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Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*

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Abstract :

The floc system has been successfully applied for shrimp grow-out, but little is known about floc contribution on reproductive performance. In a 30-day trial, spawning performance of broodstock was evaluated in floc and earthen ponds. Floc spawners achieved better results (*P* < 0.05) compared with pond spawners in terms of number of spawns per ablated female (2.9 vs. 1.3), spawns per spawning female (3.6 vs. 2.4) and number of consecutive maturation (average of 3.6 times compared with 2.5 times in ponds females). Latency period was lower for floc spawners (7.4 days) than pond spawners (10.7 days) (*P* < 0.05). The percentage of females that spawned at least once were higher in floc (77.1%) when compared with pond spawners (53.8%) (*P* < 0.05). Number of eggs per spawn per g of spawner's body weight and female mortality presented no significant differences between treatments (*P* > 0.05). Biochemical analysis as a nutritional status indicator showed no difference between treatments in hepatopancreas (*P* > 0.05), with the exception of lowest values of acylglycerides and acylglycerides/total soluble protein ratio in floc females ovary (*P* < 0.05). Results evidenced better overall spawning performance for *L. stylirostris* broodstock produced under floc condition than in earthen ponds.

Keywords : floc ; *Litopenaeus stylirostris* ; Reproduction ; broodstock

1. Introduction

The term ‘floc’ is a designation that can be defined as flocculation of organic matter present in the medium resulting in a high concentration of particulate biomass, and could also represent a culture system called ‘moulinettes’ (Aquacop 1975; Cuzon, Lawrence, Gaxiola, Rosas & Guillaume 2004). The latter is described as a water column in constant movement with strong aeration. Floc system was developed in early 70's at Ifremer-COP with different peneid species including *Penaeus monodon, Fenneropenaeus merguiensis, L. vannamei* and *L. stylirostris* (Aquacop 1975; Sohier 1986). Such culture system was compared with an ‘external rumen’ (Cuzon *et al*. 2004), but now applied for shrimp. This concept illustrated a complex interaction between organic matter, physical substrate and large range of microorganisms such as bacteria, protozoa, rotifers, nematodes and others microorganisms, providing a food source for one major species (Ray, Seaborn, Leffler, Wilde, Lawson & Browdy 2010). In 1980, the programme ‘Ecotron’ was initiated by Ifremer to better understand the floc system. Several studies enabled a comprehensive approach to the ‘floc’ and explained interrelationships between different compartments such as water and bacteria, as well as shrimp nutritional physiology.

Currently, other researchers proposed alternate appellation, such as ‘zero or limited water

exchange' (Wasielesky, Atwood, Stokes & Browdy 2006; Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brock 2007; Ballester, Abreu, Cavalli, Emerenciano, Abreu & Wasielesky 2010), suspended-growth systems (Hargreaves 2006) and more recently 'biofloc technology' (Azim & Little 2008; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008; Avnimelech 2009). In early 70's, Ralston Purina developed a system based on nitrifying bacteria while keeping shrimp in total darkness. In connection with Aquacop, such system was applied to *L. stylirostris* and *L. vannamei* both in Crystal River (USA) and Tahiti (Rosenberry 2010), and led to considerations on benefits of floc for shrimp culture.

Originally conceptualized in small volumes (12 m^3), biofloc system was extended to outdoors tanks (30 m^3 circular tanks or 1000 m^2 rectangular concrete tanks). In 1988, a world record in production (20–25 mt ha^{-1} $year^{-1}$) was obtained by Sopomer farm in Tahiti using floc in concrete tanks (Garen & Aquacop 1993; Rosenberry 2010). Currently, floc system is being applied successfully in large-scale shrimp farming in Asia and Latin America (Burford, Thompson, McIntosh, Bauman & Pearson 2004; Taw 2010), although knowledge on bacteria and phytoplankton interaction-control in large-scale shrimp ponds remains scarce. The main problems detected are fluctuations in pH, alkalinity and nitrogen compounds (Rosenberry 2010).

