



Rearing practices and early performance of migratory fish for stocking program : study of the critically endangered European sturgeon (*Acipenser sturio*)

Erika Carrera Garcia

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Doctor of Philosophy

Rearing practices and early performance of migratory fish for a
stocking program: study of the critically endangered European
sturgeon (*Acipenser sturio*)

Erika Carrera-García

4th April 2017

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"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein

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Rearing practices and early performance of migratory fish for stocking program: study of the critically endangered European sturgeon (*Acipenser sturio*).

Abstract

Stocking for conservation purposes is the practice of raising animals in captivity and releasing them into an area from which the species have either declined or disappeared, in an attempt to enhance the natural population. Fish reared in hatcheries are exposed to selective reproduction, and early life experiences in a safe setting can strongly influence the behavioral, genetic, morphological and physiological attributes necessary to respond adequately to natural conditions after stocking. Exposing naive individuals to variability during early stages –enriched environment– could reduce such deficiencies and improve fish survival after release. This approach needs to be studied to understand how it affects performance traits that will directly impact fish fitness in the wild and consequently, its effects on stocking programs efficiency for conservation. The last remaining wild sturgeon population in Western Europe, the European sturgeon (*Acipenser sturio*), is located in the Gironde (Southwest France). This species is critically endangered and subject of a recovery plan with specific action plans in France and Germany. In this study, this species was used as a model to assess effects of rearing practices on survival, growth and behavioral performance of young-of-the-year sturgeons before stocking and their behavior and survival in the wild after release. Captive-born sturgeons belonging to two crossings were reared under traditional (i.e. low spatial and temporal variability) and enriched (i.e. higher spatial and temporal variability) practices for 3 months and their performance was evaluated in laboratory. Then, the fish were released in the Dordogne River and their individual movements were tracked for 20 days using a acoustic telemetry system; after evaluating the tagging effects on fish performance. Results in the laboratory demonstrated that enriched environment resulted in bigger fish from the first month. Growth curve analysis revealed that enriched environment made both fish crossings grow in a similar manner. In contrast, crossings' growth differed in traditional rearing. Behaviorally, enriched-reared fish were slower to explore a new environment but more individuals engaged on doing so than traditional-reared fish. Post-release assessment on such small individuals was carried out for the first time for a sturgeon species. Fish overall survival was 69.3% (52.2–90.2%) where the lowest survival was found for one crossing reared under traditional conditions. After release, most movements (85.7%) occurred during the first three days after stocking and downstream direction. During the study, 82% of the fish were detected within 13.5km from the release site and no fish reached the saline estuary. Fish were mainly active during night hours, but traditional-reared fish were significantly more active during the day than enriched-reared fish during the first three days. Our findings advocate for the integration of enriched rearing practices within the juvenile production for release in order to boost the performances linked

to fitness. Stocking practices and life history research should work together to favor adaptive aquaculture approaches, which support species conservation.

Keywords: conservation aquaculture, stocking, sturgeon, rearing conditions, behavior, acoustic telemetry, survival.

**Pratiques d'élevage et performances des jeunes stades de poissons migrateurs amphihalins dans le cadre de programmes de repeuplement : cas d'étude d'une espèce en danger critique d'extinction
l'esturgeon européen (*Acipenser sturio*)**

Résumé

En biologie de la conservation, les pratiques de réintroduction consistent à élever des animaux en captivité et à les relâcher dans l'aire de répartition où l'espèce en question a décliné ou disparu, ceci dans le but de soutenir la population naturelle. Pour les poissons, l'élevage en pisciculture implique une sélection des individus au moment de la reproduction et un développement des jeunes stades dans un environnement contrôlé qui peuvent influencer fortement les attributs comportementaux, génétiques, morphologiques et physiologiques nécessaires pour répondre de manière adéquate aux conditions du milieu naturel. L'exposition de ces individus naïfs, particulièrement les jeunes stades, à la variabilité (environnements enrichis) pourrait limiter ces carences et améliorer la survie post-lâcher. Dans ce cadre, des expérimentations sont nécessaires pour comprendre comment l'environnement d'élevage affecte les performances individuelles qui impacteront directement la fitness en milieu naturel et par conséquent l'efficacité des programmes de réintroduction avec un objectif de conservation. La dernière population d'esturgeon sauvage d'Europe de l'ouest est issue de la Gironde (Sud-Ouest France), il s'agit de l'esturgeon européen (*Acipenser sturio*). Cette espèce est classée en danger critique d'extinction et fait l'objet d'un programme de restauration européen décliné en plans d'actions nationaux en France et en Allemagne. Dans ce travail, cette espèce est utilisée comme modèle afin d'évaluer les effets des pratiques d'élevage sur la survie, la croissance et le comportement des jeunes de l'année en captivité puis leur comportement et leur survie post-lâcher en milieu naturel. Des esturgeons nés en captivité, issus de 2 croisements, ont été élevés selon deux méthodes, l'élevage « traditionnel » (faible variabilité spatiale et temporelle) et l'élevage « enrichi » (augmentation de la variabilité spatiale et temporelle), jusqu'à ce qu'ils atteignent trois mois puis leurs performances ont été évaluées en conditions contrôlées. Les individus ont ensuite été relâchés en rivière et leurs déplacements individuels ont été suivis pendant 20 jours à l'aide d'une technologie de télémétrie acoustique après avoir évalué les effets du marquage sur les performances individuelles. Les résultats en conditions contrôlées mettent en évidence que dans un élevage enrichi les individus atteignent un poids et une taille supérieurs dès le premier mois de vie. L'analyse de la croissance révèle que les individus issus des deux croisements ont une croissance similaire en conditions enrichies mais celle-ci diffère dans un environnement traditionnel. L'analyse du comportement en milieu contrôlé indique que les individus élevés en conditions enrichies seraient plus lents à explorer un environnement inconnu mais plus nombreux que parmi les individus élevés de manière traditionnelle. Le suivi post-lâcher des jeunes stades de cette espèce et plus

largement des espèces d'esturgeon a été réalisé pour la première fois dans le cadre de ce travail. La survie globale des poissons était de 69,3% (52.2–90.9%), la survie la plus faible concernant un croisement élevé de manière traditionnelle. Après le lâcher, la plupart des mouvements (85,7%) se sont produits au cours des trois premiers jours et ils étaient orientés vers l'aval. 82% des poissons ont été détectés jusqu'à 13.5km du site de lâcher et aucun des poissons n'a atteint l'estuaire salé. Les poissons étaient principalement actifs durant la nuit, mais les poissons élevés de manière traditionnelle étaient significativement plus actifs pendant la journée que les poissons issus de l'élevage enrichi durant les trois premiers jours. Nos résultats plaident en faveur de l'intégration de pratiques d'élevage enrichies pour la production de juvéniles destinés au repeuplement afin d'améliorer les performances liées à la fitness. Ce travail milite pour que les pratiques de repeuplement et la recherche sur l'écologie des espèces travaillent en synergie afin de favoriser la mise en place d'une aquaculture adaptative qui soutienne la conservation des espèces.

Mots clefs: aquaculture de conservation, réintroduction, esturgeon, pratiques d'élevage, comportement, télémétrie acoustique, survie.

**Prácticas de cría y rendimiento temprano de peces migratorios en programas de repoblación:
estudio del esturión europeo (*Acipenser sturio*), especie en peligro crítico de extinción.**

Resumen

En conservación, la repoblación de especies es la práctica de criar animales en cautiverio y su posterior liberación en zonas donde la especie ha disminuido o desaparecido con la finalidad de aumentar la población natural. Los peces cultivados en piscifactorías están expuestos a la reproducción selectiva, y la experiencia temprana en ambientes seguros puede tener una fuerte influencia en los atributos genéticos, morfológicos, fisiológicos y de comportamiento, necesarios para responder adecuadamente en la naturaleza después de la liberación. La exposición de individuos sin experiencia previa a la variabilidad en el ambiente (ambientes enriquecidos) durante los estadios tempranos de su desarrollo puede reducir estas deficiencias y mejorar la sobrevivencia después de la liberación. Este tema necesita ser estudiado más a fondo para entender cómo el ambiente de cría afecta los atributos de rendimiento que influirán directamente en el “fitness” de los individuos una vez en la naturaleza y por consecuencia, en la eficiencia de los programas de repoblación para la conservación. El último relicto de esturión salvaje en el sudoeste de Europa, el esturión europeo (*Acipenser sturio*), está en peligro crítico de extinción, sujeto a un plan de recuperación y planes específicos de conservación en Francia y Alemania. En este estudio, esta especie fue usada como modelo para evaluar los efectos de las prácticas de cría en la sobrevivencia, crecimiento, y comportamiento en juveniles de menos de un año de edad antes de la liberación y, su comportamiento y sobrevivencia en la naturaleza después de la liberación. Para esto, esturiones de dos cruces diferentes fueron criados durante tres meses en cautiverio en ambientes tradicionales (baja variabilidad espacial y temporal) y ambientes enriquecidos (alta variabilidad espacial y temporal) y evaluados en laboratorio. Posteriormente los peces fueron liberados en el río Dordoña y se rastrearon sus movimientos individualmente durante 20 días usando un sistema de telemetría; los efectos de este marcado se evaluaron previamente. Los resultados de laboratorio demuestran que la cría enriquecida produce peces de mayor tamaño desde el primer mes de crianza. Los análisis en las curvas de crecimiento revelaron que la cría enriquecida hizo que los peces de ambos cruces crecieran de manera similar. Por el contrario, el crecimiento fue diferente bajo la cría tradicional. Con respecto al comportamiento, los peces de cría enriquecida tardaron más en empezar a explorar un ambiente nuevo pero más individuos se involucraron en esta actividad que los peces de cría tradicional. En la naturaleza, es la primera vez que se monitorea esturiones de tan poca edad. La sobrevivencia global fue del 69.3% (52.2–90.9%) donde uno de los cruces criado tradicionalmente tuvo la tasa más baja. La mayoría de los movimientos (85.7%) ocurrieron durante los primeros tres días después de la liberación y orientados río abajo. Durante el estudio, 82% de los peces fueron detectados a menos

13.5km del sitio de liberación y ninguno alcanzó el estuario salino. Los peces estuvieron principalmente activos durante las horas de la noche. Sin embargo, durante los 3 primeros días después de la liberación, los peces criados por medios tradicionales estuvieron significativamente más activos durante el día que aquellos criados en medios enriquecidos. Este estudio aboga por la integración de las prácticas de cría enriquecida dentro de la producción de juveniles para la liberación con el objetivo de mejorar el rendimiento ligado al “fitness”. Las prácticas de repoblación y la investigación de historia de vida deben trabajar en conjunto para favorecer los enfoques adaptativos de acuicultura que apoyen la conservación de especies.

Palabras clave: acuicultura de conservación, repoblación, esturión, condiciones de cría, comportamiento, telemetría acústica, sobrevivencia.

1

Introduction

1.1. Biodiversity and conservation

The world's biodiversity shows a decline of 58% in the last 42 years (WWF 2016). Overall, the most impacted habitats are freshwater environments with a decline of 81% (WWF 2016). Biodiversity extinction rates in the past few centuries are comparable with mass extinction events in the past and thus, it has been considered that a sixth mass extinction is in progress (Pievani 2014). Generally speaking, a decrease in species diversity alters the ecosystem robustness and resilience and, consequently its capacity to sustain humans in the biosphere (Costanza et al., 1997). To overcome this diversity's loss a wide range of actions are taken worldwide to identify its threats and to correct them. *In situ* corrective actions aim to facilitate diversity to maintain itself within the context of their natural environment as habitat protection, restoration and fishing and hunting regulations (Bain 1987; Armstrong et al., 2001; Pikitch et al., 2005). They can be targeted to populations, species and whole ecosystems. *Ex situ* actions, on the other hand, aim the conservation of biological components outside their natural habitats like zoos and captive breeding programs (Arlati and Poliakova 2009; Williot et al., 2009a). Breeding species in captivity to be released into the wild for conservation purposes has been considered a strong mean to stabilize, re-establish or enhance populations that have suffered significant decline (Jachowski et al., 2016). This strategy occurs worldwide in a wide range of fish species (Aprahamian et al., 2003; Buckmeier et al., 2005; Zhu et al., 2006; Williot et al., 2009a). In this work, this type of actions, within the fish context will be referred to as "stocking" and encompass the IUCN/SSC definitions for introduction¹, reintroduction² and reinforcement³. The ultimate goal of stocking for conservation purposes is to enhance or produce a self-sustaining (Robinson and Ward 2011) and persistent population (Seddon 1999). This action is a complex and long term process generally expensive in which several aspects like habitat requirements, behavioral and genetics issues, population dynamics and species intrinsic characteristics, citizens awareness and implementation of management protocols are implicated in its success achievement (Jachowski et al., 2016). Yet, conservation of migratory species are even more challenging due to their multiple spatio-temporal scales and wide-range movements (Shuter et al., 2011).

¹ introduction: intentional or accidental dispersal by human agency of a living organism outside its historically known native range (International Union for Conservation of Nature, 1987).

² reintroduction: intentional movement and release of an organism inside its indigenous range from which it has disappeared (International Union for Conservation of Nature/Species Survival Commission, 2013).

³ reinforcement: intentional movement and release of an organism into an existing population of conspecifics (International Union for Conservation of Nature/Species Survival Commission 2013).

Migration itself can lead to high mortality due to increased exposure to threats along the way. Often, it can place the species outside of protected areas and their routes can go through various jurisdictional and political boundaries (Shuter et al., 2011). Also, these species have annual or multi-cycles in which timing of events is critical making them vulnerable to a variable environment e.g. climate change (Mihoub et al., 2010; Shuter et al., 2011). Thus, events affecting any stage of the migratory cycle will affect the others and the whole population. This is the case of Acipenseridae species which most of them are migratory and at risk of extinction (Birstein et al., 1997; Billard and Lecointre 2001). Furthermore, there is very limited information on biology and ecology or particular life stages of critically endangered species due to lack of specimens to study. This points out the need of urgent research to improve their conservation outcomes.

1.2. Stocking programs

Through human history, stocking have been often a tool to mitigate the overexploitation of seas and inland waters for food, commerce and recreation (Olden 2006). Some can be even traced back to the Middle Ages. For example, in the 14th century, trout culture initiated when a French monk came across with the artificial fertilization of trout eggs (Pillay and Kutty 2005). Then, trout culture spread around the world and by 18–19th century, hatcheries were establish everywhere in the world and used to stock natural water bodies to improve sport fishing (Pister 2001; Pillay and Kutty 2005). By that time, fish stocking happened without management plans and it was driven by a utilitarian resource management ethic (Pister 2001) used to satisfy commercial and recreational demands. Therefore, these introductions were focused on fish species with recreational and commercial value. By this means, rainbow trout (*Oncorhynchus mykiss*) ended being the most widely distributed salmonid in the world (Stanković et al., 2015). This type of fish stocking has also been considered as one of the main threats to freshwater fish conservation (Seddon et al., 2012). Nowadays, stocking practices have evolved to address the need of ecosystem and biodiversity conservation (Pister 2001). According to the Food and Agriculture Organization of the United Nations (2005-2016), ninety four countries have reported stocking into aquatic habitats to alleviate environmental damage, compensate overfishing, maintain productivity and conservation of threatened species. Stocking, which is still a controversial practice (Araki and Schmid 2010), is also an important tool for conservation (IUCN/SSC 2013; Cochran-Biederman et al., 2015), mainly focused on population enhancement, that is gaining relevance as biodiversity continue to decline (Reading et al., 2013). Although, stocking cannot address the factors of diversity decline in first place so, it is not enough to protect species against decline or extinction. But, it can be regarded as a complementary action along within *in situ* conservation strategies like habitat protection and restoration (George et al., 2009). In these programs the fish to be released are often captive-born from a broodstock that is

wild-caught and/or of hatchery origin. In this context, aquaculture practices are important since the species' survival in early stages can be significantly increased in captive conditions over the rates found in the wild (Secor et al., 2002). Therefore, stocking programs need high breeding success and low mortality rates to allow them to provide individuals for release that will survive and breed in the wild (Reading et al., 2013). However, the successful integration of these individuals into the wild is influenced by many biological (e.g. genetic diversity, species characteristics), environmental (e.g. habitat quality) and socio-economic factors (e.g. funds) (Reading et al., 2013; Cochran-Biederman et al., 2015). A better knowledge of these aspects can increase success and can help to design strategies and methodologies that could result in baseline data to be shared and compared between current and future programs improvement.

Worldwide, 76% of sturgeon species are subjected to conservation stocking programs (Waldman and Wirgin 1998; Billard and Lecointre 2001; Jackson et al., 2002; Arlati and Poliakova 2009; Maltsev 2009; Williot et al., 2009a; IUCN 2015) mainly because it is a very vulnerable fish group as from its 25 species, 18 are listed under the endangered and critically endangered category of the IUCN red list (2015) (Table 1). Records of the use of stocking to compensate poor natural reproduction in sturgeons come as early as 1954 in the Caspian Sea (Birstein 1993) where stocking was heavily used to increase catches (Barannikova 1987). Currently, stocking is mostly focused on natural population enhancement, as a conservation measure mainly done in North America and Europe (Waldman and Wirgin 1998; Billard and Lecointre 2001; Drauch and Rhodes 2007; Arlati and Poliakova 2009; Gessner et al., 2010a; Dreal 2011; Kirschbaum et al., 2011). As sturgeons are threatened in their entire range of distribution, some species have disappeared from most of it and are on the verge of becoming extinct (Billard and Lecointre 2001). For such species captive stocks and stocking programs are the only possible means to save them from extinction (Birstein 1993).

Table 1. List of extant sturgeons species in alphabetic order. A: *Acipenser*, H: *Huso*, P: *Pseudoscaphirhynchus*, S: *Scaphirhynchus*.

Species	English name	Historical geographic distribution	Status (1)	Stocking (2)
<i>A. baerii</i> (Brandt, 1869)	Siberian	Siberia, Arctic	EN	+
<i>A. brevirostrum</i> (Lesueur, 1818)	Shortnose	From Florida to New-Brunswick	VU	+
<i>A. dabryanus</i> (Duméril, 1868)	Yangtze / Dabry's	China, Yangtze, Korea	CR	+
<i>A. fulvescens</i> (Rafinesque, 1817)	Lake	Great Lakes and lakes of S. Canada	LC	+
<i>A. gueldenstaedtii</i> (Brandt, 1833)	Osetra / Russian	Caspian, Black and Azov Seas	CR	+
<i>A. medirostris</i> (Ayres, 1854)	Green	N. Pacific	NT	
<i>A. mikadoi</i> (Hilgendorf, 1892)	Sakhalin	N.W. Pacific	CR	
<i>A. naccarii</i> (Bonaparte, 1836)	Adriatic	Adriatic, Pô, Adige, Mediterranean	CR	+
<i>A. nudiventris</i> (Lovetzky, 1828)	Ship	Caspian, Black and Azov Seas (Aral)	CR	+
<i>A. oxyrinchus</i> (Mitchill, 1814)	Atlantic Aoo / Gulf Aod	Gulf of Mexico, Hamilton/Fundy	NT	+
<i>A. persicus</i> (Borodin, 1897)	Persian	Caspian and Black Seas	CR	+
<i>A. ruthenus</i> (Linnaeus, 1758)	Sterlet	Europe, ex. USSR Caspian, Black, Azov	VU	+
<i>A. schrenckii</i> (Brandt, 1869)	Amur River	Amur River, Okhotsk and Japan Seas	CR	+
<i>A. sinensis</i> (Gray, 1834)	Chinese	Yangtze, Pearl Riv., Korea, Japan	CR	+
<i>A. stellatus</i> (Pallas, 1771)	Sevruga / Stellate	Black, Caspian Azov Seas	CR	+
<i>A. sturio</i> (Linnaeus, 1758)	European / Atlantic	Europe (North Africa)	CR	+
<i>A. transmontanus</i> (Richardson, 1836)	White	N.E. Pacific	LC	+
<i>H. dauricus</i> (Georgi, 1775)	Kaluga	Amur Riv., Japan sea	CR	
<i>H. huso</i> (Linnaeus, 1758)	Beluga / Giant	Black, Caspian Azov, Adriatic Seas	CR	+
<i>P. fedtschenkovi</i> (Kessler, 1872)	Syr Dar Shovelnose	Syr-Darya	CR	
<i>P. hermanni</i> (Kessler, 1877)	Small Amu Dar Shovelnose	Aral, Amu-Darya	CR	
<i>P. kaufmanni</i> (Bogdanov, 1874)	Large Amu Dar Shovelnose	Aral, Amu-Darya	CR	+
<i>S. albus</i> (Forbes and Richardson, 1905)	Pallid	Mississippi, Missouri	EN	+
<i>S. platyrhynchus</i> (Rafinesque, 1820)	Shovelnose	Mississippi, Missouri	VU	
<i>S. suttkusi</i> (Williams and Clemmer, 1991)	Alabama	Alabama, Mississippi	CR	+

Table taken and adapted from Billard and Lecointre (2001).

(1) CR: critically endangered, EN: endangered, VU: vulnerable, NT: near threatened, LC: least concern (IUCN 2015).

(2) Conservation stocking is practiced on the species at least experimentally (IUCN 2015).

1.3. Rearing practices

1.3.1. Aquaculture

Hatchery environments vary from ponds to high-technology recirculation systems. Most of these farming conditions are very different from natural habitats to which species are naturally adapted; fish are reared in unchallenging, invariable and structurally very simple conditions (Huntingford et al., 2012; Johnsson et al., 2014). In these conditions, fish adapt and undergo intended or unintended changes that can get accentuated generation after generation (Price 2002; Johnsson et al., 2014) and the rearing purpose will dictate the importance and direction of these changes. Adaptations to captivity are important in commercial aquaculture that aims to produce a supply for consumption and fish required to adapt to rearing conditions (i.e. density, handling, feed administration, pathogens) to maximize productivity and profit (Parker 2011). However, in conservation aquaculture, these adaptations to rearing conditions are incongruent with the aim of conservation and recovery of endangered populations along with their locally adapted gene pool and their typical phenotypes and behaviors (Anders 1998). In this context, conservation aquaculture is also contrasting with traditional supplementation programs that aimed to release large records quantities of fish to build a harvestable surplus (Anders 1998). The advantage of aquaculture practices for conservation lays in the fact that fish survival, especially in early stages, can be increased several times in the hatchery's controlled conditions over the rates found in the wild (Secor et al., 2002). Nevertheless, there are concerns associated with aquaculture practices like artificial selection and domestication.

1.3.2. Artificial selection, natural selection in captivity and relaxation of natural selection

Fish rearing for conservation faces similar problems as fish rearing for other purposes. Indeed, environmental conditions established in the hatchery are artificial, i.e. different from those found in nature (Youngson and Verspoor 1998; Huntingford et al., 2012; Johnsson et al., 2014). As a consequence, the phenotypes produced via genotype by environment interaction as well as the genotypes selected by the new environment can strongly differ from those found in the wild population (Huntingford et al., 2012; Johnsson et al., 2014). This process is often termed domestication, and can be divided in two well defined mechanisms, artificial and relaxed natural selection, often occurring simultaneously (Price 2002). Artificial selection occurs because the breeder defines the crossing pairs to select specific traits and can manipulate reproduction timing for some species (Price 2002). For example, in commercial aquaculture, high growth rates are often the trait of interest (Huntingford 2004; Huntingford and Adams 2005), as it promotes directly the fish farm productivity. Thus, fast growing fish will be given priority by the man-made selection to establish a genetic pool in the next generation. Furthermore, the more the promoted traits are heritable, the

more the effect of artificial selection will be pronounced in the offspring. Fish farm managers often have to deal with indirect selection of secondary traits, strongly linked to the traits of interest, or traits unintended selected because they are beneficial in captivity (Waples 1999; Price 2002) allowing fixation of non-adaptive traits and the accumulation of deleterious alleles (Price 2002). For example, selection for productivity traits have been linked with lower lifetime reproductive success (Youngson and Verspoor 1998). Fish rearing for conservation purposes are not under the same constraints e.g. high generational turn-over, high feed conversion ratio, as those in commercial farms. However, in many cases, where the parental stock is low, the establishment of genetic pool for the next generation is limited to the few fish able to reproduce –natural selection in captivity. In other words, to start, wild specimens that would not adapt to captivity would die, have low reproductive success or no reproduce at all (Price 2002). Those that have survived to adulthood have undergone adaptations to this environment, i.e. physiological changes, and they are able to reproduce (Price 2002). Furthermore, changes occur since the first-generation of captive fish (Siikavuopio et al., 1996; Alvarez and Nicieza 2003; Christie et al., 2016); thus, the following generations will be more fit to captive conditions, pointing out that natural selection is stronger in the first generations (Price 2002). In captivity, many traits and skills that are important for survival in the wild lose their adaptive value and therefore natural selection is not strong on them; this relaxation of natural selection leads to higher variability of phenotypic traits (Price 2002) by increasing the frequency of reaction norms that would be otherwise disfavored in the wild. Indeed, fish mortality is often higher in the wild than in the captive born fish, where the whole environment is setup for the best survival of individuals since the youngest stages. For example, survival rate of steelhead trout juveniles (*Oncorhynchus mykiss*) reach 85–95% in the hatchery (Reisenbichler et al., 2008) while in the wild it is 27–35% (Melnychuk et al., 2007). Relaxed natural selection in captive-bred fish is not a concern when they will end-up on a plate, but is not the case if fish are stocked and expected to match the wild phenotype (Waples 1999).

