EXPERIMENTS FOR THE BREEDING OF AUTHOHTONOUS SHRIMPS AT THE ROMANIAN LITTORAL, AIMING AT THE TREATMENT OF RESULTED EFFLUENTS

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Abstract

Many aquacultural operations furnish metabolic products, such as faeces, ammonia and un-consumed food, which reach in the surrounding environment. Many times, the particulate organic debris are accumulated in the substrate in the near vicinity of the farm, while the soluble debris are eventually incorporated in the supplying waters. Organic enrichment of the benthic ecosystems can produce an increase of the oxygen content by the benthic communities, and consequently it can create anoxic conditions. At the same time, the increase of inorganic soluble nutrient content leads to the increase of the primary productivity of waters. That is way, some experiments viewing the growing of authohtonous shrimps (Palaemonidae Family), in correlation with the treatment of the effluents resulted from this process, was performed. The experiments were developed in two phases:

- **phase 1**: laboratory experiments with the growing of grass shrimp (*Palaemon adspersus*) based on the capturing of individuals from nature and subjected to experimentally growing in different cultivation vases, settled in laboratory conditions;

- **phase 2**: experiments in a pilot station, developed outdoors, regarding the growing of the juveniles of grass shrimps (obtained through natural conducted reproduction in NIMRD). The mini-pilot station is constituted from an integrate system of shrimp growing, where the resulted water represents the supplying source of a basin for mussel growing (*Mytillus galloprovincialis)*; the effluents from this basin are guided toward a basin for cultivation of macrophite algae (especially *Enteromorpha* sp.).

In both growing variants, the potential of breeding the two selected species was demonstrated. The effluents (biologically characterized in NIMRD) were delivered to National Institute for Environmental Engineering Bucharest for finding the solutions for their purifying.

Key words: breeding, authohtonous shrimp, effluent, purifying, integrate system

Introduction

During the past years, shrimp consumption has registered a significant increase in many countries of the world, due to their nutritive value. Out of the 320 shrimp species, about 100 are suitable for human consumption, conducted growing and artificial reproduction.

At the level of the year 1997 (FAO statistics), 941 thousands tons of shrimps were obtained, with a value of 6,074 mil. USD $. Is obvious that this industry has impact on the environment, especially through the effluents resulted from technological processes.

In the traditional aquacultural systems, the operators have periodically discharged in the environment, especially in the sea, the nutrients, dissolved gases, phytoplankton and patogenous agents. During the last decades, 10-30% of the waters of breeding basins were discharged in the world seas and oceans, every day. Lately, shrimps breeders discharge into the sea about 2 to 5% of the basin water. In the intensive systems, mechanical aeration adds extra-oxygen, in order to prevent hypoxia in the breeding tanks.

The main chemicals added in the breeding water are fertilizers (to stimulate the phytoplankton development, which is the food for shrimps) and burned lime (which modifies the acidity of the soil and water). In Asia, the farmers have constantly added porous minerals and zeolits in order to remove the amonia, and sometimes they desinfect the ponds with hypocloryte, formaldehyde and other compounds which kill the patogenous agents (JONES, 1999).

In some areas, the pollutants from the breeding farms of the shrimps are higher then the assimilation capacity of the littoral ecosystems. In the situation when the quality of effluents is within admissible limits, the high number of coastal farms can cause only an undesirable fertilization of the waters. The effects influence the lives of all the inhabitants living on the coast, including the aquaculture workers. But, eutrophikation is not the only trouble, a long chain of reactions can occur.

That is why, it imperative to find methods and ways for the treatment of the effluents resulted from aquaculture.

The present paper deals with the results of experiments performed for authohtonous shrimps breeding, aiming the treatment of the resulted effluents.

