The influence of IMTA pond production systems on the dynamics of heterotrophic bacteria in the pond water column

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Abstract. In any aquatic ecosystem, the essential role in the distribution of heterotrophic bacteria is assigned to the main environmental parameters such as temperature, pH and dissolved oxygen. For this reason, the aim of the present study was to study the influence of the integrated multi-trophic aquaculture (IMTA) pond production systems on the dynamics of heterotrophic bacteria in the pond water column for two different on-growing systems for cyprinids, integrated multitrophic system (P1) and traditional system (P2), correlating the number of heterotrophic bacteria (log CFU mL^{-1}) with the main parameters of the technological environment, such as: temperature (T °C), pH, dissolved oxygen (DO mg L^{-1}) and chemical oxygen demand (COD mg L^{-1}) from water samples, during vegetative season, July – September 2016. The results of total number of heterotrophic bacteria of pond water of the P1 pond, during July, were found in the range of 3.42 log CFU mL^{-1} to 4.38 log CFU mL^{-1} and for the P2 pond, from 3.53 log CFU mL^{-1} to 3.89 log CFU mL^{-1}. In August, the number of bacteria ranged from 4.10 log CFU mL^{-1} to 4.66 log CFU mL^{-1} in the P1 pond and from 4.45 log CFU mL^{-1} to 4.60 log CFU mL^{-1} for the P2 pond. The count of bacteria during September were in the range 4.07 log CFU mL^{-1} to 4.59 log CFU mL^{-1} in the P1 pond and 4.26 log CFU mL^{-1} 4.03 log CFU mL^{-1} in the P2 pond. During the experiment, the highest number of heterotrophic bacteria was recorded in August in both types of carp growing systems, IMTA and traditional. There were no significant differences of total number of heterotrophic bacteria between the two ponds, P1 and P2. Fish feed consumption monitoring and stable phytoplankton microflora maintenance may lead to reduced bacterial load, especially heterotrophic bacteria.

Key Words: aquatic ecosystem, cyprinids, IMTA system, pond aquaculture, environmental parameters.

Introduction. One of the most important areas which determine pond dynamics, fish health and the hygiene of the fish farming system is bacteriology. Pond aquaculture is based on feed administration in addition to other management practices. Therefore, fish bacteria in cyprinids culture receive increased attention, some bacteria species being associated with increased stress on fish stocks. In the pond environment bacteria are to be found in water and sediment, and also on detritus and aquatic plants. Fish organism is in direct contact with the microflora of the environment and opportunistic pathogens, already present in water, overrun the host in stressful conditions.

Uddin & Al-Harbi (2012) and Razavilar et al (2013) conducted researches on the influence of bacterial composition of fish, especially on the health and growth of the host. Skjermo & Vadstein (1999) and Lavens & Sorgeloos (2000), also studied the influence of bacterial microflora on the host, which is clearly of great interest in aquaculture, especially where low productivity and/or stock losses are widespread. Thus, it is very important to know the bacterial microflora associated with the pond, as a living environment of cultured cyprinids. Cahill (1990), Otta et al (1999) and Reilly & Kaferstein (1997) have shown in their researches the importance of knowing the microflora of the environment associated with fish microflora. Thus, bacterial load and bacterial types in wild fish have been studied, but there is little research on bacterial flora of cultured fish. Zobell (1946), Kriss (1963) and Sieburth (1971) cited by Ganesh et al (2010) conducted the first studies on the distribution of large heterotrophic bacteria. Keller (1960) studied the distribution and activities of heterotrophic bacteria in polluted waters. His research has shown that the number of bacteria in the water is naturally variable and depends on a wide variety of factors (Ganesh et al 2010).

Bacteria play a vital role in the pond ecosystem. They have a positive role in recycling nutrients and in organic matter degradation, and a negative role as fish pathogens. In addition to native bacteria in the ponds, manure and fish feed, the high density in intensive and semi intensive farms leads to high bacterial density and therefore causes disease in the farming system.

The aim of the present study was to study the influence of the integrated multi-trophic aquaculture (IMTA) pond production systems on the dynamics of heterotrophic bacteria in the pond water column for two different on-growing systems for cyprinids, integrated multitrophic system (P1) and traditional system (P2).

Material and Method

Study area. The researches were conducted in the Movileni Fish Farm, Iaşi district, Romania. The farm's water source is Jijia River. The water supply and discharge are gravitationally, by using „monk" type hydraulic constructions.

Experimental design. The experiment has been conducted for 83 days, during vegetative season (July, August and September) of year 2016 in two ponds with an area of 0.45 ha each and an average water depth of 1.5 m.

The experimental design and sampling areas are presented in Figure 1.

Figure 1. Details of the study area, Movileni Fish Farm, Iaşi district, Romania.

The first pond (P1) was used for cyprinid multitrophic aquaculture system (IMTA). It has been divided by using a net as follows: first part with an area of 0.15 ha dedicated to carp monoculture (P1C) and the second part with an area of 0.30 ha dedicated to cyprinid polyculture (P1P). The second pond (P2) was used for cyprinid traditional polyculture system.

The fish stocking structure in P1P was: common carp (*Cyprinus carpio*) - 73%, grass carp (*Ctenopharyngodon idella*) – 15%, bighead carp (*Hypophtalmichthys nobilis*) – 6% and silver carp (*Hypophthalmichthys molitrix*) – 6% and in P2 it was: *C. carpio* – 93%, *C. idella* – 4%, *H. nobilis* – 1.5% and *H. molitrix* 1.5%. The average weight of the fish used in the experiment was for: *C. carpio* – 61.4±9.95 g/specimens, *C. idella* – 199.9±20 g/specimens, *H. nobilis* – 1,880.5±187.03 g/specimens, *H. molitrix* – 2,025.15 g/specimens.

Fish feed was represented by a cereals mix, distributed in P1C and P2, five days per week during the experimental period, as it was described in the work published by Petrea et al (2017).

