AGROCAMPUS OUEST

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Année universitaire : 2019 - 2020 Spécialité : Ingénieur agronome Spécialisation : Sciences halieutiques et Aquacoles Option : Aquaculture	**Mémoire de fin d'études** ☑ d'Ingénieur de l'Institut Supérieur des Sciences agronomiques, agroalimentaires, horticoles et du paysage ☐ de Master de l'Institut Supérieur des Sciences agronomiques, agroalimentaires, horticoles et du paysage ☐ d'un autre établissement (étudiant arrivé en M2)

Evaluation de l'impact de la restauration écologique du saumon Atlantique dans le bassin Garonne-Dordogne grâce aux empreintes génétiques

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Soutenu à Rennes le 18 septembre 2020

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REMERCIEMENTS

Ce projet n'aurait pas abouti sans l'aide de mes précieux encadrants que je souhaite remercier au cours de ces quelques lignes. En premier lieu je voudrais remercier toute l'équipe de MIGADO qui m'a accompagnée au cours du stage. Merci à Stéphane Bosc, toujours à l'écoute qui a pu répondre à mes nombreuses interrogations tout au long du stage, même les plus complexes. Merci aussi à Isabelle Caut pour ses encouragements et félicitations et sa visite des installations de MIGADO en Dordogne au mois de mars. Enfin merci aux techniciens et chargés de mission de MIGADO, notamment Olivier Menchi, grâce à qui j'ai pu avoir accès aux nombreuses données de ces dernières années. Je remercie aussi bien évidemment Anastasia Bestin ma maître de stage qui m'a accompagné au long de ce stage, et qui malgré le peu de contact visuel dû au confinement a toujours su m'orienter dans la bonne direction pour la rédaction de ce rapport de stage. Merci aussi à Pierrick Haffray, également membre du SYSAAF pour son implication croissante dans le projet, qui m'a permis d'aller plus loin dans ce stage et de voir au-delà du simple rapport scientifique, à savoir les enjeux écologiques mais aussi politiques et économiques de ces actions de restauration écologique. Je souhaite aussi remercier Guillaume Evanno qui m'a accompagné sur les aspects génétiques du projet et notamment qui m'a apporté de l'aide sur les nombreux logiciels à ma disposition. Il aura été de très bon conseil. Enfin merci à Marc Vandeputte qui m'a aidé sur tous les aspects du projet et qui par son exigence de correction a permis de rendre ce rapport tel qui l'est aujourd'hui.

LIST OF FIGURES

LIST OF TABLES

LIST OF ANNEX

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Table of contents

1. Introduction

This work, realized as part of a Master's degree in aquaculture science for the association MIGADO with the support of INRAE (National Research Institute for Agronomy and Environment) and SYSAAF (French Poultry and Aquaculture Breeders Technical Center), aims to assess the efficiency of the stocking program of Atlantic salmon (*Salmo salar*, Linnaeus 1758) in the Garonne-Dordogne basin since 2008 by using genetic markers. The access to this tool, allowing an individual tracking, offers an opportunity to analyze the practices and their impact to propose improvement in the stocking program.

Nearly one million tons of salmonids are caught each year since 2010. For almost all species, captures remain stable or are increasing since the 80's (FIGIS), except catches of Atlantic salmon. Since 1973, when the highest total nominal catch was reported with 12 670 tons, captures have continued to decrease, reaching only 1 090 tons in 2018. (*Figure 1*) (ICES, 2019). This species inhabits a wide range of habitats, from high Arctic to Spain (Aas, 2011) and has experienced widespread population declines over the last century (Parrish et al., 1998; ICES, 2019). Identified threats are climate change, habitat depletion (e.g destruction of spawning areas) and destruction of the river ecological continuity (Forseth et al., 2017). Also, a recent study has underlined that high density genomic data clearly segregated significantly declining and non-declining populations of North-Atlantic salmon (Lehnert et al., 2019). The rise of Atlantic salmon aquaculture (*Figure 1*) has also been seen as a threat for wild Atlantic salmon population (especially through farm escapees and spread pathogens.) (Glover et al., 2017; Wringe et al., 2018). It is nowadays performed at a large scale in both hemispheres, specifically in Norway and Chile where the production reaches respectively more than 1.2 million tons and 0.6 million tons today (FIGIS), and it is currently the most cultivated salmonid in the world (FAO, 2020).

