

## Effect of *Spirulina pletensis* Powder on Growth, Feed, Body Chemical Composition and Fatty Acids in *Mugil cephalus* Linnaeus, 1758

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### Abstract

This experiment was conducted to evaluate the effect of *Spirulina pletensis* powder on the growth performances (final weight (FW), daily growth ratio (DGR), feed indices (feed conversion rate (FCR), voluntary feed intake (VFI), protein efficiency ratio (PER), protein produce value (PPV), body chemical (protein, fat, moisture and ash) and fatty acid composition of *Mugil cephalus* for 62 days. The experiment was run in a completely randomized design with 450 of grey mullet larvae (with an average weight of  $0.72 \pm 0.02$  g) in 5 treatments with 3 replicates ( $n = 30$  in each replicate). The treatments were control group without algae extract, other groups (treatment 2, 3, 4 and 5) with algae extract, *i.e.* 5, 10, 15 and 30 g/kg food. The results showed that at the end of experiment, the highest FW ( $5.13 \pm 0.02$ g), DGR ( $3.44 \pm 0.20\%$ ), the lowest FCR ( $0.99 \pm 0.03$ ), the highest PER ( $8.61 \pm 0.27$ ), the highest PPV ( $39.48 \pm 2.54$ ), the highest crude protein ( $19.70 \pm 0.30\%$ ), the highest crude lipid ( $10.08 \pm 0.0\%$ ) and the highest poly unsaturated fatty acid (PUFA), including C18:2n-6 ( $32.60 \pm 2.09\%$ ) and C18:3n-3 ( $3.72 \pm 1.03\%$ ), were observed in the diet containing 15 g/kg algae extract in all of these parameters. The treatment 4 showed a significant difference with control treatment ( $P < 0.05$ ). Finally, the present results suggest that diet containing 15 g/kg *Spirulina platensis* could improve growth, feed performances, carcass quality and increase PUFA level in *Mugil cephalus* larvae.

**Keywords:** *Mugil cephalus*, *Spirulina platensis* Powder, Carcass Composition, Fatty Acid, Growth Promoter

## Discrimination between GH-2 and GH-1 Genes in *Rutilus Kutum* Using Sequencing and Restriction Enzymes

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### Abstract

Fish genome contains two copies of growth hormone (GH-1 and GH-2) as a result of duplication process during evolution. The aim of this study was to distinguish GH-1 from GH-2 genes in *Rutilus kutum* (Mahi Sefid) from the Caspian Sea using sequencing techniques and application of specific restriction enzymes for these loci. For this purpose, five fish were randomly selected and samples for DNA extraction were isolated from caudal fin clips. The DNA was extracted by salting-out method. Then, fragments of 333 and 410 bp from exon 4, intron 4 and exon 5 of GH-1 and GH-2 genes were amplified and, following purification, sequenced on the gel. GH-1 sequence of *R. kutum* was not different from that of common carp on the long allele showing 100% overlap with each other. On the short allele, however, they showed differences in GH-1 sequence of carp at four positions namely 89, 94, 105, and 164 bp with an overlap of 99%. The GH-1 overlapped with GH-2 sequences from both common carp and *R. kutum* as 99% and 86%, respectively. Comparison of the GH-1 and GH-2 sequences derived from *R. kutum* showed that they mainly differed in Intron 4. Other differences observed were mutations at one or more nucleotides. Despite the high similarity in the nucleotide sequences, the differences detected are applicable as specific genetic markers for the discrimination between GH-1 and GH-2. By identifying and sequencing GH-1 and GH-2 genes, the sequences obtained can be used to reconstruct the phylogenetic relations, study different allelic forms more precisely, and establish relationships between these loci with important economic traits in *R. kutum* from the Caspian Sea.

