The effects of *Pediococcus acidilactici* as a probiotic on growth performance and survival rate of great sturgeon, *Huso huso* (Linnaeus, 1758)

Zare A.[1]; Azari-Takami G.[2]; Taridashti F.[3*]; Khara H.[1]

Received: August 2015 Accepted: July 2016

Abstract

This study was accomplished to investigate the effect of *Artemia urmiana* nauplii enriched with *Pediococcus acidilactici* as probiotic on growth performance and survival rate of great sturgeon, *Huso huso*. *Artemia* nauplii were enriched with *P. acidilactici* at a final concentration of 10^{10} CFU mL^{-1} in three time dependent treatments as 3 h (T3), 6 h (T6), 9 h (T9), and one non-enriched *Artemia* as the control treatment. All treatments were considered in triplicates. Since the nauplii enriched for 9 hours (T9) had the most significant CFU/g compared to other treatments (p<0.05), juvenile beluga at the stage of first feeding with the mean body weight of 48 ± 1 mg (mean ± SE) were fed with nauplii enriched for 9 hours (T9) and the control diet, with three tanks assigned to each diet. No significant differences were observed in final weight, final length, condition factor, specific growth rate, average daily growth, and survival rate for fish fed with T9 compared to those in the control group (p>0.05). On the other hand, a decreasing trend was recorded in food conversion ratio (FCR) and final biomass changed significantly for T9 in comparison with that recorded in the control group (p<0.05). The results indicated that *P. acidilactici* had a positive effect on growth and survival of beluga larvae, and a different time of enrichment had a significant effect on LAB effect. The best time of enrichment for beluga larvae was found to be 9 hours.

Keywords: *Artemia* nauplii, Enrichment, Probiotic, Lactic acid bacteria, *Huso huso*

1- Department of Fishery, Faculty of Natural Resources, Lahijan Branch, Islamic Azad University, Lahijan, Iran.

2-Department of Animal Breeding and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

3-Department of Fishery, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran

*Corresponding author's Email: tari_fateme@yahoo.com

Introduction

Great sturgeon, *Huso huso* is one of the most important species of sturgeon in the Caspian Sea. It inhabits in the Caspian Sea, Black Sea, and Sea of Azov (Razavi, 1988). It is considered as an endangered species by the International Union for Conservation of Nature and Natural resources (Sturgeon Specialist Group, 1996) due to over fishing and destruction of habitat, which is the subject of restoration and restocking schemes (Williot *et al.,* 2001; Mohseni *et al.,* 2008). One of the major problems with restocking is the low rate of larval survival (Suboski and Templeton, 1989). The use of nonspecific immuno-stimulants (Anderson, 1992; Figueras *et al.,* 1997) and bacterial probiotics such as lactic acid bacteria (Gatesoupe, 1991, 1994, 1999; Ringø and Gatesoupe, 1998; Verschuere *et al.,* 2000) as well as several approaches such as improving husbandry nutrition (Reitan *et al.,* 1993; Roennestad *et al.,* 1999), water quality (Vadstein *et al.,* 1993; Skjermo *et al.,* 1997), disinfecting of fish eggs (Salvesen and Vadstein, 1995), and UV treatment (Munro *et al.,* 1999) have been recommended to increase larval survival.

Probiotics are defined as non-digestible food supplements (Gibson, 2004) which have a beneficial effect on the host physiology by modulating the mucosal and systemic immunity, and improving the balance of intestinal micro flora by preventing the colonization of undesirable bacteria in the intestinal tract (Naidu *et al.,* 1999). Probiotics, particularly as food formulations, are widely used to develop sustainable aquaculture practices (Verschurere *et al.,* 2000; Venkat *et al.,* 2004; Wang, 2005; Saad, 2009). It has positive effects on fish survival (Villamli *et al.,* 2003), and increases growth rate by enhancing food absorption (Fuller, 1992).

