected for Tahaa location, showing: 1) the lowest rate of grey pearls

327    ($p < 0.001$; -41% in comparison to the GMR-Atiaoa location, which showed the highest rate

328    of grey pearls together with Ahe location); 2) the highest peacock pearl rate ($p < 0.0001$;

329    +37% in comparison to Ahe location, which showed the highest rate of peacock pearls); and

330    3) the highest rate of "other" pearl colours ($p < 0.001$; +11% on average) in comparison with

331    the four other locations (Table 5). No significant environment effect was detected for numbers

332    of green pearls whatever the location (Table 5).  For [Y] phenotype used as donor, and for the

333    lustre trait, a very highly significant site effect existed ($p < 0.001$) for GMR-Taku location,

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334    which showed the lowest rate of pearls with lustre in comparison to the four other sites (-17%

335    on average) (Table 5).

336    **Predictive models of cultured pearl darkness level and colour**

337    The binary classification tree generated by CART analysis allowed us to predict the

338    pearl darkness level following the branching point from the root node down to the leaf nodes

339    (Figure 4a). These latter were associated with the pearl darkness level: dark (high level) or

340    light-medium. The first decision was whether the oyster used as donor had phenotype [Y]. If

341    the answer was "no" (i.e. the donor oyster used was then [G] phenotype), then the harvested

342    pearls would have high darkness level. Fifty nine percent of the total high darkness level pearl

343    harvested depended on this [G] inner shell criteria. By contrast, if the answer was "yes" (the

344    donor oyster used was then [Y] phenotype), the second decision was then whether the oyster

345    used as donor had the [R] phenotype. If not, (i.e., the donor oyster used had the [B]

346    phenotype), then this accounted for 23% of the total high darkness level pearls harvested. The

347    discriminative abilities of the model therefore correspond to a sensitivity of 82%. This rose to

348    91% when the grow-out location criterion was also considered in the classification tree

349    (Figure 5). Indeed, if the grow-out location was GMR-Atiaoa or Rangiroa, then the

350    corresponding pearls would be of high darkness level. Seventeen percent of the total pearls

351    with the high darkness level were dependent on this two-location criterion.

352    For cultured pearl colour irrespective of location, the classification tree showed that if

353    inner and outer shell phenotypes of the donors were [Y] and [B], respectively, then this would

354    explain 80% of the grey pearls produced (sensitivity of 80%) (Figure 4b). By contrast, if the

355    configuration was [G] and [R], this then explained 26% of the green and peacock

356    pearl production (Figure 4b). For grow-out locations, another classification tree could be built

357    and revealed that: 1) in the Gambier archipelago locations (GMR-Atiaoa and GMR-Taku), the

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358     [G] inner shell phenotype explained 65% of dark green pearl production; 2) in the Ahe

359     location, the [R] outer shell phenotype explained 80% of the medium grey pearl production;

360     3) in the Rangiroa location, 90% of the high darkness level and green + peacock pearl

361     production were explained by [Y] and [B] (Figure 6a); and 4) in the Tahaa location, 90% of

362     the high darkness level and green + peacock pearl production were explained by [G] and [B]

363     (Figure 6a).

364

## Discussion

Pearl colour determination in *Pinctada* is currently examined using high technology tools, such as candidate gene expression level studies of genes implicated in biomineralisation processes (Joubert et al., 2014), or electronic microscopy of pearl cross sections (Perez-Huerta et al., 2014). It is less clear how to analyse the phenotypic diversity of the donor oysters, which plays a key role in the pearl production process. Indeed, selection of the donor oysters is the entry step of the process, which takes place in every pearl farm and indirectly impacts their final income. This choice is of great importance as the graft tissue (from the donor) will form the pearl sac, where the biomineralization process takes place (Wada 1972). This metabolic activity consists of the secretion of an organic cell-free matrix (proteins, glycoproteins, lipids and polysaccharides) on the nucleus surface by the external mantle epithelium (Rousseau et al., 2003). In addition, the maintenance of donor family effect via the pearl sac biomineralization process has been demonstrated to persist through the first graft culture period and the first surgreffe culture period (Demmer et al., 2015). The present study was the first on *P. margaritifera* to analyse the shell colour phenotypic criteria of donor oyster selection for pearl colour prediction and selective production (to fit to the international market demand). Real traceability between a donor oyster used and the corresponding pearl sample harvested allows us to establish the preliminary "rules" of phenotypic criteria for grafters to select by; rules that to date have been mostly variable from a farm to another, and mainly empirical.

**Shell phenotype influence on cultured pearl colour determination**

Cultured pearl production could be clearly orientated by prior selection of appropriately coloured donor oysters. In the Gambier archipelago, only about 20% of the wild oysters had colourful inner shells. These were used as donors. The remaining 80% had an unattractive grey inner shell colour, exclusively associated with a black outer shell colour, and

18

390  were therefore used as recipients rather than donor oysters. Among the (colourful) wild donor

391  oysters, near two thirds had the [B] or [G] shell phenotype (farmers' pers. comm.).

392  Considering [B], [G], or [B + G] phenotypes as donors, our result demonstrates that this

393  clearly favored the production of both dark and green pearls. By contrast, including the [R]

394  and/ or [Y] phenotype as donor, alone or in combination (i.e. [B + Y], [R + Y] or [R + G]),

395  clearly favored both medium/ light darkness pearl level, with more peacock and "other" (blue,

396  aubergine and white) pearl colours. This was confirmed by the classification tree results,

397  which showed that [G] and [B] phenotypes explained 82% of the dark pearl production. By

398  contrast, for cultured pearl colour, the [Y] and [R] phenotypes explained 80% of grey pearl

399  production. Without considering the grow-out location, selection of [G] and [B] phenotypes

400  would be better than the [Y] and [R] phenotypes, both for obtaining a high darkness level in

401  the pearls produced and to avoid a high rate of unattractive grey pearls. This difference

402  between [B] and [R] in terms of production, could be attributed to the deposition difference of

403  melanin pigments (Elen, 2002; Tayale et al., 2012). Indeed, two forms of melanin, produced

404  from different molecular precursors, are present in nature: eumelanin (dark brown-black in

405  colour) and pheomelanin (orange-red in colour) (Ito, 1993). Black outer shells are

406  characterized by eumelanin deposition, whereas the [R] phenotype can be attributed to

407  pheomelanin deposition. As part of the biomineralisation activities of the donor oyster mantle,

408  which are transferred through the graft process to the forming pearl; it is then easy to

409  understand why [B + G] is associated with dark and green pearls, in comparison to [R + G] or

410  [R + Y]. For the [B + G] phenotype, this was also confirmed by the fact that the amount of

411  grey pearls was also greater than for other combinations including the [R] and/or [Y]

412  phenotype. Molecular tools must be useful to explore these differences, as in the tropical

413  abalone *Haliotis asinina* where mantle gene expression studies have characterized a number

414  of genes involved in shell construction and coloration (Jackson et al. 2006, 2007).

