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## Evaluation of floating cages as an experimental tool for marine shrimp culture studies under practical earthen pond conditions

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### Abstract:

The New Caledonia blue shrimp *Litopenaeus stylirostris* is commercially produced under semi-intensive rearing conditions. The size of the farming earthen ponds (5–10 ha) and the subsequent production constraints make it difficult to use them as experimental units for random experiments. Moreover, since every pond has its own characteristics, ponds' effects cannot be ruled out, thus making it hard to define true replicates.

In order to design future experiments under conditions resembling those used in production, we evaluated the possible use of floating cages as experimental units with the aim of assessing treatment effects with a reasonable statistical power. To this end, two sets of floating cages were placed respectively in two different ponds in a commercial farm. In both cases the zootechnical conditions in the cages were similar in post-larvae origin, management, and diet and feeding regime.

The aim of the study was to evaluate on a technical basis the possibility of rearing shrimps in floating cages set up in earthen ponds and to assess this method from a statistical standpoint. Shrimps reared in and outside the cages showed comparable growth and survival rates. The variability in the zootechnical parameters between cages, expressed as the estimated coefficient of variation (CV) for survival rate, total final biomass (g), final body weight (g), daily increment in body weight (g day<sup>-1</sup>) and FCR were 11.0, 13.7, 4.4, 5.4, and 7.0%, respectively. On the basis of these figures, we calculated for a given statistical power (80%) the number of replicates (cages) that would be required to reveal significant differences between two treatments, at a 5% level of significance. We found that for expected differences of 20% from the control mean, 3 and 6 floating cages per treatment would be reasonable to determine statistical differences for growth parameters and survival rate, respectively. Moreover, we showed a significant pond effect in regard to survival and growth between the two sets of cages. These results illustrated the within-farm variability among the ponds, and confirmed that the specific characteristics of each pond from the same farm make it difficult to use the ponds themselves as experimental units.

The study demonstrates that rearing in floating cages is an economical, powerful and sensitive experimental tool for shrimp culture studies specifically carried out under conditions close to semi-intensive production.

**Keywords:** *Litopenaeus stylirostris*; Floating cages; Pond experimentation; Replicates; Statistical power

## 1. Introduction

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In New Caledonia, the shrimp *L.stylostris* is reared in semi-intensive systems. Such systems are widely used in shrimp production countries worldwide (Chim *et al.*, 2003), and contribute significantly to the total farmed shrimp production estimated at nearly three million metric tonnes in 2004 (FAO, 2006). In a semi-intensive rearing system, earthen ponds from 1 to 20 ha are commonly used. Irrespective of their size, ponds also differ greatly in terms of their depth, hydrodynamics and the nature of their sediment. This rearing system (Clifford, 1994) is midway between an intensive agro-system, where the main parameters are under control (Shigueno, 1975; Burford *et al.*, 2003; Browdy and Moss, 2005), and an extensive system, comparable to an ecosystem where the human impact is relatively slight (Burford *et al.*, 2004). More generally, semi-intensive aquaculture systems reveal similar patterns to polycultures where natural productivity, phytoplankton, zooplankton and benthos must be taken care of as well as the cultured species. The main practical consequence is that two sources of food are therefore available for the cultured species: the artificial feed provided and the natural biomass endogenously produced by the pond (Rubright *et al.*, 1981; Tacon, 1996; Nunes *et al.*, 1997).

Scientific and technical studies carried out under semi-intensive farming conditions to assess the effects of treatments on zootechnical results are highly complex due to the many logistical, economic and/or experimental constraints involved. The most obvious constraints may be summarized as follows: (i) delivery of a high number of post-larvae (PL) coming from the same batch, which have to be seeded at the same time in all the ponds subject to experimentation, (ii) calculation of the final survival rate affected by possible under- or overestimation, particularly regarding the number of seeded PL and subsequently harvested animals, (iii) the supply of experimental feeds at an industrial scale and (iv) the high within-pond variability of zootechnical responses, deriving *inter alia* from differences in pond characteristics, the origin and quality of post-larvae, and rearing management. Consequently, when ponds are used as experimental units, setting up experiments of high power and sensitivity may lead to a large increase in replicates of each treatment (ponds in this case), which can often be difficult to found, especially for medium and low level treatment effects (Cohen, 1988). Moreover, the cost per replicate pond in such cases is high, both in terms of the capital required to purchase and house the animals and of the labour needed to rear them and to collect representative data.