Water sampling. The water samples were collected in plastic containers of 0.5 L and 1 L capacity from inlet (I) and outlet area (O) of each of the both ponds. P1 pond had four sampling points coded - I.1.1., O.1.1., I.1.2, O.1.2. and P2 pond had two sampling points coded - I.2.1., O.2.1. Also, there were collected samples on intake water source, coded A and the ponds outlet coded E1 and E2. For the estimation of different parameters, all water samples were collected every two hours from all sampling points during the experiment. The samples were pre-treated on the field to preserve them and immediately brought to the laboratory for physic-chemical and bacteriological analysis of various parameters following the standard methods (APHA 1989).

Physico-chemical parameters. The water physico-chemical parameters, such as temperature (T °C), pH, dissolved oxygen (DO mg L^{-1}) and chemical oxygen demand (COD mg L^{-1}) were determined. The equipment used for water analyses were: the HQ40d Portable Multi-Parameter (HACH-Lange), respectively Spectroquant photometer.

Microbiological analysis. The total number of coliform bacteria and total mesophilic aerobic bacteria as main bacteriological indicators for the bacteriological quality of water used for the cyprinid farm were determined. The number of total heterotrophic bacteria was carried out in pour plate using plate count agar followed by incubation at 37°C for 48 h, method provided by the Romanian Standard STAS 3001-91.

Statistical analysis*.* The one-way analysis of variance (ANOVA) has been performed on the data of bacterial and physical-chemical variables using Statistica (version 5) software. Mean values and standard errors were generated for each physico-chemical parameter and microbial group. The significance of differences was defined at $p<0.05$.

Results. The results of the total number of heterotrophic bacteria for water samples in experimental ponds (P1 – IMTA system and P2 – traditional system) are shown in Figure 2.

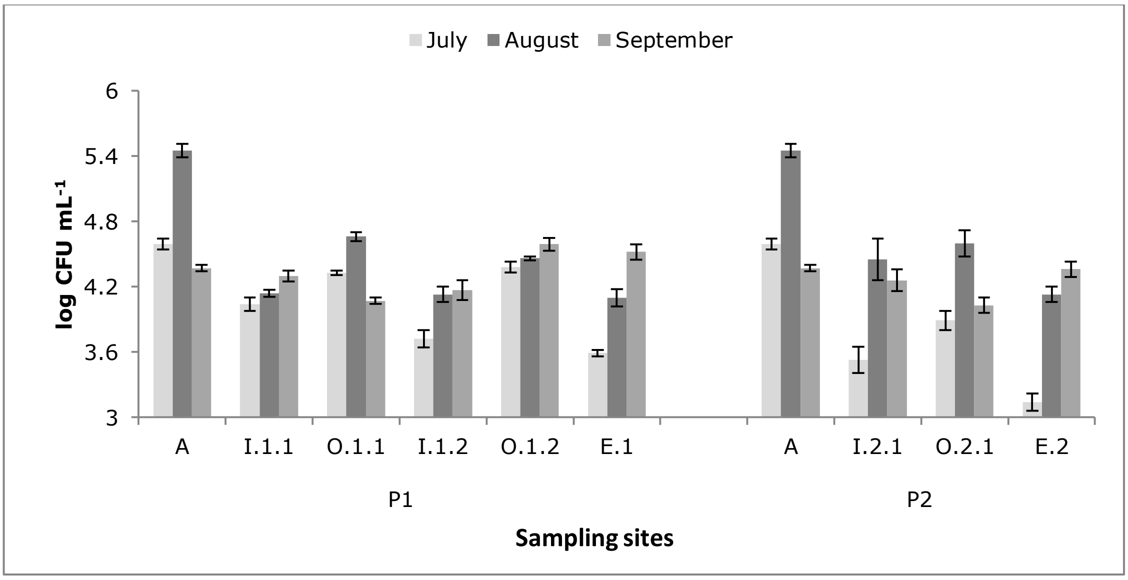

Figure 2. The dynamics of heterotrophic bacteria in the pond water column: P1 – IMTA system, P2 – traditional system.

During the vegetative season, July, August and September, there were variations in the number of heterotrophic bacteria.

The results of total number of heterotrophic bacteria of pond water of the P1 pond, during July, were found between 3.42 log CFU mL^{-1} and 4.38 log CFU mL^{-1} and for the P2 pond, from 3.53 log CFU mL^{-1} to 3.89 log CFU mL^{-1}. In August, the number of bacteria ranged from 4.10 log CFU mL^{-1} to 4.66 log CFU mL^{-1} in the P1 pond and from 4.45 log CFU mL^{-1} to 4.60 log CFU mL^{-1} for the P2 pond. The counts of bacteria during September were ranged from 4.07 log CFU mL^{-1} to 4.59 log CFU mL^{-1} in the P1 pond and 4.26 log CFU mL^{-1} 4.03 log CFU mL^{-1} in the P2 pond.

The correlation between the total number heterotrophic bacteria and some physicochemical parameters of the water samples from all the sampling points on the both ponds, P1 and P2 are presented in Figures 3-5. No significant variation was observed in the parameters between the ponds.

Figure 3 presents the correlation between temperature (Figure 3a), pH (Figure 3b), DO (Figure 3c), COD (Figure 3d) and total number of heterotrophic bacteria on all the sites from each pond in July 2016. For the P1 pond, the average temperature of water in sampling points varied between 20.30°C and 20.85°C, the average pH in the range of 8.48 to 8.57. The DO ranged from 7.76 mg L^{-1} to 10.22 mg L^{-1} and the COD from 87 mg L^{-1} to 124.5 mg L^{-1}. For the P2 pond, the temperature varied between 21.60°C and 22.45°C and the pH value between 8.24 to 8.53. The value of the DO ranged from 8.18 mg L^{-1} to 8.91 mg L^{-1} and the value of the COD from 108.5 mg L^{-1} to 136.5 mg L^{-1}.

The differences of the physicochemical parameters values were insignificant ($p>0.05$) in the analyzed cyprinid ponds P1 and P2.