415   **Environment influence on cultured pearl colour determination**

416         The grow-out environment of the recipients plays a key role in the final pearl colour

417   achieved by harvest. Our results show that if pearls with a high darkness level are the

418   objective, then the locations GMR-Atiaoa and Ahe seem to be the most appropriate and, from

419   the point of view of the donor outer shell, [B] phenotypes should be selected. This latter

420   phenotype showed no variation between grow-out locations for production of either light

421   and/or "other" pearls. By contrast, [R] phenotype showed no variation between grow-out

422   locations for green pearl colour production. From the point of view of inner shell phenotype,

423   the use of [Y] phenotype donors produced an equivalent rate of darkness pearl level whatever

424   the grow-out location, as it also did for green pearl colour. Effects of light, salinity, and

425   climate on colorations, as well as dietary influence, have been reported in some marine

426   gastropods, (Sokolova & Berger 2000; Liu et al, 2009). Specifically, the colour of pearls may

427   vary with depth as a result of light, temperature and turbidity (Gervis & Sims 1992). For

428   example, the minimum depth required for the rearing of *P. fucata* is 5 m, which is favourable

429   for the production of pearls of a pinkish colour (most valued in China), although the growth

430   rate is slow (Alagarswami 1968). In this study, the cultured pearls harvested from GMR-

431   Atiaoa and GMR-Taku were different, in terms of darkness and colour of pearl, while these

432   two sites belong to the same geographical entity (Gambier archipelago). This non surprising

433   result was explain by the geographic implantation of the rearing site, with GMR-Atiaoa

434   located in a bay where no water current was recorded, in comparison to GMR-Taku situated

435   along the coast where water current was often present. In addition, GMR-Taku received

436   nutrients providing from the near mountains after rain period, whereas GMR-Atiaoa don't

437   have de proximity of mountainous area. At the opposite, distant culture site, such as GMR-

438   Atiaoa and Rangiroa atoll (situated in different archipelago) could show near similar darkness

439   pattern of pearl darkness. This similarity was also not surprising as culture practice plays a

20

440    role in darkness level determinations, as recipient oyster washing frequencies. Recent studies,

441    showed that for colour variation, 10% more pearls have an attractive green overtone in

442    Rangiroa than in Tahaa, where more grey bodycolor were harvested (Ky et al., 2015b). Here,

443    a discriminative model was built of the production of high darkness level pearls, integrating

444    both shell colour phenotype and grow-out location to target, for example, the specificity of the

445    Asian market. Results showed a high rate of sensitivity, where 91% of the total pearls with

446    high darkness level were explained by the following successive criteria (nodes of the

447    classification tree): 1) [G] inner shell phenotype (59%), 2) grow-out location (17%), and 3)

448    outer shell phenotype (15%). Specific expression of colour was observed site-by-site,

449    revealing a notable difference between Rangiroa atoll and Tahaa Island: [Y] explained 55% of

450    the desirable green and peacock colour at Rangiroa, whereas the [G] phenotype explained

451    63% of the same colours. These opposite consequences of using [Y] or [G] inner shell

452    phenotypes highlight that: 1) the environment plays a major role in colour determination and

453    2) the donor inner shell phenotype expression in pearl colour determination is site-specific.

454    By contrast, the [B] outer shell phenotype in these two locations favored for green and

455    peacock pearl production. In addition, donors with the [R] outer shell phenotypes in these two

456    locations favored formation of "other" colour pearls. Such a predictive model could be

457    integrated into pearl farm management for the orientation of pearl colour range production. At

458    the scale of French Polynesia, statistical models could be established to make the best use of

459    local and environmental specificities to enhance production quality at each grow-out site and

460    use appropriate phenotypes (and genetic origin of collected spat) to produce specific pearl

461    colour patterns.

462    **Cultured pearl luster determination**

463        Lustre was already known to be mainly under environmental control.  Our present

464    results confirm this tendency with: 1) of all the traits studied, least significant effect was

21

465     detected when only the inner shell phenotype was considered (lustre is favored by the used of

466     the [Y] phenotype), and 2) the very highly significant effect observed between the grow-out

467     environment with GMR-Taku site showing the worst pearl lustre, in comparison to the other

468     locations, whatever the phenotype. This would rely not only on specific environmental

469     parameters (temperature, salinity, trophic level etc.) of the grow-out site at GMR-Taku, but

470     must also integrate pearl oyster culture management. Indeed, the frequency of recipient

471     cleaning throughout the culture time may impact pearl lustre (farmers' pers. comm.).

472     **Genetic control of shell colour**

473        No genetic studies have been conducted on the coloration of *Pinctada margaritifera* as

474     yet, largely because spat supply relies on natural collection rather than hatchery production

475     (where breeding designs could be applied), but also because of the long breeding cycle of the

476     species and complications caused by environmental factors. A better understanding of the

477     genetic factors underlying *P. margaritifera* shell variants would indirectly benefit the pearl

478     industry. As these colourful phenotypes were not very abundant in the wild, it would be

479     advantageous to propagate such colour lines through the hatchery process. In fact, wild *P.*

480     *margaritifera* mostly have a black outer shell colour combined with grey inner shell colour

481     (phenotype used as recipients). The pattern of geographic variation in the phenotype shows

482     evidence for natural selection (Endler, 1977; 1986). Such geographic variations cause

483     spatially differing selection and thus provoke varying levels of local adaptations. A classic

484     example of local adaptation of the phenotype is concealing coloration in animals (Benson,

485     1933; Cott, 1940; Kettlewell, 1956). Melanism is one form of concealing coloration present in

486     a wide array of organisms (Majerus, 1998). Breeding combinations between *P. margaritifera*

487     shell colour variants and segregation data analysis of the corresponding families would be

488     helpful to understand the complexity of colour determination. Genetic determination of shell

489     colour was first reported in the conch *Urosalpix cinerea*, where a single-locus genetic model

490    controls three colour types (Cole 1975). Similar simple patterns of inheritance were later

491    found in a number of bivalves such as *Mytilus edulis*, *Argopecten irradians*, *Fulvia mutica*

492    and *Haliotis discusthis* (Innes & Haley 1977, Adamkewicz & Castagna 1988, Fujiwara 1995,

493    Kobayashi et al. 2004). For other mollusc species, such as the Pacific oyster (*Crassostrea*

494    *gigas*), shell pigmentation was shown to be a polygenic, quantitative trait controlled by the

495    segregation of many genes of small-effect (Brake et al., 2004; Evans et al., 2009).