Paquotte *et al.* (1998) have studied an original shrimp farming technology in small floating cages (15m<sup>2</sup>) over three successive years. This technology has been shown to be very efficient from a zootechnical standpoint. Parameters such as growth rate, survival rate and food conversion ratio (FCR) reached the same levels as the best results obtained with intensive farming in ponds. More recently, other studies have confirmed the efficiency of this original rearing system (Lombardi *et al.*, 2006; Yusufzai et Singh, 2005; Zarain-Herzberg *et al.*, 2006).

In our study we evaluated the use of floating cages immersed in earthen ponds as an experimental tool in order to design future powerful and economical experiments focused on two main objectives: (i) to assess the zootechnical feasibility of rearing shrimp in floating cages set-up in earthen ponds, (ii) to estimate inter-cage variability for various zootechnical parameters (survival, growth, FCR, etc.) and subsequently to estimate the number of replicates that would be needed to measure treatment effects within a given statistical power.

## 2. Material and Methods

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### 2.1. Farm site and study period

The study was undertaken in two earthen ponds of about 7 ha each (pond A and pond B) in a semi-intensive farm in Bourake, New Caledonia (21°55' South; 165°57' East). The depth of the ponds was between 80 cm (water inlet) and 150 cm (water outlet). The experiment was conducted over 10 weeks from April to June 2006.

## 2.2. Floating cages

Ten floating cages of 14 m<sup>2</sup> net surface each were built by IFREMER New Caledonia (Picture. 1). The cages' rectangular frames (4 m x 2 m) consisted of four polyvinyl chloride pipes (PVC; 110 mm diameter) connected by PVC elbows and waterproofed with silicone adhesive.

The plastic net (Netlon®) of 1cm mesh size was 4.5 m long, 2.5 m wide and 0.5 m high. The non-submerged part of the net was wound round the pipes with woven metal cables. Fifty centimetres of the net remained immersed in the water. The cages were covered with nylon net to prevent the shrimp from jumping out of the cages. Each cage was anchored to the substrate at its four corners using four vertical metal bars attached by rope. The fabrication cost per cage was estimated at 300 euros. A wooden platform was built in each pond to access the cages.

## 2.3. Experimental design

An experiment design with 5 cages was duplicated in two part of the two earthen ponds (pond A and pond B), where the depth was of 100 cm, giving a total of 10 floating cages arranged in two sets of 5 floating cages tied together. The distance between two cages of the same set was about 50 cm. In the text, both sets were referred as Cages-A and Cages-B.

Shrimp were sampled weekly in one cage in pond A and pond B alternately. Each cage was sampled only once in the course of the trial. The animals were captured with a cast net spread along the surface of the cage. Each sample was set up with 30 shrimps. It is agreed that sampling less than 10% of the initial population does not affect the survival rate (Bouyer, 1997).

## 2.4. Shrimp breeding

### 2.4.1. In ponds

The animals used in this experiment were first pre-grown in the two ponds. On February 21, ponds A and B of the farm were stocked at a density of respectively 17 and 18 post-larvae (PL)-m<sup>-2</sup> with animals originating from the same hatchery batch. The animals were reared according to standard semi-intensive farming practices in New Caledonia (Clifford, 1994) until they reached the desired size ( $\pm 3g$ ).

Shrimps were fed twice a day with a commercial formulated feed. The feeding rate was adjusted by the farm's technical staff according to the weekly estimated body weight, the survival rate and the amount of remaining feed in the feeding trays.

### 2.4.2. In floating cages

Cages-A and cages-B were seeded with shrimp coming from each respective pond. Shrimps were captured using a cast net and then transferred into the cages. During the transfer the animals were counted to adjust precisely the number of stocked animals. Finally each cage was filled up with 400 animals for a final approximate density of 29 animals-m<sup>-2</sup> of net area. Shrimp initial body weights were respectively  $3.4\pm 0.6$  g and  $2.7\pm 0.7$  g in cages-A and in cages-B. The trial was conducted over 10 weeks.

One feeding tray was set up in each cage. Shrimps were fed the same commercial pellets used by the farm (purchased from the company Moulins de Saint Vincent, New Caledonia). Feed was delivered exclusively on the feeding tray in order to be able to estimate consumption. Visual estimates of feed consumption were made two hours after each meal and by the same person to avoid any skew. The animals were fed twice a day, at 8.00 am and 3.00 pm, and the feeding rate was adjusted every day for each cage according to the feed remaining.

## 2.5. Zoo-technical parameters

The final survival rates were calculated for each cage by counting the number of remaining shrimps and comparing it with the initial stock, excluding the 30 sampled animals per cage.

Individual body weights were recorded for 10% of the population at stocking (estimated mean initial weight) and for all the remaining shrimps at the end of the experiment. Apparent individual growth rate ((individual final body weight – estimated mean initial weight)/rearing days), final biomass and feed

conversion ratio (FCR = amount of given feed/shrimp wet biomass gain) were then calculated for each cage. In addition, the animals were weighed weekly in order to track their growth (mean weight and growth rate).