Materials and methods

The experiments were developed in NIMRD Constanta, in its experimental station, composed of:

- 9 outdoor concrete basins (5 x 10 x 1.5 m, supplied with sea water and under pressure air);

- wet laboratory: 2 glass fiber basins (1 x 2 x 1 m), 2 fiber glass basins (1 x 3 x 1 m), 16 PVC tabs/vats (70 l); all the experimental vases are supplied with marine water, under pressure air and supplementary light with neon tubes (Photo 1);

Photo 1 – NIMRD wet laboratory

- laboratory for algae breeding; 6 tanks of 120 l, different glass vases for intermediate culture, strains of allohtonous or authohtonous macrophitic algae (*Tetraselmis suecica*, *Nannochloris oculata* f. *atomus*, *Isochrysis* sp. (clone Tahiti), *Pavlova luthery*, *Chaetoceros calcitrans* f. *pumillum*, *Chaetoceros* sp.); the laboratory has a marine water supplying source, a filtration device, an installation for aeration with under pressure air;

- laboratory for invertebrates breeding: 14 PVC tubs/vats supplied with under pressure air and lighted with neon tubes.

The biologic material was represented by the individuals of *Palaemon adspersus* Ratke 1837 (grass shrimp) and *P. elegans* Ratke 1837 (rock shrimp) (Photos 2 and 3);

- adults captured from the natural environment;

- juveniles obtained through controlled-natural reproduction, and grown in controlled conditions.

Photo 2 - *Palaemon adspersus* Ratke 1837 (grass shrimp)

Photo3 - *Palaemon elegans* Ratke 1837 (rock shrimp)

The work methods were those used in aquaculture, namely the use “clear waters" or "green waters", according to the needs.

For the determination of the physical-chemical parameters, the standard methods were used, the samples being processed in the RENAR accredited physical-chemical laboratory.

Results and Discussions

1. Experimental variant I: *Palaemon adspersus* species

The experiments carried out between May and July 2004 were represented by:

- the creation of an experimental lot: juveniles of *P. adspersus* (about 10months of age, 3-4 cm in length, 0.222 g/specimen);
- the establishment of the captivity growing potential;
- the establishment of the optimal breeding density;
- the establishment of the feeding rations and recipes.

Sub-variant 1: intensive breeding

- breeding capacity: PVC tank, 0.050 mc (50 l); 0.200 mp (77 x 26 x 25 cm);
- breeding density:
    - a/ 1,000 ind./mp (about 4,000 ind./mc);
    - b/ 500 ind./mp (about 2,000 ind./mc);
    - c/ 250 ind./mp (about 1,000 ind./mc);
- marine water supplying: 0.35 l/min. (total replacement of the water from the tub - about 2.6 hours);
- permanent supplying with under pressure air;
- natural illumination;
- food supplying: minced fish meat, 30-10% of the body weight, once a day.

Sub-variant 2: extensive breeding

- breeding capacity: glass fiber basin, 3 mc, 3mp (3 x 1 x 1 m);
- breeding density: 500 ind./mp (about 500 ind./mc);
    - c/ 250 ind./mp (about 1,000 ind./mc);

- marine water supplying: 5.5 l/min. (total replacement of the water from the tub - about 9.5 hours);

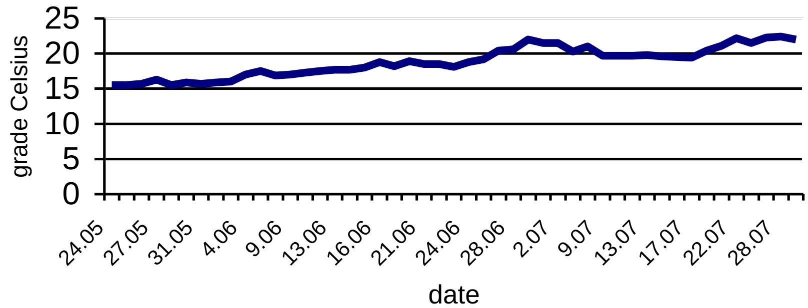
- permanent supplying with under pressure air;

- natural illumination;

- food supplying: minced fish meat, *ad libitum* (at request), once a day.

Water temperature is a very important environmental parameter in the breeding of organisms. There have been temperature variations, characteristic for the experimental period, from about 15^{o}C (in May) to 22^{o}C (in July); the temperature increase was constant, without sudden variations, which could have affected the physiology of the animals (Figure 1).

Figure 1 - Temperature variations of water in the experimental tank of grass shrimp



The physical-chemical characterization of water was carried out by the beneficiary.