Relaxation of selection can produce changes in behavior, physical and immune condition (Kronenberger and Medioni 1985; Dohm et al., 1994; Olla et al., 1998; Yampolsky et al., 1999; Berejikian 2005). For example, being reared in a predator-free environment has negative consequences in anti-predator performance; fish do not react properly or even approach the threat (Johnsson and Abrahams 1991; Berejikian 1995; Olla et al., 1998; Alvarez and Nicieza 2003; Biro et al., 2004; Meager et al., 2011). Thus, captive fish tend to be bolder (Biro et al., 2004; Sundström et al., 2004; Houde et al., 2010) and explore novel objects faster (Sundström et al., 2004) because they not necessarily associate correctly the predator presence with immediate danger. Thus, once released into the wild, predation is the main reason of mortality (Olla et al., 1998; Braithwaite and

Salvanes 2005). Homogeneous conditions in hatcheries also contribute to altered behavior in captive fish; it can decrease behavioral flexibility, i.e. adjustment of behavior in response to changes in the environment (Braithwaite and Salvanes 2005; Johnsson et al., 2014), as there is no need to maintain the potential to adapt in an unchanging environment. Others traits such cognitive skills, spatial memory and learning abilities can also be altered (Brown et al., 2003; Salvanes and Braithwaite 2005; Salvanes et al., 2013; Johnsson et al., 2014). Most of the traits are influenced simultaneously by the environment and artificial/unintended selections, giving raise to specific behavioral syndromes (boldness vs shyness) or stress coping styles (proactivity vs reactivity) (Réale et al., 2007; Stamps 2007; Biro and Stamps 2008; Réale et al., 2010).

1.3.3. The alternative approaches: pre-release training and environmental enrichment

Rearing environment of captive animals for conservation purposes is a subject rising attention as a method to improve captive breeding and release programs (Brown et al., 2003; Strand et al., 2010; Chebanov et al., 2011; Roberts et al., 2011; Reading et al., 2013; Bergendahl et al., 2016). The goal of conservation aquaculture for stocking programs is to enhance the fitness of individuals –ability to survive and reproduce (Maynard-Smith 1989) following release. Thus, these individuals require a set of morphological, behavioral and cognitive skills that will allow this to happen. These skills develop in early stages and consequently are shaped by their genetic properties and the environment they grow-up in (Reading et al., 2013; Johnsson et al., 2014). However, the individuals destined for stocking grow in artificial environments and these settings, as mentioned before, influence traits necessary to respond adequately to natural environments after release (Brown et al., 2003; Braithwaite and Salvanes 2005; Klefoth et al., 2012). It has been documented that after reintroduction, hatchery strain individuals are less successful in establishing and surviving than wild strains (Maynard et al., 1995); most of the mortality occurs the immediate days after reintroduction (Howell 1994; Svåsand et al., 1998; Blaxter 2000) and 5% or less make it to adult stages (McNeil 1991; Salvanes 2001). Some important traits that could impact success after release are territorial and feeding efficiency, social and anti-predator skills, reproductive success or locomotor skills that can differ between captive-born individuals and their wild counterparts (Olla et al., 1998; Sundström and Johnsson 2001; Aarestrup et al., 2005; Salvanes and Braithwaite 2006; Stamps 2007; Araki et al., 2008; Reading et al., 2013). For this reason, many programs try to incorporate pre-release protocols to familiarize individuals with the environment to which they will be introduced (Beck et al., 1994; Olla et al., 1998; Braithwaite and Salvanes 2005). For example, in social species, naïve individuals could learn skills from the more experienced ones. Thus, cohabitation incentives social learning and improves skills needed to survive in the wild (Suboski and Templeton 1989; Brown and Laland 2001;

Brown and Laland 2003). Other skills as predator avoidance and foraging can also be improved when captive-bred individuals are exposed to natural live preys and predators cues before release (Järvi 1990; Järvi and Uglem 1993; Hossain et al., 2002; Arai et al., 2007). Temporal allocation in semi-natural enclosures before release is also practiced in order to improve the captive-wild transition (Näslund 1992; Maynard et al., 1996). The post-release recovery time is reduced, the individuals can feed on natural prey and adjust to the new environment while being safe from predators (Brown and Day 2002). Nevertheless, studies show that these procedures do not always obtain the expected results (Johnsson and Abrahams 1991; Sundström and Johnsson 2001; Brown and Day 2002; Vilhunen and Hirvonen 2003) probably because the training period occurred too late in animals' lives or because the method may not be adapted to the species concerned. Indeed, the effects of environment occur early in life (Kotrschal and Taborsky 2010; Ebbesson and Braithwaite 2012; Salvanes et al., 2013). So, it has been suggested that exposing individuals to "environmental enrichment" in early stages can alleviate behavioral deficiencies that cause significant mortality rates (Braithwaite and Salvanes 2005; Kotrschal and Taborsky 2010; Roberts et al., 2014). Environmental enrichment consists in providing an environment with increased complexity in which animals can demonstrate their species-typical behavior and choice over their environment (Young 2003; AZA 2016); features that are absent in traditional captive environments. The purpose is to reduce atypical behavior and increase ability to cope with challenges (Young 2003). Enrichment can be provided in a variety of ways such as presence of spatial cues (e.g. novel objects, plants, logs) (Salvanes and Braithwaite 2005; Salvanes et al., 2007); variability in food items and its distribution (Brown et al., 2003; Chapman et al., 2010; Kotrschal and Taborsky 2010) and presence of other individuals (Young 2003; Strand et al., 2010). Thereby several authors suggest this type of environment would promote neural development (Brown et al., 2003; Reading et al., 2013), behavioral flexibility (Braithwaite and Salvanes 2005; Salvanes et al., 2007; Kotrschal and Taborsky 2010), phenotypic variation (Crossman et al., 2014) and physical fitness (Biggins et al., 1991; Mathews et al., 2005) which can improve post-release survival. For this reason, it has been considered as a promising approach to improve reintroduction programs outcomes.

Specifically in sturgeons, Crossman et al., (2011) has studied the enrichment approach for 8–13 weeks in lake sturgeon using a streamside hatchery (river water and natural temperature fluctuations) from the natal river. His results showed a main effect of rearing method favoring recapture rate of enriched-reared fish compared to controls (traditional hatchery). In another study, the same authors (Crossman et al., 2009), reared fish for 3 months in the same streamside hatchery and then transfer them to a traditional hatchery for 3 more months. Fish of both rearing methods were released at the age of 6 months and were acoustically monitored for 2 months. The author did

not find survival differences between the rearing methods and suggested that the enriched-reared fish could have lost any advantage developed during the first months when maintained in the traditional hatchery or because of a size threshold beyond which rearing effects may not be relevant. Boucher et al., (2014) found that white sturgeon (*A. transmontanus*) larvae reared on a combination of gravel and warm water temperature resulted in higher survival, better yolk absorption efficiency and bigger fish than the control group. Finally, Du et al., (2014) reared 15–40 days post hatch (dph) Dabry's sturgeon (*A. dabryanus*) under water current resulting in higher endurance and critical swimming speed (U_{crit}) for those fish under current conditions than those reared in static tanks. The mentioned studies in sturgeons have chosen to work with few variables, all of them directed to improve fish physical condition to increase survival but none took into account fish behavioral skills. This suggests that alternative rearing approaches for sturgeons need more study considering that stocking for conservation is done on 19 of 25 sturgeon species (Table 1).

1.4. Post-release monitoring

Once individuals are released into the wild, monitoring is a vital component and the only means to evaluate the reintroduction outcome, successful or not, is through information regarding changes in the targeted populations, its development (population trends) and their impact on the environment (Sutherland et al., 2010; Acolas et al., 2011b; Gitzen et al., 2016). The data obtained allow developing adaptive management responses that will not only fit the program specific needs but also will help to improve current and future plans (Bearlin et al., 2002). Based on this, indicators related to population size and distribution as well as parameters influencing them are often used (Gitzen et al., 2016). In the long run, reintroduction's main purpose is to produce a self-sustaining (Robinson and Ward 2011) and persistent population (Seddon 1999), and these demographic qualities can only be assessed with long term monitoring (Gitzen et al., 2016).

For post-release monitoring, individual identification is needed to distinguish stocked from wild fish and marking techniques allows it (Taylor et al., 2005; Sutherland et al., 2010). Nevertheless, to access the information that marking can provide, most of the time, recapturing the specimens is also necessary. Therefore, a combination of marking and re-capture techniques, allow the acquisition of demographic and ecological information such as recruitment, survival, growth, health, feeding habits, etc. Marking can be achieved using a wide range of methodologies and done individually or in groups. The techniques used will depend on the specimen's characteristics, the environment, the type of information to be collected and economic resources available.

Telemetry technology is the automatic measurement and wireless transmission of data from remote sources to the receiving equipment (Bégout et al., 2016). It is one of the most efficient techniques for

individual recognition and can be used to evaluate the fate of the stocked fish to improve conservation actions outcomes. So, it has been widely used for post-release monitoring in conservation stocking programs (Ebner and Thiem 2009; Grabowski and Jennings 2009; Mohler et al., 2012; Thompson et al., 2016). It is a reliable means to study spatial ecology of fish (Lucas and Baras 2000; Cooke et al., 2004; Bégout et al., 2016) and it is used in several taxa (Dieperink et al., 2001; Ingraham et al., 2014; Horká et al., 2015). In sturgeons it has been previously used in stocked population monitoring to evaluate post-release survival (Parkyn et al., 2006; Rudd et al., 2014; Eder et al., 2015), habitat use (Jordan et al., 2006; Acolas et al., submitted), dispersal and migration (Secor et al., 2000; Neufeld and Rust 2009) mainly on large juveniles. Yet, nowadays telemetry transmitters tend to decrease in size which will allow monitoring of smaller individuals in the near future (McMichael et al., 2010; Ferrari et al., 2014; Lu et al., 2016).

1.5. The European sturgeon (*Acipenser sturio*)

1.5.1. Study model context

In Western Europe, the last remaining wild population of the European sturgeon (*Acipenser sturio*) is listed in several international conventions and directives as critically endangered (Rochard 2011) (Table 2). Thus, the species is strictly protected and a subject of the European recovery strategy (Rosenthal et al., 2007) along with national action plans in France (MEDDTL 2011) and Germany (Gessner et al., 2010b). In the eighteenth century the species had almost pan-European distribution and spawning was occurring in 196 basins (Lassalle et al., 2011) (Figure 1). By 1850 the species was present in 26 basins and by 1950 only in 18 (Lassalle et al., 2011). Currently, there is only one functional population in the Gironde basin in France and its two last reproductions in the wild were reported in 1988 and 1994 (Lochet et al., 2004). The European sturgeon is a long-lived and slow-growing species that can reach 3.50m in length (Magnin 1962; Lepage and Rochard 1995). The wild population effective size is estimated to be around 20–750 individuals as consequence of habitat loss and overexploitation that reduced the population to 10% (IUCN 2015). The age at first maturation is estimated to be around 10–12 years for males and 14–15 years for females and spawning interval is reported between two and three to four years for males and females, respectively (Magnin 1962; Rochard and Jatteau 1991). In virtue of these extreme life history traits common in sturgeons: large species, late age at the first maturation, long spawning intervals, low density and endangered status (Jager et al., 2008), studies and manipulation under control and *in situ* conditions are not straightforward. But, this species is a perfect model to study behavior and ecology under the reintroduction programs context.

Table 2. Conventions and directives listing *Acipenser sturio* (from Rochard 2011).

Name	Ratification (place/date)	Listing of <i>A. sturio</i> in the text
Convention on International Trade in Endangered Species of Wild Fauna and Flora. (CITES)	Washington, 1975	Appendix I means that CITES prohibits international trade in specimens of these species except for scientific research (http://www.cites.org/eng/resources/pub/checklist08/Checklist.pdf).
Convention on Migratory Species (CMS)	Bonn, 1979	Appendix I lists endangered migratory species (http://www.cms.int/documents/appendix/cms_app1_2.htm#appendix_I).
Bern Convention on the Conservation of European Wildlife and Natural Habitats	Bern, 1979	Appendix II lists animal species requiring special protection (http://www.coe.int/t/dg4/cultureheritage/nature/bern/default_en.asp), action plans go with this listing.
Council Directive on the conservation of natural habitats and of wild fauna and flora (CEE 1992)	Brussels, 1992	Appendix II lists animal and plant species of community interest whose conservation requires the designation of special areas of conservation (Natura 2000 network) (http://www.central2013.eu/fileadmin/user_upload/Downloads/Document_Centre/OP_Resources/HABITAT_DIRECTIVE_92-43-EEC.pdf)
Convention protecting and conserving the North-East Atlantic and its resources (OSPAR)	Oslo–Paris, 1992	The list of threatened and/or declining species (http://www.snh.gov.uk/docs/B469310.pdf) is to guide the setting of protection priorities by the parties.

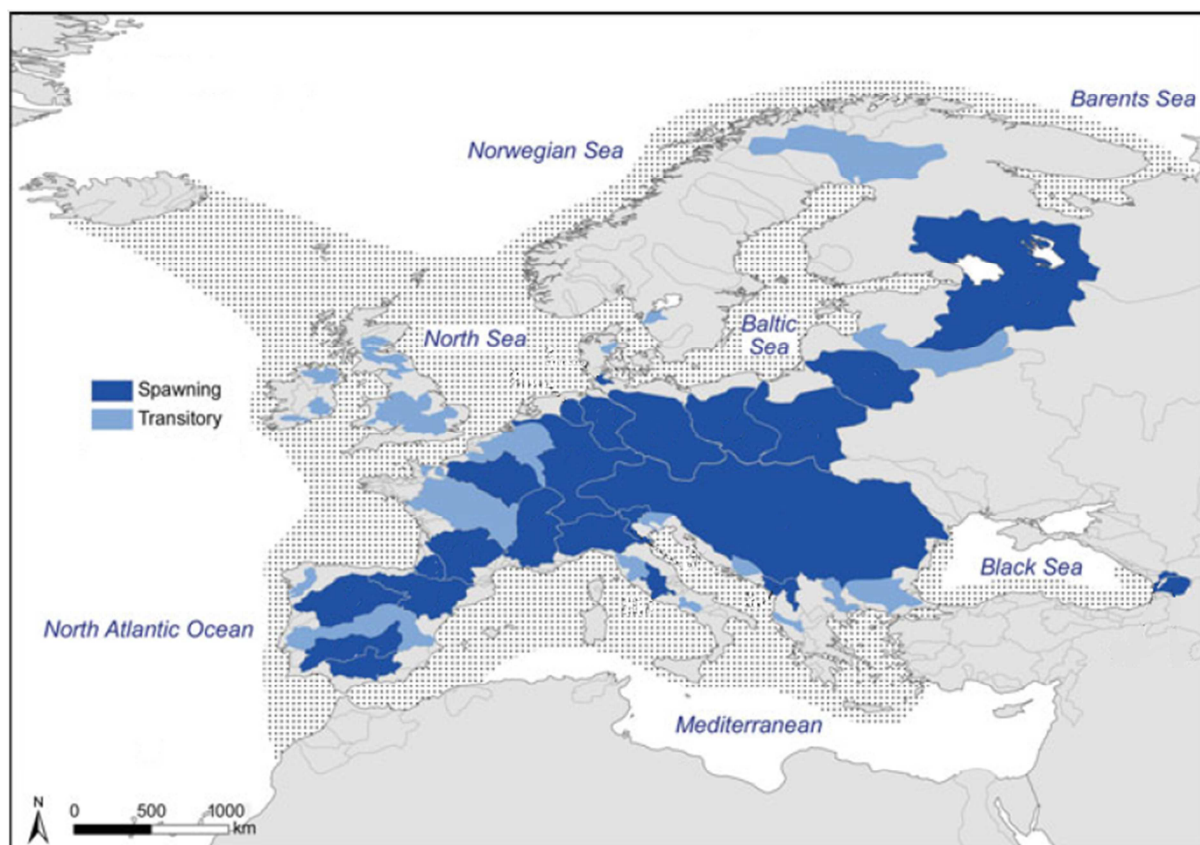


Figure 1: Distribution of *Acipenser sturio* during 1750–1850. Dotted area indicates the species marine distribution. The Nile basin and the Caspian Sea are truncated to ease visualization (adapted from Lassalle et al., 2011).

1.5.2. Life cycle

The species is anadromous. Reproduction occurs between May and June in rivers where it has been suggested that females mate with numerous males. Adults, return to the sea by the end of July (Magnin 1962; Castelnaud et al., 1991; Acolas et al., 2011a). The species early life stages are poorly documented but juveniles migrate to the estuary within the first year of life for further growth (Rochard et al., 2001; Acolas et al., 2011a). The estuarine phase is well documented (Taverny et al., 2002; Brosse 2003); after the second year of life, fish move seasonally between the estuary and the sea until they reach 3-7 years old to and then they become permanent sea residents (Castelnaud et al., 1991; Acolas et al., 2011a). Thus, most of the growth and the beginning of maturation take place at sea before they return to the rivers to spawn (Acolas et al., 2011a) (Figure 2).

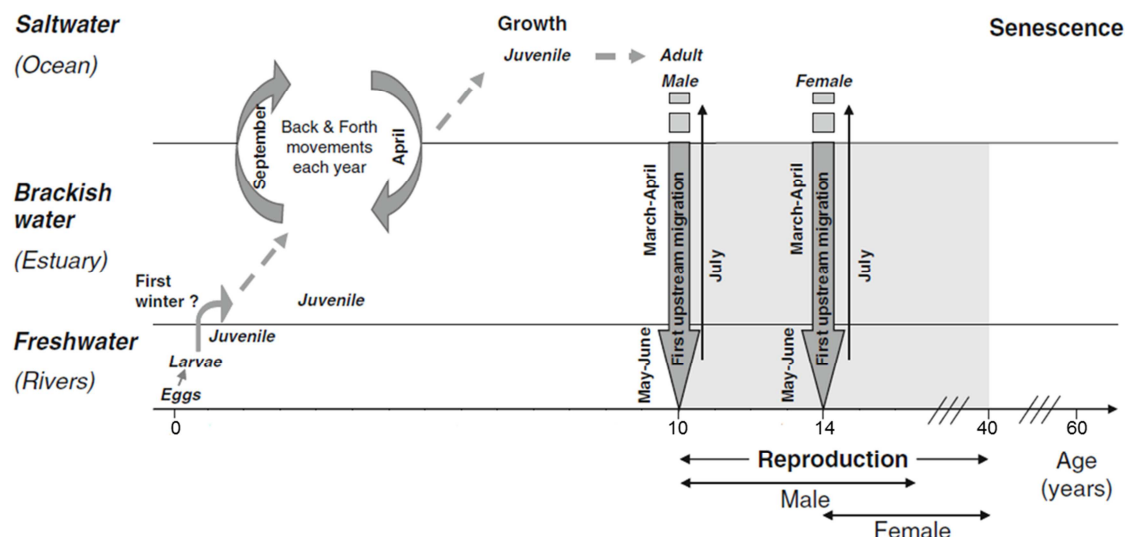


Figure 2: Biological cycle of *Acipenser sturio* (adapted from Acolas et al., 2011a).

1.5.3. The stocking program

Since the 1920s, the European sturgeon was exploited for flesh and caviar while its habitats were receding due to water dam constructions and gravel extraction in the Dordogne and Garonne Rivers (Rochard et al., 1990; Williot et al., 1997). By the 1970s, the species has disappeared from all the West Europe except France (Williot et al., 1997; Williot et al., 2009b) losing its commercial importance (Williot et al., 1997). Protection and conservation concerns lead to a total fishing, transport and trade ban in 1982 in France (Williot et al., 2009b). At the same time natural life history research on wild sturgeon and on artificial reproduction for stocking purposes started. The creation of a French captive stock started in the 1993, and the first successful artificial reproduction and larvae stocking was accomplished in 1995 (Williot et al., 2009b). Since then, the captive stock has been formed mainly by wild origin individuals from 1970s–1994 cohorts and hatchery produced fish of 1995 (Williot et al., 2009b). This broodstock has been supporting the wild sturgeon population by frequent larvae and juvenile stockings since 2007 (Acolas et al., 2016) (Figure 3). Up to date (January 2017), more than 1.5 million fish originated from assisted reproduction have been released into historical spawning grounds (Jego et al., 2002) in the Dordogne and Garonne rivers (Acolas et al., 2016). Most of the fish released are 7 dph larvae and 3-month old juveniles. Stocking of 1–2 years old fish and older is also done in lesser proportion to spread the mortality risk between different age classes (in total 3430 individuals between 2008 and 2015). Currently, the captive stock is composed of 6 females, 15 males and about 300 juveniles; the spawners number has drastically decreased in the last 4 years (39 females and 33 males in 2012 (Acolas 2013)). In addition to the French captive stock, another is held in Germany (Gessner et al., 2010a; Kirschbaum et al., 2011), which was built on hatchery reared individuals produced in France. The German stock has 6 broodstock fish of 1995 and

about 808 juveniles (Gessner 2017, pers. comm). With the aim of increasing the broodstock, individuals obtained from the different genetic crossings that are produced every year are kept and incorporated into the French and German captive stocks (Acolas 2013; Jatteau 2014).

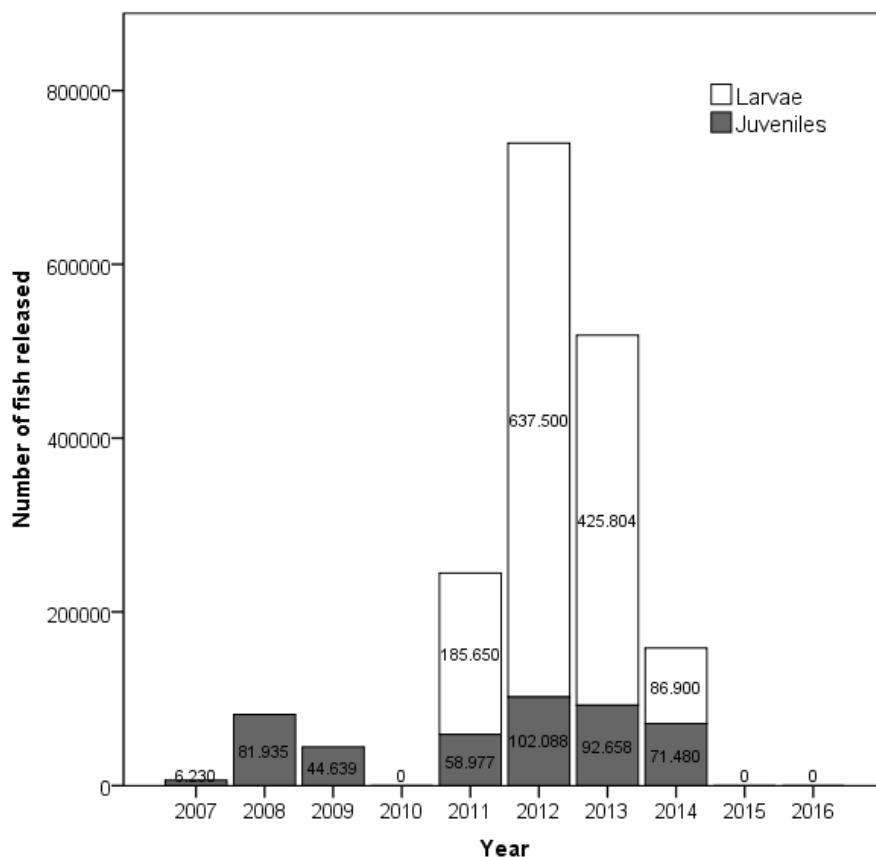


Figure 3: Hatchery fish produced by the European sturgeon stocking program since 2007. In years 2010, 2015 and 2016 artificial reproduction was unsuccessful and thus stocking did not occur (adapted from Acolas et al., 2016).

1.5.4. Monitoring the European sturgeon

The European sturgeon restoration program uses several monitoring approaches to assess the effectuation of the restoration plan: incidental capture declaration, standardized scientific monitoring in the estuary and specific studies (MEDDTL 2011; Acolas et al., 2016). For incidental capture, the program encourages the fishermen and the general population to declare sturgeon catches through awareness campaigns carried out by the fisheries association and a close partnership between managers and scientists to collect and analyze data (Rochard et al., 1997; Acolas et al., 2011a; Acolas et al., 2016; MEDDTL 2011). These data provide insights on fish distribution and habitat utilization which are consistent with the species life cycle (Acolas et al., 2016). To monitor the estuarine population since the recent stocking, trawling campaigns have been

used since 2009 (Acolas et al., 2011b) on the same line as the historical protocol that was carried out for the wild population (Rochard et al., 2001). The protocol is standardized, and the sampling occurs once every two months throughout the year in the mesohaline area of the estuary. Each sturgeon captured is measured, weighed and fin samples are taken for age estimation (Rochard and Jatteau 1991; Jatteau et al., 2011), parental assignation (Roques et al., 2016) and diet is analyzed through non-invasive methods (Brosse et al., 2002; Vega 2016). Besides, each fish is tagged externally (Hallprint tags), to ease recognition by fishermen, and internally (PIT tags) (Acolas et al., 2016). This specific monitoring allows evaluating health state of the estuarine population, demographic parameters, life history traits and to estimate stocking efficiency. The estuarine population is mainly composed of 2–3 years old fish (age range 1–7 years, mean total length 71.4cm) and the preliminary annual survival estimation at the age of two years old range between 5.5–8.9% for 2007 to 2009 cohorts (mainly released at the age of 3 months) and between 0.8–3.3% for 2011–2013 cohorts (released as larvae and 3 months old fish) (Acolas 2017, pers. comm). In the estuary the population density and spatial distribution has shown an increasing trend since 2014. Specific studies on European sturgeon aim at better understanding the species life history, the effect of environmental factors on their ecology and to evaluate the *ex-situ* practices. Recent studies include research in hypoxia and pollutant's tolerance in sturgeon of young stages (Delage 2015), migration patterns and habitat utilization of 1 year-old stocked fish (Acolas et al., 2012; Acolas et al., submitted), diet specificity and estuarine carrying capacity (Vega 2016), species repositioning within the climatic change scenario (Lassalle et al., 2010), phylogeography of the species (Chassaing et al., 2016) and experimental releases of older juveniles in the watershed where the species has disappeared were tested (Brevé et al., 2014). The *ex-situ* practices are assessed through the genetic characterization of the broodstock to optimize mate crossings (Roques et al., 2015) and to develop genetic markers for parental assignment of the recaptured individuals (Roques et al., 2016) as well as by analyzing the individual capacity of the juveniles produced (Acolas and Gesset 2014).