Artemia nauplii as one of the most important live feeds for commercial production of larvae fish, is used extensively in marine and freshwater aquaculture (Jalali *et al.,* 2009). Considering the nonselective and continuous feeding of *Artemia*, it feeds from a variety of food particles (Seenivasan *et al.,* 2012). Therefore, it has been used as a live feed and a multipurpose vector for the delivery of different materials in aquaculture such as essential nutrients (Watanabe *et al.,* 1983), antimicrobial agents (Dixon *et al.,* 1995), vaccines (Campbell *et al.,* 1993), and probiotics (Gatesoupe, 1994). The enrichment of *Artemia* nauplii with probiotic bacteria is associated with positive effects on the host organism by improving properties of the native microflora (Patra and Mohamed, 2003), the efficacy of which strongly depends on the status of bacteria, time of exposure, and the type of bacteria used (Gatesoupe, 1991, 1993). In fish, the lactic acid bacteria (LAB) were described as part of the normal microflora (Ringø and Gatesoupe, 1998; Robertson *et al.,* 2000). It has been reported to improve water quality, fish growth, and survival which in turn ultimately increases

aquaculture output (Gatesoupe, 1994; Shiri Hanceville *et al.,* 1998; Skjermo and Vadstein, 1999; Verschurere *et al.*, 2000; Planas *et al.,* 2004), and in some cases inhibits the growth of pathogenic bacteria (Gildberg *et al*., 1995). Probiotics effectively improve the survival, growth, and immunity of host organism, therefore, the present study was carried out to investigate the effects of enrichment of *Artemia urmiana* with *P. acidilactici* on growth and survival of beluga larvae.

Material and methods

Probiotic

The dried powder of Bactocell (Lallemand Co., Montreal, Canada) was used as probiotic. It is a food additive which contains 10^{10} spores of *Pediococcous acidilactici*.

Artemia nauplii

Artemia urmiana (Urmia Lake, Iran) cysts were decapsulated prior to hatching in sodium hypochloride (Hoff and Snell, 1987). Cysts were allowed to hatch in a conical tank (40 L volume) containing seawater at a salinity of 33ppt for 24h, at 28°C under strong illumination (2000 Lux) and heavy aeration. Freshly hatched nauplii were reared for 24h up to instar-II stage. The instar-II *Artemia* were harvested by exploiting the positive photoperiod behavior of nauplii.

Enrichment treatment

Instar-II *Artemia* nauplii were transferred into glass containers with a water volume of 500mL and stocked at rate of 100 naupli per mL. Three different *Artemia* enrichment treatments in a time dependant manner cultured for 3h (T3), 6h (T6), and 9h (T9), and one control treatment (non-enriched *Artemia*) were considered in triplicates. Nauplii were fed with *P. acidilactici* at a final concentration of 10^{10} CFU mL^{-1} with aeration at 15°C.

Sample processing

After 3 h enrichment, 10 mL was sampled from each replicate of control and T3 to determine bacterial load, and transferred into sterilized experiment tubes for bacteriological sampling. This procedure was repeated for T6 and T9 after 6h and 9h enrichment, respectively.

Samples were passed over a sterile 100-µm mesh to separate *Artemia* from culture water. Trapped *Artemia* in the filter were rinsed with sterile saline water (35ppt), and homogenized in 5 mL sterile saline water. Samples were serially diluted then 100 µm of each dilution was spread plated on tryptone soy agar (TSA) and de Man, Rogosa and Sharpe (MRS) agar (Liofilchem) for total viable bacteria count and LAB, respectively. Plates were incubated at 30°C for 48- 72h. After that, the number of bacteria were counted as colony-forming unit (CFU) per nauplii for each replicate.

