Gametogenic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atoll (French Polynesia)

Stéphane Pouvreau [a, b*], Aline Gangnery [c], Jérôme Tiapari [b], Franck Lagarde [b], Matthieu Garnier [d], Alain Bodoy [a]

[a] *Crema (Ifremer-CNRS), BP 5, 17137 L'Houmeau, France*

[b] *Ifremer Cop, BP 7004, Taravao Tahiti, French Polynesia*

[c] *Ifremer-Palavas, Chemin de Maguelone, 34250 Palavas-Les-Flots, France*

[d] *CNRS-Station Biologique, Place G. Teissier, BP 74, 29682 Roscoff, France*

Abstract — The gametogenic cycle and the reproductive effort of the blacklip pearl oyster, *Pinctada margaritifera*, cultivated in Takapoto lagoon were studied for a 1-year period (March 1997–April 1998) by bimonthly observations of gonadal sections, dry tissue weights and gonadal index in a population of pearl oyster composed of three age-groups. Pearl oysters attained sexual maturity in the end of their first year (height ≈ 40 mm), implying that *P. margaritifera* is a late-maturing species in comparison with other Pteriidae. This species was also confirmed to be a marked protandrous successive hermaphrodite in culture, with 100 % of males at first maturity and 75 % in older pearl oyster (height > 120 mm). The general pattern of gametogenic activity, fairly synchronous in both sexes, was comparable with that of other tropical bivalves: reproduction occurs continuously throughout the year with a maximal activity during the warm season (November–May). No resting period was observed. Quantitative growth data showed that *P. margaritifera* exhibits an annual synchronised polymodal spawning pattern, with two spawning peaks in age-group I (height ≈ 70 mm) and five in age-groups II (height ≈ 100 mm) and III (height ≈ 120 mm). Spawning was sometimes incomplete, nevertheless a clear relationship between gamete production (P_R, g) and size (height H, mm) was obtained: $P_R = 5.26 \times 10^{-7} H^{2.91}$ ($R^2 = 0.99$, $P < 0.05$). Estimation of P_R was used to calculate the annual reproductive effort in *P. margaritifera*. Reproductive effort (%) was similar to those calculated for temperate species and showed a progressive increase with the age of pearl oyster, from 7 % in age-group I to 38 % in age-group III. This study showed that, in a fairly stable tropical environment such as the Takapoto lagoon, *P. margaritifera* is a multiple spawner, which uses an opportunistic reproductive strategy, allowing investment, all year around, of any surplus energy into gamete production. Surplus energy is ensured by the high pumping rates developed by this non-symbiotic bivalve to succeed in low seston conditions. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Blacklip pearl oyster / gametogenesis / tropical aquaculture / reproductive cycle / reproductive effort / French Polynesia

Résumé — Cycle et effort de reproduction de l'huître perlière, *Pinctada margaritifera* (Bivalves: Ptériidés), en élevage dans l'atoll de Takapoto (Polynésie Française). Le cycle et l'effort de reproduction de l'huître perlière à lèvres noires, *Pinctada margaritifera*, cultivée dans le lagon de Takapoto, ont été étudiés sur une période annuelle (de mars 1997 à avril 1998), par un suivi bimensuel des stades de maturation et des indices gonadiques, sur une population en élevage composée de trois groupes d'âge (1, 2 et 3 ans). Cette étude a confirmé que *P. margaritifera* est une espèce qui se reproduit tardivement par rapport aux autres Ptériidés (elle atteint sa maturité sexuelle à la fin de sa première année, pour une hauteur approximative de 40 mm). Par ailleurs, elle présente, en élevage, une protandrie marquée (100 % de mâles à première maturité, 75 % pour des huîtres âgées de trois ans). Le schéma général de sa gamétogenèse, relativement synchrone dans les deux sexes, est comparable à celui des autres bivalves tropicaux : la reproduction est quasiment continue (aucune période de repos sexuel) avec une activité accrue en saison chaude (de novembre à mai). L'analyse des variations du poids de la gonade suggère l'existence de deux pics d'émission de gamètes chez les huîtres d'un an et cinq pics chez les huîtres de 2 et 3 ans (cycle de reproduction de type polymodal). Bien que ces émissions de gamètes soient parfois incomplètes, une relation entre la production de gamètes (P_R, en g) et la taille de l'huître perlière (hauteur, H, en mm) a été établie : $P_R = 5,26 \times 10^{-7} H^{2,91}$ ($R^2 = 0,99$, $p < 0,05$). Ces résultats ont permis de calculer l'effort de reproduction de *P. margaritifera* à Takapoto et sur un bilan annuel : il augmente avec l'âge de l'huître perlière de 7 % pour le groupe d'âge I à 38 % pour le groupe d'âge III. Ces valeurs annuelles sont similaires à celles calculées chez les bivalves de milieux tempérés. Cette étude a donc montré que, dans un environnement tropical assez stable tel que celui du lagon de Takapoto, *P. margaritifera* présente une reproduction continue, avec

* Corresponding author: spouvrea@ifremer.fr

plusieurs pics d'émission de gamètes par an. Cette stratégie « opportuniste » permet l'investissement, de tout surplus d'énergie en production de gamètes, tout au long de l'année. Malgré la faible concentration du milieu en nourriture, ces surplus d'énergie sont assurés par les fortes capacités de filtration développées par cette espèce tropicale non-symbiotique. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