1.6. Thesis outline

The European sturgeon's only population left has been under protection over 35 years and has probably not reproduced naturally in the last 22 years. Consequently, wild offspring of this species does not currently exist. Conservation and stocking actions are probably the main factors why the species is still present today. Nevertheless, current conservation actions need to constantly seek ways to improve and evolve for a better fulfillment of the ecological needs of the species. Up to date, there are many gaps in early life history of the species that cannot be studied in wild fish but that are essential for population conservation. For example, it is expected that fish stocked years ago will

start reproducing in the years to come. Yet, ecology of the larvae and early juvenile's is not well known and improving this knowledge will allow conservation action plans to better meet the requirements of these stages. Moreover, the stocking will continue to occur for several years before the natural population could sustain itself and constant evaluation to improve these practices is a key factor to success. Currently, studying the stocked juveniles is the only mean to obtain such data as the program progresses. Therefore, assessing the rearing practices within the program is needed to understand their effects on the fish produced and thus, pursue, ameliorate or adapt the practices to the program's goals. The European captive stock is small and not all individuals are available for reproduction every year. Therefore, in my study, two males and one female were used leading then to the unforeseen insights of father influence on the offspring. In the restoration program, many fish are released at three-month-old and thus, the study focused on this particular stage. However, monitoring these early stages in the wild was not only limited due to lack of sturgeon's recruitment but also because the techniques to do so were not suitable for very young fish. In this study, recent miniaturization in acoustic techniques developed in other species (McMichael et al., 2010) was tested for the first time on a sturgeon species and thus allowed us to study young of the year European sturgeons in the wild.

In this context, the aim of my work was to assess different rearing practices and early performances of young sturgeons within the frame of the restoration program (Figure 4). Specifically, my study aimed to: 1) assess the effects of rearing on the survival, growth and behavioral performance of three-month-old sturgeons belonging to two different crossings under controlled conditions (chapter 2); 2) evaluate the feasibility of intraperitoneal tagging and effects on survival, growth, and swimming behavior in young-of-the-year Siberian sturgeon to be able to transfer the results onto protected sturgeon species (chapter 3); 3) obtain a better understanding of the species freshwater early stages and assess the effects of rearing on post-release behavior and survival (chapter 4). In chapter 5, the main results will be summarized and discussed on the grounds of the study's main hypothesis: enriched rearing practices can improve fitness-related traits in European sturgeon juveniles, specifically improve growth, survival and behavior. The results insights and limits will be discussed, and some perspectives will be proposed to better understand rearing practices for conservation purposes.

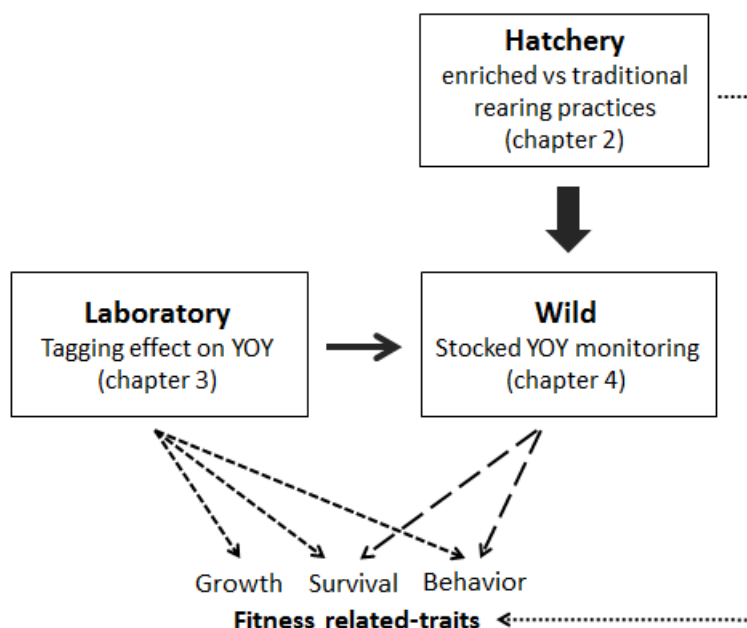


Figure 4: Scheme of the thesis outline showing the flow between the chapters that will be presented in this work.

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2

European sturgeon (*Acipenser sturio* L.) young of the year performance in different rearing environments – study within a stocking program.

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Foreword

Environmental enrichment has captured attention because increased environmental complexity could support the preservation of species characteristic phenotypes and behaviors as demonstrated in previous studies (Brown et al., 2003; Berejikian 2005; Näslund and Johnsson 2016). However, experimental studies in sturgeon species are lacking and therefore their importance in *ex situ* conservations programs are unknown.

Within this context, the first experiment focused on comparing fish performances in controlled set-ups: the traditional rearing practice used since 2007 to raise 3-month old European sturgeons for stocking in the Gironde and an alternative approach. It was decided to use modified tanks in an attempt to resemble wild conditions as much as possible. The premise was that fish will have better chances to survive if they are familiar with the diverse and variable environment they will encounter in the wild. Also, these “wilder” conditions will make release less abrupt for the fish. To evaluate fish performance linked to fitness many attributes could be evaluated such as fecundity (Murua et al., 2003; Riesch et al., 2012), size and number of offspring (Einum and Fleming 2000) and morphology and swimming capacity (Peake et al., 1997; Pakkasmaa and Piironen 2000; Smith et al., 2017). However this study focused on growth and survival because these traits can be easily measured in juvenile fish and are widely used in aquaculture to evaluate fish performance (Deng et al., 2003; Jalali et al., 2009; Falahatkar et al., 2012). Furthermore, studying behavioral traits was considered important as it's through behavior that an organism interacts with and adapts to the environment (Huntingford et al., 2012). Thus, this aspect should be taken into account in conservation programs. In the European sturgeon captive stock, few spawners are available each year and the number of offspring for experimentation can be limited, thus this work had access to the progeny of only two wild-born males and one female. So, in addition to the comparison between rearing methods, I was able to examine genotype effect also.

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2.1. Abstract

The European sturgeon is critically endangered and the French ex-situ conservation approach involves developing a captive stock to produce offspring for release to boost natural populations. The purpose of our study was to assess the effects of rearing environment before stocking on the survival, growth, and behavior of three-month-old sturgeons from two different crossings. Enriched rearing was designed to mimic the variability of the natural environment using river water, natural photoperiod, substrate, variable water current and depths. Traditional rearing was carried out with bare tanks, underground water, dark conditions, without current and at constant depth. Fish survival was determined monthly and growth was estimated weekly. Behavior was assessed with exploration and novel prey tests in solitary using video tracking. Results demonstrated that enriched condition resulted in bigger fish from the first month. Growth curve analysis revealed that enriched environment made both fish crossings grow in a similar manner. In contrast, crossings growth differed in traditional rearing which may reflect a genotype-environment interaction. Behavioral data highlighted that enriched-reared fish were slower to explore a new environment but more individuals engaged on doing so than traditional-reared fish. Results also showed that survival was high (>80%) during all the trial. However, survival was lower during the second month in enriched environment. Our findings advocate for the integration of enriched rearing practices within the juvenile production for release in order to boost the performance linked to fitness. Stocking practices and life history research must work together to favor adaptive aquaculture approaches which support species conservation.

Keywords: stocking, sturgeon, survival, growth, behavior, rearing conditions.

2.2. Introduction

Most of the 25 species of sturgeons are believed to be at risk (Birstein 1993; Billard and Lecointre 2001), and 72% of the family are considered “endangered” or “critically endangered” according to the IUCN status (IUCN 2015). Many species benefit from specific conservation measures, such as habitat restoration and protection, fishing regulations, and specific targeted stocking practices (Waldman and Wirgin 1998; Jackson et al. 2002; Arlati and Poliakova 2009; Maltsev 2009; Williot et al. 2009). The European sturgeon, *Acipenser sturio* (Linnaeus, 1758) is a critically endangered migratory species (Lepage and Rochard 1995; Lassalle et al. 2010; Chassaing et al. 2016) which is currently the subject of a European recovery plan (Rosenthal et al. 2007), along with national action plans in France (MEDDTL 2011) and in Germany (Gessner et al. 2010). The species has a complex life cycle involving migration periods associated with habitat shifts (Castelnaud et al. 1991; Rochard et al. 2001; Acolas et al. 2011a; Acolas et al. 2012). The reproduction occurs in rivers between May and

June, following which juveniles migrate to estuaries in the first year of their life. They then remain and grow in estuaries for several years. Sturgeons adopt a nomadic lifestyle by foraging within estuarine and marine habitats until finally leaving the estuary to further grow at sea before coming back to the river to breed. However, knowledge about young stages of this species in the wild is limited (Acolas et al. 2011b). The specific needs of this migratory species with regard to habitat, as well as vulnerability during migration, due to overfishing and habitat degradation are major factors involved in population collapse over the past few decades (Rochard et al. 1990). One species restoration action consists of developing a captive stock to produce offspring for release in river systems in an attempt to boost natural populations (IUCN 1987; Chebanov et al. 2011).

Elasticity analyses which calculate the potential to increase a population provided the highest values for the young of the year stage (Gross et al. 2002). Indeed, while this age-class suffers from the highest natural mortality rate, there could be a recovery of declining populations if vital rates such as growth, survival and migration capacities during early stages were increased (Secor et al. 2002). This aspect highlights the importance of hatcheries in conservation programs, since captive fish survival rates in early life stages can be significantly greater than those found in the wild (Secor et al. 2002). Therefore, stocking practices should consider knowledge provided by studies of early life history in order to develop creative and adaptive aquaculture approaches that will allow support for better species conservation (Chebanov et al. 2002; Secor et al. 2002). This type of research-based approach in aquaculture is a promising avenue for sturgeon conservation. Generally, major concerns of stocking for conservation purposes focused on the impact of genetic drift (Busack and Currens 1995; Campton 1995; Yokota et al. 2003), introgression (Susnik et al. 2004; Lamaze et al. 2012) and genotypic fitness (Zhu et al. 2002; Aprahamian et al. 2003; Jager 2005) on wild populations. Nowadays, rearing environment of hatchery fish for stocking purposes is a subject raising attention (Brown et al. 2003; Strand et al. 2010; Roberts et al. 2011; Bergendahl et al. 2016) because artificial environments may induce behavioral responses different from those expected in wild fish (Johnsson et al. 2014). Indeed, hatcheries often expose the fish to selective reproduction and early life experiences in a safe setting, which could strongly influence the behavioral (Brown et al. 2003; Klefoth et al. 2012), genetic, morphological and physiological attributes necessary to respond adequately to natural conditions after stocking (Brown et al. 2003; Braithwaite and Salvanes 2005). In other words, artificial selection enacted in hatchery conditions will produce fish suited to that environment, but which may be not well-suited to face life in the wild. Fitness value, defined as the ability of an individual to survive and reproduce in a particular environment (Maynard-Smith 1989), is difficult to quantify in nature (Arnold 1983). Instead, some traits, such as growth (Huusko and Vehanen 2011), swimming capacity (Adams et al. 1997; Adams et al. 1999), foraging abilities (Brown

et al. 2003; Massee et al. 2007), and the ability to detect and escape predators (Alvarez and Nicieza 2003; De Mestral and Herlinger 2013) are used as alternative predictors, which can be measured in controlled conditions (Arnold 1983). When comparing these fitness-related traits in hatchery and wild-born fish, the former often show behavioral deficiencies, such as reduced territorial and feeding efficiency (Aarestrup et al. 2005), reduced anti-predatory responses and social interactions (Salvanes and Braithwaite 2006), reduced reproductive success in the wild (Araki et al. 2008), risk taking behavior (Sundström and Johnsson 2001; Stamps 2007), increased vulnerability towards angling (Klefoth et al. 2012), than in wild fish. These deficits may lead to poor post-release survival (Aarestrup et al. 2005; Braithwaite and Salvanes 2005). However, exposing the fish to variability during early stages, also called an “enriched environment”, can reduce such deficiencies (Braithwaite and Salvanes 2005; Kotrschal and Taborsky 2010; Roberts et al. 2014). Enriched conditions are characterized by increased habitat complexity (Braithwaite and Salvanes 2005; Johnsson et al. 2014), often mimicking the instability of natural habitats in photoperiod, temperature, prey availability, visual and spatial cues, and could be considered as a “training” approach that would increase fish survival in the wild. Test on salmonids showed that exposure to such conditions increases the ability of fish to feed on novel prey (Brown et al. 2003) and increased survival after restocking in semi-natural conditions (Maynard et al. 1996). These results could be explained by the improvement of cognitive efficiency and behavioral flexibility as proven in other reintroduced animal groups (Hunter et al. 2002; Rabin 2003).

Studies using enriched rearing conditions need to be specifically designed for the fish species studied, because the ways they can be improved largely depend on the species’ sensitivity to environmental conditions, i.e. phenotypic plasticity. In previous studies, the majority of data obtained is related to species of commercial interest (i.e. salmonids), and were gathered under controlled conditions (Braithwaite and Salvanes 2005; Salvanes and Braithwaite 2006). However, there should be more research into the effects of enriched environment on fish performance (growth, survival and swimming capacities) under natural conditions and on other fish species. For fish species in reintroduction programs, as is the case of the European sturgeon, there is a real need to understand how rearing conditions of artificially reproduced larvae can affect life history and performance traits that will directly impact fish fitness once released into the wild (Chebanov et al. 2011). Fish performance is the product of a combination of environmental and genetic effects (Dammerman et al. 2015). The program’s European sturgeon broodstock is small, i.e. few numbers of spawners and not all fish are ready to reproduce every year, and breeding of genetic dissimilar individuals may not be enough to guarantee good offspring quality, since genetic quality (additive and non-additive genetic effects) is not assessed (Neff and Pitcher 2005; Pitcher and Neff 2007). Paternal effects on

early life history success may be more important than previously thought, and may also merit more extensive investigation (Rideout et al. 2004).

The aim of the present study was to assess the effects of enriched rearing on the survival, growth and behavioral performance of three-month-old sturgeons belonging to two different crossings. This particular life stage was chosen because it is one of the preferred stages used for stocking in the Gironde.

2.3. Methodology

Fish: Fish specimens used in this experiment came from French captive stock, and experiments were carried out at the Irstea experimentation station in St Seurin sur l'Isle. Fish for this study were raised between June and September 2014, from 6 days post-hatch (dph) until 92 dph (3-month old). Fish originated from assisted fecundation of a single female with two males (C1=crossing male 1, C2=crossing male 2) (Chèvre et al. 2011). These wild-born spawners were chosen based on availability and their characteristics are described in Table 1.

Table 1: Spawners characteristics

Sex	Cohort	Capture year	Time in captivity (years)	Weight (kg)	Total length (cm)
Female ¹	1994	2003	11	29.4	169
Male 1 ²	1994	1995	19	12.4	133
Male 2 ³	1994	2002	12	27.6	163

Fish ID: ¹3D49, ²041121E3C4, ³0411285AA5

Rearing conditions: Two sets of rearing conditions were tested: enriched and traditional. Two hundred and fifty larvae from each crossing were reared under each set of conditions (n=500 larvae per rearing conditions). During the first month, for each rearing environment, larvae from each crossing were reared separately in hatching tanks (raceways 135x50x22cm; length*width*height; water level=15cm, two raceways per rearing condition). Later, 50 fish of each crossing (n=100 per rearing conditions) were randomly chosen, mixed and reared together in larger tanks (2 replica per rearing condition) until they were 3 months old. To identify their parental background, larvae from both crossings were marked, beneath the rostrum, using visible implant elastomer tags (VIE) (Northwest Marine Technology Inc.) (Kapusta et al. 2015) at 1-month old (Photo 1). Based on a preliminary trial on 1-month old Siberian sturgeon (*Acipenser baerii*), the VIE tag was expected to last 2 months.

Traditional rearing conditions were based on the protocol used to raise fish for stocking in the Gironde and captive stock supplementation (Chèvre et al. 2011). In this experiment, we used tanks

(from month 2 tanks size 120x120x60cm, water level=40cm) run indoors in darkness ($0.43 \pm 0.51 \times 10 \text{ lux}$, mean \pm SD, Chauvin Arnoux luxmeter DA815) with a flow-through system of underground water (Photo 2). Tank conditions were maintained at a constant temperature of $18.4 \pm 0.2^\circ\text{C}$ and 30% of water renewal per hour. Temperature and oxygen (>90%) were recorded automatically once every 5 minutes with an automatic computerized system (WTW™ devices). Additionally, ammonium (0.1mg/l), nitrites (0.02mg/l), nitrates ($1.5 \pm 0.84 \text{ mg/l}$) levels (Visocolor® ECO test kit) and pH (7.7 ± 0.12) were registered weekly. Fish were fed *ad libitum* with live artemia (*Artemia salina*) since 9 dph for 12 days, a mix of artemia and unfrozen bloodworms (*Chironomus* sp.) for 9 more days and then only bloodworms as mentioned in Chèvre et al. (2011). Tanks were cleaned twice per day by flushing water away.

Enriched rearing conditions consisted of small mesocosms imitating the variability of the natural environment. Spatial cues were added and manipulated. Tanks were run outdoors to be subject to natural photoperiod, but covered with mesh to reduce brightness and strong light (natural= $346.6 \pm 634.0 \times 10 \text{ lux}$; under cover= $32.6 \pm 55.9 \times 10 \text{ lux}$) and avoid bird predation. One-month old fish were placed in 170x100x75cm tanks (2 replica, same density as in traditional rearing i.e. density= 0.2 fish/m^3) (Photo 3). A partition wall was added in the middle of the tanks to allow water current circulation using a water pump. The water supply consisted of a flow-through system of filtrated river water with the same water flow as in traditional rearing. Temperature ($21.5 \pm 1.5^\circ\text{C}$, Figure 1), oxygen (>90%), were recorded once every 5 minutes and ammonium (0.1mg/l), nitrites ($0.04 \pm 0.02 \text{ mg/l}$), nitrates ($8.83 \pm 3.31 \text{ mg/l}$) levels and pH (7.8 ± 0.08) were registered weekly. During week 6–8 of rearing, a mix of well and river water (1:1) was used to decrease water temperature due to the high records registered on week 3–5 (max. registered 25.8°C) (Table 2, Figure 1). Fish were fed in the same way as for traditional rearing. Landscapes were created using different elements, according to variability encountered in the river: sand (0–2mm), fine gravel (8–16mm), coarse gravel with pebbles (30–95mm) (standard A.S.T.M), logs (20–30cm), variable water current and depth (Table 3). These cues were modified once a week and combination levels were chosen at random (Photo 4). Tanks were cleaned twice per day by flushing water away.

The two types of rearing environment differed in terms of temperature regime (Mann-Whitney, $U=0.5$, $p<0.01$), nitrites (Mann-Whitney, $U=3.0$, $p<0.01$), and nitrates (Mann-Whitney, $U=0.0$, $p<0.01$), but had similar levels of ammonium (Mann-Whitney, $U=18$, $p=1$) and pH (Mann-Whitney, $U=2.0$, $p=0.63$) features.

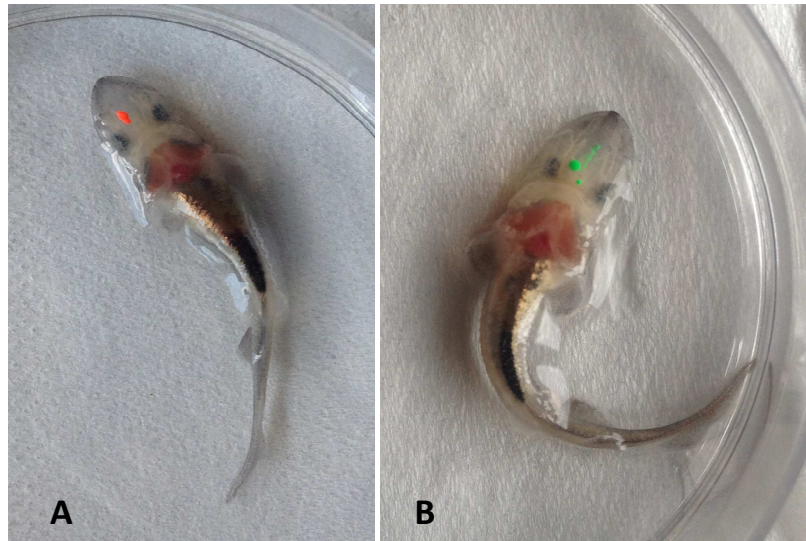


Photo 1: One month *A. sturio* marked with visible implant elastomer, dorsal view. A) Fish of crossing 1 and B) Fish of crossing 2. Fish size is approximately 3.5 cm.



Photo 2: Example of the traditional rearing set up. The rearing tanks in the photo do not represent the tank's size used in this study.



Photo 3: Enriched rearing set up.

Table 2: Temperature in enriched rearing condition.

Week	Min (°C)	Max (°C)	Daily mean (°C)	Standard deviation
1 (1 – 7 July)	18.5	21.6	19.9	0.7
2 (8 – 14 July)	18.8	22.0	20.8	0.5
3 (15 – 21 July)	20.0	24.9	23.1	1.1
4 (22 – 28 July)	22.6	25.8	24.1	0.7
5 (29 July – 4 August)	20.3	24.1	21.9	1.0
6 (5 – 11 August)	20.1	21.7	21.0	0.3
7 (12 – 18 August)	19.0	20.6	19.8	0.3
8 (19 – 25 August)	18.3	19.7	19.1	0.3
9 (26 August – 1 September)	19.3	22.7	21.6	0.7
10 (2 – 8 September)	20.5	23.1	21.9	0.6
11 (9 – 15 September)	20.4	23.1	21.9	0.6
12 (16 – 24 September)	19.8	23.0	21.8	0.7

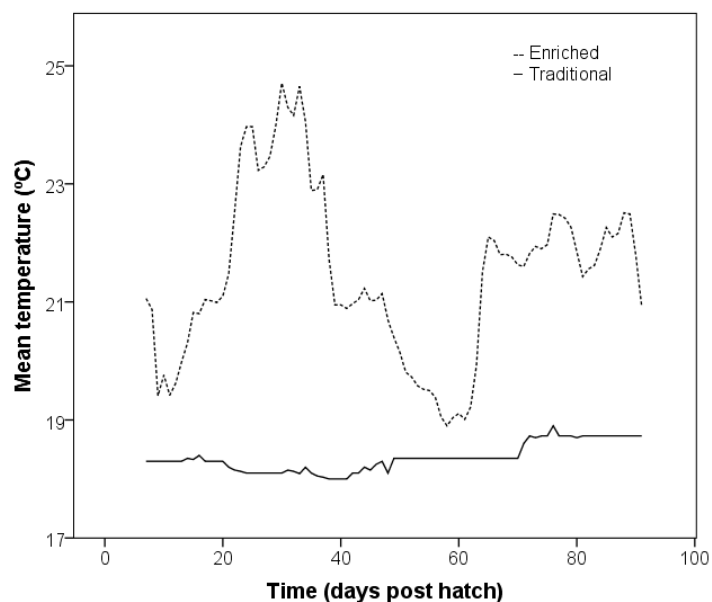


Figure 1: Mean temperature during fish rearing. Interrupted line indicates enriched rearing and continuous line traditional rearing.

Table 3: Landscape cues in the mesocosms

Water parameters			Landscape	
Level	Depth (cm)	Average velocity (cm/s) ¹	Logs	Substratum
1	0.70	8 ± 5	Present	Large/medium
2	0.57	6 ± 4	Absent	Medium/small
3	0.40	0		Large/small

¹water velocity varied across the water column and around the tank.



Photo 4: Enriched environment landscape arrangement. A) sand, gravel and logs; B) gravel and cobble.

Growth, survival and pigmentation: Each month, the total number of live fish per crossing per tank was counted to assess the mortality rate for each treatment (2 crossings x 2 rearing methods). Mortality was determined monthly as: $\text{Mortality (\%)} = (\text{number of dead fish} / \text{total number of fish}) * 100$. Survival probability for fish on each rearing method, at the end of the experiment, was calculated as the product of each month survival probability which was determined as: $\text{survival probability} = (\text{number of live fish} / \text{total number of fish})$. To assess growth and adjust the food ration, fish were weighed to the nearest 0.001g (Sartorius Praxum 513–1S) and measured for length (Mitutoyo Absolute Digimatic) individually (n=25 per treatment) every week from the second week of trial (not done before due to small larvae size) until 2 months. During the third month, fish were measured only once at the end of the experiment to avoid handling before behavioral tests. The heterogeneity index was determined at 92 dph as: $\text{Coefficient of variation (\%)} = (\text{Standard deviation} / \text{mean weight}) * 100$. Observations on fish pigmentation were done empirically as an unintended outcome of the study.

Behavioral tests: To assess differences in fish cognition, behavioral tests were carried out on randomly chosen 78 dph old juveniles. A subsample of the fish was used in the 2 different tests. The first set up was designed to evaluate fish exploratory behavior (n=35 per treatment): It was carried out in Komatex® (walls) and Plexiglas® (bottom) aquariums (90x50x30cm) divided into three

compartments (start and challenge chamber 20x50x30cm; novel chamber 50x50x30cm; Figure 2), in dark conditions and using either well or river water depending on fish rearing condition. Water in the experimental arena was replaced between every individual to eliminate chemical cues. First, fish were placed individually in the “start chamber” for 5 minutes. The divider was then removed to let the fish explore the “novel” and “challenge” chambers (both connected through a semi-circle passage of 4.5 cm radius) for 15 minutes. Behavior was video recorded (Ikegami ®) from above using EthoVision® XT 9 software (Noldus et al. 2001) using infrared light (Photo 5). The measured parameters were: latency to enter and swimming speed in the “novel chamber”, success (pass or not), frequency (number of successes) of movement into the “challenge chamber”, latency to first enter the “challenge chamber” and success in exiting from it. After the test, every fish tested was weighed and measured for length (up to 1 mm) to standardized swimming speed. The second behavioral test “novel prey” experiment was conducted after the exploration test for a subsample (n=20 per treatment). This test aimed to measure the ability of individuals to feed on unknown prey. Individuals were placed in separate glass aquaria (28x19x17 cm) in darkness, using water depending on their origin, and fasted for 24 hours (Photo 6). Afterwards, fish were provided with 10 live white worms (*Enchytraeus albidus*) and given 60 minutes to feed. Success to feed (food consumed or not) and amount of prey consumed per fish were recorded. The white worms came from a local aquarium store and were unfamiliar to the fish. We choose this worm species because it has been previously recorded in the stomach contents of wild juveniles (Acolas et al. 2011c).