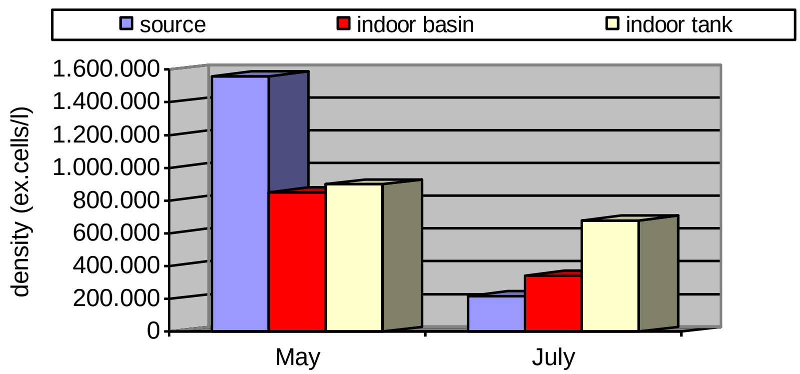
During the entire breeding period, the charges in the water, that is the content of phytoplankton, zooplankton and bacteria, was held under observation.

The phytoplankton did not realize a high development, the densities being insignificant, not-affecting the breeding environment. If in May the source had a higher charge getting diminished in experimental tubs, in July the source had a more reduced charge, which is obvious in the tanks charge (Table 1 and Figure 2).

Table 1 - The variation of phytoplankton density in variant I

Lot	Species	Density (cells/l)	
		May	July
Supplying source	Cyanophyta	$1,516 \times 10^6$	198×10^3
	Chlorophyta	$3,8 \times 10^4$	4×10^3
	Chrysophyta	-	14×10^3
	Bacillariophyta	4×10^3	-
	Total	*$1,558 \times 10^6$*	*216×10^3*
Indoor basin	Cyanophyta	$8,38 \times 10^5$	326×10^3
	Chrysophyta	-	8×10^3
	Chlorophyta	8×10^3	-
	Bacillariophyta	6×10^3	8×10^3
	Total	*$8,51 \times 10^5$*	*342×10^3*
Indoor tanks	Cyanophyta	$8,54 \times 10^5$	668×10^3
	Chrysophyta	-	10×10^3
	Chlorophyta	$2,6 \times 10^4$	
	Dinophyta	-	2×10^3
	Bacillariophyta	2×10^4	-
	Total	*9×10^5*	*680×10^3*

Figure 2 - Variation of total phytoplankton density

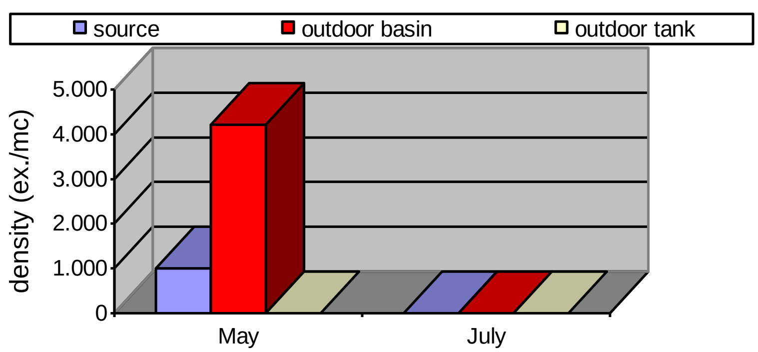


The zooplanktonic organisms were almost absent, both in the supplying source and in experimental tubs; the fact was positive for the experiment, because the animals received only inert food, in order for the animals to have an unique feeding source (Table 2, Graph 3).

Table 2 - The variation of zooplankton density in variant I

Lot.	Species	Density ex/m^3	
		May	July
1.Supplying source	Polichetes	1.000	
	Total	*1.000*	*0*
2. Indoor basin	Rotiferous	198	
	Copepodes (adults)	18	
	Polichetes	4.000	
	Total	*4.216*	*0*
3. Indoor tank	*Total*	*0*	*0*

Graph 3 - The variation of total zooplankton density



The bacterial charge, the most significant for the experiments regarding the treatment of the effluents resulted from the shrimp breeding, reflected both the quality of supplying sources and also of the breeding water. While the supplying source contained a bacterial charge within the normal limits for breeding organisms, in the experimental tub and basin there were registered situations which can be critical and plead for the necessity of effluents treatment.

