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Estimation of physical and physiological performances of blacklip pearl oyster larvae in view of DEB modeling and recruitment assessment

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Abstract :

In French Polynesia black pearl farming represents one of the dominant business sectors. However, it still entirely relies on unpredictable Pinctada margaritifera spat collection success, which is itself conditioned by larval development completion. To assess the relationship between larval development and recruitment success, we studied under controlled conditions the effect of food concentration on development, growth, ingestion rate, survival and metabolic rate at the larval stage. Larvae were exposed to four different phytoplankton densities (2,5; 7,5; 15 and 30 cell.µL−1). Larvae survived equally all over the range of phytoplankton concentration with an average survival rate of 16% at the end of experiments. Food concentration significantly affected the larval physiology throughout its development from birth to metamorphosis. Growth and feeding were close to those reported by previous laboratory observations with young spat of 210 µm long obtained in 18 days of rearing at 28 °C for the highest food concentration. Differences in length at metamorphosis and cumulated energy ingested until settlement occurred according to trophic levels with a saturation threshold close to 0.0086 J.ind−1. This level was reached at the food concentration of 15 cell.µL−1. Larval development stages could be divided on the basis of the energy balance between feeding and respiration rates. An initial mixotrophic period with a lower and constant ingestion/respiration ratio over the first three days (from birth to D-veliger larva) was followed by an exotrophic phase characterized by a sharp increase in energy balance highly dependent of food concentration. Finally two sharp decreases of feeding rates were recorded during metamorphosis before umbonate and eyed stages. This study provided numerous new clues to establish a quasi-deterministic relationship between food condition and larval development. It highlights the major effect of food concentration and how energy intake through feeding as well as behavioral and physiological transitions can optimize larval development duration and minimize “the risky phase” of their life cycle. By taking into account the observed metabolic switches, the results provide a strong foundation for Dynamic Energy Budget model development and better description of the complex interactions between *P. margaritifera* physiology and environmental conditions.

Highlights

► *Pinctada margaritifera* larval phase is studied for DEB modeling. ► Food density highly impact physical traits of larvae such as age or size at settlement. ► Low food density is not lethal for pearl oyster larvae. ► Cumulated energy ingested until settlement differs according to trophic levels. ► Energetic balance reveals critical metabolic periods, especially during the metamorphosis phases.

Keywords : Bivalve larvae, Pinctada margaritifera, Physiology, Energetics, Dynamic energy budget theory, Pearl farming, Aquaculture

1. Introduction

In marine species, questions regarding the origin of recruitment variability first appeared for fish populations (Hjort, 1926) before it was extended by Thorson (1950) to all species with a planktotrophic larval phase. The link between larval stage, recruitment and population dynamic is commonly described by the "supply-side ecology" hypothesis which focus on the number of individuals surviving to recruit in habitats occupied by adults rather than process causing death (Underwood and Fairweather, 1989; Grosberg and Levitan, 1992). Considered as a risky phase of the life cycle, the larval stage received a lot of attention. Very early on, Hjort (1926) already made the assumption of an excess of mortality when larvae start to feed by themselves. Then, the so-called optimal window theory (Cury and Roy, 1989) linked spawning output and recruitment success through larval development and environmental conditions. This theory emphasized that "match-mismatch" between the presence of trophic resource and period of larval development can explain recruitment variations for bivalves species (Olson, 1989; Menge *et al.,* 2009; Thomas *et al.,* 2011). The lack of a quasi-deterministic relationship between spawning and recruitment continues to confound attempts to fully understand and predict population dynamics. In the case of bivalve larvae, growth and survival are determined by complex interactions between their physiology and the environmental conditions (Widdows, 1991; Hofmann *et al.,* 2009) and many studies have contributed to our understanding of how environmental variables influence larval growth and development (Doroudi *et al.*, 1999a; 2000; Rico-Villa *et al.*, 2009). Such studies were also instrumental in optimizing the hatcheries conditions for growth and survival of bivalve larvae, for commercial cultivation.

In French Polynesia, pearl culture is based on the production of a single species: the black-lipped pearl oyster (*Pinctada margaritifera,* Linnaeus, 1758) (Andréfouët *et al.*, 2012). This activity has a major economic and social function since it employs about 1500 workers and represents the second income of the country right behind tourism (ISPF, 2016). The

supply of juvenile oysters to the farms is largely dependent on the natural collection of larvae on artificial substrates. This spat collection process takes place in 26 atolls and 4 islands (as in 2017). For a given site, spat collection has proven to be very variable both spatially and temporally, to the point that it may at time jeopardizes the steady supply of oysters to the local demand. French Polynesian atoll lagoons have been described as stable and homogeneous environments due to the low variations of water column characteristics in comparison to temperate semi-enclosed system (Charpy *et al.,* 1997; Pouvreau *et al.*, 2000a). However, at the intra-lagoon scale, fluctuations in temperature and food concentration occur in the water column (Fournier *et al.*, 2012; Pagano *et al.*, 2017). This spatial and temporal heterogeneity can impact larval development, survival and dispersal and ultimately the success of spat capture (Moran and Manahan, 2004 ; O'Connor *et al.,* 2007, Thomas *et al.,* 2016).

Since bivalve growth is directly linked to its environment (Southgate and Lucas, 2011), energetic models have been developed the last two decades to determine the role of biotic and abiotic parameters on growth (Hofmann *et al.,* 2009; Pouvreau *et al.,* 2006; Powell *et al.,* 2002). At the individual level, the Dynamic Energy Budget (DEB) theory (Kooijman, 2010) describes the processes of development, growth, maintenance, reproduction and ageing for any kind of organism throughout its life cycle. A DEB model gives a representation of the link between environment and physiological performances by describing the metabolic rates, but it can also relate ecotoxicology to life traits for a given organism (Jager *et al.*, 2010; Jager and Zimmer, 2012). Such models were successfully applied to well-studied species such as *Crassostrea gigas*, an intensively cultivated bivalve (Rico-Villa *et al.*, 2010). For *P. margaritifera,* temperature and food concentrations are considered to be the primary environmental factors affecting the oyster physiological processes (Southgate and Lucas, 2011), but their influence on the metabolic rates during development remain poorly documented.

