"Floc" Story

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Abstract

What makes the effectiveness of the "floc" on growth of tropical shrimps and its reproductive capacities? The "floc" as called it 40 years ago also named "particulate" biomass came from the design of sludge from that purify the water on the one hand and consideration on the power mode of the other bivalves. When we come to these concepts we arrive at the concept of floc which produces a kind of shrimp "external rumen". What are the benefits that the animals retrieve? This article gives a review of chains of explanations in terms of environmental quality and the nutritional physiology of shrimp lead to what is now the most popular growing breeding model. This model represents a future opportunity to shrimp farming in the world by saving water, increased stocking densities and lower surfaces used and probably a freedom from direct use of the coastal zone. Will therefore be considered in this article nutritional aspects, changes in phytoplankton and microbial population and the interactions between nutrients, dissolved organic matter that give this chaotic culture medium and difficult aspect to control in any case and renderers very difficult to model. But some aspects of the shrimp physiology as its food/feed supplement behavior seem fixed in this farming system called "moulinettes" and recently resumed in the acronym "BFT biofloc" technology sometimes slightly obscuring the entire history of research in this area.

Key words: floc, bacteria, plankton, water quality, sludge, intensive culture, juveniles, breeders

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Parte de la historia del “floc”

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Resumen

¿Cuál es la eficacia del "floc" sobre el crecimiento de los camarones tropicales y sus capacidades reproductivas? El "floc" como se denominó hace 40 años la “biomasa partículada", provino del diseño de recuperación de los lodos a partir de los sistemas de purificación de agua por un lado y la consideración del modo de alimentación de los bivalvos por otro lado. Cuando llegamos a estos conceptos se llega al concepto de “flóc” que se define como un "rumen externo" para las especies como los camarones. ¿Cuáles son los beneficios que los animales obtienen? Este artículo ofrece una revisión en términos de calidad del medio ambiente y la fisiología nutricional de camarones a lo que hoy es el modelo de cultivo en crecimiento más popular. Este modelo representa una futura oportunidad para el cultivo de camarón en el mundo por el ahorro de agua, el aumento de las densidades de población, superficies más bajas utilizadas y, probablemente, una libertad por evitar el uso directo de la zona costera. Por lo tanto, serán considerados en este artículo los aspectos nutricionales, cambios en el fitoplancton, la población microbiana y las interacciones entre nutrientes, materia orgánica disuelta que dan a este medio de cultivo caótico un aspecto difícil de controlar en cualquier caso y es muy difícil de modelar. Sin embargo, algunos aspectos de la fisiología de camarones como su comportamiento con suplemento alimenticio, pienso que parecen fijos en este sistema de cultivo llamado "moulinettes" y recientemente se reanudaron con el acrónimo tecnología biofloc "BFT " a veces oscurece parte de la historia de la investigación en esta área

Palabras clave: floc, bacteria, plankton, calidad de agua, lodo, engorda intensiva, juveniles, repr

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Introduction

When swimming in polynesian lagoon it can be seen through the mask some small whitish elements suspended in clear water poor in phosphorus that is trapped by zoothanthelles from coral reef; this is what is called a "light floc" support for bacteria and potential food for fish larvae, crustaceans and molluscs in addition to dissolved organic fraction that can be assimilated by oysters.

Long time without knowing, it promoted bacterial growth. Moreover originally when the COP met the Ralston Purina team in Crystal River (USA) early results in floc were due only to bacteria, bacteria of nitrification that transformed ammonia into nitrites and nitrates and then medium stood at equilibrium.

Up to 1977, COP/Tahiti used 12m^2 flat bottom circular tanks in fiberglass. Results obtained with *P.vannamei* and *L.stylirostris* were consistent, allowing to get in final 2.3kg per m^2,av. weight 22g with 108 per m^2 of shrimp in very good health. In theory, it yielded about 46mt/ha/year with a 10% daily water renewal. In 1977, CNEXO/cop launched a program on intensive shrimp farming to 100 individuals per m^2 based on permanent agitation and aeration of the medium and low turnover, the pumping cost being an important factor in the livestock economy. Results evidenced the role of bacteria in the system that produced together with organic matter (feed, leftovers, feces) in constant suspension, the formation of «activated sludge» (Ecotron, 1980). This compound was already found beneficial for *M japonicus* and incorporated up to 12% in a compounded feed (Shigeno, 1972). Actually there is a regain of single cell protein to replace fishmeal (Ali, 1992).

The shrimp were receiving so rich carbohydrate feed, low protein that favored the bacteria development; working in dark condition, it was a way to simplify the model turning into pure bacterial mode. Shrimp such as *P.vannamei* or *P.stylirostris* get accommodated perfectly. But we had to move to another level and this is where the system is complicated with the contribution of microalgae. We had to cope together to achieve significant production scale. Studies were then focused on the balance between bacteria and phytoplankton (Ecotron, 1980-85). Phytoplankton contributes to the formation of exopolysaccharides after the phyto 's phase of senescence. That's provide in the culture medium a support for bacteria, filamentous microalgae, protozoans and nematods,.. nominated as "particulate biomass". Obligatory scaling experienced some success, then the Sopomer farm used the "floc" technique but the management of culture medium faced little to small problems that took over and the farm switched to management of "green water" which failed to make this shrimp farm sustainable.

Material and methods

-The first technique was initiated by RP in Crystal River in 200L indoors tank
-Ecotron experiment in 5L, and to 10m^3
-on initial biomass,
-transfer from previous preformed floc,
-adding salts or sugar as carbon source
-mastering floc in small tanks, or floc+phytoplankton in outdoors volumes

"Five different aspects of the impact of bacterial communities in the cultures were studied: (i) the quantitative and qualitative development of bacterial communities (biomass, production, species diversity, distribution); (ii) estimation of the metabolic potential of isolated bacteria (catabolic potential, nutritional requirements, auxotrophy/prototrophy); (iii) potential for liberating telemediaters by bacteria, vitamins B_{12}, thiamine, biotine, antibacterial substances, anti-algal substances; (iv) the zoopathogenic power of certain strains and (v) particulate or dissolved primary production and an analysis of feces with heterotrophic activity" (Martin *et al.*, 1978).