Fish culture and feeding trial

One thousand and two hundred beluga larvae hatched at the Shahid Beheshti Sturgeon Fish Propagation and Rearing Complex in Rasht, Guilan, Iran, were

used for this study. They were at the stage of first feeding with the mean body weight and total length of 48±1 mg (mean ± SE), and 18.5 ± 0.1 mm, respectively. Water temperature, pH, and dissolved oxygen were maintained at 8- 10°C, 7.5- 8, and 6- 8 mg/L, respectively throughout the trial. Water flow was continuous and the outlet was at the center of each tank. Fish were randomly distributed into 6 plastic tanks with a water volume of 45 L at the density of 200 fish per tank. Since *Artemia* nauplii enriched for 9h (T9) had the most significant CFU/g compared to other treatments (p<0.05), it was fed to beluga larvae. Fish in the control group were fed with non-enriched *Artemia*. Each feeding group was run in triplicates. Juveniles were fed at 30% of body weight per day at various times including 4:00, 8:00, 12:00, 16:00, 20:00, 24:00 hours. The unfed *Artemia* nauplii were collected after the respective hours of feeding. The experiment was carried out for 11 days.

Sample collection and analyses

At the end of experiment, on the 11th day, 25 fish were randomly removed from each tank to assess the growth performance including specific growth rate (SGR), food conversion ratio (FCR), condition factor (CF), average daily growth (ADG), and percent body weight increase (PBWI) which were calculated according to the following formulae:

$WG= W_2- W_1$

$SGR= 100 (\ln W_2- \ln W_1)/ T$

$FCR= FO/ WG$ (g)

$CF=$ fish weight (g)/ (fish length cm)$^3 \times 100$

$ADG= (W_2- W_1)/ (W_1- T) \times 100$

$PBWI= 100 (BW_2- BW_1)/ BW_1$

Where:

ln= natural log, W_1= initial weight (g), W_2= final weight (g), T= time period in days, FO= feed offered (g), WG= weight gain, BW_1= initial biomass weight, and BW_2= final biomass weight. Survival rate was calculated at the end of the experiment: survival= (Nf/N0) × 100; where N_0 is initial number of fish and N_f is final number of fish.

Fifteen fish in each treatment were taken for intestinal microbiota analysis. Fish were fasted for 24h before the microbiological sampling. To open the ventral surface of fish, they were killed by brain physical destruction, and the skin was washed with 0.1% benzalkonium chloride. To isolate the autochthonous intestinal microbiological communities, the entire intestinal tract was removed aseptically, washed thoroughly with sterile saline water and homogenized (Potter–Elvehjem Tissue Homogenizer, Cole-Parmer Instrument Company, IL, USA). Then, it was serially diluted to 10^{-7} with sterile saline. Samples were spread in triplicate onto tryptone soy agar (TSA) and de Man, Rogosa and Sharpe (MRS) agar (Liofilchem), for total viable bacteria count and LAB, respectively. Plates were incubated at room temperature at 25ºC for 5 days (Mahious *et al.*, 2006) and colony-forming units (CFU/g) were calculated

from statistically viable plates (i.e. plates containing 30–300 colonies) (Rawling *et al.,* 2009).

Statistical analysis

Data are presented as means±standard error (SE). To evaluate normality of data and homogeneity of variances, one Sample Kolmogorov-Smirnov and Levene's test were used, respectively. One-way analysis of variance (ANOVA) was applied to compare the total viable bacteria in *Artemia* nauplii between all treatments. When significant differences were observed, Duncan's multiple range tests were performed. Independent Samples Test was used to determine differences between treatments in intestinal microbiota analysis and growth performance. The significance level of 0.05 was considered for data analyzes.

Results

Enrichment experiment

The LAB levels in all experimental treatments are presented in Fig. 1. As it is illustrated in figure1, there are significant differences among nauplii treatments enriched by the concentration of 10^{10} CFU mL^{-1} compared to control group (p<0.05). On the other hand, while the time of enrichment increased, the LAB levels increased, and the highest LAB level was observed in nauplii enriched for 9 h. The mean total bacterial load has changed significantly among experimental treatments (p<0.05). Only those enriched for 9 hours indicated significant difference with the control group, while nauplii enriched for 1 and 6 hours had no significant differences (Fig. 1).

