North Rhine-Westphalia
State Agency for Nature, Environment
and Consumer Protection

The reintroduction of the allis shad *(Alosa alosa)* to the Rhine system

LANUV-Fachbericht 28

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Environment and Consumer Protection

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The allis shad: a herring-like migratory fish

The allis shad *(Alosa alosa)* and its close relative the twaite shad *(Alosa fallax)* are both European fish species similar to the herring; they leave the sea when ready to spawn and travel up the rivers to breed. It shares this so-called diadromic lifestyle with what is probably the most well-known long-distance migratory fish, the salmon. Unlike the twaite shad, which mainly breeds in the lower reaches of the rivers just above the estuary, the allis shad migrates to the middle and upper reaches of the bigger rivers, although unlike the salmon, it does not penetrate as far upstream as the trout or grayling regions. The German name for the allis shad, "Maifisch", refers to the month of May, during which the majority of allis shad migrate upstream and spawn in water temperatures of more than about 16°-18°Celsius. Depending on the temperature conditions, the spawning period can begin as early as April and usually continues into June. Allis shad begin migrating when the water temperatures in the estuary and the river leading to the spawning grounds rise above 11° Celsius. Allis shad cover approximately 20 kilometres a day during their journey to the spawning grounds. Their preferred spawning grounds are the areas bridging deeper waters flowing at a leisurely pace and shallower, fast-flowing waters with gravel beds. This is where the allis shad shed their eggs and sperm into the water with loud splashing, particularly on mild May nights (in France, this behaviour is known as "bull"). The fertilised eggs sink to the river bed, where they are spread by the current rather than being attached to certain structures or even hidden, as is the case with most other freshwater fish.

Although the allis shad (shown here at adult stage) is a type of herring, it migrates to fresh water for spawning and prefers to spawn on warm May nights (hence the German name "May fish") in the middle and upper reaches of larger rivers with moderate currents and gravel riverbeds.

Like salmon, the majority of allis shad die after spawning. Depending on the water temperature, the allis shad larvae hatch after 3 to 6 days and immediately begin feeding on small plankton. That same summer, the allis shad fry reach a length of up to 13 centimetres. A few weeks after hatching, they migrate downstream and spend a few weeks in the brackish waters of the estuary before moving into the sea in winter at the latest. The period spent in the waters between the river and the sea, which thanks to the incoming tide are naturally turbid and rich in nourishment, is particularly important for them to adjust to the difference in the salt levels of fresh and sea water; the presence of natural estuaries is extre-

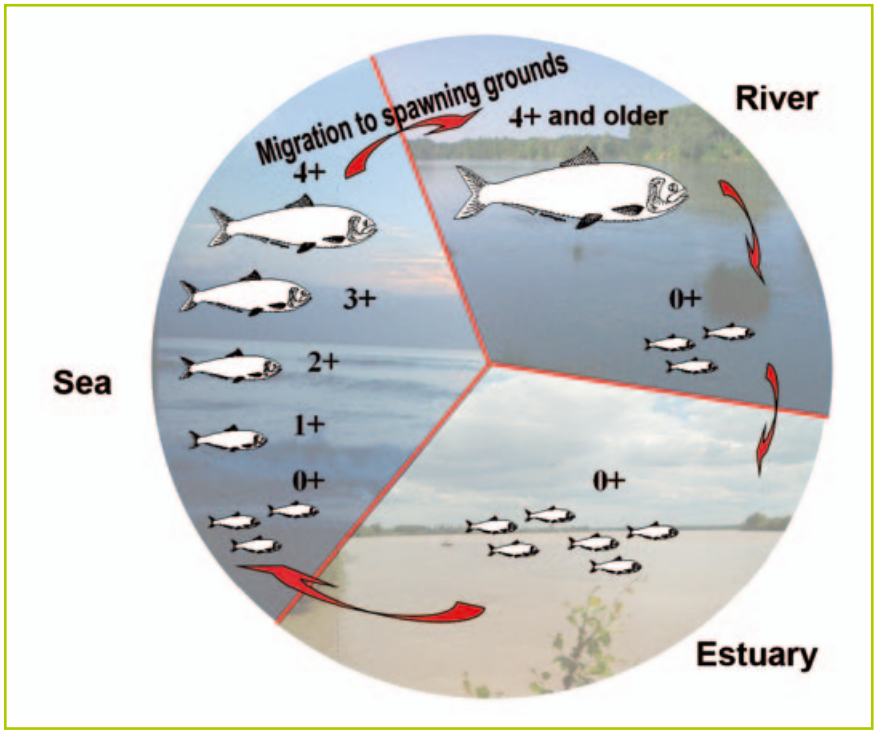
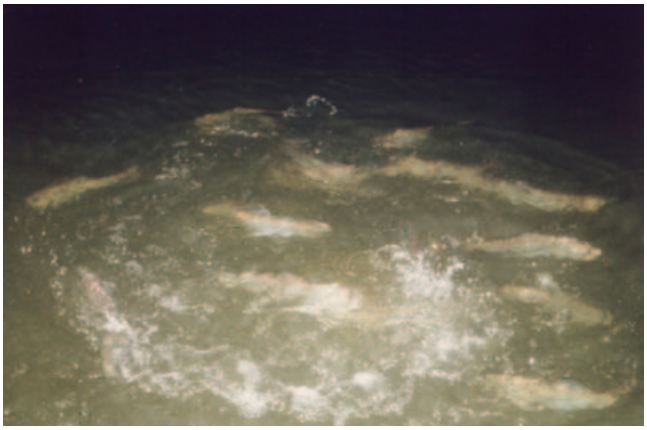


Diagram of the allis shad's lifecycle. Adult allis shad become sexually mature in their third or fourth year at the earliest, and migrate up the rivers from the sea to spawn. Most of them die after spawning. The fry hatched from the eggs spend several weeks in the river before migrating to the estuary in late summer and autumn. After a few weeks, they move into the sea during their first year of life (0+), where they spend several years before reaching sexual maturity.

mely important for the allis shad population. Once they reach the sea, the allis shad feed on the wealth of plankton and crustaceans found there. The sieve-like structures in their gills, the so-called gill rakers, make them especially suited to this form of food intake. Allis shad mostly remain in the coastal regions; unlike salmon, they do not migrate to deep waters and the arctic regions. After 3 to 8 years in the sea, they reach sexual maturity and migrate to the rivers for spawning. Sexually mature females, which are somewhat larger than males but which are usually one or two years older on reaching sexual maturity, can reach a length of more than 70 cm and weigh up to 5 kilograms. On average, allis shad reach a length of approximately 55 cm and a weight of 1.8 kilograms. The female produces 100,000 to 150,000 eggs per kilogram of body weight. This large quantity compensates for the losses which are inevitable when spawning in open waters. However, the eggs do not all ripen at the same time, which means that they are spawned in several batches over a period of a few weeks. The tasty allis shad is traditionally fished while migrating to the spawning grounds. They are fished with seine nets, driftnets and gillnets and sometimes with certain dip nets, which can only catch single fish. Allis shad are occasionally the target of anglers.

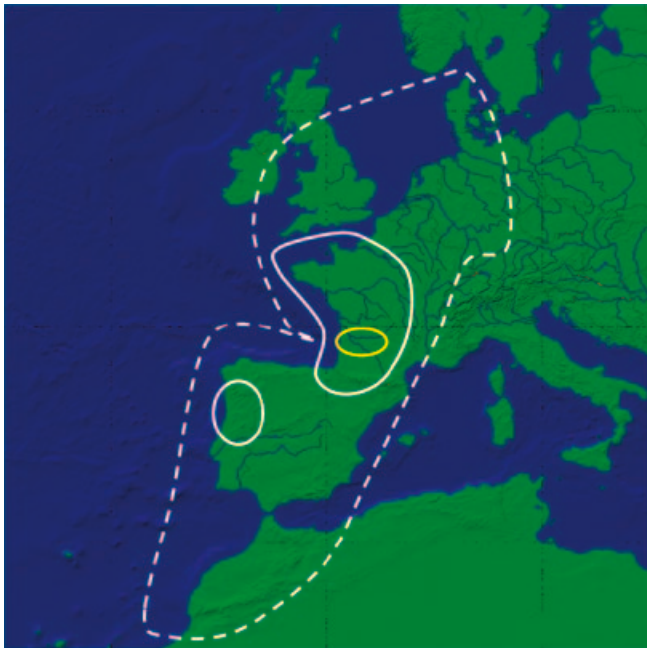
View of the gullet of an allis shad: the long, closely arranged gill rakers filter tiny plankton from the water.



