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Reproductive performance and offspring quality of non-ablated Pacific white shrimp *(Litopenaeus vannamei*) under intensive commercial scale conditions

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Abstract

This study evaluated reproductive performance of non-ablated *Litopenaeus vannamei* and the quality of their offspring under commercial conditions. Five tanks were stocked with non-ablated female and other five with ablated individuals as control. Two different larval rearing trials (Larviculture I and II) have been conducted. Six larviculture tanks (n=3) were used on the first trial (LI) and ten for the second one (LII) (n=3). Postlarvae from LII were used for nursery and grow-out. Spawning event and hatching rate per day were similar between both treatments. Mating success, mortality of female and number of eggs and nauplii per tank per day of non-ablated group were significantly lower than ablated female. Non-ablated female fecundity (number of eggs and nauplii per spawned female per day) was significantly higher than control. There was no significant difference between daily larval stage index of larvae in LI and LII. The response to the salinity stress test, and final survival and weight in LI was similar. However in LII, postlarvae derived from non-ablated had significantly higher survival

to salinity stress test. Identical survival, final weight, weekly growth, feed conversion rate and yield were observed in nursery. The same was observed in grow-out, including weight gain and specific growth rate. Overall this study demonstrates that non-ablated females can have comparable level of productivity to ablated females in intensive commercial hatchery conditions. Their offspring perform comparably in all culture stages with evidence of enhanced resistance to stress in larvae derived from non-ablated female broodstock.

Keywords: Eyestalk ablation; Natural reproduction; Growth performance; Welfare; Hatcheries.

1. Introduction

The Pacific whiteleg shrimp (*Litopenaeus vannamei*) is currently the most cultured marine shrimp worldwide with 75% of global shrimp aquaculture production in 2016 (Anderson et al., 2016). It is the single most valuable aquatic farmed species, worth 25% more than global Atlantic salmon production, (Tacon, 2017) and widely internationally traded.

Standard hatchery methods worldwide to control the maturation and reproduction of *L. vannamei* are based on the use of eyestalk ablation (Chamberlain and Lawrence, 1981; Zhang et al., 1997; Palacios et al., 1999a; FAO, 2003; Sainz-Hernández et al., 2008; Das et al., 2015). Eyestalk ablation involves the removal or constriction of one (unilateral) or two (bilateral) eyestalk through cutting, cauterizing or tying, to reduce the level of gonad inhibiting hormone (GIH) which is produced by the X-organ and sinus gland complex situated in the optic ganglia of the eyestalk (Alava and Primavera, 1979; Raviv et al., 2006; Bae et al., 2013; Treerattrakool et al., 2014). Ablation is reported to increase and improve

predictability of commercial hatchery output (Treece and Fox, 1993; Palacios et al., 1999a) but is also known to have disadvantages. Removal of the optic ganglia is known to severely impact the animal's welfare. In addition to causing trauma and stress associated with the procedure itself, physiological imbalance and a compromised immunological defense that lead to accelerated reproductive exhaustion and increased broodstock mortality are possible consequences (Palacios et al., 1999ab; Bae et al., 2013; Treerattrakool et al., 2014; Das et al., 2015). Additionally growing consumer awareness of animal welfare and ethics in food production and the related interest of choice editors to reduce commercial risk (Little et al., 2018) have made a reassessment of the use of eyestalk ablation in hatcheries a priority for producers, retailers and seafood certifiers.

The use of non-ablated females (NAF) has attracted interest for some time especially as hatchery managers report NAF have longer reproductive lifetime than ablated individuals under intensive maturation conditions (FAO, 2003). However NAF suffer from less predictable maturation peaks and spawning synchronization, making the establishment of production schedules more difficult (Palacios et al., 1999b). Few studies have comprehensively evaluated difference in productivity between systems based on eyestalk ablated (AF) and non-ablated (NAF) shrimp. Mating success, spawns, hatching rate, female mortality rate and fecundity (number of eggs and nauplii per spawn) of NAF and AF of *L. vannamei* have been assessed by Chamberlain and Lawrence (1981) and Palacios et al (1999b), and in other species (Santiago Jr, 1977; Emmerson, 1980; Chamberlain and Lawrence, 1981; Yano, 1984; Choy, 1987; Makinouchi and Primavera, 1987; Gendrop-Funes and Valenzuela-Espinoza, 1995). Zhang et al. (1997) only assessed spawning and molt cycle of *Stenopus hispidus*.

Non-ablation of *L. vannamei* female is reported to affect mating success and not eggs hatching rate, female mortality rate, number of eggs and nauplii per spawn (Chamberlain and

Lawrence 1981; Palacios et al. 1999b). In addition, non-ablation did not have a significant effect on larval survival in zoea, mysis and postlarvae 1-day-old (PL1) stages (Palacios et al. 1999b). However, Chamberlain and Lawrence (1981) evaluated only reproductive parameters under non-commercial conditions and some of their results might have been affected because NAF and AF animals were maintained in the same tanks. Furthermore the authors assume that some of their AF animals might have had regenerated eyestalk during the trial. This might have made the authors to consider some animals as NAF whilst they were AF. Palacios et al (1999b) are the only authors to date who have compared reproductive performance between both groups under intensive reproduction conditions, but their experimental design was unbalanced with respect to the numbers of AF and NAF used which might have influenced their results. Additionally, they also compared larval growth and survival to PL1 from eggs derived from both NAF and AF but not under commercial conditions. Moreover there is an urgent need for more contemporary assessments of the effects of the impact of non-ablation on broodstock performance in terms of reproductive outputs and offspring quality in the context of shrimp strains improved through selective breeding (Castillo-Juarez et al, 2015) and based on modern production practices.

This study evaluated reproductive performance of NAF and AF *L. vannamei* and the quality of their offspring in term of growth performance and survival during larviculture, nursery and grow-out under commercial conditions. The ultimate goal of this work is to provide a body of evidence for producers, regulators and retailers to support on-going dialogue around the continued use of eyestalk ablation in modern *L. vannamei* breeding practices.

2. Material and methods

2.1. Broodstock and experimental rearing conditions

The study was conducted in the Larvicultura del Pacífico (Larvipac), commercial hatchery of Seajoy company in Honduras. *L. vannamei* broodstock with average male and female weight, 39.7 ± 2.6 and 49.7 ± 4.3 g respectively, were used in this experiment. Prior to the experiment, shrimp entered pre-maturation conditioning of approximately 36 days where males and female were reared in separate systems consisting of two 41.8 m^2 geomembrane tanks. Both male and females were fed daily with chopped defrosted squid (12:00 pm and 3:00 am) at 5% of biomass and polychaete worms (7:00 am) at 2.5% of biomass. All fresh feed were defrosted and chopped to approximately 0.6 g. These were supplementary to the feeding of commercial maturation diets: a shrimp broodstock diet (40% Crude protein, Zeigler, USA) was used at 5% of biomass at 9:00 am; 3:00 pm and 10:00 pm, and EZ-mate (55% Protein, Zeigler ,USA) was supplied at 3.5% of biomass at 8:00 pm and 5:00 am.

