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Which future for stocked European sturgeon in the Gironde Dordogne Garonne system? Habitats used and movements' patterns.

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The European Sturgeon (*Acipenser sturio*) is a critically endangered species and the last population is located in the Gironde Dordogne Garonne system (South west of France). Since autumn 2007 a European restoration plan on this species has been adopted under the aegis of the Berne convention. Thanks to recent successful assisted reproductions, more than 90 000 larvae were stocked in this system in 2007 and 2008.
In this context field experiments were initiated in 2008 to assess stocking success and to document fish spatial behaviour and habitat use. Different methods and techniques are implemented to achieve these goals. Trawling sampling in the estuaries is planned to estimate fish abundance and to locate their habitats. Telemetry studies were initiated in 2008 and in 2009 to document individual movement patterns and habitat use. Knowledge acquired from these experiments is intended to bring a help to the management programme and to the conservation of this species.

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A Genetic Analysis of a Recently Rediscovered Population of the Anadromous Twaite Shad (Alosa fallax) in the Ebro River, Spain (Western Mediterranean).

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The Ebro River is the largest river in the Iberian Peninsula (18,217 Hm^3 $year^{-1}$), draining one-sixth of the Iberian Peninsula's surface area (88,835 km^2) and forming the fourth largest delta in the Mediterranean Sea (350 km^2). In spite of the rivers size anadromous fish from the Ebro River have been nearly exterminated since the 1950´s. Habitat alterations and denial of access to the upper reaches, by the construction of a dam at Xerta, have greatly diminished the available spawning habitat. This blockage concentrated what anadromous species which remained, downstream of the dam where the fishery of shads developed in the 1800´s and earlier. Further, it is likely that an additional consequence would have been forcing the normally upstream spawning species *Alosa alosa* into overlapping its spawning habitat with that of the *Alosa fallax* which normally spawns in lower reaches of their natal rivers. The appearance of mature twaite shads (*A. fallax*) in the lower Ebro River in the spring of 2005, suggested the possibility that a natural recovery of this population may be occurring. In this study, to better understand the origin of these fish, we present genetic analysis of five microsatellite loci and haplotypes from two mitochondrial loci from the shad of the Ebro River, and the closely related species *A. alosa* from a captive rearing facility. The possibility of hybridization between these two shad species and the genetic origin of the recently rediscovered population of *A. fallax* will be presented.

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Stock identification of sardine (*Sardina pilchardus*) using morphometric characters

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Morphologic differentiation among stocks of sardine, *Sardina pilchardus*, throughout the Black, Marmara and Aegean Seas, was investigated using morphometric characters. Discriminat function analysis of morphometric characters suggested that there is restricted migration of sardine among the adjacent seas. The Aegean Sea sample (İzmir Bay) was the most isolated samples from all others, which may indicate existence of distinguishable sardine stock in the area. The second overlapping group included the remaining samples from the waters off the Aegean, Marmara and Black Seas, which suggesting some degree of intermingling between this areas. Examination of the contribution of each morphometric variable to discriminant functions indicated that differences among samples seemed to be associated with the posterior part of the body. In Turkish coastal waters, sardine, is a widely exploited species, espacially in Aegean Sea. However, during the last decades, the stocks sardine in the Aegean Sea have experienced increasing fishing pressure and changing environmental conditions. Therefore, it is necessary and important to know the stock structure of explored species, as each stock must be managed seperately.
Keywords: sardine, morphometrics, stock identification, *Sardina pilchardus*.

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Influence of food distribution upon the development of feeding anticipatory activity (FAA) and growth performance in rainbow trout (*Oncorhynchus mykiss*)