The following scheme summarizes main steps of shrimp life cycle where floc is commonly set to enhance weight gain, health status and potential for reproduction in clear water (CW) compared to floc.

PI's	juveniles	comercial size	ponds	maturation area	*eyestalk ablation*	spawn
	5g	20-25g	35-40g	CW+ff		
feed+meoifauna						
feed+floc			floc+feed			
4-5months			4months	2-3weeks		

-managing as in SE Asia (Vietnam, Indonesia, Malaysia, Philippines… with aerators and enough water depth

-managing back to its initial i.e. in hyper-intensive system indoors (Poulain, com. pers. 2014).

Results

1972, Shigeno formulated diets with activated sludge for juveniles *P.japonicus.* Reported by those authors that could be considered as a pioneering work in direction of further floc utilization to participate to the supply of single cells protein source.

1972 Ralston Purina and Aquacop (1976): the first trials were conducted in a system called “moulinettes” that involved a bacterial floc. This work done in collaboration with Ralston Purina was conducted on wild specimen and shrimp fed on marine ration MR^{30} or MR^{25} showing a possibility for a low requirement for protein under floc conditions.

Table 1-Trial in “moulinettes” with *L.vannamei* for 27days.

feed	shrp/m^2	IW	FW	wt gain%	survival%	FCR
MR^{30}	40	3.3	6	81	100	1.7
MR^{25}	40	3.2	5.8	78	95	1.7
18.1.1.0	40	3.2	4.9	53	100	2.2
MR^{30}	150	3.1	5.5	77	91	-
MR^{30}	260	3.3	5.6	70	91	-

Reported results on *L. stylirostris* were similar to those observed on *L.vannamei* (Table1) and shrimp placed in 170l tanks were fed at 4%biomass daily. The set up of “moulinettes” as in Ralston Purina (13m^3 round tanks let achieve stocking densities approaching 3.5kg/m^2 at end (Aquacop, 1981).

1975-80 Ecotron program: In 1978, two circular concrete tanks 700m^2 around 0.8m depth with compacted coral in the bottom were built up at COP to change scale of production. Preliminary trials raised problems such as N-ammonia increase after 35d and no nitrification therefore nitrates did not form. Inhibition of nitrifying bacteria could be due to sterilized action of solar UV and a poor re-suspension of bacterial floc. The apparition of toxic dinoflagellates provoked massive mortalities.

In 1980 results and analysis evidenced the role of bacteria in the system and nitrifying bacteria will transform N-ammonia excreted in nitrate; the process of total nitrification lasted 29d without water renewal. Those bacteria definitely were implicated in the feeding of shrimp. Floc composition was surveyed with proximate analysis, but only few data exists on lipid classes indicative of a presence of mono and tri-acylglycerids and free cholesterol, 2.6% total lipids (Galois, 1980).(Table 2) and phospholipids remained

Table 2. floc composition for acylglycerids and sterols (Galois, 1980).

	mg/g dry wt.	%TL
acylglyc.		
mono	0.77	3.39
di	0.16	0.71
tri	0.6	2.64
sterols		
free	0.59	2.6
esterif.	0.03	0.13

largely to be taken into account because of presence of live microorganisms in constant supply.

Three stages were described by Sohier and Bianchi (1985) in a closed system with interrelationships between autotrophic nitrifying bacteria and the related heterotrophic community (Fig 1). During the first few days the bacterial community derived from seawater, shrimp and feed. Both ammonia and nitrite increase and the chemical composition of the water select a low diversity community requiring free amino acids and organic C plus energy. 20d later, the bacterial community reaches equilibrium in a so-called “non-stressed environment. The heterotrophic community on the contrary possessed a high diversity for metabolites. Few months later, there is a dominance of bacteria from feces and feed/food.

food/feed web in dark (indoors)	food/feed web w/ phytoplankton (outdoors)