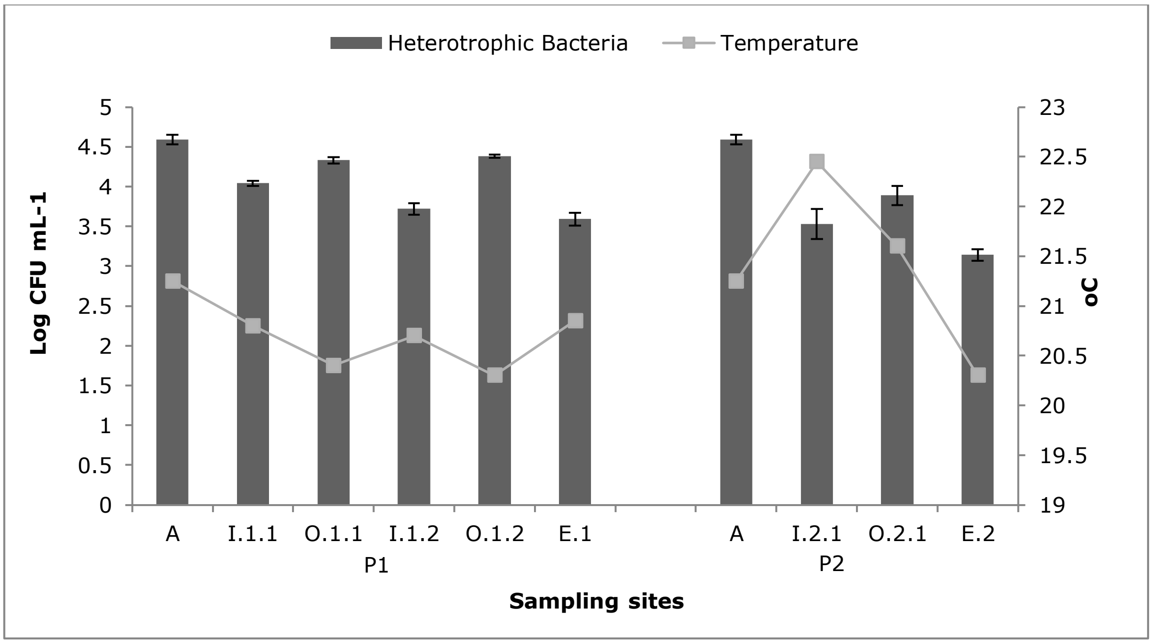

Figure 3a. Correlations between the number of heterotrophic bacteria and temperature of the water in P1 and P2 ponds, July 2016.

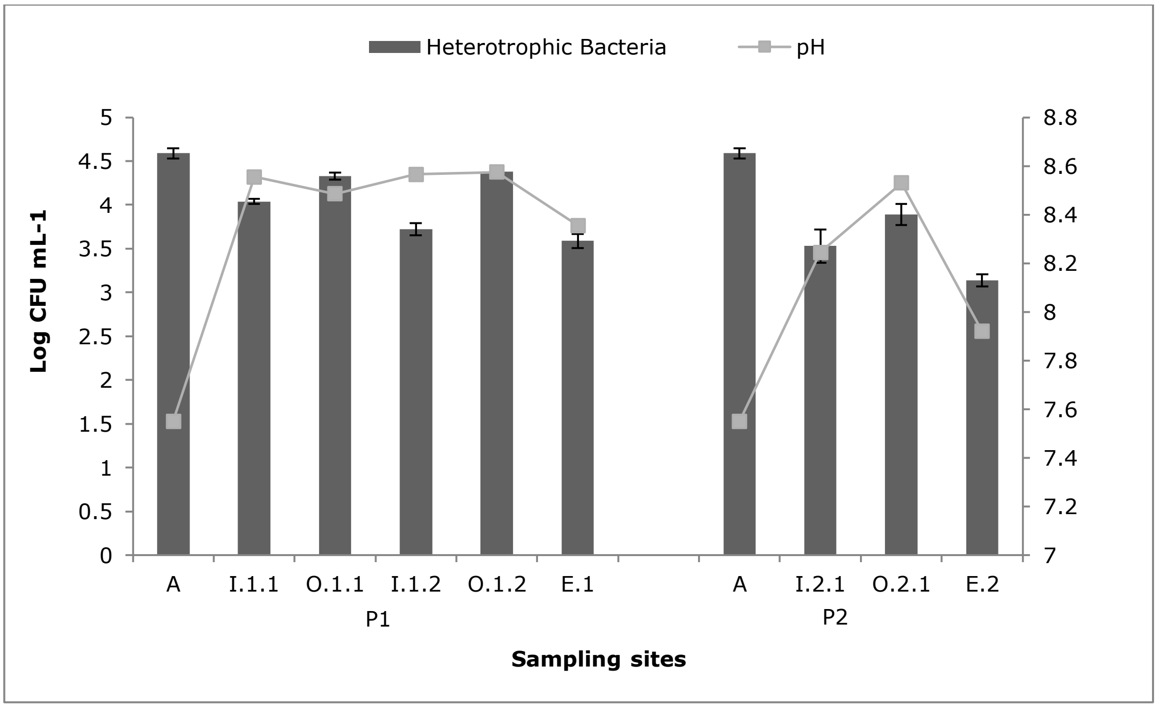

Figure 3b. Correlations between the number of heterotrophic bacteria and pH of the water in P1 and P2 ponds, July 2016.