**Conclusions**

497        This study was the first to analyse the effect of shell colour phenotypes of *P.*

498    *margaritifera* donors on cultured pearl colour and lustre.  A multisite grow out design with

499    culture locations in the three main French Polynesian archipelagos with pearl farms

500    (Tuamotus, Society and Gambier) enabled us to incorporate a study of environmental effects

501    into our experiment. The importance of a good knowledge of the impact of *P. margaritifera*

502    colour phenotype on cultured pearl colour is of great importance prior to appropriate

503    selection: 1) by grafters for pearl farm production, and 2) by breeders for hatchery

504    propagation. In the present study, we revealed that if dark green pearls were targeted in pearl

505    farm production (Asian market), it would be better to use the [B + G] phenotypes as donors.

506    A statistical predictive model was built, explaining 91% of the total high darkness level of

507    pearl production, following three successive criteria: 1) inner shell phenotype, 2) grow-out

508    location and 3) outer shell phenotype.  In contrast, if medium/ light pearl colour is targeted

509    (European market), it would be better to select donors that incorporate [R] and/ or [Y]

510    phenotypes. Selective breeding for corresponding shell pigmentation in the black-lipped pearl

511    oyster is still in its infancy, and stable strains with desired traits have not yet been produced.

512    Studies are currently being conducted through controlled mating design experiments so as to

513    improve understanding of the complexity of shell colour phenotypes in *P. margaritifera*.

514    Segregation data in full-sib families would indicated the genetic factors implicated in the

23

515     expression of shell colour. Shell colour phenotypic response for cultured pearl colour

516     expression in the present study indicated that effects of the environment and its interaction

517     with genetic factors are important in *P. margaritifera.* Overall, the use of multi-trait selection

518     approaches, taking into account quantitative genetic control, environmental effects and

519     associated correlations, may be the most effective strategy to improve pearl colour for the

520     design of future breeding programs of *P. margaritifera,* as oysters can be reared under a broad

521     range of environments.

522

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670

671    **Figures legends**

672

673    **Figure 1:** *Pinctada margaritifera* donor variants showing green and yellow inner shell

674    phenotypes and black and red outer shell phenotypes.

675

676    **Figure 2:** Experimental design of the grafting procedure of *P. margaritifera* using donors

677    with green [G] and yellow [Y] inner shell phenotypes. All 2520 graft operations were

678    performed in Atiaoa bay, Mangareva island (GMR-Atiaoa). After checking for nucleus

679    retention at 45 days, recipient oysters grafted from each donor oyster were randomly split into

680    five groups, one was left in (1) GMR-Atiaoa and the others were transferred to: (2) Taku bay,

681    another site on Mangareva island (GMR-Taku); (3) Ahe atoll; (4) Rangiroa atoll and (5)

682    Tahaa island. Black stars indicate the locations of pearl farms in the island lagoons and atolls.

683

684    **Figure 3:** Cultured pearl colours produced by *Pinctada margaritifera*: (a) grey and green

685    bodycolor and peacock overtone, with examples of three darkness level (light, medium and

686    dark) and (b) "other" colours. Pictures are harvest samples of high quality cultured pearls

687    (grade A).

688

689    **Figure 4:** Classification tree showing the characteristics of *Pinctada margaritifera* cultured

690    pearls according to donor inner and outer shell phenotypes: (a) darkness level (DARK: high

691    darkness level; LIGHT MEDIUM: light and medium darkness levels) and (b) colour. The

692    fraction (n/N) gives the classification rate at the node, expressed as the number of correct

693    classifications (n) out of the number of observations at the node (N). The rate (in %)

694    corresponds to the proportion of (a) dark pearls and (b) grey or green/peacock pearls.

695

31

696     **Figure 5:** Classification tree showing cultured pearl darkness levels (DARK: high darkness

697     level, and LIGHT MEDIUM for light and medium darkness levels) according to inner and

698     outer shell phenotypes and grow-out locations. The fraction under the leaves (n/N) gives the

699     classification rate at the node, expressed as the number of correct classifications (n) out of the

700     the number of observations in the node (N). The rate (in %) corresponds to the proportion of

701     dark pearls obtained following the node relative to the total high darkness level pearls

702     harvested.

703

704     **Figure 6:** Classification tree showing the cultured pearls colour of *Pinctada margaritifera*

705     following inner and outer shell phenotypes for the locations (a) Rangiroa and (b) Tahaa. The

706     fraction under the leaves (n/N) show the classification rate at the node, expressed as the

707     number of correct classifications (n) out of the number of observations in the node (N). The

708     rate (in %) corresponds to the proportion of dark pearls obtained following the node in

709     relation to the total high darkness level pearls harvested.