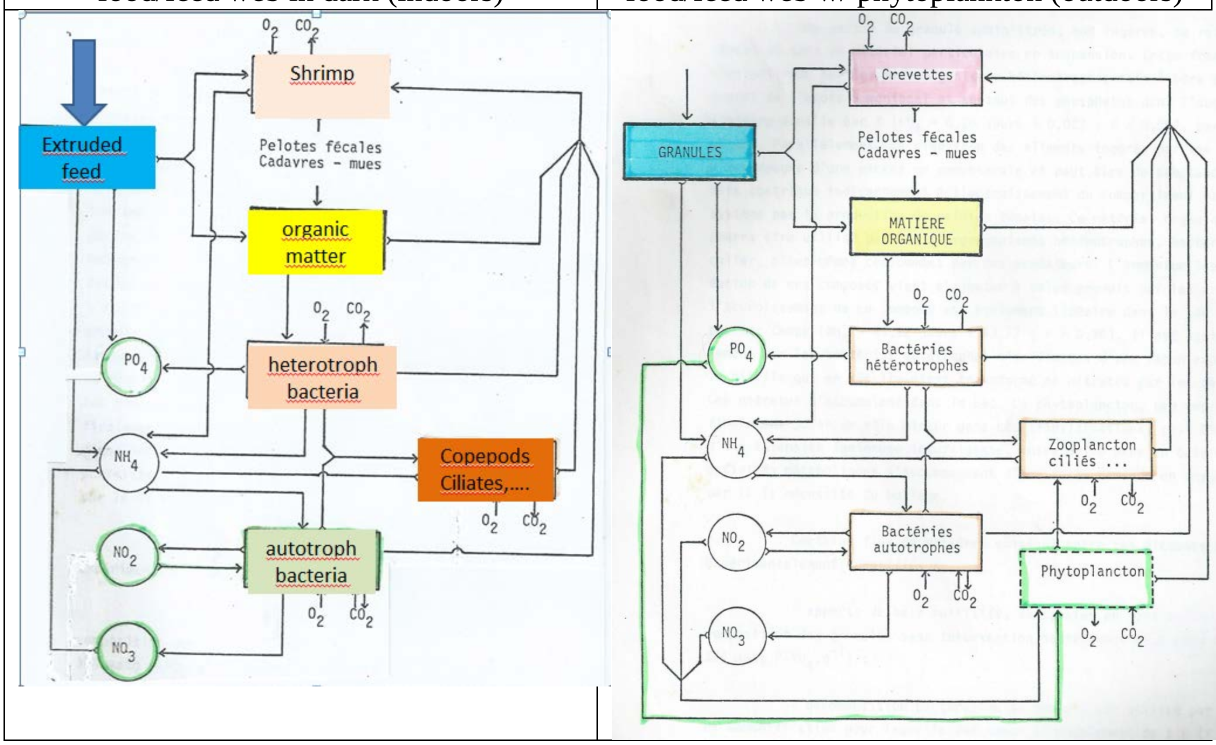

Fig 1. Ecotron, 1980

-sludge: the floc system can be managed without too much problem and with a minimum of microalgae. Yet, in routine, there is a need for flushing the tank that liberates dark black water, made of liquid sludge to be eliminated each day. In this context, the «zero water exchange» will produce foam on water surface. In practice, at lab level, during the

Ecotron program short term experiments trials at 10, 30 and 50% water renewal in "moulinettes" K, L and M produced the following:

Table 3. Effect of water renewal on survival in floc with *P.monodon* juveniles (Ecotron, 1980).

	units	K		L		M	
water renewal		0		10		50	
		t_0	t_{35}	t_0	t_{35}	t_0	t_{35}
av. wt	g	5,4	6,3	3,9	**5,8**	3,6	5,3
av. wt gain	g		0,9		1,9		1,7
daily wt gain	% j_{-1}		0,5		1,4		1,3
feed input	g d_{-1}		280		197		167
biomass	g	1036	476	1002	800	1003	770
yield	%		0		12		9
survival	%		46		**80**		77

NB. «moulinettes» is a term called to describe a tank system where water depth is enough (>1m) to allow convection movements in the medium and maintain particulate biomass in suspension all the time, shrimp can remain quite on the bottom ready to filtrate particles in suspension and catch pieces of compounded feed before it desintegrate in the water, «re-invested» in mocrobiota (heteroptophic bacteria) or/and in zooplankton when present.

On the basis of Table 3 water renewal had a significant impact on weight gain and survival. In spite of low % in moulinette K, final weight is slow taking into account its initial weight due to cannibalism probably exacerbated by water quality. Then, the low biomass was drastically low in final. In regard of such experimental result, the best compromise with juveniles *P .monodon* would have been observed in moulinette. In practice, one would consider a survey with a minimum of water renewal to maintain water level. In fact, «zero water exchange» can provoke temperature fluctuations (day/night) in outdoors conditions that could impact the weight gain. This being, in Vietnam (2014) for example, it is observed liner ponds 300-500m^2 equipped with paddle wheels that produced in floc mixed with microalgae. Therefore when a pure floc is operated, the amount of sludge is substantial but the association floc+microalgae could purify the environment to a certain extent.

In the end, the intrinsic contribution of the feed to the energy level is much higher than the "floc". The feed's energy density is unrelated to that of floc from its composition (energy density of about 15kJ g^{-1} while "floc" with its 70% water is almost 5 times less dense. The problem persists at intake that is difficult to assess. In fact, the feed on the one hand undergoes a leaching due to water movements caused by strong bubbling, and so a part of the daily shrimp ration escapes but not included heterotrophic bacteria especially during the last months of culture. On the other hand, floc appears to be ingested within 1% of body weight (Ecotron, 1980), but the data obtained in small volume (5L) remains questionable due to an extrapolation compared to shrimp in % per day. Assuming floc ingested quasi-continuously, this biomass seen its water content does not represent a substantial contribution gravimetrically compared to dry feed.

Fig 2. Bioenergetics in clear water (Gauquelin *et al.*, 1996) and in floc (2010)

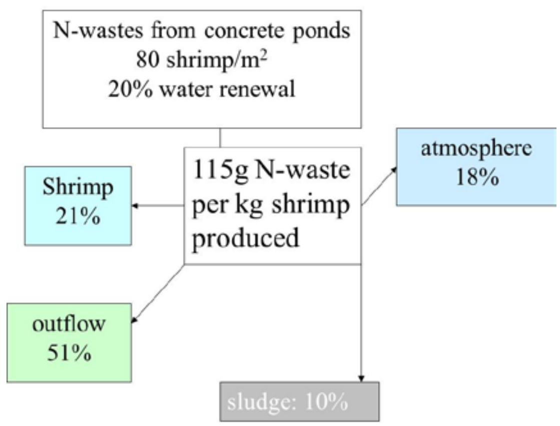

Fig 2. N partitioning from a diet containing 7g N (Burford *et al.*, 2004)

Energy content of MOD was established based on mollusks research (Février *et al.*, 1975) and pointed out values near 7,5J L^{-1} of culture medium that made in terms of digestible energy an input far from negligible aside from other nutritional sources such as phyto, bacteria and feed. Feed for example brought according to formulation something like 12kJ g^{-1} and feces were re-colonized by bacteria then ingested like a well nutritive complement thanks to a double digestion but this is not quantified at the moment (Fig 2).

However, perspectives indicate that this is a very significant contribution because it reflects a bit what the animal can find in its natural environment. It complements this way the compounded feed. Stable isotopes give comprehensive information about the contribution of each fraction (Cam *et al.*, 1999; Magaña-Gallegos et al, 2015) and allow discriminating between particle sizes of those to be absorbed preferentially on laboratory short cycles. With the feed on which one can play with the delta^{15}C it is possible to make its composition very negative (-40% ◦) with the addition of fossil ingredients such as ICI yeast and thereby obtain consistent differences in relation to the natural productivity or floc. In fact the contribution of the feed can range from 30% to 65% between the beginning and the end of a grower period (several months). In such a scheme, it is possible to infer the proportion of live microorganisms (meiofauna) actually more abundant during early rearing (Réquillart, 2004, pers com.). But also which reduces to nothing near harvest when this meiofauna practically disappeared with increasing shrimp biomass.

