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Phenotype plasticity, local adaptation, and biofouling influence on growth of the pearl oyster *Pinctada margaritifera*: A common garden approach

Hulot V. [1, 2], Saulnier Denis [2], Latchere O. [1], Maihota N. [3], Gaertner-Mazouni N. [1, *]

[1] Université de la Polynésie Française, UMR-241 EIO, Laboratoire d'excellence Corail, BP 6570 – 98702 Faa'a, Tahiti, French Polynesia
[2] Institut Français de Recherche pour l'Exploitation de la Mer, UMR-241 EIO, BP 49 – 98719 Taravao, Tahiti, French Polynesia
[3] Institut de Recherche pour le développement, UMR-241 EIO, BP 6570 – 98702 Faa'a, Tahiti, French Polynesia

* Corresponding author : N. Gaertner-Mazouni, email address : nabila.gaertner-mazouni@upf.pf

Abstract :

The purpose of our study is to investigate (1) the influence of phenotype plasticity and local adaptation on pearl-oyster physiology by testing the persistence of growth differentiation of two pearl oyster populations (Arutua and Mangareva) in common garden experiment; (2) to quantify the influence of biofouling development on the growth of each pearl oyster population. According to our observations, the growth rate in terms of total oyster weight suggested better growth performance of the pearl oyster Pinctada margaritifera in Mangareva (0.21–0.24 ± 0.01 g.day−1) than Arutua (0.14–0.15 ± 0.01 g.day−1). However, similar growth performances are observed at the Vairao common garden site for oyster stocks from Mangareva (0.07 ± 0.01 mm.day−1 or 0.15 ± 0.01 g.day−1) and Arutua (0.07 ± 0.01 mm.day−1 or 0.13 ± 0.01 g.day−1). Our results thus suggest that growth performance variability observed at the pearl farming sites of Arutua and Mangareva is due more to phenotypic plasticity than to local adaptation. This result thus accords a major importance to site selection for the pearl farming optimization process.

Biofouling dynamics on Pinctada margaritifera shells differed radically between Arutua and Mangareva sites. In Arutua, biofouling colonization was relatively slow (0.016 g.oyster−1.month−1) and was mainly composed of sponges and bivalves. On Mangareva, the colonization process appeared faster and more continuous over the study period (0.15–0.18 g.oyster−1.month−1) and the biofouling community was dominated by tunicates. On the basis of our results obtained on growth performance between cleaned and uncleaned stock in Arutua and Mangareva, biofouling development after 14–15 months of colonization does not appear to have any negative effect on P. margaritifera growth. Due to the high cost of biofouling management in pearl farming facilities, our results suggest once more that reconsideration of the pearl farming management process is needed.

Highlights

► Faster biofouling colonization was observed in Mangareva than in Arutua. ► Mangareva biofouling colonization was associated with tunicate settlement. ► Biofouling had no negative effect on *Pinctada margaritifera* growth by 14–15 months. ► Large growth differences in *Pinctada margaritifera* were mainly phenotype plasticity.

Keywords : Pearl Oyster, Growth performance, Biofouling, Endogenous factor, Environmental factor, Common garden experiment.

Introduction

In shellfish aquaculture worldwide, growth and condition indexes are the main means to assess bivalve health and farming success (Pouvreau & Prasil, 2001; Saxby, 2002). Similarly to other organisms, the growth of cultivated bivalves is the result of genotype and environment interaction (Gosling, 2015).

The environmental influence on the growth of organisms was quickly demonstrated for bivalves on the basis of several factors including temperature (Mann, 1979; Brown & Hartwick, 1988; Hiebenthal et al., 2012), salinity (Dickinson et al., 2012; Hiebenthal et al., 2012) and hydrodynamic conditions (Steffani & Branch, 2003). Among these environmental factors, food availability is certainly considered as the most important factor affecting the growth of bivalves (Gosling, 2015). This factor is so essential that it determines most farming strategies of shellfish producers regarding farming density (Fréchette, 2010), structure design (Strohmeier et al., 2008) and biofouling management (Lacoste & Gaertner-Mazouni, 2015). Finally, the genetic basis of

growth variation within bivalve populations has been demonstrated by means of reciprocal transplantation, common garden experiments or genomic tools (Gosling, 2015).

In the case of the culture of the pearl oyster *Pinctada margaritifera* in French Polynesia, the variations of growth observed between sites are most often considered as the result of the environmental effect on organisms (Pouvreau & Prasil, 2001). If several laboratory experiments are in agreement in considering temperature and food availability as important factors controlling the growth rate of *P.margaritifera* (Yukihira et al., 1998; Chávez-Villalba et al., 2013; Joubert et al., 2014), a field study carried out over the different pearl farming sites tended to prioritize the influence of temperature on growth determination (Pouvreau & Prasil, 2001). In contrast with several shellfish farming studies (López et al., 2000; Sá et al., 2007), a recent study has demonstrated that the presence of biofouling organisms (most often considered as trophic competitors and removed preventively) does not affect the growth of pearl oysters in cultivation (Lacoste et al., 2014). In pearl oyster cultivation, the influence of genetic effects on the growth variations observed is not well understood. However, recent studies on selection have demonstrated significant family effects on pearl oyster growth rates (Ky et al., 2013).

If the pearl farming industry is working with these site disparities in pearl oyster growth performance (ISPF, 2018), the industry will certainly gain from clarifying the mechanisms t work behind this variability in production. In fact, the investigation of pearl oyster growth drivers will in time make it possible to prioritize future scientific research on *P. margaritifera* growth optimization (by site prospection, genetic selection or revision of farming practices). Our study aims to investigate the importance of phenotype plasticity and local adaptation with regard to pearl oyster physiology by testing the persistence of growth differentiation of two pearl oyster populations from pearl farming sites with contrasting environments (Arutua and Mangareva) using a common garden approach. Our study was also designed to confirm recent results obtained on the role of biofouling in the pearl farming context (Lacoste et al., 2014) by quantifying the influence of biofouling development on the growth of each pearl oysters population.

1. Material and methods

1.1. Study Sites

Our *in situ* experiment was performed in two contrasting pearl farming areas (Figure 1; Table 1), located in different archipelagos of French Polynesia: Tuamotu (Arutua) and Gambier (Mangareva). A third site in Tahiti (Vairao) served for the common garden part of the study.