To assess how environment and *P. margaritifera* physiology could be linked, Fournier (2011) and Thomas *et al.* (2011) built two different DEB models for larvae and adult stage respectively, that were partially calibrated based on parameters from *Crassostrea gigas*. The larval DEB model has already been used and coupled with an hydrodynamic model to investigate recruitment variation in space and time in an atoll lagoon (Thomas *et al.*, 2016). Sensitivity analyses pointed out the major effect of the broodstock population structure, the larval mortality rate and inter-individual growth variability. However, to date, an integrative model for the full life cycle is still missing. A possible reason for this lack of integration may be that all available data thus far on this species have been collected independently for a variety of topics (genetic, evolutionary or comparative physiology and experimental bioenergetics) that were not focused on informing a single model. To move forward, a DEB

model is currently in development to explicitly encapsulate all life stages into a single bioenergetic model, and further integrate broodstock population dynamic and larval performances (stage, mortality, fixation threshold) to the larval dispersal models initiated by (Thomas *et al.*, 2016).

The accuracy of a DEB model for any given target species depends on the availability of a set of parameters specific to the modeled species. The "covariation method" developed by Lika *et al.,* (2011) provides specific sets of parameters which are estimated from standard empirical datasets. The method has formalized 10 qualitative levels of parameterization, which are directly linked to the nature of the empirical datasets on which the accuracy of the parameters will depend. As the literature now provides a wide range of relevant datasets(Doroudi *et al.*, 1999a; Doroudi *et al.*, 1999b; Doroudi and Southgate, 2003a, 2000; Pouvreau *et al.*, 2000a, 2000b; Pouvreau and Prasil, 2001), *Pinctada margaritifera* currently ranks at the fourth completeness level which refers to fits of growth (curve), age, length and weight at birth and puberty at several food levels. This apparently low level is nevertheless an achievement as well studied species such as *Magallana gigas* also reach the same level 4. The highest levels are reached when fluxes and balances for energy and elements (C, H, O and N) are characterized at several body sizes and food levels, but these fluxes remain very difficult to measure. Progress on rearing methods have contributed to enhance the knowledge of the parameters necessary for the bioenergetic models for various species. Ultimately, performances at larval stages, mortality rates, and fixation thresholds according to environmental conditions could be used to enhance larval dispersal models, however, specific ecophysiological data such as respiration rates remain rare for bivalve larvae (Gerdes, 1983; Hamburger *et al.*, 1983) and nonexistent for *P. margaritifera* larvae.

In this context, this study assesses the effects of food concentrations on development, growth, ingestion rate, survival and metabolic rate at the larval stage of *P. margaritifera*. The new experimental results ultimately provides specific data useful for the calibration of a DEB encompassing all life-stages of *P. margaritifera.* It also provides valuable information on the links between larval growth, mortality and fixation thresholds that are fundamental in the context of spat recruitment modeling.

2. Material and Methods

Experiments were conducted in hatchery at Ifremer facility (Vairao, Tahiti Island, French Polynesia) from January to early February 2018 during the austral summer. Larvae were reared from hatching to settlement over a range of four trophic levels.

148 *2.1 Production of larvae*

149

150 The spawning was obtained by thermal shock: seventy breeders (age: 6 years; mean
151 height ± SD: 140±10 mm) reared in the Vairao lagoon were progressively exposed from 26 to
152 18°C at 0.5°C per hour followed by a bath at 30°C. Four males and six females were
153 selected as genitors after microscopic observation of gametes morphology and sperm
154 motility. Fertilized eggs were stocked 24h in an aerated-seawater 500 L tank. Fertilization
155 and metamorphosis rates were measured then trochophore larvae were counted and
156 measured using a coulter counter (Beckman Multisizer 3) before being distributed in twelve
157 18 L breeding sieves at a density of 60 larvae.mL^{-1}.

158

159 *2.2 Experimental setup*

160

161 The larval rearing structure was made up of 12 cylinders of 25 L (30 cm diameter, 35
162 cm height). Then, 40 µm mesh screens were fitted to the cylinders bottom to prevent loss of
163 larvae. Each rearing sieve was placed in another larger cylinder (40 cm diameter, 40 cm
164 height), equipped with an output to allow water discharge (Figure 1). The flow-through
165 system was set at a renewal rate of 50%.h^{-1} (220 mL.min^{-1}) of 1 µm filtered and UV light
166 treated seawater. The water temperature was measured every hour with an accuracy of ± 0.1
167 °C using an iBcode 22L temperature sensor.

168

169 *2.2.1 Food conditions*

170

171 The 12 rearing sieves arranged in 4 rows corresponding to 4 trophic conditions
172 reproduced in triplicate (Figure 1) were supplied continuously with a mixture of cultured algae
173 in a 1:1 cells ratio of *Isochrysis lutea* (Ti ≈ 30 µm^3 volume diameter) and *Chaetoceros gracilis*
174 (Cg ≈ 60 µm^3). The algae diets were mixed at 4 different phytoplankton concentrations:
175 112.5, 337.5, 675 and 1350 µm^3.µl^{-1} (≈ 2,5; 7,5; 15; 30 cell.µL^{-1} TiCg equivalent diameter)
176 corresponding hereafter to 4 trophic levels respectively named C1, C2, C3 and C4.
177 According to Fournier *et al.* (2012), chlorophyll-a concentration is an accurate proxy
178 to quantify the available food for pearl oysters. The cultured algae concentrations relative to
179 the trophic levels number C1 and C2 were calibrated from available *in situ* measurements of
180 average fluorescence carried out during the 2017 austral winter (C1) and summer (C2) in the
181 lagoon of Ahe atoll (Thomas *et al.*, 2012, 2010). The trophic conditions C3 and C4 were
182 selected to follow the optimal breeding concentrations reported in the literature. According to
183 Doroudi and Southgate (2000), these concentrations represent the optimum ratio for larval
184 rearing and development ratio for the first 8 days of larval phase.