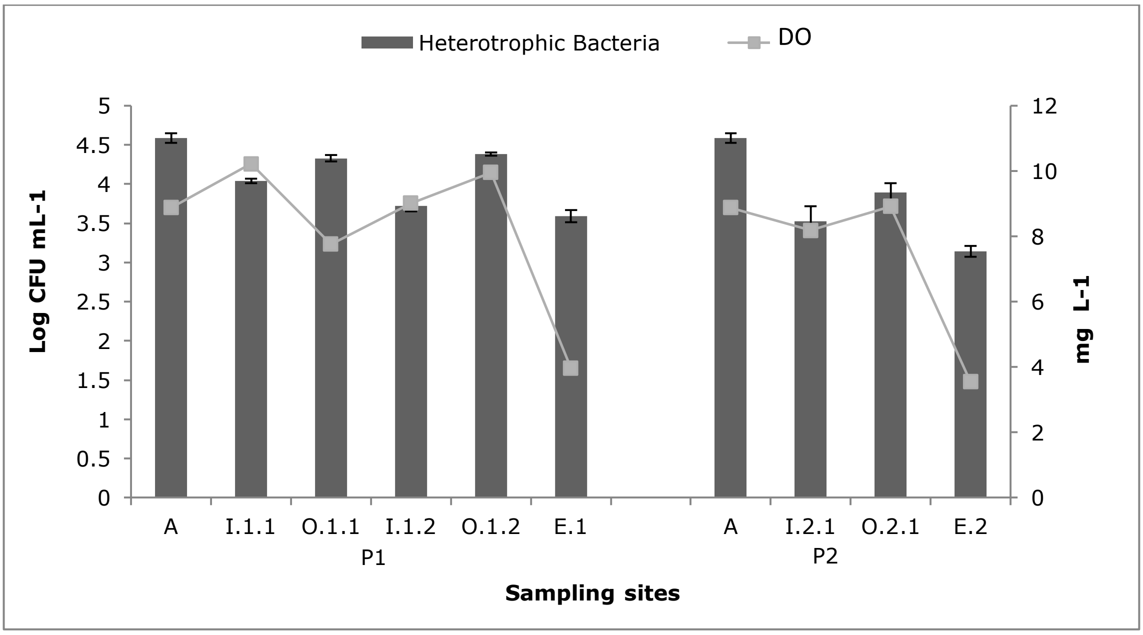

Figure 3c. Correlations between the number of heterotrophic bacteria and dissolved oxygen of the water in P1 and P2 ponds, July 2016.

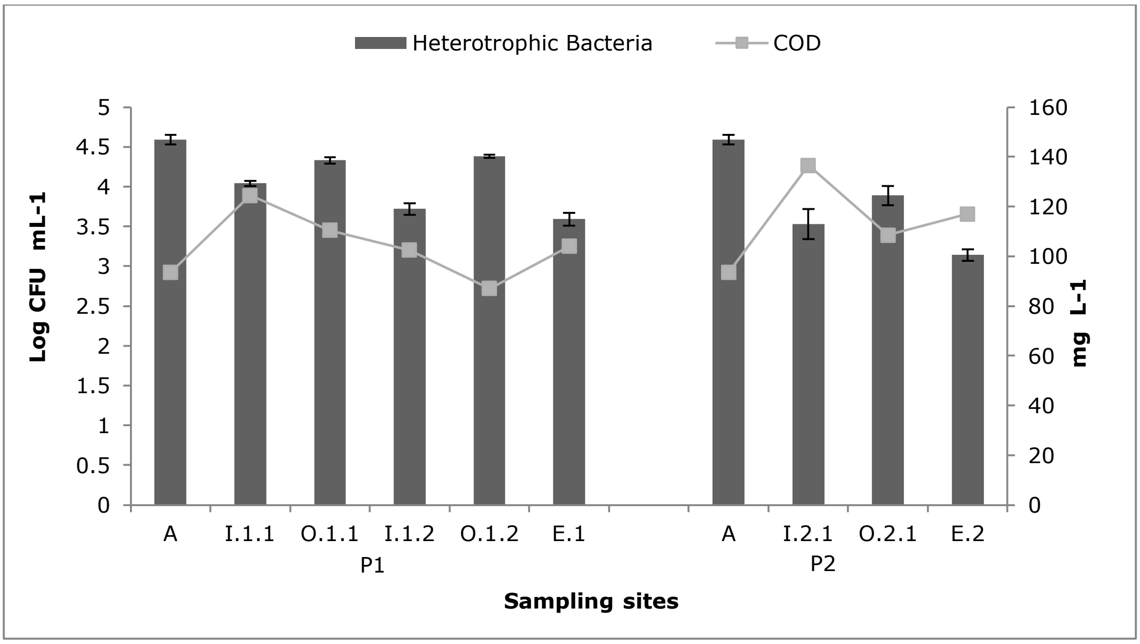

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Figure 3d. Correlations between the number of heterotrophic bacteria and the chemical oxygen demand in P1 and P2 ponds, July 2016.

For August 2016, the results of the correlation between temperature, pH, DO, COD and total number of heterotrophic bacteria is shown on the Figure 4.

For the P1 pond, the temperature of water varied between 20.10°C and 20.95°C and the value of the pH between 8.69 to 8.77. The DO content was ranged from 6.58 mg L^{-1} to 7.25 mg L^{-1} and the content of the COD, between 130.5 mg L^{-1} to 146.5 mg L^{-1}.

The physic-chemical parameters of the water samples of the P2 pond recorded variations. The temperature values recorded in the P2 pond were between 20.35°C and 20.85°C and the pH value, 8.69 to 8.77. The content of the DO varied between 6.58 mg L^{-1} to 7.25 mg L^{-1} and the content of the COD from 130.5 mg L^{-1} to 146.5 mg L^{-1}.