Growth performance and feed utilization parameters

Growth performance and feed utilization parameters of beluga larvae fed with nauplii enriched for 9 hours and non-enriched nauplii at the end of trial are presented in Table 1. There are no significant differences in final weight, final length, CF, SGR, ADG, survival rate for fish fed with T9 compared to that in the control group (p>0.05). On the other hand, a decreasing trend was observed in FCR and final biomass changed significantly for T9 in comparison with the control group (p<0.05).

Bacteriological study

LAB levels in digestive tract of fish fed with T9 were significantly elevated (p<0.05) (Fig. 2) compared to the control group. While, no significant differences was observed in total bacterial load between digestive tract of fish fed with control and T9 (p>0.05) (Fig. 2).

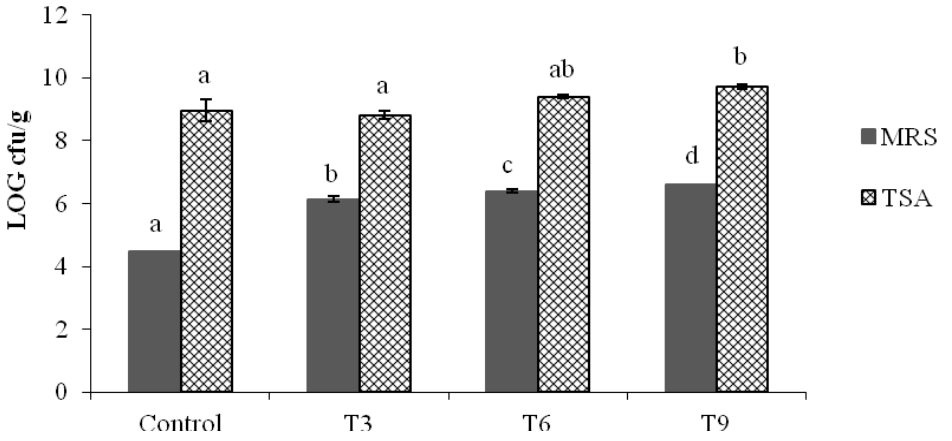

Figure 1: LAB levels (MRS agar) and total bacterial load (TSA agar) in *Aretemia* nauplii enriched for 3 hours (T3), 6 hours (T6), and 9 hours (T9) and control in trial 1. Small letters show significant difference in each treatment during time by Duncan's test (p<0.05). Values are mean±SE.

Table 1: Growth performance and feed utilization parameters of beluga larvae, *Huso huso* fed with *Artemia* nauplii enriched for 9 hours (T9) and control in trial 2.

Growth performance and feed utilization parameters	T9	Control
Initial weight (g)	0.048 ± 1	0.048 ± 2
Final weight (g)	0.11 ± 2	0.95 ± 3
Initial length (cm)	1.85 ± 0.05	1.85 ± 0.05
Final length (cm)	2.3 ± 0.0	2.1 ± 0.0
Percent body weight increase (PBWI) (%)	125.15 ± 0.21	97.72 ± 2.14
Condition factor (CF)	0.8 ± 0.01	0.94 ± 0.03
Food conversion ratio (FCR)	3.03 ± 0.12	7.31 ± 1.13
SGR (% /day)	3.92 ± 0.13	2.62 ± 0.18
ADG (%)	4 ± 0.01	3 ± 0.01
Survival (%)	83.33 ± 4.16	58.33 ± 4.17

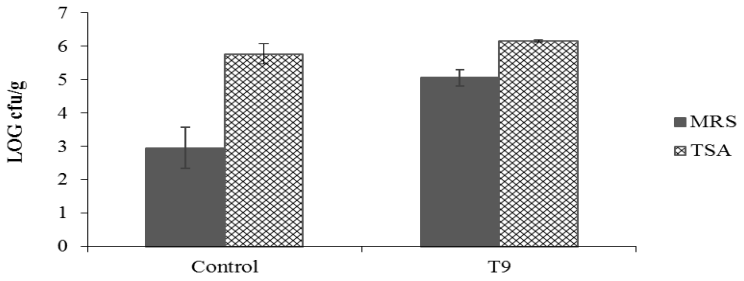

Figure 2: LAB levels (MRS agar) and total bacterial load (TSA agar) in *Aretemia* nauplii enriched for 9 hours (T_9) and control in trial 2. Values are mean ± SE, n= 15 per each treatment.