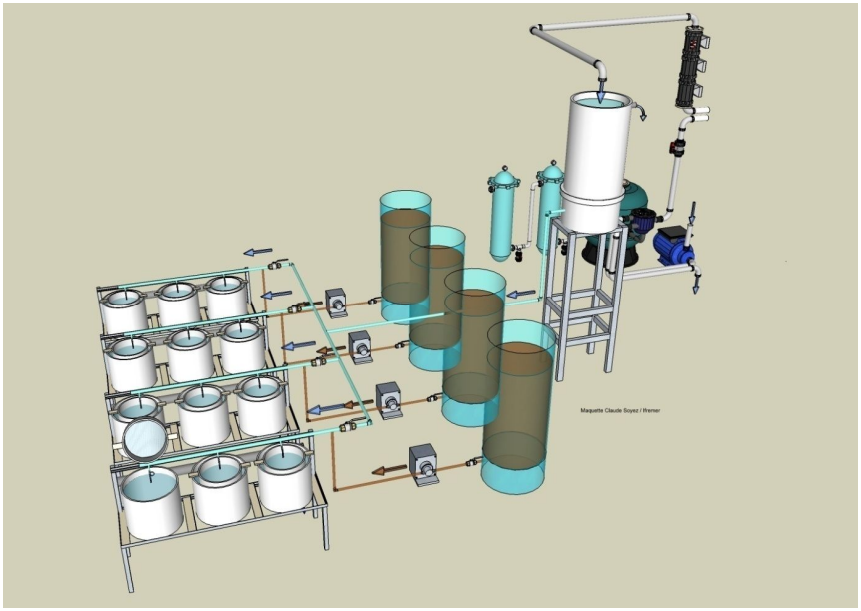

Figure 1 Experimental design used to allow continuous algae supply at the four concentrations, in a flow-through larval rearing system.

2.3 Quantification and analysis of larval development

The physical and physiological performances of the differently fed oysters were measured with similar time step until fixation for each trophic condition. One sample per tank was taken at days: 1; 3; 4; 5; 6; 8; 11; 13; 16; 18; 23; 27; 31 and 35.

Averages growth and mortality were obtained by measuring larval individual size (± 0.1 µm) and larval density. These measurements were carried out using the coulter counter and considering larvae as spherical item with 100 mL samples taken from each of the 12 rearing sieves after homogenization.

The average individual respiration rate was obtained for each trophic level from a sample of 150 000 larvae. Individuals were sifted on a 40 µm mesh in order to eliminate microalgae and then collected in a hermetic DBO Winkler bottle of 300 mL. The concentration of dissolved oxygen was measured with an optode (WTW inoLab® Multi 9310 IDS) and recorded every minute during 70 minutes. The individual hourly consumption was computed from i) the decrease of dissolved oxygen concentration and ii) the count of larvae, achieved with the coulter counter corrected by a coefficient of percentage of living individuals, which was estimated by counting a sub-sample of 300 individuals under a microscope.

The feeding rate per larva was obtained by sampling twice a day the enriched filtered sea water at the inlet and outlet of each larval rearing cylinder (Figure 1). Thus, after controlling that the algae sedimentation rate was negligible in a reference sieve, microalgae concentrations were obtained from 20 mL samples analyzed with the coulter counter. The

difference of numbers of particles between the in- and outlet was calculated and divided by the larval density. Then results were divided by the mean larval size to express feeding rate in $\mu m^3.\mu m^{-1}.h^{-1}$.

The age at settlement was assumed to be when at least 50% of the individuals were fixed on the sieves sides. For each sieve, the percentage of fixation was obtained from the ratio of swimming larvae density over total oyster density.

During oyster's development, the energetic balance calculation followed the scope for growth (SFG) concept (Bayne, 1976), with the exception of the assimilation efficiency coefficient since faecation and pseudofaecation were not measured. Thereby, the algae ingested and respiration were converted into energy values and the difference between both yielded the energetic balance. We used as conversion factors 3.81×10^{-9} J per μm^{-3} of algae mixture (González□Araya *et al.*, 2011; Yukihira *et al.*, 2000) and 14.1 J for 1 mg O_2 (Bayne and Newell, 1983; Gnaiger, 1983). In order to smooth the graphical representation of the results, linear mixed models (see below) fitted on observation data were used to estimate the missing values between observations.

Finally, the cumulated ingested energy at metamorphosis was estimated as the energetic value of the sum of the daily average number of cells ingested per individual until settlement. Despite the fact that algae consumption was recorded daily for each tanks, the density of larvae per tank was not continuously measured. Hence,linear mixed model results were used to fill the missing larval density data.

2.3.1 Statistical analysis

All analyses were performed with the software R v.3.4.1 (R Development Core Team, 2012). Considering our experimental set up; in order to assess the effects of the four different trophic levels on the evolution of size, mortality and length-normalized respiration and feeding rates as repeated measurement over time, mixed effects analyses using the 'lmer' function of R package 'lme4' (Bates *et al.*, 2017) were performed. Relationships were tested using random intercept and slope linear mixed models. The fixed effects feed ration was considered as a factor and time was a continuous numeric variable. The variable tank was set as a random factor. The 'anova' function (default) in the 'car' package (Fox *et al.*, 2012) was used to compute the significance tests and provide *p values*. In addition, a one-way ANOVA was performed to investigate the effect of food concentration on the age at settlement.

Before the mixed effects analyses, data were Box-Cox transformed to improve normality. Model predictions were back transformed to plot the results using the R packages 'MASS', 'car' and 'ggplot2' (Fox *et al.*, 2012; Ripley *et al.*, 2013; Wickham, 2010).

After mixed effects analyses, pairwise comparison by post-hoc Tukey analysis were performed thanks to the 'multcomp' and 'lsmeans' packages (Hothorn *et al.*, 2017; Lenth, 2016) to determine how trophic levels differed from each other.

The outputs were printed thanks to the 'tab_model' function of the 'sjPlot' package (Lüdecke, 2015). In any case, the normality of the residuals was checked (Shapiro–Wilk test) and the homoscedasticity of the variance of errors was visually assessed.

Note that to focus the results on the larval stage, all mixed effects analyses were strictly applied on data recorded before settlement. Finally to avoid time vector length issues related to dissimilar ages at settlement, break points over time were visually assessed.

3. Results

3.1 Temperature profile

During the experiment, the recorded mean temperature was 28.1°C with daily fluctuations of up to 0.5 °C within 24h. A general decrease of daily mean temperature occurred from 28.9 to 27.6 °C between the first and the last day of rearing (see Supplementary Figure 1). A maximum of 29.6 °C and a minimum of 26.7 °C were recorded. They appeared the fifth and 25th day of the experiment respectively. No difference in temperature was measured between the different rearing tanks.