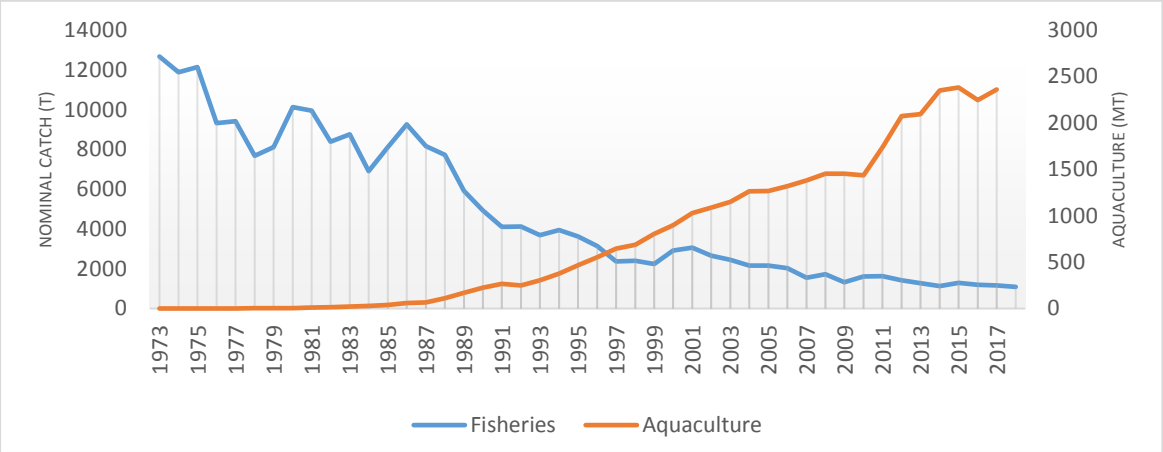


Figure 1: Evolution of Atlantic salmon fisheries and aquaculture in tons (Source: FIGIS)

To restore wild depleted or vanished populations of Atlantic salmon many solutions are tested and applied. As an anadromous fish, it spends a minimum of one year in freshwater before the downstream migration and 1 to 5 years at sea before its upstream migration *(Figure 2)*. The part of its life cycle in freshwater ecosystems is very important because the Atlantic salmon spawns in very particular area (Aas, 2011). Freshwater ecosystems are among the most threatened and the most fragile ecosystems in the world (Dudgeon et al., 2006). Moreover, a recent study on fish species sensitivity ranks the salmonids species as high sensitive species for habitat destruction (van Treeck et al., 2020). Therefore, the restoration of wild salmon habitats is one of the key options to save Atlantic salmon population.

