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Restoration of *Acipenser sturio* L., 1758 in Germany: Growth characteristics of juvenile fish reared under experimental indoor conditions

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ABSTRACT

The survival of the highly endangered *Acipenser sturio* L., 1758 depends nearly exclusively upon the establishment of captive broodstocks. Such measures were initiated in Germany in 1996, due to a transfer of 40 artificially reproduced individuals from France under a co-operation agreement. We report the results of rearing these fish for a period of 3 years under freshwater conditions. During the first year the fish being exclusively fed frozen chironomid larvae (N = 40) grew from a median of 27 cm (with range of 23-31 cm), averaging 71 g (42-112 g), to 43 cm (38-51 cm) and 280 g (168-505 g) at a mean temperature of 20 °C (13-27 °C). Following their transfer to larger tanks (approx. 5 m^3) at a mean temperature of 21 °C (17-24 °C), the fish (N = 27) reached a length of 68 cm (56-76 cm), averaging 1 281 g (512-2 097 g) at the end of the second year. Decreasing growth and increasing food conversion rates (FCR) during the first four months of the third year made us change the food composition (addition of large chironomids, krill and small marine fish). An increase in growth and a decrease in FCR was observed over the next four months resulting in a median length of 76 cm (58-89 cm), with a weight of 1 827 g (855-3 462 g) at the end of the third year (N = 27). Our fish showed rather large differences in growth, with some individuals reaching the maximum weight gain observed in wild fish, thus indicating the potential to optimise results by future testing involving additional natural food items in order to develop a formulated diet to stimulate early sexual maturation.

Key words: Growth curve, recovery, broodstock, behaviour, sexual maturation, semiclosed system.

RESUMEN

Recuperación de* Acipenser sturio *L., 1758 en Alemania: características del crecimiento de los peces juveniles mantenidos en condiciones experimentales de interior

La supervivencia del altamente amenazado Acipenser sturio *L., 1758 depende casi exclusivamente del establecimiento de stocks de cría en cautividad. Tales medidas fueron iniciadas en Alemania en 1996, gracias a la transferencia desde Francia bajo un convenio de cooperación de 40 individuos reproducidos artificialmente. Presentamos los resultados de la cría de estos peces por un periodo de tres años en condiciones dulceacuícolas. Durante el primer año los peces (N = 40) siendo alimentados exclusivamente con larvas congeladas de quironómidos crecieron desde una media de 27 cm (con rango de 23-31 cm), promediando 71 g (42-112 g), a 43 cm (38-51) y 280 g (168-505 g) a una temperatura media de 20 ºC (13-27 °C). A continuación de su traslado a tanques más grandes (de aproximadamente 5 m^3) a una temperatura media de 21 ºC (17-24 ºC), los peces (N = 27) alcanzaron una longitud de 68 cm (56-76 cm), promediando 1 281 g (521-2 097 g) al final del segundo año. La disminución del crecimiento y el incremento de las tasas de con-*

versión de alimento (TCA) durante los primeros cuatro meses del tercer año nos hicieron cambiar la composición del alimento (adición de quironómidos de gran tamaño, krill y pequeños peces marinos). Un incremento en el crecimiento y una disminución de las TCA fueron observados durante los cuatro meses siguientes resultando una longitud media de 76 cm (58-89 cm), con un peso de 1 827 g (855-3 462 g) al final del tercer año (N = 27). Nuestros peces mostraron numerosas diferencias en crecimiento, con algunos individuos alcanzando la mayor ganancia en peso observada en peces silvestres, indicando así el potencial para optimizar resultados por futuras pruebas que incluyan alimentos naturales adicionales en orden a evaluar una dieta formulada para estimular la maduración sexual temprana.

Palabras clave: *Curva de crecimiento, recuperación, stock de cría, comportamiento, maduración sexual, sistema semicerrado.*

INTRODUCTION

Over the last decade, the protection of biodiversity has become a major subject of public awareness, and is now an aim of European policies (e.g. Agenda 21 - Convention of Biological Diversity, Rio de Janeiro, 1992). This applies in particular to most of the 27 species of sturgeons, all of which are endangered (Birstein, Bemis and Waldman, 1997).