Some authors suggest to stock microalgae before floc development in small-scale experiments (Emerenciano, Wasielesky, Soares, Ballester, Cavalli & Izeppi 2007; Ballester *et al.* 2010) aiming to maintain water quality, provide food and guarantee a source of C:N, as algae growth is followed by bacterial growth (Cole 1982; Williams 2000; Hargreaves 2006). On the other hand, it is possible to leave the medium with its own strains, although phytoplankton bloom could be delayed. Initiating a floc in outdoor conditions remains a rule of thumb. As a general guide, the three considerations would be (i) a minimum of 300 g of shrimp biomass m^{-2} achieved at juvenile stage; (ii) a regular input of dry feed with low protein content (Azim & Little 2008) or application of an external carbon source (Avnimelech 2009); and (iii) limited or zero water exchange.

The potential to introduce disease into shrimp facilities, through infected wild broodstock and vertical transmission of pathogen, has placed considerable interest on penaeid breeding programmes (Ibarra, Racotta, Arcos & Palacios 2007) and production of biosecure closed-life cycle broodstock (Regunathan 2008). Penaeid breeding programmes were frequently associated with rapid growth and disease resistance as well as the enhancement of reproductive performance (Goyard, Patrois, Peignon, Vanaa, Dufour, Viallon & Bedier 2002; Racotta, Palacios & Ibarra 2003; Gitterle, Salte, Gjerde, Cock, Johansen, Salazar, Lozano & Rye 2005). Domestication of broodstock is a rule in shrimp industry to control production plan of successive generations (Coman, Arnold, Callaghan & Preston 2007). However, nutritional problems in domesticated broodstock remain unresolved (Wouters, Lavens, Nieto & Sorgeloos 2001a), and alternatives should be evaluated. Floc as a benefit for shrimp broodstock is still under investigation (Emerenciano, Cuzon & Gaxiola 2011), and its importance on the zootechnical parameters, and relationships between nutrition and different environmental conditions remain to be validated. The aim of this study was to evaluate floc contribution on spawning performance of blue shrimp, *L. stylirostris*, as a preliminary approach of floc application in shrimp broodstock.

Materials and methods

Experimental design and culture conditions

This work was carried out at Ifremer-COP, Tahiti, French Polynesia, and data were derived from three trials of each broodstock source in floc and earthen ponds.

Broodstock production protocol was based on a stock of *L. stylirostris* juveniles (~1 g) raised in 400–700 m^2 earthen ponds (1–1.2 m depth) at a density of 4 shrimp m^{-2} with 5–20% daily water exchange rate. Shrimps were fed commercial feed (40% CP) at 3–10% shrimp biomass twice a day at 08:00 and 16:00 hours. After 4–5 months at 25–30 g, two different management strategies were applied: (i) change of pond stocked at 2 shrimp m^{-2} with a new phytoplankton bloom until transfer to maturation room and (ii) stock in floc system based on 30 m^3 circular fibre glass tanks called 'moulinettes' (Cuzon *et al.* 2004) at 15 shrimp m^{-2} until transfer to maturation room.

Protocol in floc was based on water, vigorously aerated using a finely perforated ring of PVC tube (32 mm; 40 cm diameter) positioned in the centre

of the tank. Water exchange was limited (<5% flow rate), and central waste evacuation was performed daily to prevent sludge accumulation. The tanks were totally covered for an approximately 90% reduction in light by shade cloth to control excessive phytoplankton bloom. C:N ratio was based only on C:N content present in commercial feed (8:1).

After 4–5 months since stocking of PL, animals of both sources were provided a ration of fresh food consisting of frozen squid, once a week, at 2% biomass (based on dry matter) in addition to commercial feed. A summary of experimental procedure is given in Fig. 1.

Reproductive performance

Each reproductive trial (30-day) were performed in eight 4 m diameter fibre glass maturation tanks, four per origin (floc or pond), where one tank was used to stock males and the remaining three for females. The water exchange was carried out with flow-through during summer or recirculation system during winter to keep temperature at 26.5 ± 1.6. Batches of 22–32 females and 50–60 males from each source were stocked at a density of 2.5–3.5 and 5.5–6.6 shrimp m^{-2}, for female and male, respectively. Animals were fed *ad libitum* three times per day at 08:00, 11:00 and 16:00 hours, with commercial feed, frozen squid and frozen mussels.

