New species for freshwater aquaculture: preliminary results on small cyprinids rearing

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ABSTRACT

Italian freshwater fish farming is dominated by the production of salmonids, in particular rainbow trout. During the last decade trout production in Italy declined steadily for various reasons and trout farmers might be interested in the domestication of new species. Diversification is well studied for Mediterranean marine fish farming but not yet for freshwater one. In this scenario, it may be possible to establish new cold water adapted species for a niche market. The key aspects to be considered for introduction of new species are economical (production costs and market demand) and biological (easy adaptation to rearing conditions, endemic species, etc.).

Vairone Telestes muticellus (Bonaparte 1837) and bleak Alburnus alburnus alborella (De Filippi 1844) are two small cyprinids that seem to be potential candidates for diversification in North Italian fish farms. Their feeding and social habits, being not strictly predators and showing strong gregariousness, can facilitate the adaptation to captive rearing and domestication processes, too. Moreover, they are reophilic, moderately cold freshwater fish and autochthonous in the Po valley basin. Market potentials may be both consumption as food and as live bait for anglers.

The aim of this study was a preliminary evaluation of the adaptability of vairone and bleak to captive rearing conditions and to an artificial diet.

After two months of adaptation to experimental conditions, 95 specimens of vairone and 39 bleaks (mean body weight 0.52 g and 0.63 g, respectively) caught from the wild were stabulated in two 1m x 1m, 150 l tanks, supplied with well water (Temperature: 11.5±2.0°C; Dissolved oxygen: 10.8±0.5 mg/l; pH: 8.04±0.12; TAN: 0.02±0.01 mg/l; NO₂: <0.02 mg/l)

A practical diet was formulated for both species according to carp nutrient requirements (dry matter 93.6%, crude protein 38.5%, crude lipid 12.0%, crude fiber 0.3%, ash 7.4%). To make the feed suitable to all fish sizes, the dry pellets were crumbled and sieved in particles size range of 0.5-2.0 mm. Experimental diet was offered in a single daily meal to apparent satiety.

Both fish species showed good adaptation to captive rearing conditions over 91 days of rearing. Observed mortality was low for a wild species kept in captivity (5.3% for vairones and 7.7% for bleaks). The artificial diet was well accepted by both species and the final mean weight was 0.57g for vairone and 0.73 g for bleak (weigh gain 11.9% for both species).

Preliminary results point out a positive reaction to captivity and good tolerance to artificial diet. The observed performance stimulates further investigations on specific diets and rearing conditions in order to achieve optimal growth.
Novel application of LED lamp technology in crustacean farming

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ABSTRACT

The aim of this study was to investigate the effects of a new lighting technology called LED (light emitting diodes) under laboratory conditions in crayfish farming. LED lamps are ‘point sources’ that allow a sensible consume reduction of the radiant flux. It is known that exposure to polarized light is used effectively for different therapeutic purposes. Juvenile Astacus atacus (initial weight: 1.2±0.07 g) were reared in 200-liter glass tanks in a flow-through and aerated system (temperature 14.0±0.01°C and photoperiod L:D 12:12h) for four months. Effects of light intensity (150 and 600 lux) and colour spectrum (635, orange-red and 525 nm, blue-green) were compared to a control (350 lux, tube fluorescent 220V AC 40 W). Both light intensity and colour spectrum significantly (P<0.01) influenced the relative weight gain (RWG, %, [(final weight–initial weight)x100]/initial weight). The best mean RWG was observed for the crayfish cultured at 600 lux and 635nm (80.52% higher than control group). The effects of artificial lighting on body colour, chemical composition and stress parameters were analyzed. Significant effects of coloured light on body appearance were observed by means of point processing of images (ImageJ software). The optimization of artificial lighting in large scale in-door activities during reproduction and early crayfish stages would improve the quality of the environmental management. Present results suggest that light colour should be regarded as a rearing factor worth to be further investigated.
Bacterial flora associated with the digestive tract of gilthead seabreams reared in floating cages in the Alghero Bay (North western Sardinia, Italy): preliminary results

