Determination of Gender in the Pearl Oyster *Pinctada margaritifera*

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Abstract:

The pearl industry in French Polynesia is based on exploitation of natural stocks of the blacklip pearl oyster (*Pinctada margaritifera*). It generates an annual turnover of €90 million. Improvements in pearl quality need genetic studies to improve the populations. This pearl oyster is a protandric species, in which the sex ratio normally is biased toward males. There is an increasing interest in gender control to find the mechanisms to augment female proportions for management purposes. This review summarizes information on exogenous and endogenous factors regulating gender in this and other bivalves, and concludes that *P. margaritifera* is a protandric hermaphrodite, developing as a male during the first 2 y, and without evidence of an effect from abiotic and biotic factors on gender during this phase. Later, pearl oysters progressively change to females, reaching a sex ratio close to 1:1 in specimens older than 8 y. At this stage, gender is apparently influenced by environmental parameters, but particularly by stress. Future research should seek to determine accurately the effect of temperature and food on sex ratios. Studies should be performed to characterize genes responsible for expression of gender. The use of hormones is a path that might be explored to influence the gender of pearl oysters.

Keywords: pearl oyster, *Pinctada*, hermaphrodite, temperature, food, stress, sex genes, sex steroids
1. Introduction

The black-lip pearl oyster (*Pinctada margaritifera* L., 1758) is a benthic bivalve occurring in the Indo-Pacific region, particularly abundant in the South Pacific, New Guinea, Hawaiian Islands, and Polynesia (Le Pennec et al. 2010). Under natural conditions, this species lives near the bottom in coral reef waters, while cultivated pearl oysters are hung from down lines suspended on subsurface long lines, ~10 m from the surface (Pouvreau et al. 1999, Niquil et al. 2001). Farming of *P. margaritifera* started in the early 60s and today represents the main aquacultural activity in the lagoons of French Polynesia (Fig. 1). This activity plays a major economic role, thanks to the production of black pearls, the largest export industry in the region. The market for pearls increased from 86 kg in 1980 to 10 tonnes in 2003, representing 87 million euros (Cochennec-Laureau et al. 2010). The pearl culture industry employs 7,000 people at 800 farms, occupying approximately 10,000 hectares at 30 atolls and islands of Polynesia (Salvat et al. 2008).

Current studies and prospective projects of *P. margaritifera* in French Polynesia are strictly oriented to address the problems occurring on pearl farms. The Polynesian pearl culture agency (*Service de la Perliculture*), together with researchers of IFREMER (Tahiti), coordinates most of the studies dealing with pearl oysters in Polynesia. One of the main objectives for cultivating *P. margaritifera* is control of reproduction under controlled settings to guarantee a steady supply of spat (Le Moullac et al. 2003). Controlling reproduction involves broodstock conditioning, induction of spawning, cultivation of larvae, settlement of eyed larvae, and nursing of early spat. The economic viability of such tasks may require the ability to produce spat with desirable traits (pearl colour, fast growth,) either through polyploidization (He et al. 2004) or through selection (Deng et al. 2009), both strongly dependent on genetic resources in the wild (Arnaud-Haond et al. 2003).

Reproduction of pearl oysters under controlled conditions mainly depends on the availability of mature females, which unfortunately are not naturally abundant. *P. margaritifera* is protandric; females are found in low quantities and mainly among old animals in natural populations or in stocks at pearl farms. This results in low availability of eggs for laboratory management. Furthermore, the time to produce new breeding groups is too long for selection purposes. One of the main objectives of IFREMER and the Polynesian pearl culture agency is to identify mechanisms that will increase the proportion of females in a less time. Results from different lines of investigation indicate that determining gender is essential in gonad determination either in gonochoristic or hermaphrodite species, but fewer studies have been done on the mechanisms of sex ratio variations in pearl oysters. The gender-determining mechanism is one of the factors affecting sex ratio, but other genetic or non-genetic factors (genetic, cytoplasmic, or environmental factors) may affect the sex ratio (Yusa 2007). Within the animal kingdom, gender is determined by genetic or environmental factors or the interaction of both (Valenzuela et al. 2003). This report describes the factors that are potentially involved in gender determination in *P. margaritifera*.