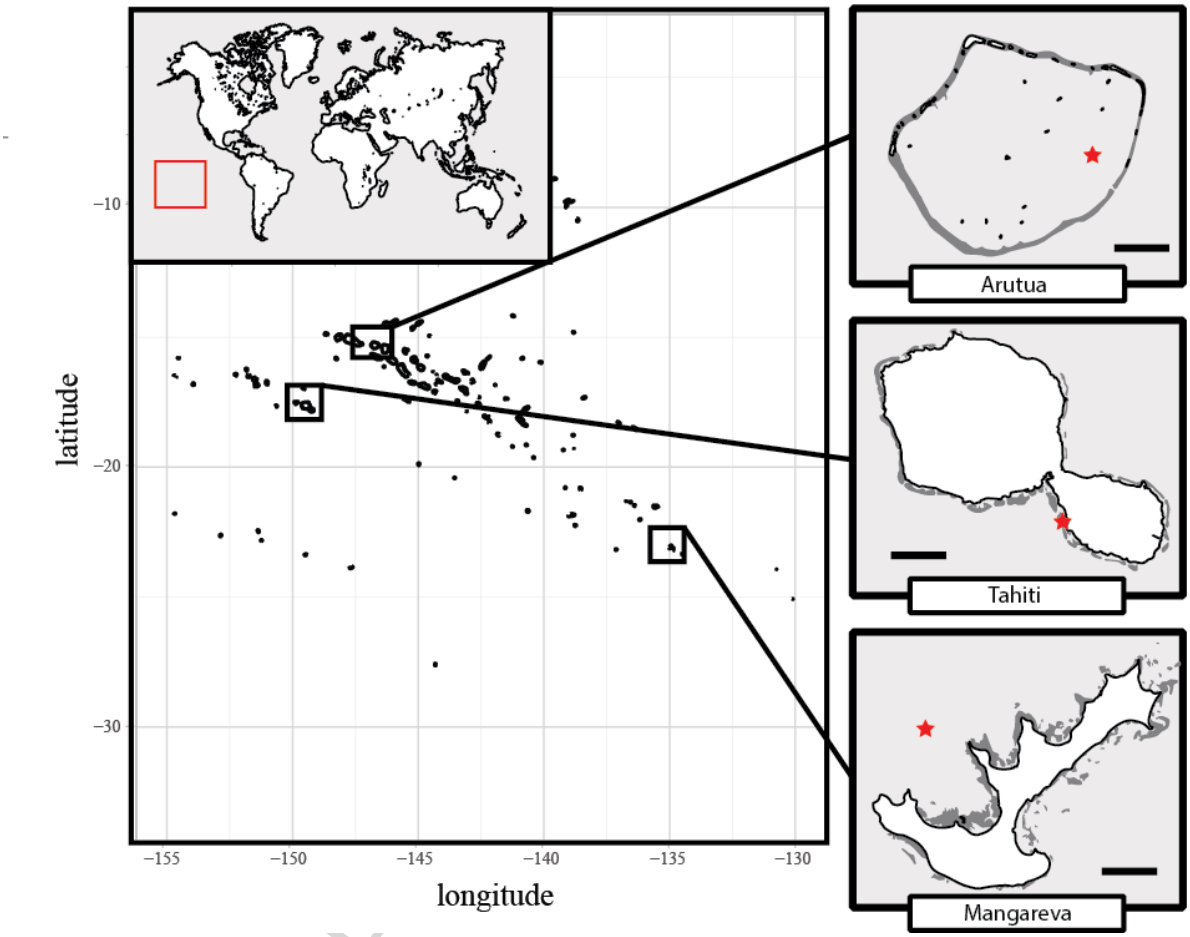

Figure 1. Location of the three study sites in French Polynesia: 1. Arutua in Tuamotu archipelago, 2. Vairao in Tahiti, 3. Mangareva in Gambier archipelago. Red stars indicate rearing locations of experimental pearl oysters. Scale-bars on the Arutua, Tahiti, Mangareva maps represent 7, 12 and 2 km, respectively.

Arutua, Tuamotu archipelago, French Polynesia

Arutua is an atoll located 368 km NE of Tahiti. This atoll has a pentagonal shape with a length of 31 km and 24 km width, for a total area of 484 km^2. The wide pass close to Rautini village is the sole opening of the atoll to the ocean (except for numerous *Hoas:* small channels crossing the coral reef). Arutua is considered as one of the four hotspots of pearl farming industry (ISPF,

2018). Our experiments were conducted in direct collaboration with farmers, using part of their oyster long lines.

Mangareva, Gambier Archipelago, French Polynesia

Mangareva is the central and largest island of the Gambier Archipelago, located 1619 km SE of Tahiti. The island is about 8 km long with a total area of 15.4 km². The coral barrier is highly fragmented and the ocean opening very wide. The island experiences strong seasonality between a dry season (from November to April) and a wet season (from May to October); (Cochard et al., 2003). According to the latest statistics from the Polynesian administration (ISPF, 2018), Mangareva is the largest pearl farming area in French Polynesia, with a 1315 Ha production area. Pearl farming is the main economic activity on the island and constitutes the main anthropic pressure in the ecosystems. Here again, our experiments were conducted in direct collaboration with farmers, using some of their oyster long lines.

Vairao, Tahiti, French Polynesia

The Vairao site in Tahiti was used for the common garden part of this study. Pearl oysters were placed on long-lines in the Vairao lagoon, at IFREMER facilities. Located close to a small river, Vairao lagoon receives an input of organic material during the wet season (from November to March) due to heavy rainfall.

Table 1. Locations of the experimental sites.

	Arutua (Tuamotu)	**Mangareva (Gambier)**	**Vairao (Tahiti)**
GPS	15°20'18.2"S 146°39'01.2"W	23°05'38.7"S 134°59'52.8"W	17°49′9″S 149°17′32″W

1.2. Biological material

In Arutua, 400 one-year-old oysters (collected in Ahe lagoon) were placed on experimental long-lines (May 2016) and 500 others were translocated to Vairao (July 2016). Similarly, in Mangareva, one-year-old locally collected oysters (N = 400) were placed on experimental long-lines (September 2016) and 500 others were translocated to Vairao (June 2016).

In Arutua and Mangareva, oysters were divided into two experimental groups to analyse the influence of biofouling. At each site, the first group of 200 pearl oysters, referred to hereafter as 'PO' (for 'Pearl Oyster') stocks, were cleaned twice a year ('MPO' and 'APO' distinguish stock from Mangareva and Arutua, respectively). In parallel, the 200 remaining pearl oysters, referred to hereafter as 'POBC' (for 'Pearl Oyster and Biofouling Community'), were not cleaned at all during the experimental period ('MPOBC' and 'APOBC' distinguish stock from Mangareva and Arutua respectively).