## 2.6. Environmental parameters

The water temperature was recorded continuously with two optic stowAway Temp® sensors immersed in cages-A and cages-B. Oxygen measurements were also carried out twice a day (7.00 am and 4.00 pm), for both sets of cages, with an oxymeter (WTW OXI315i).

## 2.7. Statistical analysis

The data were statistically analysed by the statistical package Stat View (SAS Inc., Cary, NC, USA). After checking normality and homogeneity of variance (F-test) for all the zootechnical parameters, comparisons of final results between cages-A and cages-B were made using t-test for independent comparison of means. Survival percentages were also assessed by a t-test after arcsine transformation (Sokal & Rohlf, 1995).

A priori power, post hoc power and sensitivity calculations were carried out using the free internet program G\*Power 3 (Faul *et al.*, 2007). Estimate of the standardized effect size was made in accordance with Cohen (1988), the level of significance was fixed at 5% and the desired power at 80% as commonly stated (Hayes, 1987; Cohen, 1988).

*A priori analysis* - For a priori analysis we considered two treatments in a completely randomized design with two-tailed t-test of significance. A priori analysis was carried out to estimate the number of replicates that would be required, based on CV estimates, to show significant differences with an error type I probability ( $\alpha$ ) of 5% and a power of 80%, in future floating cages experiments with a view to comparing two treatments.

*A posteriori analysis and sensitivity analysis* - Post hoc power analyses were implemented to assess a posteriori the power of the study for a completely randomized design with two treatments using a one-tailed t-test of significance. In this case the pond effect was considered as a treatment effect (fixed effect). It is important to specify that the ponds used under semi-extensive rearing conditions cannot be considered as similar entities especially in regard to hydraulic statements: (i) they have different shapes and areas (in the present case 6.9 et 7 Ha respectively for pond A and B) (ii) different orientations to the wind and (iii) different water inlet and outlet positions. The outcome variability generally obtained among the ponds on the same farm confirmed these considerations, which corroborate the assumption of pond effect as a treatment effect.

Sensitivity analysis was also conducted to determine the size of the effect that the experiment could detect given the type I error probability ( $\alpha$ ), type II error probability ( $\beta$ ), and the number of replicates for all the parameters considered. The size of detectable effect was recorded as the minimum detectable difference (%) between the two ponds.

## 3. Results

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### 3.1. Temperature and oxygen

Temperature over the rearing period was similar in cages-A and in cages-B (fig 1 (a) and (b)). The temperature, around 30°C during the first week, started to decrease from the third week to reach values around 22°C at week 7. Thereafter, water temperature remained stable until the end of the experiment.

Oxygen concentrations also changed similarly for both sets of cages (fig 1 (c) and (d)). From week 8 to the end, the variation in oxygen concentration between morning and afternoon decreased. The overall values measured never went below 2 ppm.

### 3.2. Zootechnical results.

#### 3.2.1. Ponds (Farm owner, pers. com.).

The average zootechnical results for shrimps reared in the ponds were of the same order as the farm's previous crops. According to the farmer's records, the estimated survival rates were 30.7% and 31.3%, respectively in ponds A and B. The survival rate at this particular farm since 1997 fluctuated between 22% and 38% depending on the year (Lemonnier *et al.*, 2006), which is lower than the average survival rate observed in other New Caledonian farms ( $54.7 \pm 4.4\%$ ;  $n = 39$ ; year 2004-05).

The growth of the animals was in accordance with the general growth rate recorded at this farm during the same season (fig 2 (a) and (b)). FCR were of 2.67 and 2.57 for pond A and pond B respectively.

#### 3.2.2. Floating cages

The growth of shrimps reared in cages-A and cages-B was comparable to those raised in the respective ponds (fig 2a and 2b). However, from week 10 of rearing, shrimp growth in cages A slowed in comparison with pond-reared animals.

The daily amounts of delivered feed per cage followed the same profile in cages-A and cages-B: (i) during the first four weeks animals were fed with 90 – 110 g of pellets per day and (ii) from week 5 these quantities decreased to 60-70 g per day. Finally, Figure 3 shows that rations in cages-A from week 5 to the end were slightly lower to those of cages-B.

Table 1 gives zootechnical results and estimations of variability for the zootechnical parameters between cages (experimental unit), for both sets (cages-A and cages-B). For each parameter recorded, no significant difference was evident between variances of both sets of cages. The mean coefficient of variation (CV = pool standard deviation of both ponds/mean) for survival rate, final biomass, final body weight, daily growth rate and FCR were of 11%, 13.7%, 4.4%, 5.4% and 7% respectively.