**Keywords:** GH1, GH2, Restriction Enzymes, Sequencing, *R. kutum*

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## Effects of Spirulina Algae on Growth, Survival and Coloration Demasoni Fish (*Pseudotropheus demasoni*)

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### Abstract

The successful aquaculture depends on different criteria, among them, selection of suitable food with required diet trial including control diet (without potential is of particular importance. In this study, effects of four Spirulina algae) and three diets containing Spirulina algae were investigated on the growth indices and skin color of Flower horn (*Pseudotropheus demasoni*). A total number of 180 two-month specimens were randomly allocated to 12 aquaria. They were fed for eight weeks with the experimental diets. The biometric characteristics of the specimens were measured including total length (cm), weight (g), SGR (g) and FCR (g/day). Total carotenoid quantity of the fish was measured spectrophotometrically at the end of the experiment. According to showed an increase in weight (P algae spirulina containing a diet that were fed the results, all three treatments algae different amounts of (P > 0.05). Feeding algae had no significant effect on length < 0.05). The amounts of a\* (P < 0.05). Therefore, the present study showed L\* and such as parameters had significant effects on the color Demasoni fish. growth and coloration in on positive effects had of fish spirulina algae in the diet of use that the

**Keywords:** Spirulina Algae, Growth Index, Survival. Coloration, Demasoni Fish (*Pseudotropheusdemasoni*)

# Evaluation of the Antimicrobial Activity of Sea Cucumber (*Holothuria Leucospilota*) Extract on the Shelf-Life of Rainbow Trout (*Oncorhynchus Mykiss*) Fillets during Refrigerated Storage

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## Abstract

In this study, the antimicrobial effect of sea cucumber extract (0.5, 1 and 2%) on the microbial population of rainbow trout fillets during refrigerated storage was examined. Fillets were stored for 16 days and every 4 days microbial tests (including total bacterial load, Pseudomonas, Enterobacteriaceae and H<sub>2</sub>S producing bacteria) were performed on the samples. Results showed that there was no significant difference ( $P > 0.05$ ) for the total bacterial load in all treatments during the storage time. For the samples treated with the extract, Pseudomonas, Enterobacteriaceae and H<sub>2</sub>S producing bacterial count was significantly ( $P < 0.05$ ) lower than the control samples. Also among the different concentrations, 2% of the extract showed the highest antibacterial activity. The findings of this study showed that the sea cucumber extract could be a natural preservative for the storage of fishery products in the short-term storage in cold conditions.

**Key words:** Sea Cucumber Extract, Fish, Microbial Quality, Shelf Life, Storage

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# The Role of Probiotics Supplements, *Bacillus subtilis* and *Saccharomyces cerevisiae* on Biological Parameters of *Artemia franciscana* in Mass Cultures

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## Abstract

Probiotics are beneficial microorganisms of intestine with useful effects on growth and health of the animals by the colonization. In present study, effects of two different probiotic as *Bacillus subtilis* and *Saccharomyces cerevisiae* were investigated on growth parameters, survival, and body composition of *A. franciscana*. Also the resistance against pathogenic bacteria of *Vibrio campbellii* was studied with the animals being exposed to  $1 \times 10^5$  CFU per mL of bacteria. The experiment was designed in 4 treatments: treatment one as a control (no probiotic), treatment two (food + bacteria), treatment three (food + yeast) and treatment four (food with equal amounts of yeast and bacteria). The survival and growth rate of the animals were measured on day 8 and 15 of culture period. At the end of the experiment (day 15), the Artemia was challenged with pathogenic *V. campbellii* in which survival of the animals were calculated finally. Based on the results, the maximum body size significantly was showed in treatment two that had significant difference with control ( $P < 0.05$ ). The maximum survival rate without any significant difference was found in treatments two and four ( $P > 0.05$ ). After 24, 48 and 72 hours of exposure of pathogenic bacteria, the highest and lowest survival rate were found in group four and group control (no probiotic), respectively ( $P < 0.05$ ). The body composition analyses also revealed the efficiency of probiotic on total ash of the animal by significant difference between treatment three with other treatments ( $P < 0.05$ ). The highest amount of protein and fat were found in the control and treatment four, respectively ( $P < 0.05$ ). Study on the effect of probiotic of the Artemia indicated that the resistance and survival of the exposed animal to the *V. campbellii* can be enhanced while the animal fed with probiotic supplements.