Before the start of the trial, the shrimps were acclimated for 1 week, and each female was weighed and unilaterally eyestalk ablated to enhance gonadal development and tagged with an eye ring. The spawners with fully developed ovary (ready-to-spawn) was sourced out daily, artificially inseminated with male spermatophores from the same source (manually eject by applying gentle pressure to the base of the genital pore, Arce, Moss & Argue 1999) and transferred to a 180 L conical-bottom spawning tank containing aerated seawater. The number of eggs was estimated from four replicates of 1.0 mL samples, and collected after homogenization of spawning tanks. Reproductive performance was evaluated in terms of female mortality (%), latency period (interval in days between eyestalk ablation and first spawn), number of eggs (per spawn per g of spawner's body weight), number of consecutive maturations per female, percentage of female that spawn at least once (%), number of spawns per ablated female, number of spawns per spawning female, number of spawns per female per month and cumulative spawning rate (%).

Biochemical analysis

After ablation, hepatopancreas (HP) and ovaries (OV) were collected from twelve ready-to-spawn females from each treatment. HP and OV were removed through an incision on the back of the cephalothorax and dorsal region of the entire abdomen length, respectively. Samples were weighed, placed in 1.5 mL Eppendorf tubes, immediately frozen in liquid nitrogen and preserved at −80°C. Subsequently, samples were freeze-dried and re-stocked at −80°C for further analysis.

Changes in metabolite levels of acylglycerides (AG), cholesterol and total soluble proteins (TSP) in HP and OV were used as indicators for nutritional status of female broodstock. AG/TPS (AG:TSP) and AG/cholesterol ratio (AG:C) as a condition index were calculated using metabolites data (Mourente & Rodríguez 1997; Palacios, Ibarra, Ramirez, Portillo & Racotta 1998; Anger 2001). HP and OV were homogenized in 500 ml of distilled water for 2 min. Aliquots of 20 μL were

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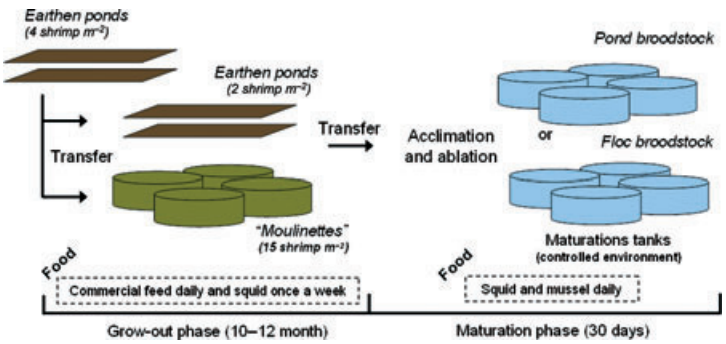

Figure 1 Summary of experimental procedure carried out for growout phase (floc or earthen pond; total of 10–12 month) and maturation phase (30 days).

taken to determine AG and cholesterol with 200 μL of reactive solution (Kits Elitech Diagnostics, Sees, France; cat. TGML-0427 and cat. CHSL-0507, respectively). The rest of the sample was centrifuged at 2500 rpm for 3 min at 4°C, and 20 μL of the supernatant was collected in Eppendorf tubes to determine TPS with 200 μL of Bradford reactive solution (Bradford 1974). Solutions were transferred to microplates in triplicate and incubated in an oven for 7, 6 and 5 min in 37, 37 and 24°C for AG, cholesterol and TPS, respectively. Immediately after, samples were read in an ELISA reader (Bio-Rad Laboratories, Richmond, CA, USA) at 500 nm and 595 nm for total protein. A blank was considered with distilled water and the final concentrations (mg g^{-1}) were calculated from a calibration curve, in which the standard was the substrate as reagent in the kit.