Discussion

Since probiotics have the ability to control potential pathogens, and increase the growth rates and welfare of farmed aquatic animals, which has been demonstrated by several studies (Carnevali *et al.,* 2004; Macey and Coyne, 2005); this study was carried out to examine the ability of *Artemia* nauplii enriched with *P. acidilactici* on the growth and survival of beluga larvae. In our study *Artemia* nauplii enriched for 9 hours had the most significant LAB level, this treatment was selected in order to transfer the maximum rate of *P. acidilactici* to intestine of beluga larvae for enhancement of growth performance. The previous study on great sturgeon larvae has shown that *Artemia* nauplii enriched for 9 hours has the highest level of LAB (Azar-Takami *et al.*, 2013).

Manipulation of bacterial load present in rotifers and *Artemia* for fish feeding may constitute a valuable mechanism to increase survival rates and larval growth (Gatesoupe, 1994; Noh *et al.,* 1994; Robertson *et al.*, 2000). Probiotic supplementation has extensively been used in aquaculture species (Suralikar and Sahu, 2001; Lara-Flores *et al.,* 2003; Ziaei-Nejad *et al.,* 2006; Shinde *et al.,* 2008; Didinen *et al.,* 2016). In the present study, although there was no significant difference in growth performance, an increasing trend was observed for fish fed with enriched *Artemia* compared to the control group, and FCR decreased significantly. The same results have been found in many species including Indian carp, *Labeo rohita*, freshwater shrimp, *Macrobrachium rosenbergii*, common carp, *Cyprinus carpio*, turbot, *Scopthlmus maxinus*, Persian Sturgeon, *Acipenser persicus*, and Zander, *Sander lucioperca*(Gatesoupe, 1991; Bogut *et al.,* 1998; Gosh *et al.,* 2003; Faramarzi *et al.,* 2011; Seenivasan *et al.,* 2012; Faeed et al., 2016). Generally, LAB are capable of attaching themselves to the epithelium and establish their colony there (Seenivasan *et al.,* 2012), and by releasing the external enzyme they can cause better digestion and growth (Ghosh *et al.,* 2002). Therefore, increased growth performance and observation of a decreasing trend of FCR in beluga larvae fed with T9 may be due to the administration of significant changes in the population of gut micro flora, in which significant elevations in establishment of colonies of LAB were recorded in the intestine of beluga larvae fed with T9. This indicates that *P. acidilactici* could eliminate harmful bacteria in the intestine of beluga or the colonization of *P. acidilactici* may be dominant over harmful bacteria by their large presence, which saturates the adhesion receptors and prevents the pathogenic bacteria from attachment and colonization (Vine *et al.,* 2004; Venkat *et al.,* 2004).

In conclusion, *P. acidilactici* had a positive effect on the growth and survival of beluga larvae. Considering the adhesion potency of *P. acidilactici* to *Artemia* nauplii, enhancement of growth performance, reduction in FCR,

and increase in number of beneficial bacteria, the best time of enrichment for beluga larvae was 9 hours. Although much work is needed on research in probiotics for aquaculture at an early stage of development, the available information is inconclusive (Gomez-Gil *et al.*, 2000).

Acknowledgements

The authors would like to thank the staff of the Shahid Beheshti Sturgeon Fish Propagation and Rearing Complex for providing the juvenile sturgeon. The authors also acknowledge Pakgostar Parand Company, the Iranian branch of Lallemand Animal Nutrition for supporting this experiment.

References

Anderson, D.P., 1992. Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. *Annual Review of Fish Diseases,* 2, 281-307.