**Keywords:** Artemia, Probiotic, Yeast, Bacteria, Biological Parameters

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# A Study on Biodiversity of Planktonic Shrimp-like Communities in Artificial Reefs of the Khuzestan Coasts, the Bahrekan Area

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## Abstract

The aim of this investigation was to study the planktonic shrimp-like communities density and diversity in artificial reefs of Khuzestan coasts, Bahrekan waters (the Persian Gulf). Sampling was conducted for one year from May 2011 to April 2012, monthly. Zooplankton were collected with plankton tows using 300 µm mesh net. Totally, two superfamily, Penaeoidea, Sergestoidea and one suborder Caridea and four families, Sergestidae, Luciferidae, Penaeidae, Alpheidae were identified. *Metapenaeus affinis*, *Penaeus indicus* and *Parapenaeopsis styliifera* species from Penaeidae family, the species *Alpheus* sp. from Alpheidae and *Acetes* sp. from Sergestidae and *Lucifer hansenii* from Luciferidae family were identified and introduced. The most abundant superfamily, Penaeoidea, with 54% and most abundant species was *M. affinis* from family Penaeidae with 32% relative abundance. Finally, two frequency peak was observed that the first peak found in September and the second November. Ecological indicators including Margalef species richness, Biodiversity Shannon, Simpson dominance index and Pielou evenness were measured in different stations and months. The most Shannon index and Pielou evenness at station B and the lowest rate at station D was observed. The most Simpson dominance index was in December (0.94) and Pielou evenness index during the same month the lowest (0.11). The Most of Margalef species richness index was in May (0.86) and lowest rate in August (0.20). These results is important for evaluation reserves Shrimp like larvae in artificial reefs Bahrekan area.

**Keywords:** Bahrekan area, Artificial reefs, Penaeoidea, Sergestoidea, Caridea

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# Effects of Environmental Factors on Community Structure of Macrobenthic Bivalves in the Marine Protected Area of Jask (Gulf of Oman) Using the Redundancy Analysis (RDA)

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## Abstract

In this study, macrobenthic bivalves were identified at 12 stations located in four regions (Markazi, Shahr no, Khalasi, and Gabrik creeks). The relationships between density of bivalves and non-biological factors were examined, as well. Sampling of sediments was done seasonally from autumn 2009 to summer 2010, by 0.04 m<sup>2</sup> Van Veen Grab, in three stations including mouth, middle and end of each creek. Water physicochemical parameters (including dissolved oxygen, salinity, temperature and turbidity), as well as sediment grain size, and TOM (Total Organic Matter), were measured at each station. A total of 24 species belonging to 20 genera and 7 families were identified that was dominated by *Paphia gallus* (36.13%), *Tellina* sp. (13.32%), and *Callista multiradita* (8.35%). At different seasons, the highest density of bivalves was observed in the autumn with total of 10900 pcs per m<sup>2</sup>. The highest density of bivalves was observed in mid station of Markazi creek, in the autumn, with total of 3650 pcs per m<sup>2</sup>. The diversity index, Shannon – Wiener, showed a range between 0 to 2.2, and the maximum value of this index was observed in mid station of Markazi creek in the spring. The results of multivariate analysis (RDA), showed a significant correlation between abundance of bivalves species and dissolved oxygen, medium sand, spring, autumn and Markazi creek.