In Vairao, pearl oyster stocks from Arutua (VAPO, n = 500) and Mangareva (VMPO, n = 500) were placed in the IFREMER experimental facilities.

Translocation operations were carried out by plane, respecting the rules imposed by the Polynesian administration authorities. Cleaned pearl oysters were put in special storage boxes and immediately placed in a fresh water bath for one night on their arrival in Tahiti (to remove any remaining epibionts). The day after their arrival, the pearl oysters were cleaned with compressors and then placed in lagoon water. Cleaning operations were repeated during the experiment for the treatments concerned (MPO, APO, VMPO, VAPO) to prevent a large accumulation of biofouling. Cleaning operation dates are summarized in Table 2.

During the cleaning operation in Arutua and Mangareva and Vairao, biofouling removal was performed using a pressure washer or manually with a knife coupled with a natural fish cleaning technique, depending on the material available at the farm site. Both types of cleaning operation are considered to have the same degree of effectiveness in removing all the biofouling organisms and therefore not to be a source of bias in the biofouling dynamic observed. The protection structures (net-cylinders) used during rearing were also cleaned of all biofouling organisms.

Table 2. Summary of cleaning operations and growth monitoring performed during the 2016–2017 period for different stocks. The Arutua (A), Mangareva (M), and Vairao (VA and VM) stocks used were divided according to cleaning treatment: Uncleaned stock: POBC (pearl oyster and biofouling community), cleaned stock: PO (pearl oyster). Crosses indicate that a group was cleaned. The periods shaded in grey correspond to the starting period of experiment and thus the beginning of biofouling colonization. For uncleaned POBC stocks, the time of biofouling colonization (in month) is specified in the table.

		2016												2017									
Site	Group	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Arutua	APO					X						X									X				

(Tuamotu)	APOBC					X						6										15				
Mangareva	MPO									X							X							X	
(Gambier)	MPOBC									X							7							14	
Vairao	VAPO							X			X				X		X			X			X		
(Tahiti)	VMPO						X				X				X		X			X			X		

Pearl oyster cultivation procedures were similar in Arutua, Mangareva and Vairao: 10 pairs of pearl oysters (randomly selected on collector) were fixed on adapted rope and then placed inside a plastic net cylinder, to be protected against predators. The net-cylinders were stocked on long lines at 6 m depth, 0.5–1 metres apart.

1.3. Growth measurements

At the beginning of the experiments, all pearl oysters were individually labelled, then measured for shell dimension (dorso-ventral measurement; DVM) with a steel rule, to the nearest 0.1 cm, as in previous studies (e.g., Lacoste et al., 2014). All pearl oysters were also weighed (total oyster weight: TOW) on a precision balance (± 0.01 g).

To document individual growth rate, 30 pearl oysters from each group were randomly collected for second monitoring at the end of the experiment in August, November and October 2017 for Arutua, Mangareva and Vairao stocks, respectively. For each pearl-oyster monitored, individual growth rate is obtained from shell height (dorso-ventral measurement; DVM) increment or total oyster weight increase (TOW). Time until harvest was 14–15 months for Mangareva (MPO and MPOBC) and Arutua (APO and APOBC), allowing sufficient time for biofouling colonization and thus stock distinction. Time until harvest in Vairao was similar for VMPO and VAPO (15 and 16 months, respectively). Individual DVM and TOW growth rate is calculated for each pearl oyster monitored as below:

$$DVM\ growth\ (in\ mm.day^{-1}) = \frac{DVM\ final\ (in\ mm) - DVM\ initial\ (in\ mm)}{Number\ of\ day}$$

$$TOW\ growth\ (in\ g.day^{-1}) = \frac{TOW\ final\ (in\ g) - TOW\ initial\ (in\ g)}{Number\ of\ day}$$

1.4. Biofouling community monitoring

Each time biometry measurements were taken at the Mangareva and Arutua sites, a survey was made of the biofouling community. To quantify the biofouling community biomass and assess its

composition, three groups of 6 pearl oysters of POBC stock were randomly collected and cleaned with a knife to collect all of the biofouling organisms on a piece of aluminium paper, which were immediately frozen at -20°C. In the lab, all organisms were identified and divided into taxonomic groups (Algae, Anthozoans, Tunicates, Bivalves, Sponges and Macrozoobenthos). To quantify the dry biomass of each group, all organisms were frozen temporarily at -80°C before being treated in a freeze dryer for 48 h at -20°C (taking care to remove all mineral parts such as shell in order to quantify only the dry flesh biofouling biomass). After the drying process, all the taxonomic groups were precisely weighed and the equivalent weight per oyster was calculated.

1.5. Data analysis

The relative physical proportions of shellfish harvested were investigated by allometric analysis. The relationship between TOW and DVM were compared (after log transformation) using ANOVA and the Tukey test. With respect to results homogeneity, DVM and TOW individual growth rates were averaged for each stock and cleaning treatment in the experiment. Mean individual growth performances were statically compared using non-parametric tests (Kruskal-Wallis and Wilcoxon-Mann-Whitney tests with associated Bonferroni adjustments). All statistical analyses were performed in R- Software (R Team, 2014).

2. Results

2.1. Biomass and composition of biofouling community

Monitoring of the development of the biofouling community in Arutua and Mangareva revealed different lengths of time until colonization (Figure 2; Table 3). In Arutua, biofouling biomass was null after 6 months of experiment and reached 0.24 ± 0.02 g.oyster^{-1} after 15 months. At this point in time (15 months), the biofouling community was mainly composed of bivalves (*Pinctada maculata, Saccrostrea cuculata*) and sponges (mainly *Dysidea* sp.), which made up 55% and 24% of the biofouling biomass observed, respectively.

In Mangareva, the biofouling biomass observed reached 1.07 ± 0.05 g.oyster^{-1} after 7 months and 2.45 ± 0.43 g.oyster^{-1} after 14 months of colonization. The biofouling community appeared different at the two sampling times. The younger biofouling community (T0 + 7 months) was mainly composed of numerous ascidians (mainly *Herdmania* sp.), which represented 79% of

standardized dry biomass observed on Mangareva (0.84 g.oyster^{-1}) (Figure 2). After 14 months of colonization, the biofouling biomass observed consisted of bivalves (*P. maculata, S. cuculata*); ascidians (mainly *Herdmania* sp.) and sponges (mainly *Dysidea* sp.), representing 37, 26 and 17% of total biofouling biomass, respectively. During the last monitoring survey in Mangareva, anthozoans (*Aptasia* sp.) and algae (*Caulerpa* sp.) appeared well established in the biofouling community even though the biomass they represented was low (0.23 and 0.14 g.oyster^{-1}, respectively) compared with the other biofouling taxonomic groups.