1. INTRODUCTION

The blacklip pearl oyster, *Pinctada margaritifera* (Linnaeus, 1758) var *cumingi* (Reeve), occurs throughout the coral areas of the Indo-Pacific, but is really abundant in atolls of French Polynesia. Apart from for the goldlip pearl oyster, *P. maxima* (Jameson), which inhabits the north Australian region, *P. margaritifera* grows to a much larger size than the other species of the genus and is able to produce larger pearls. Today, black pearl aquaculture plays a major economic role in French Polynesia; the annual production is six metric tons of pearls ($US 175 million). After the large scale mortality which decimated cultivated pearl oysters from several atolls in 1985, French Polynesia decided to set up a general research programme on the pearl oyster, the so-called PGRN. The main objective of PGRN was to gain new knowledge on the ecology of *P. margaritifera* in lagoon environment with the aim of modelling the carrying capacity of pearl farming sites. In that respect, a first step was to build a physiological model which explained growth and reproduction of the pearl oyster according to its environment. These kinds of models have already been achieved for temperate species [2, 3, 48, 55, 58, 71] but nothing was available for tropical bivalves. In order to build such a model for *P. margaritifera*, the entire feeding process was studied, using field experiments [44, 45]. Once the model was built, growth and reproduction data were required to test the adequacy and reliability of the model. In this respect, the reproductive biology of *P. margaritifera* was investigated in Takapoto atoll, providing, at the same time, useful information for the pearl industry (resource management, development of hatchery technique, selective breeding programme, etc.).

As a general rule, most species of bivalves show flexibility in their reproductive cycle according to environmental variability [30, 31]. Reproduction of bivalves was mainly studied in temperate species (e.g. for Pectinidae [1, 7, 13, 14, 32, 36, 37, 42, 53, 64]; for Mytilidae [4, 43, 51, 59, 73]; for Ostreidae [9, 12, 25, 29, 61]; for other orders [11, 20, 21, 23, 40, 49, 52, 77]) which generally exhibit a seasonal reproductive strategy, organised into four steps: storage, gametogenesis, spawning, and inactivity. However, in some species, storage and gametogenesis may tend to overlap temporally, which led Bayne [4] to divide the bivalve gametogenic pattern from temperate areas into two groups: 1) 'conservative' species, with gametogenesis occurring in autumn/winter by using energy stores previously accumulated during the summer; this strategy implies that the peak of gamete production takes place during less favourable period; 2) 'opportunistic' species, with gametogenesis delayed until spring/summer, when food is sufficient; in this case, the peak period of gamete production coincides with maximum food availability, winter corresponding to a resting period.

More recently, species inhabiting environments with low seasonal trends (tropical but also polar or deep sea areas) have been studied [8, 15, 16, 22, 24, 26–28, 60, 75] and their reproductive strategy shows marked differences: the main specificity is that breeding is generally continuous [31, 56].

Studies on gametogenesis and breeding cycles of pearl oysters are available: 1) in Australian waters for *Pinctada fucata martensii* [70], for *P. maxima* [54], for *P. albina* [66–68] and for *P. margaritifera* [69]; 2) in Japanese waters for *P. fucata fucata* [74]; 3) in Californian waters for *P. mazatlanica* [18]. In French Polynesian waters, reproduction of *P. margaritifera* has been previously investigated [65] but only for ultrastructural aspects of spermatogenesis.

The aim of this study was to complete this preliminary work by examining, on an annual basis, the gametogenic cycle and reproductive output of three age-groups of *P. margaritifera* cultivated in Takapoto lagoon. The results presented here were determined from qualitative histological observations and quantitative gonadal growth data.

2. MATERIALS AND METHODS

2.1. Sampling, biometry and histology

Reproduction of cultivated pearl oysters was studied in Takapoto lagoon. Takapoto atoll is located in the Tuamotu Island of French Polynesia. This atoll (latitude 14°30'S and longitude 145°20'W) is 18.7 km long and 4.4 km wide, and presents an area of 81.7 km^2. Mean depth of the lagoon is 23 m, with a maximum at 55 m. The volume of water is estimated between 1.3 km^3 [62] and 2.3 km^3 (Yann Morel, pers. comm.). Exchanges with the ocean are very restricted because of the absence of channels into the coral ring. Homogeneity in the body water is ensured by regular trade winds. Sometimes, calm conditions may occur,
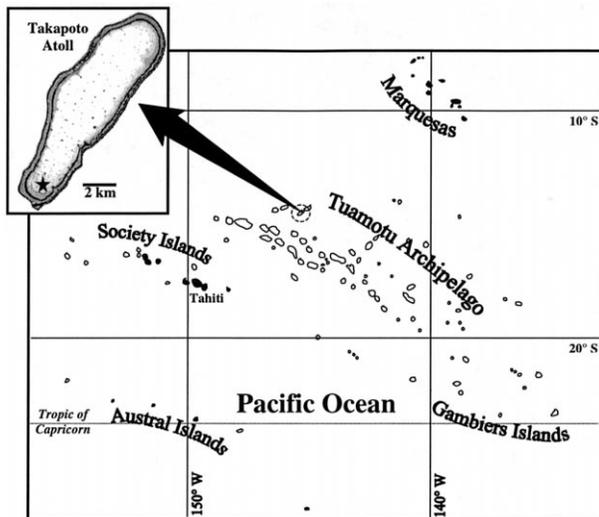
Figure 1. French Polynesia islands and location of the Takapoto atoll. Polynesian islands are in black and atolls in white. Star indicates the location, in Takapoto lagoon, of the cultivated oysters studied in this work.

especially during the warm season (November–April), causing occasional development of stratification.