Statistical analysis

For reproductive performance and biochemical analysis, Student's t-test was applied to find the differences among the treatments when data were homogeneous and normality distributed (Sokal & Rohlf 1995). Percentage data were calculated using Chi-Square method, and differences were considered significant at $P < 0.05$ (Zar 1984).

Results

The results of spawning performance are given in Table 1. Number of egg per spawn per g of spawner's body weight and female mortality presented no significant differences between treatments ($P > 0.05$). Floc spawners showed better reproductive performance ($P < 0.05$) in terms of number of spawns (per ablated female and per spawning female) and number of consecutive maturation compared with spawners from ponds (2.9, 3.6 and 3.6 for floc and 1.3, 2.4 and 2.5 for earthen pond, respectively). Latency period was shorter for floc spawners (7.4 days) than pond spawners (10.7 days) ($P < 0.05$). The percentage of females that spawned at least once were higher in floc (77.1%) when compared with pond ones (53.8%) ($P < 0.05$). Cumulative spawning rate (%) from 3rd to 10th day indicated that females from floc had a slightly higher rate than females from ponds, and then both sources showed the same trend until 30th day (Fig. 2). Number of spawns per female per month was similar during the first 2 days (Fig. 3). From the 3rd day, a difference in performance was observed that was maintained until day 30 (2–3 spawns per female per month in floc vs. <1 in pond females). Floc spawners showed a quicker response to ablation by presenting a higher rhythm of spawn in a shorter period of time (Fig. 2 and 3), as well as achieved a higher spawn order compared with pond ones (Fig. 4).

The biochemical data (Table 2) from hepatopancreas showed no significant differences for all variables between floc and earthen pond spawners ($P > 0.05$). On the other hand, in ovary, AG (64.2 mg g^{-1}) and AG:TPS (0.6) were higher for pond spawners than for floc spawners (AG: 42.2 mg g^{-1} and AG:TPS: 0.4) ($P < 0.05$).

Discussion

Reproductive performance

In a shrimp hatchery, a large percentage of the females in any production cycle spawn only once

Table 1 Spawning parameters (means ± SD) of *L. stylirostris* broodstock from floc system and earthern pond during a period of 30 days

	Pond	Floc	Significance level
Reproductive parameters			
Weight female (g)	40.5 ± 4.3[a]	53.6 ± 6.5[b]	***
Weight male (g)	35.4 ± 6.0[a]	50.5 ± 3.8[b]	***
Number of egg per spawn ($\times 10^3$) per g of spawner's body weight	3.3 ± 1.1	3.9 ± 1.9	ns
Female mortality (%)	69.9 ± 29.8	68.0 ± 15.7	ns
Number of spawn/ablated female	1.3 ± 0.7[a]	2.9 ± 1.5[b]	*
Number of spawn/spawning female	2.4 ± 0.4[a]	3.6 ± 0.9[b]	*
Number of consecutive maturation per female	2.5 ± 0.5[a]	3.6 ± 0.8[b]	*
Latency period (days)	10.7 ± 4.6[a]	7.4 ± 1.8[b]	*
Females that spawned at least once (%)	53.8 ± 27.2[a]	77.1 ± 20.0[b]	**

Within rows, superscript letters indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, no significant difference).

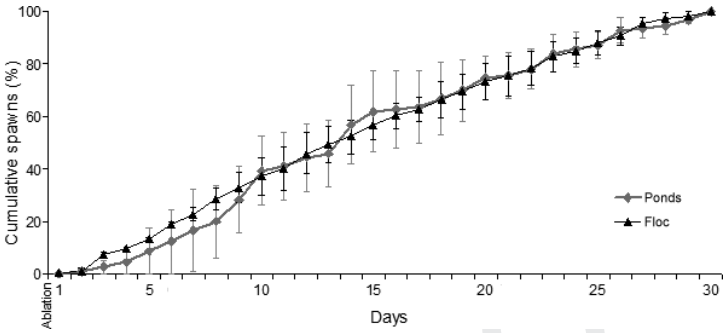

Figure 2 Percentage of cumulative spawns (means ± SE) of *L. stylirostris* spawners from floc system and earthen pond during a period of 30 days from ablation.