Table 3. Taxonomic composition of biofouling community observed during the Arutua and Mangareva monitoring campaigns in 2016–2017. ** Main taxon observed in each group (in terms of abundance).

Groups	Arutua	Mangareva
Algae		*Caulerpa* sp.
Anthozoans	*Aptasia* sp.	*Aptasia* sp.
Tunicates	*Herdmania* sp.**	*Herdmania* sp.** Undetermined ascidians *Didemnum* sp.
Bivalves	*Pinctada maculata* ** *Saccrostrea cuculata* Undetermined bivalve	*Pinctada maculata* ** *Saccrostrea cuculata* Undetermined bivalve
Sponges	*Cliona* sp. *Dysidea* sp.**	*Cliona* sp. *Dysidea* sp.**
Macrozoobenthos	Undetermined Crustacean Undetermined Polychaete	Undetermined Crustacean Undetermined Polychaete *Ocenebra inornata*

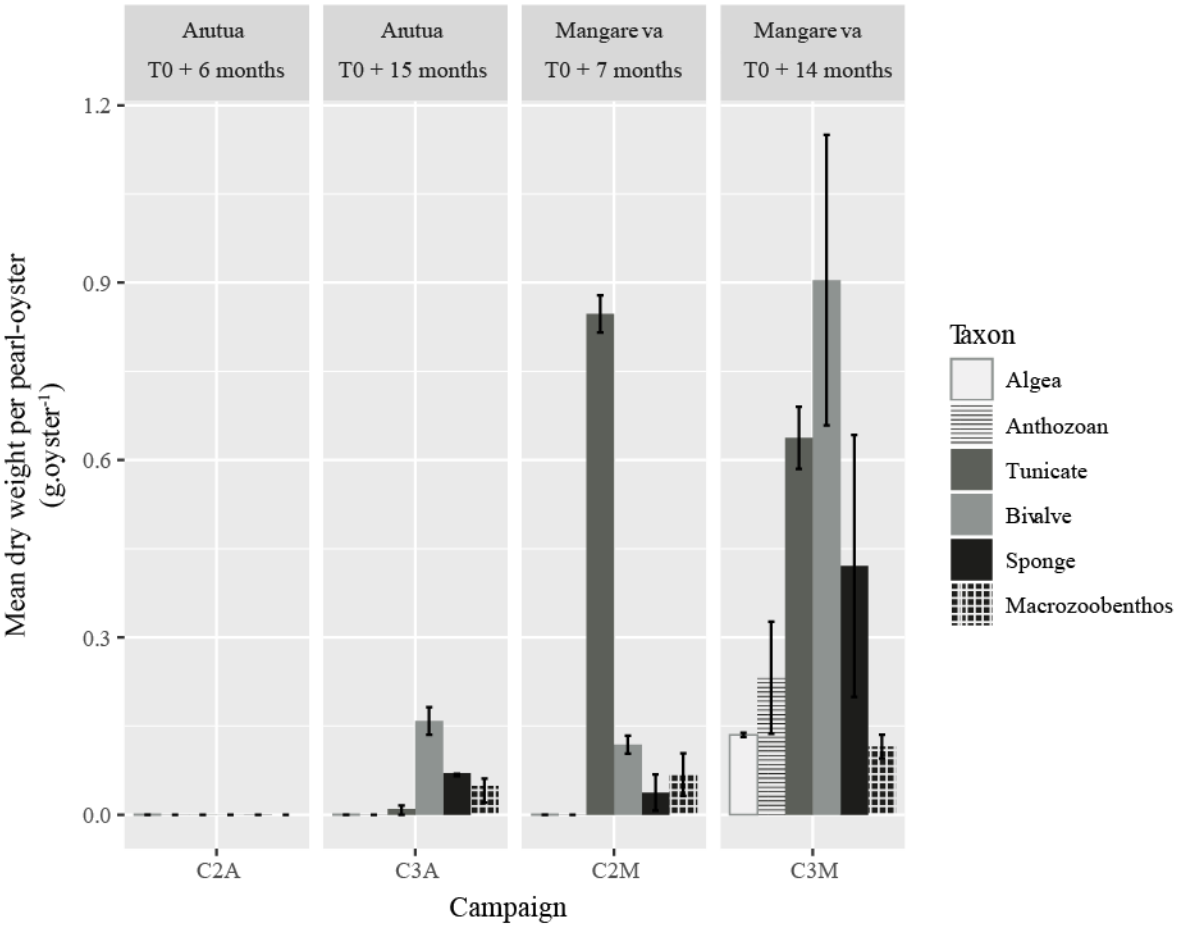

Figure 2. Total dry biofouling biomass (in g.pearl oyster^{-1}) per taxonomic group observed on *Pinctada margaritifera* shells during the 2016–2017 sampling campaign in Arutua (C2A and C3A) and Mangareva (C2M and C3M). For each date corresponding duration of colonization after initial cleaning at the beginning of the experiment (T0) is specified.

Table 4. Means of biofouling total dry weight (in g), biofouling total dry weight per pearl oyster (in g.oyster^{-1}) observed during the 2016–2017 sampling campaign in Arutua (C2A and C3A) and Mangareva (C2M and C3M).