Historically, three different sturgeon species were present in German waters (Lelek, 1987). *Huso huso* (L., 1758) (beluga) and *Acipenser ruthenus* L., 1758 (sterlet) occurred in the Danube River and its tributaries only, whereas *Acipenser sturio* L., 1758 (Atlantic sturgeon) was present in all major German rivers entering the North or Baltic Seas. In addition, this species was found along nearly all European coasts and previously was common in most of the large river systems of Western Europe, from the White Sea to the Black Sea. In the 1994 Red Book of Germany (Bless, Lelek and Waterstraat, 1994), *A. sturio* is considered threatened in the North Sea and extirpated in the Baltic Sea. Throughout Europe the species is considered threatened or extinct (Debus, 1993; Birstein, Bemis and Waldman, 1997).

A. sturio, meanwhile, occurs with certainty only in the Gironde-Garonne-Dordogne basin in France (Rochard, Castelnaud and Lepage, 1990; Lepage and Rochard, 1995; Williot *et al.*, 1997) and perhaps in the Rioni basin in Georgia (Ninua and Tsepkin, 1984). Its occurrence in the Adriatic Sea, especially in the Buna River in Albania, has yet to be proved (Gulyas, pers. comm.). The Gironde population in France has decreased dramatically since the 1970s. *A. sturio*, therefore, is a species of special concern to the European Union, being listed in Annex 2 of Directive 92/43/EEC. The recovery of the known remaining populations by natural reproduction does not seem probable. Therefore, a restoration programme should include artificial reproduction and supportive measures in order to stabilise the stocks. Additionally, habitat improvement and protection should accompany the management of the endangered stocks.

This strategy has been adopted in France by the National Agricultural and Environmental Engineering Research Centre (Cemagref) in the 1980s (Williot *et al.*, 1997). In Georgia, a similar attempt seems to be in preparation (Zarkua, pers. comm.). The first successful reproduction, including rearing of the larvae, was carried out in France in 1995 (Williot *et al.*, 2000). As part of a co-operation programme aimed at restoring *A. sturio* in German waters, 40 juveniles were obtained from the Cemagref in 1996 for behavioural studies (Staaks, Kirschbaum and Williot, 1999), and as a basis of a broodstock to be reared in captivity.

Little is known about the specific conditions for supporting optimal growth to provide a healthy stock in long-term rearing, and to achieve maturation of gonads in this species. On the contrary, the first results in France indicated (Williot *et al.*, 1997) that *A. sturio* is a species rather difficult to grow under controlled conditions, compared with most other sturgeon species, which are quite easy to grow, and can be weaned to dry food successfully (Williot *et al.*, 1988; Giovannini *et al.*, 1991; Charlton and Bergot, 1991; Ronyai *et al.*, 1991; Hung *et al.*, 1993; Jähnichen, Kohlmann and Rennert, 1999). The present paper reports on our first results of rearing *A. sturio* over a period of 3 years under indoor conditions.

MATERIALS AND METHODS

Specimens

The 40 *A. sturio* obtained from Cemagref as part of a cooperation agreement were 11 months old (Williot *et al.*, 2000) on their arrival at the Institute of Freshwater Ecology and Inland Fisheries in Berlin in May 1996. The fish were the smallest of those raised in the Cemagref facility at that time. The average length upon arrival was approx. 20 cm with a mean weight of 41 g. The 40 fish were separated for most of the time into 3 different groups of similar size.

Tanks

The fish were first stocked in three aquaria (1.2 m^2, 0.8 m^3; 0.9 m^2, 0.5 m^3; 0.8 m^2, 0.3 m^3), then transferred to small (1.4 m^2, 1.0 m^3) and later to two large rectangular tanks (6.8 m^2, 6.8 m^3) and one circular tank (3.3 m^2, 4.7 m^3), using recirculated tap water. The tanks were equipped with filters of spongy open-cell foam (10 cm thick), and filter material was cleaned every 3-5 months. Water circulation was mediated by Eheim pumps; their number was adapted to the size of the tanks to obtain effective filter systems (i.e. water content of the tank was recirculated approximately once per hour).

