

# “Floc” Contribution to Peneid Intensive Culture

Cuzon G<sup>1</sup>., Goguenheim, J<sup>1</sup>., Gaxiola G.<sup>2</sup> and Aquacop

1. Centre Océanologique du Pacifique-Tahiti, Ifremer, France

2. UMDI-Sisal, Facultad de Ciencias, UNAM, México

---

## Abstract

The bacterial “floc” is a very complex ecosystem that works in a multifactor context where each parameter needs to be considered. The bacterial microflora played initially the major role; it could stand on a unique role in total darkness. However, outdoors tanks getting larger and larger dimension induced an additional factor with the presence of phytoplankton. At start, a few programs were held in order to comprehend how such a “black box” would function, some people speaking of an “external rumen”. Implication are coming from different fields such as the evolution of each bacteria communities in the medium, feed composition, management of wastes, sizing for a commercial operation. How shrimp performed in such environment? How shrimp could derive so many nutrients for its growth; would such a culture system be explained through a model? .

First very positive results came from a zootechnical approach with considerable improvement in weight gain, healthy animals at harvest compared to regular earthen ponds production; and recently it was evidenced a potential for reproduction of *Litopenaeus stylirostris*.

That is the purpose of this paper to present a comprehensive approach of the use of “floc” ecosystem in a tank structure called also “moulinettes”. Today there is such a great attention from farmers willing to go into intensive or super-intensive culture system that a comprehension of the trophic chain is necessary. Finally, there are great expectations for a biosecurity aspect with the disappearance of diseases outbreaks, the possibility to develop culture out of the constraints of the seashore and overall an application of the concept of sustainability linked with a profitable operation.

Key words: bacteria, nitrification, phytoplankton, intensive culture, shrimp.

## Oulines

### 1. historic of floc

RP Crystal River/COP Tahiti: first “moulinettes” In total darkness, pure floc then in tanks conditions 160 liters, nitrification, bacteria involved and bacteria ecotypes. Extension to 12m<sup>3</sup> then 30m<sup>3</sup> in outdoors tanks; difference indoors vs outdoors

### 2. Ecotron program 1979-1980

### 3. Extension work

#### 3.1. growth

#### 3.2 maturation

4. What is the nutritional input of floc: particulate biomass; dissolved organic matter; phytoplankton; TEP; enrichment of pelleted or collet feeds; role of the feed; necessity of vitamin mix in the feed? constant feed intake

5. Role of floc on immune status of shrimp; antibiosis.

6. Sustainability of the system

7. Conclusions

8. References

## Introduction

”Floc” is an appellation relating to flocculation of organic matter (MO) in a medium; floc by extension represented a culture system also called “moulinettes” (Aquacop, 1975). The term would signify a water volume in constant movement with strong aeration. In 1992 such a system of culture was assimilated by a researcher on mammals to an “external rumen” for shrimp. It means a complexity of interaction between MO, bacteria, protozoa,...other researchers more recently proposed the denomination of “zero water exchange” (Samocha *et al.*, 1990). But at origin, in early 70’s Ralston Purin (Persyn HO. and Mc Grath B.) developed the system purely with bacteria nitrification by keeping shrimp in total darkness. That was the true bacterial floc ecosystem. In connection with Aquacop this system was applied for *L. stylirostris* and *L.vannamei*, both in Crystal River (USA) and Tahiti. In fact, it proceeded from previous

consideration (C. Mock, Galveston, Texas) on the potential of shrimp Pl's to sustain in seawater with high ammonia level (4mg/l but variable according to pH) reference.....