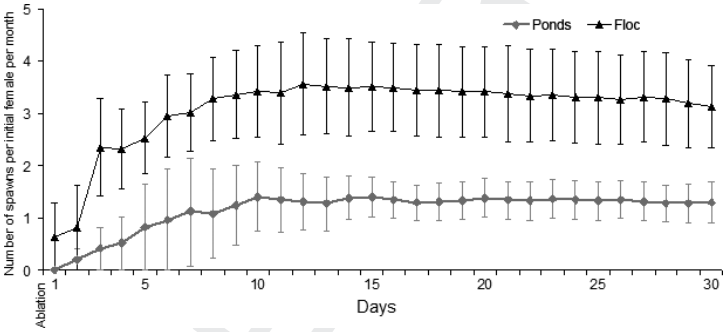

Figure 3 Number of spawns per initial female per month (means ± SE) of *L. stylirostris* spawners from floc system and earthen pond during a period of 30 days from ablation.

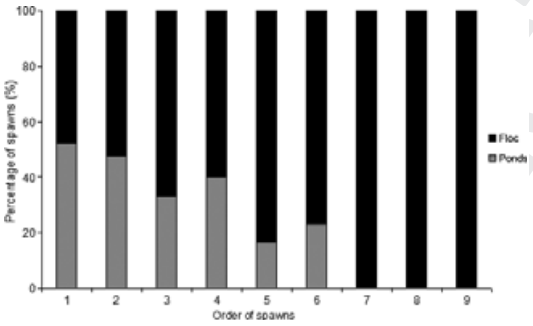

Figure 4 Percentage of spawns (%) in each spawn order of *L. stylirostris* spawners from floc system and earthen pond during a period of 30 days from ablation.

or sometimes never spawn, whereas a small percentage is able to spawn several times (Cavalli, Scardua & Wasielesky 1997; Palacios, Racotta & APSA 1999a; Ibarra *et al.* 2007). Hence, the number of spawns is considered as an important criterion to evaluate broodstock performance (Ibarra *et al.* 2007). High-quality spawners will represent higher amount of spawns per animal, and are less demanding in terms of costs and broodstock maintenance to achieve a target production.

In this study, floc females in terms of number of spawns per ablated female and number of spawns

Table 2 Biochemical composition (means ± SE based on dry matter) of hepatopancreas and ovary of *L. stylirostris* spawners from floc system and earthern pond collected during a period of 30 days after ablation

	Pond	Floc	Significance level
Hepatopancreas			
Acylglycerides (mgg^{-1})	63.3 ± 6.1	64.2 ± 6.4	ns
Cholesterol (mgg^{-1})	12.6 ± 1.7	14.3 ± 1.6	ns
Total sol. protein (mgg^{-1})	93.4 ± 15.2	85.3 ± 14.8	ns
AG:TSP	1.0 ± 0.4	1.2 ± 0.3	ns
AG:C	4.5 ± 0.6	4.5 ± 0.5	ns
Ovary			
Acylglycerides (mgg^{-1})	64.2 ± 3.7[a]	42.2 ± 3.1[b]	**
Cholesterol (mgg^{-1})	8.1 ± 0.5	11.6 ± 3.8	ns
Total sol. protein (mgg^{-1})	111.0 ± 9.1	97.4 ± 6.6	ns
AG:TSP	0.6 ± 0.1[a]	0.4 ± 0.04[b]	*
AG:C	8.0 ± 0.5	6.6 ± 2.9	ns