The allis shad spawn in groups in open waters with gravel beds and moderate-flowing currents. The actual spawning process is accompanied by splashing and noise which can be heard from far off; in France, this is known as “bull”. The intensity of the noise even gives French allis shad experts a clue as to the number of spawning fish.

The decline of allis shad stocks in the Rhine and other European rivers

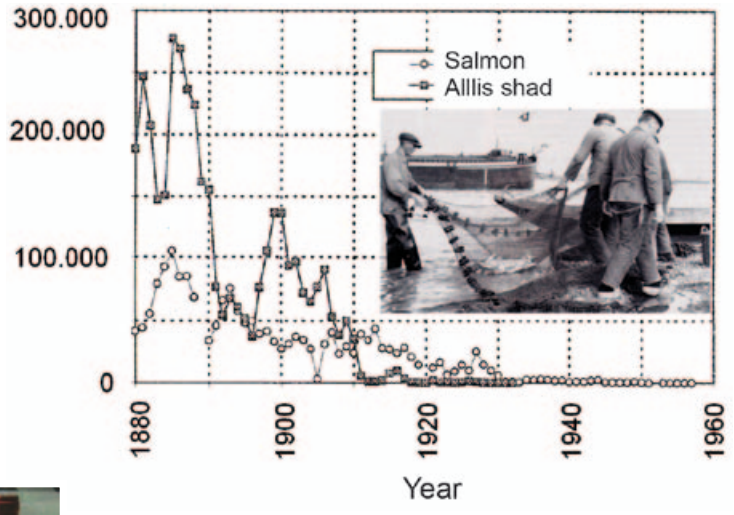
At the end of the 19th century, the eastern Atlantic and the North Sea were still densely populated with allis shad, which at spawning time migrated upstream along all larger West European and North Sea rivers in numbers barely imaginable today. The Rhine was home to probably one of the largest allis shad populations in Europe, and the several hundred thousand fish which migrated up the Rhine and its tributaries every year were immensely important for the business of local fishermen and hostelries. Relentless over-fishing, increasing pollution, the construction of weirs and other obstacles to migration and the destruction of spawning grounds during the course of progressive river development caused allis shad stocks to die out in most of the Atlantic tributaries by the middle of the 20th century. For the Rhine, this development is well documented by records of the number of fish caught in the German and Dutch stretches of the Rhine. While the number of allis shad caught at the end of the 19th century still amounted to more than 250,000 per season in the Dutch stretch of the Rhine alone, just 20 years later, this number had already declined to around 10,000 catches per season. The last major hauls of allis shad in the German part of the Lower Rhine were caught in the 1940s. Since then, the allis shad is considered to have died out in the Rhine. Similar developments occurred in the majority of rivers, in which once housed large populations of allis shad. Large spawning populations currently only exist in a few Atlantic tributaries in south-west France, particularly in the Garonne and the Dordogne, which both flow into the Gironde delta. The dramatic decline of stocks in Europe resulted in the allis shad being listed as a priority species in annexes II and V of the European Union’s Habitats Directive.



The allis shad's territory (dotted line) originally covered almost all of Europe's major Atlantic tributaries. The Rhine was one of Europe's most important allis shad rivers. Stocks practi-cally disappeared throughout Europe during the 20th century. Larger populations can still be found in some of the rivers in Portugal and the south-west of France (solid line). The largest population, which boasts more than one hundred thousand migrating adult fish each year, is found in the Garonne and Dordogne, both tributaries of the Gironde (yellow line).

Testimony to the former abundance of the allis shad and the great economic significance of allis shad fishing on the Rhine: advertise-ment from the Düsseldorfer Generalanzeiger, a newspaper printed in 1904, which refers to the sale of fresh allis shad in the local inns and hostelries, and Fr. Schnitzler's painting "Allis shad market in Düsseldorf"

Along with the deterioration in water quality, the construction of obstacles (weirs) and the destruction of spawning grounds as a result of hydrological engineering measures, the intensive fishing of allis shad was the main reason for the disappearance of stocks. The graph shows the official fishing figures for the salmon and allis shad, the two most important migratory fish for the fishing industry, in the Netherlands at the end of the 19th century (after Bartl & Troschel 1997) and is impressive evidence of how quickly the huge stocks of migratory fish in the Rhine died out.



Allis shad still migrate to spawn in the Garonne and other Atlantic tributaries in the south-west of France in such numbers that they can be fished and marketed.

Has the allis shad really died out in the Rhine?

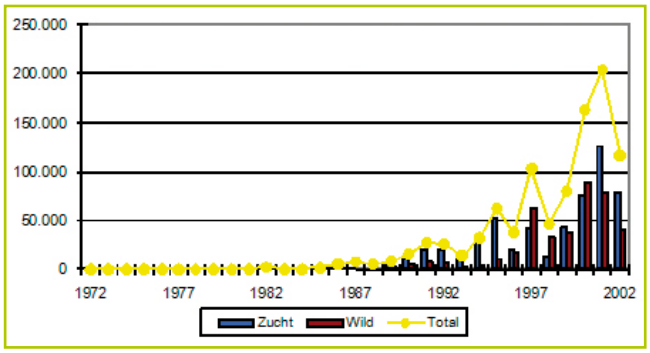
Although the Rhine's allis shad population has died out, individual sexually mature allis shad have been spotted negotiating the fish way which was constructed on the lowest Rhine weir near Iffezheim in Baden-Württemberg in 2000. However, despite intensive investigations of the fish fauna throughout the Rhine including the delta, no proof has yet been found of the presence of allis shad fry. Neither have any young allis shad been found in the North Sea over the last few decades. There is therefore no sign of successful reproduction in the Rhine or any other North Sea tributary. Moreover, the number of allis shad migrating up the Rhine has not increased over the years, and is therefore obviously not enough to guarantee the natural permanent recovery of allis shad stocks in the Rhine.

Genetic examinations of 140 allis shad from all over Europe have shown that the allis shad found in Iffezheim resemble those from the French Gironde population most closely. It must therefore be concluded that the allis shad which sporadically appear in the Rhine are so-called "strays" from the Gironde or at least very closely related to them. As the allis shad population in the Gironde is probably Europe's largest remaining allis shad colony, with several hundred thousand fish spawning every year, one obvious solution would be to use it as a donor colony for a repopulation program on the Rhine without the population itself suffering.

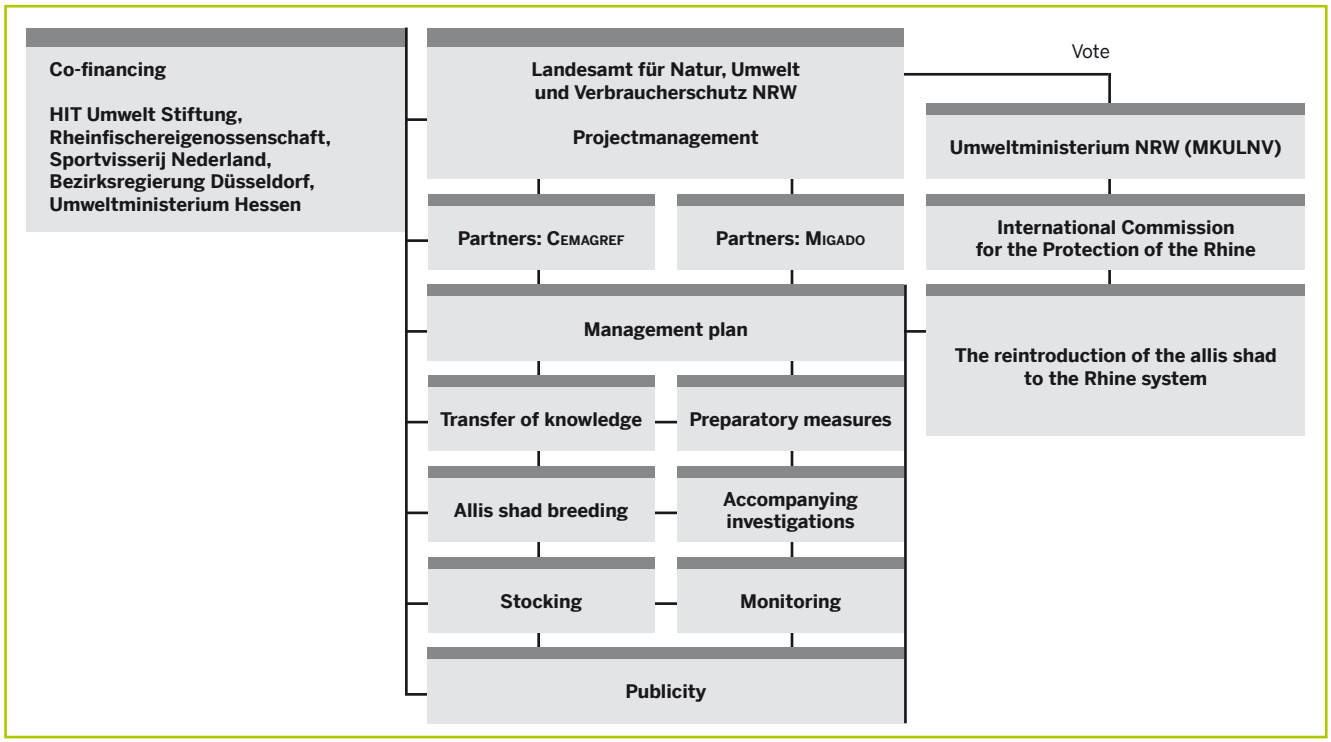
Considerations on the possible reintroduction of the allis shad