Azari-Takami, Gh., Zare, A. and Azari-Takami, S., 2013. The growth factors and survival rate of great sturgeon, *Huso huso* larvae feeding with *Artemia* nauplii enriched with *Pediococcus acidilactici*. 7th International Symposium on Sturgeon, July 21-25, Nanaimo, Canada.

Bogut, I., Milakovic, Z., Bukvic, Z., Brkic, S. and Zimmer, R., 1998. Influence of probiotic *Streptococcus faecium* M74 on growth and content of intestinal microflora in carp *Cyprinus carpio*. *Journal of Animal Science*, 43, 231-235.

Campbell, R., Adams, A., Tatner, M.F., Chair, M. and Sorgeloos, P., 1993. Uptake of *Vibrio anguillarum* vaccine by Artemia salina as a potential oral delivery system to fish fry. *Fish & Shellfish Immunology* 3, 451-459.

Carnevali, O., Zamponi, M.C., Sulpizio, R., Rollo, R., Nardi, M., Orpianesi, C., Silvi, S., Caggiano, M., Polzonetti, A.M. and Cresci, A., 2004. Administration of probiotic strain to improve sea bream wellness during development. *Aquaculture International*, 12, 377-386.

Didinen, B.I., Bahadir Koca, S., Metin, S., Diler, O., Erol, K.G., Dulluc, A., Koca, H.U., Yigit, N.O., Ozkok, R. and Kucukkara, R., 2016. Effects of lactic acid bacteria and the potential probiotic *Hafnia alvei* on growth and survival rates of narrow clawed crayfish (*Astacus leptodactylus* Esch., 1823) stage II juveniles. *Iranian Journal of Fishery Science*, 15, 1307-1317.

Dixon, B.A., Poucke, S.O.V., Chair, M., Dehasque, M., Nelis, H.J., Sorgeloos, P. and De leenheer, A.P., 1995. Bioencapsulation of the antibacterial drug sarafloxacine in nauplii of the brine shrimp Artemia franciscan. *Journal of Aquatic Animimal Health*, 7, 42–45.

Faeed, M., Kasra Kermanshahi, R., Pourkazemi, M., Darboee, M. and Haghighi Karsidani, S., 2016. Effects of the probiotic Entrococcus faecium on hematological and non-specific immune parameters and

disease resistance in zander (Sander lucioperca). *Iranian Journal of Fishery*, 15, 1581-1592.

Faramarzi, M., Jafaryan, H., Patimar, R., Iranshahi, F., Lashkar Boloki, M., Farahi, A., Kiaalvandi, S., Ghamsary, M. and Makhtoumi, N.M., 2011. The effect of different concentration of probiotic *Bacillus* spp. and different bioencapsulation times on growth performance and survival rate of Persian sturgeon (*Acipencer persicus*) larvae. *World Journal of Fish and Marine Science*, 3(**2**), 145-150.

Figueras, A., Santarem, M. and Novoa, B., 1997. In vitro immunostimulation of turbot (*Scophthalmus maximus*) leukocytes with h glucan and/or Photobacterium damsela bacterin. *Fish Pathology*, 32, 153-159.

Fuller R., 1992. History and development of probionts. In: R. Fuller (Ed), probiotics the scientific basis. Chapman and Hall, New York, N.Y: pp.1-8.

Gatesoupe, F.J., 1991. The effect of three strains of lactic acid bacteria on the production rate of rotifers *Brachionus plicatilis*, and their dietary value for larval turbot *Scophthalmus maximus*. *Aquaculture*, 96, 335-342.

Gatesoup, f.J., 1993. Bacillus sp. spores as food additive for the rotifer *Brachionus plicatilis*: improvement of their bacterial environment and their dietary value for Larval turbot, *Sophthalmus maximus* L. In: S.J. Kaushik, P. Luque (Eds), Fish Nutrition in practice. INRA, Paris, France, Les colloques, Vol. 61, pp. 561-568.