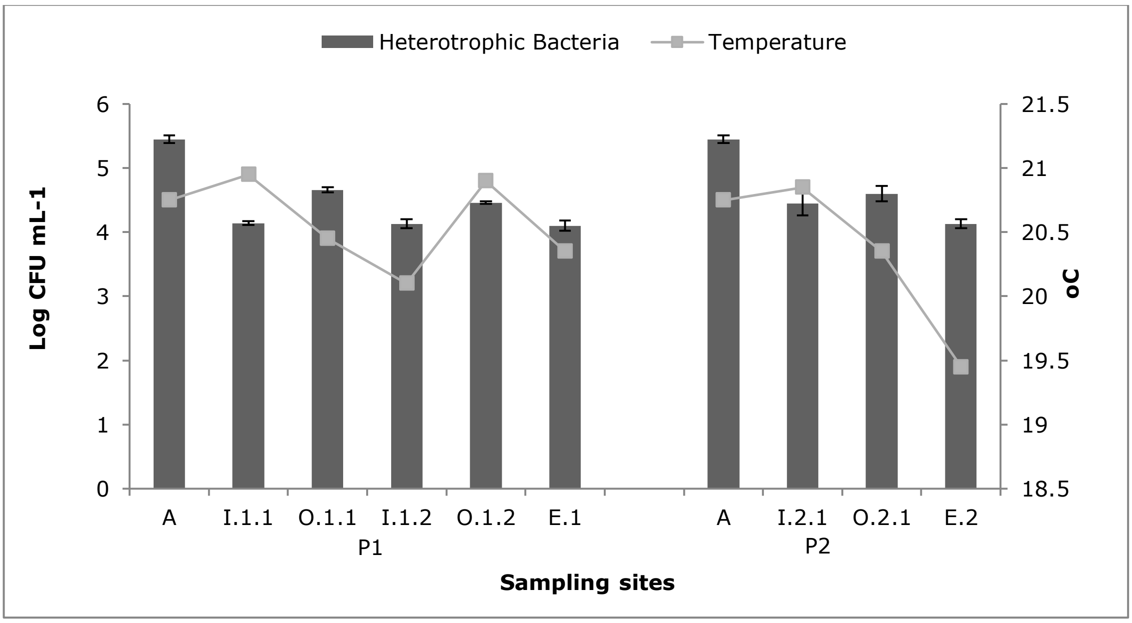

Figure 4a. Correlations between the number of heterotrophic bacteria and water temperature in P1 and P2 ponds, August 2016.

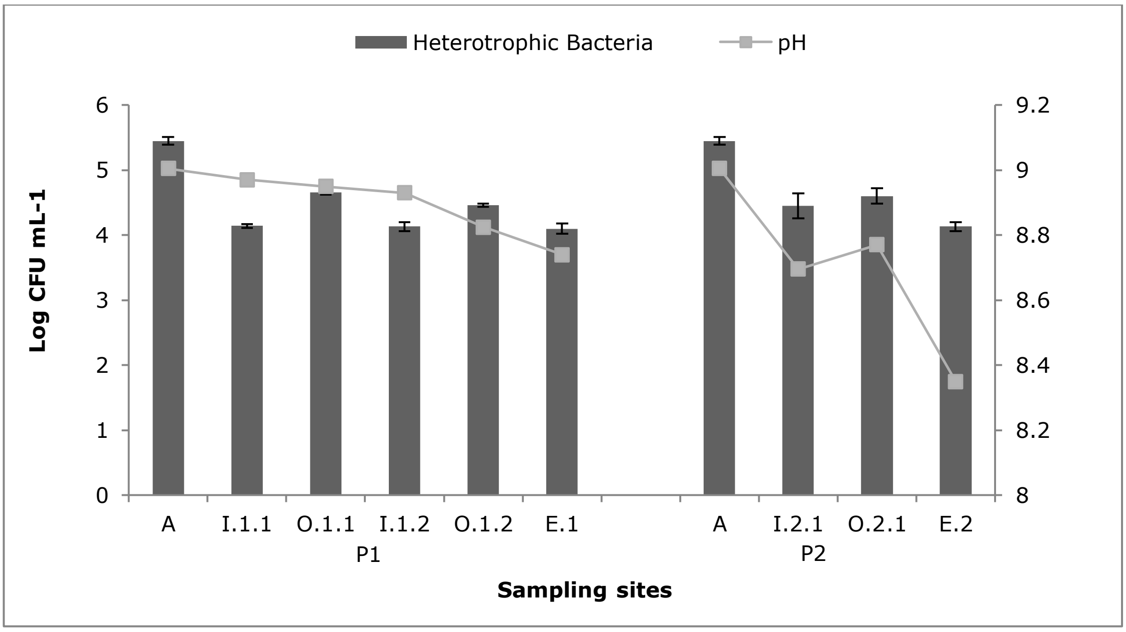

Figure 4b. Correlations between the number of heterotrophic bacteria and water pH in P1 and P2 ponds, August 2016.

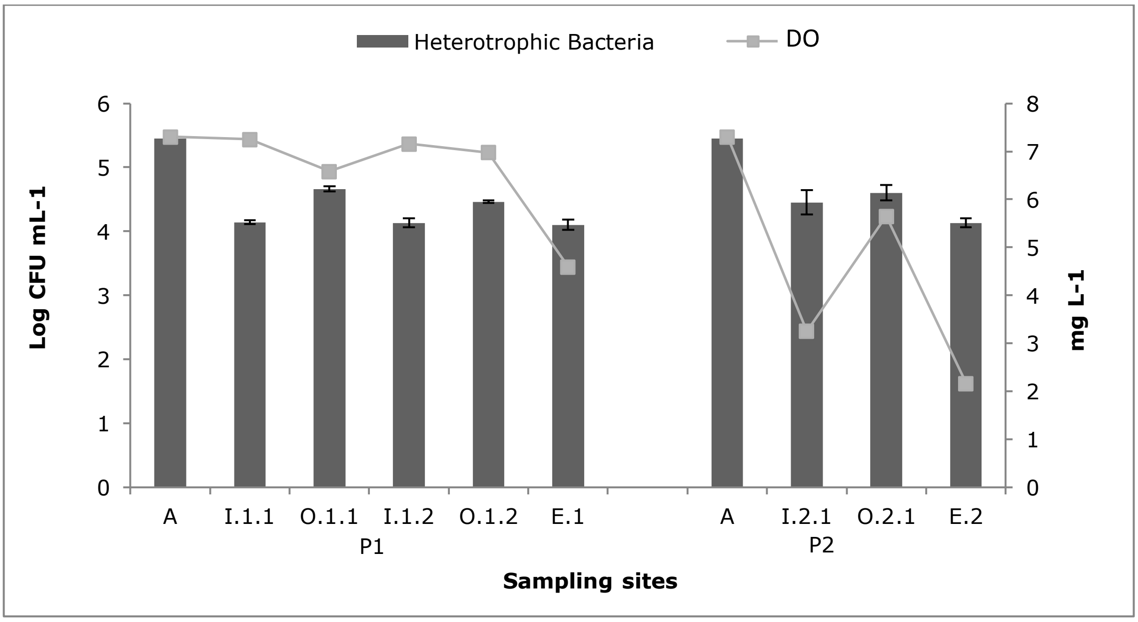

Figure 4c. Correlations between the number of heterotrophic bacteria and dissolved oxygen of water in P1 and P2 ponds, August 2016.