2. State of the art in *Pinctada margaritifera*

As with other pearl oysters, including *P. mazatlanica* (Saucedo & Monteforte 1997), *P. albina sugilata* (O’Connor 2002), *P. imbricata* (Kimani et al. 2006), *P. fucata* (Hwang 2007), and *P. radiata* (Derbali 2009), *P. margaritifera* is a protandrous hermaphrodite (Tranter 1958). Hermaphroditism or ambosexuality consists of four categories according to Coe (1943) and Kasyanov (2001): (1) functional hermaphroditism (functional ambosexuality), where an animal concurrently develops both sperm and eggs, (2) consecutive sexuality, where once in the life of the bivalve, the animal undergoes a single switch in gender, usually from male to female, (3) rhythmic consecutive sexuality, where the animal experiences an equal number of sexual phases, changing from one gender to the other and maintaining a rhythmic pattern throughout its life, and (4) alternative sexuality, whereby animals change gender depending on seasonal or environmental triggers. For example, *P. mazatlanica*, which is phylogenetic close to *P. margaritifera*, shows consecutive sexuality because individuals change gender once in their lifetime every year (Arnaud-Haond et al. 2003).

The first studies of changes in gender in natural populations of *P. margaritifera* were described by Tranter (1958a) in pearl oysters in Australian waters. The author reports protandric and protogynic gender changes, concluding that several sexual phases may occur during the life of the pearl oyster, which could correspond to rhythmic consecutive sexuality. He considers this pearl oyster a protandric consecutive hermaphrodite even if, on rare occasions, male and female phases were observed simultaneously in the same specimen; almost always they are separate phases. Thielley (1993) shows changes from male to female and from female to male when conditions, either natural (temperature or
food) or non-natural (handling or cleaning) were stressful. These observations would better correspond to alternative sexuality. In our laboratory (unpublished results), of 97 pearl oysters twice sexed through observations of gonadal smears at 6-month intervals, four females changed to males (protogynic) and one female became indeterminate, which suggests a forthcoming change to male. Pouvreau et al. (2000a) confirms findings of gender change by Tranter (1958a) and concludes that *P. margaritifera* in farm systems is a protandric successive hermaphrodite with a highly dominant male phase. In all cases, the observation of bisexuality or undetermined status was uncommon. Bisexual animals suffer a cost in energy because they maintain two reproductive systems (Heath 1976). For example, Pouvreau et al. (2000a) found only seven bisexual specimens among 3,360 pearl oysters, with no evidence that both gonads were functional, but they did not find pearl oysters of undetermined gender. Thielley (1993) found some sexually undefined specimens among the oldest individuals. It seems that gender changes occur quickly and are probably not common, so that it is difficult to detect through classical techniques (histology); therefore, it is necessary to determine whether *P. margaritifera* is a successive hermaphrodite that changes gender once in their life or whether this species has alternative sexuality, depending on seasons or environmental conditions.

At Takapoto atoll, Pouvreau et al. (2000a) found that sex ratio of cultivated specimens had a clear relationship with size, where ~25% were females among the oldest group, against 4% in young pearl oysters (<3 years). At this same atoll, Thielley (1993) sampled 30 pearl oysters each month (>130 mm height, approximately five years old) during an 18-month study where half came from wild beds and the other half from farm systems. She found that females represented up to 80% of the wild population and 50% of the cultivated population (Fig. 2). When the mean proportion of wild (47.3%) and cultivated (30.6%) female pearl oysters were compared, a significantly higher proportion of females are found in natural populations.

An analysis of a series of reports spanning more than 10 years concerning the gender of pearl oysters at farms showed differences in the sex ratio between the atolls and islands of French Polynesia. Although the gender of specimens was determined by observation of gonadal smears and histology, the proportion of females increased northward, from ~23°S to ~14°S (Table 1). The comparison of percentages detects three groups that are statistically homogenous, but sampling size was important because there were only two groups of pearl oysters >130 mm. The larger-sized group had higher proportions of females than smaller pearl oysters, but the latitudinal trend remained with more females in the atoll of Takapoto (Thielley 1993) and less in the Gambier Archipelago (Le Moullac et al., accepted) (Fig. 1).