Based on average CV, the requisite number of replications needed to determine a treatment effect (5, 10, 15, 20, 25 and 30% of the mean) according to the parameter under consideration was calculated. Table 2 shows the results: for both sets of cages and for a fixed statistical power of 80%, the number of replicates that would be necessary to highlight significant differences, if they exist, at a level of significance  $\alpha = 5\%$  (two-tailed test of significance).

Table 3 summarizes statistical differences between both sets of cages according to survival, growth rate (GR), final biomass and FCR. These results show a significant pond effect in terms of survival rate ( $P < 0.05$ ) and GR ( $P < 0.01$ ). No significant differences were recorded for biomass and FCR. An a posteriori statistical power analysis and subsequent sensitivity calculation showed the statistical power and sensitivity (% differences between means) of the experimental design. Differences between cages-A and cages-B for final biomass (5%) and FCR (0.4%) were below the experiment's sensitivity levels for these parameters (respectively 30% and 14%), making them undetectable, if they existed, at  $\alpha = 5\%$ .

## 4. Discussion

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The low survival rate during this study derived from high mortalities during the first 4 weeks of the trial (Castex *et al.*, 2008). These mortalities simultaneously applied to shrimps reared both inside and outside the cages. Water temperature and oxygen concentrations measured in the cages stayed within the preferendum of *L. stylirostris* throughout the rearing period (Wabete, 2005; Chim and al., 2004). Consequently, these parameters did not provide any information that could help explain the mortalities recorded during the trial. It is more likely that the mortalities can be attributed to "summer syndrome". Indeed, as recently observed on this farm (Goarant *et al.*, 2006; Castex *et al.*, 2008), a high prevalence and portage of the causal pathogenic agent (*V. nigripulcritudo*) were recorded in shrimp haemolymph sampled both in cages and in ponds when the mortalities occurred. Besides our last feeding trials carried out with a private farm during one month in 20 floating cages led to high survival rates ( $91.6 \pm 1.74\%$ ;  $n = 20$ ) (SICA, pers.com.). We conclude that the mortalities are unlikely to be linked to the rearing conditions in floating cages, since in other studies where no particular disease was reported, these same conditions led to survival rates ranging from 57% to 100% (Paquotte *et al.*, 1998; Lombardi *et al.*, 2006; Zarain-Herzberg *et al.*, 2006).

In the present study, as the feed remains on the feeding tray were visually quantified to adjust the size of the next meal, this frequent alteration in feed ration may have led to a reduction of waste (Smith *et al.*, 2002). After four weeks of rearing in the cages, the size of the rations (adjusted daily) was

substantially reduced in both sets. This decline in the shrimps' consumption can be attributed to (i) the high mortalities during the first four weeks of the experiment (Castex *et al.*, 2008), (ii) the drop in water temperature (Chim *et al.*, 2004) and (iii) the shift in the feeding behaviour from juvenile to sub-adult shrimps (Nunes *et al.*, 1997). Moreover, during the final three weeks, rations delivered to cages-A were lower than those to cages-B, which is consistent with the higher recorded final biomass in cages-B. Further, despite of the high mortalities recorded during the trial, the FCR were close to 2 in the cages. Interestingly, these FCR were, for cages-A and cages-B, 13% and 17% lower than those obtained in the respective ponds, where feed was adjusted on a weekly basis. This result could be accounted for by the daily adjustments of feed rations and feeding exclusively on feeding trays. This practice, which allows feed rations to be precisely quantified, is regarded as a major advantage over other traditional feed delivery methods, especially when ration adjustment is made on a daily basis (Jory *et al.*, 2001; Nunes and Suresh, 2001). These results are relevant for checking the accuracy of the daily visual adjustment method for feed remaining in the feeding trail. In addition, the bio-fouling which grew on the netting of the cages may, through its nutritional contribution to the total shrimp diet, be another relevant factor for explaining the best FCR obtained in cages (Paquotte *et al.*, 1998; Pérez-Rostro *et al.*, 1999; Yusufzai and Sing, 2005; Zarain-Herzberg *et al.*, 2006). For instance, it has been shown that natural food greatly improved growth parameters during the nursery phase of *Farfantepenaeus paulensis* in indoor systems and in cages (Thompson *et al.*, 2002; Ballester *et al.*, 2007).