3.2 Effects of food concentration

Food condition, time and their interaction had significant effects on larval development variables (*p-values* < 0.05) for each statistical test performed, with the exception of the mortality rate for which only time was significant (Table 1).

Table 1 Summary of random intercept and slope linear mixed models fitted on larval performance measurements recorded from birth until settlement. *p-values* numbers marked in bold indicate numbers that are significant on the 90% confidence limit.

	Growth			Respiration rate			Feeding rate			Mortality rate		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.99109	0.99076 – 0.99143	**<0.001**	-7.76069	-7.95216 – -7.56921	**<0.001**	0.92428	-0.09900 – 1.94755	0.105	-6.54297	-30.17951 – 17.09357	0.592
Time	0.00030	0.00028 – 0.00033	**<0.001**	0.10847	0.09867 – 0.11828	**<0.001**	0.14802	0.11158 – 0.18447	**<0.001**	9.26121	8.12855 – 10.39387	**<0.001**
Food condition 2	-0.00005	-0.00055 – 0.00046	0.862	0.43380	0.13985 – 0.72776	**0.005**	1.94855	0.48791 – 3.40919	**0.024**	19.16938	-14.25773 – 52.59649	0.272
Food condition 3	-0.00008	-0.00062 – 0.00045	0.759	0.37742	0.06804 – 0.68679	**0.020**	2.50133	1.02023 – 3.98242	**0.006**	36.30233	2.87521 – 69.72944	**0.044**
Food condition 4	-0.00108	-0.00161 – -0.00055	**<0.001**	0.23626	-0.07311 – 0.54564	0.139	1.12883	-0.35227 – 2.60992	0.161	49.13853	15.71141 – 82.56564	**0.008**
Time:Food.condition2	0.00009	0.00005 – 0.00013	**<0.001**	0.02688	0.00911 – 0.04464	**0.004**	0.01557	-0.04297 – 0.07410	0.603	-0.58612	-2.18795 – 1.01571	0.474
Time:Food.condition3	0.00016	0.00011 – 0.00021	**<0.001**	0.08092	0.05967 – 0.10216	**<0.001**	0.18553	0.11554 – 0.25551	**<0.001**	0.10257	-1.49925 – 1.70440	0.900
Time:Food.condition4	0.00024	0.00019 – 0.00029	**<0.001**	0.10102	0.07977 – 0.12227	**<0.001**	0.27397	0.20398 – 0.34395	**<0.001**	-1.10078	-2.70260 – 0.50105	0.180
Random Effects												
σ^2	0.00			0.05			0.78			999.23		
τ_{00}	0.00 $_{Tank}$			0.00 $_{Tank}$			0.63 $_{Tank}$			182.56 $_{Tank}$		
ICC	0.00 $_{Tank}$			0.00 $_{Tank}$			0.44 $_{Tank}$			0.15 $_{Tank}$		
Observations	144			72			129			180		
Marginal R^2 / Conditional R^2	NA			NA			0.765 / 0.869			0.824 / 0.851		

The post-hoc Tukey analysis exhibited different levels of significance depending on the tested physiological performance and the pair of tested trophic levels (Table 2).

Table 2 Simultaneous tests for general linear hypotheses with Tukey contrasts multiple comparisons of means, fitted on linear mixed models of food concentration and time effect on larval size, respiration, feeding and mortality and the ANOVA relating the food concentration effect on the age at settlement. *p-values* numbers marked in bold indicate numbers that are significant on the 90% confidence limit.

Physiological performance	Compared conditions	C1 - C2	C1 - C3	C1 - C4	C2 - C3	C2 - C4	C3 - C4
Growth	**Estimate**	-0.0007438	-0.0013655	-0.0010864	-0.0006217	-0.0003427	0.0002790
	Pr(>|t|)	**0.00594**	**< 0.001**	**<0.001**	**0.02419**	0.26089	0.44649
Respiration rate	**Estimate**	-0.80558	-1.49674	-1.63372	-0.69117	-0.82814	-0.13698
	Pr(>|t|)	**< 0.001**	**<0.001**	**< 0.001**	**<0.001**	**<0.001**	0.441
Feeding rate	**Estimate**	-2.0952	-4.2487	-3.7092	-2.1536	-1.6141	0.5395
	Pr(>|t|)	**0.06244**	**0.00107**	**0.00287**	**0.05667**	0.16898	0.86254
Mortality	**Estimate**	-11.16	-34.09	-37.70	-26.55	-22.93	3.61
	Pr(>|t|)	0.822	0.110	**0.074**	0.244	0.348	0.992
Age at settlement	**Estimate**	-0.3813	-0.6089	-0.7167	-0.2276	-0.3355	-0.1078
	Pr(>|t|)	0.13909	**0.01779**	**0.00721**	0.49144	0.20906	0.89429