To study reproduction of *P. margaritifera* for several age-groups, three populations of pearl oysters were cultivated at a single station (station 1) in the SW extremity of Takapoto lagoon (see *figure 1*). The first cohort was collected in March 1994 (age-group III), the second in March 1995 (age-group II) and the third in March 1996 (age-group I). The age of the three cohorts was estimated based on the March spat fall. Error in estimation was ± 2 months (uncertainty in time of spat settlement). Farming of these pearl oysters was conducted by the SRM field laboratory. The cultivation technique was very close to that commonly used in commercial farms. Suspended long-lines were immersed at 7 m deep. Pearl oysters were 'ear hung' on downline at low density (< 20 oysters m^{-3}) except for young oysters which were arranged in lantern nets where density was somewhat higher.

The reproduction cycle was followed for 1 year (from March 1997 to April 1998). A systematic sampling scheme was conducted: every 15 days, 45 individuals were randomly collected by SCUBA in each age-group, cleaned of fouling organisms and sent by aircraft to Ifremer Laboratory on Tahiti island. Then, they were stored under refrigeration at approximately 5 °C and analysed within 24 h of collection.

For all specimens ($n = 3\,360$), shell height (dorso-ventral axis, H) was first measured to the nearest millimetre prior to sexing, dissection, weighing and histology. Sex (indeterminate, male, female, hermaphrodite) was then observed. Since males and females of *P. margaritifera* are externally difficult to distinguish, sex was determined by rapid microscopic observation of fresh gonad smears. Dry shell weight (both valves,

W_{Shell}) was obtained after drying at 60 °C for 72 h, and total wet tissue weight after 5 min of draining. After flesh dissection, the same operation was conducted, separately, on adductor muscle, on retractor muscle, on (gills + mantle) and on (gonad + digestive gland). Dry weight of each part was obtained after complete freeze-drying. Tissue water content was determined by the difference between wet and dry weights, ash content was obtained on a sub-sample ($n = 10$) every 15 days after ignition in a muffle furnace at 500 °C for 12 h.

Gonad histology was performed on a sub-sample ($n = 15$) in each age-group. After being dissected, gonadal tissue samples (+ digestive gland) were placed in Bouin-Hollande's fixative for a few days, after which they were preserved in 70 % alcohol. Samples were then dehydrated through a graded series of alcohol, embedded in paraffin, sectioned at 3–4 μm on a rotary microtome, stained with Giemsa colorant and finally mounted on microscope slides. Sections were made from the gonadal area between the proximal end of the gut loop and the base of the foot. The sections were examined with a light microscope, first under low power (40×) to scan the entire gonadal area and, then, under high power (200×) to assess follicle stages.

2.2. Qualitative reproductive analysis

After preliminary examinations of a wide range of slides and preliminary review of the existing bibliography on Pteriidae reproduction, a seven-stage reproductive staging criterion was adapted from the classification cited for *P. margaritifera* [69], *P. maxima* [54], *P. fucata* [74] and *P. mazatlanica* [18]. Each of these classifications had advantages and disadvantages: Rose et al. [54], Wada et al. [74] and Garcia-Dominguez et al. [18] studied other species of Pteriidae; Tranter [69] worked on *P. margaritifera*, but his classification was somewhat complicated (nine stages) and difficult to set routinely. Using the merits of these four studies, a new classification was established for gonadal development of *P. margaritifera* in Polynesian waters (*table I*). Sometimes, more than one stage occurred simultaneously within an individual. When this occurred, stage criterion decisions were based upon the condition of the majority of the section. A total of 1 200 individuals was examined histologically to determine the gametogenic cycle.

2.3. Quantitative reproductive analysis

A simple and quantitative indicator of the reproductive state is the gonad index GI [27, 75]. This index is useful to determine potential spawning period (minimal GI-values). In our study, GI was computed for each individual, by using the following equation:

$$GI = W_{Gonad} \, / \, W_{Shell}$$

where W_{Gonad} is the dry tissue weight of gonad + digestive gland and W_{Shell} is the dry weight of shell (in

Table I. Reproductive stages in gonad development of the genus *Pinctada*. Staging criteria used in this study were adapted from those of previous works [18, 54, 69, 74]. This table shows the relationship between the stages used in our study (0, 1, 2, 3, 4, R_P, R_T) and those used in previous works. For some of them, stages have been regrouped (see the braces).

Classification used in previous studies				Classification in this study	Histological criteria
[69]	[54]	[74]	[18]		
Gonad inactivity					
i	0	–	indifferent stage	0	indeterminate or inactive, no evidence of gonadal development
Gonad development					
$d_1 - d_2$	1	1	developing stages	1	early gametogenesis, follicles small, gonia numerous
d_3	2	2		2	actively developing, but matures gametes are not observed
d_4		3		3	near ripe follicles with matures gametes
d_5	3	4	ripe stage	4	spawning ripe, follicles distended, confluent and entirely filled
Gonad regression					
$r_1 - r_2$	4	5	partially spawned	R_P	partially spawn, partially empty lumen
r_3		6 – 7	spent stage	R_T	spent, completely empty lumen

i, indeterminate; d, development; r, regression; R_P, partial regression; R_T, complete regression.

g). In some works, flesh weight is used in place of shell weight, but the calculation of the gonad index on this basis may create problems since flesh weight is known to vary periodically, with higher amplitude than shell weight. Furthermore, preliminary calculations showed that GI based on shell weight was the best discriminant index concerning reproductive activity.