With final average body weight of  $18.5 \pm 1.7$  g and final biomass of  $245 \pm 30$  g.m<sup>-2</sup> for all the cages, the results after 10 weeks growth in floating cages were comparable to those generally observed in semi-intensive ponds in New Caledonia (unpublished). To our knowledge, this study is the first reported attempt to rear shrimps in floating cages inside earthen ponds. It supports preceding studies, having shown that floating cages were well suited to this purpose (Paquotte *et al.*, 1998; Zarain-Herzberg, 2006; Lombardi *et al.*, 2006). Nevertheless, the main objective of our study was to assess this original rearing method as an experimental tool and not for production ends. For this reason we used a relatively low rearing density (29 shrimps.m<sup>-2</sup>) compared to previously cited studies (Paquotte *et al.*, 1998; Zarain-Herzberg, 2006; Lombardi *et al.*, 2006).

Overall, we conclude that the floating cages rearing method does not present any zootechnical biases which may mask the effects an experimenter wants to highlight, thereby making it a tool of choice for experiments in ponds. However, while our method enables studies to be carried out under conditions similar to those used for farming (e.g. similar water quality), there are two fundamental differences. First, animals raised in floating cages have no access to pond sediment, which can influence positively by providing living prey (Boucher, 2004) or negatively if its biochemical state is altered (Avnimelech *et al.*, 2004). Nevertheless, neither the present results nor our successive trials with floating cages to evaluate dietary probiotic effects on shrimps in commercial ponds (Castex *et al.*, 2008) suggest a potential bias due to sediment-free conditions. Secondly, as was previously discussed, the net acts as a substratum for the development of periphyton and associated benthos, both of which can contribute to shrimp nutrition.

With these limitations taken into account, trials in floating cages are very probably more representative of the pond conditions than laboratory trials in clear water and thus offer a good compromise. Indeed the results of our trials confirm that zootechnical outcomes in floating cages are comparable to those recorded in the pond.

The second objective of the study was to assess the floating cages method from a statistical point of view. The aim was to determine, based on an a priori power analysis (Aaron and Hays, 2004), the number of replicates needed for powerful experiments in order to detect potential significant differences between treatments. The term 'power' signifies the capacity of an experiment to detect real differences, if they exist, at the desired significance level. Therefore, power depends on the magnitude of the differences to be detected, the significance level, and the degree of experimental error (Aaron and Hays, 2004). Before conducting an a priori power analysis and then estimating the number of replicates needed for an experiment of known power and sensitivity, it is essential to have a good estimate of the variability among replicates. This variability is, however, generally not known in advance and must be estimated from previous experiments (Berndtson, 1991). For this reason, the present trial was used as a pre-experiment study to assess the variability between floating cages. The experiment was duplicated in two ponds in order to increase the reliability of the coefficient of variation estimate for each zootechnical indicator considered. For both sets of cages, the experiments were conducted according to the same modalities, and were independent of each other with measurements of equivalent technical precision. These criteria were reported to be necessary for appropriately estimating the CV (Berndtson, 1991). The results show that the homogeneity of variances is confirmed in both sets of cages for all the parameters under consideration. It was then possible to estimate an

average CV based on the pooled variance estimate and an estimate of the mean. For the parameters studied (mean survival rate, final body weight, daily growth rate and FCR), the number of necessary replicates decreases substantially when the expected difference of the means goes up from 5% to 10% with a statistical power of 80%. It is important to state that we considered using a two-tailed test. A one-tailed test will have reduced replication requirements but, given that the outcome of an experiment is generally unknown in advance, a two-tailed test was probably more appropriate. Based on these results, the floating cages system appears to be a reasonable tool for revealing differences of at least 10% between two treatments. Moreover, for expected differences of 20% from the control mean, 3 and 6 floating cages per treatment will be reasonable to determine statistical differences for growth parameters and survival rate respectively. However, among the parameters measured, the final biomass, with an average CV of 14%, does not appear to be a sensitive indicator in the present instance.

An a posteriori power analysis can be a useful supplement to hypothesis testing in cases where significant results are not detected (Myers and Well, 2003). In our study, this was the case for the final biomass in regard to the pond effect. The post hoc analysis showed an observed power of only 13%, which cannot rule out the existence of a real difference. An additional and more appropriate use of an a posteriori power analysis is the calculation of sensitivity (Aaron and Hays, 2004). We showed that to detect a significant difference in final biomass between sets of cages-A and cages-B, the pond effect should have been higher than 30%, whereas in the present instance it was only 5%. It can thus be concluded that the experiment was not sufficiently powerful to detect a difference in the final biomass in regard to the pond effect, if it existed.