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ABSTRACT

The intestinal microflora of fish is highly variable and can depend on many factors such as species, developmental stage, environmental conditions, trophic habits and, in the case of farmed fish, on rearing conditions (i.e., fish density, quality of rearing water, dietary regimen, etc.). Different studies showed that Gram-negative bacteria such as Enterobacteriaceae and the Vibrio-Aeromonas group dominate the fish intestine. However, since few studies have been carried out on the bacterial flora of intensively reared marine teleosts, the aim of the present study was to investigate the microflora associated with the digestive tract of Sparus aurata specimens reared in floating cages. A total of 30 gilthead seabreams (mean weight 324.9±61.4 g) starved for 48 hours was sampled in autumn 2008 from a fish farming facility located in the Alghero Bay (North western Sardinia, Italy: Lat 40°33'43.9''N, Long 8°16'09.0''E). The intestine between the pyloric caeca and the anus of each specimen was removed and analysed by means of conventional bacteriological techniques using five culture media: Plate Count Agar (PCA), Nutrient Agar (NA), Violet Red Bile Glucose Agar (VRBGA), Violet Red Bile Agar Mug (VRBA-MUG), and de Man-Rogosa-Sharpe (MRS) agar. Total viable counts on PCA and NA were quite similar, showing mean values of 126.7±109.0 colony forming units (cfu) per gram intestinal tissue (between 10.0 and 495.0 cfu/g) and 108.0±101.6 cfu/g (from nil to 350.0 cfu/g), respectively. The mean number of Enterobacteriaceae determined on VRBGA proved to be 46.3±42.8 cfu/g (from nil to 150 cfu/g) and the VRBA-MUG mean count were 30.4±29.3 cfu/g (from nil to 95.0 cfu/g). No bacterial colonies were instead detected using the MRS medium. These results, although preliminary, evidenced a relative low number of bacteria associated with the digestive tract of the fish examined, thus indicating good hygienic conditions inside the cages as well as a suitable rearing density and a balanced diet for the seabreams.
Effect of different dietary lipid sources on rainbow trout fillet fatty acid profile

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ABSTRACT

Fish products are considered as an healthy food not only for their energy and protein supply but also for the quality of the lipid profile. Its fatty acid profile, rich in polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA), confers to flesh a very high nutritional value (Palmegiano et al., 2000). Those features confer to fish an important role for human health in relation to the principal risk factors linked to diet (Corraze, 1997; Albert, 1998; Klotte, 2002). As the fatty acid composition of fish is markedly influenced by the lipid pattern of their food (Steffens, 1997; Sargent et al., 1999), it looks very important to assess the possibility to improve, by mean of feeding, the flesh lipid profile of fresh water fish. The aim of this research was to evaluate the effect of different lipid sources on the fatty acid profile of rainbow trout (Oncorhynchus mykiss). 360 rainbow trout (IBW: 141.18±1.93 g) were kept in 12 fibreglass tanks (0.5m³) with a water flow of 25 l/min, a water temperature of 12.5±0.5 °C and 7.85±0.05 mg/l of dissolved oxygen. Fish were fed 4 isonitrogenous and isoenergetic experimental diets (3 replicates): 1) cod liver oil diet; 2) linseed oil diet; 3) tuna stearina oil diet; 4) echium oil diet. Fish were fed twice a day for 107 days at 1.5% of live weight. Proximate composition and fatty acid profile of fillets were detected at the end of the trial. Total lipids were extracted according to Folch et al. (1957) and methyl esters of fatty acids were prepared by methylation procedure (Metcalfe and Schmitz, 1961) and analysed by gaschromatography. The atherogenicity (AI) and thrombogenicity (TI) indexes were calculated according to Ulbricht and Sauthgate (1991). Data were evaluated by ANOVA (SPSS, 2004). Significant differences at 5% level were determined by Newman-Keuls test.

All the fish readily accepted the experimental diets and no mortality was recorded due to the dietary treatments. No differences appeared for ether extract content of fillets that ranged from 3.3 to 5.1%.

The highest values of linoleic (C18:2n6), α and γ linolenic acid (C18:3n3 and C18:3n6) were observed in fish fillet fed vegetable oil diets reflecting the fatty acid profile of linseed oil and echium oil diets. Diets containing marine origin oils (cod liver oil and tuna stearina oil diets) showed the highest percentage of EPA (C20:5n3) and DHA (C22:6n3) on total fatty acid (% FAME). Regarding AI, fish from vegetable oils showed the lowest (0.45 and 0.54 for linseed oil and echium oil diets respectively) value, while TI resulted low in all treatments with the highest value in tuna stearina oil diet (0.42) Those results are similar to those reported by Valfré et al., 2003. The possibility to modulate the foodstuff final features of the lipid fraction is very interesting in relation to the final purpose of a product able to reduce the risk of cardiovascular and other related pathologies. In this trial the most interesting treatment, for reducing risk of chronic diseases, seems to be the linseed oil based diet. Nevertheless the highest contents of EPA and DHA were obtained using marine oils. In this sense none of the diets in this trial resulted efficient for all aspects; then a blend of different lipid sources in the same diet could optimize the results.