Physico-chemical parameters

Water quality was monitored once daily, including NH_4, NO_2, NO_3, using Aquamerck Test kits. The pH and O_2 levels were measured twice daily using an Oxyguard probe. Alkalinity of the water was tested once a week (Aquamerck). Partial water exchange was performed with chlorine-free tap water when water quality in the tanks was not within the following limits: pH 5.7-8.1 (mean 7.2); O_2 4.9-9.9 mg/l (mean 6.2); NH_4 0.8-0.025 mg/l (mean 0.05); NO_2 0.5-0.0 mg/l; NO_3 500-5 mg/l (mean 200 mg/l). Water temperature was regulated by aquaria heaters for each closed system, to obtain stability within the given limits of ± 2 °C per day at an average temperature of 20 °C (range 13-27 °C) during the first year and 21 °C (range 17-24 °C) thereafter. Photoperiod was regulated according to the continuous change of the natural photoperiod (light-dark) in Berlin (LD 16:8 in summer, 8:16 during winter), since it is well known that light has a large impact on the growth of temperate fishes (Trenkler and Semenkova, 1995; Boeuf and Le Bail, 1999).

Feeding and growth

From May 1996 to August 1998 feeding was undertaken *ad libitum* with small frozen chironomids. Each 100 g package of chironomids contained on average 44 % of water; the remaining wet biomass contained 12 % of dry weight. From August 1998 to January 1999 small frozen chironomids, large chironomids, krill and small marine fish (small elongated tropical fish, purchased from a wholesale dealer supplying aquarium shops) were given. Thereafter, the krill was left out of the feed.

Weight and length were first recorded at about 2-5 month intervals; later, at 4-week intervals, and finally every 2 weeks. Length was taken as total length, within 0.5 cm. Weight was measured using a Mettler scale with a d = 1 g for 1 below, after externally drying the fish with soft paper. Apart from sampling, the fish were handled only when graded and newly stocked. Individual growth was followed from April 1997, when the fish were tagged with a passive integrated transponder (Trovan®) for safe identification of the individuals. Tagging was undertaken under anaesthesia using a bath of 70 ppm MS222. The degree of intoxication was determined by the behavioural response of the fish following the criteria given by Conte *et al.* (1988).

Growth previous to this date was analysed for the three respective groups. The growth performance after tagging was calculated for each individual fish. Feeding rate was calculated as percentage of the body weight (weight of food administered) administered per day (% bw/d). Feed conversion ratio (FCR) was calculated as the ratio of feed administered compared to weight gain.

For the presentation of the growth characteristics, we used median and range because these variables represent more real values than mean (extreme values heavily influence the mean values) and standard deviation.

General management

Apart from handling during the procedure of weighing the fish no particular measures were taken to reduce any kind of disturbance. Our intention was to get the fish used to various kinds of manipulation, e.g. we put sufficient light over the tanks to enable continuous observation of the fish.

Statistical analysis

To locate significant differences ($p < 0.05$) between the growth of our fish and those of a wild population (Lepage, Lambert and Rochard, 1994) we used a *t*-test.

RESULTS

Growth characteristics

During the observation period of about 3 years, the fish grew from a median length of 27 cm to a median length of 76 cm (median of 43 cm after the first and of 68 cm after the second year, respectively). A length frequency distribution (figure 1) and a growth curve (figure 2) show more detailed information about these growth characteristics.

The individual growth values revealed that there was an individual who was, right from the beginning of our individual measurements, the smallest fish (36 cm), and continued to be so (58 cm) to the end of the observation period (20 May 1999). As far as the longest fish is concerned, there were 3 individuals (no. 1906, 81EF and D385) that changed position several times: for the first two weighing times after tagging fish no. 1906 was the longest (51 and 58 cm); then for the next three times it was fish no. 81EF (70, 75 and 77 cm); for nine times, fish no. D385 (80 to 88 cm), and the last time (20 May 1999), fish no. 81EF (89 cm) again.