Three days before starting of the experiment, unilateral eyestalk ablation was performed on control females by cauterization. Ten maturation tanks (round geomembrane tanks, dark-coloured; 22.9 m^2 area) were used in total; five were stocked with ablated female (control group) and five with non-ablated individuals (experimental group). Water depth was maintained at 0.5 m using a central stand pipe and tanks were aerated constantly using two air stones per tank. Males were stocked in all tanks one day before starting the experiment. Each tank was stocked with 300 animals (13/m^2) with a sex ratio of 1:2 (male to female). All experimental units were connected to a single closed recirculating system that allowed 5-10% daily water exchange. The water filtration and treatment system consisted of sand, cellulose, activated carbon and silica filters, and UV light. Photoperiod was maintained naturally by allowing exposure to ambient sunlight through translucent roof windows in the maturation room. Water temperature, salinity, dissolved oxygen, pH, alkalinity and ammonia were maintained at 28.9 ± 0.1 ºC, 32.0 ±1.0 g/L, 4.3 ± 0.0 mg/L, 7.0 ± 1.0, ≥ 100 mg/L $CaCO_3$ and

< 1 mg/L respectively. The broodstock remained in maturation tanks for 62 days and were fed daily with polychaete worms twice a day (07:00 am and 10:00 pm) at 5% of biomass, squid three times a day (9:00 am, 2:00 pm and 3:00 am) at 12% of biomass, and mussels twice a day (12:00 pm and 5:00 am) at 6% of biomass. All fresh feed were defrosted and chopped to approximately 0.6 g. In addition a commercial maturation diet EZ-mate (55% Protein, Zeigler, USA), was also applied once a day (8:00 pm) at 2% of biomass according to commercial protocol. The common practice of commercial hatcheries to replace deceased individuals in order to maintain the same stocking density throughout the experiment was followed. Females (509) and males (242) of similar conditions described above were used to restock non-ablated treatment while ablated group used 738 females and 220 males.

2.2. Reproductive performance and broodstock mortality

Reproductive performance was evaluated a minimum of three times per week over the 62-day study based on standard commercial hatchery practices and previously published recommended protocols (Crocos and Kerr, 1986; Coman and Crocos, 2003). Gonad development was monitored three times daily with a lamp, from 4:00 to 7:00 pm, by observing the size and colour of the gonad through the exoskeleton. Mature females from each treatment with an attached spermatophore were collected, transferred and pooled in three 5000 L spawning tanks per treatment. Spawning tanks were maintained at a temperature of 28 ± 1 ºC. Females from each treatment were then returned to their respective maturation tanks at midnight, and eggs were collected from the spawning tanks and pooled in a 25 L bucket. After stirring, three 5 mL subsamples of water per treatment were taken for volumetric assessment of number of eggs in each treatment. Each group of eggs were subsequently transferred to an illuminated 1000 L fibreglass hatching tank and kept for 13 h. Each hatching tank was aerated constantly using one air stone per tank and temperature and salinity were

maintained around 32 ± 0.5 ºC and 30 ± 0 g/L. Nauplii were then collected exploiting their positive phototropic behaviour, transferred to a 25 L bucket, where volumetric assessment of numbers was undertaken for each treatment using the same method as for eggs.

As the females from each treatment were collectively managed, reproductive performance parameters were estimated per unit time period (days) (Bhujel, 2008): mating success per day (number of mated female per treatment per day/number of female per treatment per day), spawning event per day (number of spawned female per treatment per day/number of mated female per treatment per day), hatching rate per day (number of nauplii per treatment per day/ number of eggs per treatment per day), fecundity in term of eggs per spawned female per day (number of eggs per treatment per day/ number of spawned female per treatment per day) and nauplii per spawned female per day (number of nauplii per treatment per day/ number of spawned female per treatment per day), and tank production parameters in term of eggs per tank per day (Number of eggs per treatment per day/ number of tanks per treatment) and nauplii per tank per day (Number of nauplii per treatment per day/ number of tanks per treatment). Spawning event is defined in this study as percentage of mated female that released eggs in spawning tanks. Mortality of females per treatment was recorded daily.

2.3. Larval rearing

Two different larval rearing trials (Larviculture I - LI and larviculture II - LII) of 16 days each were conducted using larvae (nauplii 5) produced at the beginning (day 12) and middle (day 38) of the observation period as the productivity of eyestalk ablated animals are reported to deteriorate over time (typically two to three months) because of exhaustion (Palacios et al., 1999a) and this might affect the quality of the offspring. In LI, six rectangular

geomembrane tanks (16 000L) with initial 10 000L water volume (8000 L seawater + 2000 L algae) were stocked with 2 500 000 nauplii each at a density of 250/L on the first day. Both treatments were triplicated and randomly distributed in a greenhouse. Algae (*Thalassiosira sp*) were added daily to the tanks at concentration of 100 000 – 130 000 cells/mL until the shrimp reached PL2, and then 25% of water was renewed daily until the end of the trial. The diet was also composed of artemia sp. (from Mysis 1 to PL6) and artificial liquid and dry diets (Frippak – Inve Aquaculture; liqualife ZM and MPL – Cargil; liquid hatchery feed – Epicore; and brine shrimp flake – Mackey Marine (from Zoea 1 to PL12-PL14). The amount of feed applied in the trials followed manufacture recommendations. Water temperature, salinity, dissolved oxygen, pH, ammonia, nitrate, total suspended solids and nitrate were kept in 32.7 ± 0.5 ºC, 32.9 ± 0.0 g/L, 4.4 ± 0.1 mg/L, 8.0 ± 0.0, < 1 mg/L, 1.5 ± 0.2, 34.3 ± 2.3 and 140 ± 2.6 mg/L $CaCO_3$ respectively. The procedures used in trial II were similar to the first trial except for the following details: ten rectangular geomembrane tanks (5 for each treatment) of 24 000 L each, in addition each unit had initial 12 000L water volume (10000 L seawater + 2000L algae) stocked with 1 950 000 nauplii each at a density of 162.5/L on the first day.

During both trials larval stage development was determined daily using larval stage index (LSI) which is a calculated weighted average of stage determination i.e., the number of larvae or PL at each stage were multiplied by the stage number and the sum of the products were divided by the number of larvae sampled as illustrate in equation 1 (Uno and Kwon, 1969). This index was also used to express larval growth (Uno and Kwon, 1969; Manzi et al., 1977). At the end of the trials wet weight and survival were recorded. Survival was determined by taking three 100 mL samples from each 500 L fiberglass tank (used to concentrate PL from each trial tank) and counting all live PLs. Weight was determined by weighing 1g of PLs from each tank and then dividing it with number of PLs observed in that sample. Postlarval quality was also assessed using a simple salinity stress test. 100 PLs11-12

(for LI), PLs13-14 (for LII) per tank were transferred from full strength seawater (32 g/L) to a 1 L beaker of fresh water (0 g/L) for a period of 30 minutes before being returned to a beaker of seawater. After a further 30 minutes, survival (%) was evaluated based on immobility/reaction after physically stimulating with a pipette (Palacios et al., 1999b; Racotta et al., 2004).

Equation 1: $LSI = (\Sigma niEi)/n$ in which: LSI = Larval stage index; ni = number of larvae at stage Ei; n = number of larvae analysed; Ei = larval or postlarval stage.