Recent work suggests, in floc tanks or in earthen ponds, a contribution of up to 60% for shrimp, perhaps the consumption of floc and its structure at the particle level can make it the most important while ingested in a row, isotopic labeling of shrimp muscle gave more intense signature when actually translating the greatest contribution of floc what can come from the feed. Again, further precisions on these measures will help to maintain a coherent description on the relative contributions but the parallel (Fig. 1) between feed and floc made from the work of Gauquelin *et al.* (1999) highlighted the level of energy retention (RE) with higher values based on floc.

Sopomer era (1987-2003)

This first shrimp farm operated on floc was created in 1999 and the technical background of Aquacop served to design and set up the operations at a significant scale (1ha) the culture of *L. vannamei* and *L. stylirostris*. The owners faced a few setbacks at start and then were able to run productions at two cycles per year; he even reached a world record with *L. vannamei* (24mt a year in two crops). But in the long run, some mortality appeared, the quality larvae decreased, the medium originally to evolve with a predominance of phytoplankton bloom to lead after more than 10years the farm to collapse. Reasons were technical (not enough water depth, no shaded area to limit UV action, phytoplankton bloom, feed with inconsistent quality,…and the shift from one species to another did not really improved the situation, then attempts to mixed seawater and fresh water did not bring positive trend. But this was the first attempt to transfer results from a small or pilot scale to production.

Cuzon *et al.* (2008) produced a synthesis on Ecotron program with an emphasis on results obtained at COP during 80's leading to comprehensive interrelationships between communities and the exchange of salts by means of bacteria.

1999-2012 Avnimelech & Taw, (2012) " found that N was converted into microbial protein and using ^{15}N tagging of biofloc that more than 20% protein eaten by shrimp at harvest comes from biofloc (28-49% CP); protein utilization rose from 15-25% in conventional ponds to 45% in BFT (a save-up in feed cost up to 31-36% for fish and 50% for shrimp cultivation).

2011 MaB-floc (van den Hende et al., 2011) demonstrated the association of µalgae plus bacteria that improved by a balanced C/N and used fish waste water to maintained the system feeding animals with a feed MaB-floc enough to get animals grow-up to a commercial size.

2013 in SE Asia, shrimp culture development started following the floc method and a use of green pellet to cope with benefits of particulate biomass and microalgae in ponds with liner (Emerenciano *et al.*, 2012-13).

2014. Utilization of "floc" under dry form to be incorporated in a diet for peneids-to drastically replace fish meal in formulations (Glencross, 2014).

2010-15: **UNAM-UMDI Sisal: recent results on "floc"**

A research program started at UNAM in 2012 leading to several thesis. The originality of the work was the comparison of shrimp responses to clear water compared to floc conditions (Arevalo, Valenzuela, 2010). Moreover, this approach was possible not only with *L. vannamei* but with several others native species from the Gulf of Mexico (*F. brasiliensis*, *F. duorarum*). The main trials concerned weight gain performances in "moulinettes" located outdoors with a shading zone to demonstrated the feasibility of the technique on site.

Arevalo *et al.* 2013 «Failure to improve performances of males *F.duorarum* in "floc". *F.duorarum* males were raised in "floc" or in clear water tank, fed with or without fresh food. Spermatophores formation, spiked sperm, spermatozoids (sptz) motility were not modified with the rearing conditions or feeding regime. Metabolites from (total protein, triglycerids, cholesterol), in hemolymph, hepatopancreas (HP) and gonads were measured during grow-out and maturation phases. Total soluble protein in gonad varied from 62 to 73mg g^{-1} with fresh food (eviscerated squid) or a commercial feed and in HP from 37 to 44mg g^{-1} ($p>0.05$). Neutral lipids varied from 8.to 11mg g^{-1} in "floc" conditions and clear water respectively ($p>0.05$). Such contrast in food/feed composition had incidence on fatty acids profile (% total fatty acids) in HP and gonads respectively with LOA (3*vs*7), LNA (2*vs*2), ARA (1*vs*11), EPA (2*vs*17), DHA (2*vs*11) in all rearing conditions. Similarly as with females there was a transfer of lc-PUFA from HP to gonads. Native protein+free amino acids+lc-PUFAs displayed there essentiality in the maturation phase more explicitely than during grow-out phase. "Floc" definitely provided better nutrition status than clear water but additional fresh food reinforced health status for reproductive phase.

Then a comparative study was conducted to propose an economic approach for two species as indicated in table 5 with two types of feed rations, traditional or optimal (Arbelaez *et al.*, 2013).

Simultaneously, it was examined to which extent future breeders could benefit from floc compared to floc plus fresh food (Emerenciano *et al.*, 2013; 2014) as displayed in table 5.

Table 4. Bioenergetics in clear water and in floc. Values with one digit are in kJ $shrimp^{-1}\ d^{-1}$ (up) or J/shrimp/day (below).

Gauquelin *et al.*, 1996	GE=7.5 100	DE=6 80	N- NH_4=0.4 5	ME=5.6 75	SDA=1.4 19	NE=4.2 56	~HeE=1.8 24	RE=2.4 32
floc, 2010	GE=100	DE=100 100	N-NH_4=5 5	ME=94 75	SDA=89 19	NE=89 56	~HeE=69 24	RE=69 69

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A 40-day trial was performed to evaluate the effect of short-term fresh food (ff) supplementation twenty days prior to ablation in *L. vannamei* broodstock raised under biofloc conditions. Changes in biochemical composition and fatty acids (FA) profile were used as indicators of nutritional condition. Females that received ff supplementation (floc+ff) achieved better eggs production, spawned more promptly and presented higher levels of HUFA in eggs as compared to those ones that did not received ff (floc alone). Proximate analysis in biofloc and microorganisms assessment showed a higher crude protein and lipid content from(floc+ff) tanks (26.3 and 0.7% respectively) as compared to floc tanks (18.4 and 0.3%) and also demonstrated a higher concentration of filamentous cyanobacteria and nematodes.