As far as weight is concerned (figure 3), the fish grew from a median of 71 g, when they were all first

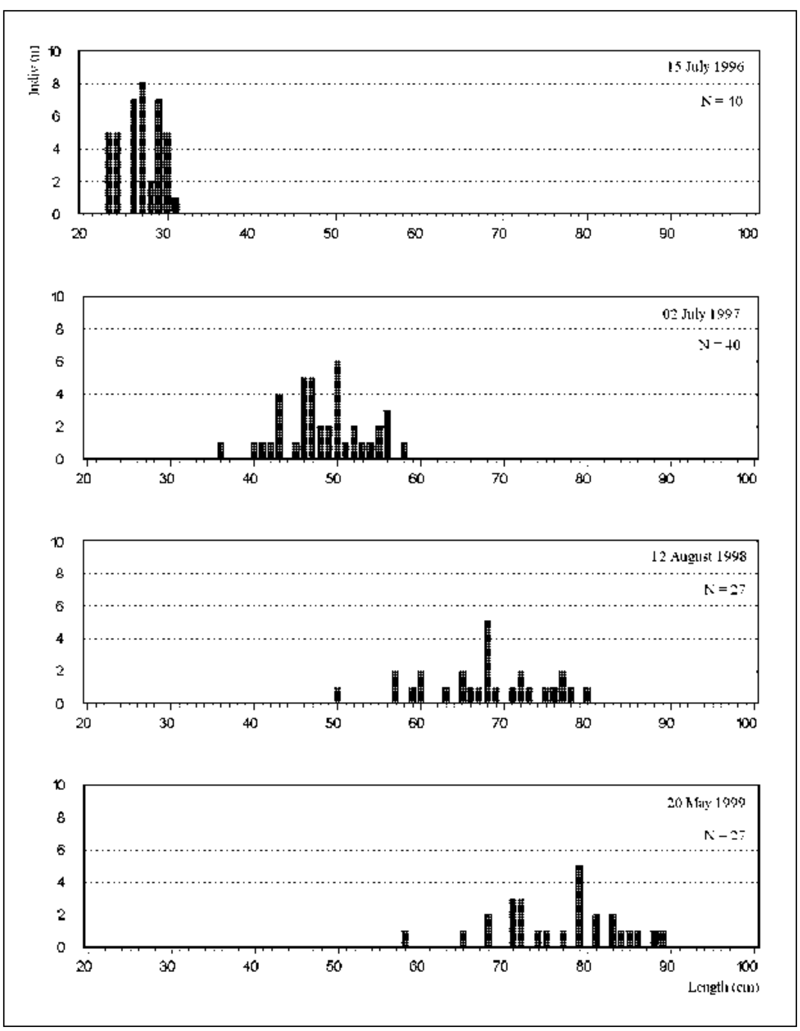

Figure 1. Length-frequency distribution of *A. sturio* representing the observation period of 3 years

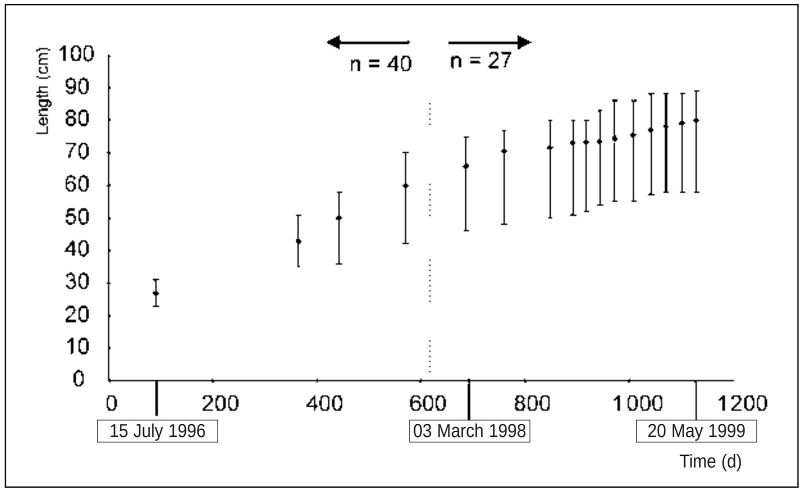

Figure 2. Growth characteristic of *A. sturio* concerning length (median and range) representing the observation period of 3 years. The dotted vertical lines represent the time of transfer of fish from one rearing unit into a larger one