### 3. Size and age

Sex ratios in bivalve populations are usually close to 1:1 (Morton 1991), but as animals grow and age, the gender of young specimens may bias toward females or males. According to Allsop & West (2004), the sex ratio is biased toward the gender that individuals first reach at reproductive maturity. As a general rule, male cells mature in advance of the female cells, so the initial phase of functional sexuality is male (Coe 1943). Examples include *Arca noae* (Peharda et al. 2006), *Crassostrea corteziensis* (Chávez-Villalba et al. 2008), and *Pinctada radiata* (Derbali et al. 2009), where males predominate in the small shell categories and females becoming predominant as size increases. Less frequently, the initial phase of a young population is female and individuals are protogynous (Coe 1943), such as the mussel *Mytella charruana*, where females are more abundant than males in all size classes (Stenyakina et al. 2010). Tranter (1958a) found that young specimens of *P. margaritifera* from a wild population were composed mainly of males (>70%), and later, the proportion of females increased to 50%. Pouvreau et al. (2000a) found that small, cultivated pearl oysters (<80 mm) were male and that females began to appear in pearl oysters >90 mm, reaching ~25% of the population at 120 mm. In this case, *P. margaritifera* behaves as a protandric species under natural and cultivated conditions.

Data from unpublished reports and recent information from our laboratory, allowed us to observe different farms and determine the proportion of males, females, undifferentiated, and hermaphrodites according to shell size. Similar to Pouvreau et al. (2000a), individuals <80 mm were exclusively male and gender change appeared when specimens were ~80 mm. The proportion of females continued to increase as the pearl oysters grew, reaching 65% in specimens >180 mm (Fig. 3). Undifferentiated pearl oysters occurred only in pearl oysters >130 mm and this increased as pearl oysters become older (from 4.7% at 130 mm to 11.1% at >160 mm). Of 1067 pearl oysters that were examined, only one was bisexual (110 mm; 0.01% of this population). These data were related to the approximate age of *P. margaritifera*, with gender change occurring after their second year and a dominant proportion of females in groups older than eight years (Fig. 4).
Age at maturity may affect sex ratios because of the stock of individuals available for mating (Yusa 2007). Age at maturity of *P. margaritifera* has not been clearly determined; size at initial sexual maturity, corresponding to the smallest individuals with mature gonads (stage 4), is <40 mm (Pouvreau et al. 2000a). Other pearl oysters, such as *P. albina* and *P. fucata* become sexually mature within the first 6 months and probably spawn twice in the first year (Tranter 1958b, 1959). A population of *P. radiata* (average height 54.5 mm) of Gulf of Gabes, Tunisia, showed a sex ratio (female:male) of 1:1.27 and the size when 50% of the population reached maturity was 28 mm for males and 38 mm for females (Derbali et al. 2009).

In the case of *P. margaritifera*, there is the possibility that sex ratios might be affected by metabolic expenditures during development. Gamete formation demands a lot of energy; many marine invertebrates invest heavily in gametogenesis and spawning so that reproduction represents a major stress (Barber & Blake 1991). During the early stages of development, most of the energy is used for growth; after some time, up to two years, more reserves are directed toward reproduction. Pearl oysters are producing sex cells at the age of one year, but the gonad begins to develop after two years (Le Pennec et al. 2010). From this scenario, the first expression of gender will be males since they need less energy than females to produce gametes, and females appear once the energy demands for growth decrease and more energy is available for development of gametes. Since there is no stored reserves for gametogenesis in this pearl oyster, Le Pennec et al. (2010) suggests that energy for gamete development comes directly from food. Given the material and energy costs of gametogenesis and spawning, metabolic expenditures are likely to change considerably during development, particularly in species with high reproductive output (Kraffe et al. 2008). In *P. margaritifera*, the energy allocated to reproduction is 22% in two-year-olds, but 50% in those over five years (Pouvreau et al. 2000a).

It appears that the critical phase for determining gender in *P. margaritifera* occurs when the pearl oysters are more than two years old, since there is no evidence of gender changes before this age. It would be advantageous to determine the age at first maturity, particularly in females, to identify those that are reproductively active.

### 4. Genetic determination of gender

Genes that determine gender are of two-factor oligogenic and polygenic systems. Gender determination by heterogamety, XY (male heterogamety) or ZW (female heterogamety), is a well-known system involving two genetic factors (Yusa 2007). The majority of gonochoric molluscs with known gender-determining systems, including some bivalves (Allen et al. 1986; Guo & Allen 1994), are XY. In the oyster *Crassostrea gigas*, gender determination is mainly influenced by males because there is a feeble female effect (Guo et al. 1998). The authors suggest that a familial sex ratio is controlled by a small number of genes expressed at a small number of loci. They found two genotypes for males; true males (MF), protandric males (FF), and one single genotype for females (FF), assuming that the allele M is a dominant male allele and allele F a recessive protandrous allele.