Sampling campaign	C2A	C3A	C2M	C3M
Culture Site	Arutua	Arutua	Mangareva	Mangareva
Biofouling Colonization Time (in months)	6	15	7	14
Number of pearl oysters monitored	6	6	6	6
Biofouling total dry weight (in g)	0	1.43 ± 0.09	6.42 ± 0.29	14.67 ± 2.60
Biofouling total dry weight per pearl oyster (in g.oyster^{-1})	0 ± 0.00	0.24 ± 0.02	1.07 ± 0.05	2.45 ± 0.43

2.2. Pearl oyster growth allometry in experimental stocks

To test for differences in morphometry between the different experimental stocks, the relationship between shell length and total oyster weight was compared (Figure 3). A linear relationship is observed between these two variables for all stocks analysed (R^2 = 0.60–0.87; Table 5). Allometric parameter b estimated with the curve slope [log(TOW) = log(a)+b*log(DVM)] ranged from 2.00 to 2.74. A significant difference in morphometry was observed between oyster stocks according to site (ANOVA test, df = 2, F = 3.10, p-value = 0.05) and the origin of stock considered (ANOVA test, df = 1, F = 7.95, p-value < 0.01). According to allometric b parameter examination, Mangareva pearl-oyster stock demonstrates for the same shell length a lower total oyster weight (TOW) than Arutua stock. In contrast, biofouling biomass did not appear to have any influence on the observed morphometry (ANOVA test, df = 1, F = 0.86, p-value = 0.35). Despite the significant effect of site and stock origin, paired comparison revealed no significant differences in allometric parameter b between the stocks examined (Tukey's test p-value > 0.63; Table 5).

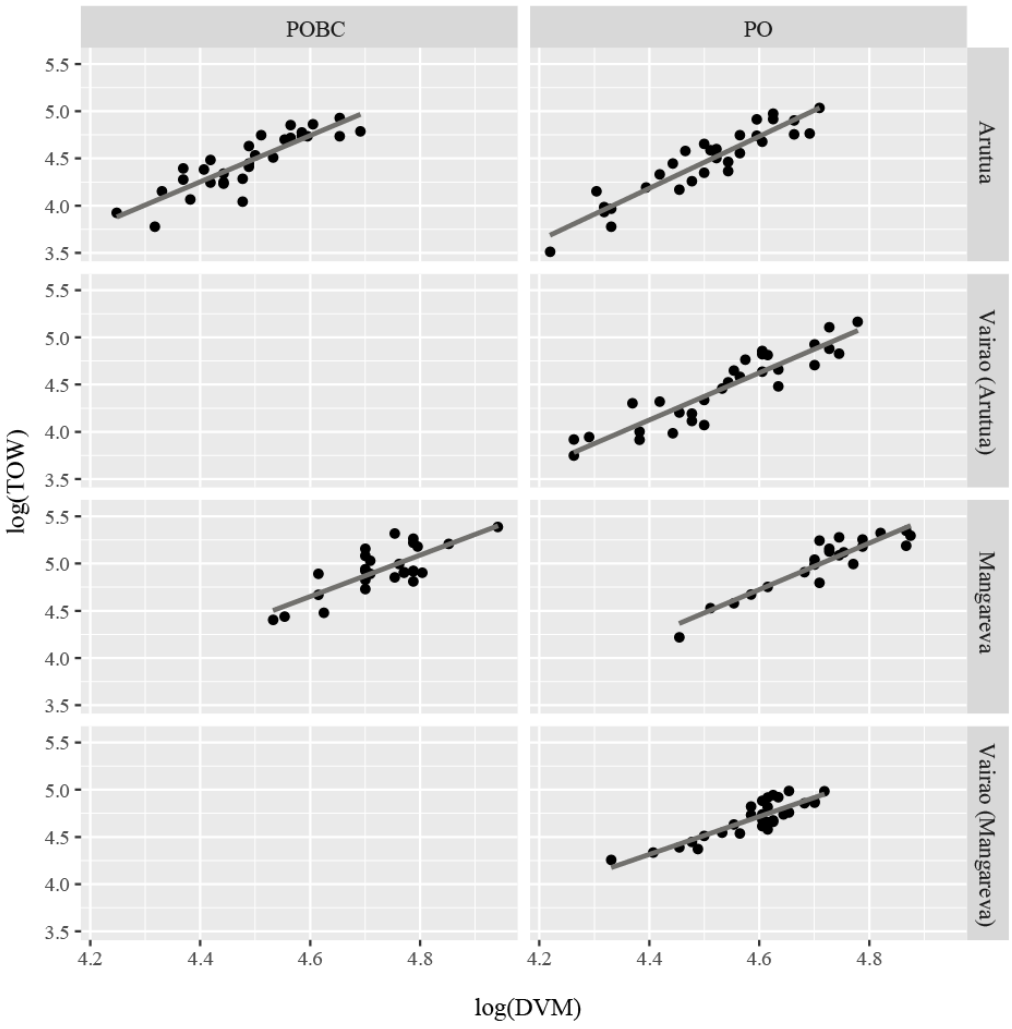

Figure 3. Linear relationship between log(dorso-ventral measurement: DVM; in mm) and log(Total Oyster Weight: TOW; in g) observed in each experimental pearl oyster (*Pinctada margaritifera*) stock (n = 30) reared in Mangareva (MPO and MPOBC), Arutua (APO and APOBC) and Vairao (VMPO and VAPO).

Table 5. Linear correlation parameter (R^2) between log(dorso-ventral measurement: DVM; in mm) and log(Total Oyster Weight: TOW; in g) observed in each experimental pearl oyster (*Pinctada margaritifera*) stock (n=30) reared in Mangareva (MPO and MPOBC), Arutua (APO and APOBC) and Vairao (VMPO and VAPO). Slope curve represents the b coefficient in the allometric relationship linking oyster weight and length ($TOW = a \times DVM^b$).

Group	Allometric coefficient b (slope)	R^2
APOBC	2.45	0.76
APO	2.74	0.86
MPOBC	2.19	0.60
MPO	2.46	0.85
VAPO	2.50	0.85
VMPO	2.00	0.77

2.3.Growth rate comparison based on DVM

Growth rate based on DVM observed between the start and the end of experiment for each experimental pearl oyster stock is plotted as a function of harvesting site, origin and biofouling treatment in Figure 4. Mean DVM growth rate ranged from 0.064 to 0.076 ± 0.004 $mm.day^{-1}$. Statistical analysis showed no significant difference for mean DVM growth rate among the experimental pearl oyster stocks (Kruskal-Wallis Chi-squared = 4.2918, df = 5, p-value = 0.51).