The high number of heterotrophic saprophitic bacteria in the indoor basin, up to 600 thousands per ml of water, proves the existence of a high organic matter quantity (originated in the provided food - minced mussel flesh) (Table 3). Densities of 2,000-6,000 germs/ml pertaining to *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio anguillarum* or *Vibrio* sp. represent a real threat to the health of marine organisms, including shrimps.

Table 3 - Bacterial charge of the water used in grass shrimp breeding

Crt. No.	Groups (No./ml)	Supplying source May	Supplying source July	Indoor tank May	Indoor tank July	Outdoor basin May	Outdoor basin July
1	Saprophytic heterotrophic species	500	200	1.000	500	600.000	400
2	Total coliforms	20	20	70	70	170	20
3	Faecal coliforms	absent	absent	absent	20	absent	absent
4	Faecal strepococcs	absent	absent	absent	absent	absent	absent
5	*Aeromonas*	absent	absent	500	20	2.000	10
6	*Pseudomonas*	absent	absent	900	absent	6.000	absent
7	*Vibrio*	absent	absent	100	150	6.000	300

Growing of experimental lot, sub-variant I (growing in tanks):

The shrimps passed through an accommodation period, then they were put in the growing vases. The daily maintenance consisted in:

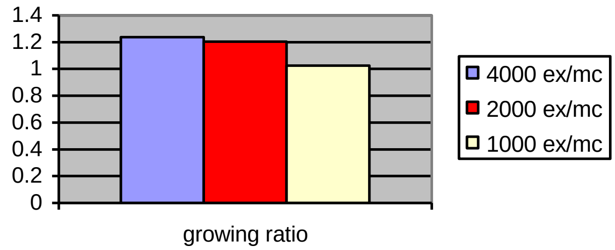
- food supplying (minced fish flesh, 30% from the body weight once a day);
- cleaning of food scraps;
- providing of a daily maintenance flow capacity.

The growing (expressed in relation growing/period = final weight/initial weight) is presented in Table 4.

Table 4 - Results in the grass shrimp's breeding

Crt. No.	Sub-variant	Density	Initial weight (25/05/2004) (g/ind.)	Final weight (30/07/2004) (g/ind.)	Growing Ratio (final/initial)	Survival (%)
1.	1a	200 ind./tub (about 4,000 ind./mc)	0.2225	0.2785	1.237	42
2.	1b	100 in./tub (about 2,000 ind./mc)	0.273	0.3288	1.204	35
3.	1c	50 ind./tub (about 1,000 ind./mc)	0.286	0.2931	1.024	58

Graph 4 - Variation of growing ratio of experimental lots of grass shrimp



Although the growing is almost uniform for all the experimental lots, the higher densities are preferred; higher growing ratios are registered at a density of 4,000 ind. /mc, with a survival rate of 50% (rather small, due to the cannibalism phenomenon, manifested at sloughs stages).

The supplied food consisted of small-sized marine fish, 30-10% from the total body weight (changed depending on the consumption).

2. Experimental variant II: Palaemon elegans

The experiment was realized outdoors, with natural lightening, the integrate breeding system of shrimps consisting in (Photo 2):

- 1 PVC tank (0.9 x 0.9 x 0,8 m), 0.648 mc, 0.810 mp, continually supplied with marine water ($Q_{maintenance}$ = 8 l/min.), and under pressure air; 2,000 specimens of grass shrimp, aged 6 weeks, (meaning a density of 3,086 ind. /mc or 2,469 ind./mp) were placed in the tank; food was provided daily, consisting in small-sized marine fish mixed with fish flour.