In *Mytilus edulis*, *M. trossulus*, and *M. galloprovincialis*, there is a large sex ratio bias in favour of males or females. The degree of bias is a characteristic property of the female parent because mating of a female with different males produces the same sex ratio, but mating of a male with different females produces different sex ratios (Kenchington et al. 2002). The sex ratio is more biased in protogynous (female first) than in protandrous (male first) species (Allsop & West 2004). At sexual maturation (2–3 months) of the dwarf surf clam (*Mulinia lateralis*), all gynogenetic diploids were female, indicating that this species has XX females and XY males determined by Y domination (Guo & Allen 1994). In *C. gigas*, artificial gynogenesis is a way to obtain female lines (Guo et al. 1993). The method involves irradiating spermatozoids with ultraviolet rays; after fertilization, cytochalasine B is applied to block the second polar body during meiosis II. This approach has not been applied to *P. margaritifera*, where investigations have focused on population genetics (Le Moullac et al. 2003, Herbinger et al., 2006, Arnaud-Haond et al. 2008). It would be interesting to identify gender-determining genes in *P. margaritifera* and determine if there is familial dominance controlled by maternity or paternity (Hedrick & Hedgecock 2010, Powell et al. 2010).

### 5. Hormonal control

Some sex steroids that play important roles in vertebrate reproduction have similar physiological effects on molluscs (Janer & Porte 2007). Estradiols, androgens, progestins, 17ß-estradiol, testosterone, 11-keto-testosterone, 5α-dihydro-testosterone, and progesterone occur in the blue mussel *Mytilus edulis* (Reis-Henriques & Coimbra 1990), Pacific oyster *C. gigas* (Le Curieux-
Belfond et al. 2001), and Japanese scallop Patinopecten yessoensis (Matsumoto et al. 1997). These studies show that variations of sex steroids are related to the sexual maturation cycles in these species. In bivalves, estrogens are more abundant in females and androgens in males (Reis-Henriques et al. 1990, Siah et al. 2002, Croll & Wang 2007). In organ culture experiments of several molluscs, internal controls (Lubet & Mathieu 1982), in vitro effects of heterologous molecules (Pazos & Mathieu 1999), or endogenously active molecules have been clearly demonstrated. Among the putative active molecules, the GnRH pathway, mediated by specific receptors for GnRH or GnRH-like peptides, occurs in C. gigas and M. edulis (Pazos & Mathieu 1999); also indicated is insulin signalling (Gricourt et al. 2003, 2006).

Estradiol creates a feminizing effect in bivalves (Mori et al. 1966, Varaksina & Varaksin 1991) and other invertebrates and vertebrates (Schoenmakers et al. 1981). Mori et al. (1966, 1969) show the effects of estradiol on seasonal gender reversal in C. gigas, finding that injections of estradiol at early stages of seasonal gender maturation induce gender reversal from males to females; Li et al. (1998) show that injections of estradiol stimulate vitellogenesis. Moss (1989) shows that methyltestosterone fed to the cute clam Mulinia lateralis before spawning increases the male/female ratio from 0.8 to 1.6 in. Injections of estradiol, testosterone, progesterone, and dehydroepiandrosterone (DHEA) into the Atlantic sea scallop Placopecten magellanicus accelerated gonadal differentiation, stimulated morphological and sexual differentiation, and in some cases shifted sex ratios toward males (Wang & Croll 2004, Croll & Wang 2007). Despite suggestive evidence for effects of sex steroids on seasonal maturation, there are no reports of possible effects of these substances on determining gender at the juvenile stage, before the first signs of sexual maturation.

6. Environmental effects on gender

In gonochoric molluscs, an individual’s gender is determined environmentally, genetically, or both (Yusa 2007). Expressions of gender has a strong environmental component, resulting from abiotic factors (temperature and length of day) or biotic factors (availability of food, symbionts, parasites, or exposure to nearby individuals of a specific gender (Korpelainen 1990). In most bivalves, the main factors affecting reproduction, and probably gender, are temperature and availability of food (Mann 1979, Chávez-Villalba et al. 2003, Dutertre et al. 2009), and photoperiod, but in a lesser degree (Saout et al. 1999). In general, reproductive phases (storage, gametogenesis, spawning, and inactivity) in temperate zone bivalves are particularly controlled by variations in temperature (Chávez-Villalba et al. 2002). For the temperate zone Pacific oyster C. gigas, the reproductive cycle is entirely modulated, accelerated, or delayed by temperature and photoperiod. Temperature plays a key role in regulating gonial mitosis (Fabioux et al. 2005). Reproduction in P. margaritifera occurs, as in other tropical bivalves, in regions with stable temperatures without showing significant seasonal variations (Narváez et al. 2008). In French Polynesia, an area as large as Europe, the most important differences in temperature are found between archipelagos, where the annual range is 3 °C in the northern Tuamotu Archipelago and 8–10 °C in the Gambier Islands (Le Moullac et al. 2003). Although gametogenesis in P. margaritifera takes place throughout the year, a reproductive cycle exists; the most intense spawning occurs during the warmer season of December–April (Pouvreau et al. 2000b).