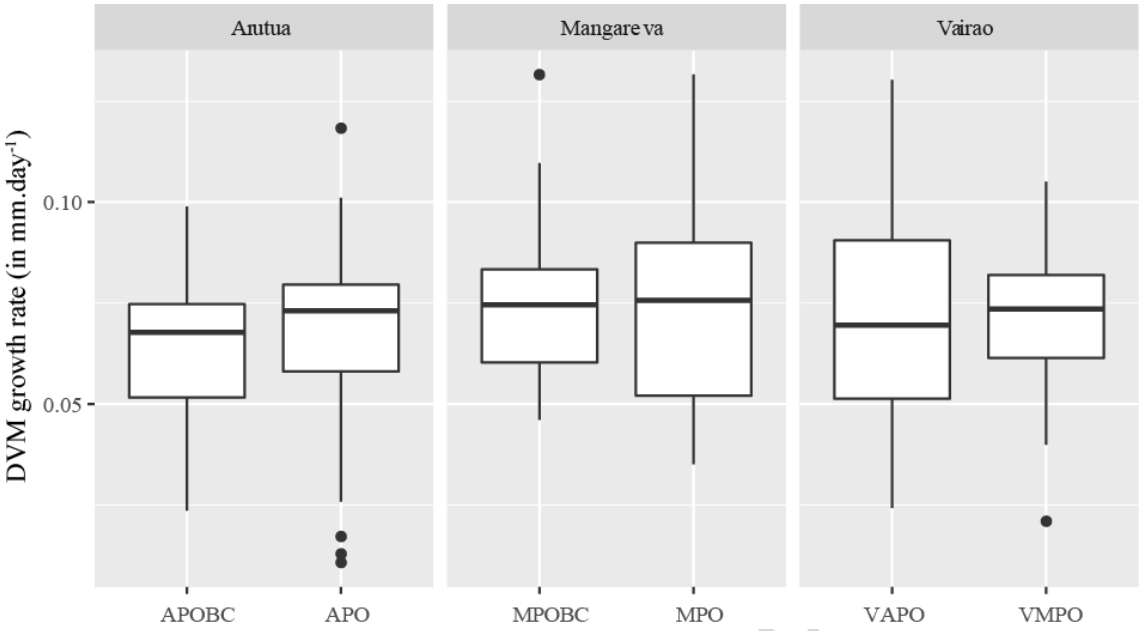

Figure 4: Boxplot of individual dorso-ventral measurement (DVM) growth rate (in mm.day^{-1}) for each experimental pearl oyster (*Pinctada margaritifera*) stock (n = 30) reared in Mangareva (MPO and MPOBC), Arutua (APO and APOBC) and Vairao (VMPO and VAPO).

2.4.Growth rate comparison based on total weight

Using the same individually tagged pearl oysters as for DVM, mean individual TOW (g.day^{-1}) was used to estimate growth rates of the different experimental stocks, revealing a range from 0.13 to 0.24 g.day^{-1} (Figure 5). Stocks from Mangareva (MPOBC & MPO) had higher mean individual TOW-based growth rates, 0.21–0.24 ± 0.01 g.day^{-1} (Wilcoxon test; p-value < 0.05), than those from Arutua and Vairao (APOBC, APO, VAPO, and VMPO) (0.13-0.15 ± 0.01 g.day^{-1}), which could not be distinguished from each other (Wilcoxon test; p-value > 0.46). Furthermore, in Arutua and Mangareva, we found no significant differences (Wilcoxon's test; p-value 0.99–1.00) for TOW growth rates between cleaned (PO) and uncleaned stocks (POBC). The development of biofouling therefore does not appear to have an effect on pearl oyster growth.

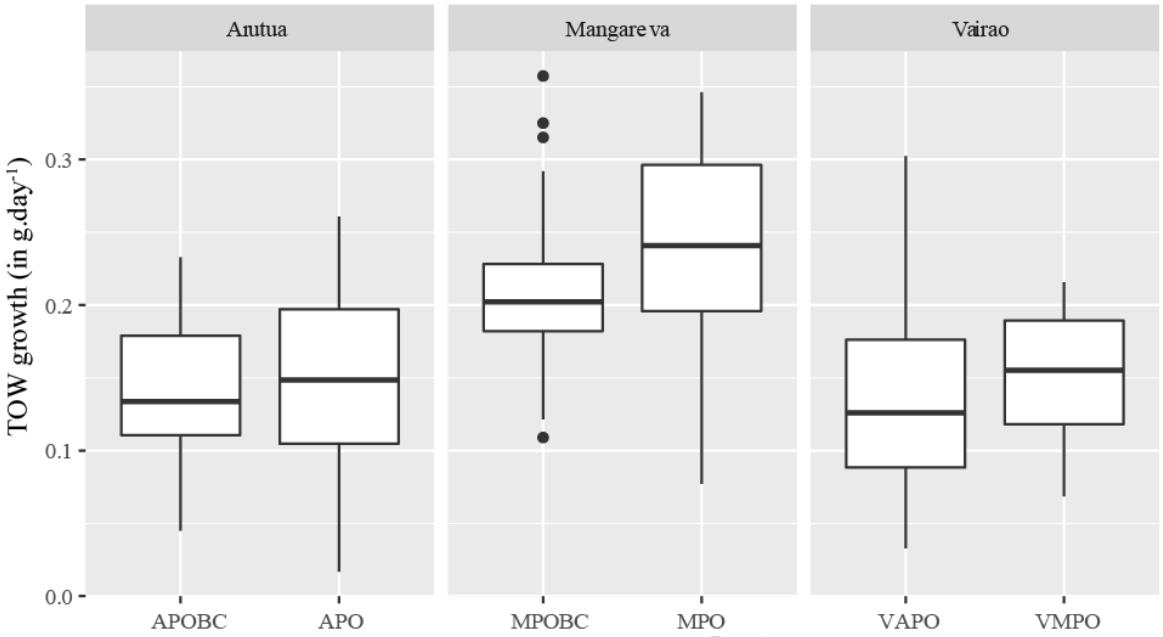

Figure 5. Boxplot of individual total oyster weight (TOW) growth rate (in g.day^{-1}) in each experimental pearl oyster (*Pinctada margaritifera*) stock (n = 30) reared in Mangareva (MPO and MPOBC), Arutua (APO and APOBC) and Vairao (VMPO and VAPO).