2.4. Nursery and grow-out

PLs from larviculture trial II were used for nursery and grow-out trials in a commercial farm (Biocultivos Mariños - BIOMAR) of Seajoy Company. Four rectangular earthen ponds of 1.50 ha each were stocked with PLs13-14 from the two treatments (two ponds per treatment) at a density of 123/m^2 and reared for 20 days. During this stage the animals were fed only artificial feed Diamassa (35% Protein, Gisis, Ecuador) 7 times a day and at a rate of 15 – 20% of total biomass. Two feed trays of 50 cm^2 each were placed 1 m in the water column. Water temperature, dissolved oxygen, salinity, pH and ammonia were monitored and no significant difference was observed between the treatments (Table 1). Final survival [100 x (final shrimp number/initial shrimp number)], final weight (g), weekly growth (final weight/time in weeks) and yield [(final shrimp number x final weight)/area] and feed conversion rate [consumed feed (dry weight)/total weight gain] were used to assess the culture performance (Maicá et al. 2012) of PLs generated by NAF and AF broodstock.

After the nursery period, juveniles from each treatment were transferred to triplicate rectangular, semi-intensive earthen ponds of 1.82 ha each and stocked at 25 juveniles/m^2 for final grow-out (86 days). Artificial feed (Diamassa, 35% Protein, Gisis, Ecuador) was offered

three times a day at rate of 2 – 10% of total biomass. The amount of feed offered at the beginning of the trial was equal in all replicates, but subsequently adjusted based on weekly animal growth and biomass. Two feed trays of 50 cm^2 each were placed 1 m in the water column. Final survival [100 x (final shrimp number/initial shrimp number)], final weight (g), weekly growth (final weight/time in weeks) total weight gain (final weight – initial weight), yield [(final shrimp number x final mean weight)/area], feed conversion rate [consumed feed (dry weight)/total weight gain] and specific growth rate [100 x (ln final weight – ln initial weight)/time in days] were determined to evaluate their performance (Maicá et al. 2012). Water temperature, dissolved oxygen, salinity, pH, ammonia, nitrate, silicate, phosphate and alkalinity were monitored and were similar between the treatments (Table 1).

2.5. Statistical analysis

One-way ANOVA followed by a Tukey test (Zar, 2010) was used to compare the treatments in significance level of 0.05. Normality and homogeneity were tested using Shapiro-Wilk and Levene tests, respectively. Reproductive outputs such as number of eggs and nauplii per spawned female per day were analysed by two-way ANOVA using first variable as time (days) and second one as non-ablation and ablation. All statistics were run using STATISTICA 7 software.

3. Results

3.1. Reproductive performance

No effect of time was observed on the number of eggs and nauplii per spawned female per day but interaction between the two factors was recorded. Spawning event per day

(percentage of mated female that released eggs in spawning tanks) and hatching rates per day were similar for both treatments (Table 2). The mating success, mortality, number of eggs and nauplii production per tank of NAF per day were significantly lower than AF (Table 2). The fecundity (number of eggs and nauplii per spawned female per day) from NAF was significantly higher than AF (Table 2). A significant difference in egg production per spawned female per day between treatments became apparent two weeks after the onset of the trial (Figure 1), and after four weeks for nauplii production per female per day (Figure 2). Within each treatment no significant difference was observed in fecundity over time (Figures 1 and 2).

3.2. Larval rearing, nursery and grow-out

In both larviculture trials (Figures 3 and 4) there was no significant difference in the daily larval stage index between the treatments. Similarly there was no significant difference between treatments in response to a salinity stress test, and final survival and weight in Trial 1 (Table 3). In contrast in Trial 2, postlarvae derived from NAF had significantly higher survival to salinity stress test than AF.

At the end of the nursery period, juvenile survivals were similar between the two treatments (Table 4) and also in final weight, weekly growth, feed conversion rate (FCR) and yield. No significant differences between treatments were observed for all considered parameters during grow-out trial, including weight gain and specific growth rate (Table 4).

4. Discussion

This is the first study to compare reproductive performance between non-ablated and ablated female under intensive commercial conditions, and to follow the impact of non-ablation on offspring development, growth and survival in larviculture, nursery and grow-out at commercial scale. Non-ablation of *L. vannamei* female eyestalk did not affect spawning event and hatching rate. It has previously been suggested that mated *L. vannamei* females did not or only partially release the eggs under typical hatchery conditions, possibly due to stress from a range of factors, such as inappropriate handling from maturation to the spawning tanks, noise in spawning rooms and poor water quality (FAO, 2003; Treece and Fox, 1993). Our results suggest that mated non-ablated *L. vannamei* female (with attached spermatophore) will spawn (release the eggs in spawning tanks) in the same way as ablated individuals. Hatching success of eggs from non-ablated and ablated *L. vannamei* female were not significantly different which corroborated the earlier results reported by Chamberlain and Lawrence (1981) and Palacios et al. (1999b) with the same species, and in *Penaeus stylirostris* (Gendrop-Funes and Valenzuela-Espinoza, 1995), and *Penaeus canaliculatus* (Choy, 1987). This indicates that hatching rate may not be related to non-ablation of female shrimp but depend on other factors like the spawning tank environment (Ogle, 1995; FAO, 2003; Primavera, 1988; Treece and Fox, 1993), variable fertilization rate, and deterioration of the female during breeding time,. The impact of broodstock ablation on subsequent hatching rate may also depend on the method used for ablation. The hatching rate of eggs from non-ablated *Penaeus indicus* were significantly higher than from females ablated by pinching the eyestalk but not from those ablated by tying and cauterizing (Makinouchi and Primavera, 1987). Choy (1987) also observed lower hatching rates of eggs from female *Penaeus canaculatus* ablated by pinching. This method is stressful for the broodstocks as pinched eyestalk takes longer to heal and hemolymph loss is greater than by cauterization and tying

(Makinouchi and Primavera, 1987). In the present study, where no difference in egg hatching was observed, cauterization was used on AF.

Eyestalk ablation acts to reduce the level of gonad inhibiting hormone which is responsible for limiting the reproduction process of shrimp in captivity (Alava and Primavera, 1979; Bae et al., 2013; Das et al., 2015; Raviv et al., 2006; Treerattrakool et al., 2014). This leads the *L. vannamei* female to mature and spawn frequently within a short time interval, thus the mating success in the current study was more than double the level for AF compared to NAF, as was previously demonstrated by Chamberlain and Lawrence (1981) and Palacios et al. (1999b) with similar species and in other marine shrimp species (Choy, 1987; Gendrop-Funes and Valenzuela-Espinoza, 1995). In contrast, mortality rates of AF females were almost double that of NAF confirming the trauma and stress associated with the technique (Bae et al., 2013; Choy, 1987; Taylor et al., 2004). Ablation has been linked to causing physiological imbalance, reducing immunological defense, and to reduced reproductive productivity of female shrimps over time (Das et al., 2015; Palacios et al., 1999b; Treerattrakool et al., 2014). Depleted nutrient stores have been observed in AF which could explain lower survival rates of ablated to non-ablated female (Treece and Fox, 1993). The higher mortality in the current study has also been observed in other research on ablated females. Mean mortality of non-ablated was considerably lower (3.7%) than ablated female (33.3%) of *Penaeus stylirostris* after 97 days. Santiago Jr. (1977) also observed lower mortality for non-ablated (61%) than unilateral (73%) and bilateral (100%) ablated *Penaeus monodom* over 265 days. Chamberlain and Lawrence (1981) and Palacios et al., (1999a) found similar mean and overall mortality rate on their study with *Litopenaeus vannamei*, 41.7% and 48.3%, and 13% and 17% to non-ablated and ablated female after 97 and 90 days respectively.