Gonad index variation was also useful to analyse synchronicity of gametogenesis: if heteroscedasticity is detected between samples, then gametogenesis could be suspected to be asynchronous. To check homogeneity of GI variance between samples, Cochran's C test, within Statgraphic's plus Software, was performed for each age-group and for each sex of pearl oysters. The value of this test increases when homoscedasticity decreases.

Once potential spawning periods were determined and gametogenesis synchronicity was tested, gamete production (P_R) was estimated on the basis of the gonad dry weight losses, as follows:

$$P_R (t) = W_{Gonad} (t - 1) - W_{Gonad} (t + 1)$$

where $W_{Gonad} (t - 1)$ and $W_{Gonad} (t + 1)$ are the dry tissue weight of gonad + digestive gland just before and just after spawning, respectively.

For a whole year, the total gamete production (P_R, g) was then compared to annual soft tissue production (P_G, g) and annual organic shell production (P_S, g) using two indices, called reproductive effort, ER_1 and ER_2, expressed in per cent and calculated as follows:

$$ER_1 = 100 \times P_R/(P_R + P_G)$$

$$ER_2 = 100 \times P_R/(P_R + P_G + P_S)$$

The reproductive effort is known to increase with age, and a logistic model ($ER = ER_\infty/[1 + a.e^{(-kt)}]$) was proposed for *P. margaritifera* in Takapoto atoll. This model was fitted by the Marquardt algorithm within Statgraphic's plus Software.

3. RESULTS

3.1. Sexuality

No indeterminate stage was observed during the study and mature individuals were found in all size classes sampled (*figure 2*). In this respect, initial sexual maturity size, corresponding to the smallest individuals with mature gonads (stage 4), is presumably below 40 mm.

Samples taken biweekly were combined into monthly values in *table II*. The month-by-month analysis of this table indicated that the sex-ratio was always significantly different from 1:1 (χ^2 test, $P < 0.05$) for all samples. More precisely, sex-ratio showed a clear relationship with the size of individuals (*figure 2*): individuals < 90 mm were exclusively males and sex-inversion appeared around 90 mm. Above this size, the proportion of females increased progressively
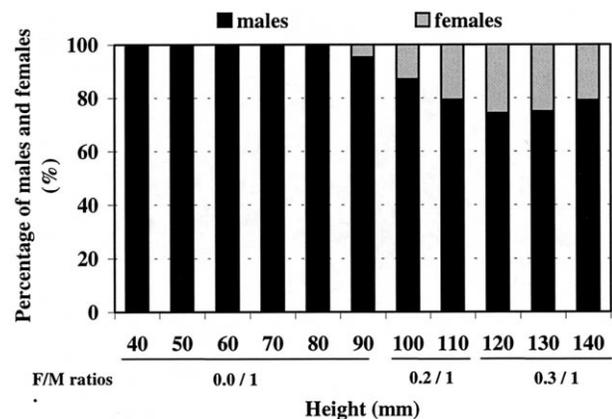
Figure 2. Relative size frequency distributions for *P. margaritifera* females and males cultivated in Takapoto lagoon. Sex-inversion occurs for specimens > 80 mm.

Table II. Sex ratios in three age-groups of *P. margaritifera*, from March 1997 to April 1998.

	F/M ratio		
Months	Age-group I	Age-group II	Age-group III
March 1997	0.0/1	0.0/1	0.2/1
April 1997	0.0/1	0.1/1	0.2/1
May 1997	0.0/1	0.1/1	0.3/1
June 1997	0.0/1	0.1/1	0.4/1
July 1997	0.0/1	0.2/1	0.2/1
August 1997	0.0/1	0.1/1	0.4/1
September 1997	0.0/1	0.2/1	0.4/1
October 1997	0.0/1	0.3/1	0.3/1
November 1997	0.0/1	0.3/1	0.3/1
December 1997	0.0/1	0.2/1	0.5/1
January 1998	0.0/1	0.3/1	0.6/1
February 1998	0.0/1	0.3/1	0.3/1
March 1998	0.0/1	0.5/1	0.3/1
April 1998	0.0/1	0.3/1	0.5/1
Mean	0.0/1	0.2/1	0.3/1

F, females; M, males.

Table III. Test for homogeneity in variance of gonadal index (GI). Values on the Cochran's C test showed that variance was homogeneous ($P > 0.05$) in all age-groups independently of the sex or the age of pearl oyster.

	Cochran's test	Probability level
Age-group I	0.060	0.39
Age-group II		
Males	0.062	0.36
Females	0.102	0.33
Age-group III		
Males	0.063	0.41
Females	0.138	0.05

and reached approximately 25 % in oldest individuals. In this respect, *P. margaritifera* in culture systems is a protandric successive hermaphrodite species with a highly dominant male phase. Bisexuality was uncommon: only seven simultaneous hermaphroditic pearl oysters were observed among the 3 360 analysed specimens, with no evidence that both gonads were functional.

3.2. Gametogenic cycle

The temporal distribution of pearl oysters in various maturity stages during the year is illustrated in *figure 3*. From these data, it is apparent that: 1) sexually undifferentiated individuals were never encountered during the course of this study; 2) gametogenic activity was clearly evident throughout the year in all specimens > 1 year old, without any clearly defined seasonal reproductive cycle; and 3) no resting period was apparent.