Finally, it seemed interesting to point out the advantage of the floating cages method compared to experiments carried out with earthen ponds. To do so, we compared the replication requirements for a fixed power (80%) and significance level ( $\alpha = 5\%$ ) for both systems (rearing in ponds and in cages). Such a comparison depends greatly on the estimation of the coefficient of variation of the parameters considered in both systems. For the pond system, we estimated a CV for each parameter (mean survival, final body weight, daily growth rate and FCR) from results obtained in six similar earthen ponds (1500 m<sup>2</sup>) from our facilities. The ponds were seeded at the same time with larvae from the same hatchery batch, harvested on the same date, and managed in the same way in accordance with commonly used practices in New Caledonia. The number of replicates needed, taking all zootechnical parameters into consideration, is shown in Table 2. Whatever the expected differences for survival (from 5% to 30%), the number of replicates needed is greater if ponds are used as experimental units, which is directly linked to higher CV between ponds than cages. The same trends are observed for final body weight and FCR up to 15% difference between treatments. Beyond that, the number of replicates becomes similar for both cages and ponds, all parameters taken into consideration.

These results thus show that floating cages are a more powerful and sensitive experimental tool for studies undertaken in practical pond conditions. It is important to specify that the CV determined in the present case for the pond system cannot be an accurate estimate of the true variability in and between the farm's earthen ponds. Indeed, the present study sought to minimize sources of variability (ponds of the same surface and the same shape, same origin for PL, same period of breeding, same management, etc.). However in within- and/or between-farm experiments the variability of sources cannot usually be controlled in the same way. As a direct consequence and in view of the variability between the semi-intensive systems, the CV will be fatally increased, leading to a rise in the number of replicates necessary for experiments of same power. These conditions would make such experiments unrealistic, both logistically and economically.

In conclusion, our study shows that floating cages provide a reliable method for carrying out powerful experiments under near-pond conditions, and are an economical and handy experimental tool to assess, on a pilot scale, scientific results obtained under laboratory controlled conditions.

## **Acknowledgments**

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This work was supported by research grants from the Northern and Southern Provinces of New Caledonia. The authors would like to thank the whole IFREMER staff from the Département Aquacole Calédonien. They kindly provided data from the six rearing experiments used for replicate calculation and helped us in the design, building and setting up the cages and the final shrimp sampling process. We would also like to thank the owner of the private farm and his employees for their assistance throughout the trial period.

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## Tables

**Table 1.** Zootechnical results of rearing in cages for both sets in ponds A and B. <sup>(1)</sup> Different superscript letters within the same line indicate significant differences among treatments variance with Fisher test for variance equality.

	Survival (%)		Total cage biomass (g)		Final shrimp weight (g)		Growth rate (g.day <sup>-1</sup> )		FCR	
	Cages-A	Cages-B	Cages-A	Cages-B	Cages-A	Cages-B	Cages-A	Cages-B	Cages-A	Cages-B
n	5	5	5	5	5	5	5	5	5	5
Mean	31.1	37.8	2291	2408	19.91	17.22	0.226	0.199	2.29	2.28
SD	3.4	4.2	346	299	0.99	0.63	0.014	0.009	0.19	0.13
Variance <sup>(1)</sup>	0.001 <sup>a</sup>	0.002 <sup>a</sup>	120323 <sup>a</sup>	89707 <sup>a</sup>	0.98 <sup>a</sup>	0.4 <sup>a</sup>	0.0002 <sup>a</sup>	0.0001 <sup>a</sup>	0.036 <sup>a</sup>	0.016 <sup>a</sup>
Coef Var	0.108	0.111	0.151	0.124	0.05	0.04	0.06	0.044	0.083	0.056
Mean CV* (%)	11.0		13.7		4.4		5.4		7.0	
<sup>(1)</sup> Fisher test for variance equality										
*Coefficient of variation (CV) = (pooled standard deviation/mean) x 100										

**Table 2.** Estimated number of replications needed in shrimp experiments depending of which rearing system is used <sup>a</sup>.

Experimental design	Parameters	Average CV <sup>b</sup>	Expected differences, % of mean <sup>a</sup>					
			5	10	15	20	25	30
Cages	Survival (%)	11	71	20	10	7	5	4
	Final body weight	5	18	6	4	3	3	2
	Daily growth rate	6	23	8	4	3	3	3
	Final Biomass	14	126	32	15	9	7	5
	FCR	7	32	9	5	4	3	3
Pond	Survival (%)	16	207	48	21	12	8	6
	Final body weight	8	43	12	6	4	3	3
	Daily growth rate	5	21	6	4	3	3	3
	FCR	8	40	11	6	4	3	3

<sup>a</sup> Assumes a randomized design with two treatments, two-tailed test of significance, and power of 80% at P<0.05

<sup>b</sup> Coefficient of variation = (pooled standard deviation/mean) x 100.

