



**HAL**  
open science

# Phenotype plasticity, local adaptation, and biofouling influence on growth of the pearl oyster *Pinctada margaritifera*

V. Hulot, D. Saulnier, N. Maihota, Oihana Latchere, Nabila Gaertner-Mazouni

## ► To cite this version:

V. Hulot, D. Saulnier, N. Maihota, Oihana Latchere, Nabila Gaertner-Mazouni. Phenotype plasticity, local adaptation, and biofouling influence on growth of the pearl oyster *Pinctada margaritifera*. *Aquaculture*, 2019, 512, pp.734309. 10.1016/j.aquaculture.2019.734309 . hal-03187317

**HAL Id: hal-03187317**

**<https://hal.science/hal-03187317v1>**

Submitted on 31 Mar 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

## Authors

Hulot V<sup>1-2</sup>, Saulnier D<sup>2</sup>, Latchere O<sup>1</sup>, Maihota N<sup>3</sup>, Gaertner-Mazouni N<sup>1\*</sup>

<sup>1</sup> UNIV. POLYNESIE FRANCAISE, IFREMER, ILM, IRD, EIO UMR 241, Tahiti, POLYNESIE FRANCAISE.

<sup>2</sup> Institut Français de Recherche pour l'Exploitation de la Mer, UMR-241 EIO, BP 49 - 98719 Taravao, Tahiti, Polynésie Française

<sup>3</sup> Institut de Recherche pour le développement, UMR-241 EIO, BP 6570 - 98702 Faa'a, Tahiti, Polynésie française.

\*Corresponding author

## 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 ( $0.21\text{--}0.24 \pm 0.01 \text{ g.day}^{-1}$ ) than Arutua ( $0.14\text{--}0.15 \pm 0.01 \text{ g.day}^{-1}$ ). However, similar growth performances are observed at the Vairao common garden site for oyster stocks from Mangareva ( $0.07 \pm 0.01 \text{ mm.day}^{-1}$  or  $0.15 \pm 0.01 \text{ g.day}^{-1}$ ) and Arutua ( $0.07 \pm 0.01 \text{ mm.day}^{-1}$  or  $0.13 \pm 0.01 \text{ g.day}^{-1}$ ). 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 ( $0.016 \text{ g.oyster}^{-1}.\text{month}^{-1}$ ) and was mainly composed of sponges and bivalves. On Mangareva, the colonization process appeared faster and more continuous over the study period ( $0.15\text{--}0.18 \text{ g.oyster}^{-1}.\text{month}^{-1}$ ) 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.