3. Discussion

3.1. Allometric growth parameter

Allometric parameter *b* and linear correlation parameter R^2 between log(TOW) and log(DVM) observed during our experiments (2.00–2.74 and 0.60–0.87, respectively), indicate overall isometric growth in *P. margaritifera* ($TOW = a \times DVM^b$). The *b* value is in accordance with previous studies on this species in Egypt (b = 2.83; El-Sayed et al., 2011), Taiwan (b = 2.79; Hwang et al., 2007) and French Polynesia (b = 2.79; Pouvreau et al., 2000). Despite the difficulty of distinguishing the stocks, the influence of their origin on allometry determination could suggest a genetic influence on the shell shape variability observed. Similarly, the significant influence of growing site on allometry demonstrates the influence of the environment on shell shape. These results are in accordance with a previous study, which considers morphometric shell variability in bivalve populations to be partly driven by both genotype and local environmental conditions (Caill-Milly et al., 2012). Studies on other bivalve species have demonstrated a link between environmental conditions, especially the food availability or stock density, and shell shape (Alunno-Bruscia et al., 2001; Caill-Milly et al., 2012; Bonel et al., 2013). Despite this encouraging result, our study was not appropriately designed to distinguish between the different

environmental factors affecting pearl oyster allometry (Telesca et al., 2018). However, our results suggest we should undertake a dedicated study to investigate the allometric response across a panel of environmental conditions from mediocre to optimum growth environment. In fact, linking environmental conditions with the allometric response of pearl oysters could make it possible to assess the potential ecological conditions in the field by simple shell morphometry analysis.

3.2. Influence of biofouling on growth performance

In spite of common biofouling taxonomic groups observed between the sites, the dynamics of biofouling colonization on the *P. margaritifera* shell surface differed greatly between Arutua and Mangareva. In Arutua, colonization by the biofouling community was relatively slow (0.016 g.oyster^{-1}.month^{-1}) and made up of sponges and bivalves, which developed only after 15 months of immersion (no sign of these organisms was detected at 6 months). On Mangareva, the colonization process seemed faster and more continuous over this time period (0.15–0.18 g.oyster^{-1}.month^{-1}). The pioneer colonizers here were tunicates (*Herdmania sp*.), which constituted the majority of the biofouling biomass on *P. margaritifera* shells. Later (T0 + 14 months), a more diverse biofouling community had formed, with the appearance of multiple taxonomic groups. It is important to note the appearance of polychaete worms, demonstrating that soft soil benthic organisms had joined the epibiont community. Their presence could indicate the existence of a suspended sediment compartment associated with the pearl oyster shell, probably inducing functional interactions with the water column, especially in Mangareva where the biofouling biomass appears greater.

The high level of biofouling development in Mangareva has already been reported (Lacoste et al., 2014), although we cannot make a direct comparison with these previous results because they were recorded in a different way (wet biofouling weight per pearl oyster). Our understanding of the difference in biofouling between Arutua and Mangareva is very limited. According to the literature, biofouling settlement in aquaculture may vary spatially and temporally. Environmental conditions, as important drivers of biofouling settlement and development, are probably the cause of these differences (Holloway & Keough, 2002a, 2002b).

According to our growth performance results obtained in Arutua and Mangareva on cleaned and uncleaned stock (PO: Pearl Oyster vs POBC: Pearl Oyster with Biofouling Communities),

biofouling development after 14–15 months of colonization did not have any significant negative effect on *P. margaritifera* growth. Evidence of the neutral effect of biofouling presence on bivalve metabolism has already been reported in the literature (Royer et al., 2006; Sala & Lucchetti, 2008; Fletcher et al., 2013). This result also supports the findings of an earlier study in which 20-month experiments also showed no influence of biofouling on *P. margaritifera* growth (Lacoste et al., 2014). According to these authors, this neutral effect of biofouling may be explained by several factors, such as food resource partitioning, or planktonic promotion, preventing food limitation between harvested species (Lacoste & Gaertner-Mazouni, 2015). On this basis, the extent of biofouling accumulation on pearl oyster shells does not appear to be a valid argument to explain the growth variability of *P. margaritifera* observed among different French Polynesian pearl farming sites.

3.3. Growth performance variability : phenotypic plasticity or local adaptation

Pearl oyster growth performance was significantly different between Arutua and Mangareva sites. The TOW growth rate observed suggests better growth performance of *P. margaritifera* in Mangareva (0.21–0.24 ± 0.01 g.day-1) than Arutua (0.14–0.15 ± 0.01 g.day^{-1}). Results relative to TOW growth rate are in agreement with reports in the literature of higher performances observed on Gambier Island compared with the Tuamotu atolls (Pouvreau & Prasil, 2001). However, our results contradict the existing literature (Lacoste et al., 2014; Pouvreau & Prasil, 2001) by highlighting lower growth performances in Vairao (mean 0.14 ± 0.01 g.day^{-1}) than Mangareva (mean 0.22 ± 0.01 g.day^{-1}). Examination of *P. margaritifera* growth performance in previous studies tends to suggest a scenario of exceptional growth in Mangareva island in our study rather than poor growth in Vairao or Arutua. This result is also supported by comparison of growth performance observed with seasonal monitoring of Mangareva pearl oyster growth quantified by Cochard et al. (2003). In fact, these authors reported significant seasonality in their results, indicating a period of fast growth (September–March) following by a period of halted growth (April–August). Thus, during the fast growth period, Cochard et al., (2003) noted a significantly higher growth rate (0.07 mm.day^{-1}), equivalent to our mean daily growth rate observed throughout the year (0.08 mm.day^{-1}). Moreover, in our study, at the 6-month subsampling in Mangareva

during the cleaning operation, the result obtained suggests constant growth during the September–April and April–November periods (see 7. Appendices section, Appendix 1).

On the basis of similar growth performance observed in the Vairao common garden site between stocks from Mangareva (VMPO: 0.07 ± 0.01 mm.day^{-1} or 0.15 ± 0.01 g.day^{-1}) and Arutua (VAPO: 0.07 ± 0.01 mm.day^{-1} or 0.13 ± 0.01 g.day^{-1}), we suggest that growth performance variability observed in both pearl farming sites (Arutua and Mangareva) is the result of phenotypic plasticity, not local adaptation. These results are in accordance with the study of Pouvreau et al. (2001) demonstrating high variability in growth response of a similar *P. margaritifera* population after a transplantation experiment between Vairao (29.6 mm.year^{-1}) and Takapoto lagoon (19.7 mm.year^{-1}). These results are also supported by a more recent experiment (Le Pabic et al., 2016), which reported high variability in growth performance obtained for the same oyster population reared in five different localities.