3.2.1 Survival and settlement

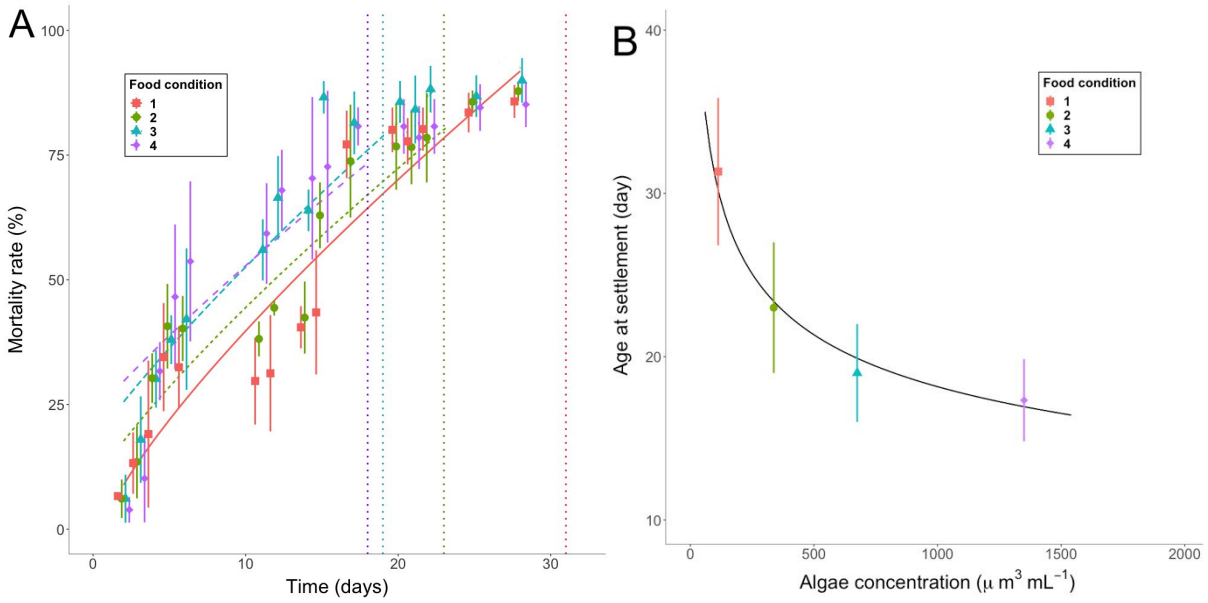

Figure 2 (A) Evolution of the percentage of dead oysters across time under different feeding conditions. (B) Mean age at settlement according to food concentration. The continuous curves are predictions from the models that were fitted to the observations (symbols), with the standard errors (vertical lines). The vertical dotted lines mark the age at settlement for each trophic level.

Regardless of the time dynamic, larval survival was low on day 22, with mortality ≈ 78% corresponding to a drop from 60 to 10 ind.mL^{-1} (Figure 2A) and no significant difference between conditions (ANOVA, p = 0.201). During the rearing, different dynamics occurred between trophic levels. On day 12, larval mortality ranged from 28% to 43% at the lowest food concentrations (C1 and C2) and from 64% to 68% for the highest food concentration C3 and C4, with significant differences between contrasted food conditions C1 and C4 (Table 2).

The age at settlement increased exponentially with the diminution of the food concentration. From the lowest to the highest trophic level, the age at settlement was 31, 23, 19 and 18 respectively (Figure 2B), with significant differences only between the lowest and the two highest food conditions (Table 2).

3.2.2 Growth

Significant differences in growth rate between food levels were recorded mainly from day-12. Differences were significant between diets, excepted for the comparison of the conditions C2 versus C4 and C3 versus C4 (Table 2). In addition to growth speed variations related to the food concentration, the size at settlement varied with the diet, reaching an average size of 210, 200, 190, and 180 µm at the trophic levels C4, C3, C2 and C1, respectively. After settlement, growth increased sharply. For instance, for the highest food

level C4, the size went from 67 to 210 µm during the first 18 days and from 210 to 950 µm between the following 9 days (Figure 3). Despite the apparent reciprocity between growth and food concentration, it appeared that larvae grew faster within the first 8 days at C3 (675 $\mu m^3.\mu l^{-1}$) than C4 (1350 $\mu m^3.\mu l^{-1}$). This trend changed when larvae reached a mean size of 100 µm after which the fastest growth was recorded for the highest trophic level.

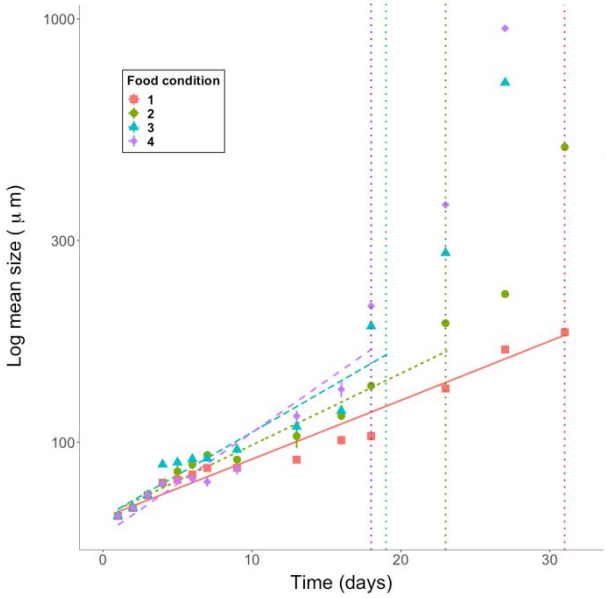

Figure 3 Oyster growth (log scale) over time for the different food conditions. The continuous curves show predictions from the linear mixed models that were fitted to the observations (symbols) with the standard error (vertical lines), for each food condition. Vertical dotted lines represent the age at settlement. The values after settlement are not taken into account in the models.

3.2.3 Feeding rate

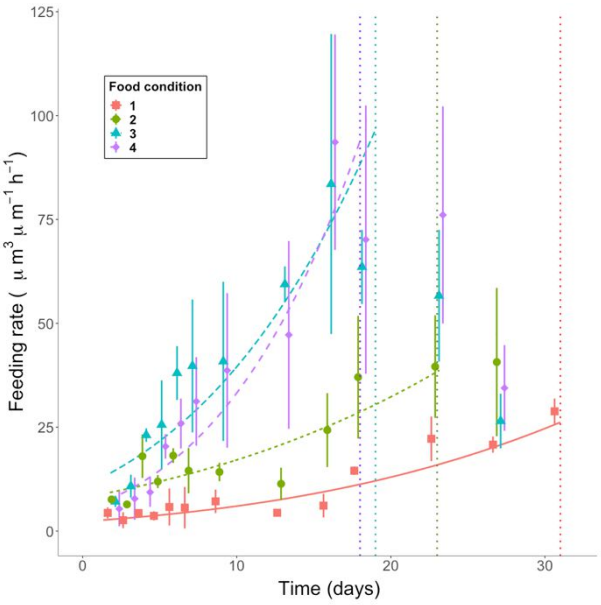

Figure 4 Oyster algae consumption per hour as a function of age for different food conditions. The feeding rate is scaled by unit of length. The continuous curves show predictions from the linear mixed models that were fitted to the observations (symbols) with the standard error (vertical lines). Vertical dotted lines represent the age at settlement for each trophic level. The values after settlement are not taken into account in the models.