More precisely, *figure 3* showed that ripe (stages 3–4) and partially spawned (stage R_P) individuals were observed all through the year with a high percentage, generally above 70 %, except at the end of the austral winter (September), where this percentage decreased to approximately 40 %. In contrast, developing stages (stages 1–2), found every month, represented generally less than 40 %, except at the end of the austral winter (September), where the value was somewhat higher (approximately 60 %) for both sexes. *Figure 3* showed also that no resting period (indeterminate stage) occurred during the gametogenic cycle of *P. margaritifera* in this environment. Total regression stage (R_T) was very scarcely observed during the study period.

The fact that 70 % of the population were in spawning-ripe or in partially spawned stages indicated that reproduction of *P. margaritifera* in Takapoto

lagoon is fairly synchronous. To test this global synchronicity in reproductive activity, homogeneity in gonad index (GI) variances between sampling was checked by using Cochran's C test. Asynchronicity in reproductive activity was rejected since the Cochran's C test values showed that variance was homogeneous ($P > 0.05$) in all age-groups, independent of the sex or the age of pearl oysters (*table III*).

To quantify in a more precise way the reproductive activity, variations in dry weight of gonad (W_{Gonad}) and in gonad index (GI) were analysed. Since theses variations were not significantly different between males and females, data were pooled. Concerning W_{Gonad}, *figure 4* showed several monthly cycles, especially in age-groups II and III, underlying rapid reproductive cycle. For example, five significant decreases were observed in W_{Gonad} for age-group III pearl oysters. Three of them represented approximately 30 % of W_{Gonad}: in May 1997 (-0.58 g $\pm$ 0.19 CI), in January 1998 (-0.70 g $\pm$ 0.23 CI) and in March 1998 (-0.61 g $\pm$ 0.29 CI), and two of them were lower (approximately 20 %): in September 1997 (-0.39 g $\pm$ 0.29 CI) and in November 1997 (-0.36 g $\pm$ 0.36 CI). These variations were presumably due to gamete emissions or digestive gland resorption.

To determine the origin of these losses, GI monthly variations were analysed since GI and maturity stages were clearly related as described by *figure 5*: GI increased progressively with the development of maturity to reach a maximal value (1.02 $\pm$ 0.02 CI) in ripe (stage 4) pearl oysters, and decreased afterwards with regression stages to a minimal value of 0.84 ($\pm$ 0.08 CI) for complete regression stage (high CI, scarcely observed). Concerning these variations, *figure 6* shows a great irregularity, especially in age-groups II and III. Taking into account the relationship between GI and maturity stages, increases in GI should indicate developing gametogenesis, whereas obvious GI decreases should indicate principal spawning. In this respect, if some small irregularities in curves were not significant, and were presumably due to sampling variability and/or minor spawning or gamete resorption, five major and statistically significant variations, supporting those observed in gonad weight, could be determined in age-groups II and III, and two in age-group I. For example, in age-group III, during the month of
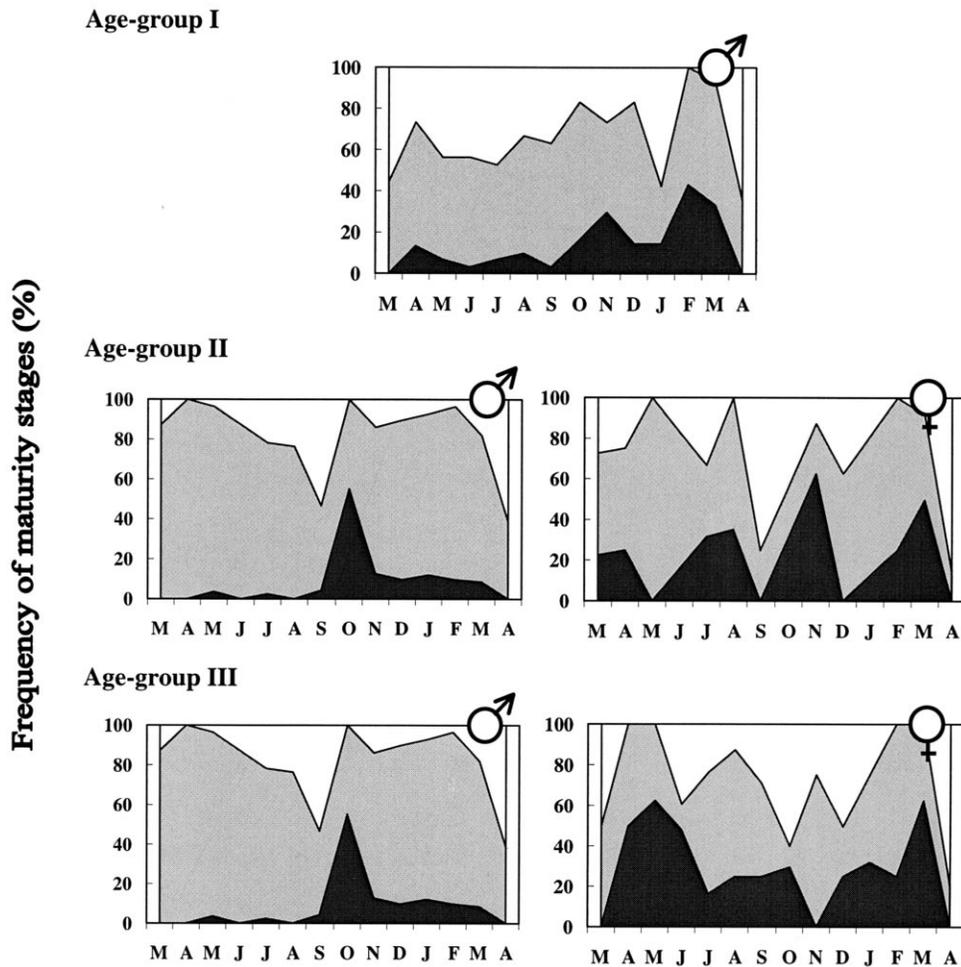
Figure 3. *P. margaritifera*. Monthly evolution of gonadal development stage frequency in males and females, for three age-groups. □, early gametogenesis (stages 1–2); ▨, spawning-ripe (stages 3–4); ■, partially spawned to spent (regression stages).