Table 5 Comparative performances of *L.vannamei* and *F.duorarum* pre-breeders set in two different conditions (rations traditional or optima in floc or clear water CW) for further reproduction in “floc”

L.vannamei					*F.duorarum*			
	floc		CW		floc		CW	
rations	optima	tradition	optima	tradition	optima	tradition	optima	tradition
IBW	9.4	9.5	12	12	1.7	1.8	2.4	2.5
FBW	17	19	14	15	5	4	4	4
wt gain	8	10	1	3	3.2 [a]	2.7 [a]	1.6 [b]	1.7 [b]
g wk^{-1}	1[a]	1[a]	0.2[b]	0.4[b]	0.4	0.3	0.2	0.2
biomass	89 [a]	97 [a]	66 [b]	66 [b]	25	22	20	21
survival	86 [a]	89 [a]	66 [b]	66 [b]	63	69	66	71
FCR	1.3	2.0	1.3	2.0	1.3 [b]	2.0[a]	1.5 [b]	2.1 [a]

The better outcomes obtained with females that received short-term ff supplementation justified its employ in *L. vannamei* broodstock. Effect of short-term fresh food on reproductive performance, biochemical composition and FA profile of *L. vannamei* (Boone) reared under biofloc conditions (Emerenciano *et al.*, 2011).

Table 6. *L.vannamei.* and reproduction in "floc"

	floc	floc+FF	*p*
female weight (g)	35.0 (±3.0)	36.2 (±2.9)	0.2
mortality (%)	11.1	8.9	
total spawns	50	50	
spawns between ablation & day 20	6	12	
spawns between day 21 and 40	44	38	
unfertilized spawns (%)	88	60	
Nb of spawn/ablated female	2.8	2.8	
Nb of spawn/spawning female	3.1	2.9	
Maximum spawn order	5	7	
latency period (days)	22 (±5)	23 (±8)	0.8
females that spawn at least once (%)	88.9	94.4	
Nb of eggs per spawn ($\times 10^3$)	94.1^B (±33.3)	111.2^A (±36.4)	0.03
fertilization rate (%)	73.1 (±13.1)	79.5 (±17.0)	0.4
Nb of nauplii per spawn ($\times 10^3$)	50.3 (±33.3)	62.1 (±36.0)	0.6
hatch rate (%)	51.5 (±21.7)	61.8 (±18.2)	0.4

The theme of comparative situation in floc and in clear water was maintained in order to examine the potential effect when an exogenous probiotic was incorporated into the feed. An explanation came from the poor level of adaptation while shrimp shifted directly from "floc" to CW, impacting severely on its microflora. Adding probiotics increase the level of complex interactions; at this stage, out of bioreactor this medium will remain favorable to shrimp for nutrition, immune response, activation of quorum sensing, therefore health status and general aspect (hard shell, long antennae, good pigmentation) and the variety of *Vibrios* (common to all organic rich media) even though some of then turn to pathogens would not affect animals because of bacteria ecology aspect, leading to few symptoms when compared to animals remained in clear water (Aguilera *et al.,* 2014). "floc" was a chaotic ecosystem sometimes called a "black box" with a fragile equilibrium between two communities. On the contrary CW abruptly changed environmental conditions and increase daily variations unfavorable to some bacteria encountered an osmotic shock; animals became more susceptible to infection form opportunist bacteria (Fig 3).

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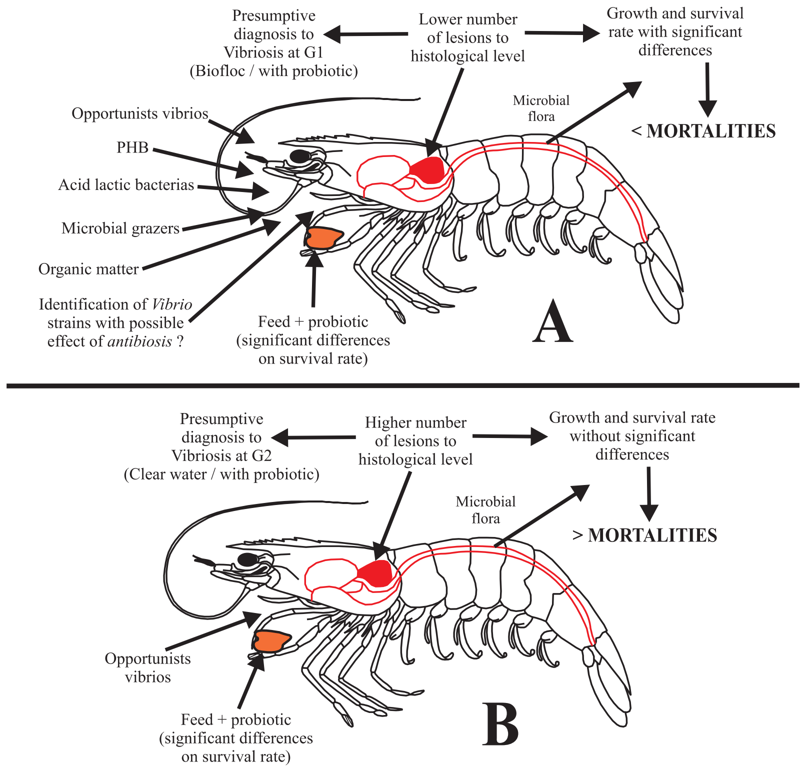

Fig 3. Interactions related to floc-probiotic effect (A) in comparison with results obtained in CW system (B) in the second study (after Aguilera *et al.*, 2014).

Finally, and according to the fact that each site of culture could be different, and various management especially with such a chaotic system, it was useful to examine once again the contribution of floc in two distinct species and in comparison with clear water as well as the incidence not only during the growing period but also when animals entered in pre-maturation (Magaña-Gallegos *et al.* 2015). Stable isotopes indicated some distinct situations according the type of fresh/frozen food aside from a compounded feed. Concerning *F.brasiliensis* it was evidenced a distinct situation when considering F^1 generation and wild specimen (Fig. 4).