Feeding rate was highly dependent on food concentration and could be described visually by three regimes. For high food concentration, standard deviations were important and statistically no significant differences were reported between the trophic levels C3 versus C4 and C2 versus C4 (Table 2). At low food concentration, the increased consumption was closely related to phytoplankton density. At high food concentration, feeding rate was higher at 675 $\mu m^3.\mu l^{-1}$ (C3) than 1350 $\mu m^3.\mu l^{-1}$ (C4) within the first 8 days of the rearing then this trend switched and the feeding rate increased faster at high food concentration. A microalgae uptake decrease corresponding to settlement was thereafter observed when the feeding rate was length-normalized (Figure 4) but this trend did not occur if the feeding was expressed as hourly uptake per individual (not shown). Moreover, independently of the size, a slight decrease in the feeding rate also appeared during the transition in the umbo and eyed stages, respectively close to days 7/8 and 17/19 for conditions C4 and C3. At low food level (conditions C1 and C2) this trend is less obvious for the umbo stage but clearly appeared few days before the metamorphosis of eyed larvae. Finally, a generalized drop of food intake clearly appeared on day 15 which matched the main peak of mortality.

3.2.4 Metabolic rate

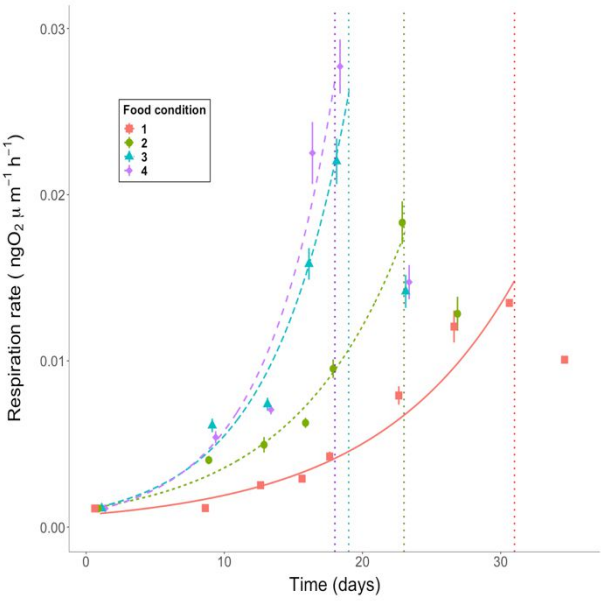

Figure 5 Oyster respiration rate according to their age under different feeding conditions. The respiration rate is scaled by unit of length. The continuous curves show predictions from the linear mixed models that were fitted to the observations (symbols) with the standard error (vertical lines). Vertical dotted lines represent the age at settlement for each trophic level. The values after settlement are not taken into account in the models.

Over time, the length-normalized respiration varied significantly between trophic levels except for the food concentrations C3 and C4 (Figure 5). There was an exponential increase in respiration rates with time with a marked decrease in metabolic rate after fixation of the eyed larvae. This decrease in breathing was apparent when the respiration was not length normalized and coincided with the cessation of the active search for food. Indeed, this is the moment when individuals stopped swimming and attached themselves to a substrate from which they started filtering the surrounding water.

During the first 3 days, the balance between energy consumed and ingested was close to 0 ($\approx 4\times 10^{-6}$ J.day^{-1}) (Figure 6A). Then, it slowly increased at food concentrations C3 and C4 while it remained in a 2×10^{-5} to 1.3×10^{-4} J.day^{-1} range during the entire larval phase at low food concentrations (C1 and C2). Similar to the feeding rate dynamic, drops in energy balance occurred at time of the switches to the umbo and eyed stages as well as during major mortality events. This resulted in possible peak or plateau of negative energetic balance. The extent of these periods depended on the time required by the whole batch to fully move toward the next larval development stage.

The cumulated ingested energy at settlement increased with food concentration but a saturation threshold close to 0.0086 J.ind^{-1} is observed at C3 concentration (675 µm^3 µl^{-1} ≈ 15 cell µL^{-1} TiCg equivalent diameter) (Figure 6B).

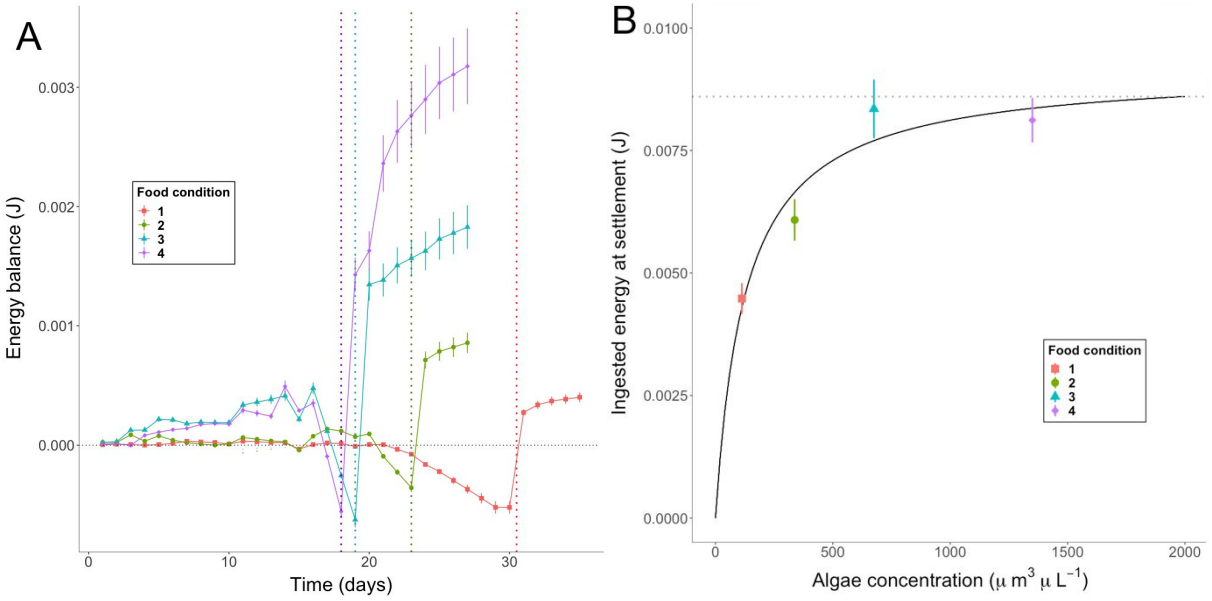

Figure 6. (A) Energy balance between food intake and respiration over time, vertical dotted lines represent the age at settlement for each trophic level. (B) Cumulated energy consumed at metamorphosis against food concentration. The continuous curve shows a Holling type II functional response fitted against the observations. Vertical bars are standard errors (A & B).