## 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).

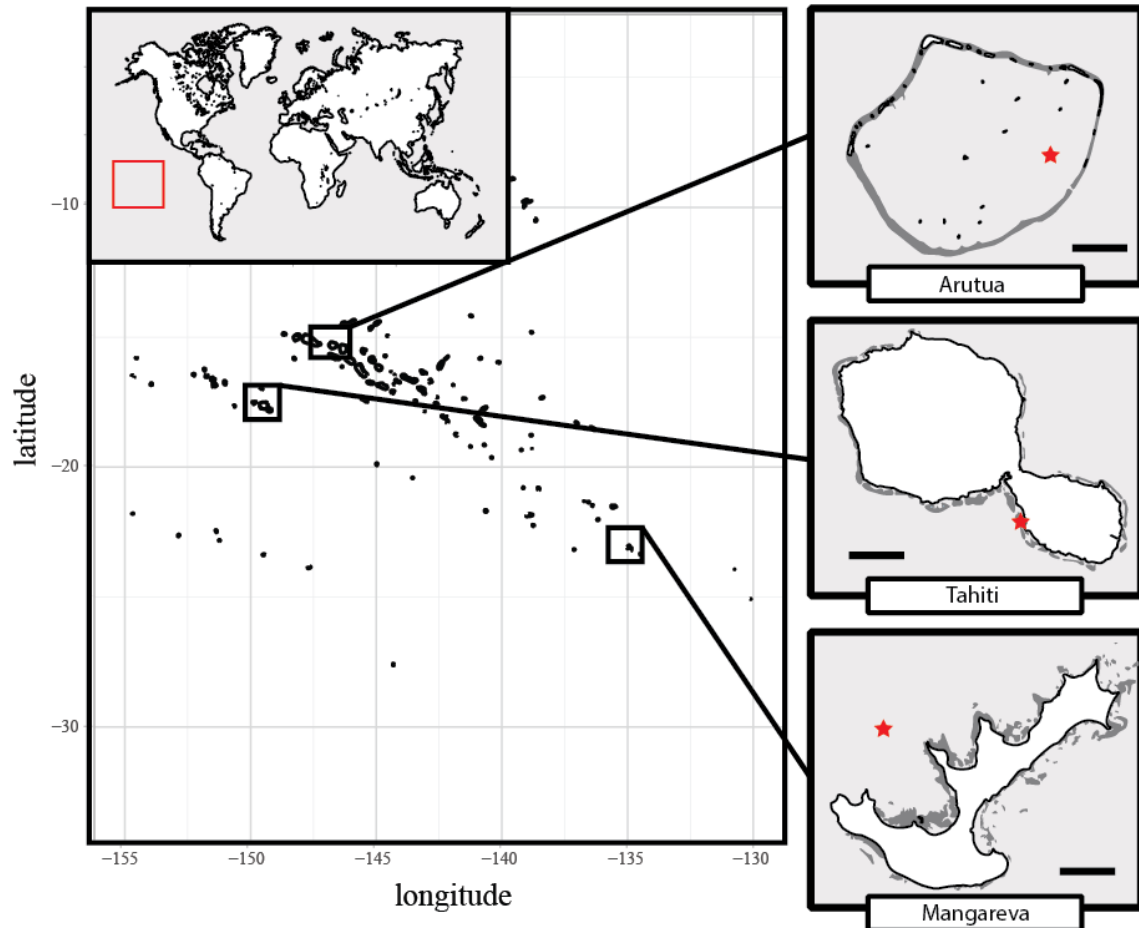
In the case of the culture of the pearl oyster *Pinctada margaritifera* in French Polynesia, the variations of growth observed between sites are most often considered as the result of the environmental effect on organisms (Pouvreau & Prasil, 2001). If several laboratory experiments are in agreement in 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 that 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<sup>2</sup>. 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 km<sup>2</sup>. 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.**

	<b>Arutua (Tuamotu)</b>	<b>Mangareva (Gambier)</b>	<b>Vairao (Tahiti)</b>
<b>GPS</b>	15°20'18.2"S	23°05'38.7"S	17°49'9"S
	146°39'01.2"W	134°59'52.8"W	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				





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  $-20^{\circ}\text{C}$ . 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  $-80^{\circ}\text{C}$  before being treated in a freeze dryer for 48 h at  $-20^{\circ}\text{C}$  (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  $0.24 \pm 0.02 \text{ g.oyster}^{-1}$  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  $1.07 \pm 0.05 \text{ g.oyster}^{-1}$  after 7 months and  $2.45 \pm 0.43 \text{ g.oyster}^{-1}$  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 ( $0.84 \text{ g.oyster}^{-1}$ ) (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  $0.14 \text{ g.oyster}^{-1}$ , 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		<i>Caulerpa</i> sp.
Anthozoans	<i>Aptasia</i> sp.	<i>Aptasia</i> sp.
Tunicates	<i>Herdmania</i> sp.**	<i>Herdmania</i> sp.** Undetermined ascidians <i>Didemnum</i> sp.
Bivalves	<i>Pinctada maculata</i> ** <i>Saccrostrea cuculata</i> Undetermined bivalve	<i>Pinctada maculata</i> ** <i>Saccrostrea cuculata</i> Undetermined bivalve
Sponges	<i>Cliona</i> sp. <i>Dysidea</i> sp.**	<i>Cliona</i> sp. <i>Dysidea</i> sp.**
Macrozoobenthos	Undetermined Crustacean Undetermined Polychaete	Undetermined Crustacean Undetermined Polychaete <i>Ocenebra inornata</i>