**Keywords:** Environmental Factors, Bivalves, Creek, Jask, RDA

# Effects of Feeding Adult Artemia Enriched with Synbiotic on Growth Indices, Intestinal Microbiota and Stress Resistance of Angel Fish (*Pterophyllum scalare*)

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## Abstract

During the recent years, there has been an increasing interest toward dietary administration of synbiotic to improve growth, immunity and physiology of fish. The aim of this study was to evaluate the effects of feeding on synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) enriched adult *Artemia franciscana* on growth indices, intestinal microbiota and stress resistance of angel fish (*Pterophyllum scalare*) juvenile. Three hundred and sixty fish with initial weight of  $3.2 \pm 0.13$  g were randomly divided into 12 aquaria (50 L) assigned to four groups with three replicates. Fish were fed for 48 days with dietary treatments, including treatment 1: feeding adult artemia without enrichment (control group), treatment 2: feeding adult artemia enriched with probiotics *P. acidilactici* (700 mg), 3: feeding adult artemia enriched with prebiotics fructooligosaccharide (100 mg), group 4: feeding adult artemia enriched with a synbiotic (700 mg *P. acidilactici* and 100 mg prebiotics fructooligosaccharide). Growth indices, intestinal microbiota as well as resistance of fish against environmental stress (acute increase of temperature and salinity) were measured. Artemia enriched with synbiotic significantly improved the growth performance compared to other treatments ( $P < 0.05$ ). The highest body weight gain and specific growth rate (SGR) was observed in synbiotic fed fish ( $P < 0.05$ ). Compared to the other treatments, the population of lactic acid bacteria was significantly higher in the intestinal microbiota of fish fed Artemia enriched with synbiotic ( $P < 0.05$ ). In the environmental stress challenge, the maximum resistance to abrupt decrease of temperature ( $17^\circ\text{C}$ ) or elevation of salinity (12 g per liter) was found in the synbiotic treatment. These results revealed that enriching Artemia with the synbiotic showed better result compared singular enrichment with probiotics or prebiotics.

**Keywords:** Synbiotic, *Artemia franciscana*, Angel fish, Intestinal Microbiota, Stress Resistance

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## Effects of Malathion on DNA Breakage in the Liver and Gill of Rainbow Trout (*Oncorhynchus Mykiss*) Using Weighted Averaging

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### Abstract

This study aimed to investigate effects of malathion on DNA breakage in the liver and gill of rainbow trout (*Oncorhynchus mykiss*) in the laboratory condition. The experiment was performed with four concentrations of malathion, *i.e.* 0 (control), 0.025, 0.05 and 0.075 mg L<sup>-1</sup> and three replicate for nine days. The liver and gill were sampled on the first, fifth and ninth days after the start of the experiment and stored in microtubes containing ethanol. The DNA was extracted using a kit (Cinnapure), electrophoresed and photographed using the gel doc system. The percent of DNA breakage was determined using fuzzy logic and the weighted average. Malathion concentration and time had significant effects on the DNA breakage. Also there was a significant interaction between malathion concentration and time on the DNA breakage. The greatest damage in the liver was found in specimens exposed to 0.075 mg L<sup>-1</sup> and for the gill, in those exposed to 0.05 mg L<sup>-1</sup> nine days after the start of the experiment indicating a lack of a mechanism in both tissues to repair the damage malathion. The highest level of DNA damage was found in the liver. Thus, DNA breakage can be used as an appropriate method for detecting genetic damage of environmental pollution that may serve as a biological marker in ecotoxicological studies.

**Keywords:** Malathion, DNA breakage, Weight averaging, Rainbow trout (*Oncorhynchus mykiss*).

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# Effects of Zeolite Nano-Structure on Experimental Chronic Toxicity of Aflatoxin B1 in the Diet of Rainbow Trout (*Oncorhynchus mykiss*) and Evaluation of Growth and Physiological Indices