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In cultured fish, scheduled feeding appears to act as a zeitgeber, producing feeding anticipatory activity (FAA). Using indefensible (ID) and defensible (D) pattern of food delivery, the aims of the present study were to investigate whether rainbow trout (*Oncorhynchus mykiss*) can anticipate three daily meals, and to compare growth performance in relation to the different methods of food delivery. The experimental set-up consisted of three glass aquaria each with eight fish for two treatments, i.e. ID and D. Feed was delivered by hand through a submerged outlet tube in the aquaria for the D pattern of food delivery. No tube was used for the ID treatment. Fish were subjected to restricted feeding (RF) for 28 days. Growth performance was conducted at the end of the experiment and following parameters were measure: feed intake (FI), condition factor (CF), feed conversion efficiency (FCE), specific growth rate (SGR), coefficient of variation of weight (CVw (%).The results show that only using an indefensible pattern f food delivery, rainbow trout are able to show FAA in three different locations. Also, most of the growth parameters were higher in D than ID pattern. It was concluded that only using ID pattern of food delivery, rainbow trout may predict the timing of food delivery and appear in the location of food delivery before feed is made available and that D pattern of feeding can lead to hampering and/or suppressing the growth.

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Experimental studies on juvenile European sturgeon *Acipenser sturio* habitat preferences

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The European sturgeon *Acipenser sturio* is a threatened species, which is one of the 5 species identified as a priority by the Council of the European Community (Directive 92/43 – Annex II). Conservation and restoration of this species is then a challenge in order to preserve European biodiversity. With the goal of protecting essential sturgeon habitat, the standing committee of the Berne Convention underlined that an urgent knowledge is needed concerning habitat preference, and especially larvae and juvenile habitat during their freshwater phase (Draft Action Plan for the European sturgeon, 2007, component 2 – objective 3). We carried out experiments with 3 month old juvenile sturgeon in experimental mesocosms (SCOLA II) to determine substrate preference and daily rhythm activity. Three different granulometries were tested by pair in 3 mesocosms. One minute video was recorded in each mesocosm, every hour on a 24 hours basis, during the 2 7 days replicate experiments. Results show a clear activity pattern, with an intense swimming activity during night. During daylight, juveniles preferred fine substrate, where they were observed static on the bottom, while they swam all time on cobbles. Data presented are a first step on juvenile habitat preference knowledge, and they must be completed, particularly on earlier phase.

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First characterization of the spawning habitat and mating behaviour of twaite shad in the Ebro River (western Mediterranean, Spain)

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After nearly 40 years of scientific reports with few records of twaite shad in the lower Ebro River, this species seems to be increasing in numbers as a seasonal member of the fish fauna of this river, appearing to be the only anadromous species in the Ebro River. The presence of suitable spawning grounds seems to be the main reason for the survival of this species. We conducted the first survey to document the spawning habitat selection and the mating behavior by means of direct observations of the mating behaviour and validated by means of a DIDSON® camera (Dual frequency IDentification SONar) during two consecutive years (2007-2008). Between late March until late June, we periodically surveyed the river banks for spawning episodes (tail-splashing activity) and characterizing the physical habitat where spawning took place. Two main spawning areas were identified, located at 1 km and 4 km below the insurmountable weir of Xerta-Tivenys (located 64 km upstream from the river mouth). These strictly riverine areas are far from any tidal influence, and were characterized by fast-flowing water currents (ca. 0.7-1.5 m/s) and a substrate composition dominated by coarse gravel bottoms. The daily rhythm of spawning episodes was consistent between years, with recorded mating episodes registered between 23:30 h and 04:30 h with a peak of activity around 01:00-02:00 h in the early morning. Details of the spawning behaviour have been recorded with imaging sonar for the first time for this species. Sub-aquatic images show an intense interaction between individuals with apparently erratic fast movements, interpreted as an active search by the males for females that culminates with the congregation of groups of specimens for mating (tail-splashing and circular surface swimming). This information will be of value for managing and preserving the migrating populations of this species.