Unfortunately, our methodological approach limits our understanding of environmental influence on phenotypic growth expression (due to the limited environmental gradient at the site observed). Among environmental factors driving phenotypic growth plasticity, according to the literature available temperature is suspected of being particularly determinant (Pouvreau & Prasil, 2001). Paradoxically, our results show an optimum growth of *P.margaritifera* occurring at the Mangareva site, described as a site marked by a strong seasonality (Cochard et al., 2003) and low temperatures (23-26°C in our study), far from the optimum growth at 30°C predicted by the laboratory experiment (Le Moullac et al., 2016). This notable divergence with the literature may highlight the importance of other environmental factors in phenotypic plasticity of growth. Even if food availability is often cited as an important driver for bivalve growth, the Pouvreau & Prasil (2001) study suggested on contrary the exclusion of food availability from the list of drivers acting on growth site phenotypic plasticity. These authors suggested a strong adaptation of *P.margaritifera* to food deprivation, to with the ability to grow effectively in poor oceanic waters. Further analyses on the planktonic communities at these two sites, could be helpful to discriminate the ecological factor responsible for this site-growth variability. The discrepancy observed between the laboratory experiments and the field observations demonstrates that the

determinants of growth are not trivial, and might be due to synergetic effects of the different environmental factors (temperature, food availability, salinity) on bivalve growth (Gosling, 2015).

4. Conclusion

By using a common garden approach, our results demonstrate the importance of phenotype plasticity in pearl oyster growth variability observed in French Polynesia. This result accords a major importance to site selection for the pearl farming optimization process. Even if environmental factors have demonstrated their major importance in growth determination of *P.margaritifera*, the ecological drivers acting on the variability observed still need to be clarified. Finally, the allometry observed for *P.margaritifera* shows interesting variations with regard to the ecological conditions encountered and could prove a promising tool. However, further investigations are needed to confirm this result

This work confirms, the neutral influence of biofouling on oyster growth, as pointed out in previous studies (Lacoste et al., 2014). Due to the high cost of biofouling management in pearl farming facilities, this suggests, once more, a major reconsideration of the management of the pearl farming management process (for at least the growing phase) is needed. However, before extending our results to the whole pearl farming process, supplementary experiments are needed to confirm the influence of biofouling on the biomineralization process, which enables the formation of pearls. Due to the additional energy requirement during pearl formation, it is primordial importance to confirm this neutral influence of biofouling development on the final stage of the pearl farming process (the post-grafting phase).

5. Conflict of interest

The authors have declared no conflict of interest.

6. Acknowledgment

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7. References

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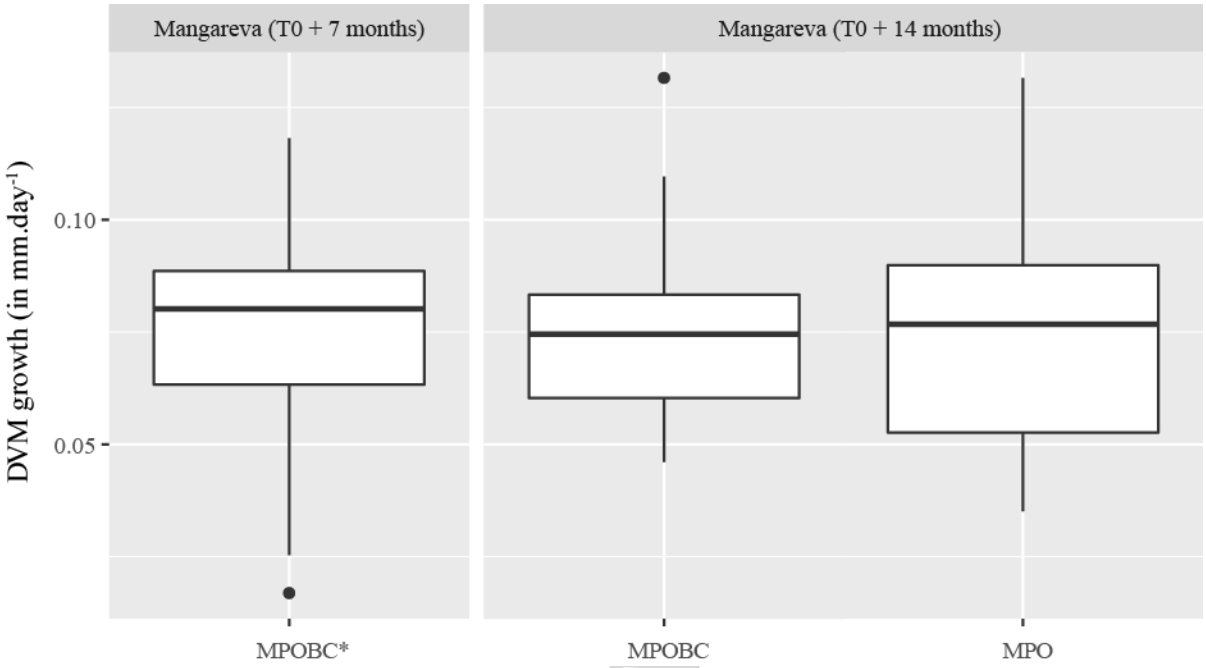
8. Appendices



Appendix 1 : Boxplot of *Pinctada margaritifera* individual dorso-ventral measurement growth rate observed (in mm.day^{-1}) in different stocks in Mangareva (Pearl Oyster= PO; Pearl Oyster with Biofouling Communities =POBC) after 7 and 15 months culture. * indicates that at this date MPO stock and MPOBC stock were undifferentiated. Statistical analysis demonstrate s that growth rates are similar for all dates and stock observed during the experiment.

9. Graphical abstract

FIG1.EPS = figure 1 (colored version is suitable)

FIG2.EPS = figure 2 (non-colored version is suitable)

FIG3.EPS = figure 3 (non-colored version is suitable)

FIG4.EPS = figure 4 (non-colored version is suitable)

FIG5.EPS = figure 5 (non-colored version is suitable)

AP1.EPS = figure 5 (non-colored version is suitable)

10. Highlights

- Faster biofouling colonization was observed in Mangareva than in Arutua
- Mangareva biofouling colonization was associated with tunicate settlement
- Biofouling had no negative effect on *Pinctada margaritifera* growth by 14-15 months
- Large growth differences in *Pinctada margaritifera* were mainly phenotype plasticity

CRediT author statement

Vivien HULOT:
Methodology, Validation, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization.

Nabila GAERTNER-MAZOUNI:
Conceptualization, Methodology, Validation, Investigation, Resources,Writing – Original Draft, Writing – Review & Editing,Visualization
Supervision, Project Administration, Funding Acquisition

Denis SAULNIER: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition.

Oihana LATCHERE:
Formal Analysis, Investigation, Writing – Review & Editing, Visualization.

Nicolas MAIHOTA
Methodology, Formal Analysis, Investigation.