In the present study, non-ablated female produced more eggs and nauplii per spawned female per day (>20% and >16% respectively) than ablated animals which is in agreement

with Choy (1987) and Emmerson (1980) with *P. canaliculatus* and *P. indicus*. In contrast, Makinouchi and Primavera (1987), Gendrop-Funes and Valenzuela-Espinoza (1995), Chamberlain and Lawrence (1981) and Palacios et al. (1999b) observed no significant difference in the number of eggs per spawn in *P. indicus, P. stylirostirs* and *L. vanammei*. Furthermore Gendrop-Funes and Valenzuela-Espinoza (1995) and Palacios et al. (1999b) reported no difference in nauplii per spawn between non-ablated and ablated female of *P. stylirostirs* and *L. vanammei*.

Eyestalk ablation accelerates maturation and spawning process in shrimp, but it also affects many other aspects of shrimp physiology that are regulated by the X-organ Sinus Gland Complex (Choy, 1987; Das et al., 2015; Palacios et al., 1999a). For instance, levels of hormones such as molt inhibitory hormone (MIH) and crustacean hyperglycemic hormone (CHH) are found to be reduced (Sainz-Hernández et al., 2008; Racotta et al., 2003). Hence, a drop of MIH forces the female to molt more frequently, demanding additional energy, at the same time as nutritional demands associated with reproduction are elevated (Emmerson, 1983; Racotta et al., 2003). This will influence the reproductive performance of ablated female, especially under intensive commercial conditions, as nutritional resources required to support reproduction are likely to become limiting. This undoubtedly explains the reduced fecundity (lower amount of eggs and nauplii per female per day or per spawn) and lower reproductive productivity associated with ablation observed here and in other studies.

Tank production parameters of NAF were 44% and 45% lower than AF for eggs and nauplii, respectively. This was directly affected by higher mating success observed in AF animals and suggests that a timing to meet a hypothetical daily production target for larvae would require either more broodstock or tanks or require rescheduling because of the lower level of breeding intensity in NAF than AF. For example, to produce 10 million nauplii using 1000 broodstock, AF would require 2 days and NAF 3 days. However, an interesting

phenomenona has been observed in the industry; specifically the company where this study was conducted is the trend of increasing % of mating success over increasing generations of NAF.

Indeed, the rapid, consecutive maturation and spawning characterizing the reproductive performance of ablated female *P. indicus* (Emmerson, 1980) and *L. vannamei* (Browdy and Samocha, 1985; Palacios et al., 1999a) was shown to lead to exhaustion-related deterioratation, over 75 days in *L. vannamei*. Data from this study did not find significant deterioration in fecundity of AF over our experimental period but there was a trend towards increasing reproductive output with NAF. When comparing both groups at each time point, non-ablated female produced more eggs per spawned female per day than ablated animals from the third week onwards and more nauplii per spawned female per day from fifth week onwards. This has important implications for broodstock management and costs. Increasing productivity over time and improved longevity for NAF might compensate for the higher short-term productivity of AF. Such tradeoffs in reproductive performance are well known in the terrestrial livestock breeding literature e.g. De Vries (2017).

Shrimp broodstock condition is considered to be crucial for production of good quality of offspring (Racotta et al., 2003). Broodstock condition has both nutritional (Braga et al. 2015; Cardona et al. 2016; Emerenciano et al. 2013; Emerenciano et al., 2014; Hoa et al., 2009; Naessens et al., 1997; Perez-velazquez and Gonzaez-feliz, 2003; Racotta et al., 2003; Wouters et al., 2001a; Wouters et al., 2001b;) and physiological components (Chamberlain and Lawrence, 1981; Lawrence, 1983; Palacios et al., 1999ab; Racotta et al., 2003). Theoretically, non-ablated female broodstock would demonstrate better overall condition than ablated and this would be reflected in differential quality of their offspring. However, the two larviculture trials presented little evidence that larval growth and development over time was affected by broodstock ablation, based on larval stage index (LSI), and final survival and

weight of PLs. Palacios et al. (1999b) also did not find any difference when comparing larval survival from ablated and non-ablated *L. vannamei* females in Zoea (from stage 1 to 3), Mysis (from stage 1 to 3) and PL1. There was some evidence for improved tolerance to salinity stress test in NAF derived PLs with a significant improvement in one of two independent tests. However, a better evaluation of PLs stress resistance would require multi-assessment approach in the future as the difference in salinity stress survival (2.4%) would not be relevant for the industry.

Nursery and grow-out trials were conducted using PLs of the second larviculture trial, where the results of salinity stress suggested NAF produced larvae were more robust. Salinity stress tests are commonly used to date by farmers to evaluate postlarvae quality (Álvarez et al., 2004; Palacios and Racotta, 2007) as it is assumed that higher survival from this test would mean better performance during nursery and grow-out (Racotta et al., 2004; Palacios and Racotta, 2007). However, performance during nursery and grow-out phases demonstrated no differences between PLs from NAF and AF. This is consistent with the findings of Alvarez et al. (2004) who found no relationship between survival to a salinity stress test of PL15 and survival during grow-out. Apparently the effect of non-ablation is reflected in early shrimp development stage but not at the nursery and grow-out stage. Clearly other factors (environment, nutrition and management) affect survival (Van Wyk et al., 1999; Alvarez et al., 2004) and growth (Van Wyk et al., 1999; Wickins and Lee, 2002) of shrimp during nursery and grow-out more than broodstock condition alone. Our findings also support the idea that a better evaluation of PLs stresses resistance would require multi-assessment approach in the future as the difference in salinity stress survival (2.4%) was not reflected in further farming stages.

Overall, our results indicate that a comparable level of productivity (eggs and nauplii per spawned female) can be realised by an intensive commercial hatchery based on NAF

system. However, the calculation has to take into account changes in productivity as well as mating success and mortality of female. The managers should also consider other factors such as the sex ratio used in their production tanks. Normally, hatcheries based on AF system use 1:1 male to female ratio and if they switch to NAF system would require increasing sex ratio to 1:2 male to female in order to get same productivity observed in 1:1 sex ratio. It might be expected that hatchery scale would determine the impact of a switch from AF to NAF with larger scale hatcheries most likely to invest in more capacity. A major advantage of using NAF would be the extended productive lifespan of these expensive animals but a full analysis of this potential advantage would require commercial level data over several years of production. Furthermore, there has been observed in the industry a trend of increasing percentage of mating success of NAF over increasing generations of closed-cycle breeding. This demonstrates the potential of selective breeding program for rapid maturation of non-ablated female. The best evidence for the commercial viability of the approach is the wholesale switch of Seajoy to using NAF.