4. Discussion

The present study aimed to assess, in view of DEB modeling, the effect of food concentration on *Pinctada margaritifera* larval performances and development efficiency. This study also incidentally identified metabolic traits that can be explained by behavioral and physiological changes at key moment of larval development. These new observations are fundamental in view of recruitment modelling.

4.1. Effects of food density on larval development

A close relationship between performances and feeding regime was demonstrated during larval development within the range of tested food densities (Table 1). During the larval phase, the amount of food consumed is an essential factor in successful settlement as larvae must accumulate sufficient reserves to meet the energy demands required during metamorphosis (Strugnell and Southgate, 2003). The dynamic of the cumulated energy ingested at settlement was in accordance with previous studies that reported differences in

post settlement size and biochemical composition of bivalves raised at different trophic levels (Holland and Spencer, 1973; Pechenik, 1990). These physical dissimilarities were closely related to the different ages at settlement that ranged in our case from 18 to 31 days. Our observations substantially differed from those observed by Doroudi and Southgate, (2003a) for the maximum age at settlement who reported settlement from 16 to 21 days in laboratory at 28.1 °C, but they agree with the 29 days reported by Thomas *et al.*, (2011) during *in situ* larval survey, which corresponds to our low food level conditions.

Here, larval growth was directly correlated with food density up to 20 cells μL^{-1} and the optimal algal ration remained within a range of 4.5–11.5 and 15–32 cells μL^{-1} for 7- and 20-day-old larvae respectively. These results are in agreement with Doroudi *et al.*, (1999b) and Doroudi and Southgate, (2000). Similarly, the recorded feeding rates per individual were in the range of those reported by Doroudi *et al.*, (2003b): 8.7 to 165 cells h^{-1} $larvae^{-1}$ respectively for larvae with shell length of 89 and 188 µm. Here feeding rates were highly dependent of the shell length and varied from 11.4 to 279.1 cells h^{-1} $larvae^{-1}$ from birth to settlement for well-fed larvae.

The respiration rates measured here (Figure 5) add to the rather shortlist available data reported for bivalve larvae. Our range of respiration rates was consistent with the rates measured for well fed *Crassostrea gigas* larvae (Gerdes, 1983) which rank from 0.4 to 6.1 nlO_2 h^{-1} $larva^{-1}$. Here, during larval development, *P. margaritifera* larvae respiration rate increased from 0.1 to 4.5 ngO_2 h^{-1} $larva^{-1}$. The DEB theory (Kooijman, 2010) itself does not use respiration rate as a primary variable, however, this information can be used to test in fine the model parameters accuracy.

The energetic balance calculation, with the exception of the assimilation efficiency coefficient, was based on the scope for growth (SFG) concept (Bayne, 1976) and the respiration-energy equivalent is directly subtracted from the energy derived from assimilated food. The energy left remains available for growth. Such calculations remain inexistent for bivalve larvae in the literature so far, respiration rates were measured over short periods and the interpolation of missing value may not be suitable for such calculation so these data have to be used meagerly. Moreover, discrepancies can arise because subtracting from the ingested food energy the energy-equivalent from respiration is only an approximation as respiration rates should compensate for both metabolic and growth costs (Kooijman, 2010).

The survival rates obtained here partially also agreed with the early studies (Doroudi *et al.*, 1999b; Doroudi and Southgate, 2000). We observed high mortality rates up to 7.5 cells μL^{-1} (≈ 337.5 μm^3 μL^{-1}) at day 12 but no significant differences occurred between the four tested trophic levels within 22 days of rearing. Moreover, mortality showed important standard deviation corresponding to large differences of mortality episode within the replicates of one trophic level. As suggested by Asmani *et al.* (2017), mortalities may be due

to increased microbial activity or reduced water quality associated with unconsumed food decomposition and larvae excretion. The synchronicity between mortality and feeding decreases suggests that mortality episodes may relies on exogenous factors such as microbial infection accompanied by a non-feeding behavior

4.2 Influence of behavioral and ecological breaks

As reported in the literature, development and metamorphosis were closely related to the trophic levels, nonetheless this study suggests several behavioral and physiological particularities, or breaks, that occurred sooner or later depending on food concentrations. This is apparent here with the decrease of food consumption before settlement, confirming that metamorphosis is a metabolic singularity within larval development. As for embryonic development, metamorphosis strictly rely on reserve storage, for which environmental food concentration and reserve accumulation are required for an optimized larval period duration (Doroudi and Southgate, 2003). It appeared that reserve accumulation was directly linked to food concentration, and as a consequence, the eyed stage could occur at different ages and sizes depending on food availability. Therefore, well fed larvae will settle earlier and at a bigger size than underfed larvae, and with differences of accumulated total energy ingested until settlement. This energetic pattern agreed with the assumptions described by the DEB theory (Kooijman, 2010) and the influence of available energy on delayed metamorphosis (Doroudi and Southgate, 2003; Holland and Spencer, 1973; Pechenik, 1990) It can be stated that larval development for a given temperature relies primarily in the available energetic flux, and thus food quality/quantity, rather than genetic or other exogenous factors. Note that temperature may delay metamorphosis but this is also dependent on the energetic flow since no difference in size and cumulated ingested energy at settlement occurred for bivalve larvae reared at different temperatures but with similar food density (Doroudi *et al.*, 1999a; Rico-Villa *et al.*, 2009).

Settlement marks the end of the planktonic phase and comes with profound physiological changes. The post settlement metabolic rate drop can be related to the behavioral and morphological changes undergone by the pediveliger larvae. Indeed, when the competences for metamorphosis are reached, pediveliger larvae enhance their crawling behavior using their foot to find a suitable substrate to settle on, hence reducing their filtration activity (Cole, 1937). Movement and feeding are also inhibited because the velum is absorbed and replaced by the gills (Cole, 1938) and further developments need to rely on previously accumulated endogenous reserves (Holland and Spencer, 1973). Once the larvae are fixed, these drastic anatomic changes may cause the decrease in respiration rate observed at the end of the larval life. In other words, whilst the growth accelerates, the

respiration rate decreases at the same time. In fact, by stopping swimming, the part of the energy allowed to growth can increase. This suggests that settlement allow oysters reducing their global metabolic activity.