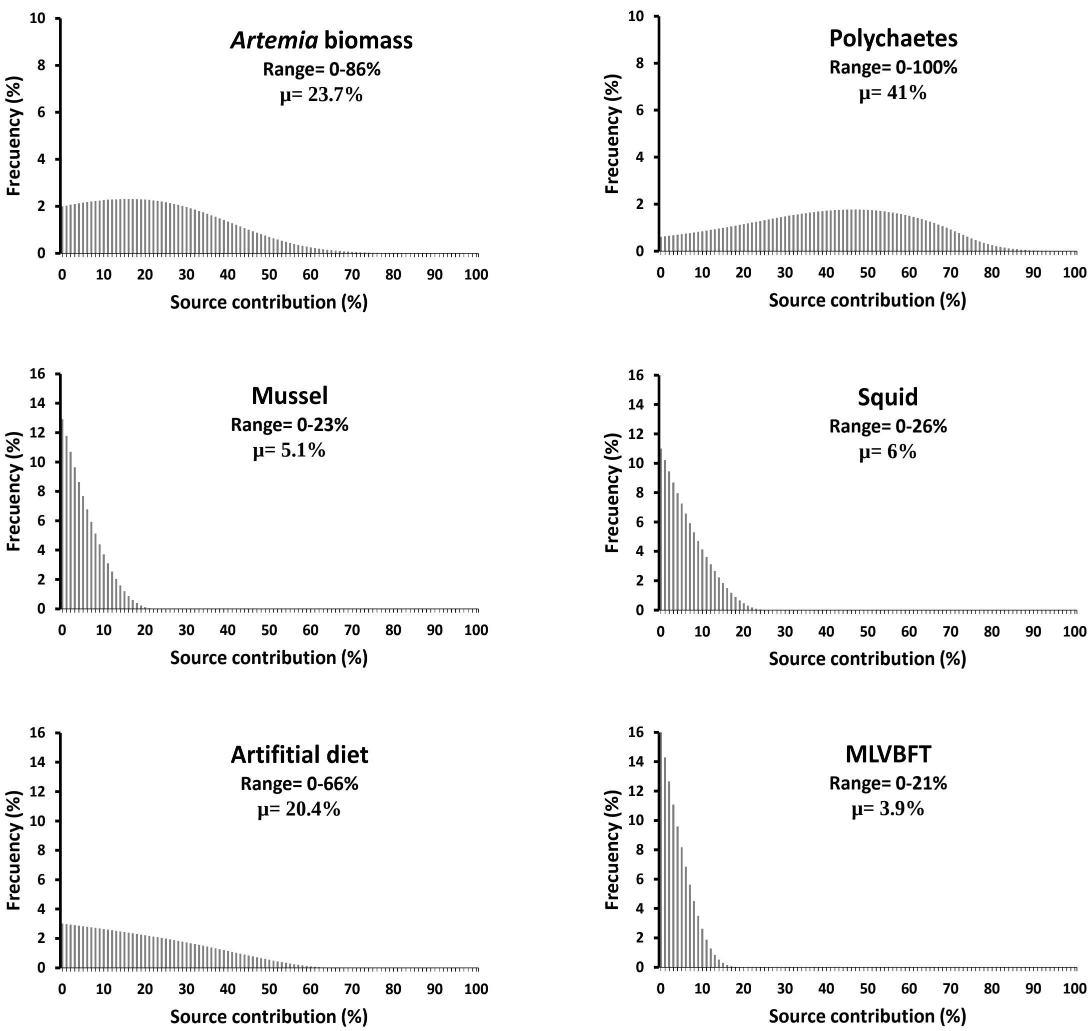

Fig 4 Contribution of 5 foods/feed sources from isotopic sign to *L. vannamei* eggs and muscle breeders raised in floc (mLv/BFT). (μ=media)

2015: back to original "floc" in dark (indoors) conditions with an example of an area of production near Madrid using artificial seawater, and indoors in order to benefit only from the bacterial community of "floc" plus a regular feed, and close to a great market place. Interestingly this approach derived from original work conducted in early 70's.

Discussion

The share of single food can be low, however leaching helping to fuel the medium (bacteria) so the feed plays a "double trigger" role and carbon intake is significant due to carbohydrates ($IME_{glucides}$ around 48%) then and the end of a rearing cycle floc dynamics integrates feces production as it is another food source for shrimp (coprophagy) plus contribution of native protein originally single cell (heterotrophic bacteria). Finally secondary production is established only rich microorganisms (protozoans). It is without doubt a key to the explanation of the nutritional benefits associated with floc. One could say that the feed supplies not only shrimp but it enriches the environment at the same time boosting the formation of these micro-ecosystems (particulate biomass) shrimp will filter at will. In fact shrimp raised in "floc" find conditions of the natural environment in which animals are in constant feeding activity, recovering these tiny amounts of particles that ultimately provide all nutrients that animals molt and grow so near their genetic potential, close to shrimp in the wild can express weight gain and reproductive performances. Also, always in these conditions, animals do not attack each other (survival>80%) thus almost no cannibalism in such stress-free environment despite high stocking density. 40 years later, floc rediscovered gives the best chance to respond a request quality shrimp, presentable, and have an analytical composition probably close to that of farmed shrimp, unlike what one has seen for farmed fish such as trout for example. Let's say that feed distributed 2 or 3 days time will not produce the same effects that a tiny contribution of small particles and highly digestible "a release of energy by small change" as wrote Szent-Georgyi, A. (1957). In addition to floc, one is facing a triphasic diet supply [bacteria+protozoaires+nematodes+copepods] plus a dry pelleted feed and finally soluble substances (MOD) source of amino acids, vitamins, glucose, lipid, minerals, nucleotides... In fact, the simultaneous contribution of these groups that brings the best supply to meet the demand for weight gain as well as breeding. This suggests that such needs are not so different. However, if growth with dry feed must be supplemented via "floc" for example, during maturation phase adding fresh products (mussels, squid, ...) will need less than floc contribution give less need to floc intake which allowed in its time (Kawahigashi, 2010-15) and advocate reproductive penaeid in clear water. Note that these authors have recently revised their position and support the idea of conditioning floc before the entry into maturation.