The concept originating in small volumes (200liters) and the association of shrimp, dry feed and bacteria could not stand for large volumes. In small volumes 12m<sup>3</sup> for example the identification of bacteria for nitrification was observed as well as the evidence for the following sequence NH<sub>4</sub>--NO<sub>2</sub>---NO<sub>3</sub> and a stabilization of the medium (Bianchi and Bianchi, 1986). And with the extension to outdoors system (30m<sup>3</sup> round tanks up to 1000sq meter) obviously another compartment had to be taken into consideration that is to say microalgae. Today some authors suggest to seed with *Chaetoceros* (Wasielevsky *et al.*, 2007) or others leave the medium with its own strains for example *Isochrysis* or *Monochrysis* (Goguenheim *et al.*, 2007). But it remains a kind of rule of thumb to initiate a floc in outdoor conditions. Basically, two considerations would be (i) a minimum of 300g/m<sup>2</sup> biomass and (ii) a regular input of dry feed. Initially, dry feed played a considerable role as it was low in protein (RP<sup>20</sup>, RP<sup>25</sup>) and it opened up great perspectives for the culture of such non-grooved species. There are a numerous amount of work related to intensive (Samocha *et al.*, 2003) or super intensive culture system Sopomer, 1989; Garen *et al.*, 1990). World record in production (25mt/ha/year) was obtained in 1988 by Sopomer Farm. However extension to production systems remains is low (Belize, 1995).

However, from an experimental point of view, in order to find basic information, a whole program called Ecotron (1980) was set by Ifremer to get a comprehensive approach of the floc system and explained interrelations between different compartments such as water, bacteria, microalgae on one side, shrimp nutritional physiology on the other (Sohier, 1986).

Basically, applied for the growing period (1-20g) the floc system in “moulinettes” remained for long on the basis for an extension at a production level (Racotta *et al.*, 2008; unpublished results). And it is recently that a benefit for breeders of shrimp was under investigation (Goguenheim *et al.*, 2007; com.pers.). But explanation on the physiological impact through nutrition and environmental conditions still remained to be expressed clearly.

In summary, we try to provide information on how it originates? There was a constant aim at intensify the culture. And appellation changed from “floc” to “microbial floc”, and the concept of

external rumen was put forward. “Moulinettes” described the tank system and explanations turned around the comprehension of nitrogen cycle. Nitrification includes two steps (i) transformation of  $\text{NH}_3$  to  $\text{NO}_2$  (nitrosation) and (2)  $\text{NO}_2$  to  $\text{NO}_3$  (nitratation).

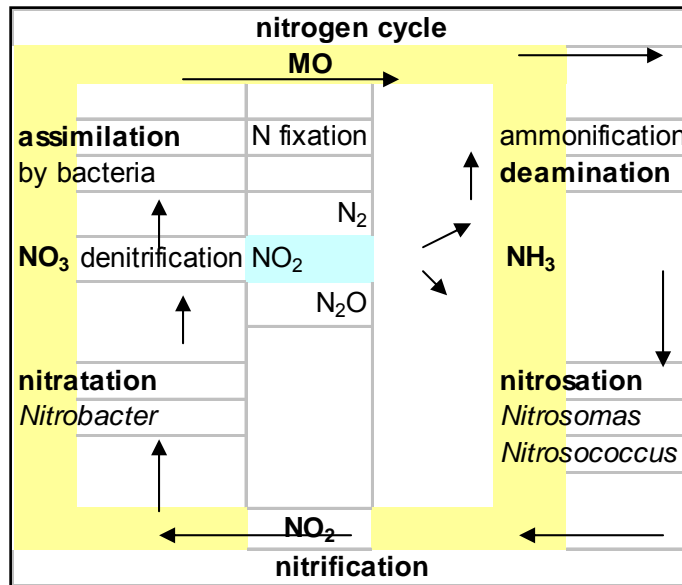


Fig 1. Nitrogen cycle.

More recently it was studied some aspects of shrimp earthen pond production that were considered as an ecosystem or more precisely an “agrosystem” (Styli 2006) and according to Funge-Smith *et al.* (1994), a great proportion of feed consumed by shrimp (>80%), only 25% of the total nitrogen and 9% of the phosphorus were assimilated; largest loss (i.e. 45%) went through final drainage. Sedimentary removal N reached 161kg/ha/cycle (26%) and sedimentary loss of  $\text{PO}_4$  was accounted for 243kg/ha/cycle (84%).

## Material and methods

5 liters flasks and tanks of several dimensions 1, 12, 30, 100, 1000m<sup>2</sup> were set for explaining the overall functioning of this complex ecosystem and sort out main trends. The method for starting a “floc” included a supplementation in ammonium nitrogen ( $\text{N-NH}_4$ ) or organic-N (coconut-molasses-artificial feeds) as described in Ecotron program (1979).