In view of the progressive restoration of linear connectivity, the marked improvement in the quality of the water in the Rhine and its tributaries, the growing success of endeavours to repopulate these waters with long-distance migratory fish such as salmon and North Sea houting and the positive independent development of river and sea lamprey stocks, the possibility of reintroducing the allis shad to the Rhine system also became the focus of interest. It was essential to start by clarifying how far conditions currently prevailing in the Rhine would allow the sustainable replenishment of stocks.

Even though it had meanwhile been found that the Gironde allis shad would be particularly suitable as a potential source for the reestablishment of allis shad stocks in the Rhine system, no experience had yet been gained as to how sufficient quantities of allis shad could be bred artificially. In this context, cooperation with the French institution Cemagref, which has considerable experience with the protection and management of stocks of migratory fish species in France, turned out to be a tremendous help in showing the way forward: Cemagref was the only European research institution, which had succeeded in breeding allis shad, even though the quantities were relatively small. The applied method was a further development of the breeding of the American shad *(Alosa sapidissima)*. Unlike Europe, where the lack of experience meant that the competent authorities were largely helpless to deal with the collapse of local allis shad populations, the USA had been enjoying increasing success with the support and replenishment of stocks of this closely related shad species since the mid-1970s. Stocks of this species in the former spawning waters had declined in a manner similar to that of the European allis shad. However, the competent authorities succeeded in developing methods of breeding shad artificially and producing larvae for stocking purposes. Numerous waters were successfully repopulated with shad using this method; large, self-sustaining stocks were also developed from residual colonies. As a technique to mark the tiny larvae had been developed at the same time, it was possible to determine the percentage of adult shad grown from these larvae returning to spawn years later.



Results of an American shad repopulation programme (Susquehanna River in Pennsylvania, eastern USA) which after adaptation served as a model for the reintroduction of the allis shad in the Rhine. After the stocks died out, the first artificially incubated larvae were released in 1976. However, the number of adult fish migrating to the spawning grounds only began to rise when fishways were constructed on existing weirs in 1991; the weirs had previously prevented the allis shad from migrating. The fish were marked so that artificially bred fish released as larvae could be distinguished from the wild fish which were the result of natural reproduction. The number of naturally bred fish returning to the spawning grounds rose in the course of the restocking programme, and are to replace larval stocks in the future.



Organigram showing project coordination and project activities

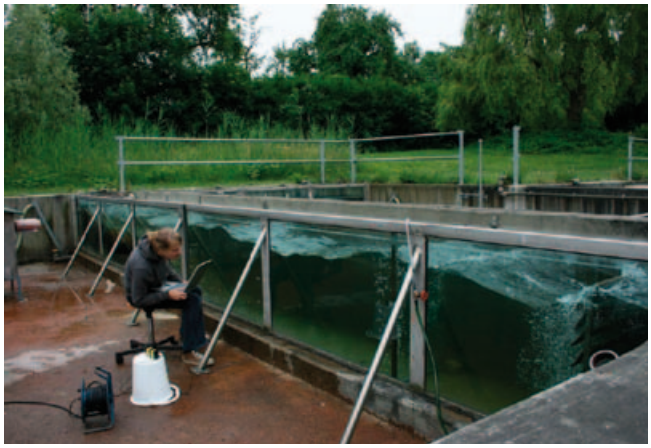
With this, the number of returning adult fish could be related to the number of the previously released larvae. Experience gained in America shows that approximately 200-500 larvae have to be released for one adult shad to return and spawn in the colonised waters 3 to 5 years later.

Even though the American shad differs from its European relative in some respects and the conditions in the rivers in the USA, which have generally been less subject to reshaping by humankind, are not comparable with those in the Rhine, the American model became the guideline for the strategy to reintroduce the allis shad to the Rhine system. CEMAGREF and University of Cologne collaborated to conduct extensive preliminary studies in Germany and France in order to clarify whether the general conditions for the reintroduction of the allis shad stipulated in the internationally binding IUCN directives for the reintroduction of threatened species could be guaranteed. At the same time, the plan to breed allis shad artificially and to re-establish them in the Rhine system attracted co-financers in Germany and the Netherlands, with whose support the foundation was laid for the development of a LIFE project application for the reintroduction of allis shad to be submitted to the European Union. The main aims of the project were to develop methods for mass breeding of allis shad, to build on and disseminate the knowledge already acquired and to release 5 million allis shad larvae into the Rhine system within 3 years. The repopulation model, the individual measures formulated in the project application and the international cooperation convinced the European Commission's experts, with the result that the project became one of 66 chosen from a total of 229 applications as being worthy of subsidisation, and was approved as a LIFE nature project in Autumn 2006. Thanks to the co-financing from the EU, the project budget was doubled to about 956.000 Euro over the four years of the project.

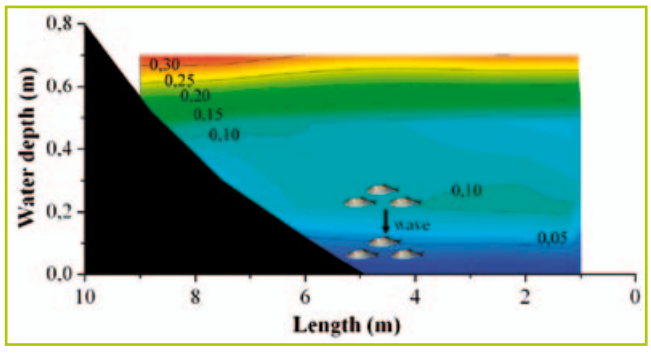
Does the Rhine's current condition make it a suitable habitat for the allis shad?