In conclusion, commercial intensive hatcheries can use non-ablated production based system as long as some hatchery management practices are changed, i.e. on sex ratio, selective breeding program. The hatchery managers and farmers should be confident that offspring derived from non-ablated female of *L. vannamei* perform comparably in all culture stages and in addition there is in fact evidence of greater resilience to stress in NAF produced PLs.

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Table 1: Nursery and grow-out water quality parameters (mean ± SD).

Nursery*		
Variables	**NAF**	**AF**
Temperature (◦C)	31.4 ± 0.6	31.3 ± 0.9
Dissolved oxygen (mg/L)	4.2 ± 0.7	4.0 ± 1.0
Salinity (g/L)	43.0 ± 1.7	44.0 ± 1.5
pH	7.0 ± 1.0	7.0 ± 1.0
Alkalinity (mg/L $CaCO_3$)	31.3 ± 0.9	31.3 ± 0.9
Ammonia (mg/L)	< 0.1	< 0.1
Grow-out**		
Temperature (◦C)	31.1 ± 1.1	31.1 ± 1.0
Dissolved oxygen (mg/L)	3.9 ± 1.2	3.9 ± 1.1
Salinity (g/L)	31.9 ± 8.0	32.3 ± 7.9
pH	7.0 ± 1.0	7.0 ± 1.0
Alkalinity (mg/L $CaCO_3$)	98.8 ± 7.4	97.7 ± 15.2

Ammonia (mg/L)	0.1 ± 0.0	0.1 ± 0.1
Nitrate (mg/L)	5.7 ± 0.9	4.9 ± 0.5
Silicate (mg/L)	3.6 ± 1.5	3.7 ± 1.2
Phosphate (mg/L)	1.8 ± 0.8	2.2 ± 0.8

*n=2, **n=3

Table 2: Reproductive performance (mean ± SD) of non-ablated (NAF) and ablated (AF) *L. vannamei* female (n = 35).

Reproductive Performance		
Variables	**NAF**	**AF**
Mating success per day (%)	3.2 ± 1.8[a]	7.6 ± 2.8[b]
Spawning event per day (%)	90.0 ± 7.2[a]	95.5 ± 2.5[a]
Hatching rate per day (%)	78 ± 10.1[a]	81.7 ± 7.8[a]
Mortality of female per day (%)*	1.3 ± 0.8[a]	2.3 ± 0.2[b]
Number of eggs/spawned female/day	142 413 ± 26 968[b]	116 752 ± 21 113[a]
Number of nauplii/ spawned female/day	112 610 ± 29 129[b]	95 127 ± 17 476[a]
Number of eggs/tank/day	811 004 ± 506 465[a]	1440 286 ± 678 397[b]
Number of nauplii/tank/day	653 004 ± 428 378[a]	118 6450 ± 605 564[b]

Rows with different superscript letters are significantly different. *n = 62

Table 3: Growth performance, final survival and survival to SST (mean ± SD) of *L. vannamei* PLs in larviculture trial I (n = 3) and II (n = 5) after 16 culture days.

Larviculture trial I

Variables	**NAF**	**AF**
Survival to SST* (%)	94.7 ± 4.5 [a]	85.7 ± 10.5 [a]
Final survival (%)	48.0 ± 15.0 [a]	41.7 ± 12.1 [a]
Final weight (mg)	3.4 ± 0.5 [a]	3.7 ± 0.4 [a]
Larviculture trial II		
Variables	**NAF**	**AF**
Survival to SST (%)	97.4 ± 0.5 [b]	94.5 ± 1.1 [a]
Final survival (%)	48.8 ± 5.8 [a]	43.9 ± 12.5 [a]
Final weight (mg)	6.5 ± 1.4 [a]	6.7 ± 1.9 [a]

*SST – salinity stress test. Rows with different superscript letters are significantly different

Table 4: Growth performance and final survival (mean ± SD) of *L. vannamei* offspring from non-ablated (NA) and ablated female (ABL) in nursery (n = 2) and grow-out (n = 3) after 20 and 86 culture days, respectively.

Nursery		
Parameters	**Juveniles – NAF**	**Juveniles – AF**
Final Weight (g)	0.5 ± 0.1	0.6 ± 0.1
Weekly Growth (g)	0.2 ± 0.0	0.2 ± 0.0
Final survival (%)	40.2 ± 12.9	45.4 ± 5.2
FCR*	0.9 ± 0.2	0.7 ± 0.2
Yield (Kg/ha)	273.7 ± 56.8	340.6 ± 71.2
Growth out		
Parameters	**Sub-adults - NAF**	**Sub-adults - AF**
Initial weight (g)	0.6 ± 0.0	0.6 ± 0.0
Final weight (g)	14.7 ± 0.9 [a]	14.9 ± 0.3 [a]
Weight gain (g)	14.1 ± 0.9 [a]	14.2 ± 0.3 [a]

Weekly Growth (g)	1.2 ± 0.1 [a]	1.2 ± 0.0 [a]
SGR (%)*	3.7 ± 0.1 [a]	3.7 ± 0.0 [a]
Survival (%)	51.7 ± 1.6 [a]	47.7 ± 4.4 [a]
FCR	1.3 ± 0.1 [a]	1.3 ± 0.1 [a]
Yield (Kg/ha)	1875.2 ± 47.9 [a]	1776.6 ± 143.5 [a]

*FCR – Feed conversion rate. ** SGR – Specific growth rate. Rows with different superscript letters are significantly different

Figures

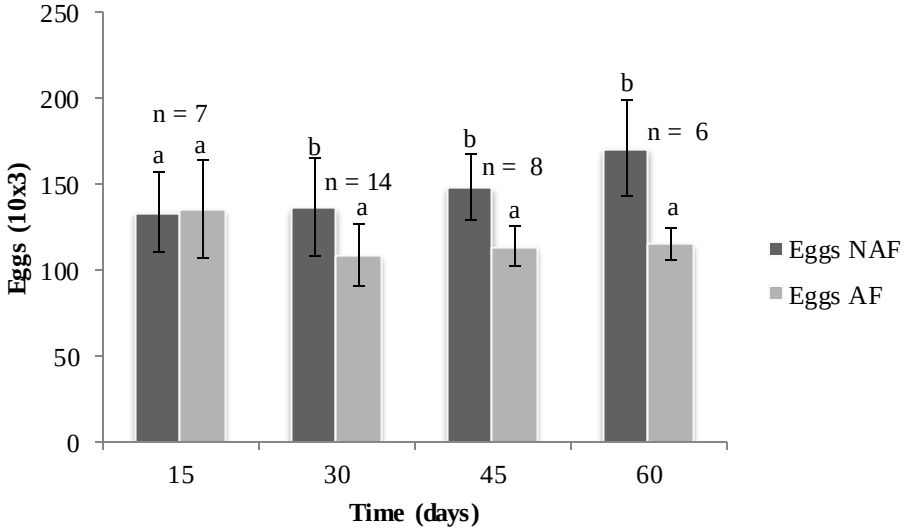

Figure 1: Egg production (mean ± SD) per non- ablated (NAF) and ablated spawned female (AF) over time. Different superscript letters mean the graphs are significantly different.