All techniques were previously described in papers listed in reference section with bacteria associated to main plankton algae (Martin et al., 1980), the optimization of rearing methods for shrimp in intensive techniques with strong aeration (Ecotron program), the contribution of bacteria communities to problems linked with rearing system at COP and short term variation of bacterial communities (Bianchi and Bianchi, 1980) and activity in aquaculture ecosystems (Bianchi and Bianchi, 1981) then the role of quantitative and qualitative microorganisms to detoxify culture medium and biomass production in intensive rearing tank (Sohier, 1983).

How to control the phytoplankton bloom? There are several approaches, renewal of the water increasing from 5% per day higher, covering the tank with a shading sheet (90%) even though an alternative exists to put the shading sheet directly on top of the tank that gives an advantage to avoid after a few months the development of filamentous algae on the walls.

Feeding methods and feed composition were based on Aquacop (1987). Basically a practical experimental diet was used to feed animals during most experiments.

Techniques of microbiology: it concerned techniques of numeration at the microscope or culture medium to obtain the CFU values.

Zootechnical parameters such as survival rate related to health-weight gain-FCR were followed during different tests (Aquacop, 1987).

## Results

Application of “floc” system: many species received attention for improving growth performances primarily using the floc conditions and trials occurred in tropical or sub-tropical conditions (Tahiti, Florida) and then under temperate climates such as South of France, South Korea, South Brazil).

*P. monodon* CNEXO COP Tahiti

*P. indicus* (Palavas les flots, France)

*M. japonicus* (Palavas les flots, France)

*L. vannamei* CNEXO COP Tahiti

*L. stylirostris* CNEXO COP Tahiti

*F.paulensis* (U o Rio Grande)

*L. stylirostris* Ifremer COP Tahiti

Stages of culture:

Pl's and juveniles as well as breeders performed very well in floc conditions and it fitted perfectly well with the need at this stage of constant feed supply then there was an adequation between stages and food supply.

### Evolution of bacterial communities (Bianchi and Bianchi, 1980)

During the Ecotron program (1979-1980) three surveys were conducted to explain the floc ecosystem.

In a first step, it was studied bacteria associated to marine plankton algae with dynamics of communities and relation with dissolved organic substances (DOS). This work included a study of primary production and mineralization of carbon compound with labeled elements in 1m<sup>3</sup> tanks. Then, a preliminary study of regeneration conditions for N-ammoniacal heterotrophic bacteria communities linked with phytoplankton in 5liter flasks. In flask #5 enriched with amino acids (aa), enrichment in organic-N jointly occurred with a carbon enrichment.

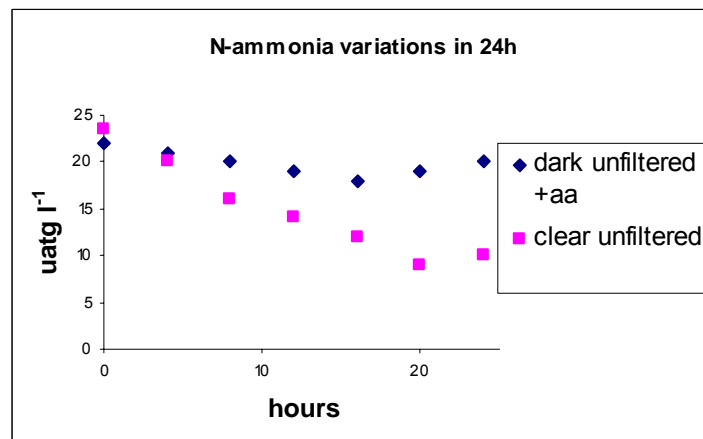


Fig 2. production in flask of ammonia from unfiltered "floc" of flask kept in dark.