Along with the excessive fishing of allis shad stocks in Germany and the Netherlands, the rapidly progressing anthropogenic reshaping of the Rhine and its tributaries was the main reason why the allis shad and other long-distance migratory fish died out during the 20th century. Besides the immense stress caused by the pumping of untreated waste water into the Rhine, which reached its peak in the 1970s and regularly caused mass fish deaths as well as drastically reducing the variety of species, hydraulic engineering measures destroyed habitats which had formerly been important spawning grounds and fry biotopes for the allis shad. The quality of the Rhine has now improved so much that it no longer poses an obstacle for the allis shad or other fish species. However, the hydraulic engineering measures are still having repercussions today. The construction of weirs and dams in the Upper Rhine and all of the major Rhine tributaries, rendering them impassable to migratory fish, increasingly prevented the allis shad from travelling upstream to the formerly so valuable spawning grounds. Although the two lowest weirs on the Upper Rhine (Iffezheim and Gambsheim) have since been equipped with modern fish ladders, the impounded stretches of the Upper and High Rhine, the Main, Moselle, Lahn and Neckar are still impassable for fish needing to travel to these levels. Moreover, the reservoir areas contain hardly any fast-flowing stretches with gravel beds such as those preferred by the allis shad for spawning. Unlike the French rivers with intact allis shad stocks, the free-flowing section of the Rhine between Iffezheim and Rotterdam, which is Europe's most important inland waterway, has been submitted to considerable hydraulic restructuring and exposed to intensive freight and leisure shipping. The demands of shipping are still given priority over those of water ecology. The banks of the Rhine are accordingly largely shored up with riprap, gravel banks are regularly demolished to create waterways with homogenous depths and the deep washouts and scour holes in the riverbed are filled. Moreover, the shipping itself must be seen as a potential hazard to larvae and fry. The passage of a ship (the central Lower Rhine is navigated by approx. 500 ships a day) is associated with the creation of strong currents and an immediate, unnaturally powerful wash. Fry with still undeveloped swimming capacities in particular are practically incapable of resisting the current, let alone avoiding the wash. The wash therefore places them at high risk of being stranded on the banks and suffering injuries. In order to assess the potential influence of this artificial wash on allis shad larvae, experiments were set up to observe the behaviour of larvae of various ages when about to be subjected to

The Rhine is Europe's most important inland waterway and has been radically altered by hydraulic engineering; this has significantly reduced the habitats of many fish species. Along with the destruction of spawning grounds and fry habitats by hydraulic structures and bank development, the wash caused by shipping is seen as a major hazard for fish larvae.



Behavioural experiment recording the reaction of allis shad larvae to sudden wash in a mesocosm.

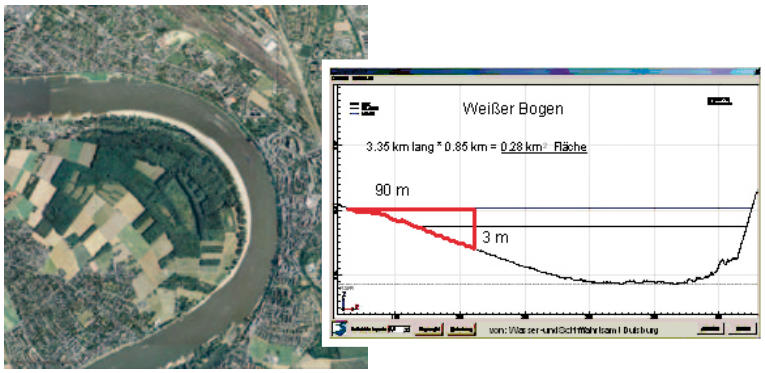


After just a few hours, the allis shad larvae began adapting their behaviour by moving to areas close to the ground with less current as the wash began (areas with the same current demarcated by shaded colours and lines). As they also avoided the areas close to the banks, which are particularly exposed to wash, the wash is obviously less of a hazard to young allis shad than anticipated.

Experts cruising the Rhine. In the opinion of French and American shad experts Eric Rochard, David Clavé, Matthieu Chanseau and Richard St. Pierre, the Rhine still contains sufficient well-structured sections which could be used as spawning grounds and habitats for growing fish, thus guaranteeing the survival of an independently breeding population.

Potential allis shad spawning ground on the Upper Rhine below the Iffezheim dam. The Rhine below Iffezheim is free of transverse constructions as far as the Netherlands and from here down the Rottederamer and the Nieuwe Waterweg delta arm. Diadromic migratory fish such as the allis shad can migrate from the North Sea to this point with no obstacles. The great, formerly brackish areas such as the Ijsselmeer and the Haringsvliet are separated from the sea by flood barriers; like the area above Iffezheim and the larger tributaries are regulated by dams and weirs with inadequate fishways. These areas are still inaccessible to migratory fish such as the allis shad.

wash and to ascertain the percentage of stranded fish. The results indicate that the influence of artificial wash on the mortality rate of allis shad larvae may be relatively low. Even when they were just a few days old, allis shad larvae which had been exposed to a breaking wash for the first time adapted their behaviour within just a few hours, causing them to move into the middle and lowest depths of the water and thus reducing the risk of them being stranded or injured in the bottom substrate. However, this type of adaptation increases as the allis shad grow older. It was also found that light and temperature conditions could exert a considerable influence on the larvae's orientation in the water column and thus on their risk of being stranded. Valuable conclusions relating to particularly suitable stock locations and the best time for stocking could be drawn from these insights. To supplement these empirical studies, French and American shad experts working in waters with intact allis shad populations were invited to assess the situation on the Rhine. After an excursion lasting several days along the entire stretch covered by the project, the team of experts came to the conclusion that the Rhine's meandering curves contained areas which in terms of current, depth and the granularity of the bottom substrate were almost identical with the spawning grounds recently used in the French and American shad rivers. Despite the large-scale development of the Rhine into a navigable waterway, mapping work carried out on these stretches showed that there is still a very large number of spawning habitats. The habitat area available is seen to be more than sufficient to guarantee the long-term development of an allis shad population created solely by natural breeding. However, the development of such stocks will take decades and can only be achieved by extensive restocking with allis shad bred artificially.

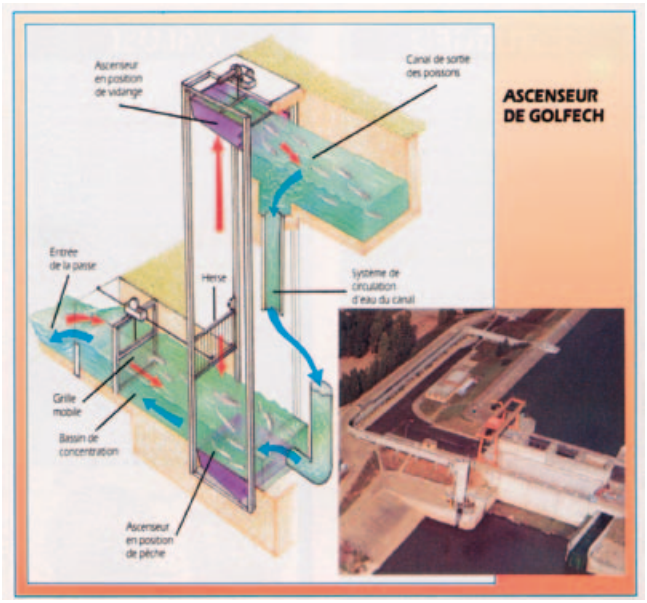


Historically, the point bars and the great gravel and pebble banks below the mouths of the larger tributaries were the allis shad's preferred spawning grounds in the Rhine. The flattening of the gravel banks to make the waters navigable for shipping and the construction of weirs in the tributaries, which not only prevented fish migration but also the transport of sediment from the tributaries, significantly reduced the potential allis shad spawning territories compared to earlier times. Today, such stretches are mainly found in undeveloped inner bends of meander curves (so-called point bars, the illustration shows the "Weisser Bogen" near Cologne) which offer the same depths, currents and substrates as intact allis shad rivers. This diagram shows a cross-section of the Rhine. The depths shown in the cross-section can be used to determine suitable allis shad spawning areas (red) and to ascertain the area of the corresponding habitat. The total area of such sections of the Rhine is still so large that allis shad stocks would still be able to maintain themselves by unassisted reproduction.

Catching allis shad ready for spawning: the principles of allis shad breeding

The basic requirement for breeding allis shad artificially is to catch a sufficient number of parent fish for spawning. However, allis shad are exceptionally sensitive and suffer high mortality rates when caught using conventional methods (nets or electric fishing). Moreover, allis shad spawn over a lengthy period of time, and the female's eggs therefore ripen asynchronously. Unlike salmon, for example, stripping the fish to extract the spawn would only utilise a very small proportion of the quantity of eggs which could potentially be used for artificial breeding.

In various preliminary projects, French project partner Cemagref has succeeded in developing processes and methods facilitating both the capture of parent fish, their safe transport to breeding facilities and above all the extraction of the gametes required for breeding. The parent fish are caught at existing fish lifts, which were actually constructed to help the fish overcome the lowest transverse obstacle on the Garonne (Golfech) and the Dordogne (Tuilières – in operation since 2009) but which also function as monitoring facilities and provide information about the chronological stages and intensity of the allis shad's migration. The removal of spawning fish from the fish lifts has the particular advantage that the fish can be caught in migration. In this way, a large number of parent fish, whose gametes are already virtually ripe, can be recruited in a relatively short time. Moreover, the fish can be transferred directly from the fish lift into suitable transport containers without having to remove them from the water, thus reducing mortality rates caused by capture and transport. The controlled removal of spawning fish at the transverse structures also ensures that the necessary proportions of each sex can be removed and that only a few vital representatives of the entire spawning population are caught. In order to guarantee smooth transportation of the sensitive allis shad from the fishing ground to the breeding facilities several hours' drive away, special spacious round channel containers were developed in which the fish could be kept in the dark and transported particularly carefully while ensuring oxygen inflow and keeping salt concentrations low.