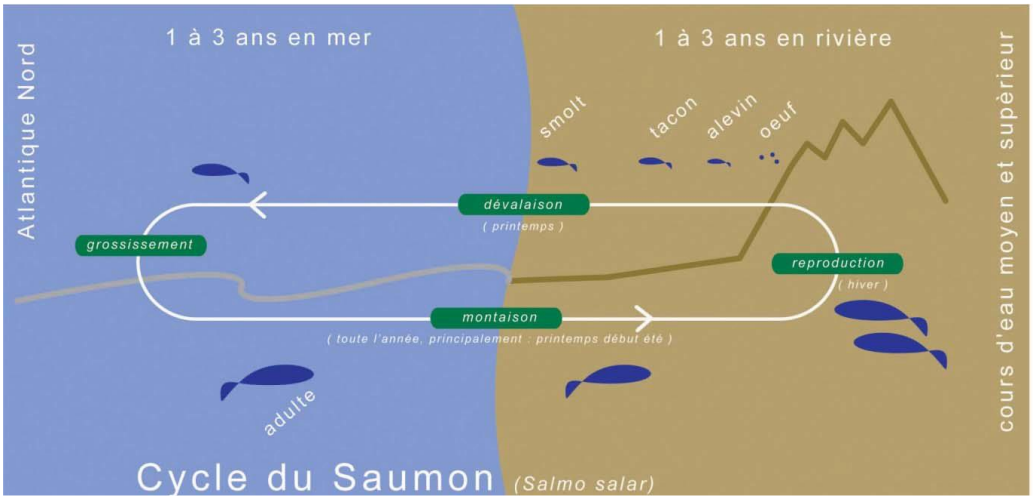


Figure 2: Atlantic salmon life cycle (Source: MIGADO)

In case there is still wild salmon in the river, restocking is hazardous and the top priority is to restore the salmon habitats (Waples & Do, 1994). It has also been recently demonstrated that stocking fails to increase fish production where wild fish populations and suitable habitat remains (Bacon et al., 2015). However, in case of an extinct population, captive-breeding and re-stocking are very important tools to restore the population. Leber in 2013 defines re-stocking as the "release of cultured juveniles into wild population(s) to restore severely depleted spawning biomass to a level where it can once again provide regular, substantial yields" (Leber, 2013). Re-stocking is described here with terms that are used for production. Indeed, hatchery programs were firstly made to compensate for fisheries depletion (Saltveit, 2006). Nevertheless, nowadays many re-stocking programs have as first aim the ecological restoration because populations are on the edge of extinction or even became extinct. Hatchery programs to restore local salmonids populations have accumulated the last 50 years.

The first documented genetic study on a re-stocking program was on brook trout (*Salvelinus fontinalis*, Mitchill 1814) (Flick & Webster, 1964). This study was conducted with trout domesticated for many generations, as were later studies on other salmonids (Allendorf & Phelps, 1980; Hansen, 2002; Jonsson & Jonsson, 2006; McLean et al., 2003). A loss of both genetic diversity and fitness in the wild was observed in all those studies because of supplementation with domesticated fish. Although Dannewitz et al., (2004) reported no difference in the reproductive success in the wild between hatchery produced and wild-born brown trouts (*Salmo trutta*, Linnaeus 1758) most of studies conducted on this subject led to rather negative outcomes with less marine survival, and less reproductive success for hatchery-reared fish. (Jonsson et al., 2003; Miller et al., 2004; Waples & Drake, 2004; Araki et al., 2007; Thériault et al., 2011; Satake & Araki, 2012). To reduce issues with hatchery fish, re-stocking programs have attempted to reduce the time and the number of generations spent in captivity (Reisenbichler & McIntyre, 1977; Frankham, 2008). Studies showed that re-stocking with only first generation hatchery fish lowers potential negative impacts on fitness (Schroder et al., 2008; Berejikian et al., 2009; Ford et al., 2016). According to some studies, using wild fish as broodstock has no negative impact on fitness and results in lower adaptation to captivity (Hess et al., 2012; Baskett & Waples, 2013). The best approach is therefore to minimize genetic adaptation to hatchery conditions by using individuals caught in the wild (Williams & Hoffman, 2009). However, even with a single generation of hatchery breeding, some studies noticed that hatchery-origin fish produced less juveniles per parent when spawning naturally than did wild-origin fish (Williamson et al., 2010; Christie et al., 2014) but also less survival between life

stages (Kennedy et al., 2012). However, a study conducted by Chilcote et al., (2011) even suggested the impact of using hatchery fishes descending from "wild-type" brood stock (with 1 generation in captivity) is not less negative than using domesticated breeders. This short-term adaptation of salmonids to captivity was demonstrated by Christie et al., (2012). They estimated that fish from first generation hatchery had twice the reproductive success of wild fish when spawning in captivity, which is clearly an adaptation to captivity. Also, juveniles from hatchery reared parents with only six months in captivity showed significant differences in terms of growth, migratory behavior and genetic variability than offspring of wild fish (Christie et al., 2016; Horreo et al., 2018). A short-term captivity may thus be enough to initiate changes that are usually related to domestication.