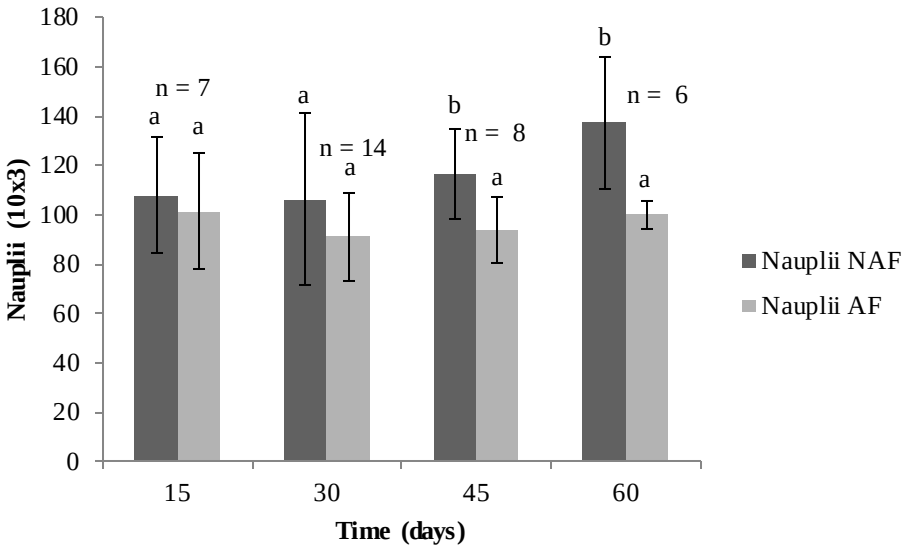

Figure 2: Nauplii production (mean ± SD) per non-ablated (NAF) and ablated spawned female (AF) over time. . Different superscript letters mean the graphs are significantly different.

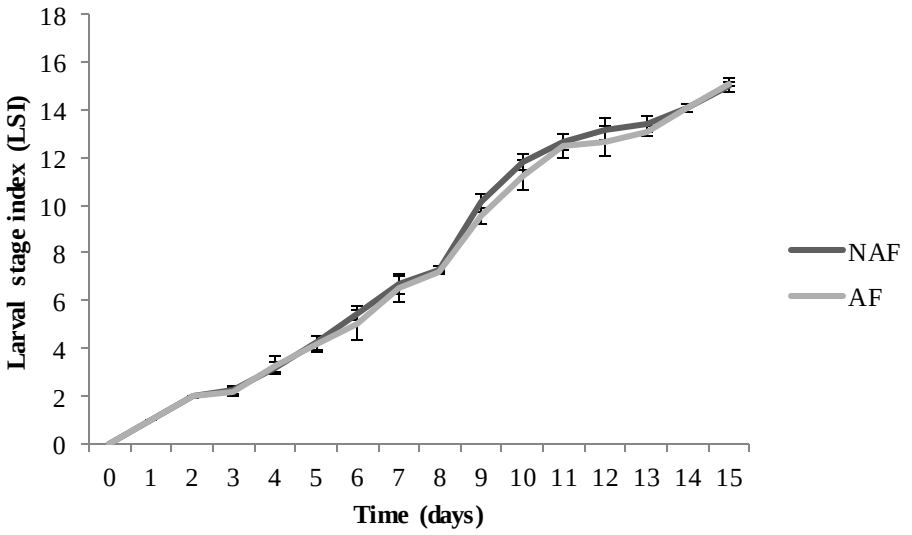

Figure 3: Larval stage index (LSI) (mean ± SD) of *L. vannamei* from non-ablated (NAF) and ablated female broodstock (AF) during larviculture trial I. (n = 3).

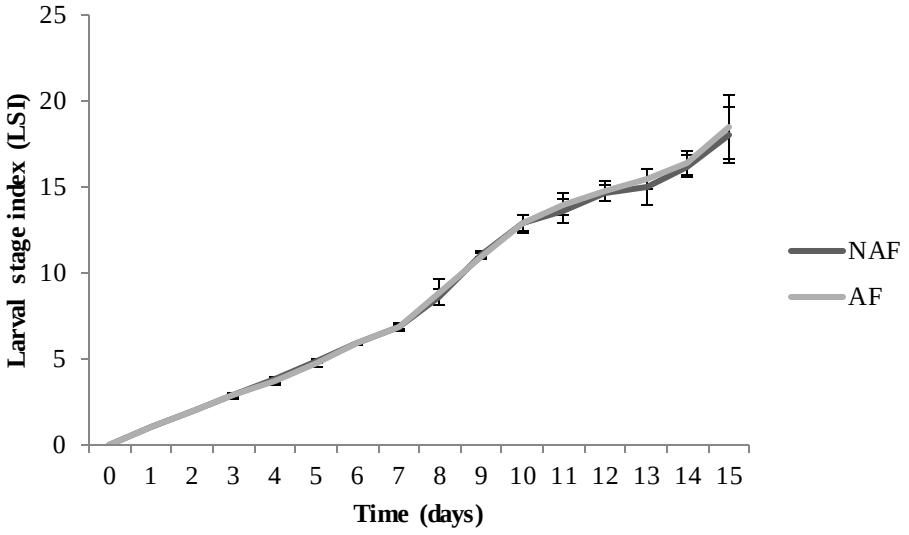

Figure 4: Larval stage index (LSI) (mean ± SD) of *L. vannamei* from non-ablated (NAF) and ablated female broodstock (AF) during larviculture trial II. (n = 5).

Highlights

- Non-ablated Pacific white shrimp (*Litopenaeus vannamei*) females produce more eggs and offspring (>20% and >16%), and have higher survival in hatchery than conventionally ablated broodstock.
- Offspring of non-ablated female perform similarly to those from ablated group from early development stage to consumed size.
- A comparable level of productivity (eggs and nauplii per female) can be realised by a hatchery using non-ablated broodstock.