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Alpine and Northern European arctic charr (*Salvelinus alpinus*) populations analyzed by means of mitochondrial control region sequences and nuclear AFLP markers

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**ABSTRACT**

The Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), is a Holarctic Salmonid species adapted to cold-water habitats and whose either anadromous or sedentary populations are characterized by an extreme morphological variability.

The presence of *S. alpinus* in Southern Europe is interpreted as a relic of the Pleistocene glaciations which strongly influenced both the distribution and the genetic variability of the species, due to repeated isolation and bottleneck events. Besides the Pleistocene climate changes, during the last centuries also the anthropogenic activities had a strong impact on *S. alpinus* populations, leading to fish translocations and change in population densities due to exploitation/overfishing.

Previous genetic studies on European Arctic charr revealed a contrasting scenario, with European populations being characterized by a very low mitochondrial diversity (e.g. only three control region haplotypes were identified in Alpine specimens) and a high genetic variability at nuclear microsatellite loci (6-49 alleles per locus and overall expected heterozygosity between 0.72±0.09 and 0.87±0.04).

In this study, mtDNA control region sequence and AFLP (Amplified Fragment Length Polymorphism) markers were characterized in 592 specimens from 40 populations sampled in 35 Alpine and five Scandinavian lakes, aiming at the detection of possible autochthonous, introduced and hybrid stocks.

The control region sequences (n=112) were aligned with GenBank data to build a Median-joining network. AFLP profiles were obtained by digestion with EcoRI and TaqI endonucleases and selective amplification with four primer combinations. Polymorphic loci were scored to build a final data matrix including 537 individuals and 77 markers. This dataset was analyzed by Factorial Correspondence Analysis (individual level) and Multi-Dimensional-Scaling (population level), while hidden genetic structures were investigated by a Bayesian clustering approach.

MtDNA sequence data confirmed the existence of a low level of genetic variability: newly produced sequences included only five different haplotypes, two of which newly identified in this study.

Multivariate statistical analyses and the Bayesian assignment test on AFLP data showed that individuals and populations cluster into three main groups, corresponding to specimens of Italian, Austrian/German and Scandinavian geographic origin. Furthermore, some populations displayed low levels of genetic polymorphism which is probably due to either past demographic fluctuations or to a strong impact of anthropogenic activities in the recent decades.
Nutritional and quality traits of *Chamelea gallina* (Linnaeus, 1758) collected along the coastal area of Barletta (Adriatic Sea, Italy)

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**Abstract**

The bivalve *Chamelea gallina* (Linnaeus, 1758) is commonly found in the Mediterranean Sea at depths between 2 and 15 m where it inhabits well-sorted fine sands. This species has a considerable commercial importance, representing an important fishery resource in Italian inshore waters. Its commercial size corresponds to a minimum length of 25 mm, as established by the Council of the European Union in 1994. The aim of this study was to investigate the morphometric traits and the nutritional qualities of *C. gallina* harvested on the southern Adriatic coast of Italy, during different seasonal periods. Samples of commercial size of *C. gallina* were collected with an experimental hydraulic dredge from 2 to 5 m water depth, in 3 fishing areas along the coastal area of Barletta, every season from July 2007 to May 2008. The specimens collected were examined for size (length, height and width) and weight (total, flesh and shell). Their edible part percentage was calculated and flesh was analyzed to determine its content in moisture, protein, fat and ash. One-way ANOVA was used to test the differences in morphometric characteristics and proximate composition of the clams collected at the 3 sampling areas, in different seasons. Morphometric characteristics of *C. gallina* from the 3 areas showed the same trend throughout the year. Edible part index showed a peak values in spring coinciding with gamete release (mean value 20.44±2.36). Proximate composition showed a similar pattern in clams from all the areas, thus highlighting the importance of reproductive cycle and physiological features of the species in determining its biochemical composition. Lipids and proteins contents were minima in autumn (mean value 0.49±0.01 and 12.89±0.30 g/100 g wet weight, respectively) and maxima in spring (mean value 1.46±0.14 and 16.63±0.07 g/100 g wet weight, respectively), while ash percentage showed an irregular trend. The fluctuations of nutrient contents observed during the year have an impact on the commercial and nutritional qualities of the clams. In fact, at the end of winter (i.e. before gamete release), *C. gallina* from the southern Adriatic Sea, reached its highest commercial and nutritional values.
Establishment of primary hepatocyte cultures from marine Teleostei as a new experimental in vitro model for aquaculture research