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First experience of artificial reproduction of the European grayling (*Thymallus thymallus*) in Ukraine

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European grayling (*Thymallus thymallus*) within Ukraine historically inhabited a number of mountain rivers of the Dniester and Danube river basins. Grayling biotopes in Carpathian rivers are similar to brown trout biotopes but are shifted to valley parts of rivers and areas, where timber cutting has occurred. This is connected with feeding behavior of the grayling, since an important part of its ration belongs to insects, which prefer more illuminated and sunny parts of rivers. In the beginning of the XX century, productivity of the grayling in Carpathian rivers was almost the same as that of brown trout and by 15-20% more in open area that composed 60-100 kg/km of the river. During the second half of the XX century, grayling stocks catastrophically declined. As a result, this species was listed as endangered in the Red Data Book of Ukraine (1994). Current state of the grayling in Ukrainian Carpathians is on the edge of extinction, it can be sporadically found in 5-7 rivers. Thus, the conservation and restoration of this species within its historical range is possible only based on its artificial reproduction. Artificial reproduction of grayling was conducted at trout farm of the Sinevir National Park. We used age-3-4 broodfish taken from the Chorna river (Tereblya tributary). Average weight of females was 185 g, males – 130 g. Average working fecundity of females was 558 eggs (160-1107 eggs). Average weight of unfertilized eggs was 10.3 mg with diameter of 2.1 mm. Ejaculate volume of males was 0.05-0.2 ml, sperm mobility was in average 24 sec. Fertilization rate was 53%, incubation lasted 18 days. Incubation output was 30%. The majority of embryos died during gastrulation. Fry was grown in a pond using extensive technology. During vegetation period (155 days) fingerling reached the bodyweight of 4 g with body length of 7 cm.

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Artificial Breeding of *Cottus gobio* in a full recirculation system

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Artificial breeding of *Cottus gobio* in a full recirculation system - *Cottus gobio* is a native Italian species, widely distributed in Italian freshwater drainages, from the Alps to some central-northern Apennine drainages. In order to protect this ecologically valuable species, a research project was started in the framework of the “Osservatorio dell’idrofauna” of the Alpi Marittime Natural Park, in collaboration with the Dept. of Biology of Parma University and Acqua & Co S.r.l. for the preparation of an aquaculture facilities. The purpose of this project was to develop a standard aquaculture protocol to produce bullhead fingerlings to be reintroduced in the natural environment and obtain new eco-ethological information under controlled conditions.

P

Nitrites Toxicity studies for the conservation of endemic Mexican goodeinae fish

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Anthropogenic impacts such as urbanization, farming and industrial activities have modified land and water ecosystems, affecting present and future resources. The problem is conspicuous in developing countries like Mexico, which has high biodiversity and also an increasing number of species in danger of extinction and threatened. This is occurring to the fish inhabiting the central plateau of the country, such as the Goodeinae sub-family. Concentrations of NO_2 and PO_4 have incremented in superficial and subterranean water as a result of the intensification of agricultural and farming activities and both have become common contaminants in water reservoirs. If a species is consider for reintroduction, it will be essential to know the tolerance to the NO_2 and PO_4 concentration, particularly in fry, which are more sensible to contamination. At the Universidad Michoacana de San Nicolas de Hidalgo (Mexico) a conservation program of endemic Goodeidae fish has been developed to maintain a live collection to guarantee the survival and perform studies in basic biology. In the long term this knowledge will be used to reintroduce the fish to the original environment. *Skiffia multipunctata* (considered as an endangered species according to IUCN 1998) particularly “La Luz spring” population, was selected for this research because the area has been transformed into a recreational area, where people are washing clothes, taking showers and in summer is a camping area. Around the spring are some cultivated lands, which use agrochemical products. For this reason, the nitrites lethal dose 50 (LC50) were performed. The concentrations: 0.016, 0.028, 0.049, 0.085, 0.150 mg/L and 0.00 mg/L NO_2 were determined after a preliminary essay and a literature revision for other species. For each concentration, aquariums of 6L were used, and 10 fry were tested with a triplicate. Once the fish were in the aquarium the toxic substance was introduced and observations of behavior and survival were recorded at 30 min, 1, 2, 6, 12, 24, 36, 48, 72 and 96 hours. The LC50 range has been calculated to be between 0.02 to 0.11 mg/L NO_2. The NO_2 sub-lethal concentration (chronic effect) and its effects are in research. This will allow determining safe concentrations of NO_2 that allow the establishment of reintroduced fish. However, further investigation is necessary to bring up a reintroduction program.