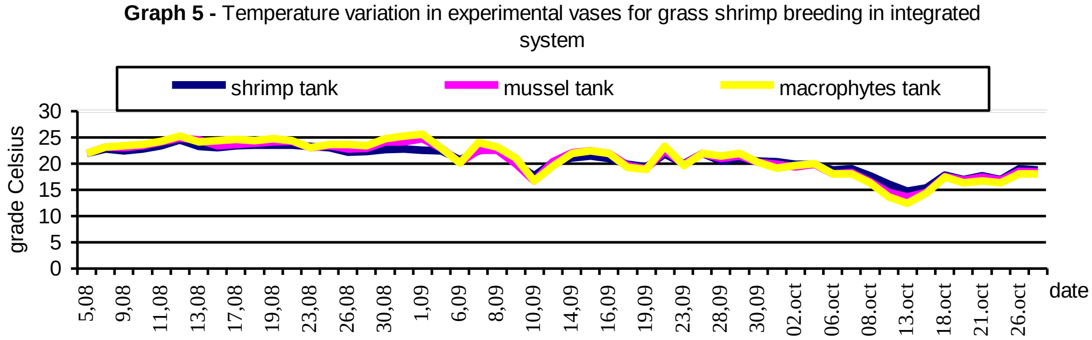
- 1 fiber-glass basin (3 x 1 x 0.4 m) was populated with mussel *Mytillus galloprovincialis:* 60 Kg/basin, 20 Km/mp; continually supplied with marine water resulted from the shrimps breeding system ($Q_{maintenance}$ = 8 l/min.).

- 1 fiber-glass basin (3 x 1 x 0.4 m) was populated with macrophytes, prevailing *Enteromorpha* sp.; continually supplied with marine water resulted from the mussel growing system ($Q_{maintenance}$ = 8 l/min.).

Photo 2 – NIMRD integrate breeding system of shrimps

The experiment started on August 5, 2004 and ended on October 28, 2004, with the aim of monitoring the effluents from the system (shrimp growing, mussel growing and macrophyte algae growing), and also of monitoring the effect of bio-filtration on these species.

Water temperature oscillated in limits characteristic for experiment, namely among 14.4 and 25.2°C, with uniform oscillations within 24 hours (Graph 5).



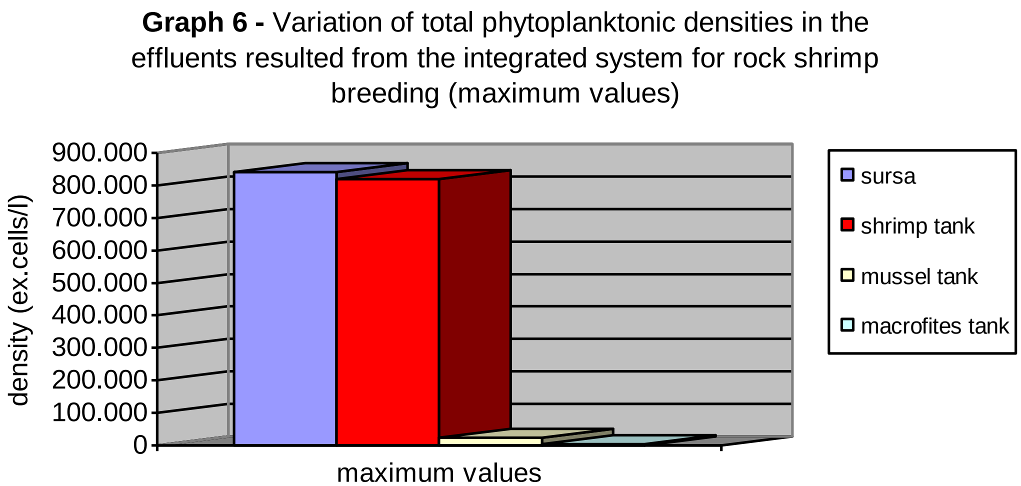
Graph 5 - Temperature variation in experimental vases for grass shrimp breeding in integrated system

Throughout the whole breeding period, the charges in the water (phyto-, zooplankton and bacteria) were under observation.

The phytoplankton did not have high developments, its densities being insignificant, without modifications on the environment quality (Table 5, Graph 6).