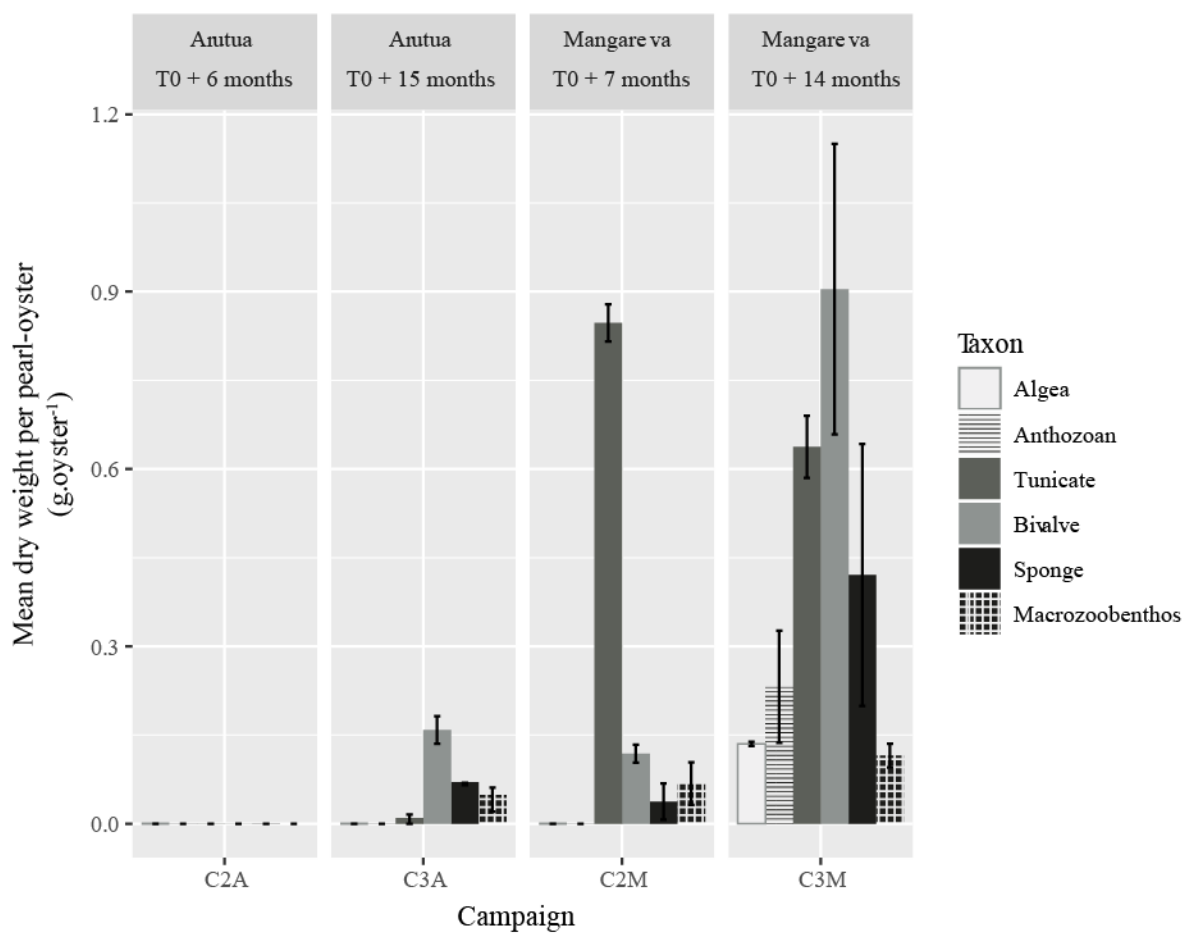


Figure 2. Total dry biofouling biomass (in g.pearl oyster<sup>-1</sup>) per taxonomic group observed on *Pinctada margaritifera* shells during the 2016–2017 sampling campaign in Arutua (C2A and C3A) and Mangareva (C2M and C3M). For each date corresponding duration of colonization after initial cleaning 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<sup>-1</sup>) 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 <sup>-1</sup> )	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 ( $R^2 = 0.60\text{--}0.87$ ; Table 5). Allometric parameter  $b$  estimated with the curve slope [ $\log(\text{TOW}) = \log(a) + b \cdot \log(\text{DVM})$ ] ranged from 2.00 to 2.74. A significant difference in morphometry was observed between oyster stocks according to site (ANOVA test,  $df = 2$ ,  $F = 3.10$ ,  $p\text{-value} = 0.05$ ) and the origin of stock considered (ANOVA test,  $df = 1$ ,  $F = 7.95$ ,  $p\text{-value} < 0.01$ ). 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,  $df = 1$ ,  $F = 0.86$ ,  $p\text{-value} = 0.35$ ). 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  $p\text{-value} > 0.63$ ; Table 5).