Temperature above or below a threshold value plays an important role in gender differentiation of eggs of many amphibians and reptiles. Progeny developing under a particular temperature regime consists solely of males or females. Although Pouvreau et al. (1999) did not demonstrate an effect of temperature on the physiology of P. margaritifera, it is not certain what the effect of temperature is on determining gender, although it does affect some bivalves, particularly oysters. While determination of gender in oysters is genetically controlled, it is influenced by environmental factors (Guo et al. 1998). At three sites of France, the high proportion of C. gigas females changed during summer to low or no females in winter; males were present throughout the year (Lango-Reynoso et al. 2006). The authors found increasing proportions of hermaphrodites (probably not functional) as temperature increased in spring, suggesting a transitional phase from male to female in preparation for reproduction and spawning. Constant low water temperature (~8 °C) during a one-year experiment, under controlled conditions, led to more C. gigas males (Fabioux et al. 2005). The tropical Cortez oyster (C. corteziensis) in different areas had different patterns of gender change related to the local temperature range. When the temperature range is ~18 °C, there are more males when the oyster is small and more hermaphrodites, as an intermediate stage (Chávez-Villalba et al. 2008); there are more females throughout the year and no hermaphrodites when the temperature range is 9 °C (Rodríguez-Jaramillo et al. 2008).

Availability of food, particularly particulate organic material, seems to affect gametogenesis in P. margaritifera in tropical environments (Pouvreau et al. 2000a). Tranter (1958) and Pouvreau et al.
relate protogynic changes to food supply. Using three food concentrations, Lango-Reynoso (1999) finds that the sex ratio in *C. gigas* changes with trophic level; during a 60-day winter, more females (63%) were present at the highest trophic level and more males (62%) at the lowest trophic level. These observations are similar to results with the Charru mussel *Mytella charruana*, where mussels from different locations were raised in the laboratory, with or without food. Under starvation conditions, the sex ratio shifted toward a male bias within one month (Stenyakina et al. 2010). This suggests that females have different metabolic needs than males, requiring more food energy to develop.

Social factors may modify gender ratios in some species. In the paradise fish *Macropodus opercularis*, isolation of specimens favours males (89%) and aggregating paradise fish increases the proportion of females (Francis 1984). In the dusky grouper *Epinephelus marginatus*, gender change does not depend on age or size, but on population structure, particularly the density of groups (Zabala et al. 1997). This behaviour has not been described in bivalves, but there are examples in molluscs. Small specimens of the slipper limpet *Crepidula fornicata* that settle on larger individuals mature as males and solitary limpets mature as females (Hoagland 1978). In the Hikueru Atoll, Ranson (1961) found *P. margaritifera* females varied from 8% to 81% within 10 different groups that were part of a single population. Although there is no evidence that gender in bivalves could be determined by the population structure, previous observations suggest that variations in sex ratios may be related to the way animals are located in the natural beds; this idea should be tested.

Frank & Swingland (1988) show that one gender will be more abundant under relatively unfavourable conditions. For example, *C. gigas* males predominate when the environment is less favourable for reproduction and females predominate when conditions favour gamete production and spawning (Steele & Mulcahy 1999). Although this theory has not been definitively demonstrated (Baghurst & Mitchell 2002), similar results occur in *P. margaritifera* in French Polynesia (Thielley 1993, Pouvreau et al. 2000b).