-Nutritive value

Aside from protein content of floc (30-40%DW), carbohydrates min. or lipid (1-2%) primarily it derives from native aspects of those compounds compared to processed ones delivered by a regular dry pellet. The focus on minerals and energy appears of great interest to cover up. Based on floc composition indications, that can help to explain some aspects of its nutritive value.

There is one point on which we can return on shrimp nutrition in floc is that of mineral requirements, based on the observation that a shrimp (50g live weight) for example will release at molt its exoskeleton that makes ~20g representing approx. 40% fresh weight. This shows how the synthesis of new body tissues will be active and the intake of essential minerals necessary. Or precisely, the floc is an environment that is characterized by a very substantial mineral content; it is only to see the data on dry matter composition of floc that is around 40%. And paradoxically, shrimp feed trend in downward; in the 70’s indeed, there were formulations or nutritional data on extruded feed bags that showed up to 19% ash while today it is more in the range of 11-12% that is a substantial decrease but that would not be harmful to the shrimp "floc" while in clear water or cage culture that could be limiting. One would not return to the form of phosphorus in the feed that was present (via dicalphos) with a low level of assimilation for shrimp while mono-P form would have-been much more digestible. Again "floc" will play its full role to meet the shrimp requirements.

But there exists again a point to elucidate, the one of energetic value of floc? What is its contribution in terms of energy supply daily and in final the retention (RE) for growth?

-energetic values:

It is a key point with floc. There exists a large contrast between dry feed supply (kJ) and live preys (J) due to respective water content. Intake cannot precisely be determined but an attempt is made to explain the energy intake that sounds plausible taking into account a putative intake but especially when considering the exceptional weight gain correlated directly to RE (retained energy); that is one of key points to explain performances in floc.

The characterization of the media is important. Indeed it is in the presence of particulate biomass MOD (dissolved organic matter) and live and dead microorganisms. The particle is the essence of the floc we say literally and figuratively because that environment is well established all the positive properties of the "floc". However, we have long forgotten the component MOD. This component was described long ago for mollusks (Février *et al.*, 1975) and the parallel between what was described and what one is studying is striking and illuminates the concept of scope for "floc" as applied today at penaeid shrimp culture. This is MOD that provides nutritive elements we had not sufficiently considered by focusing too much on particulate fractions, yet it is a compartment of the environment and its contribution is probably greater than one could imagine including prior moulting (*see next paragraph*) or spawning. It is a permanent contribution since comes from degradation of phytoplankton and bacterial communities. MOD (dissolved organic matter) based on mollusks studies (Février *et al.*, 1975) produced data such as 7,5J L^{-1} of medium representing in terms of DE a substantial input aside from other sources such as phyto, bacteria, and compounded feed. Feed, for example, brought according to the formulation around 12kJ g^{-1} and feces re-colonized by bacteria then ingested with a beneficial effect of a double digestion but no data exist at this date.

The energy efficiency of a diet based "floc" should at least be excellent with a digestibility ($ADC_{nutrients}$) close to 100% DE, and less than 6% excretion. a minimum extra heat because of the low solicitation digestive enzymes on native nutrients, a BMR protected from disturbances due to a bit stressful environment. Finally retention (RE) should be good to excellent; however, it remains very difficult to make an assessment in metabolic chamber. What one can say is that *a priori* energy expenditure would be reduced in "floc" compared to clear water on one hand and reducing stress on the other by the simple fact that shrimp landing on the bottom of the tank can thanks to water movement (in “moulinette”) continuously received food/feed: pellets or collets, suspended particulate biomass, water (a ”forgotten nutrient”) rich in dissolved organic substances. This is true for small scale basins (see the “moulinette” of $700m^2$ at COP) of sufficient height (1.20^m) but perhaps less true in tanks with liner where the water will flow in a horizontal plane under the action of paddle wheels but probably that environmental conditions remain favorable in such modules like raceways.

-Antibiotics in floc and antibiosis effect of some bacteria (Ecotron). Bacterial diversity increased in the stomachs of white shrimp *L. vannamei* adminstered oxytetracycline via feed, although diversity decreased in the hepatopancreas and digestive tract. *Vibrio* and *Achromobacter* genus bacteria persisted in the presence of OTC, but species in the order Lactobacillales, and *Citrobacter koseri* dissappeared. The bacteria present in the clear water and floc system differed, although OTC exposure had similar effects in shrimp cultured in either system It seeks to determine if the antibiotic had a detrimental influence on the bacterial community structure of *L.vannamei* under different culturing subtropical conditions. By comparing clear water and biofloc-reared shrimps microbial load may have played a role in determining the extent to which the bacteria community responded to a perturbation of the medium (Arena *et al.* 2015).

Then after years of R&D and taking account of advances in knowledge about ecosystems, one can just repeat an adaptation of such system allowing (i) a reduction of water utilization (ii) a possibility to use artificial seawater (iii) intensification of shrimp culture up to super-intensive and hyper-intensive (iv) fitting such medium for one species in particular, *L .vannamei* (v) less pressure on the quality of compounded feed whether pellet or “collet” (vi) a microbiota favorable with the settlement of various bacteria ecotypes and then less chance for pathogens to grow (vii) a depuration of toxic compounds such as nitrites in a medium in its stable form (viii) in low volume, floc can settle in after 20d. (ix) an opportunity to work in dark area with a pure bacterial floc or in a mix bacteria+µalgae in large outdoors ponds (x) a large advantage to produce shrimp not only along seashore but inland near food markets, while reducing the carbon footprint (xi) in an ideal situation (i.e. indoor+artificial SW+pure floc) where the risk of diseases would be largely reduced (xii) the principle of water treatment plants and microbiology mix well at least for primitive non-grooved species well adapted to this culture medium, where a set of fundamentals to manage it would change radically production methods with a care for coastal environment.