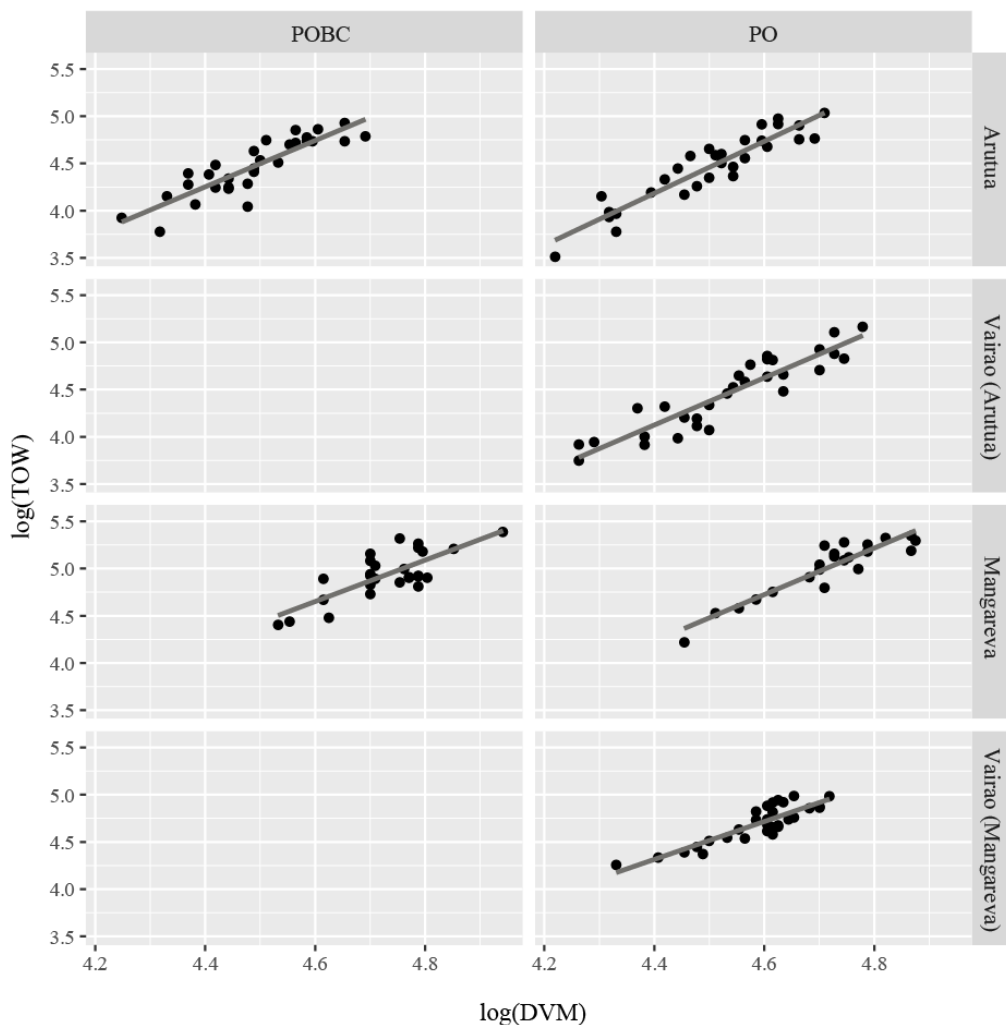


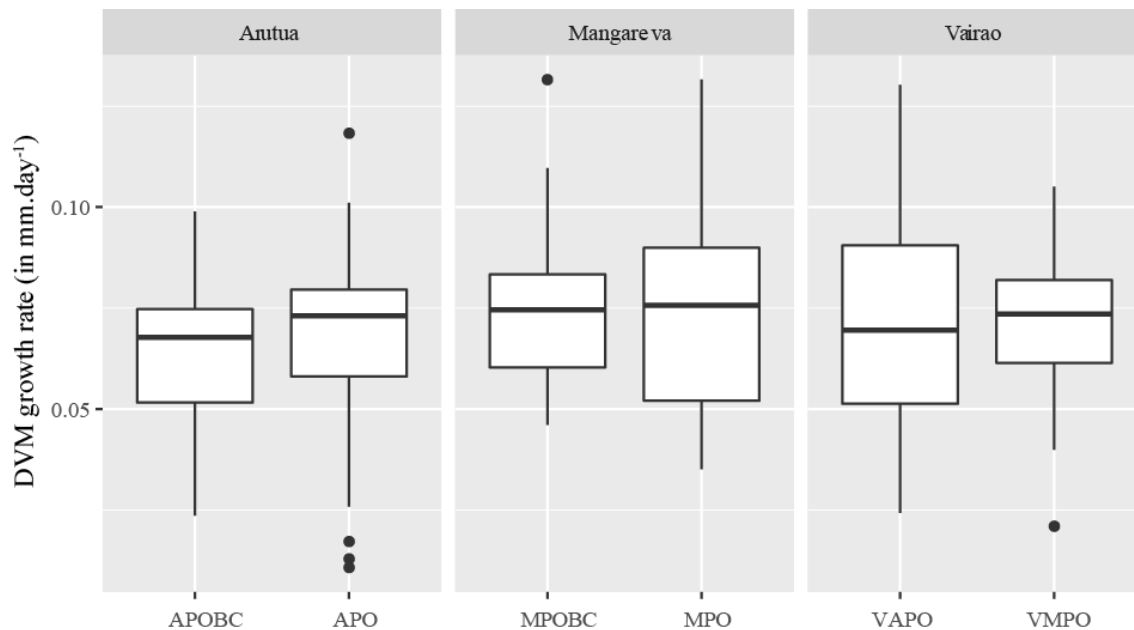
Figure 3. Linear relationship between log(dorso-ventral measurement: DVM; in mm) and log(Total Oyster Weight: TOW; in g) observed in each experimental pearl oyster (*Pinctada margaritifera*) stock (n = 30) reared in Mangareva (MPO and MPOBC), Arutua (APO and APOBC) and Vairao (VMPO and VAPO).