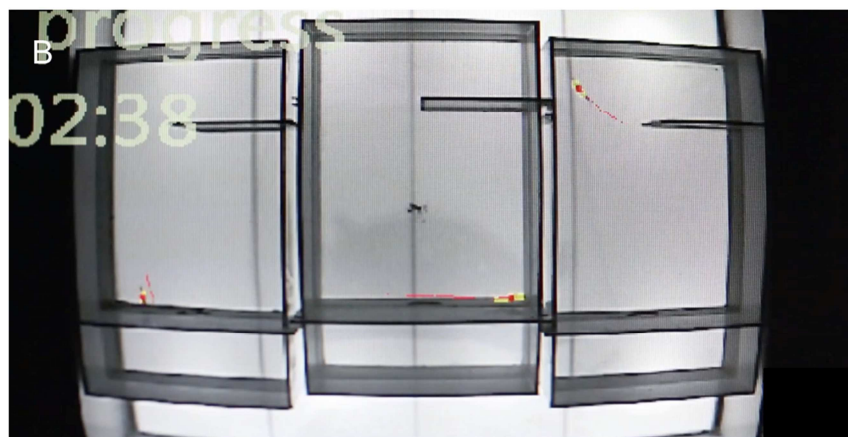
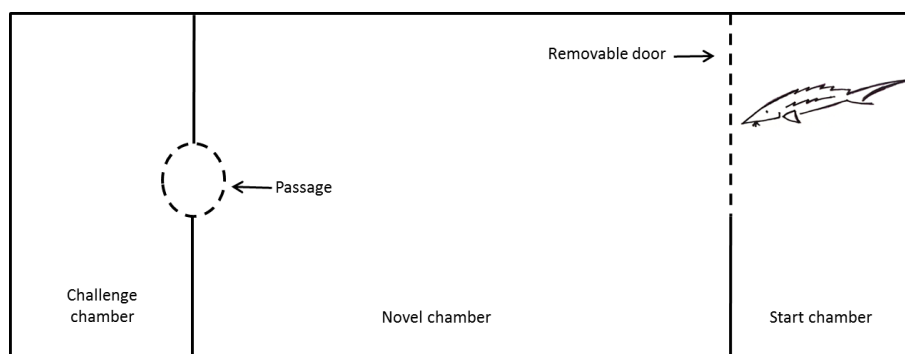


Figure 2, Photo 5: Behavioral experimental arena, 3-chamber aquarium. A) 3-chamber aquarium and B) computer view of the 3 experimental arenas, while actively recording. Red tracks represent fish in movement.

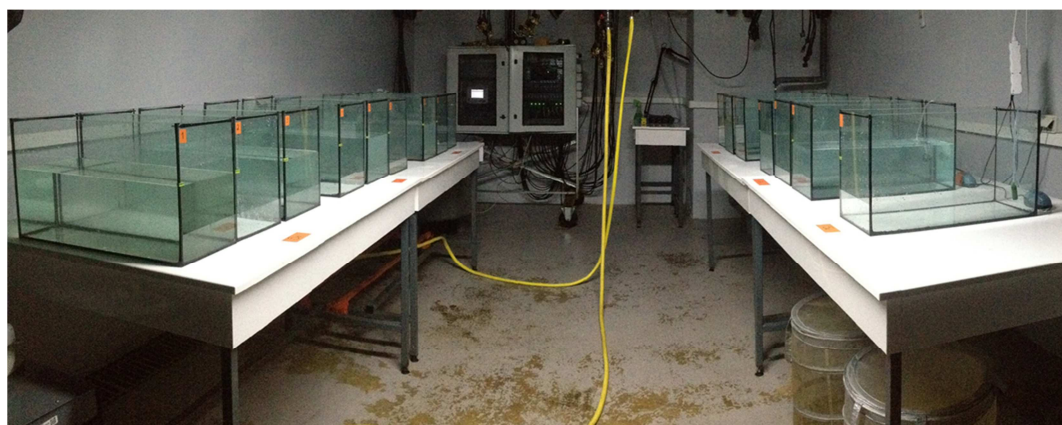


Photo 6: Space and arrangement of aquariums for novel prey tests.

Statistical analysis: The X^2 test of independence was used to explore differences in mortality between treatments, after determining no differences among replicas (i.e. tanks). A stepwise multinomial logistic regression model was used to assess the best predictors of growth (i.e. weight was used as proxy of growth) by testing the effects of the rearing method, crossings, replicas, and temperature. Analysis of variance and Mann-Whitney and Scheirer-Ray-Hare tests (when

assumptions on normality and homoscedascity were not met) were used to compare weight and length between treatments at 14, 35, 64 and 92 dph. In addition, growth for the different treatments was compared using general lineal models (GLMs) with a Gaussian distribution considering age in accumulated thermal units to compare fish at the same ontogenic stage. Behavioral discrete data were analyzed using negative binomial and binary regressions, continuous data using Scheirer-Ray-Hare test and behavioral data correlations were analyzed using Spearman correlations as assumption on normality and homoscedascity were not met. Statistical analyses were performed using R statistical software (R Core Team 2013) and p-values < 0.05 were considered significant.

2.4. Results

Survival: No fish during the experiment died or exhibited symptoms due to obvious bacterial infection or any other visible pathogen. During the first month of rearing, mortalities were observed from 12 dph of rearing in all treatments. The mortality rate during the first month varied between 12.8% and 18%, and there was no significant difference in mortality between treatments ($X^2=2.05$, $df=3$, $p=0.15$) (Table 4). For the second month of rearing, mortality in enriched rearing was 10.15% and significantly different from the value of 0.49% obtained in traditional rearing ($X^2=18.65$, $df=1$, $p<0.01$). In enriched rearing, a positive correlation was identified between temperature and fish deaths (Spearman, $rs=0.54$, $p=0.04$). Mortality in enriched rearing conditions decreased to 1.12% during the third month, and increased to 3.48% for traditional rearing, but no differences between both were found ($X^2=2.24$, $df=1$, $p=0.13$). Survival probability during all the experiment was higher for traditional-reared fish (0.8) than for enriched-reared fish (0.7).

Table 4: Rate of monthly survival according to treatment. Replicas have been pooled together because there were no significant differences between them. Different letters represent significant differences.

	Survival rate (%)	
	Enriched	Traditional
Month 1	83.8 ^a	87.0 ^a
Month 2	89.9 ^a	99.5 ^b
Month 3	98.9 ^a	96.5 ^a

Growth: For growth measurements, larvae were chosen randomly from all treatments and replicas data were pooled together because no differences between them were found. Initial weight and length were not measured because larvae were too small to be accurately measured alive. On this

basis, we assume no initial difference due to random sampling in the same hatching tanks for all treatments. The multinomial logistic regression model pointed out “rearing” and “crossing” as the best growth predictors explaining 82% of data variation (AIC=1950.6, Cox-Snell=0.82, Table 5), tank and temperature effects were not considered to be significant explanatory variables.

Differences in growth between rearing environments favored enriched rearing from 14 dph onwards ($H_2=59.7$, $p<0.01$). Both crossings had similar body weight (Mann-Whitey; enriched, $U=298.5$, $p=0.8$; traditional $U=281.5$, $p=0.6$; Table 6) and length (one-way anova; enriched $F(1,45)=0.85$, $p=0.36$; traditional $F(1,48)=2.87$, $p=0.09$; Table 6) within each rearing environment. By 35 dph until 92 dph, both crossings in enriched conditions performed similarly but their weight (Scheirer-Ray-Hare; method, $H=37.1$, $p<0.01$; cross, $H=11.4$, $p<0.01$; Table 6, Figure 3) and length (Scheirer-Ray-Hare; method, $H=42.5$, $p<0.01$; cross, $H=8.2$, $p<0.01$; Table 6) differed under traditional conditions. Moreover the final heterogeneity index was higher for traditional rearing (6 to 13% higher than in enriched condition). Further analysis to compare juveniles of the same ontogenic stage (age expressed in degree days), was consistent with results previously obtained: similar growth for both crossings enriched rearing (GLM, $p=0.81$, Figure 4) but different growth among crossings for traditional rearing (GLM, $p<0.01$, Figure 4). In traditional rearing, fish from crossing 2 showed similar growth performance to the fish from both crossings in enriched rearing (GLM, $p=0.06$, Figure 4) for the same ontogenic stage. In traditional rearing, crossing 1 had the lowest growth performance of all. In addition, empirical observations on pigmentation showed that fish coloration differed between rearing conditions from 14 dph: fish reared in enriched tanks were dark colored, while traditional fish were pale, although coloration was not precisely measured (Photo 7).

Table 5: Multinomial logistic regression models. Rearing refers to traditional or enriched rearing conditions, crossing refers to C1 and C2 parental background and tank to replicas.

Variables	Significant variables	AIC	p-value	Pseudo-square (Cox-Snell)
Rearing, crossing, tank, temperature	rearing, crossing	2628.69	0.00	0.83
Rearing, crossing, temperature	rearing, crossing	2304.31	0.00	0.83
Rearing, crossing	rearing, crossing	1950.58	0.00	0.83

Table 6: Growth performance of two crossings of juvenile European sturgeon (*A. sturio*) exposed to two different rearing conditions. C1 (male 1) and C2 (male 2) indicate the fish crossings. Letters indicate, for each line of the table, the comparison between treatments: different letters represent significant differences.

Parameter	Traditional rearing		Enriched rearing	
	C1	C2	C1	C2
Weight at 14 dph (g)	0.04 ± 0.0^a	0.04 ± 0.0^a	0.06 ± 0.0^b	0.06 ± 0.0^b
Length at 14 dph (cm)	1.9 ± 0.0^a	1.9 ± 0.1^a	2.1 ± 0.1^b	2.2 ± 0.1^b
Weight at 35 dph (g)	0.1 ± 0.0^a	0.3 ± 0.1^b	0.4 ± 0.1^{bc}	0.4 ± 0.1^c
Length at 35 dph (cm)	3.1 ± 0.3^a	3.5 ± 0.4^b	4.0 ± 0.4^c	4.2 ± 0.4^c
Weight at 64 dph (g)	1.5 ± 0.7^a	2.1 ± 0.9^{ab}	2.9 ± 1.2^c	2.7 ± 1.0^{bc}
Length at 64 dph (cm)	6.5 ± 1.3^a	7.2 ± 1.3^a	8.3 ± 1.2^b	8.2 ± 1.2^b
Final weight at 92 dph (g)	4.4 ± 2.0^a	7.2 ± 2.8^b	9.6 ± 3.0^c	9.6 ± 3.2^c
Final length at 92 dph (cm)	9.8 ± 1.5^a	11.5 ± 1.7^b	13.0 ± 1.3^c	12.9 ± 1.5^c
Heterogeneity at 92 dph (%)	46.7	39.9	31.9	33.7

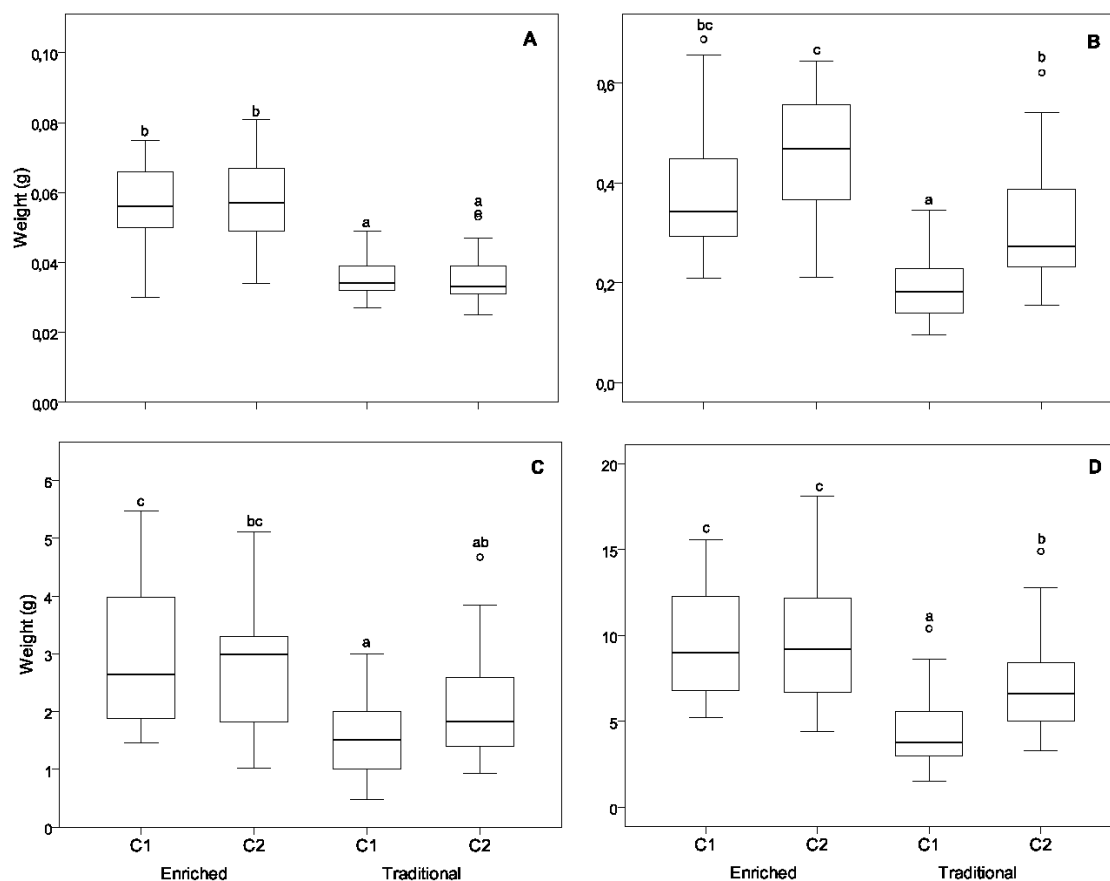


Figure 3: Box plot of juveniles weight of two crossings (C1 and C2) of European sturgeon at a) 14 dph, b) 35 dph, c) 64 dph and d) 92 dph reared in enriched and traditional conditions. Circles represent outliers, whiskers denote minimum and maximum values, box indicates interquartile range and line indicates median. Within each panel, different letters represent significant differences.

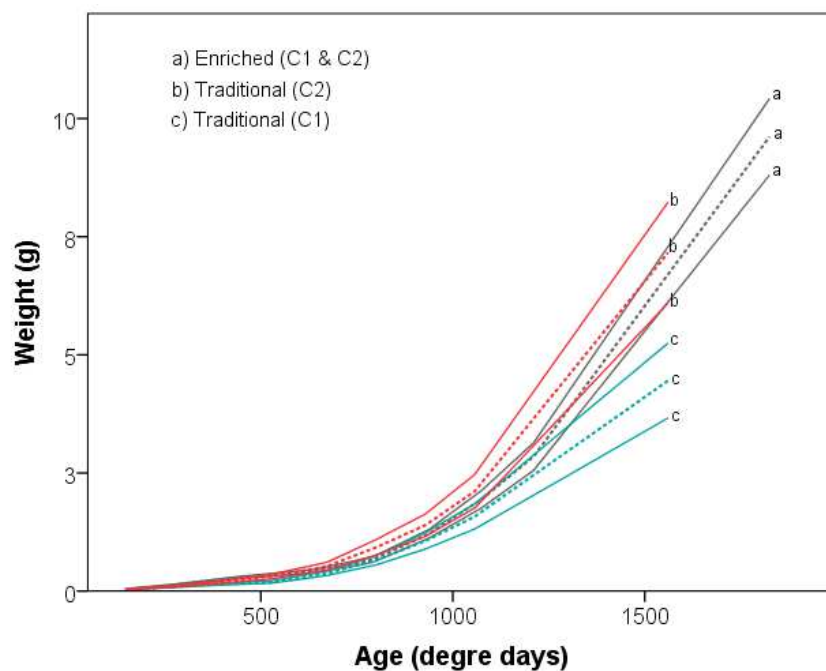


Figure 4: Juveniles growth of two crossings of European sturgeon (*A. sturio*) during 3 months (92 dph) of rearing in enriched and traditional methods. Interrupted lines indicate mean values and continuous lines 95% confidence intervals. a) indicate enriched rearing fish, both crossings plotted together, b) indicates C2 crossing in traditional rearing and c) indicates C1 crossing in traditional rearing.



Photo 7: Fish pigmentation of juvenile *A. sturio* reared under enriched and traditional conditions.

Behavior: During exploratory tests, fish expressed different behavior according to the rearing environment. First, no differences between crossing and rearing conditions were found in latency to enter the novel chamber (Scheirer-Ray-Hare; method $p=0.17$, cross $p=0.11$, interaction $=0.73$) and in swimming speed within this novel chamber (Scheirer-Ray-Hare; method $p=0.29$, cross $p=0.3$, interaction $p=0.14$). Fish reared under enriched conditions took longer to enter the challenge chamber for the first time (452 ± 243 seconds) than traditionally reared fish (437 ± 309 seconds) (Scheirer-Ray-Hare, method, $H=6.8$, $p=0.01$), but 88.4% of them engaged at least once into the challenge chamber against 67.8% of the traditionally reared fish (GLM binary logistic, $X^2=9.1$, $p=0.02$; method, $p<0.01$). Considering the fish that engaged in the challenge chamber, enriched-reared fish made an average of 1.53 entries while traditional-reared fish entered an average of 1.29 times. Also, 81.1% of the enriched-reared fish and 71.4% of the traditional-reared which entered the challenge chamber manage to get out of it. However, no significant differences were found in the number of entries (GLM negative binomial, $X^2=6.4$, $p=0.09$) or success to exit (GLM binary logistic, $X^2=6.2$, $p=0.1$) the challenge chamber between rearing methods.

In the novel prey test, 81.9% of fish ate at least one worm (mean 5.59 ± 3.84 worms eaten), consequently, no differences in feeding success (GLM binary logistic, $X^2=2.8$, $p=0.2$) or the number of worms consumed (GLM negative binomial, $X^2=3.5$, $p=0.3$) between treatment was found. The

amount of worms consumed was not correlated to fish size (Spearman, $r_s = -0.10$, $p = 0.35$). Further correlations between number of preys eaten and exploratory test parameters (latency to enter novel chamber, latency to enter challenge chamber and frequency to enter it) revealed a negative association between latency to enter to the challenge chamber and the amount of prey consumed for traditional reared individuals (Spearman, $r_s = -0.53$, $p < 0.01$) but no significant correlation was detected for enriched-reared individuals or other tested correlations on any rearing condition (Spearman, $p > 0.05$).

2.5. Discussion

When breeding fish for stocking purposes, it is important to optimize early stage survival in order to reduce the early mortality that would otherwise occur in the wild due to predation, starvation or disease. Fish survival gives a good initial quantitative insight into the efficiency of hatchery conditions from a conservation point of view. Observed early mortality was most likely produced by the adjustment from endogenous to exogenous feeding, as reported in other sturgeon studies at similar developmental stages (Charlon and Bergot 1991; Gisbert et al. 2000; Williot et al. 2005; Boucher et al. 2014). Following the exogenous feeding transition, mortality usually declines (Gisbert et al. 2000). In our experiment, for the first and the third month of rearing, mortality rates were similar between crossings and rearing conditions, which is a promising result for the enriched rearing approach. However, we highlighted a higher mortality in enriched rearing during the second month. This can be explained by the higher water temperatures observed during the summer time. Even though the precise optimal growth temperature for juveniles of *A. sturio* is unknown, 20°C have been reported to maximize survival, hatching and metabolism capacities on larvae of this specie (Delage et al. 2014). Deleterious effects on larvae have been seen between 23–26°C, (Delage et al. 2014). These results suggest that survival during the first three months can be impacted by temperature. Enriched rearing, as proposed in this study, can be a promising approach as long as the river water temperature can be maintained below 22 °C as high mortality was not observed below this temperature.

The growth of fish in rearing conditions is another key life history trait considered by aquaculture practice studies. During rearing of larvae, many biotic (parental effects, genetic background, density, hierarchical interactions) (Huntingford et al. 2012) and abiotic parameters (temperature, salinity, pH, etc.) (Stickney 2005) are intensively studied to create an optimal setup for fish growth. We found that growth performance of European sturgeon juveniles was influenced by crossing and rearing methods. However, temperature is one of the factors included in the “rearing method”. When

comparing growth, achieved in different conditions at the same number of dph, temperature variation could partly explain these differences, since temperature is known to have a significant influence on fish growth (Jobling 2002). Separating the effects of each parameter was not the goal of this study but to test how environmental heterogeneity could affect fitness-related traits instead. In our study, fish in enriched environment tended to grow better than those reared in traditional hatchery conditions. All the parameters used in enriched conditions were expected to have a positive impact on fish performance. In different species, it has been demonstrated that a minimal light threshold is required to develop and grow correctly (Boeuf and Le Bail 1999). In sturgeons, photoperiod treatment increases fish growth rates and weight gain compared with fish reared in darkness (Ruchin 2007; Ghomi et al. 2010); Kryuchkov and Obukhov 2010, as cited in Ghomi et al., 2010). Differences in fish growth in enriched rearing could also be explained by substrate occurrence. For example, white sturgeon larvae reared on substrate resulted in improved growth and survival and these effects remained after larvae were transferred to a bare environment (Boucher et al. 2014). Such observations have been attributed to reduction in fish activity and stress (Boucher et al. 2014) because substrate provides shelter. Better growth of enriched-reared fish can also be explained by exercise conditioning caused by water currents. Some studies suggest that the effects of training on fish significantly increase final weight, growth rates and food conversion efficiency (Young and Cech Jr 1993; Davison 1997). This is explained by an increment in muscular development and heart performance that can be beneficial in stocking programs because it might improve survival in the wild (Young and Cech Jr 1993; Davison 1997). Being bigger can also improve survival in the wild (Hutchings 2002; Wilke et al. 2014) because bigger fish have wider size ranges of prey items and have a lower predation risk than smaller fish (Juanes et al. 2002).

When analyzing juvenile growth at the same developmental stage we highlighted that one crossing performed differently depending on its rearing environment. This result might be explained by interaction between genotype and the environment. Based on hematological indices hatchery settings can be considered as unnatural and stressful environment (Ruchin 2007; Grant 2015). Exposure to stress can separate genotypes according to their capacity to cope with it (Wu et al. 2003), which can explain the differences in growth of both fish crossings and the higher heterogeneity in traditional rearing. Within the spawners, male 1 was the smallest with the longest time in captivity and the lowest growth performance in captivity (12.2 kg vs 27.6 kg for male 2 of the same age). This could suggest maladaptation to captivity conditions; a tendency which appears to be mirrored through its offspring which perform better in enriched environments (phenotypic plasticity) (West-Eberhard 2003; Monaghan 2008). To be able to conclude on this genotype-environment

interaction on European sturgeon, more families for common garden experiment studies are needed in the future.

In addition to the well-established parameters (i.e. survival and growth) directly assessed on fish during the rearing period, other characteristics could play an important role in their fitness once released in the wild. For example, body pigmentation may provide with good camouflage within the environment, reducing predation risk and increasing survival (Svanbäck and Eklöv 2011). In sturgeons, it is believed that body pigmentation is linked to behavioral traits and early life foraging and migration (Kynard et al. 2005). We observed differences in pigmentation that may be caused by the light differences between both methods they could have a significant influence on habitat-specific fitness of individuals after release. Another aspect to highlight is the homing behavior occurring in anadromous species (McDowall 2001; Metcalfe et al. 2002). Sturgeon is considered to have strong homing skills, although there are few data regarding their imprinting and homing processes (Bemis and Kynard 1997; Waldman and Wirgin 1998). Nevertheless, given the homing behavior of other migratory fish groups such as salmonids (Metcalfe et al. 2002), there is likelihood that hatchery-reared sturgeons may exhibit a higher straying rate than wild counterparts if the imprinting occurs at an early stage. In this regard, rearing fish using river water from the release-sites, as in the present study, may produce better-imprinted fish than those produced in well water that is not found in the wild. In addition, rearing on release-site water may overcome the introduction of parasite-naïve individuals that can fail to join the natural population due to local pathogens as have been documented previously (Work et al. 2000; Antolin et al. 2002).

Individual fish behavior may play an important role in fish survival in the wild. Indeed, individual response to challenging environments (e.g. stress coping style) which correspond to potentially risky situations can have a direct consequence on fish survival or growth by influencing fish physiological and behavioral state (Wingfield 2003; Øverli et al. 2007). Habitat heterogeneity promotes behavioral flexibility (i.e. ability to adjust their behavior to the new conditions) (Braithwaite and Salvanes 2005; Salvanes et al. 2007; Kotrschal and Taborsky 2010) and exploratory behavior (Nilsson et al. 1999; Zimmermann et al. 2001; Braithwaite and Salvanes 2005) required to deal with unpredictable environmental conditions as the conditions found in the wild. In our study, we found that more individuals from enriched rearing entered the challenge zone of our experimental device, when compared to the group of traditionally reared fish. This can be interpreted as more pronounced exploratory behavior within the individuals reared in enriched environment and corroborates previous studies where exploratory tendency of individuals could be a consequence of higher habitat

heterogeneity (Nilsson et al. 1999; Zimmermann et al. 2001; Meehan and Mench 2002; Braithwaite and Salvanes 2005). However, we found that fish from enriched conditions took longer to enter the challenge zone. This apparently paradoxical result can be explained by higher tendency towards environmental assessment developed under enriched conditions. Indeed, it has been suggested that environmental variability improves individual reaction to novel circumstances (Meehan and Mench 2002; Salvanes et al. 2007; Strand et al. 2010). In this case, the fish require longer time lapses in order to perceive the cues, assess them and adjust the behavior accordingly. In this perspective, we could associate the fish from different rearing conditions to behavioral strategies with different stress coping style, known to have a genetic background but also to be under high influence of early life environment (Frost et al. 2007; Chapman et al. 2010; Roberts et al. 2011; Jonsson and Jonsson 2014). In this case, behavior of enriched-habitat reared fish may be better suited to the conditions found in the wild. Environmental heterogeneity also increases phenotypic variation (Crossman et al. 2014) and this is a key feature that enhance population fitness and facilitates its establishment and persistence (Whitman and Agrawal 2009; Forsman 2014; Forsman 2015). Therefore, the success of stocking programs with conservation purposes relies on genetic and phenotypic diversity (Forsman 2014) that we can promote under adaptive aquaculture approaches.