Breaks in metabolic rates are not visible only at the end of the larval stage. During the first 2 days, while shell length was increasing, feeding rates were relatively low and independent from the food concentration. This observation suggests that the development of newly released *P. margaritifera* larvae relies not only on exogenous source of food but also on maternal reserves and so on initial gamete quality. This is in agreement with Ehteshami *et al.*, (2011) who showed that diet during broodstock conditioning was influencing greatly gonad composition, reproductive output and embryonic development. This mixotrophic phase could therefore explain the low ingestion activity observed in the first days.

From day 2 to day 8, feeding was correlated with microalgae concentration except at the 675 (C3) and 1350 (C4) μm^3 μL^{-1}concentrations, where growth and respiration rate of larvae in C3 condition showed higher performance than in C4. This result agreed with previous studies (Doroudi *et al.*, 1999a; Doroudi and Southgate, 2000) and could be explained by a temporary increase of microbial activity due to the decomposition of unconsumed algae. This may lower the larvae performances. This hypothesis is confirmed by the fact that at the same period mortality increased with the rise of phytoplankton concentration. Nevertheless, after 8 days of rearing, when larvae reached a length up to 100 µm, growth and feeding rates correlated with food density again.

Finally, after settlement, the decrease of the length-normalized feeding rate may just come from the increasing growth rather than behavioral or morphological changes, since the feeding rate per individual increases continuously. This behavior may also be reinforced by some physiological adaptation that allows oysters to support easily low food conditions that they cannot offset if they actively research food in the water column.

We suggest, as future perspectives, that all these behavioral patterns should be investigated in details by conducting similar experiences focusing on the oyster response to environmental possible constraint by measuring metabolic changes at settlement and minimal reserve dynamic. In addition, weight measurements and vibrio activity control should be added to assess mortality causes and metamorphosis reserve requirements.

4.3 Implications for spat collection

The behavioral and physiological patterns observed here provide new clues to interpret spat collecting success in tropical atoll lagoons, like it is commonly practiced in French Polynesia by the pearl farming industry. Commonly described as environmentally homogenous, the semi enclosed atoll lagoons in fact present relatively low temperature

variations (3 °C during the year), but not negligible food concentration variations in space and time (Thomas *et al.*, 2016, 2010). Food variability is the main driving factor for larval development success. First it affects broodstock gonad composition and reproductive output that sets egg size and quality (Ehteshami *et al.*, 2011); then it determines the success of the mixotropphic phase, the period of larval development (Rico-Villa *et al.*, 2009) and the size at settlement. Low food condition is not lethal for larvae but highly impact growth speed, which delays settlement and ultimately increases *in situ* mortality rate by predation (Marshall *et al.*, 2009). Furthermore, after settlement the individual size might represent a competitive advantage in term of accessibility to the trophic resource. Spat survival may also depends on its size at settlement and its ability to redirect the energy allowed to the swim for the benefit of the growth speed. Thus, spat collecting effectiveness depends on a suite of processes that can be easily affected by a disruption of food supply as our experiments suggest. It also indirectly confirms pearl farmers empirical observations who have reported better spat collecting during the austral summer, a period generally with more favorable environmental conditions with suitable food concentrations.

4.4 Implications for DEB modeling

In this study, the two trophic levels C1 and C2 were calibrated to match extremes *in situ* food conditions reported from the field (in atoll lagoons). The results matched field data recorded by Thomas *et al.*, (2011) with a larval size of 180 µm reached in 20 days at 29.4 °C against 190 µm in 23 days at 28.1 (C2) in this laboratory experiment. Despite expected differences in term of food quality between field and laboratory, chlorophyll-a is confirmed as a good proxy to model the growth of pearl oyster larvae. Furthermore, this contribution creates a tangible basis for a future *P. margaritifera* DEB model able to represent the full life cycle of the black lipped pearl oyster, with better parameterization of the larval stages. The DEB will be much more robust after detailing, like here, physiological processes directly relevant for Dynamic Energy Budget model parameterization (e.g., through the maximum surface-area-specific ingestion rate or the half saturation coefficient) and by providing data sets to accurately estimate values for the parameters controlling processes such as growth and respiration rate.

5. Conclusion

This work highlighted the effect of food concentration on the processes related to pearl oyster population recruitment success. Supported by previous laboratory observations, it demonstrates how energy intake through feeding as well as behavioral and physiological

551 transitions allow optimizing the larval development duration and minimizing "the risky phase"
552 of their life cycle. This study provided numerous new clues to establish a quasi-deterministic
553 relationship between temperature and food condition in one hand and larval development
554 and recruitment success in the other hand. By taking into account the metabolic switches
555 that we could characterize, this study provides a strong foundation for DEB modeling
556 development and for a better description of the complex interactions between *P.*
557 *margaritifera* physiology and environmental conditions.
558
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568

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Supplementary material

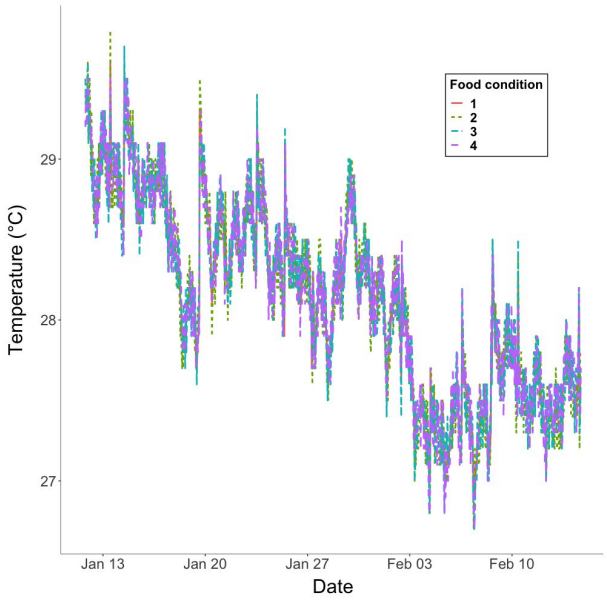

Figure 1:Temperature profiles across time for each feeding condition