710

**Figure 1:**



|  | Outer shell phenotype | |
|---|---|---|
|  | Black [B] | Red [R] |
| **Inner shell phenotype** Green [G] | [B + G] | [R + G] |
| **Inner shell phenotype** Yellow [Y] | [B + Y] | [R + Y] |

**Figure 2:**

**Figure 3:**

**(a)**

| | Grey | Green | Peacock |
|---|---|---|---|
| Light | | | |
| Medium | | | |
| High | | | |

**(b)**

| | Blue | Aubergine | White |
|---|---|---|---|
| Other | | | |
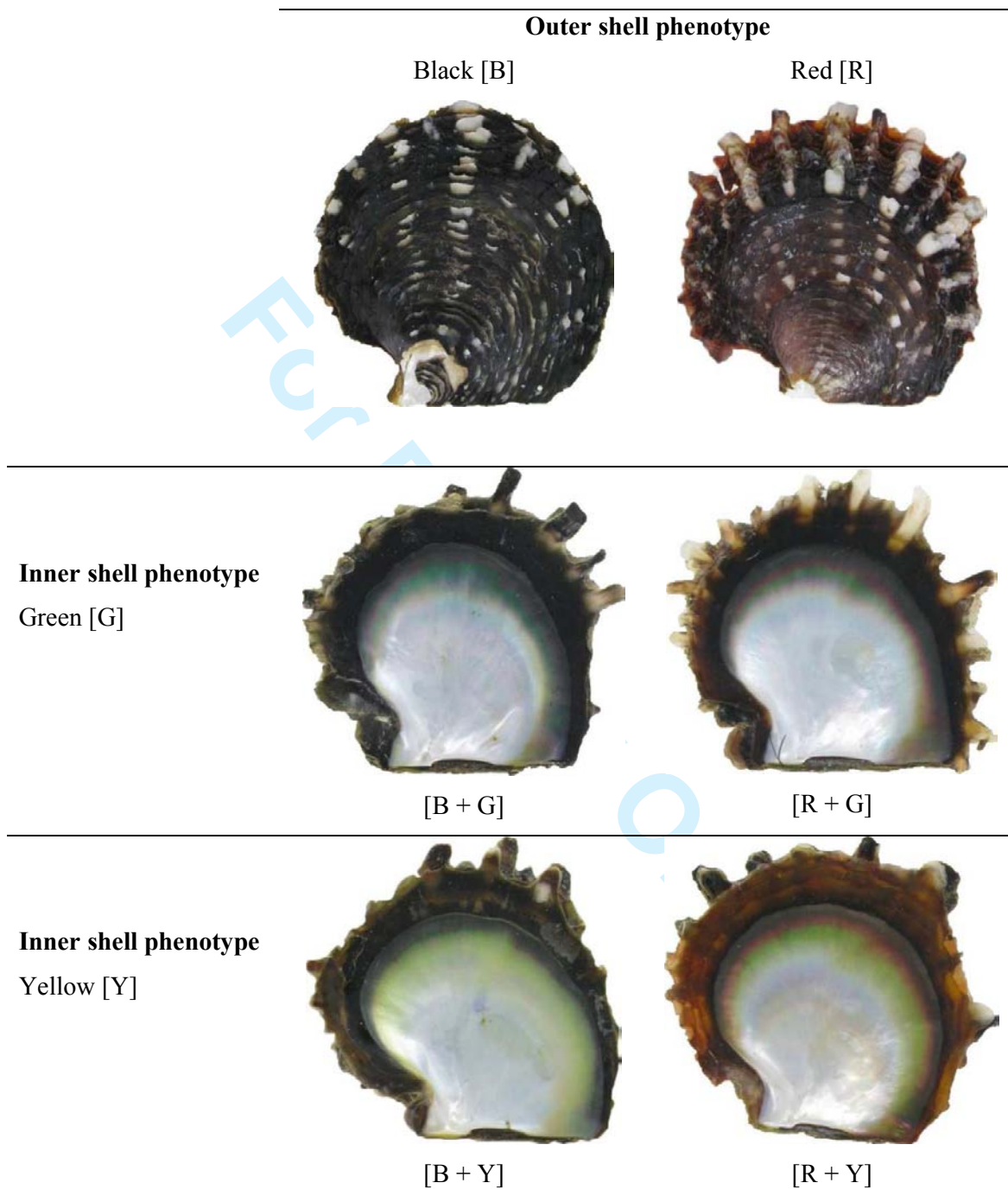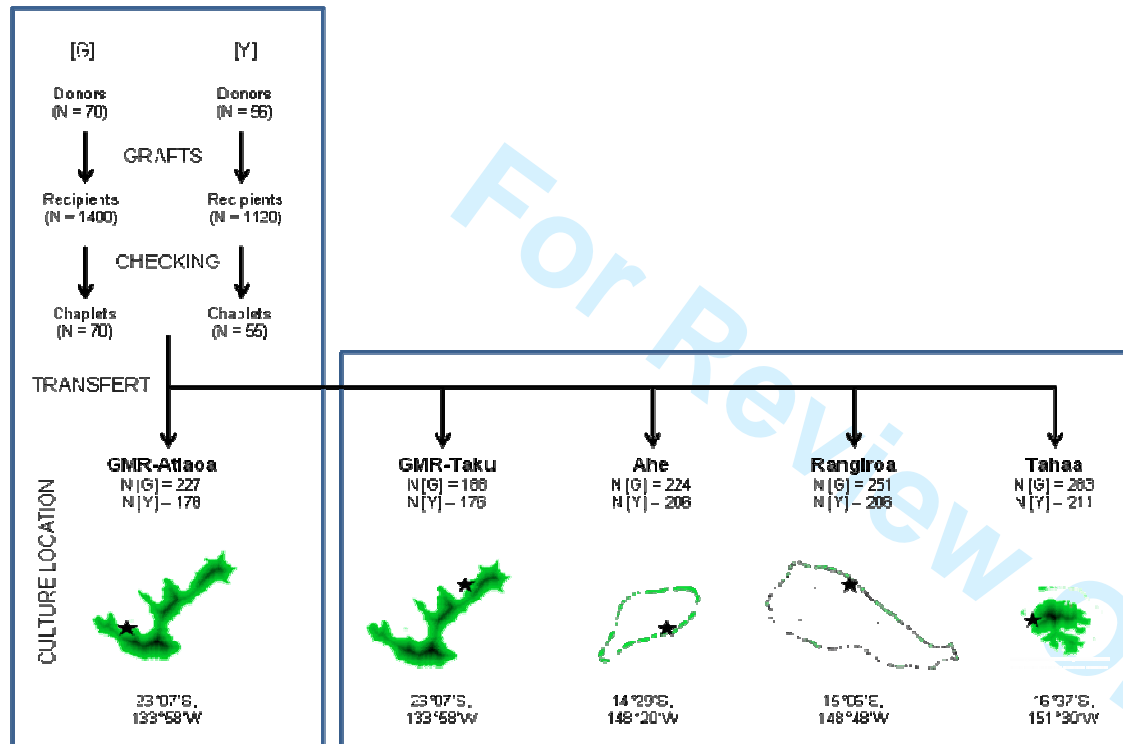
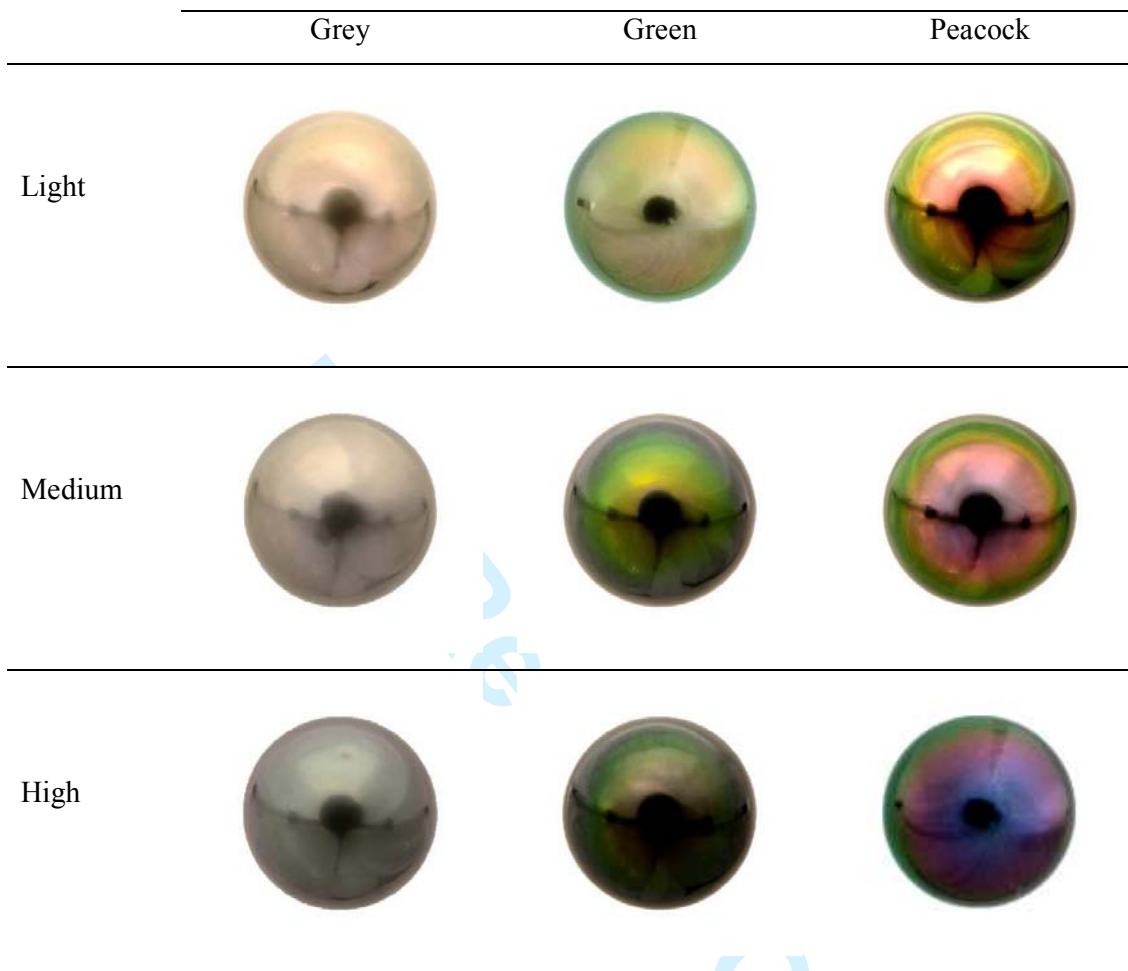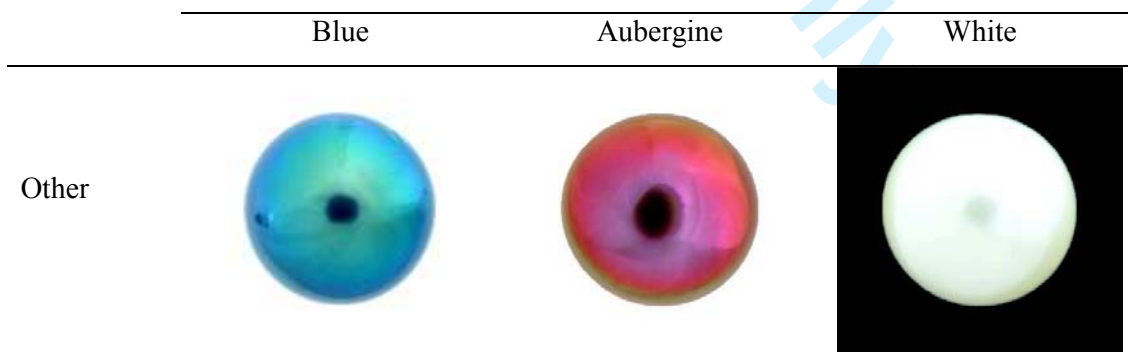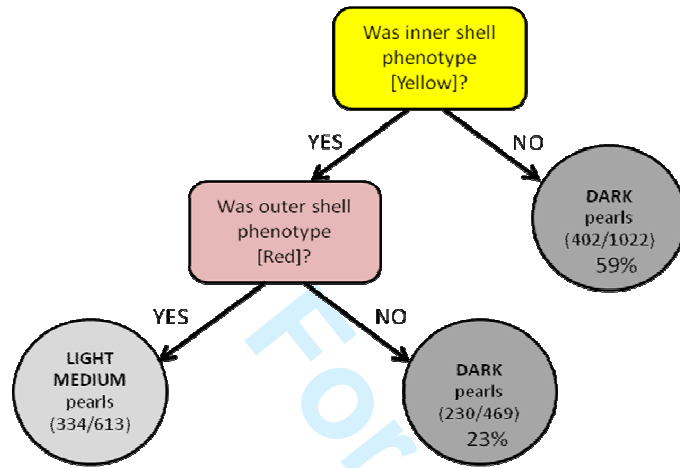**Figure 4:**