The great dams in the lower reaches of the Garonne and the Dordogne are equipped with fish lifts which facilitate the migration of allis shad and other migratory fish guided by the current. Diagram of a fish lift (in the Golfech dam on the Garonne): an artificial current encourages the fish in the water below the dam to swim into the lift. The opening where the fish swim into the lift is regularly closed and the fish transported in the water-filled lift to the water above the weir, where the exit sluice opens and the fish can continue their migration above the dam. The fish lifts can also be used to select and catch parent fish for breeding allis shad. In this case, the fish are not released into the waters above the dam after swimming into the lift, but transferred into containers where they can be kept until being transported to the fish farm.

As soon as enough of each sex of the allis shad have been caught for spawning, the fish are transferred from the basins into special transport containers and transported by van to the fish farm in Bruch.

Spawning and rearing allis shad larvae

The first successful attempts to make captive allis shad spawn artificially and thus to obtain fertilised eggs took place in the Cemagref research facility in St. Seurin on the Isle, a tributary of the Dordogne. The principle is to inject a stimulating hormone into both sexes to initiate spawning. The gentlest method found was to transfer the fish to a transparent, water-filled plastic bag and to inject them through the bag.

After this treatment, the spawning fish are transferred to special round channel spawning pools. In the wild, allis shad only spawn on warm nights in parts of the river with a moderate current; the fish are therefore kept in darkened containers with water flowing through them. The eggs and sperm are released synchronously by both sexes at a temperature of 20°C about 24 to 72 h after the hormone is injected. The highest fertilisation rates are achieved by a ratio of 2:1 to 3:2 (males to females) between the sexes. The fertilised eggs then sink to the floor, where they are siphoned off through a bottom outlet and collected in net sacks before being transferred to special incubation jars. These are integrated into a water circuit to ensure that the eggs receive sufficient oxygen, thus guaranteeing an optimum embryonic development and high hatching rates. The first allis shad larvae hatch after just four days. Hatching can be synchronised by exposing the eggs to mechanical stimuli and light pulses. Moreover, the incubation jars are placed next to the breeding basin just before the eggs hatch. Once hatched, the allis shad larvae swim to the top of the incubation jars and reach the breeding basin by passing over the rim of the jar with the current.

The hatching rates largely depend on the maturity of the parent fish and the quality of the spawn.

As soon as the larvae have hatched and consumed the yolk, they must be supplied with enough food to prevent starvation, as this inevitably results in high mortality rates among fish larvae. Newly hatched salt-water crustaceans (nauplii of the *Artemia* species), which show similar properties to the food eaten by "wild" allis shad larvae (freshwater plankton) are readily accepted and have shown themselves to be particularly suitable for feeding the larvae. Special breeding processes and containers were therefore designed for the *Artemia* used in the studies carried out by Cemagref with the aim of optimising the mass production of allis shad larvae. Sufficient quantities of these are transferred to the breeding containers by means of an automatic dosing device. Due to the considerable time and effort expended in breeding Artemia, the allis shad larvae are introduced to a special but cheaper dry food as they grow older; this is also fed into the breeding basin several times an hour by an automatic dosing device.

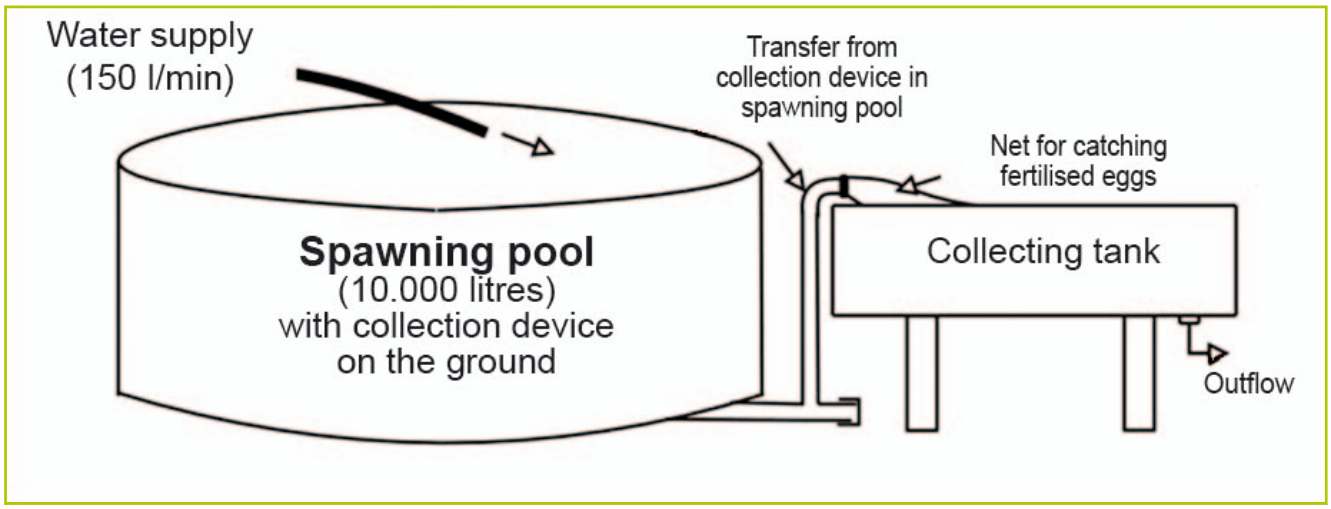


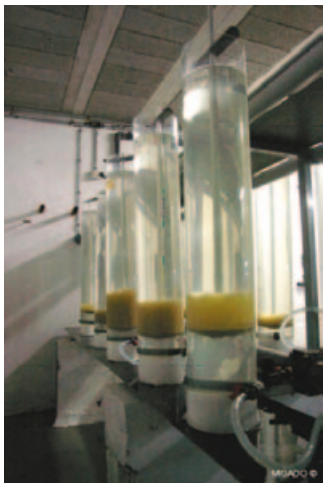
Diagram of the spawning facility

The allis shad are injected with a hormone to stimulate spawning in captivity. This injection is carried out carefully in a water-filled bag.

A close-meshed net is attached at the transition to collect the fertilised eggs.

An automatic dispensing device washes Nauplii stages of tiny salt-water crustaceans (*Artemia salina*) from the brown containers into the rearing pool at regular intervals; the allis shad larvae feed on these. As Artemia production is expensive and complicated, the allis shad larvae also receive a special dry food after the first few days. This is administered by the automatic food dispenser in the foreground.

Once the allis shad have been injected with the hormone, certain proportions of each sex are transferred to the darkened spawning pool. The fish spawn 1 to 3 days after the hormonal induction. The fertilised eggs collect at the bottom of the pool, where they are caught by a collecting device and transported with the current through a pipe into a collecting basin.