When analyzing the results on the novel prey test, no significant differences between treatments were found. We assume that this is an outcome of similar feeding protocols in both rearing environments. In enriched rearing no cues were manipulated during feeding, and unpredictability on food supply has been shown to shape behavioral traits (Chapman et al. 2010). Also, it is possible that the fish motivational state were not enough to elicit different feeding behavior. Prey characteristics as color, movement, size, shape, fish stomach fullness (Croy and Hughes 1991; Gill and Hart 1994) and prey density (Ioannou et al. 2009) have an important role on foraging behavior and our test conditions may not match the thresholds required for our species to initiate differential feeding on novel prey. However a negative correlation between latency to enter the challenge zone and amount of prey consumed was highlighted on traditional-reared fish which is difficult to interpret with the elements we have and would require further experiments.

Overall, our results suggest that rearing conditions for fish early stages should be taken into account for stocking purposes. We found that enriched rearing conditions influence positively fitness related traits of fish such as growth and behavior. They would benefit from better growth but they would also exhibit more exploratory but cautious behavior when facing a new environment. We also suspect a genotype-environment interaction in favor of enriched rearing environment that would be promising to study on further research. These are important results that encourage enhancing fish

performance within restocking programs with enriched rearing practices.

Ethical approval

This study was carried out in an approved experimental hatchery facility by the French Department of Agriculture (authorization A33-478-001) and was revised by the regional ethic committee of animal use for scientific purposes.

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3

Tracking juvenile sturgeon in the wild: miniature tag effects assessment in a laboratory study on Siberian sturgeon (*Acipenser baerii*).

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Foreword

In conservation programs, post-release monitoring is crucial. Within the context of this restoration program, assessing the rearing practices outside the laboratory constrains will provide a better and more complete understanding of its effects on the fish produced. This type of evaluation will allow pursuing, ameliorating or adapting the practices to the program's goals. However, monitoring these early stages in the wild has been limited not only for the lack of sturgeon's recruitment but also because the telemetry techniques available were not suitable for very small fish. In the last few years, a new miniature acoustic tag has been available to monitor very young salmon. This technology provided the opportunity for further assessment of the effects of rearing practices in the wild and to obtain information on the early life stages in this species that is poorly known. This technology has never been used before in sturgeons thus, points out the relevance of doing feasibility study for posterior use in the program. Moreover it has constituted an opportunity to obtain surgical skills.

The experiment of chapter 2 allowed highlighting significant differences under controlled conditions for fish raised very differently. In 2014, the next step was to follow up the first experiment in the natural environment after release. Assessing the same individuals in controlled conditions and in the wild make this work have a very complete approach which is uncommon. However, to carry out the fish monitoring *in situ*, a way to monitor the fish in the wild is needed. Sampling constitutes an option as it is used in many species (Fjellheim et al., 1995; Chiasson et al., 1997; Crossman et al., 2011) with electrofishing, drift nets or gillnets but considering the risks to injure the fish and their low number limiting the probability to recapture them it was not considered. Acoustic telemetry technique seemed the most relevant considering the site configuration and the availability of the recent small tags.

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3.1. Abstract

Acoustic telemetry is commonly used to study movements of fish within their natural environment. Telemetry studies on sturgeons have focused mainly on large individuals; research on juveniles is scarce and tagging-effect studies on young-of-the-year are needed considering the species threatened status and their poorly known freshwater ecology. To study the feasibility of acoustic tagging in juveniles, a trial on Siberian sturgeon (*Acipenser baerii*) was carried out. The purpose of our study was to assess the effects of intraperitoneal acoustic tagging on survival, growth, swimming behavior and tag retention in young-of-the-year sturgeons. Fifty fish were tagged with dummy-acoustic transmitters (1.07x0.54x0.31cm, 0.28 g) and compared to 55 control individuals that were handled and anesthetized but not tagged. Fish ranged between 14–19.1 cm in total length (TL) and the tag burden for implanted fish was 1.3–2.6% body weight. Fish growth was estimated 15 and 30 days after tagging. Swimming behavior was assessed at 2, 7, 12, 21 and 26 days post-tagging using video tracking. All fish were also tagged with Radio Frequency Identification microtags (RFID microtags) to allow individual recognition during the trial. After one month of rearing, survival and tag retention rate of dummy-tagged fish were both 98%. Tag implantation had no effect on length or weight either 15 or 30 days after tagging. Specific growth rate was influenced positively by fish initial weight 15 days after tagging but this influence disappeared by day 30. Under stress, swimming performance was influenced by tagging: during stressful swim treatments, dummy-tagged fish moved greater distances and had higher swimming speeds at 12 days after tagging. Also, their swimming paths were straighter at 21 days after tagging compared to the pre-stress behavior. RFID microtag loss probability was 22.6% for control and 46.9% for dummy-tagged fish; RFID microtag loss was influenced by surgeon on dummy-tagged fish. These results suggest that loss rate of RFID microtags is acceptable when applied alone on juveniles of this species but the tagging procedure should be improved. Implantation of miniature acoustic transmitters can be successfully applied to young sturgeons as small as 14 cm TL and 10.7 g taking into account a possible higher sensitivity to stress during the first 21 days after surgery.

Keywords: acoustic transmitters, survival, growth, swimming behavior, RFID microtags, stress.

Introduction

3.2. Introduction

Sturgeons constitute a group of 25 species and most of them are considered to be under threat (IUCN 2015). Their conservation status and the lack of information in many aspects of their biology, life and evolutionary history make them a priority for research, management and conservation actions (Birstein 1993; Waldman and Wirgin 1998; Billard and Lecointre 2001; Williot et al. 2011). Improvement of species basic knowledge in their natural environment can be obtained by telemetry techniques. This approach is one of the most common and reliable means to study fish spatial ecology (Lucas and Baras 2000; Cooke et al. 2004; Bégout et al. 2016). Telemetry has previously been used for sturgeon species, mainly on large juveniles and adults, to characterize behavior such as movement patterns (Hall et al. 1991; Dionne et al. 2013), habitat use (Auer 1999; Erickson et al. 2002; Taverny et al. 2002; Sulak et al. 2009; Peterson et al. 2013), dispersal and migration (Foster and Clugston 1997; Neufeld and Rust 2009; Brevé et al. 2014). In order to obtain information through telemetry techniques, fish need to be identified individually and this lead to the development of a variety of transmitters and tagging procedures (Bridger and Booth 2003). Studies have revealed that fish physiological, behavioral and growth responses to tagging procedures are influenced by transmitter size and attachment technique and seem to be species-specific (Cooke et al. 2011; Crossman et al. 2013). In view of this, preliminary tests are needed to adapt the transmitters and tagging method to a particular species and life stage (Jepsen et al. 2002) in order to minimize adverse effects on the animal being studied. In sturgeons, surgically implanted transmitters with external antennas can injure the fish as the transmitter migrates inducing tag loss (Collins et al. 2002). Externally attached transmitters can reduce sturgeon swimming performance (Counihan and Frost 1999) and result in poor growth and survival (Johnson et al. 2014). Internal implantation of tags without anchoring has a highly variable retention rate depending on the sturgeon species (Crossman et al. 2013). Gastric implantation has shown high rates of expulsion as well as mortality (Neely et al. 2009). Finally, peritoneal implantation is the most recommended method (Neely et al. 2009; Crossman et al. 2013; Miller et al. 2013) because it causes fewest adverse long-term effects (Mulcahy 2003). Up to now, due to transmitter design, the smallest sturgeons tracked in the wild have been restricted to individuals of 9 months of age of 27–38 cm with transmitters weighing 2.9 g in the air (Acolas et al. 2012). To our knowledge, the smallest sturgeon successfully tagged in laboratory with telemetry transmitters (0.2 g in the air) were age-0 pallid sturgeon (*Scaphirhynchus albus*), 17–26 cm, which were either given surgical or external attachments (Johnson et al. 2014). Recent reductions in the size and weight of telemetry transmitters, as seen for the Juvenile Salmon Acoustic Telemetry System (JSATS) tags (McMichael et al. 2010), may enable monitoring smaller sturgeons movements for approximately 30 days (tag expected life). JSATS have been successfully used, without effects on

growth and survival, on juvenile salmon species of 9–9.9 cm and 6.8–12.4 g (Brown et al. 2010) but its feasibility and effects in young sturgeons is unknown. Miniaturization has been also developed on radio frequency identification tags (RFID). These tags have been successfully used on several taxa from small species to young individuals of larger ones, allowing long term individual identification studies (Moreau et al. 2010; Cousin et al. 2012; Ferrari et al. 2014; Ouedraogo et al. 2014; Podgorniak et al. 2015) without any impact on growth or survival for fish as small as 1.6–4.2 cm and 0.14–0.78 g (Cousin et al. 2012).

In Western Europe, the last remaining wild sturgeon population, the European sturgeon (*Acipenser sturio*), is strictly protected and subject to a European restoration action plan (Rosenthal et al. 2007). Fish are usually stocked at a young stage in rivers and means to assess the efficiency of the stocking program are required (UICN reintroduction specialist group, 1998). Most of the current knowledge on the species traits concerns their estuarine life phase i.e. 3 to 7 year-old fish (Rochard et al. 2001; Brosse 2003). The ecology of the young-of-the-year in rivers is largely unknown (Acolas et al. 2011b) and it's a priority to improve the species conservation efforts (Gross et al. 2002; Acolas et al. 2011b). Considering the protected status of this species and the low number of specimens available, assessing the tagging impact directly on European sturgeons is not suitable and a model species must be used. For our study, Siberian sturgeon (*Acipenser baerii*) was used as it has anatomical and physiological similarities to other sturgeon species (Boone et al. 2013), as well as being available in local hatcheries. This species is not protected under French regulations. The aim of the present study was to evaluate the feasibility of intraperitoneal tagging and effects on survival, growth, and swimming behavior in young-of-the-year Siberian sturgeons to be able to transfer the results onto protected sturgeon species.

In the present study, also RFID microtags were used to identify individual fish reared in common garden conditions to assess individual growth.

3.3. Methodology

Fish rearing: The fish specimens used in this experiment were 2-month old hatchery-reared Siberian sturgeons obtained from a local commercial hatchery. The fish ranged from 14–19.1 cm TL (16.3 ± 1.0 cm, mean \pm SD) and 9.9–22.1 g (15.0 ± 2.5). The fish ($n=105$) were raised between February and March 2014 at the Irstea experimentation station of Saint-Seurin-sur-l'Isle (Southwest France). Fish were reared in 2 cylindrical tanks of 471L capacity (100x60 cm; diameter*height) under common garden conditions (half dummy-acoustic tagged fish and half control fish in each tank considered as replicas). Tanks were indoors under 12L: 12D photoperiod but covered with mesh to reduce brightness (light under cover = 10 lux). Tanks were supplied with a 5 L min^{-1} flow-through system of

underground water and temperature was recorded automatically (Tinytag®) every half hour and maintained constant at 17.4 ± 0.5 °C (no significant differences between tanks, Wilcoxon test=475, $p=0.16$). Additionally, ammonium (<0.05 mg/L) and nitrites ($0.01\text{--}0.025$ mg/L) levels were kept below toxic levels (JBL® kit) and oxygen over 95% saturation (WTW™ multi 3430). Fish were fed at 2.5% body weight twice per day using 0.2 cm pellets (Coppens International®, Steco Pre Grower-14). Tanks were cleaned once a day by scraping the bottom and food leftovers were flushed away twice per day.

Fish tagging: Dummy acoustic tags (0.28 ± 0.2 g in air) were made-up using fishing lead immersed in molded epoxy resin that aimed to replicate the JSATS L-AMT-1.416 acoustic tags (Lotek®) which are $1.07 \times 0.54 \times 0.31$ cm (length*width*depth) and 0.28g in air (Photo 8). Fish had one week of acclimation period and were food-deprived for 24 hours before tagging which occurred on February 26, 2014. Sedation was done using clove oil (0.5 ml L⁻¹ of clove oil diluted in 5ml of ethanol 75% per 10L of water). Once anesthetized, fish were measured ($TL \pm 0.1$ mm) and weighed ($W \pm 0.1$ g). Control fish were $14.2\text{--}19.1$ cm TL (16.4 ± 1.1) and $9.9\text{--}20.8$ g (15.1 ± 2.6); dummy-tagged fish were $14\text{--}18.9$ cm TL (16.1 ± 1.0) and $10.7\text{--}22.1$ g (14.9 ± 2.5). Transmitters represented 1.3–2.6% of the tagged fish body mass in air. Fish were then placed on their backs in a V-shaped ruler and gills were regularly irrigated with water containing a half dose of anesthetic to maintain an anesthetized condition and keep gills moisted as described in Acolas et al. (2012) for older sturgeons. To allow fish individual recognition during the trial, RFID microtags (Nonatec™, diameter=0.1 cm, length=0.6 cm, weight≈0.01 g) were inserted in all individuals (control + dummy-tagged) (Annex 8). All equipment used during the tagging process was autoclaved and sterilized before surgery and disinfected between each fish. Tags were cleaned in ethanol and air-dried before use. Fish skin was disinfected before any incisions with a sterile pad with 10-time diluted hydrogen peroxide. RFID microtag insertion was done by piercing the abdominal cavity, slightly left of the midline and anterior to the pelvic girdle, using a 21 gauge needle (Terumo Neolus standard®) and the tag was pushed into the cavity as described in Cousin et al. (2012) (Annex 9). Dummy acoustic transmitters were surgically implanted ($n=50$) through a $\approx 0.7\text{--}0.8$ cm ventral incision, in anterior position to the RFID microtag insertion site (Photo 9). Incisions were closed with surgeon knots using absorbable monofilament (Ethicon®, PDS™ II 4–0). Antiseptic cream was placed on the closed incision and fish were transferred to a recovery tank (water without anesthetic) before being returned to their rearing tanks once they had recovered their balance. Control fish ($n=55$) were handled similarly to the dummy-tagged fish: sedated, weighed, measured and RFID-tagged but they did not received dummy transmitters neither suturing. Three surgeons carried out the tagging: surgeon A manipulated 41 fish and surgeons B and C manipulated 32

individuals. Fish biometrics (TL and W) did not differ between surgeons (ANOVA; W: $F(2,102)=0.15$, $p=0.86$; TL: $F(2,102)=0.06$, $p=0.94$) as well as the proportion of dummy-tagged vs control fish manipulated by each one ($\chi^2(2)=0.11$, $p=0.95$).



Photo 8: RFID nanotag (left) and acoustic dummy-tag (right) used to mark juvenile *A. baerii* (©Acolas ML, Irstea).

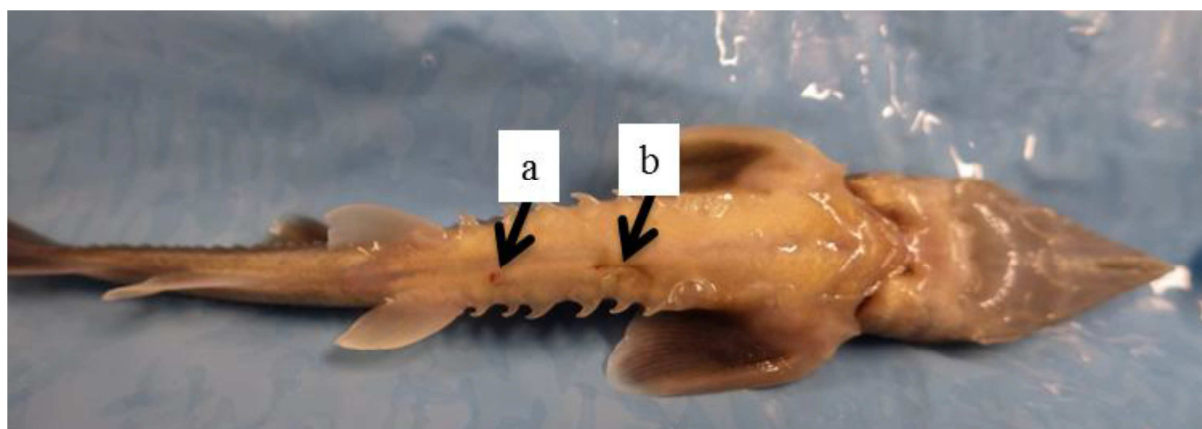


Photo 9: Insertion site of the RFID microtag (a) and implantation site of the acoustic dummy-tag (b) on juvenile *A. baerii* (©Acolas ML, Irstea).

Post-surgery monitoring: Fish mortality was monitored daily during tank inspection. To assess growth, fish biometrics (TL and W) were measured at 15 and 30 days post-tagging on fasted and sedated fish, as mentioned in the tagging procedure. In addition, wound healing, dummy-tag and RFID microtag retention rates were checked on these two occasions. To evaluate the influence of the tagging procedure (surgery + tag) on fish behavior, swimming activity was used as a proxy. For swimming tests, fish ($n=6$) were placed in solitary arenas (50x25x30 cm; length*width*height) at low light intensity (10 lux). Arenas had their sides covered to prevent fish seeing neighboring fish. Behavior was video recorded (Ikegami® camera) from above with an infrared light from below the

arenas using EthoVision® XT software (Noldus et al. 2001) to analyze swimming variables. Fish were filmed for 20 minutes: 10 minutes of acclimation and 10 minutes of testing. Within the test time frame, a stressor stimulus (bright light =110 lux) was applied after 5 minutes during 30 seconds. For each testing session, 12 tagged and 12 untagged individuals were observed. In total, 5 sessions were performed at 2, 7, 12, 21, 26 days after tagging. Selected variables, such as total distance moved (body length, BL in cm), mean swimming speed (BL/s) and mean turn angle (degrees, °/s) (i.e. amount of turning per unit time that quantify trajectory complexity (Benhaïm et al. 2012)) were measured every 10 seconds as the experiment progressed for each individual. At the end of the trial, all fish were euthanized by exposure to a sedative dose of clove oil first and then to a lethal dose of the same anesthetic according to the French legislation in force (act 02.01.2013 Art. R. 214-98). Afterwards, dead fish were dissected to determine the location of the dummy-tags in the intraperitoneal cavity and to detect possible lesions caused by it or by the surgery.

Data analysis: Survival was determined at the end of the trial as: $\text{Survival (\%)} = (\text{number of alive fish} / \text{total number of fish}) * 100$. After verification of parametric test assumptions, fish initial weight and total length were compared between treatments (i.e. dummy-tagged fish vs control fish) using a T-test of independence. A two-way analysis of variance (ANOVA) was used to assess the effect of the tagging procedure (dummy-tagged or not) and tank on fish growth (TL, W) between treatments at 15 and 30 days post-surgery. Specific growth rates (SGR) were assessed on individuals that retained their RFID microtags and calculated as: $\text{SGR (\%/d)} = 100 * (\ln W_2 - \ln W_1) / t$, where W_1 and W_2 were weight (g) at the first and last experimentation day respectively, and t was time (in number of days). SGR was calculated for the entire experiment duration and for the growth phase between days 1–15 (SGR_1) and between days 15–30 (SGR_2). Initial condition factor (K) was calculated as: $K = W / \text{TL}^3 * 100$, where W is fish weight (g) and TL is fish total length (cm). A logistic regression was used to estimate RFID microtags loss rate probabilities and to test the effects of tagging and surgeon. A stepwise linear regression model (GLM) selection by AIC was used to analyze the effects of tagging, tank, fish initial weight, initial total length and initial condition factor on the SGR at 15 and 30 days post-tagging. Then, a Pearson correlation was used to explore the relationship between SGR_1 and the explanatory variables of the model. Concerning behavioral data, as assumptions for parametric statistics were not met, Wilcoxon and Friedman tests were used. Wilcoxon tests were used to compare each swimming variable before and after the stressor for each group (control and dummy-tagged) separately and in each post-tagging time (2, 7, 12, 21, 26 days post-tagging) (Supplementary figure 1A). Friedman test was used to check for differences between fish groups before the stressor and another after the stressor in each post-tagging time (Supplementary figure 1A). Additionally, Wilcoxon test with

Bonferroni correction was used to compare each behavior variable along post-tagging time for each fish group and test frame (before and after the stressor) (Supplementary figure 1B). Statistical analyses were performed using IBM SPSS statistics 22.0 software (2013), p-values <0.05 were considered significant and the Bonferroni corrected p-value was <0.005.

3.4. Results

Survival and tag retention: Of the 105 individuals, 1 dummy-tagged fish died one day post-surgery. Its tag burden was 1.9% and no damaged organs were found after further inspection. Additionally, 2 control fish died within the last 3 days of the trial due to swim bladder disorder. Accordingly, survival rate was 98% for dummy-tagged fish and 96.4% for the controls. Fish dissection revealed that one individual lost its dummy-tag through a wound in the anus resulting in a retention rate of 98%.

Most RFID microtag losses (n=37) occurred through the insertion spot in control fish and through the dummy-tag implantation site on dummy-tagged fish within the first 15 days. This resulted in 35.6% loss rate at 15 days that reached a total of 41.2% loss rate (n=42) by the end of the experiment. RFID microtag loss rate was higher for dummy-tagged fish (46.9%) than for controls (22.6%). The probability of RFID microtag loss within the 15 days post-tagging was related to the interaction between dummy-tagging and surgeon (logistic regression, $F=5.6$, $df=2$, $p<0.01$, $n=102$) (Figure 1). Further analysis revealed that for dummy-tagged fish, surgeon was a significant factor influencing RFID microtag loss (GLM, $F=8.4$, $df=1$, $p<0.01$) but it was not the case for the control group (GLM, $F=3.6$, $df=1$, $p=0.06$). RFID microtag loss rate probability within the 15–30 days post tagging was not further analyzed because very few fish lost their tag on this time period (n=7).

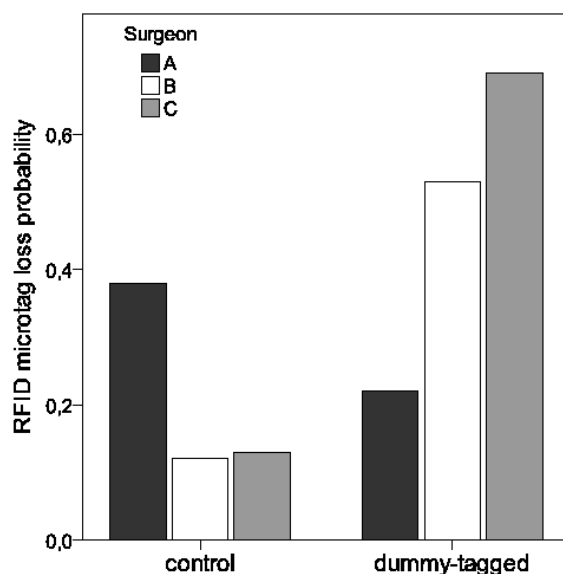


Figure 1. Probability of RFID's microtags loss according to surgeons for juvenile Siberian sturgeons (*A. baerii*) 15 days after implantation.

Healing: Fifteen days after tagging, most dummy-tagged fish had external wounds healed. However, three individuals still had wounds with separate edges and visible monofilament. Scars were hardly noticeable by the end of the study, yet 17 fish had slight hypertrophy at the incision site and 15 individuals had the monofilament still visible. Dissection revealed that 23 fish had mild tissue adhesions, one had an internal infection, another had hypertrophy inside the coelom and the remaining fish had smooth healed wounds (n=24) (Photo 10). Except for the dummy-tag which was not found and considered expelled -most of them were located close to the implantation area and free in the peritoneal cavity. RFID microtags were found at their insertion point except for one case in which it has joined the intestine.



Photo 10: Juvenile *A. baerii* showing completely healed wounds after 30 days of being marked.

Growth: At the beginning of the study, no significant differences in biometrics between the fish allocated as controls or dummy-tagged (W: $t(103)=0.49$, $p=0.62$; TL: $t(103)=1.26$, $p=0.21$) or between the tanks (W: $t(103)=-0.28$, $p=0.78$; TL: $t(103)=-0.67$, $p=0.51$) were found (Table 1). Tagging effects were not detectable on weight ($F(1,100)=2.46$, $p=0.12$) or length ($F(1,100)=4.0$, $p=0.05$) 15 days post-tagging; tank effects were not found either (W: $F(1,100)=0.39$, $p=0.54$; TL: $F(1,100)=0.02$, $p=0.90$) (Table 1). By the end of the study, no significant differences between treatment appeared (W: $F(1,99)=1.14$, $p=0.29$; TL: $F(1,99)=2.50$, $p=0.12$) but tank effects on weight did (W: $F(1,99)=4.53$, $p=0.04$; TL: $F(1,99)=2.92$, $p=0.09$) (Table 1). The mean SGR of fish ($n=39$ control, $n=24$ dummy-tagged) from the start until the end of the trial was $3.0\pm0.6\%$ per day for control and $2.9\pm0.5\%$ per day for dummy-tagged fish. The GLM revealed that fish SGR_1 was influenced by initial weight ($F(1,63)=10.6$, $p<0.01$) and SGR_2 only by tank ($F(1,55)=5.0$, $p=0.03$) favoring fish of tank 1 (Table 2, Figure 2). For both GLMs (SGR_1 and SGR_2), tagging, initial length and condition factor were not selected as explanatory variables by the model (Table 2). Further analysis showed that bigger individuals had higher specific growth rate during the first 15 days of the experiment (Pearson, $r=0.4$, $p<0.01$).