Within rows, superscript letters indicate significant differences (*$P < 0.05$; **$P < 0.001$; ns, no significant difference). Hepa-

per spawning female performed an average of 1.6 and 1.2 times more spawns than pond females, respectively. Females in floc spawned 3.6 times per spawning female, which is much more than observed in wild or domesticated *P. esculentus* (Keys & Crocos 2006), *Farfantepenaeus paulensis* (Peixoto, Wasielesky, Martino, Milach, Soares & Cavalli 2008) *F. indicus* (Regunathan 2008) and *L. stylirostris* (Mendoza 1997). The percentage of females that spawned at least once, was 20% higher in floc females than pond ones (77.1 vs. 53.8%). It is in agreement with the spawning performance of wild *P. monodon* (78.2%) (Menasveta, Piyatiratitivorakul, Rungsurpa, Moree & Fast 1993). Furthermore, females from floc maturated in an average of 3.6 times, with a maximum of nine consecutive maturations per female compared with 2.5 times, with a maximum of six maturations from pond females. These values are higher than wild and pond-reared *P. monodon* performances with a maximum of seven and three maturations per female, respectively (Menasveta *et al.* 1993) and pond-reared *L. stylirostris* with a maximum of four (Wabete, Chim, Pham, Lemaire & Massabuau 2006).

Latency period is related to the initial time required for biochemical and physiological changes after eyestalk ablation (Cavalli *et al.* 1997; Peixoto *et al.* 2008), and these changes were based on nutrient storage transport from hepatopancreas to ovary for egg development. Eyestalk ablation causes severe hormonal changes, reducing or suppressing moult-inhibiting hormone (MIH) and gonad-inhibiting hormones (GIH) (Sainz-Hernández, Racotta, Dumas & Hernández-López 2008). Latency time can vary, as some females are able to develop full ovaries and spawn in 3–5 days, but if moulting occurs soon after eyestalk ablation, the maturation period could extend to 2–3 weeks (Aquacop 1979). In addition, the latency period is an indicator of females with multiple spawning capacities and a criterion to establish the cut-off period of non-spawning females (Palacios, Ibarra & Racotta 2000; Racotta *et al.* 2003). In this study, females from floc had a shorter latency period by 3 days compared with pond spawners (7.4 vs. 10.7 days). This is less than 24–40 and
17–33 days reported in wild and pond-reared *L. vannamei*, respectively (Palacios *et al.* 2000), 10 and 30 days reported in captive and sea-caught *F. paulensis* (Cavalli *et al.* 1997; Peixoto, Wasielesky, D'Incao & Cavalli 2003; Peixoto, Cavalli, Wasielesky, D'Incao, Krummenauer & Milach 2004) and finally 9.4 and 16.8 for wild and pond-reared *P. monodon* (Menasveta *et al.* 1993). This result is probably related to a better nutritional physiology condition of eyestalk ablated females from floc. The same trend was observed in wild *F. paulensis* females that started spawning earlier and presented a higher spawning frequency than in ponds conditions (Cavalli *et al.* 1997). According to Muthu (1983), species such as *F. merguiensis, F. indicus, L. vannamei* and *L. stylirostris*, generally mature more rapidly (3–4 days) than *P. monodon* and *F. aztecus* (3 weeks).

Mortality rates were high in both treatments with 69% and 68% for ponds and floc, respectively. The reason was possibly due to a strong physiological stress resulting from eyestalk ablation followed by multiple spawning rates. Mendoza (1997), evaluating the same species, reported an overall mortality of 49% in wild and domesticated females, but with less number of spawns per female when compared with our results. Therefore, a high mortality, aside from stress, was closely related to a high number of spawns followed by progressive decrease of reserve levels (Palacios, Perez-Rostro, Ramirez, Ibarra & Racotta 1999b).

Weight differences observed in our study did not affect the overall result. Menasveta, Sangpradub, Piyatiratitivorakul and Fast (1994) observed that larger *P. monodon* females (>120 g) underwent more advanced stages of maturation and spawned with greater success than small (<110 g) spawners. On the other hand, Coman, Arnold, Peixoto, Crocos, Coman and Preston (2006) suggested that broodstock size was not the sole factor affecting the spawning performance of wild and domesticated *P. monodon*. In our study, shrimp weight was in a range of 40–53 g, an adequate size for *L. stylirostris* broodstock (Chamberlain & Lawrence 1981; Bray, Lawrence & Lester 1990; Mendoza 1997). Hence, it was assumed that animals were in the same physiological condition. Differences in maturation observed after ablation were due to previous nutritional status in relation to floc or pond conditions. Results in terms of eggs production were similar to wild *L. stylirostris* (Alfaro, Zúñiga & Komen 2004), pond-reared (Bray *et al.* 1990) and followed consecutive years of massive selection performed in Ifremer-COP (Goguenheim *et al.* 1999) .