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

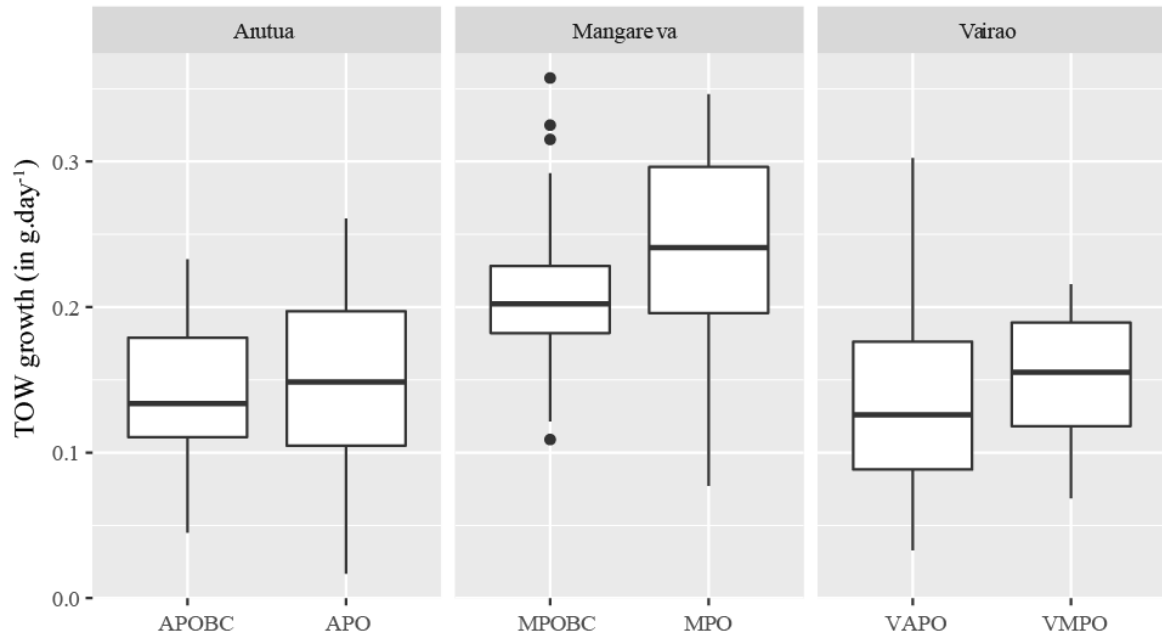
Growth rate based on DVM observed between the start and the end of experiment for each experimental pearl oyster stock is plotted as a function of harvesting site, origin and biofouling treatment in Figure 4. Mean DVM growth rate ranged from 0.064 to 0.076  $\pm$  0.004 mm.day<sup>-1</sup>. Statistical 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<sup>-1</sup>) 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<sup>-1</sup>) was used to estimate growth rates of the different experimental stocks, revealing a range from 0.13 to 0.24 g.day<sup>-1</sup> (Figure 5). Stocks from Mangareva (MPOBC & MPO) had higher mean individual TOW-based growth rates, 0.21–0.24 ± 0.01 g.day<sup>-1</sup> (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<sup>-1</sup>), 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  $\text{g}\cdot\text{day}^{-1}$ ) 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  $\log(\text{TOW})$  and  $\log(\text{DVM})$  observed during our experiments (2.00–2.74 and 0.60–0.87, respectively), indicate overall isometric growth in *P. margaritifera* ( $\text{TOW} = a \times \text{DVM}^b$ ). 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 ( $0.016 \text{ g.oyster}^{-1}.\text{month}^{-1}$ ) 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 ( $0.15\text{--}0.18 \text{ g.oyster}^{-1}.\text{month}^{-1}$ ). 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 ( $0.21\text{--}0.24 \pm 0.01 \text{ g.day}^{-1}$ ) than Arutua ( $0.14\text{--}0.15 \pm 0.01 \text{ g.day}^{-1}$ ). 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  $0.14 \pm 0.01 \text{ g.day}^{-1}$ ) than Mangareva (mean  $0.22 \pm 0.01 \text{ g.day}^{-1}$ ). 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 ( $0.07 \text{ mm.day}^{-1}$ ), equivalent to our mean daily growth rate observed throughout the year ( $0.08 \text{ mm.day}^{-1}$ ). 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:  $0.07 \pm 0.01 \text{ mm.day}^{-1}$  or  $0.15 \pm 0.01 \text{ g.day}^{-1}$ ) and Arutua (VAPO:  $0.07 \pm 0.01 \text{ mm.day}^{-1}$  or  $0.13 \pm 0.01 \text{ g.day}^{-1}$ ), 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 ( $29.6 \text{ mm.year}^{-1}$ ) and Takapoto lagoon ( $19.7 \text{ mm.year}^{-1}$ ). 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 ( $23\text{-}26^\circ\text{C}$  in our study), far from the optimum growth at  $30^\circ\text{C}$  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