April 1997, the gonadal index increased from 0.9 to a maximal value of 1.2 in May 1997, and then decreased to a minimum of 0.8 in early June 1997. The decrease was statistically significant (Student's *t*-test, $P < 0.05$) and corresponded to a major spawning event. In the following months, GI showed similar significant variations on four occasions (see arrows in *figure 6*). At least five major spawning events were observed in age-groups II and III, and two in age-group I. The particular months were: May 1997, September 1997, November 1997, January 1998 and March 1998.

3.3. Reproductive output

Losses in gonad weight corresponding to each of the five spawning periods previously described were calculated for each age-group (see *table IV*). Values varied from 0.09 to 0.15 g in age-group I, from 0.16 to 0.55 g in age-group II and from 0.36 to 0.70 g in age-group III. Theses losses corresponded approximately to a decline between 4 and 11 % of the total dry tissue weight. A clear relationship with pearl oyster

height (H, mm) was demonstrated (*figure 7*): P_R values increased with H by following the equation: P_R (g) = 5.26×10^{-7} $H^{2.91}$ ($R^2 = 0.99$, $P < 0.05$).

Reproductive effort (ER, %), i.e. the fraction of total growth allocated to reproduction, was calculated (*table V*) on the basis of the annual production values by using two different formulae (ER_1 and ER_2). ER_1 (which does not take into account shell production) increased with the age of pearl oyster from 16.5 to

Table IV. Mean gametes losses (P_R, g) ± CI (in brackets) in each age-group for the major spawning periods.

|	Gamete production (P_R, g)		
	Age-group I	Age-group II	Age-group III
May 1997		0.16 (0.14)	0.58 (0.19)
September 1997	0.09 (0.04)	0.43 (0.21)	0.39 (0.29)
November 1997		0.54 (0.23)	0.36 (0.36)
January 1998	0.15 (0.05)	0.42 (0.20)	0.70 (0.23)
March 1998		0.55 (0.27)	0.61 (0.29)
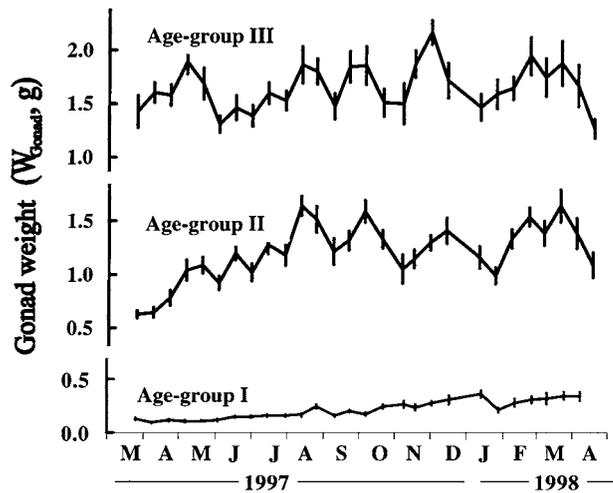
Figure 4. *P. margaritifera*: monthly evolution of dry weight of gonad (+ digestive gland), W_{Gonad}, for three age-groups. Preliminary insights into the data showed that W_{Gonad} evolution of males and females followed the same pattern, so that all the data were pooled. Vertical bars represent the 95 % confidence intervals.
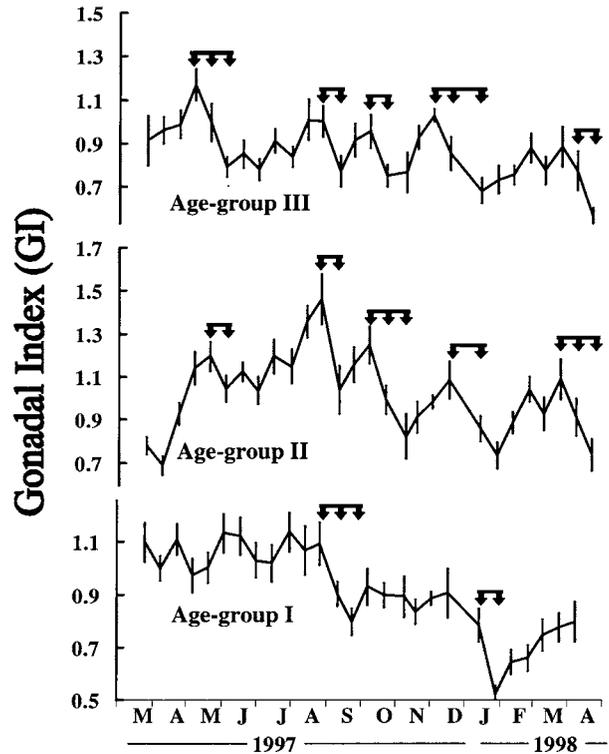
Figure 6. *P. margaritifera*: monthly evolution of gonad index, GI, for three age-groups. Preliminary insights into the data showed that GI evolution of males and females followed the same pattern, so that all the data were pooled. Vertical bars represent the 95 % confidence intervals.
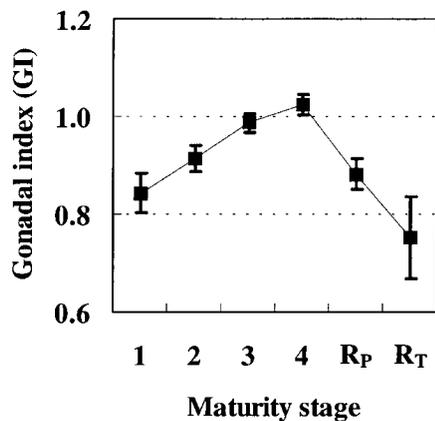

Figure 5. Relationship between GI gonad index and gametogenic stage computed for all age-groups. Vertical bars represent the 95 % confidence intervals.