Besides the reduced population fitness through adaptation to captivity, Aprahamian et al., (2003), summarized others risks associated with stocking, including the competition between wild and hatchery juveniles in terms of habitat use and food (underlined in Kostow & Zhou, (2006)) and threats to local genetic integrity. Indeed, the high level of local adaptation of salmonids has to be considered in restocking programs, that must be managed considering that the hatchery broodstock is "a genetic integrated component of an existing natural population" (Mobrand et al., 2005).

Therefore, many hatchery programs have underlined the importance of the use of wild but also local broodstock. The frequency of local adaptation in salmonids was evaluated between 55% and 70% (Fraser et al., 2011).Using local and wild fish had already been suggested by Reisenbichler & McIntyre, (1977) : "The genetic difference can be reduced by using native wild fish which are adapted to that particular stream system for brood stock to initiate the hatchery program and in subsequent generations". The efficiency of selecting native wild stock to build the brood stock has been shown in many studies. A study showed that local population had 1.2 times average fitness advantage relative to foreign population (Fraser et al., 2011). Survival rate of non-natives from ova to return as an adult is in general less than the survival rate from native salmon (McGinnity et al., 2004; Araki et al., 2007; Araki et al., 2008). Whereas intense stocking of non-native fishes can alter a population genetic structure and cause a disturbance of allelic variability (Marie et al., 2010), the use of local wild fish for hatchery brood stocks can minimize those effects (Berejikian et al., 2009; Berntson et al., 2011; Christie et al., 2014; Bacon et al., 2015).

Selecting a brood stock by taking only wild and local fish does not avoid all issues for the conservation of an extinct or depleted wild population. Another important parameter to consider is the effective population size of the broodstock of the remaining population (hatchery and wild), called N_e. It is one of the several concepts introduced into population genetics by Sewall Wright (Wright, 1931). N_e is crucial in determining the level of conservation of the genetic variability between two generations in a population, and the effectiveness of selection relative to drift (Charlesworth, 2009). Ne is often far lower than the number of breeders in the population when pedigree information and adapted management of mating to avoid inbreeding are missing. (Frankham, 1995). N_e is linked with the Ryman-Laikre effects (R-L) which is "an increase in inbreeding and a reduction in total effective population size in a combined captive-wild system, which arises when a few captive parents produce a large number of offspring" (Ryman & Laikre, 1991). The R-L effect has been widely documented (Aho et al., 2006; Blanchet et al., 2008; Horreo et al., 2008; Anderson et al., 2013; Mestral et al., 2013) and has to be avoided to not deplete the genetic make-up of the population (hatchery and wild populations). Some studies have examined the effective number of breeders in the context of hatchery programs. For example McLean et al., (2008) calculated an effective number of

breeders ranging from 11% to 31% of the census size of the population. The number of F0 parents that contribute to the released F1 generation is therefore very important. A small brood stock size will also create more inbreeding. As mentioned by Duchesne & Bernatchez (2002) : "The census size of captive populations is the single most important parameter determining the genetic consequences of supportive breeding". It was supported by later studies that advised a high refreshment rate of the brood stock each year (Blanchet et al., 2008; Favé et al., 2008). A recent study (Christie et al., 2014) found that inbreeding could account at most for a 1-4% reduction in the fitness of hatchery fish relative to wild fish under the genetic practices applied in this case.