C/N ratio of amino acids was 4 on average, the one for bacteria biomass reached 6. Such difference implied that part of organic nitrogen will add to the rest and was found back in excreta under the form of NH<sub>4</sub><sup>+</sup> by heterotrophic microflora, the remaining would participate to build-up

of bacteria biomass during the growth period considered. Good correlations between maximum rate of apparition of N-NH<sub>4</sub> and bacteria growth rate from direct counting would confirm this hypothesis (Sohier 1983; p45).

### **Effect of mineral or organic enrichment on microbial communities in a volume of water (1m<sup>3</sup> tanks) and in final, study of chemical telemiators and quantitative analysis of DOS during an experimental bloom of phytoplankton.**

In 1980, rearing techniques optimization of shrimp in intensive ecosystems included the set up of a rearing dispositive and following parameters were taken into account such as climatology, physico-chemical factors, chemical composition of particulate matter and bacteriological analysis. The results gave the structure and evolution of different compartments, an analysis of “floc” *inoculum* with the survey of three “moulinettes” called K, L and M. an experimental approach of the mechanism led to an appreciation of production/consumption of O<sub>2</sub> by “floc” and partition of particle matters from pond water. Physiological aspects were related to growth of fractions, effect of nutritive salts, effect of shrimp and feed on the medium. Kinetics of nutritive salts included inputs through aeration and autodegradation of pelleted feed (76.1.1.0) and for a microbial view point, a bacterial degradation of pellets and rate of transformation of nutritive salts by “floc”. Some aspects of the physiology of shrimp (*P. monodon*) concerned the relation size/weight, fresh vs dry weight, ash content and C, H, N content. Respiration and excretion led to metabolic rate and weight, respiration could be measured in light and at darkness; respiration in presence of N-NH<sub>4</sub> was studied and in relation to nutrition too as well as respiration and excretion. Nutrition concerned feed ration and feces-forming.

In 1980, the contribution of bacteria communities to problems linked to shrimp rearing at COP were studied in 12m<sup>3</sup> “moulinettes”. Sampling gave an idea of the quantitative estimate of bacteria microflora and qualitative appreciation as well as the activity of bacteria communities. Results allowed to classify microflora from different biotopes i.e. lagoon water, earthen ponds and artificial feeds. Bacterial community in “moulinettes” was studied through different parameters. Heterotrophic bacteria were compared in water and digestive tract on starved shrimp.

### **From pure bacterial floc to floc+phytoplankton**

Flux of nutrients nutritive salts were measured and ammonia and  $\text{PO}_4$  ranged 2.5-104  $\mu\text{atg/g}$ . Utilization of pellet by bacteria and ammonification led to rapid growth of nitrification bacteria, increase in nitrites and nitrates. «Floc» intake was found to be around 0.07% live wt/day at 5g and fecal strands from the feed consumption by shrimp participated on “floc” forming some kind of pellet particles.

### **Bacterial ecology, trophic chain**

In  $5\text{m}^3$  it was possible to describe the complexity of the system. Based on bacteria of nitrification the equilibrium of the medium came progressively after apparition of nitrates and the seeding of postlarvae or juveniles in sufficient biomass to attain  $300\text{g/m}^2$ . Such a biomass will require a daily amount of feed that is going to enhance the productivity of this ecosystem. Organic matters based of feces from shrimp, and other residual fractions of artificial feed would support a population of bacteria, then filamentous microalgae would get a biotope and then vorticelles, ciliates and copepods. Such balls of living organic matter would produce an excellent source of food for postlarvae. It was observed that in experiments conducted at COP (Soyez, com.pers.) that it was possible to stop the aeration for at least 8hours without affecting the equilibrium of the ecosystem. Color of the water became transparent; but the interesting point was that all the material was resuspended immediately after injected aeration in the system. The reference point was 6ppm and through a regulation mechanism it was possible to function without a constant aeration supply and a quality of medium that led to high survival rate among shrimp population and good health. The point was to notice a quasi absence of microalgae in the medium and in final an absence of sedimentation on the bottom. This observation confirmed the good mineralization process while managing a “floc” in optimum (and ideal?) conditions. The right conformity of the tank was a priority that meant sufficient water depth, round tank, low light intensity, aeration in a central position.