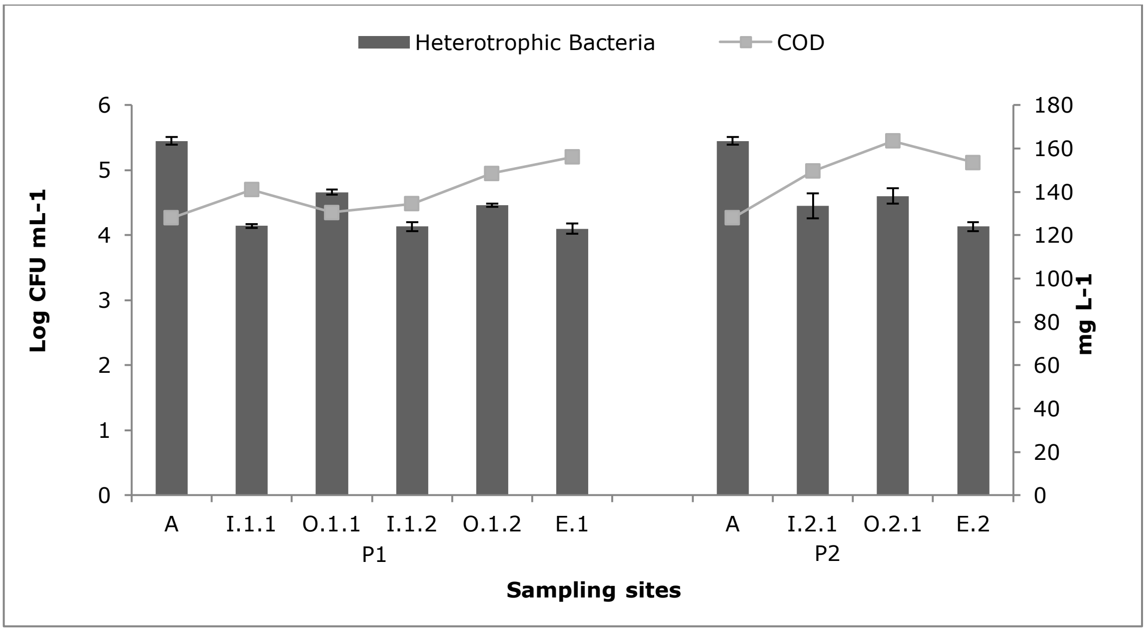

Figure 4d. Correlations between the number of heterotrophic bacteria and the chemical oxygen demand in P1 and P2 ponds, August 2016.

The correlation between the physicochemical parameters and the heterotrophic bacteria in September 2016 is presented in the Figure 5 (temperature, pH, DO and COD).

The value of the temperature of water from the P1 pond varied between 16.05°C and 16.35°C and between 16.25°C and 16.75°C in the P2 pond. The determinate value of the pH was situated between 9.21 to 9.38 for P1 pond and 9.22 to 9.41 in the P2 pond. In the P1 pond, the value of the DO varied from 8.15 mg L^{-1} to 11.96 mg L^{-1} and in the P2 pond, from 9.42 mg L^{-1} to 9.97 mg L^{-1}. For each of the ponds, P1 and P2, the content of the COD ranged between 156.5 mg L^{-1} to 183 mg L^{-1} respectively, in the range of 173.5 mg $L^{-1}$and 190 mg L^{-1}.

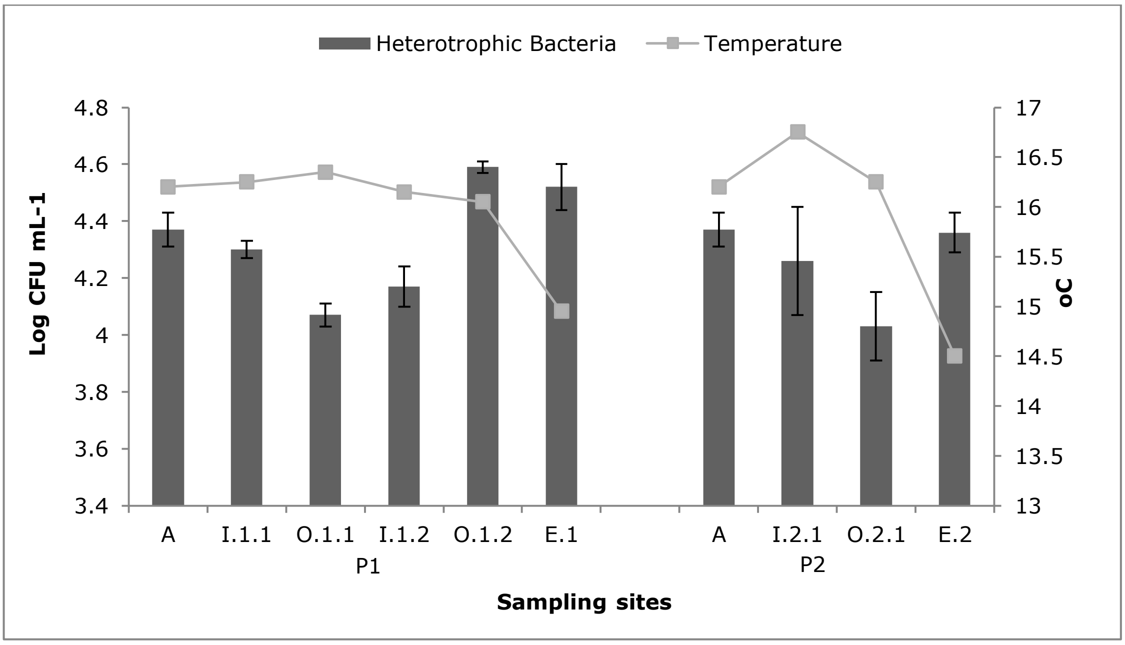

Figure 5a. Correlations between the number of heterotrophic bacteria and water temperature in P1 and P2 ponds, September 2016.