Our thanks to DRMM, UPF and IFREMER for financial support (QUALISANT research project). Vivien Hulot, was supported by a PhD fellowship from the 'Ministère de l'Enseignement Supérieur et de la Recherche' at the 'Université de la Polynésie Française' (ED 469) and by the 'Institut Français de Recherche pour l'Exploitation de la Mer' (IFREMER).

## 7. References

- Alunno-Bruscia, M., E. Bourget, & M. Fréchette, 2001. Shell allometry and length-mass-density relationship for *Mytilus edulis* in an experimental food-regulated situation. *Mar. Ecol. Prog. Ser.* 219: 177–188.
- Bonel, N., L. C. Solari, & J. Lorda, 2013. Differences in density, shell allometry and growth between two populations of *Limnoperna fortunei* (Mytilidae) from the Río de la Plata basin, Argentina. *Malacologia* 56: 43–58.
- Brown, J. R., & E. B. Hartwick, 1988. Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*: II. Condition index and survival. *Aquaculture* 70: 253–267.
- Caill-Milly, N., N. Bru, K. Mahé, C. Borie, & F. D'Amico, 2012. Shell shape analysis and spatial allometry patterns of Manila clam (*Ruditapes philippinarum*) in a mesotidal coastal lagoon. *Journal of Marine Biology* 2012:.
- Chávez-Villalba, J., C. Soye, H. Aurentz, & G. Le Moullac, 2013. Physiological responses of female and male black-lip pearl oysters (*Pinctada margaritifera*) to different temperatures and concentrations of food. *Aquatic Living Resources* 26: 263–271.
- Cochard, J.-C., J. Tiapari, H. Teissier, & G. Le Moullac, 2003. Maturation de l'huître perlière *Pinctada margaritifera* aux îles Gambier. .
- Dickinson, G. H., A. V. Ivanina, O. B. Matoo, H. O. Pörtner, G. Lannig, C. Bock, E. Beniash, & I. M. Sokolova, 2012. Interactive effects of salinity and elevated CO<sub>2</sub> levels on juvenile eastern oysters, *Crassostrea virginica*. *Journal of Experimental Biology* 215: 29–43.
- El-Sayed, A., F. A. A. Razek, M. Abou-Zaid, & S. M. Taha, 2011. Measures of allometric growth of black-lip pearl oyster *Pinctada margaritifera* (Linnaeus, 1758) Red Sea, Egypt. *International Journal of Zoological Research* 7: 201.
- Fletcher, L. M., B. M. Forrest, & J. J. Bell, 2013. Impacts of the invasive ascidian *Didemnum vexillum* on green-lipped mussel *Perna canaliculus* aquaculture in New Zealand. *Aquaculture Environment Interactions* 4: 17–30.
- Fréchette, M., 2010. Hierarchical structure of bivalve culture systems and optimal stocking density. *Aquaculture internationale* 18: 99–114.
- Gosling, E., 2015. Growth Marine Bivalve Molluscs. *Wiley-Blackwell*: 203–242, <https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119045212.ch6>.
- Hiebenthal, C., E. Philipp, A. Eisenhauer, & M. Wahl, 2012. Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. *Aquatic Biology* 14: 289–298.
- Holloway, M. G., & M. J. Keough, 2002a. An introduced polychaete affects recruitment and larval abundance of sessile invertebrates. *Ecological Applications* 12: 1803–1823.
- Holloway, M. G., & M. J. Keough, 2002b. Effects of an introduced polychaete, *Sabella spallanzanii*, on the development of epifaunal assemblages. *Mar. Ecol. Prog. Ser.* 236: 137–154.
- Hwang, J. J., T. Yamakawa, & I. Aoki, 2007. Growth of wild pearl oysters *Pinctada fucata*, *Pinctada margaritifera* and *Pinctada sugillata* (Bivalvia: Pteriidae) in Taiwan. *Fisheries science* 73: 132–141.
- ISPF, 2018. Le bilan de la perle en 2016. , <http://www.ispf.pf>.