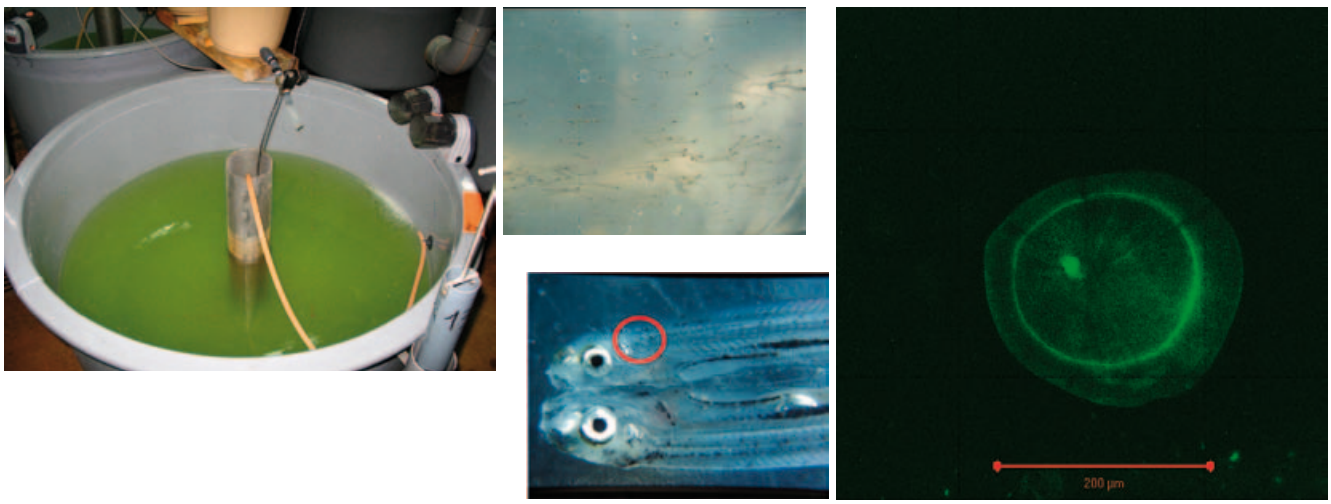


The eggs are then placed in special incubation jars with water circulating through them at a temperature of approx. 20°C. This ensures that the eggs receive enough oxygen and cuts the percentage of losses. The eggs are transferring to special hatching jars just before they hatch. The newly hatched larvae are washed by the current into the rearing pool via an overflow.

Marking young allis shad: essential for testing the success of stocking measures

In order to test the efficiency of supportive stocking measures or repopulation measures undertaken after stocks have died out, it is necessary to mark the fish so that they can later be identified as originating from this stock. This facilitates subsequent estimates of population size and the proportion of artificially to naturally bred fish, with which the stocking strategy can be evaluated and optimised. However, due to their high sensitivity, it is not economically viable to raise young allis shad to a size which allows them to be visibly marked on the outside.

Parallel to the reintroduction of the American shad, methods were therefore tested for the LIFE project with which the allis shad can be mass-marked while they are just a few millimetres long. One particularly efficient method proved to be the immersion of allis shad larvae in a fluorescent dye solution. During this bath, the substance is deposited in bony structures such as the ear bones (otoliths) and can later be recognised under the fluorescence microscope as ring-shaped inclusions. Like the annual rings in trees, temporal growth is shown in various layers deposited in the otoliths, which means that the day on which the marking was made can be deduced even years later, provided an appropriate microscopic resolution is available. However, as the marking process may be associated with a certain larval mortality, which increases with the concentration of the dye solution and the time for which they are exposed to it, experiments were carried out to ascertain the most suitable concentration and the maximum exposure time necessary to achieve optimum marking quality with minimum mortality. Oxytetracycline (OTC) is used as a dye. Five-day-old allis shad larvae were immersed in three different concentrations (200, 250, 300 ppm) of an OTC and a control solution for 4 or 6 hours and the quality of the otolith markings statistically compared once the allis shad had reached an age of 15 and 32 days respectively. In general, the best markings were obtained with higher concentrations of OTC and longer periods of exposure, which however led in individual cases to slightly higher mortality rates (< 1% on average) or lower larval growth over the duration of the experiment. The best results (highest marking quality, lowest mortality) were obtained by exposing the fish to a 300 ppm OTC solution for 4 hours. Examinations of juvenile allis shad kept in an outdoor pond for the 4 months following the marking showed that the markings can also be easily identified in allis shad which mature in the wild.



At the age of 2 to 20 days, the allis shad larvae are marked by immersing them in an oxytetracycline (OTC) solution for several hours. The OTC is absorbed by bony structures such as the ear bones (otoliths) and is later recognisable as a fluorescent ring when examined under a fluorescence microscope. For this, the otoliths (location marked in red) must be prepared by embedding in synthetic resin, grinding and polishing. Good quality markings can still be seen years later and can be used to determine whether the allis shad were artificially bred. The longer the larvae are immersed in the dye bath and the higher the concentration of the tetracycline solution, the better the markings.

Towards the mass breeding of allis shad

Detailed knowledge about the capture, transport, artificial breeding, incubation and marking of allis shad lays the foundations for the mass breeding of allis shad. The knowledge acquired by Cemagref was passed on to personnel employed by project partner MIGADO and its fish breeder clientele in workshops and training events. The intended mass production of allis shad can only take place in spacious, specially equipped professional facilities. For this purpose, an extension was added to a fish-breeding facility run by the Fédération départementale de la Pêche 47 (FD47) in Bruch, close to the Garonne. An established fish-breeding company, La Ferme du Ciron, was contracted to carry out the seasonal production work; the company's employees cooperate with experts from Migado to ensure that the production processes run smoothly. The necessary infrastructures and facilities were financed from project funds, as were special transport containers for the parent fish. Round channel containers of huge dimensions are necessary to keep the fish where they are caught and to transport them to the fish farm once the numbers required have been captured. Project partner Migado is responsible for catching the parent fish and transporting them to the fish farm; they also and most importantly organise the work phases on site.

The Federation du Pêche's existing fish farm was expanded for use as Europe's first allis shad breeding facility (left: before expansion, right: after expansion).

Experience gained during the project immediately flowed into the optimisation of production phases, as a result of which the efficiency of the allis shad production process levels was successively improved throughout the project. Spacious, optimally equipped compartments were for example designed for the fish farm chosen for the mass production of allis shad. Along with departments for parent fish and spawn production, egg incubation and hatching larvae, a separate food production department, laboratory and disinfection facilities were set up to ensure that the stringent hygiene requirements associated with mass production could be complied with. Along with filter systems to treat the water from the nearby Canal du Midi, water circuits and temperature regulation systems, light sources were integrated into individual compartments in the fish farm to allow the lighting to be adjusted in line with daily variations. In this respect, both the capacities and professionalism of each institution exceed those of project partner Cemagref's

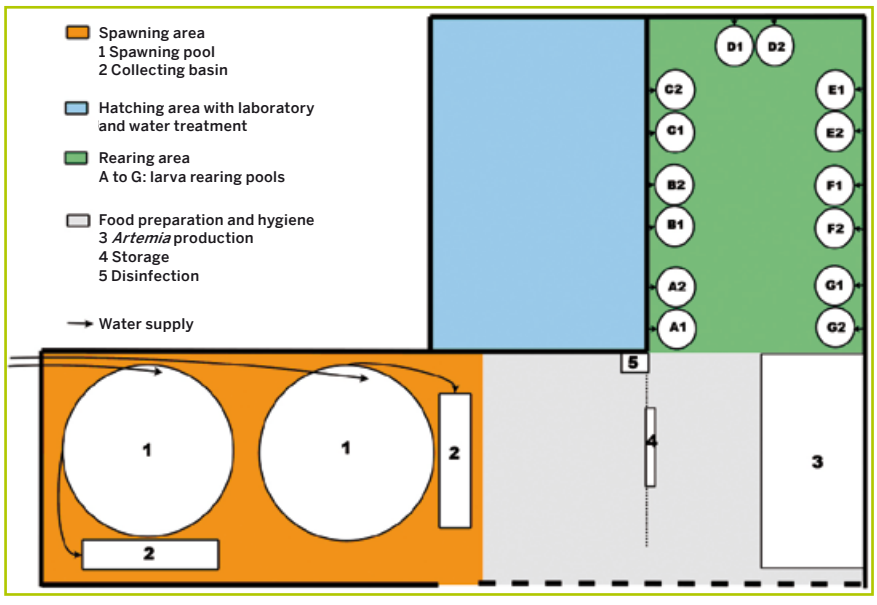


Diagram of the fish breeding facilities in Bruch.

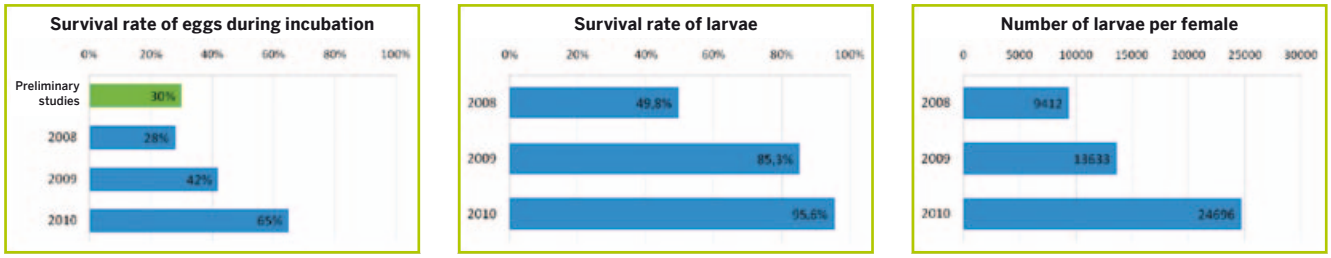
experimental facility by far and offer ideal conditions for the mass production of allis shad.