**Table 3.** Pond effect and a posteriori analysis for zootechnical results in cages from pond A and pond B.

Parameters	Differences (%)	P value <sup>b</sup>	Statistical power <sup>c</sup>	Sensitivity <sup>d</sup> (%)
Survival (%)	22	<0.05	>80%	11
GR (g.day <sup>-1</sup> ) <sup>a</sup>	14	<0.01	95%	10
Final Biomass (g)	5	n.s	13%	30
FCR	0	n.s	6%	14

<sup>a</sup>GR = growth rate

<sup>b</sup>t-test for independant means comparison

<sup>c</sup>Post Hoc test achieved for a randomized design with two treatment (Pond A and B), five replicate per treatment, one-tailed test of significance, and  $\alpha=5\%$ .

<sup>d</sup>Results of sensitivity analysis, critical detectable population differences (%) with a power  $1-\beta=0.80$ , 5 replicates per treatment and  $\alpha=5\%$ .

## Figures



Fig. 1. Set-up of a cage (4 m x 2 m) in the shrimp pond.

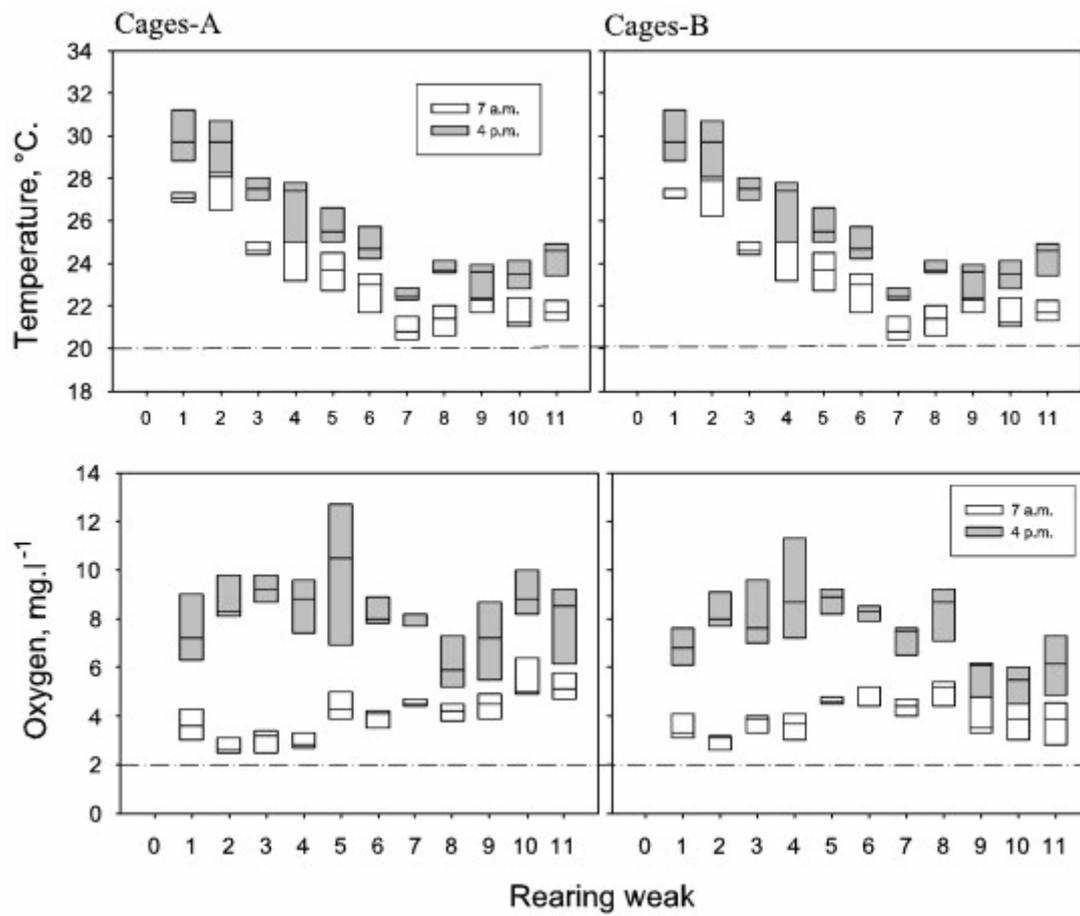


Fig. 2. Box plots for weekly water temperature (°C) and oxygen concentration (mg l<sup>-1</sup>) in Cages-A and Cages-B measured at 7.00 am and at 4.00 pm during the period of the experiment.