### **Nutritional value of floc (Sohier, 1983).**



Proteins native and processed ones but also lipids (13%) and in particular sterols (Galois, 1989) participate to overall nutritive value of particulate biomass. Among lipid classes, acylglycerols were well represented in comparison to other sources of food such as squid (mantle, viscera or both), mussel or troca and particularly monoacylglycerols (7% of total lipids).

Table1. Glycerids composition for “floc” (Galois, 1980).

	mg/g dry wt.	%TL
<b>acylglyc</b>		
mono	0.77	3.39
di	0.16	0.71
tri	0.6	2.64

Table 2. Glycerids composition of “floc” (Galois, 1980).

	mg/g dry wt.	%TL
<b>sterols</b>		
free	0.59	2.6
esterif.	0.03	0.13

Flora that proliferated on tank wall had a slightly higher monoacylglycerols content compared to floc. Another radical change was observed with sterols. And free sterols were largely represented in squid (11%) and especially in viscera compared to “floc” (3%) but similar to mussel (10%) another food source for reproduction of shrimp. Such nutrients play an essential role in gonad maturation. But “floc” compartment that is constantly available could be able to replace mollusks. Phospholipids and probably long chain polyunsaturated fatty acids (EPA but especially DHA) were present in large quantities in squid that predisposed again this kind of food to produce an enhancement of ovary development (Cahu *et al.*, 1987). Again flora from tank walls and probably particulate biomass from “floc” that contained lower amount of such fatty acids but a predominance of EPA (20:5n-3) had a role in gonad formation due to a constant supply to shrimp through permanent circulation of the water in the tank. Other neutral lipids such as waxes, alcohol and hydrocarbons were found in a proportion similar to sterols (around 2% TL):

Table. 3. Values of different parameters with characteristics of mud under survey

tank	days	biomass g/m <sup>3</sup>	water m <sup>3</sup>	feed in kg	sludge g DM	DM	OMg /l	C orga	N orga.	C/N
6	7	1000	22	2.6	408	27	6	70	12	6
6	19	1337	61	7	601	13	2.5	89	14	6
6	40	1700	128	15	1362	17	3	70	12	6

Carbohydrates composition of “floc” included mucopolysaccharides containing glucosamine, rhamnose, glucose, mannanes, galactanes. The transparent exopolysaccharid particles (TEP) can be produced by the phytoplankton under stress and agglomerated on a substrate in the medium to produce nutritive particles for the postlarvae and would contribute to the overall nutritive value of “floc” rich in vitamins, minerals or growth factors?.

“Floc” was found as a complex and composite medium, including fecal strands bacteria, protozoa (ciliates, flagellates), artificial feeds (pellets or collets), soluble substances, aggregates of organic matter, exoskeletons, carcasses...Such a complex media and in particular presence of numerous bacteria ecotypes led to compare this medium to an external rumen. This medium contained proteins, some coming from the feed (processed ones), some qualified as “native” proteins derived from live food such as protozoa or bacteria. This last category was less abundant than protein derived from feed input but it brought benefit for shrimp that digested and derived all essential amino acids.

Sugars were present in the medium; carbohydrates and especially starch from the feed to bring energy to shrimp and bacteria. Interestingly it was observed that artificial feed could be used twice because shrimp tended to adopt a coprophagy behavior. Also due to the richness of the medium in nutrients shrimp could have access permanently to a source of food or feed. Feed collets can stand so long in water that it could serve as a support for bacteria and this combination enhanced its nutritive value to the benefit of shrimp. Common sense and daily observations were useful to manage this complex system to obtain an effective environment for shrimp and get them in full appetite and healthy.

### Shrimp physiology in “floc” system

It was measured the amount of bacteria in the water in the gut of shrimp after on a practical diet (76.1.1.0) or maintained unfed. The number of bacteria changed from  $10^3$  per ml in the water to  $10^6$ - $10^8$  in the gut after feeding. Ecotypes were sometimes different between water and shrimp gut but with an absence of pathogens. However the presence of bacteria played a role in the nutrition of animals and probably as a probiotics by colonizing the gut and avoiding the development of pathogen bacteria.

It was verified in “moulinettes” under precise survey, a survival rate that reached sometimes 98% after three months trial in “floc”.