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## Abstract

The present study was carried out to evaluate the effects of dietary nano-structured zeolite (NZ) levels on reduction of aflatoxin B1 (AFB1) in the diet of rainbow trout (*Oncorhynchus mykiss*) for 56 days. For this purpose, 270 fish ( $23 \pm 3.7$  g) were randomly distributed into 6 groups including C (control), NZ0.5 (basic ration + 0.5 % nano-structure zeolite), NZ1 (basic ration + 1 % nano-structure zeolite), AF5 (basic ration + 5 mg aflatoxin B1), AF5NZ0.5 (basic ration + 5 mg aflatoxin B1+ 0.5 % nano-structure zeolite), AF5NZ1 (basic ration + 5 mg aflatoxin B1+ 1 % nano-structure zeolite) each with three replicates. Juveniles were fed satiated 4 times a day. At the end of the experiment, there was no significant difference among the treatments in growth indices. The hepatosomatic index, number of white blood cells and neutrophil decreased significantly in treatment AF5NZ0.5 and lymphocyte level increased in comparison to NZ0.5 treatment. On the other hand, eosinophil level increased significantly in treatment NZ0.5 compared with AF5 treatment. Other hematological parameters showed no significant differences among the treatments. Total protein and albumin concentrations in the treatments containing aflatoxin had a significant decrease compared with the other treatments. Also globulin level in treatment AF5 decreased significantly compared to the control group. Phosphorus content in serum was significantly higher in group NZ0.5 compared with C group, but other biochemical parameters such as sodium, calcium and cholesterol were not significantly different among the specimens that were fed different diets. According to the present study, it can be suggested that 0.5 % of zeolite nano-structure in the diet can reduce the harmful impacts of aflatoxin B1 and improve health status of trout.

**Keywords:** Aflatoxin, Zeolite, Growth, Hematology, Rainbow Trout

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## Effects of *Origanum Vulgare*, Probiotic and Florfenicol on Cellular Immunity, Some of Digestive Tract Bacteria and Histomorphology of the Gill and Liver in Rainbow Trout

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### Abstract

The purpose of this study was to determine changes in the normal flora of the gastrointestinal tract and blood parameters of rainbow trout after taking probiotics and oregano extract. This study was completely randomized design with 5 treatments and 3 replicates each with 40 fry 22 g of the rainbow trout. Treatments consisted of basal diet, florfenicol, 0.5%, 1% and of oregano extract, 2% Bactocell probiotic. After an 8 week experimental period, blood smear for cellular immunity, content of digestive tract for useful and unusual bacterial counts, the liver and gill for detection of histomrphological changes were collected. The results showed that the number of lymphocytes in antibiotics group was significantly more than that of the control. The percentage of monocyte in extracts treatments increased significantly. Total number of bacteria in the digestive system did not change much, but the numbers of lactic acid bacteria in the treated extract 0.5 and 1% were more than that of the control group. In florfenicol groups, hepatocytes were vacuolated and ratio of lipids and glycogen in hepatocytes were increased. It seems that use of organo extract in fish diet have not bad effects on the liver and gill histology and may show good effects on the liver performance and detoxification roles and increased counts of lymphocytes, monocytes and lactobacillus bacteria in the digestive tract.

**Keywords:** Rainbow trout, *Origanum vulgare*, Lactobacillus, Florfenicol, Probiotics, Histomorphology

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## Effect of Nanosilver Particles on Bacterial Gut Flora in Common Carp (*Cyprinus carpio*)

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### Abstract

The use of nanotechnology is expanding. The majority of nanoparticles are made of silver nanoparticles. These materials are known for their antimicrobial properties. As these materials are used in aquatic ecosystems and their release in the environment, the present study investigated the median lethal concentration (LC50) and the impact of these particles on the populations of the gut bacterial flora in *Cyprinus carpio*. For this purpose, 84 fish were exposed to concentrations of (0, 0.1, 0.5, 1, 2.5, 5, 10 ppm) for 96 hours and their mortality were recorded. Using probit analysis, the LC50 was calculated as 0.23 ppm. Next, 84 specimens in four treatments with three replicates were exposed to (1/2 LC50, 1/5 LC50, 1/10 LC50 and control treatment) for 14 days. After 14 days, 2 fish in each replicate was selected randomly and anaesthetized with clove powder. Their intestines were extracted in sterile conditions. Then total mesophil bacteria, acid lactic bacteria and antrobacteriacea were counted. Results showed no significant effect between nanosilver treatments and control treatments ( $P > 0.05$ ).

**Keywords:** Nanosilver Particles, *Cyprinus Carpio*, Mesophil Bacteria, Acid Lactic Bacteria, Entrobacteriacea

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