7. Pollution and stress

There are factors that may have stressing effects on marine animals, including pollution, habitat degradation, over-fishing, pathogens, and global warming. One of the main problems affecting natural and cultivated populations of bivalves is pollution. Changes in gametogenesis and sex ratios have been correlated with environmental contaminants (Hellou et al. 2003, Ortiz-Zarragotía & Cajaraville 2010). Widespread use of organotin compounds, such as tributyltin (TBT) and triphenyltin, as anti-fouling biocides for ships, stabilizers for plastics, pesticides, and catalysts in industry has severely affected marine animals (Morcillo et al. 1998, Gagné et al. 2003). Tributyltin is known as an endocrine disruptor that interferes with steroid receptors and acts as an agonist; it has similar effects as endogenous hormones or as antagonists interfering with the effects of endogenous steroids (De Rosa et al. 1998). TBT causes imposex, development of male organs in female gastropods (Matthiessen & Gibbs 1998). In a population of the soft-shell clam *Mya arenaria*, abnormal increases of males were caused by exposure to TBT (Gagné et al. 2003). Organotin compounds also produce other broad biological effects on bivalves, such as increased activity of cytochrome P450 reductase in the grooved carpet shell clam *Ruditapes decussata* (Solé 2000) or atrophy of digestive cells in the European flat oyster *Ostrea edulis* (Axiak et al. 2000). Tributyltin is monitored in many marine environments; in many places, the concentrations cause imposex (Le Blanc & Bain 1997). There is only one study dealing with expression of gender in isolated populations of *P. margaritifera* related to environmental stress (Dolgov 1991). The author found three patterns of gender structure and different sex ratios: a natural population appeared to be typically protandric (31% females); a colonizing population on an off-shore platform appeared gonochoristic (36% females); and an established population on another off-shore platform appeared to have pseudoprotogynic gender changes from oil pollution (15% females).

8. Cultivation practices

Cultivated bivalves suffer from stress from handling, cleaning, and transport. Handling stress seems to have important consequences on reproduction, the immune system, and survival. This is particularly important for pearl oysters; under natural conditions, they live close to the substrate, fixed through their byssal threads to coral or rock. Shells of cultivated pearl oysters are drilled so they can be tied to ropes that hang from long-lines. Under cultivation, these pearl oysters are frequently exposed to mechanical disturbances throughout their life: removed from water, cleaned, and then grafted to produce a cultivated pearl. Grafting involves implantation of a spherical bead of shell
of this procedure on sex ratios, although many thousands of black-lip pearl oysters are grafted every year for production. Since female 
P. margaritifera are very sensitive to stress, males predominate when the pearl oysters are subjected to unfavourable environmental or artificial conditions.

Genetic approaches are needed to increase knowledge of gametogenesis, as well as gender determinism in 
P. margaritifera. Transcription analysis of gametogenesis may help to identify genes specific to gender or reproductive stage and identify the molecular basis of reproductive traits, such as reproductive effort. We already understand that gender is determined by a cascade of molecular signals that trigger differentiation of germinal cells into oocytes or spermatozoids. Within this cascade, several genes are key in determining gender because the major gene SRY carried by the Y chromosome is a initiator of the early stages of testicular determinism in mammals or the FOXL2 and SOX9 genes that are crucial to ovarian and male differentiation, respectively (Koopman & Loffler 2003, Ottolenghi et al. 2005). This approach might be developed quickly, thanks to advances in characterizing genomic resources in the black-lip pearl oyster (Joubert et al. 2010) and the recent findings in 
C. gigas on conception, fabrication, and utilization of DNA tags (Jenny et al. 2007, Fleury et al. 2009, 2010, Sussarellu et al. 2010). Research to date has clearly demonstrated that sex steroids affect reproduction in molluscs, as well as determine gender (Croll & Wang 2007). So far, studies have focused on a few species; there is no information on the possible roles of sex steroids in 
P. margaritifera.

Studies of how environmental parameters determine gender in 
P. margaritifera are underway at IFREMER-Tahiti, but much additional research is needed to understand genetic and hormonal

9. Conclusions and perspectives

This review shows that 
P. margaritifera is a protandric hermaphrodite that is a male during the first two years of life, changing progressively to female over several years to reach a sex ratio close to 1:1 in populations older than eight years (>180 mm of shell height). Gender changes appear to occur rapidly; populations have a very low proportion of bisexual and undifferentiated pearl oysters. Minority specimens have only been detected in large-sized pearl oysters and these may represent a change-in-gender phase. Thielley (1993) associates this phase with pearl oysters in poor physiological condition. Further studies may determine the age at first sexual maturity of males and females, and as indicated, verify how and how many gender changes occur after the second year. There is no conclusive evidence that this species has successive hermaphroditism (single change of gender from male to female). This is important for controlling reproduction in laboratories because each form of hermaphroditism requires a different management approach.