a) Cultured pearl darkness level



b) Cultured pearl colour



4
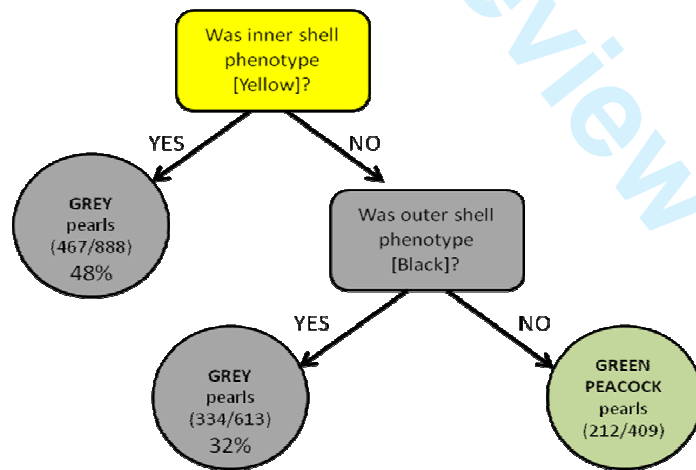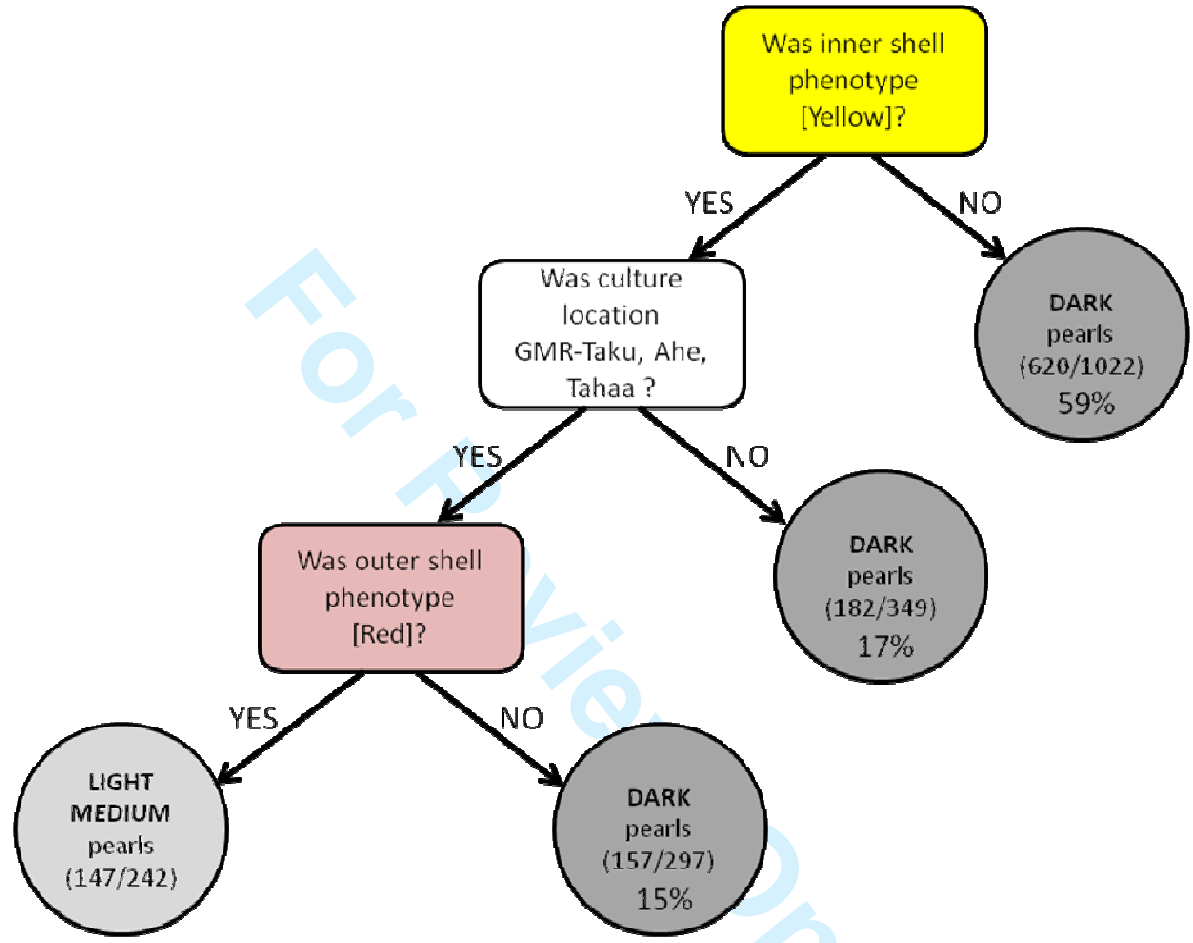
**Figure 5:**

**Figure 6:**

a) Rangiroa site
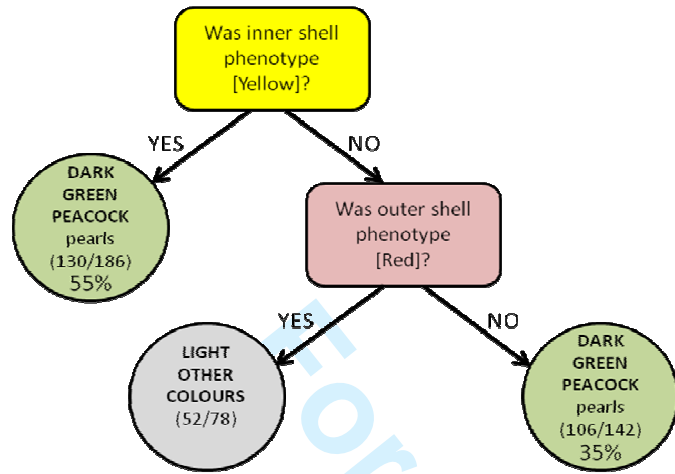


a) Tahaa site
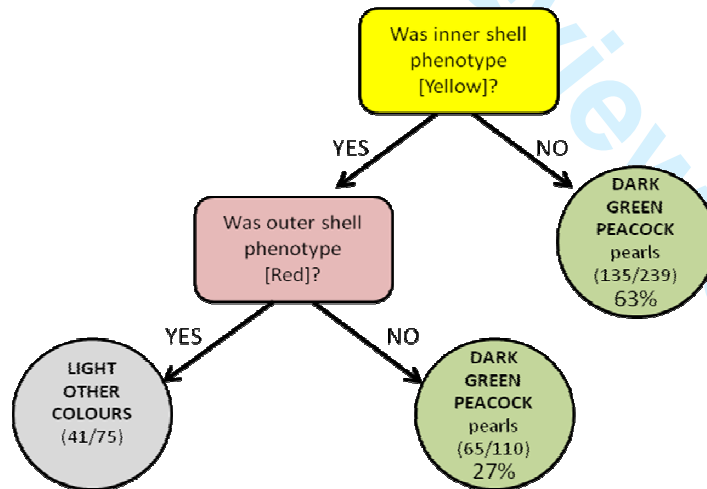
1    **Table 1**. Cultured pearl darkness levels (high, medium and light), colour categories (grey,