After the success of the preliminary experiments, the fish farm's first brood of a large number of allis shad larvae for introduction to the Rhine system was planned for 2008. However, flooding in winter 2005 meant that the fish lift in Tuilières on the Dordogne could not be used to catch parent fish as planned. Moreover, the number of migrating fish had been declining sharply throughout the Gironde system since 2005. The insufficient number of parent fish meant that only 500,000 marked allis shad larvae could be transported to Germany during the first production year rather than the 1,000,000 planned.

However, in subsequent years, the more professional facilities and the expertise gained brought about steady improvements at all production levels, as a result of which net larva production rose considerably. The survival rate of incubated eggs, for example, rose from 25% in 2008, the year in which larvae were first produced to restock the Rhine system, to 65% in 2010. Over the same period, the larva survival rate rose from 49% to a good 95%. With reference to the number of larvae produced per allis shad female, this means that numbers were multiplied by a factor of 2.6 between 2008 and 2010 alone. These figures are several times higher than the production rates achieved in the USA, despite the huge investments made in the American shad repopulation programme. Moreover, as the fish lift in Tuilières again became available for catching spawning fish in 2009 and the number of fish returning in 2010 showed a slight improvement for the first time since 2005, it was possible to deliver 1.7 million marked allis shad larvae to Germany in 2009 and no less than 2.6 million in 2010. Despite the unexpectedly low numbers of spawning fish caught, it was therefore possible to produce 4.8 million allis shad larvae and send them to Germany. The success of the allis shad breeding programme means that in future it will be possible to obtain a higher contingent of larvae while capturing the same number of spawning fish. This is extremely significant, especially in the event of the number of fish returning to the Gironde system continuing to decline.

View of the allis shad breeding facility's larva rearing area.

The expansion of the allis shad breeding facility was subsidised by LIFE project funds. The expertise required for allis shad breeding is provided by project partner CEMAGREF's research work and flowed into the design of the breeding facility and the team training sessions on site. The work on site is carried out by employees of a professional fish breeder and of project partner MIGADO, which is also responsible for capturing the parent fish and for works organisation and coordination. Thanks to MIGADO's implementation of the knowledge acquired by practical experience, improvements were made at all stages of production and larva production increased significantly.



Production processes improved significantly when the allis shad breeding facility in Bruch went into operation in 2008. Among other factors, the survival rate of the eggs during the incubation phase improved considerably compared to the preliminary studies, rising even further during the course of the project. The survival rate of the larvae after hatching also increased, thus multiplying the net larval production figures.

Transportation and introduction of the larvae to the Rhine system

The larvae are transported to Germany in large plastic sacks; each sack is filled with one-third water and two-thirds pure oxygen. In this way, up to 12,000 larvae can be kept in each sack for many hours and survive the long journey to North Rhine-Westphalia and Hesse in Germany without damage even in high summer temperatures, in which conditions they are stored on ice packs. The sacks are transported in vans, which can cover the distance cheaply and within a relatively short time. Because of the lower temperatures and traffic volume, it has turned out to be beneficial if the larvae are transported during the night, usually arriving in Germany early in the morning. The fish are released at previously selected locations which firstly have to be within easy reach and secondly have to be particularly suitable in terms of current and depth as well as being away from currents and wash caused by shipping. This is why the larvae have not yet been released directly into the Rhine current. Instead, the fish were released into the Sieg, a relatively natural Rhine tributary in NRW, the Rhine channel at Erfelden, a moderate-flowing and also very natural branch of the Rhine in Hesse, and former gravel-pits connected to the Rhine, all sites which allow the allis shad larvae to become accustomed to their new habitat before they have to tackle the strong currents in the Rhine itself. Factors taken into account when making the preliminary selection included the existence of structures as natural as possible in the habitats in question, low current, changing depths and the lack of potential predators. From the end of May onwards, the fry of indigenous fish species is often so developed that they may prey on the tiny allis shad larvae. As the allis shad larvae also have to receive nourishment fairly quickly after their long journey so that they can find their way and survive in their new habitat, two different release strategies have been followed over the years. If possible, the larvae are only released at dusk or in complete darkness, as the risk of being eaten is much lower at night. Shady round channel containers have turned out to be very suitable for keeping and feeding the larvae during the day; in these, the allis shad larvae can adapt to the water into which they are to be released and be fed with Artemia nauplii. In this way, the larvae can adjust well to local conditions before they are actually released. If there is no interim storage possibility, the larvae are released into open waters during the day after adjusting to the prevailing temperature. This strategy is used at the gravel-pits connected to the Rhine, which contain a wealth of plankton on which the allis shad can feed immediately after their release. Observations made by divers have shown that the larvae also actively avoid the areas close to the banks and the levels close to the surface, presumably to minimise the risk of being preyed upon, as the fry of most other species prefer to lie in wait for their prey close to the banks.

The allis shad larvae are placed in plastic sacks and transported from France to Germany in vans. On arrival at the stocking site, the transport sacks are placed in the water so that the larvae can adjust to the temperature. In order to minimise losses from predators and relieve exhaustion from the long journey, the larvae are kept in round channel containers until darkness and fed with *Artemia* nauplii. The pink coloration of the intestinal content shows that the larvae are eating. Once fortified, the larvae are released as darkness falls. If it is not possible to store and feed the larvae, they are released in areas with few potential predators but large quantities of plankton for food (e.g. in open waters or the delta channels of still waters which are linked to rivers).

Parallel investigations of the behaviour of the allis shad and the efficiency of the stocking measures

The release of the allis shad was monitored and accompanied by investigations aiming to draw conclusions concerning the best sites for release and the behaviour of the larvae. In general, hardly anything is known about the behaviour and habitat use of allis shad fry in the river, as corresponding investigations have not yet been carried out on the French populations. All that is known to date is when and where the adult allis shad prefer to spawn and the fact that young fry can be found in the Gironde delta in late summer and autumn, from where they move on to the sea by spring of the following year. The LIFE allis shad project broke new ground with the release of allis shad larvae. Because the allis shad larvae are still so tiny at the time they are released, the presence of fry can only be proved using special methods such as close-meshed driftnets and seine nets. Diving and drift observations were also made. However, it is almost impossible to track the almost transparent larvae for longer periods without losing sight of them, at least when the water is turbid.

The accompanying investigations carried out to date indicate that the allis shad larvae allow themselves to drift with the current soon after being released and settle below the places where they were released, especially at night. Drift investigations accordingly found numerous allis shad larvae close to the place where they were released, whereas no allis shad larvae were found in the driftnets placed much further downstream. An experiment during which observers in a boat watched the allis shad shoals drifting downstream after their release revealed an identical picture: the size of the shoals, which initially drifted close to the surface, decreased steadily as they moved further from the place where they were released. After several kilometres of drift, there were no more allis shad larvae to be seen. However, no allis shad larvae were found in the immediate vicinity of the place where they were released over the days which followed. The results of the monitoring work were unable to reveal where the larvae spent the following weeks and grew into fry before migrating down to the delta. As they spend weeks in the waters where they were released or at least in the Rhine, young allis shad were found in the lower Rhine months later.

Accompanying investigations using various methods indicate that the allis shad larvae drift downstream under cover of darkness and settle in suitable habitats. No allis shad larvae were found in the immediate vicinity of the place where they were released over the following days. Observations made by divers and snorkellers in current-free areas showed that after being released from the bank, the larvae swim towards the open water and avoid the areas close to the surface. This behaviour presumably helps them avoid potential predators.