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Application of the PHABSIM (Physical HABitat SIMulation) for the study of twaite shad habitat availability in the lower Ebro River (western Mediterranean, Spain)

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In the lower Ebro River, despite the large population decline of twaite shad since the sixties, this anadromous species seems to show some recovery during the last 5 years. Fish migration is still mostly restricted to downstream of the weir of Xerta-Tivenys (60km upstream from the river mouth). This is the first insurmountable obstacle which the fish encounter in their migratory path upriver. The historical weir is part of a relatively complex area further composed by two deviation channels, a boat lock, and a hydroelectric power plant. A fish ladder exists but of improper design for this and many other species. Thus, it is important to know the effects of different hydrological regimens on habitat selection for mating twaite shads in such a high flow-regulated river. The physical habitat simulation (PHABSIM) system is a well-established hydro-ecological model that provides a suite of tools for the numerical modelling of hydraulic habitat suitability for fish and invertebrate species. In this study, we used PHABSIM modelling to assess temporal changes in spawning habitat availability up- and down-stream of the weir at Xerta-Tivenys, and evaluate the potential use of up-stream spawning habitats if fish were able to overcome the obstacle of the weir. Characterization of spawning habitats indicates that twaite shad in the Ebro River prefer fast-flowing reaches (ca. 0.7-1.5 m/s) with a coarse gravel-bottom and well oxygenated water. Modelling indicates that if twaite shad were able to overcome the weir (high river flow conditions), which only may have happened 6 times during the last ten years, fish would have access to a further 60 km of river upstream of the weir, representing a 200% increase in the amount of freshwater habitat and potential spawning areas. Improved access for fish migration, together with restrictive fishery regulations and a renewed interest in the fishery will assist conservation of the species.

P

Diadromous Fishes and the European Water Framework Directive – Opportunities and Limits for River Basin Management

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The good ecological status or potential is the general environmental objective for surface water bodies demanded by the European Water Framework Directive. The fish fauna is one of four biological elements assessing the ecological status. In the Weser River Basin District diadromous species represent approx. 20 % of all fish and lamprey species in surface waters. But hydromorphological alterations, intensive agriculture and potash mining derogate the quality of habitats as well as the longitudinal connectivity of migration routes. Therefore, the development of sustainable populations of diadromous fish species in the catchment needs strategic reasoning to substantiate management objectives and to set priorities of measures.

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Natural spawning nests of Atlantic salmon as a model for designing in-stream egg incubators

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About 300 Atlantic salmon redds were examined in the rapids of the highly productive River Varzuga (Kola Peninsula, Russia) in order to develop incubation nests to be installed in rivers for a prolonged time period (7-8 months). Salmon spawners were found to choose river stretches with depths of 0.4-1.2 m and current speed of 0.4-1.1 m/sec. The particle size composition of their preferred gravel-boulder bed is ~65% of pebbles (0.5-5.0 cm), ~30% of boulders (5.0-10.0 cm), no more than 5% of sand. The spawners deposit eggs at a depth of 15-55 cm from the river bed surface. Each redd contains 150-600 eggs. The velocity of flow in the substratum was 0.2-0.5 cm/sec. Oxygen content in the underflow was 10.2-12.9 mg/l. These parameters were used to design incubation nests and choose the locations for their installation in rivers. The size of the work chamber, type of the artificial substratum, diameter and number of perforations in the bottom inlet and the substratum, as well as the diameter of the short tube through which larvae leave the nest and the water washing the eggs escapes were selected by trials. Operation of the incubation nests is based on utilization of the flow in the river bed, which is naturally cleaned by sediments. Water enters the device via the bottom piece and passes through the perforated substratum on which the eggs lie. The water then escapes via the short tube designed as the outlet for hatched larvae. In the trials, 50 to 150 eggs were placed into the nests. The condition of the eggs and the embryo development process were controlled at the final phase. The results show that the devices are highly effective in clear-water rivers with low sediment load.