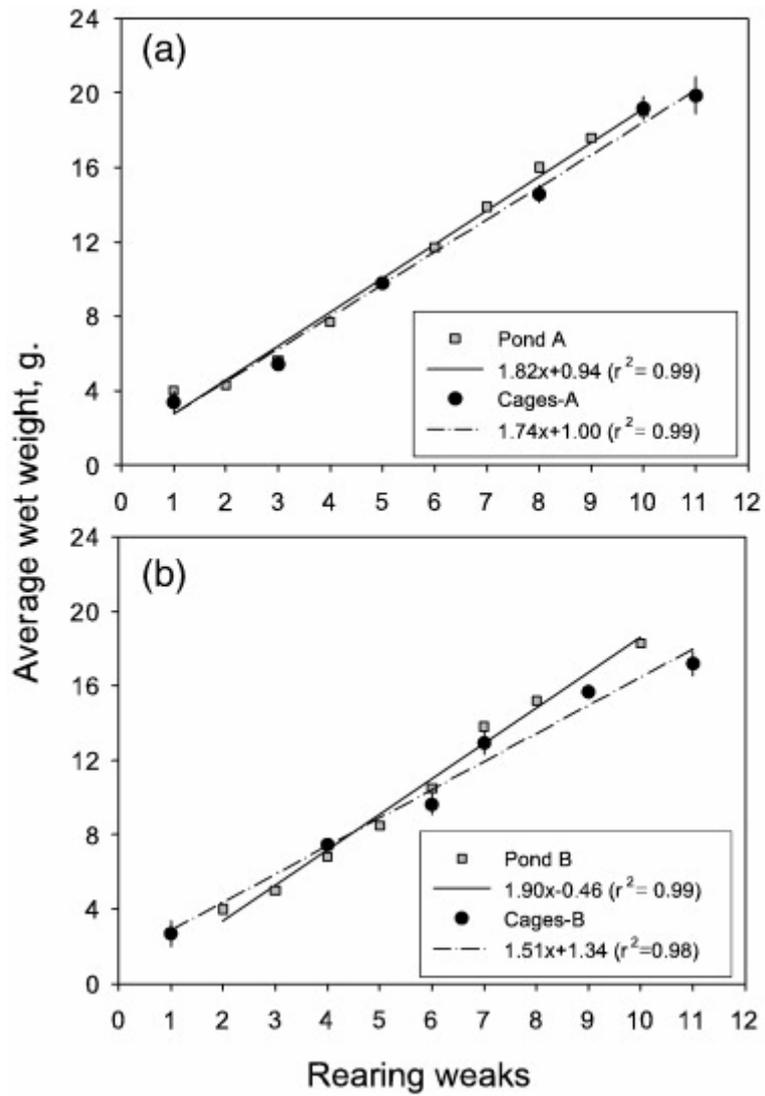


Fig. 3. Comparative weekly average wet weight (g) of shrimps reared in Cages-A (a) and Cages-B (b) and in their respective ponds. Straight line equations obtained by linear regressions of average wet weight function of the rearing weeks are indicated. Error bars indicate standard errors ( $P < 0.05$ ).

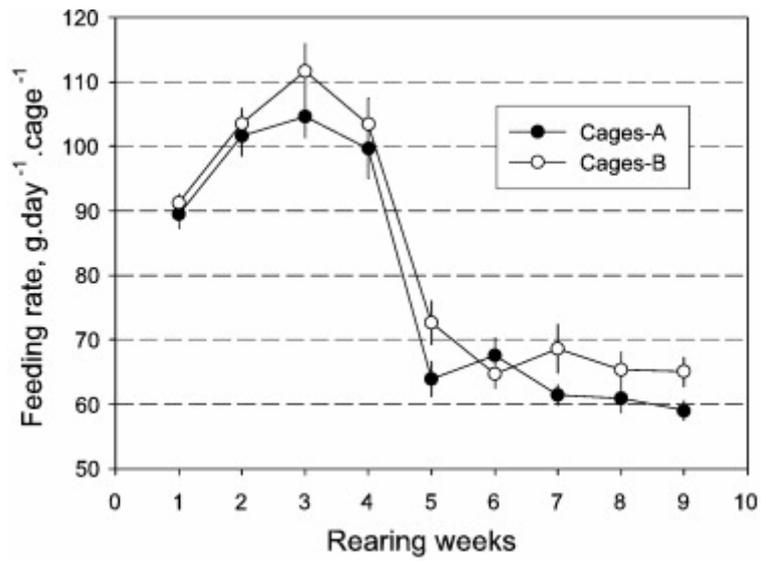


Fig. 4. Comparative weekly mean amounts of feed delivered in Cages-A and Cages-B during the trial. Error bars indicate standard deviations ( $P < 0.05$ ).