In practice it takes a lot of vigilance to maintain a floc respecting the basic principles namely, water depth, ventilation, convection cells, sieving the light, blackish discharge of sludge daily ...). However, one speaks of "zero water exchange" but in practice it seems that we compensate for the less evaporation and a central purge can be made.

Conclusion and perspectives

These studies on “floc” with an identification of size particles help understand about the point of floc qualification on a basis of its nutritive value. “Floc” particle sizes of course will depend of the intensity of aeration, the best being when convection circulation, keeping shrimp on the tank bottom positioned to filtrate with a minimum of energy expenditure, these kind of micro-ecosystems. But one needs to keep in mind the dynamic aspects of “floc” and its main characteristic as a chaotic medium (May, 1976). Because phytoplankton when present and bacteria followed a cyclic development, sometimes with a prevalence of one community, the ideal being a majority of bacterial flora that attract protozoa, copepods,…such situation is fully nutritive for juveniles. Shrimp gut will be colonized by bacteria from “floc”, and a hypothesis would be tract would contain generalist bacteria (Bolnick *et al.*, 2014). But the medium of culture is so rich (10^6) that shrimp cope with a diversified flora. And in final, after 2-3months rearing, shrimp in high stocking density produce large amount of feces, colonized by heterotrophic bacteria, which will be re-ingested by shrimp (coprophagia) and in a sense the evolution of the culture medium is such that one can figured out that heterotrophic bacteria will prevail in gut flora.

The level of interpretation of “floc” is quite complex, including a chaotic aspect, exemplified by a succession of communities, and the most simple situation will be to have shrimp in “floc” without light to avoid microalgae and to have a successive of autotrophic bacteria (nitrification) that will stabilize the system, followed by heterotrophic bacteria during the ultimate two or three months when the production of feces reaches a maximum. Would we consider C/N, “floc” in its stable status will contain large particles with 250µm (this paper) and aeration will be strong enough to let convection inside the medium and keep particles expanding to form a kind of ecosystem more attractive, surrounded by live organisms such as copepods bearing a microbiota rich in *Vibrios* (Bianchi, 1976). This complex medium not only can be assimilate to an external rumen but possess a capacity of auto-depuration to a certain extent that can limit wastes. Understanding “floc” in its complexity will remain a hard exercise but species such as *L.vannamei* adapted quite readily to such medium during grow-out as well as pre-maturation; next step could be to examine the transcriptome in “floc” compared to clear water and identify those genes from intermediary metabolism that could be up or down regulated under conditions of feeding on a basis of a triphasic diet live food+bacteria, extruded feed+bacteria, MOD.

Perspectives du “floc”

The evolution of techniques of "floc" after the concept water treatment plants was long and discontinuous. We had to face lot of settings, rather strict breeding conditions, and we faced a chaotic system as described previously by May (1976) about the copepods populations. The launch of the program Ecotron (1980) with the participation of a group of enthusiast’s researchers in microbiology portrait in itself a lot of hope for modeling the "floc" system but it was not counting the levels of variation and interactions water - microorganisms-shrimp-waste. Technological constraints remained to meet. The gestation was long because in 70-80 years the aquaculture was rather oriented semi-intensive. Indeed intensification of attempts in Taiwan for example, with the species *P. monodon* had proved catastrophic in terms of results with densities that reached more 44 shrimp/m^2 in 1988. The water level was an important factor to hold a phytoplankton layer in the first 50cm below the surface.

This was the time when Greenpeace was opposed to the massive destruction of mangrove areas along the Pacific coast, Ecuador, Baja California who presided over the construction of huge basins for the establishment of semi-intensive technique. Over the years, these techniques have proved catastrophic showing episodes of severe mortality related to the occurrence of pathogens (viruses and *Vibrios*) and it took a long hard realize awareness for the limitations of these productions (often to export so with a high carbon footprint) despite hopes to resistant strains (Super-shrimp with Persyn, H.O., SPR[43] at COP) or antibiotics treatments prove risky (Arena *et al.*, 2015).

The industry has taken the lead ultimately and expert help to make quite substantial investments giving possibility to bring intensive or super-intensive shrimp farming with much better biosecurity conditions, low water utilization and fishmeal in the grower feed therefore a path to sustainability of the sector (sustainable aquaculture).

It is clear that the shrimp are in a “on-stressful environment”, rich in specific nutrients and convener for shrimp physiology (possible isosmoticity, digestion from native proteins, provision of long-chain fatty acids , vitamins, ...) leading to optimal nutrition, leading to a limitation of cannibalism (high survival), less energy expenditure devoted to immune .response so strong retention for growth. In this environment, the approach to the formulation of an artificial diet will change, simplify proteins including a low rate (20-25%CP), but also carbohydrates which will also contribute to maintaining a C:N ratio ad hoc (around 6), less vitamins, minerals load to adjust, but with inputs such hydrolysates (from fisheries co- products) and final basic corn-wheat-soya-alfalfa that will be transformed by shrimp. Incidentally it may be interesting from an economic standpoint, while maintaining FCR which may surprise while on conventional bases was difficult to see changes in composition to meet relative’s values 1:1. In such a set, do not forget the organoleptic characteristics of shrimp and maintaining an abdominal muscle texture similar to that of wild shrimp. Will I need to check on the bright pigmentation or staining after cooking? ... without having an absolute use of synthetic carotenoïds, the presence of alfalfa showed a positive effect in the past.

Going further down, will one take into account aspects of organic (BIO)? Currently there is a farm in the world that relies on BIO production, based in Madagascar, the OSO farm. There will be then to distinguish between high-quality products and feed a population in staggering growth (9MM by 2052).

It's probably like a little revolution that is brewing in the world of industrial production of shrimp near the markets where consumption of large cities exist in order to reduce the carbon cost in the process. The vow of Addison Lawrence was to make shrimp the "chicken of the sea" and with support from the food industry and investors, perhaps we are heading slowly towards the realization of this dream.

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