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Determination of sexual maturity and reproductive season of anchovy ((*Engraulis encrasicolus* (Linnaeus, 1758)) in the Edremit Bay

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Changes in age and size at maturation influence both the numbers and characteristics of individuals in the spawning stock. Earlier age and smaller size at maturation give shorter generation time, but have also negative impacts: fisheries yield is likely declining as individuals invest more to reproduction and less to body growth, and recruitment may be negatively affected because of size and age effects on spawner quality. A total of 59 gonads of anchovy, *Engraulis encrasicolus*, were collected from Edremit Bay on July 2007. These gonads were examined in order to determine maturity and other reproductive characteristics (Sex-ratio, oocyte developmental features, etc) of this species. The fishes collected ranged from 114 cm to 150 cm. The sex-ratio was 1:1.08 M/F. All female fishes over 125 mm was sexually mature. The first maturity (50%) occurs from 120 to 125 mm in this area. The males are very scarce in the sample so we cannot discuss about male first maturity size in this paper. These and other reproductive aspects (GSI; fecundity etc.) will be discussed in this paper. The aim of this paper is to increase our knowledge about sexual maturity, reproductive season of anchovy in the Edremit Bay as an important tool for determining a correct policy for the management of fish stock. Keywords: Anchovy, *Engraulis encrasicolus*, maturitysize, reproductive potential, reproduktive season

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Presentation of a labelling tool for CITES implementation in caviar trade

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Sturgeon *(Acipenseridae*) worldwide are under intensive fisheries pressure due to their highly-priced eggs, known as caviar and sold at prices of up to US$ 4000/kg. Today, all 27 species are threatened of extinction and have been included on the CITES Appendices, regulating international trade. Still, illegal trade continues to threaten wild populations, often camouflaged as caviar from legal aquaculture. Consequently, tools to discriminate wild and aquaculture origin have to be developed to allow for the enforcement of CITES. Here, feed supplementation with two fatty acids, fed shortly before caviar production was evaluated. For this purpose, farmed female sterlet *Acipenser ruthenus* were fed on a diet supplemented with caprylic and capric acid at 4 and 20 mg/g and marker accumulation in the caviar was quantified from ovarian biopsies over a period of 90 days (0d, 10d, 60d, 90d) and compared to a control feed supplemented with sunflower oil. Individual marker accumulation was studied in maturing (early vitellogenesis, 4 year old fish) and mature (late vitellogenesis) sterlets. In the control and the treatment group, no accumulation of caprylic acid was detectable by GC-MS (detection limit <0,005 mg/g). In contrast, capric acid was detected as early as 10 d at concentrations between 0,01-0,03 mg/g in the caviar, whereas concentrations in all control fish were all below the detection limit. Therefore, use of capric acid as labelling tool allows for the discrimination of wild and farmed caviar and thus provides an identification system under CITES as recently claimed by the IUCN Sturgeon Specialist Group.

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Status of sturgeon populations in Lower Danube Region and possibilities for their better investigation and protection

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Populations of six sturgeon species in the Danube River (beluga, Russian sturgeon, stellate sturgeon, sterlet, ship sturgeon and Atlantic sturgeon) have experienced severe decline during the last several decades, mostly due to the unsustainable fishery, river fragmentation and water pollution. Unsustainable fishery levels in the Danube have been further compounded by a significant illegal fishery. It is believed that the amount of illegal fishery has decreased in recent years, but there are no studies made that could confirm this. In 2006, Romania introduced a ten year moratorium on commercial sturgeon fishery, and Bulgaria started introducing moratoria by decrees issued every year. Stellate sturgeon and Russian sturgeon catch was banned from 1993 in Serbia, while catch of beluga is prohibited in 2009. Sturgeon aquaculture is developed in Bulgaria, Romania and Moldova. Some attempts for sturgeon rearing were developed in Serbia but it is still not fully established. Scientific research of sturgeon in Lower Danube Region is performed mainly in every country separately without broader exchange of information. Countries in Lower Danube Region participated for research activities in EU-FP6 as well as in the SEE Programme 2007-2013 but proposals were rejected. So, there is still a lack of knowledge on basic sturgeon demography, life history and relative effects of different negative factors. Due to that it is necessary to find other funding opportunities and connect scientists and other stakeholders in Lower Danube Region and Danube Delta as well as in Black Sea to conduct detailed analysis of sturgeon populations in this region.