Table 5 - Variation of phytoplanktonic densities in variant II

Lot	Groups	Density (cells/l) (limits: min.-max.)
1. Supplying source	Cyanophyta	100-682 x 10^3
	Dinophyta	50-154 x 10^3
	Chlorophyta	0-4 x 10^3
	Total	150-840 x 10^3
2. Shrimps tank	Cyanophyta	300-650 x 10^3
	Dinophyta	120-158 x 10^3
	Bacillariophyta	0-12 x 10^3
	Total	420-820 x 10^3
3. Mussels tanks	Dinophyta	0-14 x 10^3
	Bacillariophyta	0-10 x 10^3
	Total	0-24 x 10^3
	Dinophyta	0-2 x 10^3
	Bacillariophyta	0-2 x 10^3
4. Macrophytes tank	Total	0-4 x 10^3
	Cyanophyta	100-682 x 10^3
	Dinophyta	50-154 x 10^3
	Chlorophyta	0-4 x 10^3



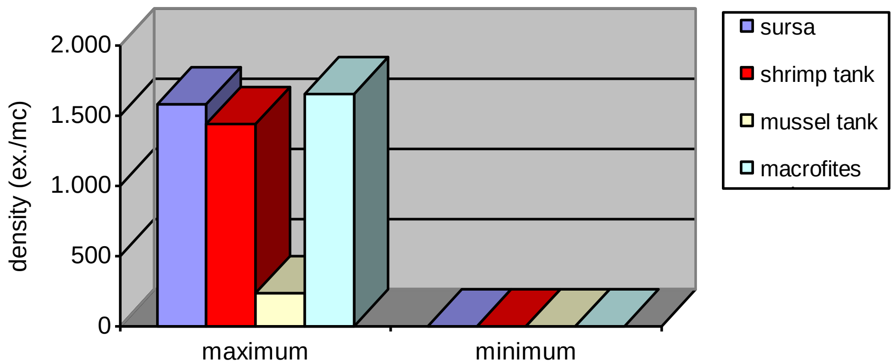
Graph 6 - Variation of total phytoplanktonic densities in the effluents resulted from the integrated system for rock shrimp breeding (maximum values)

The zooplankton was almost absent, both in supplying source and in experimental tanks, a positively fact for inert food distribution (Table 6, Graph 7).

Table 6 - Variation of zooplanktonic densities in variant II

Lot	Group	Density (ind./mc) (limits: minim-maxim)
1. Supplying source	- copepods	0 - 240
	- meroplankton	
	- mollusk larvae	0 - 240
	- *Balanus* sp. larvae	0 - 12
	- nematods	0 - 2
	- polychaets	0 - 1.080
	- chironomids	0 - 7
	- Total	0 - 1.581
2. Shrimp tanks	- copepods: - *nauplii*	0 - 320
	- *exuvii*	0 - 640
	- meroplankton	
	- polychaets	0 - 480
		0 - 1.440
3. Mussels tank	- copepods	0 - 22
	- meroplankton	
	- *Balanus* sp. larvae	0 - 164
	- polychaets	0 - 40
	- chironomids	0 - 10
	- Total	0 - 236
4. Macrophytes tank	- copepods	0 - 608
	- meroplankton	
	- *Balanus* sp. larvae	0 - 984
	- ostracods	0 - 48
	- polychaets	0 - 8
	- nematods	0 - 8
	- Total	0 - 1.656

Graph 7 - Variation of total zooplanktonic densities in rock shrimp breeding



The surveillance of the bacterial charge of the effluents, comparatively with the supplying source (marine water, non-filtered, only decanted) revealed the following aspects (Table 7):

- the marine water (the source) had a reduced bacterial charge;
- a significant depletion of the bacteria content was registered after its passing through mussels tank (heterotrophic bacteria and total coliforms);
- the *Vibrio* germs were present in all the samples; no diminution was noted at any level, maybe due to the fact that even the supplying source had a reduced number of germs.

The breeding of experimental lot, sub-variant I (breeding in tanks):

The shrimps passed through an accommodation period; then, they were placed in growing basin. The daily maintenance consisted in:

- food supplying (minced fish flesh, 30% from the body weight, once a day);
- cleaning of food scraps;
- providing of a daily maintenance flow capacity.