Table 1. Total length and weight evolution of juvenile Siberian sturgeons (*A. baerii*) after tagging. Time means number of days post-tagging. Letters indicate, within each time period and treatments, the comparison between treatments: different letters represent significant differences. 1 & 2 between brackets indicate the fish tank; tank's data were clustered when no differences between them were found.

Treatment	Control				Dummy-tagged			
Time (Tank)	0	15	30 (1)	30 (2)	0	15	30 (1)	30 (2)
Sample size	55	55	26	27	50	49	27	21
Weight (g)	15.1 ± 2.6^a	24.7 ± 6.4^a	39.7 ± 10.2^a	34.1 ± 10.7^b	14.9 ± 2.5^a	22.9 ± 5.9^a	36.4 ± 9.0^a	32.9 ± 10.0^b
Length (cm)	16.4 ± 1.1^a	19.5 ± 1.8^a	23.2 ± 2.4^a	22.1 ± 2.3^b	16.1 ± 1.0^a	18.8 ± 1.7^a	22.2 ± 2.2^a	21.6 ± 2.3^b

Table 2. Specific growth rate (SGR) modelling in juvenile Siberian sturgeon (*A. baerii*) after tagging. GLM test SGR in function of tagging, tank, initial weight (Wi), initial total length and initial condition factor variables. AIC means akaike information criterion and df is degree of freedom. The best model selected (bold) corresponds to the smallest AIC with the lowest number of parameters.

Model	AIC	df
SGR₁~ Wi	190.9	63
SGR ₁ ~ Wi + tank	189.6	62
SGR₂~ tank	181.5	55
SGR ₂ ~ Wi + tank	180.8	54

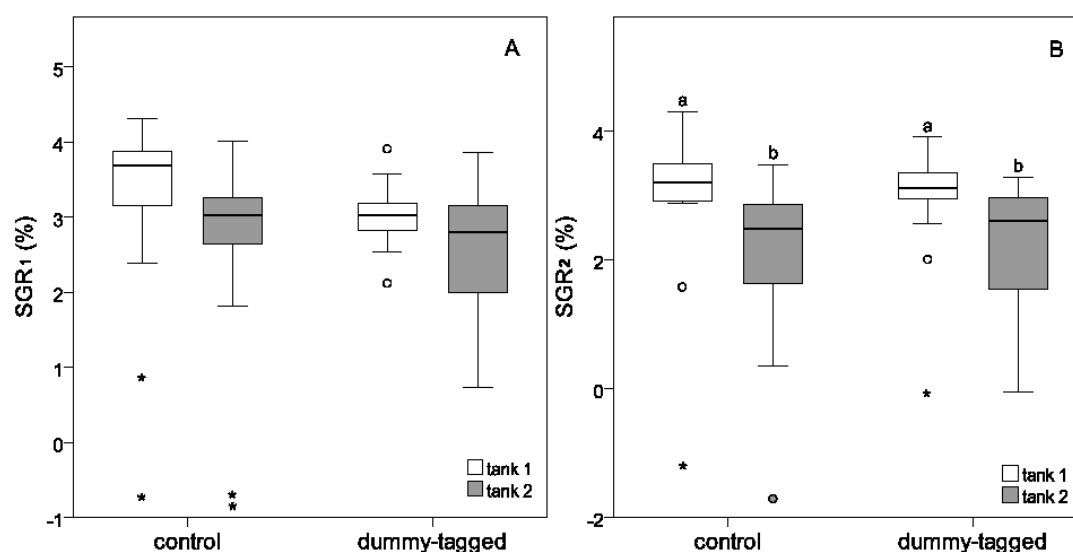


Figure 2. Growth performance of juvenile Siberian sturgeon (*A. baerii*) after tagging. A) specific growth rate between 0–15 days after tagging (SGR₁) and B) specific growth rate between 15–30 days after tagging (SGR₂). Asterisks represent extreme values and different letters indicate significant differences on the model explanatory variable “tank” for SGR₂.

Swimming activity: Swimming behavior in control fish did not differ in any of the variables measured: distance moved (body length, BL in cm), mean swimming speed (BL/s) and mean turn angle (degrees, °/s) before or after the stressor was applied at 2 days (W, $p>0.24$), 7 days (W, $p>0.12$), 12 days (W, $p>0.15$), 21 days (W, $p>0.72$) and 26 days (W, $p>0.18$) after tagging (Table 3). For dummy-tagged fish, distance moved (W, $Z=-2.27$, $p=0.02$) and speed (W, $Z=-2.48$, $p=0.01$) were higher under stress at 12 days after tagging and fish path (turn angle) were straighter also under stress (W, $Z=-2.22$, $p=0.03$) at 21 days after tagging (Figure 3). No significance differences were found for the remaining variables at 2 days (W, $p>0.53$), 7 days (W, $p>0.68$), 12 days (W, $p>0.14$), 21 days (W, $p>0.07$) and 26 days (W, $p>0.40$) after tagging. No effects on swimming behavior between

treatments were found before the stressor was applied (Friedman, $p>0.21$) or after (Friedman, $p>0.08$) regardless of the number of days after tagging. Moreover, no post-tagging time effects were found before (control, W, $p>0.008$; dummy-tagged, W, $p>0.005$) or after (control, W, $p>0.01$; dummy-tagged, W, $p>0.009$) the stressor on either fish group in any of the behavioral variables.

Table 3. Swimming performance of control and dummy-acoustic tagged juveniles Siberian sturgeons before and after a stressor stimulus was applied. Variables represent median and interquartile range (between parentheses) values of all the sessions examined combined between 2 and 26 days after tagging.

Variable	Control fish		Dummy-tagged fish	
	Before stimuli	After stimuli	Before stimuli	After stimuli
Distance moved (BL)	77.1 (44.2)	70.5 (51.9)	63.4 (41.2)	72.8 (35.7)
Mean swimming speed (BL/s)	0.3 (0.2)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)
Turn angle ($^{\circ}$ /s)	132.1 (69.0)	127.5 (50.1)	132.3 (66.0)	141.3 (50.1)

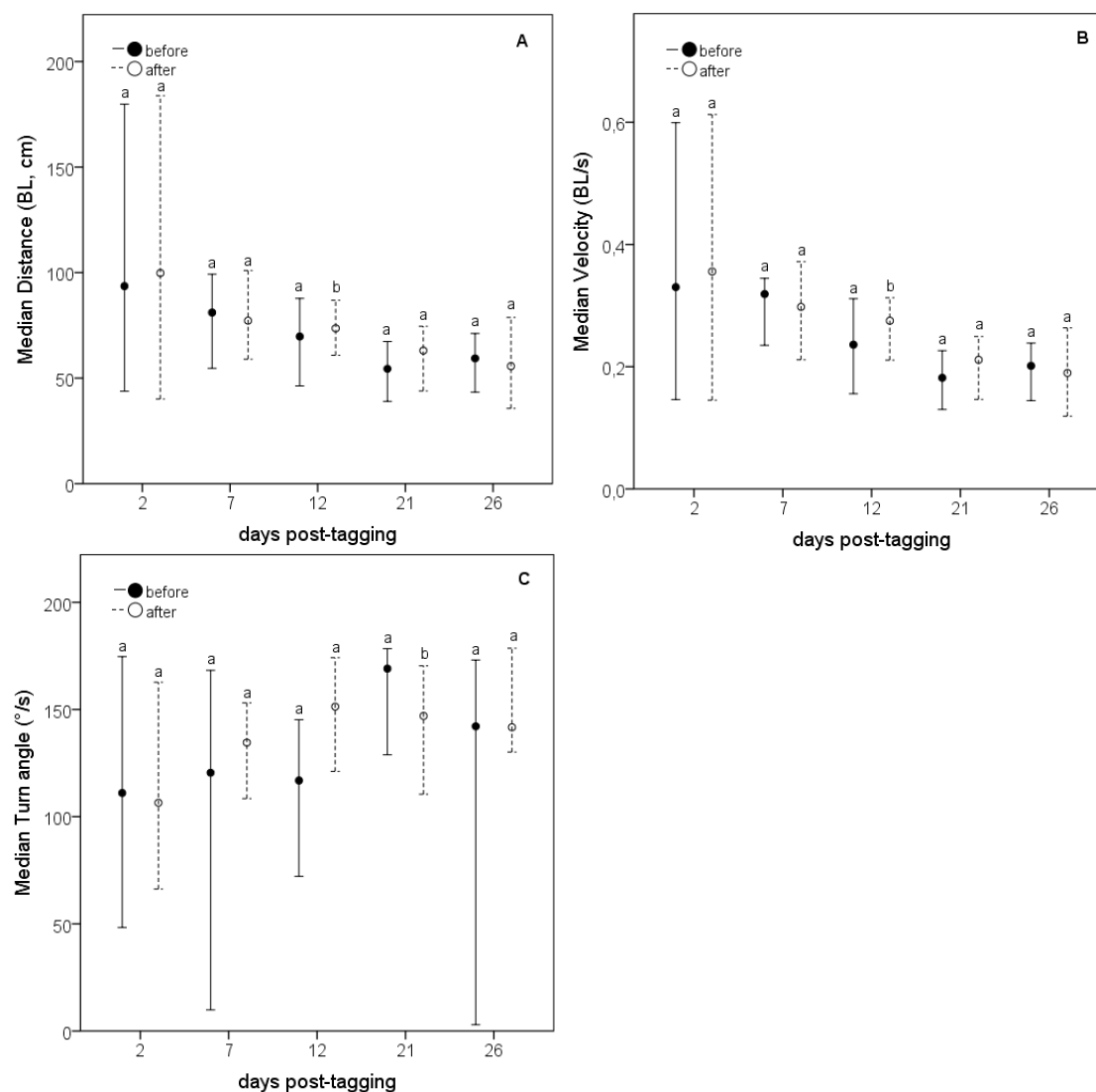


Figure 3. Swimming behavior of dummy-tagged juvenile Siberian sturgeon (*A. baerii*) before and after stressor was applied. (A) Total distance moved, (B) swimming speed and (C) turn angle during the experiment. Different letters represent significant differences within the same post tagging time.

3.5. Discussion

In our study, no differences in weight and length were found between dummy-tagged and control sturgeon during the experiment. In addition, fish growth in the first 15 days after surgery was influenced only by fish initial weight; bigger fish grew faster during those 15 days. These results suggest that the tagging procedure did not impact fish growth. However, initial weight influence, during the first half of the study, could suggest that bigger fish were less impacted by the whole manipulation than the smaller ones. The lack of tagging effects in our study are consistent with Miller et al. (2013) that found no tagging effects on green sturgeon but this study had no midterm evaluation. However, most tagging studies in juveniles of fish of other species found differences that

are noticeable for some weeks that disappear later on (Adams et al. 1998; Robertson et al. 2003; Neely et al. 2009). At 30 days, difference in growth between the tanks (replicas) was also found where tank 2 ended up with smaller fish. This tank was unintentionally placed under the room's air conditioning device which produced air flow over it and presumably disturbed the fish at feeding time. Although sturgeons are bottom dwellers, the fish used in this study come from lines that have been in captivity for generations and are therefore also accustomed to swim close to the surface. However, comparisons between dummy-tagged and control fish had the same trend in both replicas, which supports our results.

Our study revealed that dummy-tagged fish had different swimming behavior before and after the stressor. There are several approaches to study fish swimming behavior (Kawabe et al. 2004; Bolliet and Labonne 2008; Martin et al. 2012; Walker et al. 2016) ; critical swimming speed (U_{crit}) being the most popular practice for comparing tagging effects in fish swimming capabilities (Cote et al. 1999; Counihan and Frost 1999; Johnson et al. 2014). However, the goal of our study was to compare spontaneous swimming behavior between treatments before and after a stress stimulus. The methodology chosen, using video tracking, is simple to implement, can test multiple individuals at a time, and has been used successfully on other species (Milot et al. 2009; Benhaïm et al. 2012; Benhaïm et al. 2013; Ferrari et al. 2014). Using this approach, Ferrari et al. (2014) were able to demonstrate differences in swimming behavior between tagged and untagged juvenile seabass (*Dicentrarchus labrax*) and such differences disappeared 41 days after tagging. In our study, swimming behavior of young Siberian sturgeons was affected between 12 and 21 days after surgery after a stressor was applied, which may suggest a hypersensitivity to stress conditions. This effect does not appear right after the tagging procedure, probably because it is linked to the healing process and not to the manipulation itself. Similar effects on tagged fish have been found by Ferrari et al. (2014) and was suggested that fish anxiety after surgery could lead to higher activity under stress conditions (Bégout-Anras et al. 1998). In both, the Ferrari et al. (2014) study and our study, effects did not extend until the end of the trial suggesting lack of permanent effects and successful fish recovery.

As an unexpected result, our study observed higher loss rate of RFID microtags on dummy-tagged fish than for controls. This result can be explained by the fact that dummy-tagged fish had a nearby 0.7 – 0.8 cm opening, closed by 2 stiches, which facilitated the exit of the RFID microtag. Although, it was considered that both implantation sites were distant enough from each other. In addition, RFID microtag loss was also influenced by surgeon. In this study, the tagging procedure was done by three

surgeons with different expertise level suggesting that the implantation of the tags and wound closure were not always optimal. Surgeon performance, have been reported before as an significant factor on suture and tag retention (Deters et al. 2010) and important enough to be assessed on data analysis (Wagner and Cooke 2005). The RFID microtags loss of dummy-tag fish in this study was higher compared with loss rates in other studies while controls rates for two of the surgeons were similar. Expulsion rates of 11–28% have been observed in zebrafish (*Danio rerio*) of 1.6–4.2 cm and seabass (*Dicentrarchus labrax*) juveniles of 3.1–4.1 cm (Cousin et al. 2012; Ferrari et al. 2014). These two surgeons have probably inserted the RFID microtag deeper under the skin explaining the comparable loss rate between control fish and the literature. Also, justifying the higher loss rate they had on the dummy-tagged fish. The third surgeon, on the contrary, have probably placed the RFID microtag too superficially or very close to the insertion point which explains a higher loss rate for the control than for the dummy-tagged fish compared to the two other surgeons. The RFID micro-tagging of sturgeons of the size tested in this study is not recommended at the same time as an intraperitoneally tagging because the rate loss is high during the first 15 days after tagging. Although the rate loss diminishes after, the use of a smaller needle gauge or surgical glue to seal the insertion site could result in improved retention.

Conclusions

The high dummy-tag retention and negligible mortality obtained in this experiment suggest that intraperitoneal acoustic tagging is adequate and can be applied successfully to sturgeon juveniles ranging between 14–18.9 cm TL and 10.7–22.1 g. However, the influence of initial weight on growth within the first 15 days should be considered. RFID microtags can be successfully applied for this species but the procedure can be improved and it is not recommended at the same time as intraperitoneal surgical tagging.

Ethical approval

This study was carried out in an approved experimental hatchery facility by the French Department of Agriculture (authorization A33-478-001) and this study was approved (file 01738.02) and followed the standards of the national ethic committee of animal use for scientific purposes.

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Supplementary information

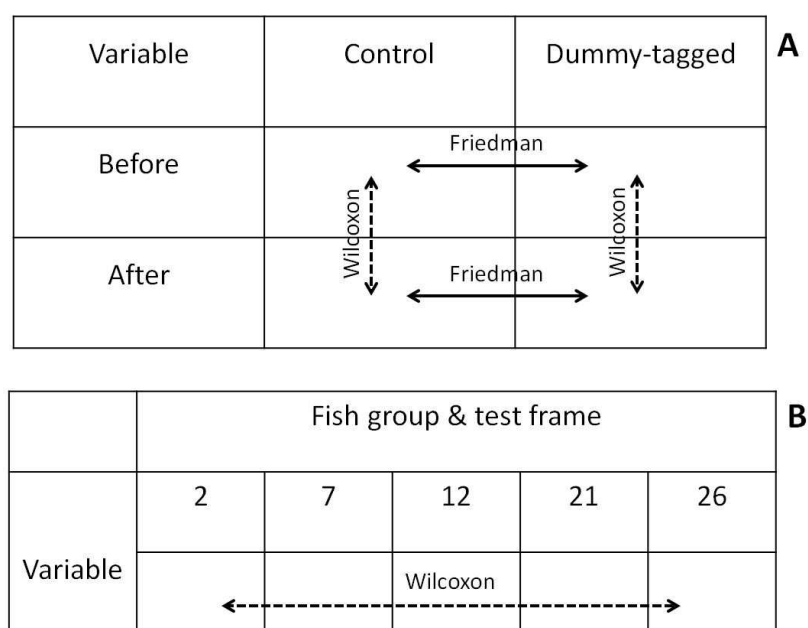


Figure 1. Swimming behavior analysis on 3 month old Siberian sturgeon. A) Wilcoxon tests were used to compare behavior (distance moved, swimming speed and turn angle), within each fish group, (dummy-tagged or not) before and after the stressor was applied. Friedman test were used to compare fish groups within each test frame (before and after stressor). B) Wilcoxon test were used to compare behavior among all the experiment duration (2, 7, 12, 21, 26 days post-tagging).

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4

Post-release young of the year behavior according to rearing practice: insights on *Acipenser sturio* early life in freshwater.

Submitted: Erika Carrera-García, Eric Rochard, Marie-Laure Acolas. Post-release young of the year behavior according to rearing practice: insights on *Acipenser sturio* early life in freshwater.

Foreword

In the context of this study, the assessed and endorsed tagging method allowed to observe the fish in the wild (chapter 3). Therefore, it was possible to evaluate the effects of rearing on the post-release behavior of young sturgeons. Moreover up to date, there are many gaps in early life history of the species while in freshwater and this study will help to acquire knowledge on this phase that is essential for population conservation.

For this last chapter, the juvenile sturgeons produced in the first experiment (chapter 1) were tagged and released in the Dordogne River. Fish are stocked since 2007 in the Garonne and Dordogne rivers. In total, 23 historical spawning grounds have been described and used as release sites for stocking. The Dordogne River release site was chosen, relatively upstream in the river, to assess the freshwater section as a first step in the monitoring. Despite the fact that following up the fish in both rivers would have been relevant, the material used is extremely costly and only allowed to survey one river. We choose the Dordogne River because we had some previous experiments in this area and then a good knowledge of this area. Lack of knowledge in freshwater offers a large possibility of specific study and, as a first step general movement's patterns according to diel were assessed.

4.1. Abstract

Stocking is an important tool for conservation that is gaining relevance as biodiversity continue to decline. The European sturgeon (*Acipenser sturio*) is a critically endangered species subject to a stocking program in which a better understanding of its natural life history and stocking achievement is crucial for population conservation. In this study, acoustic assessment on post-release behavior of 3-month old fish was carried out for the first time on a sturgeon species. We aimed to improve the knowledge of movement, distribution and survival of the species' early juveniles stocking. Fish belonging to two different crossings were reared in enriched and traditional hatchery conditions, tagged and monitored for 21 days in the Dordogne River, France. Fish overall survival estimate was 69.3% (52.2–90.9); the lowest survival was found for one crossing reared under traditional conditions. After release, most movements (85.7%) occurred during the first three days after stocking and were oriented in a downstream direction. During the whole study, 82% of the fish were detected within 13.5km from the release site. Three enriched-reared fish reached the tidal freshwater area (26 to 32 km downstream from the release site) but no one reached the saline zone of the estuary. Fish were mainly active during night hours, but traditional-reared fish were significantly more active during the day than enriched-reared fish during the first three days. Nevertheless, no differences in mean total distance covered ($7.5 \pm 7.0\text{km}$) were found between treatments. The study highlights the importance of post-release monitoring and early life rearing conditions on stocking programs for species conservation.

Keywords: Acoustic telemetry, stocking, behavior, sturgeon, rearing condition.

4.2. Introduction

The world's biological biodiversity is on decline and the most impacted habitats are those in freshwater (Dudgeon et al., 2006). To mitigate diversity loss a wide range of actions have been taken worldwide; fish species benefit from specific conservation measures such as habitat protection and restoration, fishing regulations and specific targeted stocking practices (Waldman and Wirgin 1998; Arlati and Poliakova 2009; Williot et al., 2009). Stocking is considered an important tool for conservation that is gaining relevance as biodiversity continue to decline (Reading et al., 2013). In stocking, the released individuals are often captive-born. This highlights the importance that aquaculture has on species conservation as survival in early stages can be significantly increased compared to those in the wild (Secor et al., 2002). However, there are major concerns regarding the quality of fish as a consequence of selective reproduction (Campton 1995; Zhu et al., 2002; Yokota et al., 2003; Susnik et al., 2004; Jager 2005) and early life experiences in artificial environments

(Braithwaite and Salvanes 2005; Salvanes and Braithwaite 2006; Johnsson et al., 2014) that may not produce well-suited fish to face life in the wild. Some studies (Braithwaite and Salvanes 2005; Johnsson et al., 2014) have suggested that enriched conditions, increasing habitat complexity could be considered as a “training” approach that would ease adjustment and increase survival after stocking. The European sturgeon (*Acipenser sturio*) is a critically endangered species (IUCN 2015) subjected to a stocking conservation program. All but one of this species populations have been extinct during the last century (Lassalle et al., 2011). This has resulted in scarce retrospective information available on traits of their natural life history that are indispensable for their population conservation (Acolas et al., 2011b). In this case, monitoring stocked populations is the only mean to obtain such data while the stocking programs progresses (Nichols and Armstrong 2012; Gitzen et al., 2016). The European sturgeon is an anadromous species; it reproduces in rivers and juveniles migrate to estuaries in the first year of life. They remain and grow in estuaries for several years until they migrate to sea before returning to the river to breed (Castelnaud et al., 1991; Acolas et al., 2011a). Most of the current knowledge on the species concerns their estuarine life phase (Rochard et al., 2001; Brosse 2003) but the species freshwater ecology during the first year of life is poorly known (Acolas et al., 2011b). Telemetry techniques are one of the most common and reliable approaches to obtain such data through an individual based analysis of movements (Lucas and Baras 2000; Cooke et al., 2004; Bégout et al., 2016). However, up to now, this technique has been restricted to large juveniles and adults (Kynard et al., 1995; Bramblett and White 2001; Lepage et al., 2005; Neufeld and Rust 2009; Sulak et al., 2009) and due to transmitter design the smallest sturgeon acoustically tracked in the wild was 9-month old *Acipenser sturio* (Acolas et al., 2012). Nevertheless, a recent telemetry system (Juvenile Salmon Acoustic Telemetry System, Lotek®) initially developed to acoustically track young salmonids (McMichael et al., 2010), allowed the use of miniature tags for 3-month old sturgeons (Carrera-García et al., 2017). In our study, post-release behavior assessment, on such small individuals, was carried out for the first time on a sturgeon species. We aim to improve the understanding of movement, distribution and survival on the species early juveniles within stocking practices. Therefore, we assessed the effects of two rearing conditions on post-release behavior and survival of juveniles belonging to two different crossings. This specific life stage was chosen because it is one of the most commonly used stages used for stocking in the Gironde-Garonne-Dordogne basin, France (MEDDTL 2011).

4.3. Methodology

Study area: This study was carried out in a 73km section of the Dordogne River, South-West France, between September 29 and October 20, 2014 (Figure 1). The river is influenced by the tide which

upstream limit is located 162.5km from the sea. In this solely freshwater area, the tide influences water depth and flow but not salinity. The fish were released in Le Fleix, a non-tidal river zone, known as an historical spawning site (Jego et al., 2002). The section surveyed comprised 27 km in non-tidal influence and 46 km under tidal influence. During the study, river flow was $106.2 \pm 19.4 \text{ m}^3/\text{s}$ (mean \pm SD) (HYDRO 2015). Temperature was recorded 3 times per minute using dataloggers (Tinytag®) placed in gates 5 and 9 of the studied river section (Figure 1). The registered temperature was $17.88^\circ\text{C} \pm 0.55$ (mean \pm SD) and similar in both gates (Mann-Whitney, $U=992108$, $p=0.05$). Additionally, every 30 minutes a multi-parameter water quality probe (YSI 6600 V2), located at the release site, monitored oxygen ($9.05 \text{ mg/L} \pm 0.55$), pH (7.73 ± 0.25) and conductivity ($173.61 \mu\text{S/cm} \pm 136.75$).

Fish origin and rearing: Fish originated from controlled reproduction of a single female with two males (C1=crossing male 1, C2=crossing male 2), all wild born spawners from the French captive stock (Williot et al., 2005). Fish were reared in captivity from 6 day-post-hatch (dph) until 92 dph (3 months old) using two rearing methods: enriched and traditional (Carrera-García et al., 2016). Traditional rearing was based on the protocol used to raise fish for stocking in the Gironde and captive stock supplementation (Chèvre et al., 2011). This method consisted in rearing fish in bare tanks with a flow-through system of underground water in darkness with no current. Enriched rearing consisted of small mesocosms imitating the natural environment using modified tanks under natural photoperiod with a flow-through system of river water, variable water depth, current and spatial cues (Carrera-García et al., 2016). Fish in both rearing methods were fed *ad libitum* with live artemia (*Artemia salina*) since 9 dph for 12 days, a mix of artemia and defrosted bloodworms (*Chironomus* sp.) for 9 more days and then only bloodworms as mentioned in Chèvre et al. (2011).