### **Some aspects of energetics: digestibility, excretion, respiration.**

From a bioenergetic viewpoint, some measures were made on 5g average weight shrimps placed in “floc” conditions. The results obtained were based on nitrogen excretion and oxygen consumption: Nitrogen excretion (UE+ZE) was found at  $2.5\mu\text{atg N-NH}_4/\text{g/h}$  or  $0.045\text{mg/g/h}$  and  $\text{O}_2$  consumption by shrimp= $0.45\text{mg/g/h}$  compared to  $\text{O}_2$  consumption by floc= $0.20\text{mg/g/h}$ . in final there was a possibility to make a rapid energy budget leading to a retained energy (RE) calculated for ~16% and such value appeared in agreement with previous ones even though shrimp were not in exactly the same conditions. It is an indication for further precise energy partitioning studies.

### **Characterization of wastes (Kerviel, 1994)**

Bio-depots were studied in experiments conducted in  $10\text{m}^2$  rectangular concrete tank to allow the collection of sludge every day. *L. vannamei* was stocked at  $100/\text{m}^2$  and fed a diet contained 88% organic matter (35% CP). pH varied between 7 and 8.

Table. 4. Values of different parameters with characteristics of mud under survey.

tank	days	biomass g/m <sup>3</sup>	water m <sup>3</sup>	feed in kg	sludge g DM	DM	OMg /l	C orga	N orga.	C/N
6	7	1000	22	2.6	408	27	6	70	12	6
6	19	1337	61	7	601	13	2.5	89	14	6
6	40	1700	128	15	1362	17	3	70	12	6

The ratio C/N that signed the degree of mineralization of the sludge varied between 5 and 7, relatively stable it took values indicating a rapid degradation of organic matter (OM) through mineralization.

Feed wastes made the main incidence on mud composition but no correlation between feed amount and mud composition (Kerviel, 1994). It was found that effluents could be recycled for the same type of rearing on short period provided that a recirculation system and a treatment of wastes could be done

## Extension

First trials in 1000 m<sup>2</sup> were monitored in a 1ha farm located in Tautira (Tahiti) and after some problems to manage the tanks made of concrete with a compacted coral bottom layer, phytoplankton blooms were controlled and wastes removed at regular intervals. This farm has alternatively two species in production and the best results were achieved to reach 25mt/ha/year without extrapolation (Sopomer, 1980). In relation to the fragility of the system: tanks need a constant supply with strong aeration, stocking density should be controlled but remain high in such ecosystem and temperature has to be controlled because of the low level of water renewal that leads to a drop of few degrees during the night. However, at large scale i.e. 1000sq meter or more this is not feasible to maintain water temperature constant and restrict to a certain extent the location to tropical areas.

## Belize aquaculture

Using SPF–Hawaii *L. vannamei* were raised in 2 ha ponds. The initial stocking density was 175 shrimp/m<sup>2</sup>. The preparation of the culture system was done with an addition once a week of about 200 l sugar cane molasses as a carbon source added to maintain the floc in good physiological conditions with a minimum of water renewal. Intensive culture (30 ton/ha/year) with two feeds, one at 25% CP for the period 1-12g average weight and the other with 18%CP under pellet form and containing soybean meal and corn. FCR=2.0 with 5days of balanced pellet feed and 1 day of grain pellet (18%CP). Feed consumption averaged 800ton/month (Emerenciano, 2008, com.pers.)