The growing (expressed in relation growing/period) was as follows:

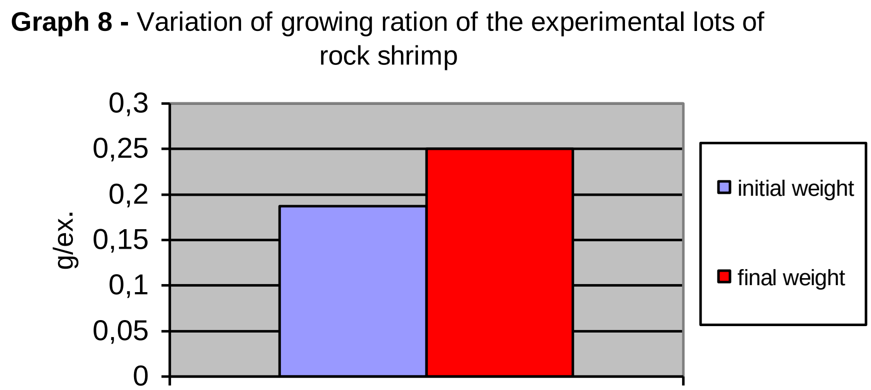
- for the population: juveniles of rock shrimp (*P.elegans*), reproduced in NIMRD laboratories, aged 35 days, had a mean weight 0.187 g/ind.;
- at the end of the experiment (28.10.2004): age 106 days, mean weight 0.250 g/ind., mean length 32 mm (max. 41 mm, min. 26 mm.

Table 7 - Bacterial loading of water used for rock shrimp (integrated system) breeding (limits: minim-maxim)

Crt. No.	Groups of bacteria	Source	Tank with shrimps	Tank with mussels	Tank with macrophytes
1	Saprophytic heterotrophic species (No./l)	1.000-18.000	1.000-20.000	0 - 1.000	0 - 1.000
2	Total coliforms (No./l)	170 - 1.090	170 - 1.090	0 - 170	0 - 170
3	Faecal coliforms (No./l)	abs.- 260	abs.- 260	abs.- 70	abs.- 70
4	Faecal streptocos (No./ml)	abs.	abs.	abs.	abs.
5	*Aeromonas* (No./ml)	abs.	abs.	abs.	abs.
6	*Pseudomonas* (No./ml)	abs.	abs.	abs.	abs.
7	*Vibrio* (No./ml)	abs.- 50	abs.- 50	abs.- 40	abs.- 50

Table 8 - Results obtained in rock shrimp breeding

No. crt.	Sub-variant	Density	Initial weight 05.08.2004 g/ind.	Final weight 28.10.2004 g/ind.	Growing ratio (final/initial	Survival (%)
1.	Integrated system	3.086 ex. /m^2 or 2.469 ex. /m^3	0,187	0,250	1,337	57,65



Graph 8 - Variation of growing ration of the experimental lots of rock shrimp

The survival is similar to that of the experiment carried out with the grass shrimp (57.65% comparatively with 35-58%).

The supplied food consisted in small-sized marine fish, 30-10% from the total body weight (quantity modified depending on the consumption).

Conclusions

1. The experiments dealt with the breeding of authohtonous shrimp (Palaemonidae Family), aiming to carry out experiments for the treatment of the effluents resulted in this process.

2. The experiments comprised two stages:

- *stage 1*: laboratory experiments regarding the grass shrimp *Palaemon adspersus*, based on the specimens captured from the natural environment and subjected to experimental breeding in different tanks, settled in conditions of laboratory;

- *stage 2*: experiments in a pilot station, developed outdoors, regarding the growing of rock shrimp *P.elegans* (obtained in natural controlled reproduction within NIMRD). The mini-pilot station consisted in an integrated system of shrimp breeding, in which the water obtained in shrimp breeding was uses as supplying source for a basin for mussels (*Mytillus galloprovincialis*); the effluents resulted in this basin were conducted towards a basin for macrophyte algae growing (especially *Enteromorpha* sp.).

3. The potential for breeding the two species was demonstrated through the two variants. The effluents (biologically characterized in NIMRD) were delivered to the beneficiary (NIEE Bucharest), in order to develop some technologic solutions for their filtration.

Note: The researches were developed within the sub-contract concluded between NIMRD Constanta and NIEE Bucharest; the title holder PN 03 04 02 06 (NUCLEU Programme), between 2003 and 2005: "Researches regarding the development of technologic solutions for purifying the waste waters obtained in the shrimp breeding on the marine littoral, aiming the protection of production and the environment." Cooperation with Vietnam Institute of Water Resources Research - Ha Noi - Vietnam"

References

1. JONES A., 1999 – Environmental Management of Aquaculture Effluent: Development of Biological Filters, Ph.D. thesis, The University of Queensland, Australia, 238 p.