- Joubert, C., C. Linard, G. Le Moullac, C. Soyez, D. Saulnier, V. Teaniniuraitemoana, C. L. Ky, & Y. Gueguen, 2014. Temperature and food influence shell growth and mantle gene expression of shell matrix proteins in the pearl oyster *Pinctada margaritifera*. *PLoS One* 9: e103944.
- Ky, C.-L., C. Blay, M. Sham-Koua, V. Vanaa, C. Lo, & P. Cabral, 2013. Family effect on cultured pearl quality in black-lipped pearl oyster *Pinctada margaritifera* and insights for genetic improvement. *Aquatic Living Resources* 26: 133–145.
- Lacoste, E., & N. Gaertner-Mazouni, 2015. Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. *Reviews in Aquaculture* 7: 187–196.
- Lacoste, E., G. Le Moullac, P. Levy, Y. Gueguen, & N. Gaertner-Mazouni, 2014. Biofouling development and its effect on growth and reproduction of the farmed pearl oyster *Pinctada margaritifera*. *Aquaculture* 434: 18–26.
- Le Moullac, G., C. Soyez, O. Latchere, J. Vidal-Dupiol, J. Fremery, D. Saulnier, A. L. Yat, C. Belliard, N. Mazouni-Gaertner, & Y. Gueguen, 2016. *Pinctada margaritifera* responses to temperature and pH: Acclimation capabilities and physiological limits. *Estuarine, Coastal and Shelf Science* 182: 261–269.
- Le Pabic, L., S. Parrad, M. S. Koua, S. Nakasai, D. Saulnier, D. Devaux, & C.-L. Ky, 2016. Culture site dependence on pearl size realization in *Pinctada margaritifera* in relation to recipient oyster growth and mantle graft biomineralization gene expression using the same donor phenotype. *Estuarine, Coastal and Shelf Science* 182: 294–303.
- López, D., V. Riquelme, & M. González, 2000. The effects of epibionts and predators on the growth and mortality rates of *Argopecten purpuratus* cultures in southern Chile. *Aquaculture International* 8: 431–442.
- Mann, R., 1979. The effect of temperature on growth, physiology, and gametogenesis in the Manila clam *Tapes philippinarum* (Adams & Reeve, 1850). *Journal of Experimental Marine Biology and Ecology* 38: 121–133.
- Pouvreau, S., & V. Prasil, 2001. Growth of the black-lip pearl oyster, *Pinctada margaritifera*, at nine culture sites of French Polynesia: synthesis of several sampling designs conducted between 1994 and 1999. *Aquatic Living Resources* 14: 155–163.
- Pouvreau, S., J. Tiapari, A. Gangnery, F. Lagarde, M. Garnier, H. Teissier, G. Haumani, D. Buestel, & A. Bodoy, 2000. Growth of the black-lip pearl oyster, *Pinctada margaritifera*, in suspended culture under hydrobiological conditions of Takapoto lagoon (French Polynesia). *Aquaculture* 184: 133–154.
- Royer, J., M. Ropert, M. Mathieu, & K. Costil, 2006. Presence of spionid worms and other epibionts in Pacific oysters (*Crassostrea gigas*) cultured in Normandy, France. *Aquaculture* 253: 461–474.
- Sá, F. S. de, R. C. Nalesso, & K. Paresque, 2007. Fouling organisms on *Perna perna* mussels: is it worth removing them?. *Brazilian Journal of Oceanography* 55: 155–161.
- Sala, A., & A. Lucchetti, 2008. Low-cost tool to reduce biofouling in oyster longline culture. *Aquacultural engineering* 39: 53–58.
- Saxby, S. A., 2002. A review of food availability, sea water characteristics and bivalve growth performance at coastal culture sites in temperate and warm temperate regions of the world. *Fisheries Research Report. Department of Fisheries, Western Australia* .
- Steffani, C. N., & G. M. Branch, 2003. Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. *Mar. Ecol. Prog. Ser.* 246: 197–209.