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ABSTRACT

Among the experimental animal models based on in vitro systems, hepatocytes in primary culture from aquatic organisms are considered the gold standard for the study of cellular, biosynthesis, metabolic functions and toxicological investigations. The liver perfusion is the only method used for isolation of hepatocytes from various animal species from over thirty years, and freshwater fish species are the most commonly studied. However, the method of liver perfusion has several limits and up to date a modest number of marine cell types have been isolated and cultivated. The development of a new method for isolation of hepatocytes derived from marine Teleostei is needed. Accordingly, the aim of the present study was to establish for the first time primary hepatocyte cultures from marine fish, such as D. labrax and S. aurata. Liver cells were isolated by tissue physical disaggregation combined with enzyme digestion. Purified hepatocytes were then seeded at a density of 3x10⁴ cells/cm² in collagen-coated 96 well-plates (100 µl/well). Cells were cultured in Leibovitz L-15 medium with 10% fetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin/100 µg/ml streptomycin/100 µg/ml amphotericin, supplemented with insulin, aminoacids, vitamins, growth factors, and cultivated in a refrigerate incubator at 18°C in atmosphere of 3% CO₂/97% air. Such a scientific innovation allows to carry out in vitro studies on those marine Mediterranean species, until now little investigated, and to translate the know-how obtained from in vitro systems to in vivo. The application fields of this novel in vitro model are vast and important: aquaculture, marine biotechnology, animal science, toxicology, genetics, animal pathology and infectious diseases.
Growth performance of common sole (*Solea solea*) fed semi-moist diets supplemented with different feeding attractants-stimulants

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ABSTRACT

A 111-day experiment was performed to compare the growth performance of common sole (*Solea solea*) fed a dry commercial diet or semi-moist feeds added or not with feeding attractants-stimulants.

Three semi-moist preparations (75% dry matter) were obtained from a basal, complete dry diet including marine protein and oil sources (crude protein, 52%, total lipid, 13%) by adding just water (B), water plus betaine, 4.5% w/w (BB) or water plus a commercial feeding enhancer additive based on yeast extract and protein hydrolysates, 1.5% w/w (BC). A dry commercial diet (crude protein: 50%; total lipid: 20%) which was shown to ensure acceptable growth rates in previous experiment with sole, was used as a control treatment. The semi-moist preparations were obtained after cold pelleting the doughs through a dye, so as the resulting feed items were of the same size as those of the dry commercial diet (2 mm in diameter). The trial used 160 fish (mean initial body weight, 58±3 g) equally and randomly divided among 8 groups stocked in rectangular vessels (100x33x25 cm) kept in an indoor re-circulating marine water system ensuring nearly optimal water quality to sole (T: 20±1°C; Salinity: 35±1 g/L). The 8 groups were allotted to the 4 diets according to a random design (each diet fed to duplicate units). The diets were offered in a single daily meal equalised so as all units received the same amount of feed dry matter (i.e. 1% biomass). Response variables recorded at the end of the trial were subjected to monofactorial ANOVA and subsequent Duncan’s test for mean comparisons, considering fish groups as the experimental units. Overall mortality was low (6%) and not affected by the dietary treatment. Relative to fish groups fed with the control dry diet, those fed the semi-moist preparation-B resulted in higher final weight (94.3 vs. 76.4 g, P<0.05), specific growth rate (SGR, 0.43 vs. 0.24 % day⁻¹, P<0.05), improved dry matter conversion rates (DMCR, 2.4 vs. 4.4, P<0.05) and productivity (gain in biomass, 15.5 vs. 7.6 g sqm⁻¹day⁻¹, P<0.05). The semi-moist feeds supplemented with feeding attractants-stimulants, resulted in a further improvement of the rearing performance. Diets BB and BC were equally effective over the unsupplemented preparation, in enhancing final weight (103.0 vs. 94.3 g, P<0.05), SGR (0.51 vs. 0.43 % day⁻¹, P<0.05) and productivity (18.9 vs. 15.5 g sqm⁻¹day⁻¹, P<0.05). However, better growth with the supplemented feeds led just to a marginal improvement of DMCR (2.2 vs. 2.4, P>0.05), thus suggesting that increased weigh gain did not result from improved feed utilisation efficiency but, primarily, from higher feed intake induced by the feeding enhancers added to the diet. The results obtained here stress the importance of the sensory attributes of a complete feed (physical form, presence of chemio-attractants-stimulants compounds) in improving the farming performance of this slow growing fish species.

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