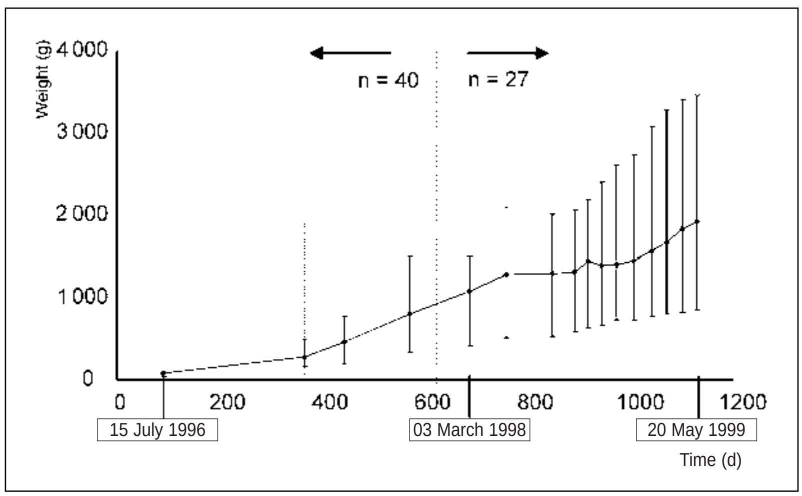

Figure 3. Growth performance of *A. sturio* concerning weight (median and range) representing the observation period of 3 years. The dotted vertical lines represent the time of transfer of fish from one rearing unit into a larger one

measured on 15 July 1996 (13 months old) up to a median of 1 827 g at the end of the third year (median of 280 g after the first and of 1 281 g after the second year, respectively).

The individual growth values concerning weight again showed the shortest fish (fish no. DB80) to be also the fish with the lowest weight (167 g), right from the beginning of the observation period up to the end (855 g). The heaviest fish after tagging was individual no. 1906 for a period of two weighing times (504 and 774 g), but afterwards fish no. 81EF (also the longest fish at the end of the observation period, see above) continuously gained more weight and remained the heaviest fish (1 498 g) from then on, reaching 3 462 g at the end of the observation period.

Figure 4 shows that the feeding rate varied between 2.5 and 4.8 % during the entire observation period. FCR, also shown in figure 4, reveals a drastic increase, from values of about 20 up to 100 at the beginning of the third year, and a decrease thereafter down to the values observed before.

Diseases and mortality

Two observation times (middle of December 1998 and of August 1999) showed the fish to be free of parasite infection. However, several times we observed haemorrhages of the ventral and lateral scales, skin, and ventral part of the pectoral girdle, in particular at the end of the observation period, when the fish, due to the transfer of the rearing tanks from one building to another, were insufficiently fed for several weeks.

No mortality was observed during the observation period due to regular rearing conditions. However, a combination of technical defects and insufficient backup devices caused the loss of 13 fish in January 1998.