74.4 %, ER_2 (which takes into account all production) increased in the same way from 6.8 to 37.8 %. A general asymptotic model was proposed on the basis of these data to express ER_2 in relation to the age of pearl oyster. Two a priori assumptions were made to assess the model: ER_2 tends to zero in very young oysters and reaches an asymptotic value of 60 % in old oysters. The asymptotic model obtained was the following (age is expressed in years): $ER_2 = 60 / (1 + 74 e^{-1.4\ age})$ with $n = 5$, $F_{ratio} = 205.6$, $R^2 = 0.99$ and $P < 0.05$. Since sample size is low, this model needs to be confirmed by additional measurements.

Table V. Somatic production (P_G), organic shell production (P_S) and reproductive outputs (P_R) for the period from March 1997 to April 1998 for each age-group.

	P_S	P_G (g·year^{-1})	P_R	ER_1	ER_2 (%)
Age-group I	2.04 (0.27)	1.20 (0.18)	0.24 (0.09)	16.5	6.8
Age-group II	3.83 (0.63)	2.32 (0.49)	2.10 (1.05)	47.5	25.4
Age-group III	3.44 (0.88)	0.90 (0.72)	2.64 (1.38)	74.4	37.8

Reproductive effort was calculated on the basis of productions values obtained ($ER_1 = P_R/(P_G + P_R)$; $ER_2 = P_R/(P_S + P_G + P_R)$). The 95 % confidence interval is indicated in brackets.
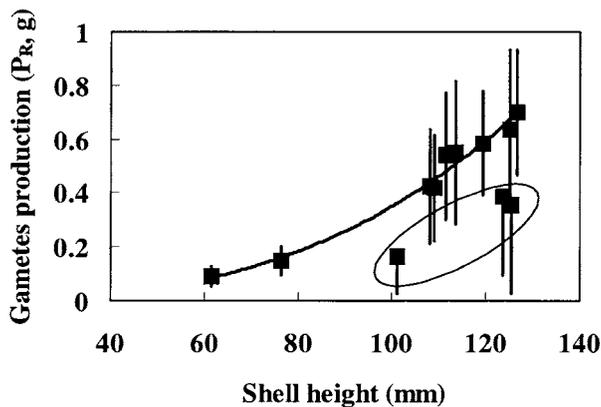
Figure 7. Relationship between gamete production (P_R, g) and height (H, mm) of *P. margaritifera*. Vertical bars represent confidence intervals. Encircled data correspond presumably to partial spawning events. A general model was fitted for maximal P_R values: P_R (g) = 5.26×10^{-7} H(mm)$^{2.91}$ ($R^2 = 0.99$, $P < 0.05$).

4. DISCUSSION

4.1. Sexuality

The present investigations have revealed that *P. margaritifera* becomes sexually mature at the end of the first year (below 40 mm), and this confirms results of earlier studies [69]. Since *P. albina* and *P. fucata* become sexually mature within the first 6 months and probably spawn twice in the first year [66, 70], *P. margaritifera* and also *P. maxima* [54] are, by comparison, late-maturing species, implying that smaller species (*P. albina* and *P. fucata*) mature more rapidly than larger ones (*P. margaritifera* and *P. maxima*).

The majority of young *P. margaritifera* in culture were mature for the first time as males, as is generally the case in pearl oysters [68–70]. Afterwards, the sex ratio of *P. margaritifera* tends increasingly towards femaleness as the population becomes older, and this phenomenon can only be explained satisfactorily on the basis of extensive protandric sex change. This finding supports those of Tranter [69] who considered *P. margaritifera* to be a protandric consecutive hermaphrodite. If, on rare occasions, male and female phases were observed simultaneously in the same specimen, they were typically separated temporally. The ability of *P. margaritifera* to change sex above a certain size appears to be typical of Pteriidae [54, 57, 69]. Tranter [69] also observed rare protogynic changes in this species and assumed that several sexual phases occur in the life of an individual. These sexual changes are known to be potentially related to food availability. As a general case, good conditions will favour femaleness, whereas bad conditions, or stress, retard it.

4.2. Reproductive cycle

It is apparent from the data presented herein that *P. margaritifera* in culture displayed a continuous synchronised polymodal breeding pattern throughout the year, with no 'inactive' period. In that respect, *P. margaritifera* can be classified into the 'continuous spawner' bivalve group.

Continuous breeding is common in the genus *Pinctada* (reviewed by Gervis and Sims [19]). Concerning histological development, such continuous reproductive competence is explained by the fact that: 1) follicles in a same gonad are not generally at the same stage; 2) gametogenesis is rapid and always active [24, 27, 75]; and 3) spawning is generally incomplete. Concerning the third point, incomplete spawning has been frequently observed in Pteriidae, with some resorption of gametes [19, 68, 70]. However, Tranter [69] found *P. margaritifera* emitted almost all of their gonad material. Our results tend to show that the two possibilities can occur for *P. margaritifera* in Takapoto lagoon; for example, 3-year-old pearl oysters spawned five times during our study: three spawnings were significantly higher in quantity than the other two.