Fish Tagging: Acoustic transmitters were surgically implanted on 88 fish (Table 1). Tagging was done using JSATS L-AMT-1.416 acoustic tags (Lotek®; $1.07 \times 0.54 \times 0.31 \text{ cm}$, length*width*thickness, 0.28g in air, delay rate: 5 seconds). Fish were fasted for 24 hours before surgery and sedation was done using clove oil (0.5 ml/L of clove oil diluted in 5ml of ethanol per 10L of water). Following anesthesia, fish were measured for total length (range 12.0–16.3cm; $14.2 \text{ cm} \pm 0.9$) and weighed ($6.6\text{--}15.1 \text{ g}$; $10.8 \text{ g} \pm 2.0$) (Table 1). Transmitters represented 1.9–4.2% of the tagged fish body mass (Table 1). They were implanted into the peritoneal cavity using surgical procedure as described in (Carrera-García et al., 2017) through a $\approx 7\text{--}8 \text{ mm}$ incision, slightly left of the ventral midline and anterior to the pelvic girdle. Incisions were closed with 2 surgeon knots using absorbable monofilament (Ethicon®, PDS™ II 4–0). The fish were kept in captivity for 3 additional days for post-surgery recovery. Most fish

(n=65) were released on September 29, 2014. The remaining fish (n=23) were released on September 30. These individuals needed additional stitches because the wound opened (stiches were intact but torn in one side of the wound). The proportion of these fish did not differ between treatments (χ^2 (3) =2.91, p=0.40) and did not differ in survival estimation (χ^2 (1) =0.31, p=0.58) or mobility (MW, U=345.5, p=0.65) compared with the fish released the day before.

Table 1: Juvenile European sturgeon (*A. sturio*) tagged before stocking in the Dordogne River. C1 and C2 indicate the fish crossings. Letters indicate, for each line of the table, the comparison between treatments: different letters represent significant differences.

Parameters	Traditional rearing		Enriched rearing	
	C1	C2	C1	C2
Individuals tagged and released	23	21	22	22
Individuals detected in the wild	12	14	20	15
Recapture rate (%)	52.2	66.7	90.9	68.2
Weight (g)	9.4 ± 1.4 ^a	10.8 ± 2.0 ^b	11.8 ± 2.0 ^b	11.1 ± 1.6 ^b
Total length (cm)	13.4 ± 0.7 ^a	14.0 ± 0.8 ^b	14.8 ± 0.8 ^c	14.4 ± 0.6 ^{bc}
Tag burden (%)	3.1 ± 0.5 ^a	2.7 ± 0.5 ^b	2.4 ± 0.4 ^b	2.6 ± 0.4 ^b

Telemetry survey: Twenty-six receivers (Lotek®, JSATS WHS4000) were placed along the 73km river section (Figure 1). Receivers were placed about 0.8m above the river's bottom in homogenous areas to hang the material in a vertical position and to avoid shadow zones in tag detection. Preliminary detection tests were performed from a boat 3 weeks in advance of stocking. An activated tag was moved gradually away from a receiver. Detections could be obtained at 200m around the receivers but satisfactory detection range was considered at 100m (distance at which 79.4% of the theoretic transmissions (every 5s) were detected; detections also occurred each 10, 15 and 20s). Based on these tests results, each acoustic gate consisted of two receivers arranged according to river width. When river width was below 100m, the receivers were located one behind the other in the river longitudinal axis; when width was above 100m, receivers were located side by side transversally to allow detection range overlapping. River's mean width, in the studied area, was 150.2m and maximum width didn't exceeded 300m. Additionally, the receivers were checked once every week to ensure their correct position and location and cleaned to restraint dragging by biofouling. Data were collected once per week on 4 gates to orientate the active tracking (Figure 1). Active tracking was done two times per week with a hand-operated receiver from a boat and performed in the river

sections where fish were detected by the nearby gates. For each fish detected, its depth and the mid-column water flow were recorded. Each passive receiver and each fish localized in active tracking were geo-localized using a differential GPS (Magellan MobileMapper CX). Fish tracking lasted 21 days due to tag battery's life expectancy.

Data analysis: After verification of parametric test assumptions, fish initial weight, length and tag burden were compared between treatments (2 rearing methods x 2 crossings) using a two-way analysis of variance (ANOVA). Differences between the number of fish detected and the number of fish released for each treatment were expressed as percentage and used as a survival estimate because the released conditions and the tracking effort were similar for all treatments (same number of fish, same tracking period). χ^2 test of independence was used to compare this survival estimation between treatments. Logistic regressions were used to determine if fish survival could be explained by fish weight or tag burden at release for each treatment. To compare the total number of detections per fish between treatments, a Kruskal-Wallis test was used.

To analyze movement data, a database was constructed including both passive (99.8%) and active (0.2%) tracking data. Our acoustic data, obtained with the JSATS technology (frequency= 416 KHz), contained considerable amount of false detections. Thus, data required post-treatment using a receiver interface application (WHS Host, Lotek®) and R statistical software (R-Core-Team 2013). WHS Host application filtered the detections by known fish identification codes and using R software, we selected consecutive detections spaced of 5, 10, 15 or 20s which were considered as “true detections” according to the preliminary tests. Then the database consisted of date and time of detection associated to a location (either the passive hydrophone's geographical coordinates or the active tracking's) for each fish. Hydrographical distances between two consecutive locations and their orientation (upstream, downstream, lateral, no movement) were estimated using ArcGis 10.2 (ESRI 2013) and Anaqualand 2.0 (Le Pichon et al., 2006) software as in Acolas et al. (2012). We considered “no movement” to two consecutive locations of the same individual within the detection range of one receiver. Also, fish trajectories were smoothed using an estimation of fish swimming speed (maximum set at 0.8 m/s) between two consecutive locations as an approach to discern overestimated data, which mostly happened on “lateral movements”. It concerned individuals detected by the two hydrophones of the same gate, at the same time or within seconds of difference, for which the hydrographical calculations estimated distances as if the fish was going from one hydrophone location to the other. In this case, the “lateral movement” identified was considered as “no movement”. The speed threshold was calculated using the maximum speed that a three-month-old sturgeon (14.3 cm, average size in this study) can sustain according to a preliminary

current speed test (Jatteau 2014, pers. comm) and the average current's velocity estimated during the study (0.09 m/s). Day, night and twilight time were determined weekly using a sunlight phase web application (SunCalc, Agafonkin 2009) using the study date and location. Sunrise and sunset together were considered as twilight (i.e. 6h22–8h02 and 19h40–21h21, respectively). Movement data were analyzed according to two time scales: during the whole study ($n=21$ days) and during the three first days and remaining days separately. At each period, Scheirer-Ray-Hare and Mann-Whitney tests were used to compare total distance covered (i.e. the sum of the distances between consecutive locations for each fish) between treatments and within each diel cycle. Spearman correlation was used to test fish total distance moved and fish length for the whole study. Statistical analyses were performed using IBM SPSS statistics software (2013), p -values <0.05 were considered significant.

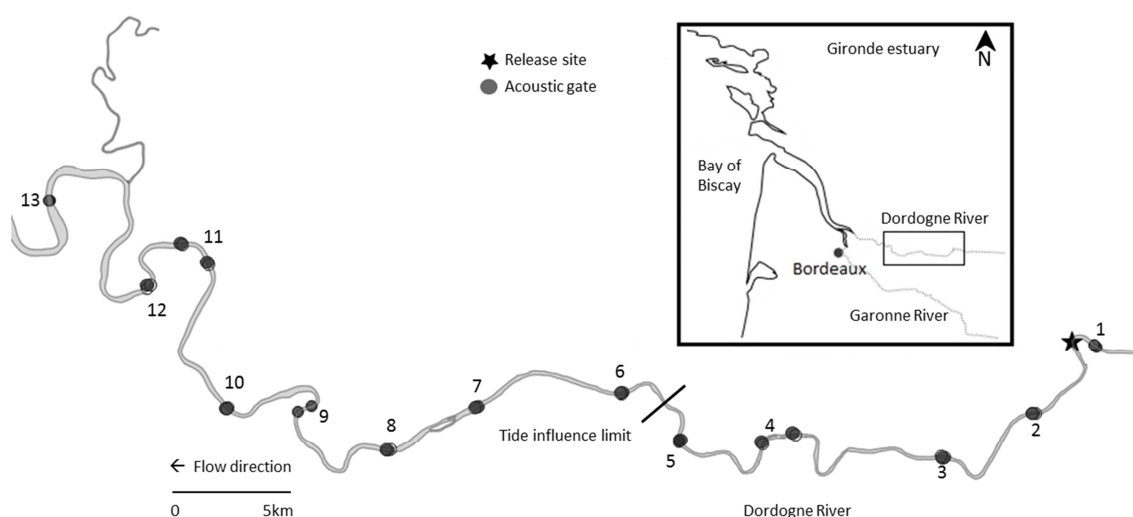


Figure 1: Study area in the Dordogne River, Southwest of France. The star shows the stocking location of 3-month-old sturgeons. Circles represent acoustic gates, each consisting in two receivers.

4.4. Results

Fish initial condition: Fish initial weight at tagging was significantly different between treatments (method, $F(1,84)=13.5$, $p<0.01$; cross, $F(1, 84)=0.89$, $p=0.34$; method*cross, $F(1,84)=7.78$, $p<0.01$) and similar results were found for initial total length (method, $F(1,84)=32.4$, $p<0.01$; cross, $F(1,84)=0.54$, $p=0.46$; method*cross, $F(1,84)=10.7$, $p<0.01$) and tag burden (method, $F(1,84)=14.98$, $p<0.01$; cross, $F(1, 84)=1.62$, $p=0.20$; method*cross, $F(1,84)=7.05$, $p<0.01$) (Table 1). Further inspection revealed that fish belonging to crossing C1, reared in traditional environment, were the smallest of all groups in weight (enriched, $F(1,42)=1.63$, $p=0.20$; traditional, $F(1,42)=7.27$, $p=0.01$), length (enriched, $F(1,42)=3.32$, $p=0.08$; traditional, $F(1,42)=7.80$, $p<0.01$) and had the highest tag burden (enriched, $F(1,42)=1.33$, $p=0.25$; traditional, $F(1,42)=7.19$, $p=0.01$) (Figure 2).

Survival estimates: Fish overall survival estimate (number of fish detected) was 69.3% (n=61) and it was significantly different between treatments ($\chi^2=8.08$, $p=0.04$). No differences were highlighted between the enriched-reared crossings (C1=90.9%, C2=68.2%) and traditional-reared C2 (66.7%) ($\chi^2=4.33$, $p=0.11$) but the traditional-reared C1 treatment had significantly lower survival than the other treatments (52.2%) ($\chi^2=8.21$, $p<0.01$) (Table 1). Survival probability was not explained by initial weight (logit reg., $p>0.08$), length (logit reg., $p>0.10$) or tag burden (logit reg., $p>0.09$) for any of the treatments.

General distribution patterns: The total number of detections per fish were highly variable; they ranged from 2 to 25878 (473.3 ± 838.4) detections and no differences between treatments were noticed (KW, $H(2)=3.03$, $p=0.39$). During the whole study, 82% of the fish (n=50; enriched: C1=14, C2=14; traditional: C1=12, C2=10) were detected within the first 13.5km (from the release site to half way beyond acoustic gate 3) and no differences in proportions of fish between treatments were found ($\chi^2=6.94$, $p=0.07$). Moreover, among these fish, 30% (n=15; enriched: C1=4, C2=2; traditional: C1=5, C2=4) settled within 1.2km downstream of the release site. From the remaining individuals, 13.1% (n=8; enriched: C1=2, C2=2; traditional: C2=4) moved downstream into the 13.5–26.5km river section (half way beyond acoustic gate 5) and only 4.9% (n=3; enriched C1=3) moved more than 26.5km, into the freshwater tidal area, but not beyond 32km (half way before gate 7). The 3 fish recorded in the freshwater tidal area were registered there 3 days after release. Most of the fish movement was oriented downstream (n=60, $7.88 \pm 7.02\text{km}$) with few lateral (n=4, $0.05 \pm 0.02\text{km}$) and upstream incursions (n=12, $0.58 \pm 0.88\text{km}$); only one individual (enriched C1) was detected upstream the stocking site one day after release. During active tracking alone, 35 fish were detected and 2 of them could not be located because of rapid signal attenuation. All the remaining fish (n=33) were found in areas of $3.3 \pm 1.2\text{m}$ in depth and $0.09\pm0.09\text{m/s}$ of current velocity.

Movement characterization

Whole study: During the whole study period, fish total distance covered did not differ between treatments (Scheirer-Ray-Hare, method, $p=0.87$; cross, $p=0.58$, method*cross, $p=0.17$; distance covered = $7.5 \pm 7.0\text{km}$). Within each diel cycle, no difference in total distance covered between treatments were found in diurnal (Scheirer-Ray-Hare, method, $p=0.06$; cross, $p=0.25$, method*cross, $p=0.67$) or nocturnal movements (method, $F(1,26)=0.44$, $p=0.51$; cross, $F(1,26)=0.16$, $p=0.70$; method*cross, $F(1,26)=0.94$, $p=0.34$). As very few fish were detected during twilight (enriched, n=11; traditional, n=8), data were only compared by rearing method and no differences were found (MW,

U=38, $p=0.66$). However, for all fish the distance covered was significantly different along the diel cycle (night vs day, MW, $U=282$, $p<0.01$; night vs twilight, MW, $U=112$, $p<0.01$; day vs twilight, MW, $U=365$, $p=0.02$); considerably higher during night hours than during day and twilight (Figure 3). Overall fish movements also revealed that bigger fish travel longer distances for both enriched-reared treatments (Spearman, C1, $r=0.57$, $p<0.01$; C2, $r=0.66$, $p<0.01$), but this relationship was not found for the fish reared using the traditional method (Spearman, C1, $r=-0.03$, $p=0.94$; C2, $r=0.29$, $p=0.33$) (Figure 4). According to the individual trajectory analysis (Figure 5), fish were moving mainly during the three first days after stocking, i.e. 85.7% of the whole distance covered on average, and they seemed to settle after those three first days as few movements were recorded later on.

Three first days and the remaining days separately: During the 3-day period after release, fish of the different treatments didn't differ in the total distance moved (Scheirer-Ray-Hare, method, $p=0.54$; cross, $p=0.45$, method*cross, $p=0.12$). However, during the day, enriched-reared fish were less active than traditional-reared fish as well as fish belonging to crossing C1 compared to those belonging to C2 (Scheirer-Ray-Hare, method, $p<0.01$; cross, $p=0.01$, method*cross, $p=0.62$) (Figure 6). Movements during night were not different between treatments (Scheirer-Ray-Hare, method, $p=0.82$; cross, $p=0.74$, method*cross, $p=0.66$; distance covered = $7.9 \pm 5.2\text{km}$) and at twilight, movements were scarce and concerned few fish (enriched, $n=11$; traditional, $n=8$) where no differences were found between rearing methods (MW, $U=38$, $p=0.66$; distance covered = $4 \pm 2.2\text{km}$). When comparing distances achieved during the remaining days after stocking, we didn't find any differences between fish treatments in total distance moved during this period (Scheirer-Ray-Hare, method, $p=0.79$; cross, $p=0.72$, method*cross, $p=0.74$), during day (Scheirer-Ray-Hare, method, $p=0.89$; cross, $p=1.00$, method*cross, $p=0.59$) and analyses on night and twilight movements were not done due to insufficient data.

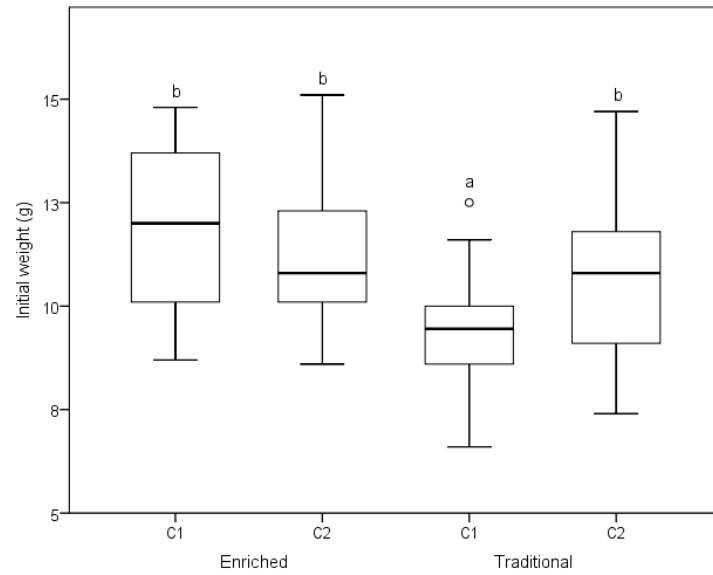


Figure 2: Weight at tagging for 3-month-old sturgeons belonging to two crossings and reared in enriched and traditional environments. Different letters indicate significant differences.

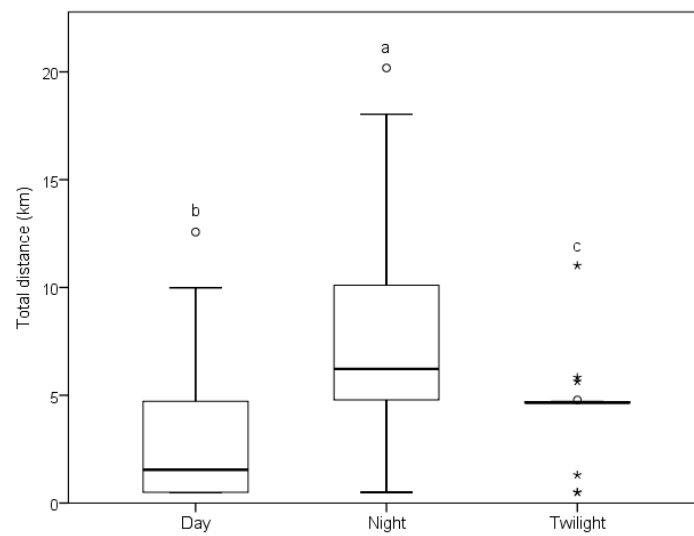


Figure 3: Total distance covered for 3-month-old sturgeons stocked in the wild according to the moment of the day during the whole study period (21 days). Asterisks represent extreme values and different letters indicate significant differences.

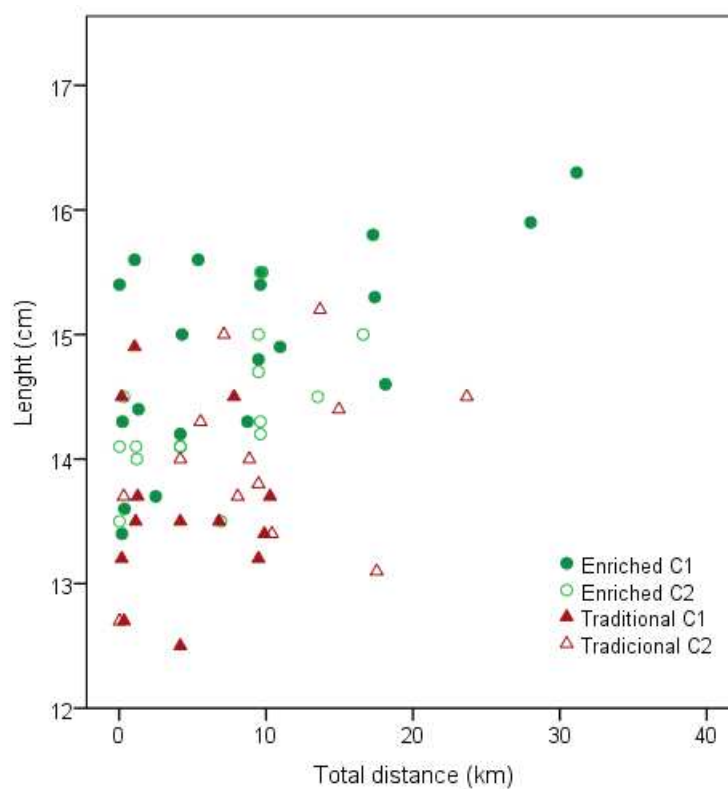


Figure 4: Total distance covered (21 days) according to fish length for 3-month-old sturgeons, originated from two rearing methods and crossings, stocked in the wild.

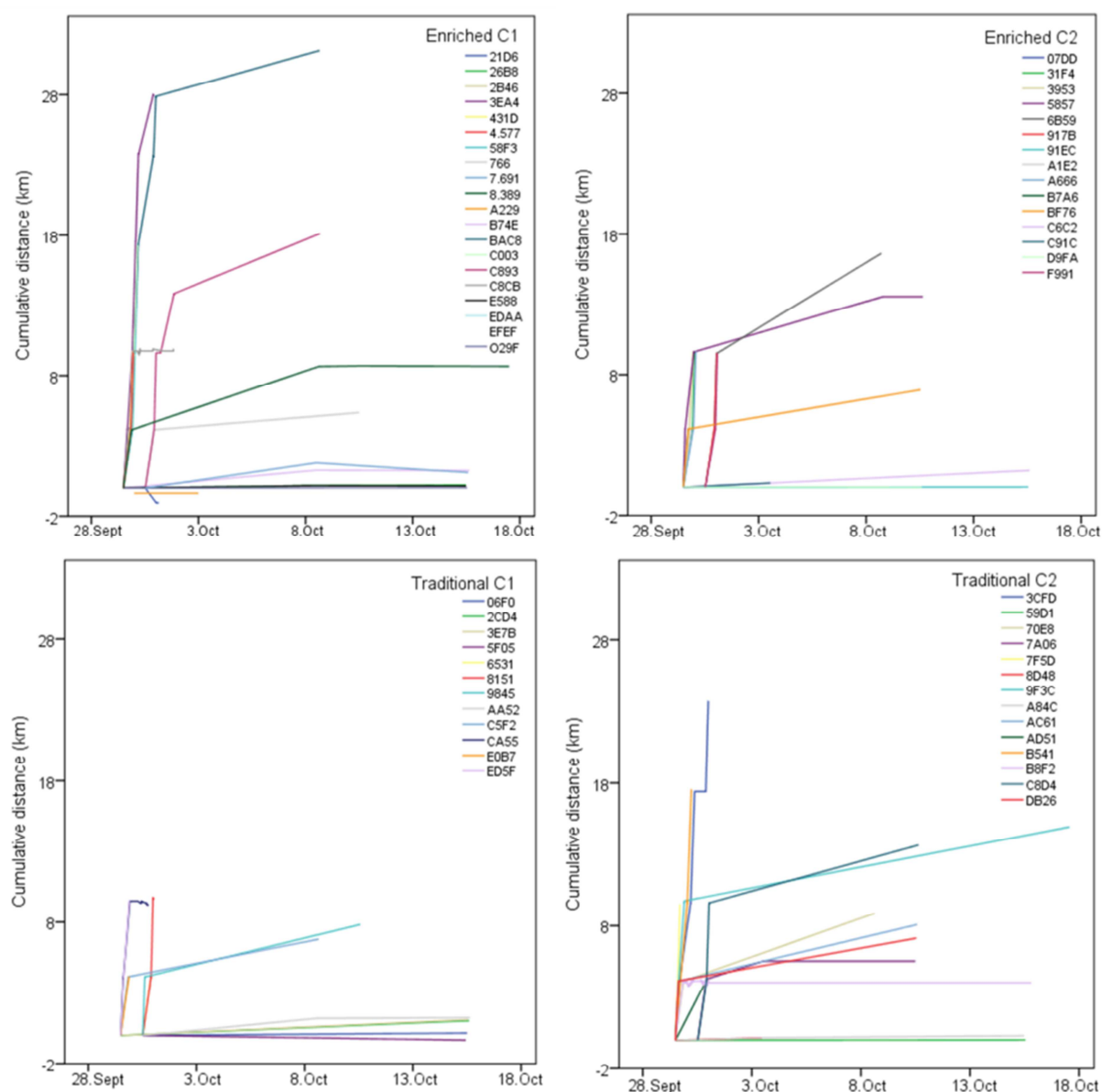


Figure 5: Individual movement's trajectories of 3-month-old sturgeons belonging to two crossings (C1 & C2) and reared in two different environments (traditional and enriched). Fish were acoustically-tagged, stocked in Dordogne River and tracked during 21 days. In each panel Y axis represents the cumulative distance from the release site, X axis represents tracked days (date) and each color corresponds to one individual.

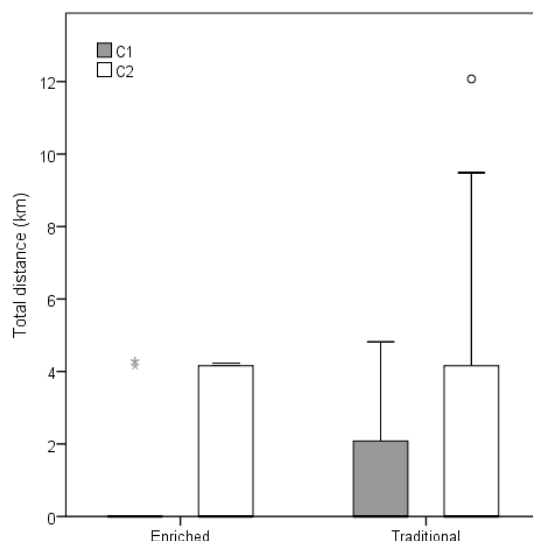


Figure 6: Box plot of total distance covered by 3-month-old sturgeons during the day for the first 3 days after release. Fish belong to two crossings (C1 & C2) and were reared in two different environments (traditional and enriched). Enriched-reared fish were less active than traditional-reared fish as well as fish belonging to crossing C1 compared to those belonging to C2.

4.5. Discussion

For European sturgeon, the last natural reproductions registered in the Gironde to have happened in 1988 and 1994 (Lochet et al., 2004). Therefore, young of the year (YOY) individuals are currently inexistent in the watershed. Fish in this study represent the youngest European sturgeon (3-months old) ever tracked and thus, our study revealed for the first time distribution and activity patterns in this age class. Even more, up to date, fish in this study are the smallest sturgeons (12.0–16.3cm TL) tagged and monitored in the wild. These results are important at providing basic knowledge of post-stocking behavior in freshwater. One particularity of our study was to take into account the rearing practices to explain fish fate and thus the results can help in designing stocking programs.