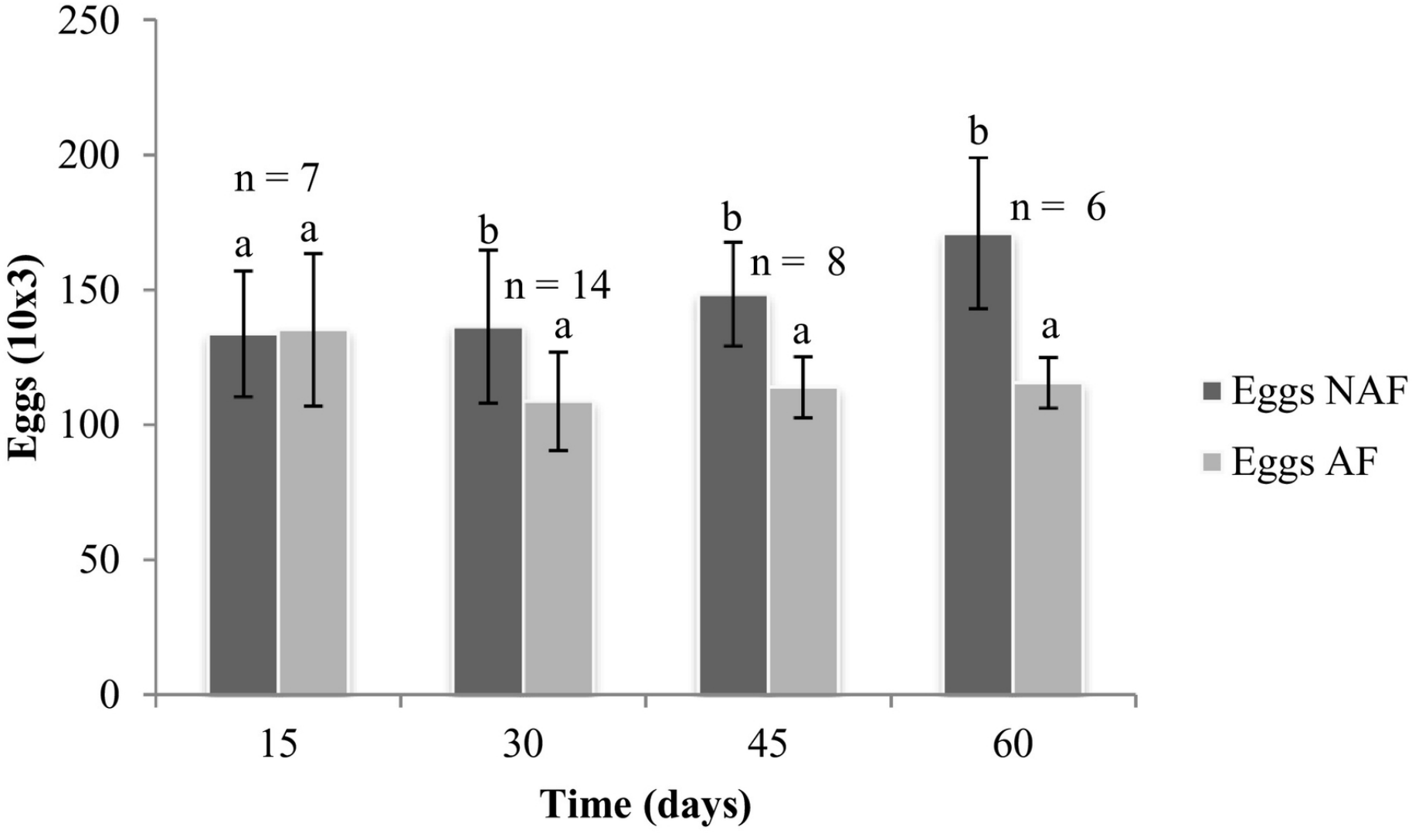
250
200
150
100
50
0
Eggs (10x3)
n = 7
a
a
b
n = 14
a
b
n = 8
a
b
n = 6
a
15
30
45
60
Time (days)
Eggs NAF
Eggs AF

Figure 1

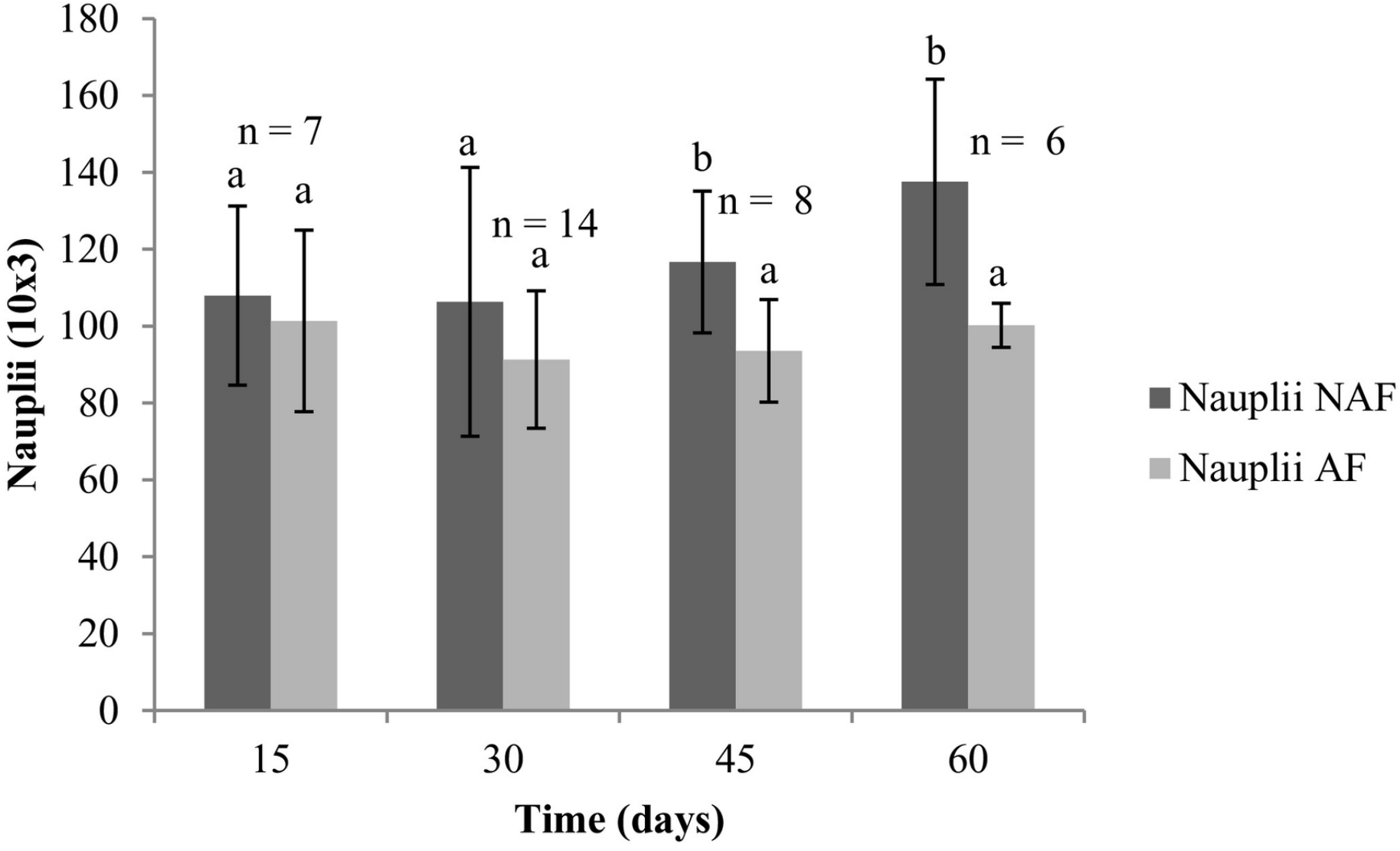
180
160
140
120
100
80
60
40
20
0
Nauplii (10x3)
n = 7
a
a
a
n = 14
a
b
n = 8
a
b
n = 6
a
15
30
45
60
Time (days)
Nauplii NAF
Nauplii AF