2    peacock, green and "other") and lustre rates in % and numbers (in brackets) in pearls issued

3    from grafts made using donor oysters with black [B] and red [R] external shell colour

4    phenotypes. Pearl traits significantly different between the two variants at *0.01 < p ≤ 0.05,*

5    *0.001 < p ≤ 0.01*, and *p ≤ 0.001* are indicated with 1, 2 or 3 asterisk(s) (*), respectively, and

6    NS for not significant.

7

| | | [B] (1082) | [R] (828) | Significance |
|---|---|---|---|---|
| Darkness | High | 59 (637) | 50 (417) | *** |
| | Medium | 31 (332) | 37 (305) | ** |
| | Light | 10 (113) | 13 (106) | **NS** |
| Colour | Grey | 48 (517) | 43 (352) | * |
| | Peacock | 23 (252) | 29 (243) | ** |
| | Green | 16 (168) | 12 (100) | * |
| | Other | 14 (145) | 17 (133) | **NS** |
| Lustre | Yes | 67 (728) | 67 (558) | **NS** |

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1

10   **Table 2**. Cultured pearl darkness level (high, medium and light), colour categories (grey,

11   peacock, green and "other") and lustre rates in % and number (in brackets) in pearls issued

12   from grafts made using donor oysters with green [G] and yellow [Y] inner shell colour

13   variants in *Pinctada margaritifera*. The traits significantly different between the two variants

14   at *0.01 < p ≤ 0.05, 0.001 < p ≤ 0.01*, and *p ≤ 0.001* are indicated with 1, 2 or 3 asterisk(s) (*),

15   respectively, and NS for not significant.

16

|  |  | [G] (1022) | [Y] (888) | Significance |
|---|---|---|---|---|
| Darkness | High | 61 (620) | 49 (434) | *** |
|  | Medium | 30 (306) | 37 (331) | *** |
|  | Light | 9 (96) | 14 (123) | ** |
| Colour | Grey | 44 (448) | 47 (421) | **NS** |
|  | Peacock | 28 (284) | 24 (211) | * |
|  | Green | 16 (164) | 12 (104) | ** |
|  | Other | 12 (126) | 17 (152) | ** |
| Lustre | Yes | 65 (662) | 70 (624) | * |

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2

19    **Table 3**. Cultured pearl darkness level (high, medium and light), colour categories (grey,

20    peacock, green and "other") and lustre rates in % and number (in brackets), in relation to

21    donor oyster combination of both external (black [B] and red [R]) and inner shell (green [G]

22    and yellow [Y]) colour  variants in *Pinctada margaritifera*. The traits significantly different

23    between the two variants at *0.01 < p ≤ 0.05, 0.001 < p ≤ 0.01*, and *p ≤ 0.001* are indicated with

24    1, 2 or 3 asterisk(s) (*), respectively, and NS for not significant. The data points significantly

25    different at *p < 0.05* between the phenotypes are indicated with letters.

26

| | | [B + G] (613) | [B + Y] (469) | [R + G] (409) | [R + Y] (419) | Significance |
|---|---|---|---|---|---|---|
| Darkness | High | 65 $^a$ | 51 $^b$ | 54 $^b$ | 47 $^b$ | *** |
| | | (398) | (398) | (222) | (195) | |
| | Medium | 27 $^b$ | 36 $^a$ | 35 $^a$ | 40 $^a$ | *** |
| | | (164) | (168) | (142) | (163) | |
| | Light | 8 $^b$ | 13 $^a$ | 11 $^a$ | 15 $^a$ | ** |
| | | (51) | (62) | (45) | (61) | |
| Colour | Grey | 46 $^{ab}$ | 51 $^a$ | 41 $^b$ | 44 $^{ab}$ | * |
| | | (279) | (238) | (169) | (183) | |
| | Peacock | 24 $^b$ | 23 $^b$ | 34 $^a$ | 25 $^b$ | *** |
| | | (145) | (107) | (139) | (104) | |
| | Green | 17 $^a$ | 13 $^{ab}$ | 14 $^{ab}$ | 10 $^b$ | * |
| | | (106) | (62) | (58) | (42) | |
| | Other | 14 $^b$ | 13 $^b$ | 11 $^b$ | 21 $^a$ | *** |
| | | (83) | (62) | (43) | (90) | |
| Lustre | Yes | 66 | 69 | 63 | 71 | **NS** |
| | | (403) | (325) | (259) | (299) | |

**Table 4**. Cultured pearl darkness level (high, medium and light), colour categories (grey, peacock, green and "other") and lustre rates in % and number (in brackets), harvested in five grow-out locations (A: Atiaoa, T: Taku; Ahe: Ahe; Rgi: Rangiroa and Tah: Tahaa), in relation to the black [B] and red [R] external shell colour variants in *Pinctada margaritifera*. The traits significantly different between the two variants at *0.01 < p ≤ 0.05, 0.001 < p ≤ 0.01,* and *p ≤ 0.001* are indicated with 1, 2 or 3 asterisk(s) (*), respectively, and NS for not significant. The data points significantly different at *p < 0.05* between the locations are indicated with letters.