The technology used allows tagging very small individuals but low frequencies signals used in these tags experience more distortion than higher frequencies and have transmissions limitations associated with underwater noises and habitat characteristics (Bégout et al., 2016) that result in high proportion of false detections. Nevertheless, filters settings reduce significantly the amount of errant detections. We have obtained 4–9 fold more false positives on receivers that were setup to work without filter. Thus, the data required substantial cleaning even after using the brand's associated software. Active tracking with the technology used was not as efficient as passive tracking, it was very time consuming as some fish were difficult to locate due to prevalent signal attenuation. There

are several factors in aquatic environments that can imperil the range of acoustic signals (Ireland and Kanwisher 2012) but we were not able to point out what could have produced it. Due to the telemetry arrangement design chosen (coupled receivers with overlapped detection range), we were not able to assess fish lateral movements. The lateral movements corresponded mainly to detections on both hydrophones of the same gate and we have removed them to avoid overestimating movements. This probably leads to underestimate the total distance covered per fish. Nevertheless, this does not affect the aims of the study which intended to explore general patterns of fish movement and fish survival after stocking.

In this study, data were obtained from 69.3% of the fish released and it has been used as an estimation of survival as all fish were stocked and monitored during the same period of time (Heisey and Fuller 1985; Acolas et al., 2012). We considered that the fish overall survival in this study (69.3%) is acceptable when compared with other studies on YOY hatchery-reared sturgeons: 40% (Crossman et al., 2009) and 5-15% (Crossman et al., 2011) in lake sturgeon (*A. fulvescens*) released at 6 and 2–4 months old respectively, 87% in European sturgeon released at the age of 9-12 months (Acolas et al., 2012). Nevertheless, monitoring duration and fish age vary among those studies and an accurate comparison is not possible. Of the 88 fish released, 27 were never detected and one was found upstream which is suspicious considering the swimming capacity of such small individuals. Although, tag malfunction could not be discarded for the fish never detected, it is probable that predation was the main reason of fish disappearance or rapid upstream move as cormorants and herons are commonly present along the river bank as well as big fish such as the European catfish (*Silurus glanis*). Many authors have confirmed predation as the main reason of mortality of stocked fish after release; 27% in largemouth bass (Buckmeier et al., 2005), 46% in Florida bass (Thompson et al., 2016), 65% in sea trout (Dieperink et al., 2001).

In our study, survival of both crossings under enriched rearing (90.9% & 68.2%) and traditional C2 (66.7%) had better survival when compared with traditional C1 (52.2%). Fish size is also an important factor influencing predation –smaller fish are more at risk than bigger ones (Dieperink et al., 2001; Juanes et al., 2002) and traditional-reared C1 fish were the smallest with the lowest survival of all treatments. However, we demonstrated in our study that fish size was not linked to the survival and there is the possibility that low survival in this treatment is better explained by rearing conditions (Carrera-García et al., 2016). These results would be congruent with studies done on YOY lake sturgeon where 3-month old fish had lower recapture rates when reared traditionally than under more enriched conditions (approx. 2–4 fold higher) (Crossman et al., 2011). Nevertheless, there were no differences due to rearing for 4 and 6-month old fish suggesting that the influence of rearing

decreases with fish age (Crossman et al., 2009; Crossman et al., 2011). Besides this, there are other studies where improved post-release survival has been obtained using environmental enrichment during rearing; 1.9 higher in common snook (*Centropomus undecimalis*) (Brennan et al., 2006), 6.4% and 19% higher in Atlantic salmon (*Salmo salar*) (Hyvärinen et al., 2013; Roberts et al., 2014). The enriched approaches of rearing would allow the fish to develop morphological, behavioral and cognitive traits that are necessary to respond adequately to wild environments which in consequence will increase survival (Braithwaite and Salvanes 2005; Kotrschal and Taborsky 2010; Roberts et al., 2014).

The fish of this study had preferendum for areas of 3.3m mean depth (1.4–6.2m) and 0.09m/s mean water current (0.004–0.4m/s). Previous records on 5-month old European sturgeon confirm their preference for low velocity areas (<0.25 m/s) and report also preferences for fine substrate in the wild (Acolas et al., 2009) as in controlled conditions (Charles et al., 2009). Fredrich et al., (2008) reported that 9-month old Atlantic sturgeon's were mainly located in zones of 3–5m depth in the Drawa River.

Our study revealed that YOY European sturgeons are mainly nocturnal while in riverine systems: they move downstream during night hours. This is congruent with a previous study in this species under controlled conditions (Charles et al., 2009), and with observations on the closely related Atlantic (*A. oxyrinchus*) (Fredrich et al., 2008) and Gulf of Mexico (*A. oxyrinchus desotoi*) (Kynard and Parker 2004) sturgeons, as well as for other less related acipenserids (Benson et al., 2005; Kynard et al., 2005). Increase activity in most species of the group could be considered as an evolutionary response to predation while in freshwater habitats (Wishingrad et al., 2015). It's possible this is so as clear water in the river could ease their exposure to predators during the day, while the nocturnal behavior is less important in the estuary where the channel is deeper and the water is turbid. Acolas et al., (2012) found no differential diel movements on 10-month-old European sturgeon in tidal area and once the saline estuary is reached.

We also observed that European sturgeon's orientation was mostly downstream. Such patterns, mainly downstream migration with occasional upstream movements, are also reported on YOY Atlantic sturgeon (Kynard and Horgan 2002; Fredrich et al., 2008; Kapusta et al., 2011). During the whole study, 82% of the fish didn't move more than 13.5km downstream from the release site. Also, most movements occurred during the three days after release after which, the fish apparently settled. It could be suggested that right after release, fish move to suitable areas and then at some

point they will start migrating again. An experimental study done by Kynard and Horgan (2002) on Atlantic sturgeons pointed out that free embryos hide, then, start migrating at 8 days post hatch (dph) and cease migration at 19 dph. Later on, fish were reported to swim actively but never resume migration while under observation –approx. 4 months. This could suggest that after release, young European sturgeon reach favorable patches, stay in them and later on continue going downstream. This behavior has also been reported on 10-month old Atlantic sturgeon (Fredrich et al., 2008) and European sturgeon (Acolas et al., 2012).

By the end of our study, fish aged 4 months, 95.1% of them settled in freshwater and only 4.9% reached the tide influence zone; no fish reached the oligohaline zone of the estuary. Development of salinity tolerance has not been studied in YOY European sturgeon. Nevertheless, all anadromous sturgeon species need to develop such tolerance (Altinok et al., 1998; Bain et al., 2000; Kynard and Horgan 2002; Allen et al., 2011) which, is age dependent (Magnin 1962; Altinok et al., 1998) and as far it is known, occurs at the age of 9–12 months at the earliest (Kynard et al., 2005). Hatin et al., (2007) pointed out that habitat use in Atlantic sturgeon juveniles is controlled by salinity and distance to the salt wedge where YOY use freshwater zones only. Furthermore, from previous telemetry studies, we know that 10-month-old stocked European sturgeons reach the saline estuary soon after release (Acolas et al., 2012). Thus, we can infer that the downstream migration towards saline waters occurs at the age of 4 and 10 months for stocked European sturgeons.

Our study revealed behavioral differences related to fish crossing and rearing methodology for the first days after release. Traditional-reared fish were more active during the day than enriched-reared fish. As the former fish were maintained under complete darkness, their circadian clock probably didn't match the outdoors light–dark cycles. In the long run, fish reared under traditional rearing would acquire a diel rhythm because the environment induce rhythms' synchronization (Reebs 2002; Zhdanova and Reebs 2006). But, it takes some time for the adjustment to happen and, during entrainment, those fish may be more at risk to predators than the enriched-reared fish, affecting survival during the first days of release. For example, these fish could have been more exposed to cormorants (*Phalacrocorax carbo*), which are diurnal and feed mainly on fish within the size range of those of this study (Kirby et al., 1996). Even more, Carrera-García et al., (2016) highlighted that traditional-reared fish were light-colored compared with enriched-reared fish. Fairchild and Howell (2004) demonstrated that unnatural light-color and abnormal behavior of hatchery-flounders made them more conspicuous to predators, explaining flounder's low survival after stocking.

In our study the total distance covered was not significantly different according to fish origin; however the only fish reaching the tidal area were reared under enriched conditions. We may suggest that being reared under water current conditions (Carrera-García et al., 2016) may have helped them to cope with current variability in the wild and thus, travel further. Moreover, a correlation between fish size and distance covered was found only on enriched-reared fish. This could be explained also by the same fact; enriched-reared fish had better swimming performance which is often positively correlated with size (Wardle 1977). On the other hand, traditional-reared fish were not trained to cope with water current and whatever their size, their swimming capacity was similar at release.

Conclusions

Our results expose the first behavioral data for such young European sturgeon in the wild which improve knowledge on early life histories of a sturgeon species. We consider that rearing conditions for fish early stages is an aspect that should be contemplated in stocking for conservation programs. Our results highlight the importance of rearing effects and post-release monitoring for identifying factors that could be undermining the stocking success to guide decisions to mitigate them.

Ethical approval

This study was carried out in an approved experimental hatchery facility by the French Department of Agriculture (authorization A33-478-001) and followed the standards of the national ethic committee of animal use for scientific purposes (authorization 01738.01).

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5

Discussion and perspectives

5.1. Summary results

In this study, during the controlled experiment in the hatchery (chapter 2), we obtained high fish survival in both rearing environments (>83.8%) and enriched-reared fish were affected negatively by high temperatures during the second month of rearing. Results showed that enriched rearing conditions influence positively fitness related traits of fish such as size and behavior. Fish size was determined by fish crossing and their rearing environment. Enriched-reared fish of both crossings resulted in bigger final size (weight and length) than the traditional-reared fish. Considering ontogenic age, both crossings in enriched conditions and one of those reared traditionally (C2) had similar growth performance while the remaining traditional-reared crossing was considerably smaller (C1). In addition, empirical observations on pigmentation showed that enriched-reared were dark colored while traditional-reared fish were pale. Fish from different rearing conditions also differed behaviorally. Enriched-reared fish had more pronounced exploratory behavior than traditional-reared fish but they took longer to initiate such behavior than fish reared traditionally. On the other hand, fish didn't react differently when exposed to a novel prey, probably because the feeding method was the same in both rearing practices.

The acoustic dummy-tagging assessment (chapter 3) revealed that intraperitoneal acoustic tagging is adequate and can be applied successfully to 3-month old sturgeons. In this study, fish survival (98%) was not affected by the dummy-tagging procedure and in dummy-tagged fish, RFID microtags loss was related to the surgeon that performed the procedure. The study showed that most fish healed well, that growth of dummy-tagged and control were alike by the end of the study and that swimming behavior of dummy-tagged fish were more reactive between 12 and 21 days after surgery after a stressor was applied.

Up to date, fish in this study are the smallest sturgeons acoustically tagged and monitored in the wild (chapter 4). Once stocked, fish overall survival estimate was 69.3% where both crossings under enriched rearing and traditional C2 had better survival than traditional C1. The study confirmed that YOY European sturgeons are mainly nocturnal while in the river and move mainly downstream. After release, most fish remained within 13.5km downstream from the release site and most of the movements occurred during the first three days after release. None of them reached the saline estuary. Behavioral differences related to fish crossing and rearing methodology were also found; traditional-reared fish were more active during the day than enriched-reared fish during the three first days after release. Total distance covered was similar in all treatments but distance covered was related to fish size only for enriched-reared fish. The tidal area was reached by very few individuals, all of them enriched-reared fish.

5.2. Discussion

Why is this project original and what are its contributions?

Individuals in this study were assessed in controlled conditions and also in the wild, giving this work a very integrated approach which is uncommon. Also, this fish represent the youngest European sturgeon (3-months old) ever tracked in the wild and thus, revealed for the first time the diel cycle activity, river distribution and post stocking movements of this age class. Basic knowledge on life history is important because it is based on the most fundamental components of life and fitness: survival and reproduction (Hutchings 2002). Thus, life histories traits determine individual's fitness, population persistence and growth rate at low abundance (Hutchings 2002). This highlights the vital role it has in population management and conservation of fish. Moreover, the study has taken into account rearing practices within the stocking context which can help designing and improving conservation programs. Furthermore, it is also important to study rearing practices as it has consequences on fish quality. Nowadays, it is recognized that enriched rearing has an important role in *ex situ* actions because this approach helps to preserve behavioral diversity in captive populations and ensures behaviorally viable populations (Shepherdson 1994). In consequence, it could contribute to stocking programs' success through the production of fish with the skills to live and adapt to the new environment that consequently will be able to survive and reproduce. Therefore, this study states the importance and effects of rearing conditions in fish supporting that the European sturgeon stocking program can improve the quality of the fish produced to ameliorate post-release survival and have a better understanding of the species.

Is the enriched practice tested the best choice?

The enriched rearing as practiced in this study was done in modified tanks which fulfilled the experiment's needs during this work i.e providing a contrasted environment compared to traditional rearing. These tanks were deep and big enough to be able to use different water depths and arrange water current. Nevertheless, they presented some drawbacks: first, the tanks were difficult to clean because they were too deep and due to substrate presence. Second, probably fish were excessively handled due to the choice of modifying the tank's landscape every week. Using these modified tanks without landscape will probably make things easier from a manipulation point of view. However, spatial cues are indispensable for behavioral and cognitive skills improvement on the fish (Salvanes and Braithwaite 2005; Salvanes et al., 2013). What could be reconsidered is how often the landscape was changed. Less recurrent adjustment will decreased fish manipulation and still achieve the positive effects that spatial cues could generate. Finally these modified tanks on the long term are probably not the best suited for implementing a new rearing method in the program which needs

large scale structures. Therefore, other reservoirs should be tested. Currently, at the Irstea experimentation station two artificial rivers were implemented, each equipped with a propeller to create water current (Photo 11). These infrastructures could harbor bigger fish quantities, provide them with even more naturalistic rearing conditions without handling the fish and with less daily handwork required. Indeed, during this study, two of them were setup with substrates and ready to be used but left untested because of failed reproduction on 2015–2016. Yet, it is worth pointing out that in these bigger reservoirs equipped with substrate, capturing the fish would be harder and more time consuming than in the tanks and predator control has also to be refined. Many fish species have been studied in enriched rearing environments for conservation and reported positive results e.g. salmon (Brown et al., 2003; Roberts et al., 2011; Hyvärinen et al., 2013), rainbow trout (*Oncorhynchus mykiss*) (Maynard et al., 2004; Berejikian 2005; Bergendahl et al., 2016), Coho salmon (*Oncorhynchus kisutch*) (Maynard et al., 2004), cod (Braithwaite and Salvanes 2005; Salvanes and Braithwaite 2005), June sucker (*Chasmistes liorus*) (Rasmussen et al., 2009), striped knifejaw (*Oplegnathus fasciatus*) (Makino et al., 2015). In previous studies in sturgeons (Crossman et al., 2011; Boucher et al., 2014; Du et al., 2014), the authors have chosen to work with few variables. Surely, fish quality can be improved when particular variables are used to enrich the rearing conditions but it does not mean that it is optimal. For example, water current as used by Du et al., (2014) make the fish physically more fit and probably in the wild they are able to cope with river current variability. Nevertheless, if the fish behavior is not adequate then probably it would not be able to survive long. The opposite situation would be also possible; a fish could be behaviorally competent but its physical condition could put it in disadvantage once released. Certainly, considering many parameters simultaneously as used in this study does not allow understanding how each variable affect fish. However making the rearing conditions as similar as possible to those found in the wild will assure to optimize the physical and behavioral quality of the fish. Furthermore, the variables themselves affect fish quality but surely, it cannot be denied that synergistic effects are possible as found by Boucher et al., (2014) and this subject is poorly understood.

Also, an aspect to highlight is that variables to use for enrichment could be chosen accordingly to the species needs. For example, water currents would be of much importance for riverine fish than for lacustrine species. In the case of migratory fish, for which most species undergo the imprinting process, using water of the natal river would be a meaningful variable to consider—if imprinting occurs during captivity. In the case of European sturgeon, the timing of imprinting is unknown. In this work, the fish were stocked in the Dordogne River and the water used came from one of its tributaries –l’Isle River. However, the program also releases fish in the Garonne River and obtaining water from there to use in recirculated systems is costly or moving a hatchery to the Garonne

surroundings, certainly is not realistic. In this case, the available running water options i.e l'Isle or underground sources, for rearing the fish constitute local constraints.

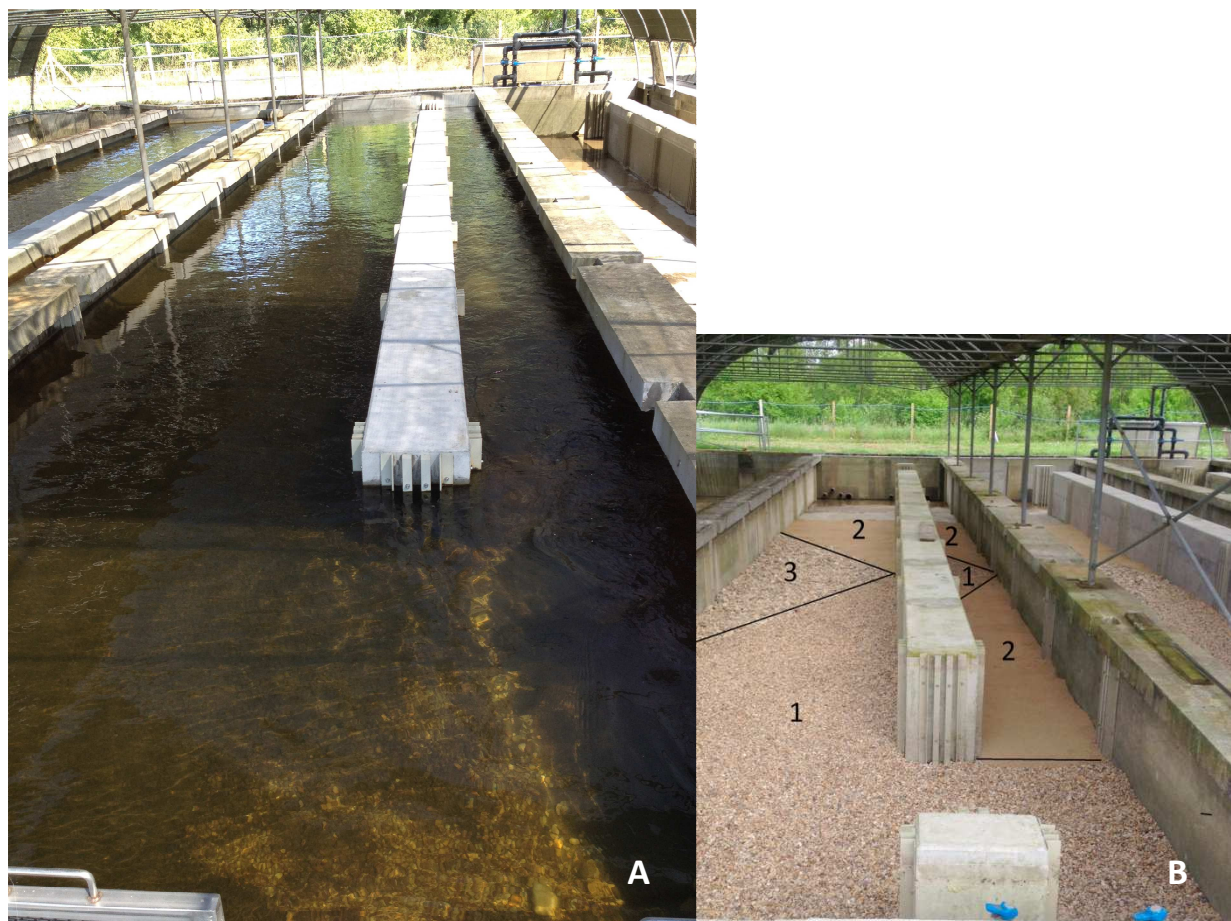


Photo 11: One of two artificial rivers prepared (left) for rearing juveniles of *A. sturio* using sand, gravel and cobble as substrate. The rivers were left untested due to failed reproduction on 2015–2016 (©Gentil A, Irstea).

Are the traditional-reared fish maladapted?

In this study, the results showed that rearing environment has effects on fish fitness-related traits. Specifically, it's noted physical and behavioral improvements in fish reared under an enriched rearing approach. All this suggest that enriched-reared fish are more suitable to face life in the wild. Nevertheless, it is not possible to state that these fish will indeed survive long enough to join the natural population, reach adulthood and reproduce. It is still poorly understood how long hatchery effects may last once in the wild (Svåsand et al., 1998). With the methodology used in this work it would not be possible to identify the individuals used in the future. However, our results do not suggest that traditional-reared fish could not adjust and succeed in the same environment, at least

not for this program. The lack of differences in certain features studied as overall survival in the hatchery, similar survival in the wild for 3 of the 4 fish treatments, alike general movement patterns in the wild could be linked to the fact that the fish belonging to the first generation descendants of wild-born fish are still adapted to the natural environment. It has been stated that adaptation to captive environment can be minimized by reducing the numbers of generations that the species spend in captivity (Price 2002; Williams and Hoffman 2009). Thus, these results are encouraging considering the amount of first-generation fish stocked since 2007. Although, this is something to take into account in the future, as the program's wild stock (wild-born spawners) is reducing and the first-generation captive individuals increasing, suggesting that the program will eventually start stocking with second generation fish in the near future.

Can the program be improved towards conservation aquaculture?

Conservation aquaculture is nothing more than the use of aquaculture for conservation and recovery of endangered species which goal is to preserve and work adaptively with the local gene pool, provide diversity to the target populations and avoid domestication effects to protect characteristic phenotypes and behaviors (Anders 1998). It doesn't imply any particular hatchery techniques to do so. Nevertheless, the standard hatchery practices cannot satisfy these goals and thus, conservation aquaculture practices need to create and adapt its rearing techniques to match these goals. Based on that, the European sturgeon conservation program currently does what is on hand to work with the local gene pool and maintain its genetic diversity. However, to advance towards conservation aquaculture, improvements are still to be done on augmentation of fish morphological, behavioral and cognitive characteristics that could be lost in the standard hatchery environment. Currently, implementation of enriched rearing would be the way to achieve so.

5.3. Recommendations and perspectives

Main priorities

Conservation program's key to success is constantly seeking ways to improve and adapt it. In this work, there were several aspects that were not assessed but that would be important to take into account in the future to improve the current releases. For example, in this study, enriched rearing was done considering many parameters simultaneously. Certainly this approach does not allow understanding how each variable affect fish and it's relevant not to dismiss the importance to understand them separately. So, I would suggest studying fish performance using these variables independently. Initially, in this study it was planned to assess the influence of water source and natural photoperiod. However, failed reproduction on 2015–2016 left the system ready to be used

but untested. Furthermore, as there are studies that show that type of rearing can affect fish morphology (Berejikian 2005; Pulcini et al., 2014), it was also planned to rear the fish in the artificial rivers and make a morphometric study to compare the fish of both (enriched and traditional) rearing methods. In the same case as before, it wasn't possible due to lack of biological material to test. Based on this, it is recommended to follow up with these plans to provide a better understanding of rearing effects on fish quality.

Also carrying out further post-release monitoring would be relevant. In my study, it was possible to monitor fish acoustically for only 21 days. Nevertheless, marking the fish externally (e.g. branding) in addition to acoustic tagging, may allow having further information about their survival during the habitual estuary monitoring to understand better rearing conditions effects in the wild. Even more, this study was done in the Dordogne River but in this restoration program, fish are also stocked in the Garonne River. So, it's also proposed to run similar studies in this river. Late last year, Lu et al., (2016) launched a new acoustic tag that lasts 98–365 days. It could be used on young fish but since the tag is 2.5 fold heavier than the tag used in this study, it should only be considered suitable for approximately 6–7 months old fish.

As mention before, stocking for conservation practices is done in many species, even though it is difficult to know if these programs have indeed implemented the enriched rearing approach in a permanent basis and if so, the caveats that they have found. Studying and sharing this information is essential as studying the approach itself because it would help to improve and maximize the implementation process and success in other programs. Based on this premise, it would be relevant to run an engineer study on the feasibility of rearing a large amount of fish using an enriched approach. A study like this would provide information about how realistic it is to implement this rearing according to our facility capacity and constraints, the costs of it, workload, space and equipment requirements.

Secondary priorities

This study did not assess the predator-prey relationship but the survival estimated after fish release in the wild suggested important predation component. Therefore, it is suggested to study the predators stocks in the release areas to optimize the release method to reduce predation. Nowadays, releases are done during the day to decrease predation by the European catfish (*Silurus glanis*) which has a strongly nocturnal feeding activity (Boujard 1995; Carol et al., 2007). Nevertheless, it remains unknown if other fish and avian predators would be more relevant on post-release mortality. Under the same context, it could be suggested to train the fish to avoid possible predators before stocking. Concerning fish predators, the process of pairing a predator cue and an alarm cue of heterospecific

prey guild members (Chivers et al., 1995; Berejikian et al., 1999) or prey alarm cue on the predators diet/faeces (Mathis and Smith 1993; Brown et al., 1995b,a; Mirza and Chivers 2003), have been proven effective to acquire predator recognition.

Even though this work focuses on juvenile rearing practices, the welfare of the captive stock which will constitute the future spawners is also relevant. Chronic stress and under-stimulation in captive conditions has inhibitory effects on reproduction and may be responsible of failure to achieve it in these populations (Beck et al., 1994). It has been suggested that simple forms of enrichment can reduce or eliminate this negative effects and aide reproduction (Shepherdson 1994; Young 2003). Improving the stock welfare and in consequence the conservation program could be something to consider in the program's future.

In this study basic information about the species early life history has been collected but there is still almost no information about the behavior of *A. sturio* before the age of 3 months. However, this information is valuable as most of the fish released by the program are 7 dph. It is not possible to mark and monitor such small fish but research on habitat selection, ontogenic behavior and migration of larvae to 2–3 month old fish would be possible under controlled conditions.

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