Gatesoupe, F.J., 1994. Lactic acid bacteria increase the resistance of turbot larvae, (*Scophthalmus maximus*) against pathogenic vibrio. *Aquatic Living Resources*, 7, 182-277.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. *Aquaculture*, 180, 147-165.

Ghosh, K., Sen, S.K. and Ray, A.K., 2002. Growth and survival of rohu, *Labeo rohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora. *Acta Icthologyca Piscatorial*, 32(**1**), 83-92.

Gibson, G.R., 2004. Fiber and effects on probiotics (the prebiotic concept). *Clinical Nutrition*, 1, 25-31.

Gildberg, A., Mikkelsen, H., Sandaker, E. and Ringø, E., 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). *Hydrobiologia*, 352, 279-285.

Gomez-Gill, B., Roque, A. and Turnbull, J.F., 2000. The use and selection of probiotic bacteria for use in the growth in rotifer *Brachionus plicatilis* (Muller). *Aquaculture Research*, 29, 411-417.

Hoff, F.H. and Snell, T.W. 1987. Plankton culture manual. Florida Aqua Farms, Inc, Dead City, Florida, 126P.

Jalali, M.A., Ahmadifar, E., Sudagar, M. and Azari Takami, G. 2009. Growth efficiency, body composition, survival and haematological changes in great sturgeon (*Huso huso* Linnaeus, 1758) juveniles fed diets supplemented with different levels of Ergosan. *Aquaculture Research*, 40, 804-809.

Lara-Flores, M., Olvera-Novoa, M.A., Guzmanadjuvants- Mendez, B.E. and Lopez- Madrid, W., 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia *Oreochromis niloticus*. *Aquaculture*, 216, 193-201.

Macey, B.M. and Coyne, V.E., 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. *Aquacultur*, 245, 249-26.

Mahious, A.S., Gatesoupe, F.J., Hervi, M., Metailler, R. and Ollevier, F. 2006. Effect of dietary and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima*. *Aquaculture International*, 14, 219-229.

Mohseni, M., Ozorio, R.O.A., Pourkazemi, M. and Bai, S.C., 2008. Effects of dietary L-carnitine supplements on growth and body in beluga sturgeon (*Huso huso*) juveniles. *Journal of Applied Ichthyology*, 24, 646-649.

Munro, P.D., Henderson, P.J., Barbour, A. and Birkbeck, T.H., 1999. Partial decontamination of rotifers with ultraviolet radiation: the effect of change in the bacterial load and flora of rotifers on mortalities in start feeding larval turbot. *Aquaculture*, 170, 229- 244.

Naidu, A.S., Bidlack, W.R. and Clemens, R.A., 1999. Probiotic spectra of lactic acid bacteria. *Critical Review in Food Science and nutrition*, 39, 13-126.

Noh, S.H., Han, K., Won, T.H. and Choi, Y.J., 1994. Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. *Korean Journal of Animal Science*, 36, 480-486.

Parta, S.K. and Mohamed, K.S., 2003. Enrichment of *Artemia* nauplii with the probiotic yeast *sacharomyces boulardii* and its resistance against a pathogenic vibrio. *Aquaculture international*, 11, 505-514.

Planas, M., Vazquez, J.A., Marques, J., Perez Loba, R., Gonzalez, M.P. and Murado, M.A., 2004. Enhancement of rotifer (*Brachionus plicatilis*) growth by using terrestrial Lactic acid bacteria. *Aquaculture*, 240, 313-329.

Razavi, S.B., 1988. Analysis on great sturgeon stocks in the South Caspian Sea. Iranian Fisheries Research and Training Organization Publications.

Rawling, M., Merrifield, D.L. and Davies, S.J. 2009. Preliminary assessment of dietary supplementation of Sangrovit on red tilapia (*Oreochromis niloticus*)

growth performance and health. *Aquaculture,* 294, 118-122.

Reitan, K.I., Rainuzzo, J.R., Oie, G. and Olsen, Y., 1993. Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus*) larvae. *Aquaculture,* 118, 257-275.