Figure 2

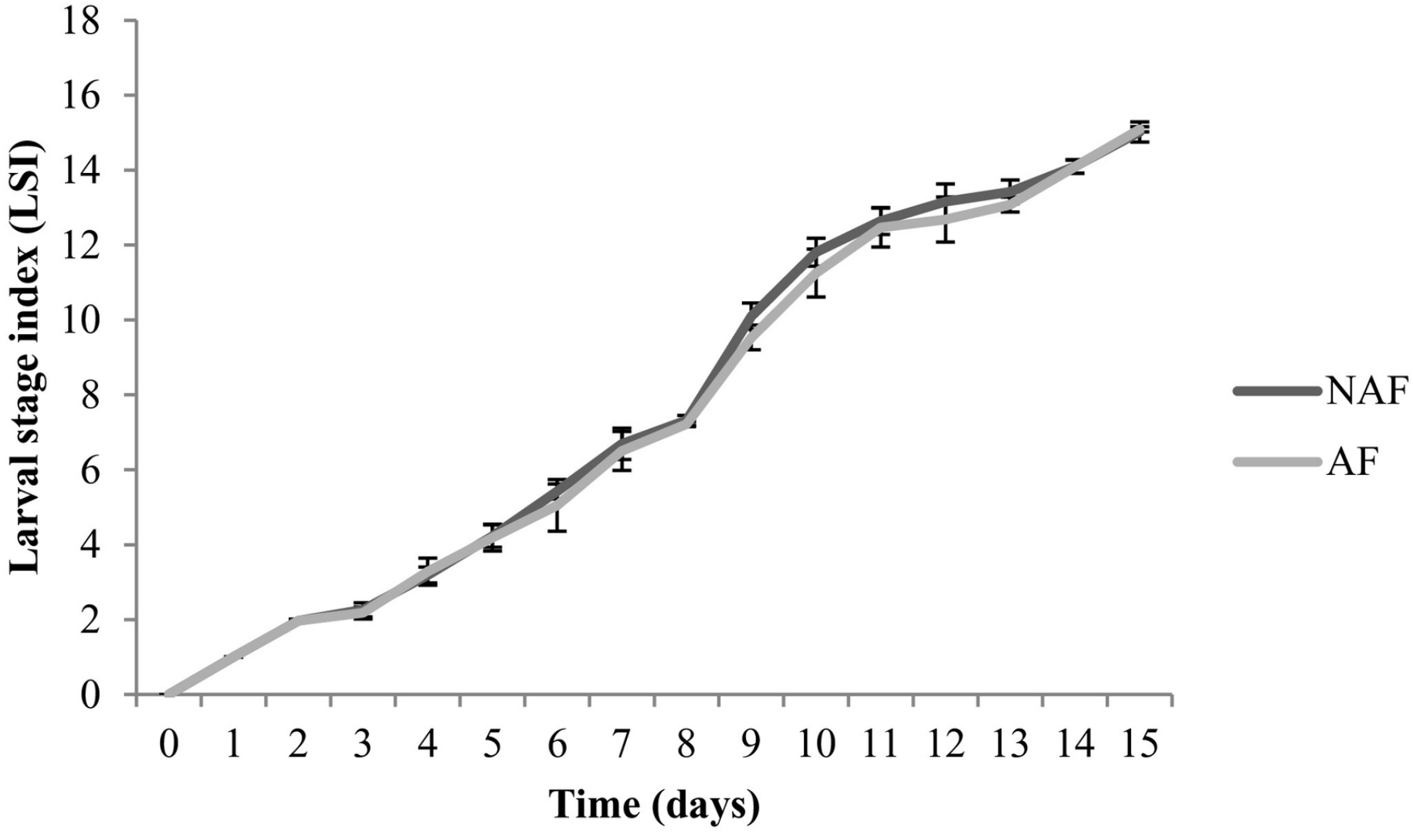
Larval stage index (LSI)
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time (days)
NAF
AF

Figure 3

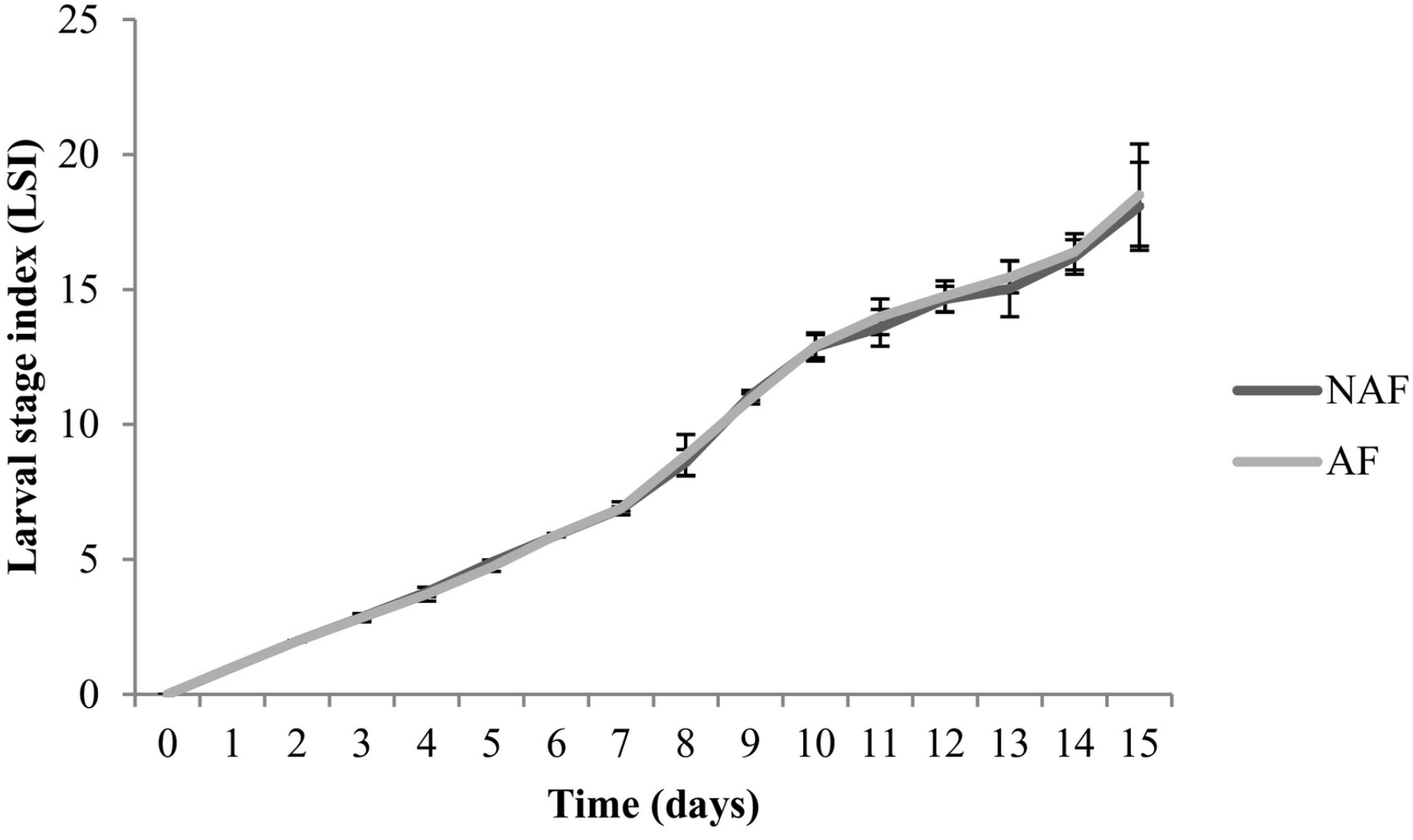
Larval stage index (LSI)
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time (days)
NAF
AF

Figure 4