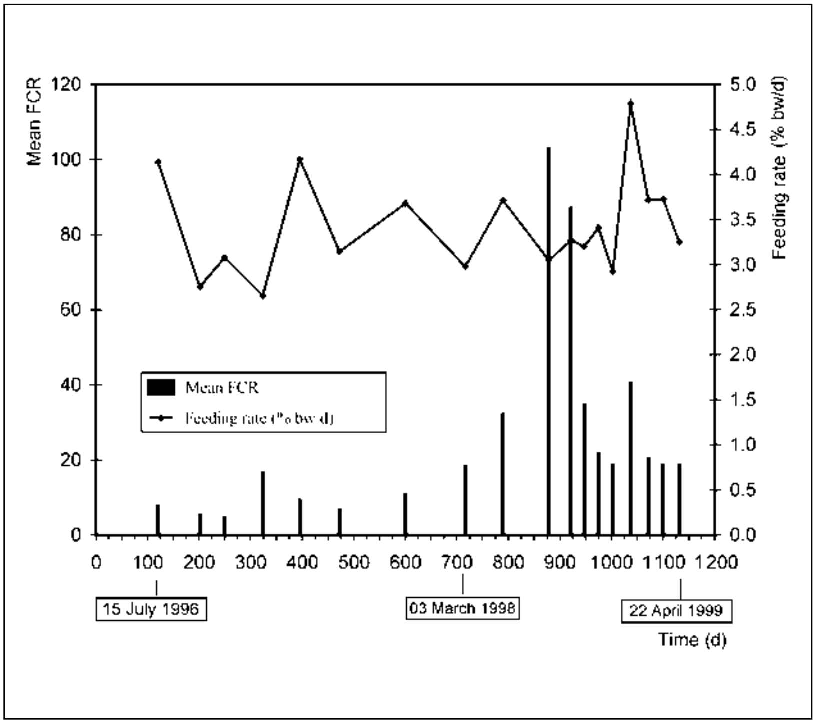

Figure 4. Feeding rate (in % body weight/ day) and mean feed conversion ratio (FCR) observed in *A. sturio* during the rearing period of 3 years

Abnormal morphology and behaviour

Two fish showed a missing integumental nose cover (figure 5), and one fish had only a single eye. Two fish showed abnormal swimming movements, apparently due to insufficient buoyancy. One fish (which died accidentally in January 1998, see above) had a distorted backbone, apparently caused by an accidentally broken backbone, which resulted in abnormal swimming movements. One large fish performed regularly circular movements at the water surface. A few fish showed caudal and ventral fins bent at 90° angles (figures 5 and 6).

Figure 5. Example of a specimen of *A. sturio* showing a missing nose cover

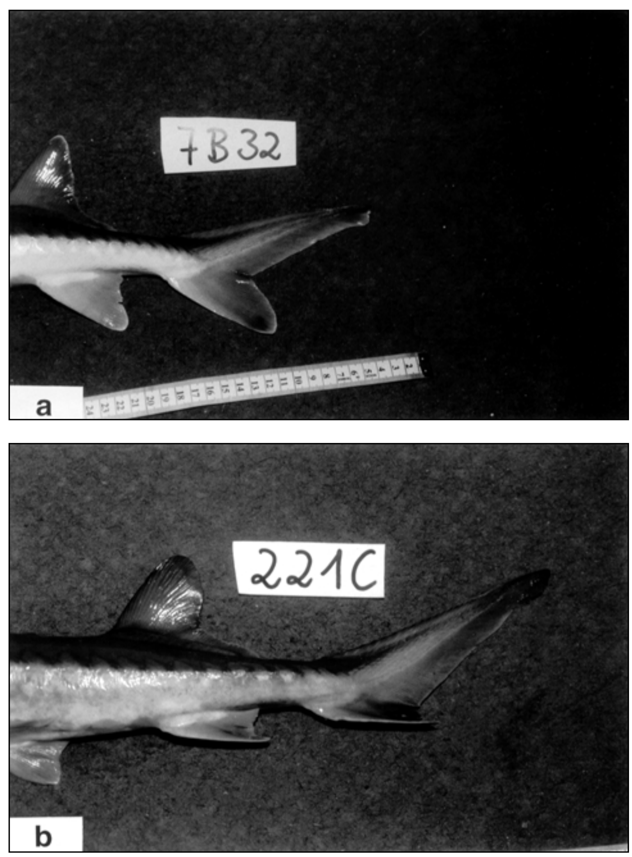

Figure 6. (a): Detail of normally shaped anal and caudal fins in a specimen of *A. sturio.* (b): Specimen of *A. sturio* with anal and ventral part of caudal fin abnormally bent at 90° angle

DISCUSSION AND CONCLUSIONS

Keeping conditions

Sturgeons seem to tolerate quite large variations in physico-chemical factors, and are more tolerant than teleosts (Salin and Williot, 1991), though exact values are scarce. Toxic levels of ammonia in *Acipenser baerii* Brandt, 1869 (Salin and Williot, 1991) are more than a magnitude higher than the maximum ammonia levels we tolerated in our tanks (0.8 mg/l). Intoxication in sturgeons is generally related to special behaviour (Salin and Williot, 1991); we never found any indication of intoxication based on this specific behaviour: our keeping conditions were apparently quite favourable for the fish.

The considerable increase in growth after the first transfer of the fish into larger tanks (figures 2 and 3, around day 360) could be interpreted as a reaction to more favourable keeping conditions in general, as the only major change seemed to be the increase in water volume and surface area per fish.