Biochemical analysis

No significant differences were found in metabolites from HP and OV, except in AG and AG:TSP ratio of ovary. AG is a readily accessible source of energy that can be used during periods of food shortage (Fraser 1989), and its reduced levels (42.2 vs. 64.2 mg g^{-1} for floc and ponds, respectively) are related to the large amount of energy required for spawning activity (Palacios, Perez-Rostro, Ramirez, Ibarra & Racotta 1999b). Successive spawns following a decrease in nutrient storage induced a progressive decrease of spawning rates, well explained as reproductive exhaustion (for reviews, see Lumare 1979; Palacios *et al.* 1999b).

According to Anger (2001) AG:TSP is an energetic ratio that evidence AG storage when higher values present differences between treatments. On the other hand, AG:C is a nutritional ratio that could indicate nutritional deficiency in shrimp when values are quite close to zero (Anger 2001). These ratios as a condition index are largely affected by energy availability (Mourente & Rodríguez 1997). In the present study, AG:TSP measured in ovary indicated a significantly better response in females from pond than those from floc. Similar metabolites trend was obtained in *F. paulensis* with or without sand substrate in maturation tanks (Nakayama, Peixoto, Bianchini, Robaldo & Cavalli 2008), however, with decrease in another source of energy (glucose).

AG, cholesterol and TSP content can just give a preliminary information on nutritional status of shrimp, but nutritional indicators such as fatty acids profile and different classes of lipids (polar and neutral lipids) are more indicative. These nutrients are strictly related to the reproductive performance (Teshima & Kanazawa 1983; Teshima, Kanazawa, Kushio & Horinouchi 1988). Cahu, Guillaume, Stéphan and Chim (1994) showed that conditions with low dietary phospholipids concentration associated with a low HUFA level induces a decrease in the spawning rate of *P. vannamei*, as female has limited ability for their biosynthesis. On the other hand, the knowledge on specific nutrient requirement for successful reproductive performance is still limited, and a better spawning income could not be attributed to any single nutrient (Regunathan 2008). Meanwhile, it could be presumed that a deficit in essential aminoacids, fatty acids (mainly represented by PUFAs and HUFAs) or vitamins can contribute to decrease on embryogenesis of *M. rosenbergii* (Clarke, Brown & Holmes 1990), vitellogenesis of *L. vannamei* (Wouters, Piguave, Bastidas, Calderón & Sorgeloos 2001b) and affect the overall reproductive performance (Teshima *et al.* 1988). The benefit of floc is difficult to clarify due to the lack of precision of its composition, as well as the variation in the amount of particulate biomass ingested, apart from nutrients derived from pelleted commercial feed . Furthermore, more research efforts are needed to evaluate different floc protocols and food management (with or without fresh food in a short-term prior to the reproduction season) with an aim to improve *L. stylirostris* reproduction fed only on dry feed.

Floc vs. pond-reared cultured systems

The better spawning performance of females from floc could be explained from a nutritional and 'susceptibility to stress' points of view, where conditions differed from pond to floc systems (Table 3). Besides the stocking density, variations in abiotic factors, such as pH and dissolved oxygen, caused by phytoplankton blooms could deplete broodstock health in ponds (Wabete *et al.* 2006). Also, temperature fluctuations in ponds due to water stratification and water exchange could create stress on animals during daytime. Moreover, limited natural productivity in ponds could not represent a

Table 3 Summary of factors that could influence the broodstock health and nutrition in general earthen ponds and floc system, and its respective score

	Pond	Floc
Abiotic and crowding		
Temperature, DO and pH fluctuations	***	*
Ammonia control	*	**
Salinity variance	*	**
Crowding	*	***
Nutritional factors		
'Native protein' and essentials aminoacids[1]	*	***
Phospholipids	*	**(?)
Lipids, PUFA's and HUFA's[2]	*	***
Cholesterol	*	**(?)
Vitamins	*	**(?)
'Unknown growth factor'	*	***(?)