Resounding success of the allis shad project: evidence of the first migrating fry

At the beginning of September 2010, a part-time fisherman caught the first juvenile allis shad from the LIFE project's stocks near Kalkar on the Lower Rhine by means of an anchored stow-net. This was the first verified proof of the presence of juvenile stages of the allis shad in the Rhine since the 1920s. The otolith markings meant that the fish, which were obviously on their way to the Rhine delta, could clearly be identified as originating from the 2010 stocks. The discovery of the fish, which at 12 to 14 cm in length were very well grown, is impressive evidence that the stocking strategy is proving effective and the allis shad are growing from tiny larvae to imposing young fish. Between September 3 and October 22, the fisherman, who is carrying out investigations in cooperation with the NRW migratory fish programme and the University of Cologne, caught 30 young allis shad in his net. As the net only "filters" a marginal proportion of the Rhine water, it must be assumed that only a fraction of the migrating allis shad was caught. Along with the achievements in the area of allis shad breeding, it is therefore becoming clear that the second primary objective of the allis shad project, the reintroduction of the species to the Rhine system, is also proving to be successful, particularly as the young allis shad have already surmounted the critical larval phase, which normally has the highest mortality rates in the fish's life cycle. This provides grounds for optimism that the first spawning fish from this stock may migrate up the Rhine and breed independently over the next few years.

The first verified evidence of young allis shad in the Rhine for decades: between September 3 and October 22, 2010, the part-time fisherman Rudi Hell caught 30 juvenile allis shad from the artificially bred stock from his eel boat "Anita II"in the Lower Rhine near Kelkar, not far from the Dutch border, as they were migrating to the sea.

Exchange of experience, knowledge transfer and public awareness

One core element of the LIFE allis shad project is not only to make the scientific knowledge as well as the practical achievements, experiences and their applicability available to experts in the field, but also to inform the general public about its objectives, content and the progress made and to make them widely accessible.

The project's approach and its eminently international orientation – involving three EU member countries – were decisive for the allis shad project being acclaimed as the best maritime LIFE project at the European Regional Champions Awards in Brussels in 2008.

Project activities included the organisation of an international scientific congress on protecting the fish population in September 2009, which attracted more than 100 participants from 22 countries; the results of the congress were published in a special volume of a scientific journal. The allis shad project is part of the international DIADFISH network (**www.diadfish.org**), a platform for information exchange and the protection and management of the remaining populations of diadromic migratory fish species in Europe. The project manager was invited by the *IUCN Freshwater Fish Specialist Group* to attend a conference of experts in Adelaide (Australia), where he spoke about experiences with and the perspectives for reintroducing extinct fish species. Once the allis shad project has been concluded, the main results, i.e. the experience gained with incubation, breeding and fish marking, will be published in a manual and passed on to biologists and fish breeders in workshops. The insights gained during the project have also been published in several scientific journals.

Düsseldorf's Aquazoo-Löbbecke Museum, which is also the office of the allis shad project, put on a special exhibition which provided more than 120,000 visitors with information about the allis shad. The Aquazoo and the La Rochelle Aquarium both succeeded in rearing impressive young fish from allis shad larvae which will in future be made accessible to visitors. It has never before been possible to display allis shad living in captivity.

Various media projects have been realised with the aim of presenting the project to a wide public in a convenient, easily comprehensible manner. Along with information

Participants in the international conference on the "restoration of fish populations" organised by the LIFE allis shad project in Düsseldorf in September 2009.

The environmental ministers of the states involved in the project, NRW and Hesse, attracted considerable media interest as they released allis shad larvae into the Rhine near Wiesbaden.

boards set up at prominent sites, free flyers and a DVD about the allis shad project, news about the allis shad project can be retrieved from a project website (all available in English, French, Dutch and German). The great response to the allis shad and the repopulation project can be seen from the intensive reporting in the mass media. Along with hundreds of articles in local and national daily newspapers and magazines, the topic was addressed in numerous radio and TV slots, all of which raised awareness of the allis shad as a species which is actually extinct among the general public throughout the area covered by the project. The internet cites more than 7000 sources referring to the allis shad project in Germany alone. A symbolic release of allis shad larvae performed every year by the environmental ministers of the German states involved in the project, North Rhine-Westphalia and Hesse, by school classes in the region and involving representatives from France and the Netherlands attracts great media interest. The official release of allis shad stocks into the Rhine in 2008 and 2009 took place in the state capitals Düsseldorf and Wiesbaden respectively. In 2010, the former fishing village of Poll, now part of Cologne, was chosen; here the first allis shad catch of the year is still commemorated in a folk festival, the Maispill, which is organised and hosted by a local association, the so-called Poller Maigeloog. The Rhine water meadows around Poll, which were formerly a well-known spawning and fishing ground for the allis shad and which are still popular with the people of Cologne as a place to relax on lovely summer days, feature an information board reminding visitors that this was once a major breeding site for this practically forgotten fish species. There is a good chance that in a few years, the splashing of spawning allis shad will again be heard in the Rhine on warm May nights.

Award for the allis shad project team: presentation of the European Regional Champions Award in Brussels.

Allis shad exhibition in the Aquazoo-Löbbecke Museum, Düsseldorf

Juvenile allis shad in the aquarium

Presentation of the allis shad project to the public: in Cologne-Poll, the Poller Maigeloog puts on a traditional folk festival every year, the so-called Maispill, which is a reminder of the first allis shad catch in the good old days (here with allis shad supplied from France). The procession goes down the "Maifischgasse" (Shad Street), whose name bears witness to the former significance of allis shad fishing in Poll. The project incorporated a cooperative agreement with the Poller Maigeloog, through which public awareness of the allis shad was raised considerably not only because of the great regional significance of the festival, but also nationally through the participation of prominent politicians and the marked media interest. Events in this context included the release of allis shad by the environmental ministers of the partner states and by school classes in the region, which had sponsored allis shad for this purpose. The sponsorship certificates were formally handed over in the presence of the environmental ministers of NRW and Hesse.

Board providing information about the allis shad project on the banks of the Rhine in Cologne

What happens after LIFE?

The LIFE allis shad project included the compilation of a so-called After-LIFE Conservation Plan which sets forth how the project's aims, achievements and measures can be implemented, developed and optimised in the future. Even though the LIFE allis shad project will officially end in 2010, the project's main aims of continuing to develop allis shad breeding and releasing approximately 2 million allis shad larvae into the Rhine every year to guarantee the growth of a population which will in future be able to sustain itself will be continued. In the next few years, this will be carried out as part of a LIFE+ follow-up project. This entails the implementation of further measures necessary to repopulate the Rhine with allis shad in the long term and to protect the donor population in the Gironde-Garonne-Dordogne system, which has been declining rapidly since 2006. This includes not only the identification of the reasons for the decline and the improvement of conditions for migration, reproduction and reaching maturity, but also the identification of migratory movements and routes with the help of radiotelemetry, the optimisation of fish migration aids and the identification and protection of the habitats in which the fry grow up to juveniles before they migrate to the estuary. This will ensure that the largest European population of allis shad currently in existence will be preserved for future generations. Moreover, technical opportunities for rearing allis shad in captivity until they reach sexual maturity are to be explored with the aim of building up stocks of parent fish and making allis shad breeding independent of stocks living in the wild. Combined with developments and further improvements in the field of allis shad breeding and, this method can minimise the use of the wild population even further in the long term.

In order to pursue the LIFE allis shad project's long-term objectives of repopulating and promoting independent breeding in the Rhine system, the measures on the Rhine aim to document the number of fish returning to spawn and the spawning activities, which in view of the restocking measures carried out from 2008 on can theoretically be expected from 2013 onwards. For this purpose, the existing fish migration systems are to be monitored and attempts at capturing allis shad in the free-flowing stretch of the Rhine are to be made. If the conditions are favourable, previously selected potential spawning grounds are to be observed at night to facilitate the documentation of spawning activities. In order to evaluate the migratory conditions in the delta, which has been subjected to radical anthropogenic change, adult allis shad which are ready to migrate are to be transported living from the Gironde-Garonne-Dordogne system to the Netherlands, where they will have transmitters implanted and be released into the Rhine delta. The fixed receiving stations in the branches of the Delta, the German section of the Rhine and in some of the tributaries are to be used to identify the migratory routes of the adult allis shad in the delta and determine the influence of the transverse constructions in the delta on allis shad migration. The variety of measures in France, the Netherlands and Germany aim to improve conditions for the allis shad in Europe, safeguard French stocks and promote repopulation in the Rhine.

The LIFE project
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