Ringø, E. and Gatesoupe, F.J., 1998. Lactic acid bacteria in fish: a review. *Aquaculture,* 160, 177-203.

Robertson, P.A.W., O'Dowd, C., Burrells, C., Williams, P. and Austin, B., 2000. Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss,* Walbaum). *Aquaculture,* 185, 235-243.

Roennestad, I., Thorsen, A. and Finn, R.N., 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. *Aquaculture,* 177, 1201-1216.

Saad, S.A., Habashy, M.M. and Sharshar, M.K., 2009. Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), to diets having different levels of Biogen. *World Applied Sciences Journal,* 6, 550-556.

Salvesen, I. and Vadstein, O., 1995. Surface disinfection of eggs from marine fish: evaluation of four chemicals. *Aquaculture International,* 3, 155-177.

Seenivasan, C., Saravana Bhavan, P., Radhakrishnan, S. and Shanthi, R., 2012. Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for enhancing the survival, growth and levels of biochemical constituents in the post-larvae of the freshwater prawn *Macrobrachium rosenbergii*. *Turkish Journal of Fisheries and Aquatic Sciences,* 12, 23-31.

Shinde, A.N., Mulye, V.B., Chogale, N.D., Bhatkar, V.R., Bondre, R.D. and Mohite, A.S., 2008. Effect of different probiotics *Macrobrachium rosenbergii* (De- Man) post larvae. *Aquaculture,* 9, 7-12.

Shiri Hanzevili, A.R., Van Duffel, H., Dhert, P., Swings, J. and Sorgeloos, P., 1998. Use of a potential probiotic Lactococcus lactic Ar21 strain for the enhancement of growth in rotifer *Brachionus plicatilis* (Muller). *Aquaculture Research,* 29, 411-417.

Skjermo, J., Salvesen, I., Oie, G., Olsen, Y. and Vadstein, O., 1997. Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. *Aquaculture International*, 5, 13-28.

Skjermo, J. and Vadstein, O., 1999. Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture,* 177, 333-343.

Sturgeon Specialist Group., 1996. *Acipenser persicus*. In: IUCN 2006. 2006 IUCN red list of threatened species. www.iucnredlist.org. Accessed April 27, 2007.

Suboski, M.D. and Templeton, J.J., 1989. Life skills training for hatchery fish: social learning and

survival. *Fisheries Research,* 7, 343-352.

Suralikar, V. and Sahu, N.P., 2001. Effect of feeding probiotic (*Lactobacillus cremoris*) on growth and survival of *Macrobrachium rosenbergii* post-larvae. *Journal of Applied Animal Research,* 20, 117-124.

Vadstein, O., Oie, G., Salvesen, I., Skjermo, J. and Skjak-Braek, G., 1993. A strategy to obtain microbial control during larval development of marine fish. In: H. Reirtesen, L.A. Dahle, L. Jorgesen, K. Tvinnereim (Eds), Fish farming technology-proceedings of the first international conference on fish farming technology. Balkema, Rotterdam: pp. 69-75.

Venkat, H.K., Sahu, N.P. and Jain, K.K., 2004. Effect of feeding Lactobacillus-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (De Man). *Aquaculture Research,* 35, 501-507.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology,* 64, 655-671.

Villamil, L., Figueras, A., Planas, M. and Novoa, B., 2003. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. *Aquaculture,* 219, 43-56.

Vine, N.G., Leukes, W.D. and Kaiser, H., 2004. In vitro growth characteristics of live Canadian aquaculture probiotics and two fish pathogens grown in fish intestinal mucus. *FEMS Microbiology Letter,* 231, 145-152.

Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture,* 269, 259-264.

Watanabe T., Kitajima C. and Fujita S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34, 115-143.

Williot, P., Sabeau, L., Gessner, J., Arlati, G., Bronzi, P., Gulyas, T. and Berni, P., 2001. Sturgeon farming in Western Europe: recent developments and perspectives. *Aquaculture Living Resources,* 14, 367-374.

Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R. and Shakouri, M. 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture,* 252, 516-524.