| | | Black external shell | | | | | | Red external shell | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Location | A | T | Ahe | Rgi | Tah | Signif. | A | T | Ahe | Rgi | Tah | Signif. |
| | | (200) | (144) | (303) | (214) | (221) | | (176) | (171) | (86) | (192) | (203) | |
| Darkness | High | 59 $^{ab}$ | 54 $^{b}$ | 60 $^{ab}$ | 52 $^{b}$ | 67 $^{a}$ | * | 63 $^{a}$ | 47 $^{b}$ | 36 $^{b}$ | 50 $^{b}$ | 48 $^{b}$ | *** |
| | | (118) | (78) | (181) | (111) | (149) | | (118) | (81) | (31) | (96) | (98) | |
| | Medium | 28 $^{b}$ | 40 $^{a}$ | 29 $^{b}$ | 36 $^{ab}$ | 24 $^{b}$ | ** | 33 $^{b}$ | 37 $^{b}$ | 55 $^{a}$ | 32 $^{b}$ | 37 $^{b}$ | ** |
| | | (55) | (58) | (88) | (78) | (53) | | (58) | (63) | (47) | (61) | (76) | |
| | Light | 14 | 6 | 11 | 12 | 9 | NS | 4 $^{b}$ | 16 $^{a}$ | 9 $^{ab}$ | 18 $^{a}$ | 14 $^{a}$ | *** |
| | | (27) | (8) | (34) | (25) | (19) | | (7) | (27) | (8) | (35) | (29) | |
| Colour | Grey | 55 $^{a}$ | 47 $^{a}$ | 58 $^{a}$ | 45 $^{a}$ | 31 $^{b}$ | *** | 48 $^{b}$ | 44 $^{b}$ | 73 $^{a}$ | 41 $^{b}$ | 25 $^{c}$ | *** |
| | | (109) | (67) | (175) | (97) | (69) | | (84) | (75) | (63) | (79) | (51) | |
| | Peacock | 25 $^{a}$ | 33 $^{a}$ | 7 $^{b}$ | 21 $^{a}$ | 41 $^{a}$ | *** | 31 $^{b}$ | 31 $^{b}$ | 5 $^{c}$ | 23 $^{b}$ | 43 $^{a}$ | *** |
| | | (49) | (48) | (20) | (44) | (91) | | (55) | (53) | (4) | (44) | (87) | |
| | Green | 9 $^{b}$ | 11 $^{ab}$ | 20 $^{a}$ | 19 $^{a}$ | 15 $^{ab}$ | ** | 12 | 13 | 10 | 14 | 11 | NS |
| | | (17) | (16) | (61) | (41) | (33) | | (21) | (22) | (9) | (26) | (22) | |
| | Other | 13 | 9 | 16 | 15 | 13 | NS | 9 $^{b}$ | 13 $^{b}$ | 11 $^{b}$ | 23 $^{a}$ | 21 $^{a}$ | *** |
| | | (25) | (13) | (47) | (32) | (28) | | (16) | (21) | (10) | (43) | (43) | |
| Lustre | Yes | 75 $^{a}$ | 52 $^{b}$ | 69 $^{a}$ | 66 $^{a}$ | 69 $^{a}$ | *** | 78 $^{a}$ | 49 $^{b}$ | 83 $^{a}$ | 70 $^{ab}$ | 65 $^{ab}$ | *** |
| | | (149) | (75) | (209) | (142) | (153) | | (137) | (83) | (71) | (135) | (132) | |

4

**Table 5**. Cultured pearl darkness level (high, medium and light), colour categories (grey, peacock, green and "other") and lustre rates in % and number (in brackets), harvested in five grow-out location (A: Atiaoa, T: Taku; Ahe: Ahe; Rgi: Rangiroa and Tah: Tahaa), in relation to [G] and yellow [Y] inner shell colour variants in *Pinctada margaritifera*. The traits significantly different between the two variants at *0.01 < p ≤ 0.05, 0.001 < p ≤ 0.01*, and *p ≤ 0.001* are indicated with 1, 2 or 3 asterisk(s) (*), respectively, and NS for not significant. The data points significantly different at *p < 0.05* between the locations are indicated with letters.

| | | Green inner shell | | | | | | Yellow inner shell | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Location | A | T | Ahe | Rgi | Tah | | A | T | Ahe | Rgi | Tah | |
| | | (213) | (169) | (181) | (220) | (239) | Signif. | (163) | (146) | (208) | (186) | (185) | Signif. |
| Darkness | High | 67 [a] | 54 [ab] | 65 [a] | 50 [b] | 66 [a] | *** | 53 | 46 | 46 | 52 | 49 | **NS** |
| | | (143) | (92) | (117) | (111) | (157) | | (86) | (67) | (95) | (96) | (90) | |
| | Medium | 27 [b] | 38 [a] | 25 [b] | 38 [a] | 24 [b] | *** | 34 | 39 | 43 | 30 | 39 | **NS** |
| | | (57) | (64) | (45) | (83) | (57) | | (56) | (57) | (90) | (56) | (72) | |
| | Light | 6 | 8 | 10 | 12 | 10 | **NS** | 13 | 15 | 11 | 18 | 12 | **NS** |
| | | (13) | (13) | (19) | (26) | (25) | | (21) | (22) | (23) | (34) | (23) | |
| Colour | Grey | 43 [ab] | 42 [ab] | 51 [a] | 51 [a] | 33 [b] | *** | 63 [a] | 49 [b] | 70 [a] | 34 [c] | 22 [d] | *** |
| | | (91) | (71) | (93) | (113) | (80) | | (102) | (71) | (145) | (63) | (40) | |
| | Peacock | 37 [a] | 34 [a] | 8 [b] | 15 [b] | 42 [a] | *** | 16 [c] | 29 [b] | 5 [d] | 30 [b] | 42 [a] | *** |
| | | (78) | (58) | (14) | (33) | (101) | | (26) | (43) | (10) | (55) | (77) | |
| | Green | 11 [b] | 15 [ab] | 23 [a] | 17 [ab] | 15 [ab] | * | 9 | 9 | 14 | 16 | 10 | **NS** |
| | | (24) | (25) | (41) | (38) | (36) | | (14) | (13) | (29) | (29) | (19) | |
| | Other | 9 [b] | 9 [b] | 19 [a] | 16 [a] | 10 [b] | ** | 13 [b] | 13 [b] | 12 [b] | 21 [ab] | 26 [a] | *** |
| | | (20) | (15) | (33) | (36) | (22) | | (21) | (19) | (24) | (39) | (49) | |
| Lustre | Yes | 77 [a] | 46 [c] | 74 [ab] | 63 [b] | 66 [b] | *** | 75 [a] | 55 [b] | 70 [a] | 75 [a] | 69 [a] | *** |
| | | (164) | (77) | (134) | (138) | (157) | | (122) | (81) | (145) | (139) | (128) | |

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