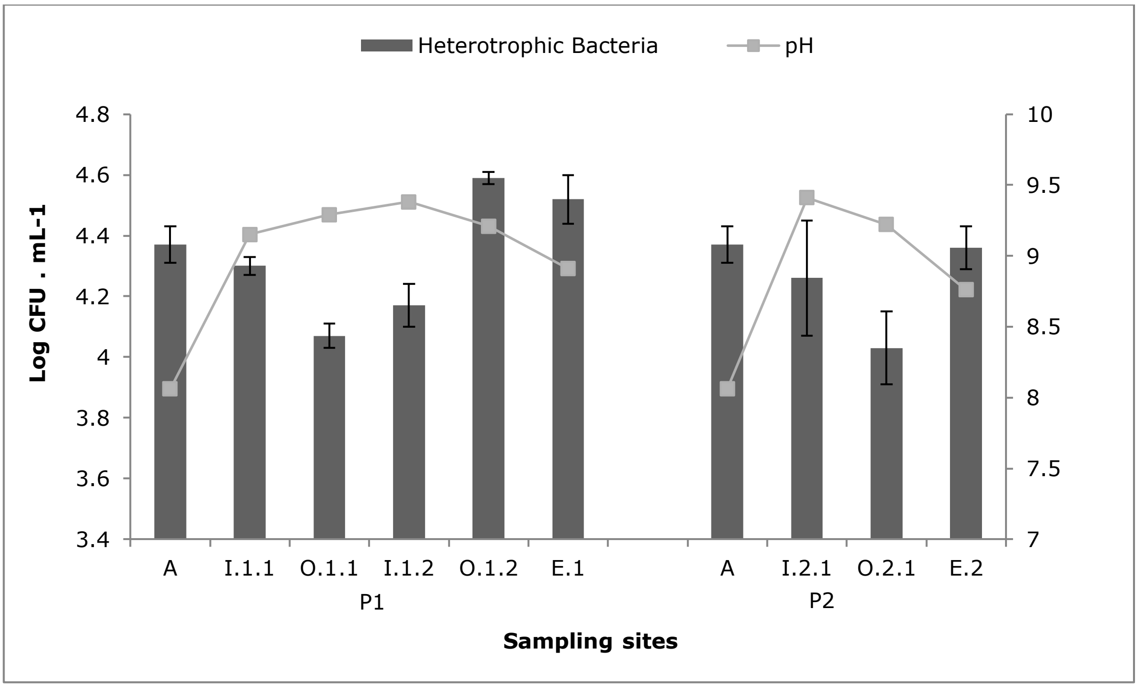

Figure 5b. Correlations between the number of heterotrophic bacteria and water pH in P1 and P2 ponds, September 2016.

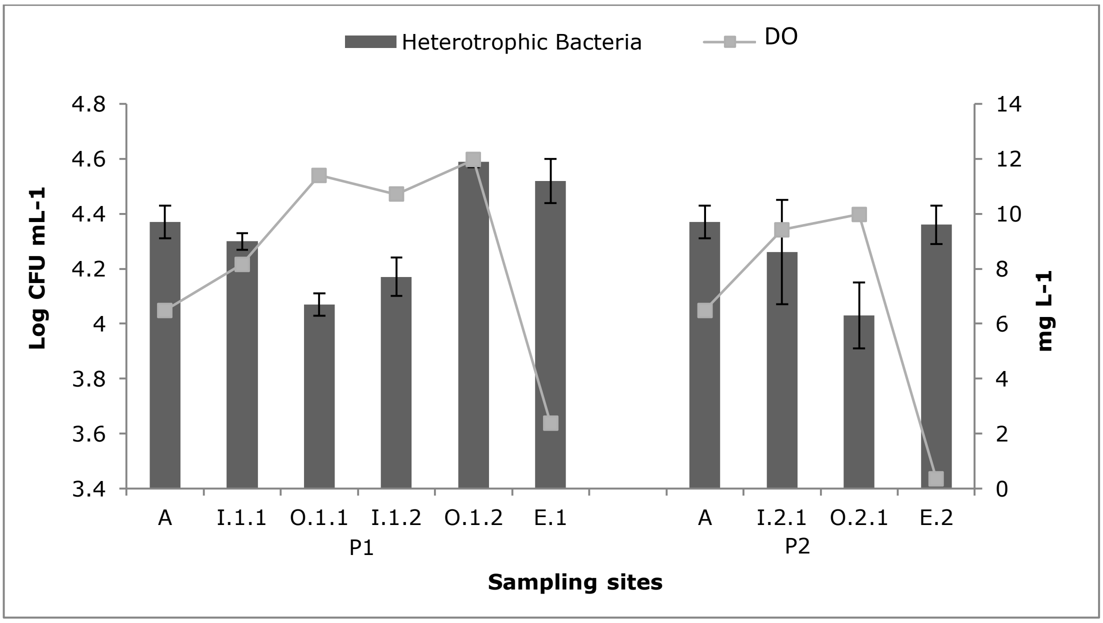

Figure 5c. Correlations between the number of heterotrophic bacteria and dissolved oxygen of the water in P1 and P2 ponds, September 2016.