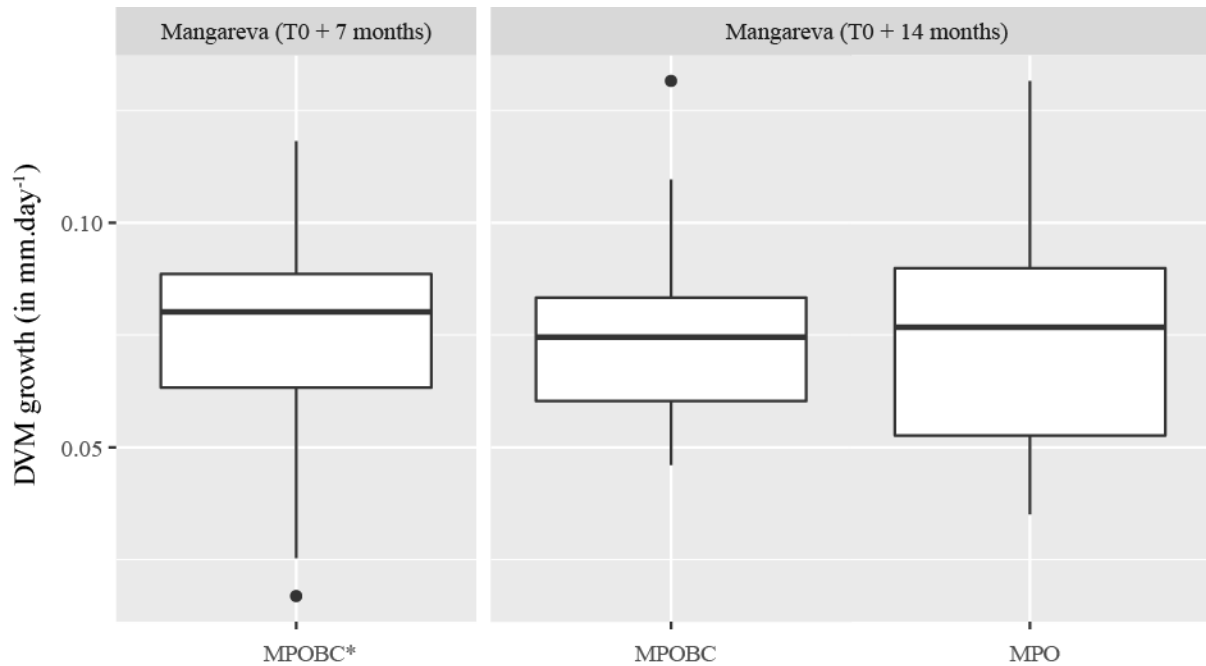
Strohmeier, T., A. Duinker, Ø. Strand, & J. Aure, 2008. Temporal and spatial variation in food availability and meat ratio in a longline mussel farm (*Mytilus edulis*). *Aquaculture* 276: 83–90.

Team, R. C., 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. .

Telesca, L., K. Michalek, T. Sanders, L. S. Peck, J. Thyrring, & E. M. Harper, 2018. Blue mussel shell shape plasticity and natural environments: a quantitative approach. *Scientific reports* 8: 2865.

Yukihira, H., D. Klumpp, & J. Lucas, 1998. Comparative effects of microalgal species and food concentration on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima* (Bivalvia: Pteriidae). *Mar. Ecol. Prog. Ser.* 171: 71–84.

## 8. Appendices



**Appendix 1 : Boxplot of *Pinctada margaritifera* individual dorso-ventral measurement growth rate observed (in mm.day<sup>-1</sup>) 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 demonstrates 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.