## Breeders

Hudinaga (1942) opened the way of the modern aquiculture with the reproduction in captivity of *P.japonicus* by taking animals of the natural environment. However the dependence of wild parents could last only a time. The reproduction of shrimps was one of the first subjects of investigation of the experimenters when the aquiculture taken his rise with upright of the seventies (Laubier, 1972; Aquacop, 1975, Primavera, 1974). The facility to obtain the reproduction in captivity will quickly become one of the first selection criteria of the species candidates for breeding. Starting from a natural food (mussels) the very first results were obtained while playing with the abiotic factors such as temperature, photoperiod (Laubier, 1975). Then, in fact the hormonal factors took the top in the inventory control of reproducers; one rediscovered “the Panouse effect” (1943). This technique opened immense prospects for the aquiculture (Aquacop, 1972). This maintenance of reproducers in captivity will produce various constraints among which the nutrition (Wouters and Al, 2001; Cuzon and Al, 1995). One will realize importance of the lipids and of two vitamins in particular, E and C. Meanwhile, the development of known food as for maturation will meet various fortunes. And the difficulty in testing on future reproducers will limit in a certain manner the research effort. To do without “fresh food” will remain an objective during years. Then, according to the availabilities of earthen ponds, one will take the option of breeding to low density (2/m<sup>2</sup>) in order to make benefit the animals from the natural productivity. The improvement of the formula of known food helped “enlargement”, that will involve also better performances of the reproducers; the history of the

animals to some extent became data to take into account. In spite of the relative stability of the environmental conditions in tropical medium, one realized difference in performances of reproduction at the same time of males and females. And the concept of warm season (29-30°C) and cold season (25°C) took all its importance. In the same way, the water quality for breeding seemed being a determining factor. After years of trials and errors, one put the question of the relevance of breeding of future parents in conditions of “bacterial floc” or floc mixed bacteria + microalgae. Within sight of the performances of the animals, extrapolation in the conditions of maturation proved to be useful. This work result in showing an increase in the performances of reproduction of animals maintained in conditions of captivity since years and placed in conditions of floc compared to those which come from the traditional method of breeding out of earthen ponds. By doing this, the management of breeders was simplified with in particular the possibility of holding shrimps in conditions of controlled temperature, in a way easier than out of ponds. Would nutritive contribution related to the floc bring a significant change at the biochemical level (Racotta et al, 2003) and at the physiological level with an extension of the lifespan of the females and thus of their prolonged use in maturation area? in the same way eyestalk ablation will still be essential in the long term? The eyestalk ablation involved a premature ageing of the animals and could be replaced essential under new conditions of breeding? This report was made at the same time on *L. stylirostris* and *F. paulensis* in conditions of floc. Will it be possible to identify a new maturation food? All these questions at the same time for zootechnical procedure, biochemical and physiological aspects as well as genetic should be approached through experiments.

After several trials and some impossibility to get breeders spawning when they came from a pond situation, it was decided to shift to “floc” system that allowed shrimp to mature again. Then the floc situation for breeders was found in perfect adequation with the need for native protein, fatty aci

ds and even vitamins plus a fraction of mucopolysaccharides.

Table 5. *L.stylostris* breeders performances after several months conditioning in 2 types of environment.

	“floc”	non-floc “clear water”
nb spawns /female/month	5	2-3

At end it will become feasible in such an ecosystem to get rid of what we used to call “fresh food supply” made of squid, oysters or mussels, polychaetes, *Artemia* biomass and as shown above (Table5) the difference in performances could open the way for a management of breeders in the future without eyestalk ablation. Avoiding this operation on animals would probably enhance the period during which females could mature.

## Conclusions

Four important points were revealed from a study (Sohier, 1985) conducted after Ecotron program:

-“The role of enrichment during preparation of the ecosystem

Enrichments in ground pellets (56%CP) generated a development of micro-organisms in the ecosystem. There was an increase in counting on Zobell medium, heterotrophic bacteria degraded macromolecules and utilized primarily sugars (IME=28%) and it evidenced a colonization of feed pellet particles by a detritus microflora. Then, an increase of counting on TCBS and EMB, strains were utilizing fatty acids and amino acids and a low IME (28%) signed a heterotrophic microflora grazing on dissolved organic matter (DOM). A shift from attached detritus microflora to a free microflora utilizing DOM produced a phytoplankton bloom with a presence of nanoplankton (nanoflagellates) preying on bacteria (as observed with countings on TCBS, EMB and Zobell). Inorganic N source under N-ammonia form was linked to protozoa increase in the medium. An accumulation in N-NH<sub>4</sub> was linked to a decrease in heterotrophic bacteria. This nitrogen source was utilized by primary production usually refrained by a lack of mineral N in the sea water”.