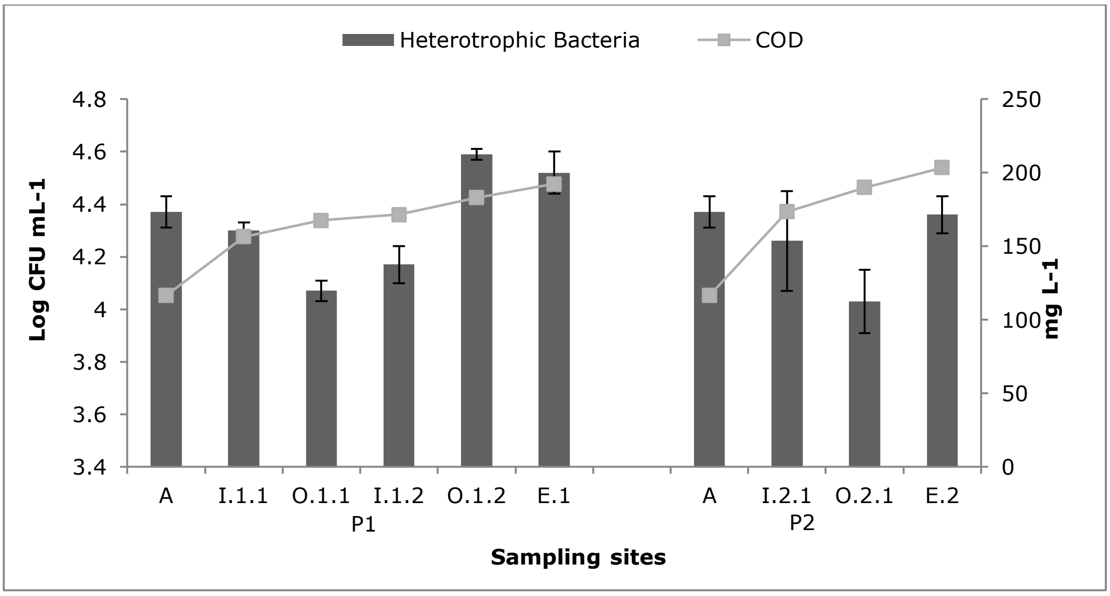

Figure 5d. Correlations between the number of heterotrophic bacteria and chemical oxygen demand in P1 and P2 ponds, September 2016.

Discussions. In any aquatic ecosystem, the essential role in the bacteria distribution is determined by environmental parameters such as temperature, pH and DO. Generally, the pond is a limited environment, the optimum environmental parameters (temperature 27-31°C, pH 7.8-8.6 and DO 5.1-5.9 mL^{-1}) could be maintained throughout the growing season by properly managing the pond physico-chemical parameters which involves lime treatment and permanent water exchange (Ganesh et al 2010).

Sharmila et al (1996) argued that bacterial load changes due to organic matter stored on the pond bottom, regardless of environmental factors and water exchange. Because turbidity, heavy organic matter plays a major role in the distribution of the bacterial population in pond water. Lloberra et al (1991) and Moriarty (1997) reported that in the ponds where cyprinids are farmed, there is high organic matters loading because of the organic fertilizers, high fish density, food waste, faeces, algae infiltration and human interference.

Ayyappan & Pande (1989) cited by Rekhari et al (2014) reported the bacterial population in water column in Indian ponds ranged from 2.07 to 3.66 log CFU mL^{-1}. Also, Das & Mukherjee (1999) found the bacterial counts between 2.90 and 4.39 log CFU mL^{-1} in the polyculture ponds. Sharmila et al (1996) reported that the mean total viable number of bacteria of the pond water was 3.25–3.65 log CFU mL^{-1} during the summer period in a semi intensive system (Rekhari et al 2014). Allen et al (1983) demonstrated that in general, the heterotrophic bacteria populations increased at high temperature. It has been found that seasonal distributions of bacterial number were related to the water temperature changes (Du et al 2002).

In the present study it can be stated that the level of heterotrophic bacteria was over the range. Bacterial density was higher during August (P1 – from 4.10 log CFU mL^{-1} to 4.66 log CFU mL^{-1}, P2 – from 4.45 log CFU mL^{-1} to 4.60 log CFU mL^{-1}) as compared to July and September, in each of two aquaculture systems, P1 (IMTA) and P2 (traditional). Uddin & Al-Harbi (2012) demonstrated that the composition and quantity of the microorganisms vary with water temperature. Our results support this observation. This was due to the higher temperature in August (20.45°C) that favors the higher rate of metabolic activity and the growth of heterotrophic bacteria. Thus, it was observed that the bacterial density was higher with 1 log in August, compared to July (P1 – from 3.42 log CFU mL^{-1} to 4.38 log CFU mL^{-1}, P2 – from 43.53 log CFU mL^{-1} to 3.89 log CFU mL^{-1}), in each of the two ponds. Also, the maximum number of heterotrophic bacteria in August was correlated to pH 8.95, DO of 6.58 mg L^{-1} and COD of 130.5 mg L^{-1}, for the water samples from the O.1.1. sampling point on the P1 pond.

It was observed the insignificant variations in the heterotrophic bacterial load of the water from the two ponds, P1 and P2. The bacterial load was high but it is not a disadvantage if the bacteria are not pathogenic. Uddin & Al-Harbi (2012) demonstrated that the high bacterial abundance may indicate a potential of organic matter recycling, self-cleaning potential and re-mineralization.

Conclusions. The present study concludes that proper water quality management and continues monitoring of heterotrophic bacteria are the key factors to avoid bacterial and parasitic diseases in culture ponds. During the experiment, the highest number of heterotrophic bacteria was recorded in August in both types of carp growing systems, IMTA and traditional. There were no significant differences of these numbers between the two ponds. Also, feed consumption monitoring, and maintaining a stable phytoplankton microflora may led to reduced bacterial load, especially for the heterotrophic bacteria.

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