There is no evidence of biotic and abiotic parameters and other factors influencing a change of gender in the first two years of life; all the variations associated with temperature, food supply, or stress seem to affect specimens older than two years, particularly females. Since 
P. margaritifera occupy areas of relatively homogenous temperature and food environments, the effect of these parameters on expression of gender has not been well established; however, the evidence suggests that temperature influences sex ratios in this species because there are more females in northern populations, where there is a narrower temperature range as well has higher average temperatures. The effect of food on protogynic gender changes was proposed a long time ago (Tranter 1958) and more recently by Pouvreau et al. (1999). Female pearl oysters probably have higher metabolic rates than males and this might affect expression of gender. This possibility needs investigation to find strategies to supply food energy to support development of females.

Stress seems to be the most important factor controlling gender expression since evidence shows that females are present in significantly lower numbers in pearl oysters subjected to culturing or pollution than undisturbed populations. Pearl farms in French Polynesia have a declining proportion of females, probably related to the greater effort to produce pearls of superior quality to meet the standards of the Polynesian pearl culture agency to stabilize the industry. This creates high stress in pearl oysters. Knowing that grafting has an effect on gender, there is no documentation of the effects of this procedure on sex ratios, although many thousands of black-lip pearl oysters are grafted every year for production. Since female 
P. margaritifera are very sensitive to stress, males predominate when the pearl oysters are subjected to unfavourable environmental or artificial conditions.

Material (the nucleus) and a piece of mantle tissue. The graft (about 4 mm²) comes from a sacrificed donor; it is placed in the pearl pouch of a recipient (Acosta-Salmon et al. 2005, Acosta-Salmon & Southgate 2005). In 
C. gigas, extreme mechanical disturbances lead to neuroendocrine responses that cause a transient state of stress (Lacoste et al. 2001, 2002). According to Saucedo et al. (2001), grafting modifies the sex ratio in the Calafia pearl oyster 
P. mazatlanica. When they grafted a piece of mantle without a nucleus in the gonadal tissue of 70 pearl oysters, the male/female sex ratio was 1:2, compared with an untreated control group. There is no detailed information on the effects of grafting on the sex ratio of 
P. margaritifera, but in recent surveys of French Polynesian pearl farms, some stocks contain as little as 5% females.
effects in expression of gender. Identifying the effects of environmental parameters, gender-specific genes, and steroids is particularly important to understand the biology of the black-lip pearl and apply this knowledge to reproduction management.

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11. Literature cited


Table 1.: Proportions of female pearl oysters (*Pinctada margaritifera*) at different latitudes in French Polynesia

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<tr>
<th>Site</th>
<th>Latitude</th>
<th>Size (mm)</th>
<th>Female (%) ¹</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takapoto (1990–1991)</td>
<td>14°38′S</td>
<td>&gt;130</td>
<td>30 (25–35)</td>
<td>294</td>
</tr>
<tr>
<td>Rangiroa (2002)</td>
<td>15°06′S</td>
<td>&gt;130</td>
<td>27 (15–49)</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>22 (14–27)</td>
<td>144</td>
</tr>
<tr>
<td>Vairao (2007)</td>
<td>17°47′S</td>
<td>&gt;130</td>
<td>18 (17–24)</td>
<td>517</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95–185</td>
<td>17 (12–18)</td>
<td>585</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70–148</td>
<td>7.4 (8–12)</td>
<td>712</td>
</tr>
</tbody>
</table>

¹Confidence interval in parentheses
Figure 1. Study sites in French Polynesia and some of the main pearl farms (white circles).

Figure 2. Proportion of female pearl oysters (*Pinctada margaritifera*) >130 mm found in samples (n ≈ 30) taken monthly from a natural population and a pearl farm at the Takapoto Atoll (*Thielley 1993*). Females were identified by histological examination.
Figure 3. Frequency distribution of relative size of males, females, hermaphrodites, and undifferentiated pearl oysters (Pinctada margaritifera) identified by gonadal smears or histological examination of specimens from different farms and natural populations in French Polynesia (unpublished data from IFREMER). Sex change occurs in specimens >80 mm.

Figure 4. Proportion of female pearl oysters (Pinctada margaritifera) in relation to age and size. Data were pooled from different natural populations and farms in French Polynesia (unpublished data from IFREMER).