Abnormal morphology and behaviour

What was the cause of the malformations observed in some of our fish? The ventral and anal fins bent at 90° angles apparently resulted from frequent contacts with the tank bottom: those fish that showed very well-developed buoyancy had normally-shaped fins, whereas those that had slightly less well-filled gas bladders showed these malformations. These abnormalities were independent of size or condition of the fish. The one fish that had serious problems with buoyancy showed extreme malformations on all ventrally located fins. A few fish had very slightly bent pectoral fins: those fish that swam in the tanks in the clockwise direction showed these malformations on the left side only, and vice versa.

The missing integumental nose cover observed in a few large fish apparently did not alter the feeding behaviour of these specimens.

The malformations observed in general did not influence the growth of the fish, and were caused by various kinds of physical contacts with the wall of the tanks.

Growth and feeding

Comparing the overall growth of our captive reared fish with wild-caught fish (figure 7), we see that our fish showed similar growth characteristics to the wild fish (Lepage, Lambert and Rochard, 1994), taking into account that our fish (received from the Cemagref facility in 1996) were the smallest of all the 1995-born specimens available at that time.

During our experiments, however, constant, high water temperatures of approx. 20 °C were applied. Our *A. sturio*, therefore, should have shown better growth characteristics (assuming that *A. sturio* reacts in the same way as other sturgeons) than the wild fish, as has been shown for other species (Rosenthal and Gessner, 1992). Consequently, we

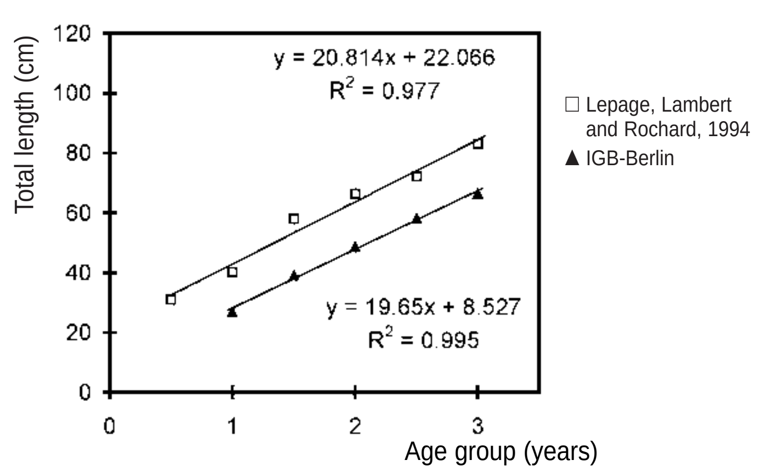

Figure 7. Growth characteristics of the *A. sturio* reared in Berlin (IGB) compared to Gironde fish (Lepage, Lambert and Rochard, 1994) based on mean values for age groups

have to conclude that our fish showed suboptimal growth. One explanation for this could be the suboptimal food supplied to our fish (see also Williot *et al.*, 1997). The natural food of *A. sturio*, however, is not yet well known. The food we supplied, chironomid larvae and marine tropical fish, cannot be considered a natural food, at least not for our larger fish, which would live, in nature, in a temperate marine environment. Future experiments, therefore, will include feeding trials with additional natural food items, experiments concerning the optimal temperature for growth, significance of tank size on growth characteristics, and the attempt to develop a formulated diet to maximise weight gain and thus stimulate early sexual maturation and gonadal development, as has been shown for other sturgeon species (Filippova, 1999; Doroshov, Van Eenennaam and Moberg, 1999).

The individual growth characteristics showed rather large individual differences. Some individuals, for short periods of time, had a growth rate comparable to the maximum length increase of about 20 cm per year, found in the wild (Lepage, Lambert and Rochard, 1994). Why this growth period in captivity is so short will be investigated in future experiments.

The decrease in growth performance at the beginning of the third year was probably due to the bad quality of the small frozen chironomids. These benthic insect larvae could have contained organic or inorganic pollutants, with adverse effects on growth; we have found a similar effect with live chironomids in feeding tropical fish (unpublished results).

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