Scale are given by *, ** and *** that represent low, intermediate and high levels or effects; DO, dissolved oxygen;[1] Ju *et al.* (2008); [2]Azim and Little (2008), Crab *et al.* (2010) and Ekasari *et al.* (2010); ?, unclear levels.

significant portion of essentials nutrients for shrimp, in particular, at high biomass.

In contrast, floc conditions present a regular availability of food in a form of highly diverse 'native protein' that include bacteria (Ballester *et al.* 2010), microalgae, protozoa, nematodes (Azim & Little 2008), copepods and rotifers (Decamp, Conquest, Forster & Tacon 2002; Ray *et al.* 2010). The concept of 'native protein' is related to protein source without previous treatment mainly including live food. Bacteria play an essential role in this equilibrium and re-ingestion of particulate organic matter and faeces (coprophagia) left by shrimp results in a form of constant food supply. The colonization of shrimp gut by bacteria is another benefit with a possible probiotic effect. Bacteria biomass intake could be responsible for better reproductive performance under floc conditions. Moreover, floc is a source of free aminoacids (Ju, Forster, Conquest, Dominy, Kuo & Horgen 2008), total lipid (Azim & Little 2008) and essential fatty acids (Crab, Chielens, Wille, Bossier & Verstraete 2010; Ekasari, Crab & Verstraete 2010). It is well known that these nutrients significantly affect the reproductive performance in peneid broodstock (Paibulkichakul, Piyatiratitivorakul, Sorgeloos & Menasveta 2008), and could also influence the initial gonad tissue formation at juvenile stages.

Abiotic factors are better controlled by tank shading and low or zero water renewal (Crab, Kochva, Verstraete & Avnimelech 2009) providing a more stable medium for broodstock (Wabete *et al.* 2006). Heterotrophic bacteria might control phytoplankton blooms as well as reduce pH-dissolved oxygen fluctuations, typically presented in ponds. Moreover, nitrogen compounds such as ammonia is constantly recycled and uptaken by heterotrophic bacteria (Avnimelech 1999). Although male quality was not the aim of this study, it is important to note that male from pond presented more frequent melanized spermatophore than floc males, even though undamaged spermatophores were used. This trend seems to be associated with dissolved oxygen-temperature fluctuations that could deplete male health in ponds (Sánchez, Pascual, Sánchez, Vargas-Albores, Moullac & Rosas 2001).

Conclusion

Floc is a difficult concept to explain, because it is a complex system with continuous physical, chemical and biological interactions. From a nutritional point of view, there is no clear evidence as to how floc participates in the nutritional requirements. Incidence on weight gain was shown in the past (Burford *et al.* 2004; Wasielesky *et al.* 2006), unfortunately no specific nutrient has been detected. One postulation is related to 'growth factor' similar to that found in squid (Cruz-Ricque, Guillaume & Cuzon 1987; Guillaume, Cruz-Ricque, Cuzon, Wormhoudt & Revol 1989). Nevertheless, one could emphasize the fact that floc is a source of 'native proteins', fatty acids preserved from oxidation and vitamins (Crab *et al.* 2010). During the drying processing, the floc was produced in bioreactors and included in compounded feeds, and the nutritional characteristics could be affected by high temperatures.

This study contributes to a better understanding of the role of floc on spawning performance of shrimp. Floc females had higher spawn activity in a shorter period of time compared with pond females. The spawners from floc require less time to start spawning as see by the shorter latency period. Spawning performance is also attributed to a better nutritional status and medium conditions experienced by floc females in grow-out phase (Table 3). Ovary AG content was lower in floc females than pond ones due to a higher need of energy for multiple spawns and consequently, reproductive exhaustion experienced. There is a need for further research to achieve a better understanding of the role of floc in broodstock nutrition.

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