A consequence of seeding with PI’s in such ecosystem, a massif transfer can induce an imbalance in the medium and as a result heterotrophic bacteria community will be reduced in terms of ecotypes diversity. Risks of dystrophia exists for the ecosystem in case of shortage of aeration

especially after 100days culture, an excess of heterotrophic activity was noticed with a drop in O<sub>2</sub> concentration and in pH.

Table 6. Main characteristics obtained in a 100m<sup>2</sup> moulinette with *P.indicus*.

100m <sup>2</sup> moulinette w/ <i>P.indicus</i>					
	day 0	day 50	day 56-63	day 70	day 100
<b>renewal</b>	0	N loss	5		
			1/2 N input for biomass prod.		
Nsource		feed			
qty g		300			
change		oligo-eutroph			
N gain g	15	160			
N-NH <sub>4</sub> g		25			
			PI's introduced		
impact			clarification of the medium		
			decrease of organic sources		
			decrease of nb of ecotypes		
biomass gain			20mg		
N fixation			25g		
feed input			90g	xxx	
excretion			100µatg l <sup>-1</sup>	xxx	
<b>diversity index</b>					
nanopk				increase	
bacteria					excess activity
ecotypes			down	up	
DOM				xxxx	
<b>C/N</b>		5.5	3	3.5	4

## The problem of nitrification

At this time dystrophia started with an accumulation of N-ammonia because its flux was no more regulated with phytoplankton. Still some empirism persists in the management of such ecosystem linked to its complexicity and multi factorial influences on the medium. That leads sometimes to a rule of thumbs to control a “floc”. The difficulty to keep steady even if in some conditions it was reported that the system could run more than a year in equilibrium (Wasielewski, com. pers.). All studies conducted on the bacteria communities would aim for the future at controlling the ecosystem in a way that could allow running an “expert system” with many captors. It seemed possible to make a model in the phase of nitrification in particular to run a “commercial tank”. There could be envisaged a model of tank or pond with characteristics such as renewal ratio, stocking density, aeration, agitation of the volume,...that would fit to ecological conditions for intensive shrimp production in a sustainable approach.



## References

- Aminot A et Chaussepied M., 1983. Manuel des analyses chimiques en milieu marin. CNEXO, 395pp.
- Aquacop, 1984. Aquaculture en milieu tropical. Ifremer publications, Brest, France, 47pp.
- Aquacop et Garen, P. 1992. Elevage intensif de crevettes peneides. Le point des techniques au COP. Rapport interne COP, DRV/AQ/TAH 92.018, 18pp.
- Avnimelech Y., Kochva M. and Diab S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. *The Israeli Journal of Aquaculture*, 46:119-131.
- Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*. 176 (3-4) 227-235.
- Bianchi M. and Bianchi A, 1982. Evolution a court terme des effectifs et de l'activite des communautes bacteriennes dans les ecosystems d'aquaculture. 2eme Colloque Microbiologie Marine, Marseille, 1981. CNEXO ed. Actes colloques, 13:61-70.
- Funge Smith SJ. and Briggs, MRP. 1998. *Aquaculture*, 164: 117-133.
- Jousselin Y., Garen, P. y Aquacop, 1993. Engorde intensivo del camarón: el rigor del manejo permite doblar la producción. II Congreso Ecuatoriano de Acuicultura, Guayaquil, Ecuador, 20-25 Octubre.
- Racotta IS., Palacios E. and Ibarra AM. 2003. Shrimp larval quality in relation to broodstock condition. *Aquaculture*, 227: 107-130.
- Samocha, TM; Patnaik, S; Speed, M; Ali, A-M; Burger, JM; Almeida, RV; Ayub, Z; Harisanto, M; Horowitz, A; Brock, DL 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacult. Eng.* 36(2)184-191.
- Sohier, L. 1983. Participation des micro-organismes marins au maintien des capacites de production des milieux eutrophes a faible taus de renouvellement utilises pour l'elevage intensif de crevettes peneides. These Univ. Aix-Marseille II, 134pp.
- Wasielesky, W., Atwood, H., Stokes, A., Browdy, CL. 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*. 258(1-4) 396-403.