This study showed that having permanently mature gonads, ready for spawning, is the reproductive strategy of *P. margaritifera*. This 'opportunistic strategy' consists in investment of any surplus energy into gamete production, since no physical factors are limiting for gametogenesis (see below). Surplus energy is permitted by the very high pumping capacity of *P. margaritifera* [44, 45, 76], which allows rapid growth but also continuous breeding of *P. margaritifera*, in spite of the low food concentration of Takapoto lagoon [10].

This reproductive strategy differs from those observed in temperate areas [31], where most marine bivalves have an annual reproductive cycle with well-defined periods of storage, gametogenesis, spawning and inactivity controlled by the variation of several environmental factors. However, a reproductive cycle still exists in *P. margaritifera* but concerns the intensity of the spawning. In this study, major reproductive events occurred during the warm season (December–April) and this is in total agreement with other studies that have investigated reproduction in tropical bivalves [18, 20, 27, 75].

4.3. Environmental factors

Gamete maturation and spawning in bivalves is mainly controlled by two factors, water temperature and food supply, and secondarily, by salinity and photoperiod. In temperate waters, temperature is the major factor in the regulation of bivalve reproduction (e.g. [38, 72]) but this environmental parameter is considered to be less effective in regulating gametogenesis in tropical populations [15, 16, 26, 27, 61]. Working on *P. fucata*, Wada et al. [74] concluded that tropical temperatures make the reproductive cycle less

Table VI. Reproductive effort, calculated as $P_R/(P_R + P_G + P_S)$, in six bivalve species related to age. In all cases, reproductive effort increases with increasing age. Interspecific variability is low.

	Reproductive effort (%)			References
	Age-group I	Age-group II	Age-group III	
Temperate areas				
Chlamys varia	0	–	29	[34]
Patinopecten yessoensi	3	–	38	[17]
Mytilus edulis	0	10	25	[6]
Ostrea edulis	0	18	30	[50]
Crassostrea gigas	–	18	–	[12]
Tropical areas				
Pinctada margaritifera	7	25	38	this study

distinct, and continuous spawning becomes prevalent. This is also the case in Takapoto lagoon, where the continuous reproduction of *P. margaritifera* could be explained by the fact that temperature is always above the critical level (temperature is around 28 °C and annual thermal amplitude is around 4–5 °C). This conclusion fits well with results of Tranter [66–70] showing that, in higher latitudes, the breeding time for pearl oysters is more restricted and occurs preferentially during warmer months, while in lower latitudes (such as Takapoto atoll) there is a less restricted spawning season and major spawning can also occur outside the warmer months. In order to really test the temperature effects, it would be of interest to investigate variation in reproductive physiology of *P. margaritifera* within various Polynesian atolls, since they exhibit graded conditions of temperature range according to their latitude.

The importance of food availability has also been emphasised in the timing of bivalve reproduction [5]. A relationship between reproductive cycle and food availability has been demonstrated by several authors (e.g. [60]), and is thought to ensure adequate nutrition for developing planktonic larvae. In Takapoto atoll, potential food for pearl oysters varies on a monthly basis, but does not present any seasonal trend [10, 46]. The breeding cycle is presumably influenced by these monthly variations. In that respect, a deterministic model of growth and reproduction based on ecophysiological concepts and particulate organic matter (POM, potential food for pearl oysters) has now been built [47] and proposes a link between POM and gonad weight.

Two others environmental parameters are also supposed to have some effect on gonadal maturation: salinity [24, 63] and photoperiod [15, 41]. Nevertheless, in Takapoto atoll, these two parameters are nearly constant (see [10, 46]), and consequently their influence on gametogenesis is probably not significant.

4.4. Reproductive effort

Surplus energy is shared between somatic and reproductive compartments [21]. The fraction of total growth allocated to reproduction is called the reproductive effort [33, 35, 39]. In temperate species, sur-

plus energy is first stored and is afterwards transferred to reproduction at the most favourable time for larval development and recruitment success. In tropical species, this allocation is presumably continuous based on the comments above. Calculated on an annual basis to permit inter-specific comparison, reproductive effort (ER, %) exhibited in *P. margaritifera* a progressive increase with age: from 6.8 to 37.8 % if all production is take into account, or from 16.5 to 74.4 % if shell growth is ignored. Available values for reproductive effort for bivalves are scarce in the literature. However, the review described in *table VI* showed that 1) inter specific variations of reproductive effort are low, and 2) differences in ER between tropical and temperate species are insignificant when calculated on an annual basis.

As a conclusion, this study confirms that, as it is the case for most of tropical bivalves, *P. margaritifera* has developed a continuous reproductive pattern, which differs from seasonal reproductive cycles observed in temperate species. This continuous breeding activity is permitted by the very low fluctuations of environmental parameters in Takapoto. Nevertheless, on an annual basis, the annual energy investment of *P. margaritifera* in reproduction remains approximately as expensive as those in temperate species. In spite of the low food concentration of Takapoto lagoon, this energetic cost is ensured by the high pumping capacity, previously demonstrated in this bivalve. Building a deterministic growth model for *P. margaritifera* was expected to give more insight into the relationship between somatic growth, gametogenic activity and environmental parameters (mainly food supply). This model is the scope of another paper [47].

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