Although the estimation of the effective population size or the effective number of breeders is difficult to determine in natural populations as pedigree is unknown and impossible to trace back (Araki et al., 2007), estimating it in the context of stocking program became easier thanks to the development of genetics in recent decades. The genomics revolution has improved our understanding of the Atlantic salmon population with a large increase in the number of genetic markers for parentage assignment, from dozens to 10^4 - 10^6 (Waples et al., 2020). Parentage assignment is used since the end of the 80's to build the pedigree of plants or animals (Meagher, 1986; Adams et al., 1992; Kaufman et al., 1998; Randall et al., 2007; Waser & Hadfield, 2011; Peterson et al., 2014) and in aquaculture (Vandeputte & Haffray, 2014). The monitoring of wild fish populations by using genetic tools has been reviewed by Araki & Schmid (2010) and the study showed that microsatellite loci are widely used for parentage assignment since the 2000's,. This method provides a good way to assign offspring to their parents and therefore to determine their origin, hatchery or wild (Jeong et al., 2007; Eldridge & Killebrew, 2008; Shikano et al., 2008). It is then easier to know how the stocking program is working and to estimate reproductive success, survival rate by distinguishing both groups. Microsatellite parentage analysis is also useful to estimate the effective population size that contributes to the census size of the juvenile population (Jeong et al., 2007). This method minimizes the two types of error identified by (Araki & Blouin, 2005) : Type A or 1 when failing to assign a true parent, or Type B or 2 error when assigning a false parent. The more microsatellite loci, the more this method is accurate (Harrison et al., 2013) to maximize parentage assignment. Several software for parentage assignment have been developed based on two different methods, either on exclusion (e.g Vitassign (Vandeputte et al., 2006)) or maximum likelihood (e.g CERVUS (Kalinowski et al., 2007), AccurAssign (Boichard et al., 2014), and recently APIS (Griot et al., 2020)). Progress in parentage assignment with microsatellites enable to monitor stocking programs and their efficiency. For example Horreo et al., (2011), following all recommendations, meaning large-sized, wild and local brood stock, calculated that re-stocking yielded only a 10% increase of the wild Atlantic salmon population size. Another study observed a long term demographic boost of the population in Chinook salmon (mean of 4.56 times in the first generation and mean of 2.52 in the second generation) (Janowitz-Koch et al., 2019). According to these results overgeneralization on efficiency of stocking program must be avoided. The present study focuses on a stocking program that takes place in the Garonne-Dordogne basin in France.

In France, Atlantic salmon is on the red list of IUCN (International Union for Conservation of Nature) since 1995 and ranks as "near threatened" (IUCN). Indeed, the distribution range of this species has been seriously reduced as it collapsed in most of the large rivers systems like the Rhine, the Seine or the Garonne and Dordogne during the 19th century (Thibault, 1994). In the 2000's the Atlantic salmon was mainly found in three areas only: in the Armorican Massif (Brittany and Normandy), in the Loire River and in the Basque Country (Baglinière et al., 2010). To curb the stock depletion, the French government took measures in

the late 70's and created a plan to preserve the Atlantic Salmon in 1976. Following those measures, obligatory reporting of catches was established in 1987 and in 1994, COGEPOMI (Comité de Gestion des Poissons Migrateurs: Management Committee of Migratory Fish) has been created in 8 French basins to set up PLAGEPOMI (Plan de Gestion des Poissons Migrateurs: Management Plan of Migratory Fish). As established by (Perrier et al., 2013), the French Atlantic salmon populations are clearly delineated in 5 genetic groups (Upper Normandy, Lower Normandy, Brittany, Loire-Allier and Adour). Therefore, in France, salmon populations are managed per basin with the COGEPOMI and with the help of local or regional associations to conduct the PLAGEPOMI. Many rivers and basins are today concerned with salmon restoration as shown in Figure 3. In the Garonne-Dordogne basin following industrialization and the building of hydropower dams, the salmon population totally vanished between the end of the 19th century and the beginning of the 20th century (Caut et al., 2018). As the autochthonous population had disappeared, it was therefore necessary to introduce salmons from other sources. The first wild strains used were those available from Canada, Scotland and Norway. This strategy was rapidly changed to favor local wild strains from Loire-Allier and Adour-Gaves. This stocking strategy was successful and a small population of Atlantic salmons has established. Therefore, in 1995, a breeding center for salmon conservation was built in Bergerac (Dordogne) to develop a captive broodstock with trapped wild migrating spawners from Dordogne and Garonne. Since then, local fish, all supposedly originating initially from hatchery-reared salmon from Loire-Allier and Adour-Gaves populations and caught in the Garonne-Dordogne basin, are being used to produce juveniles to supplement the population in the two basins.

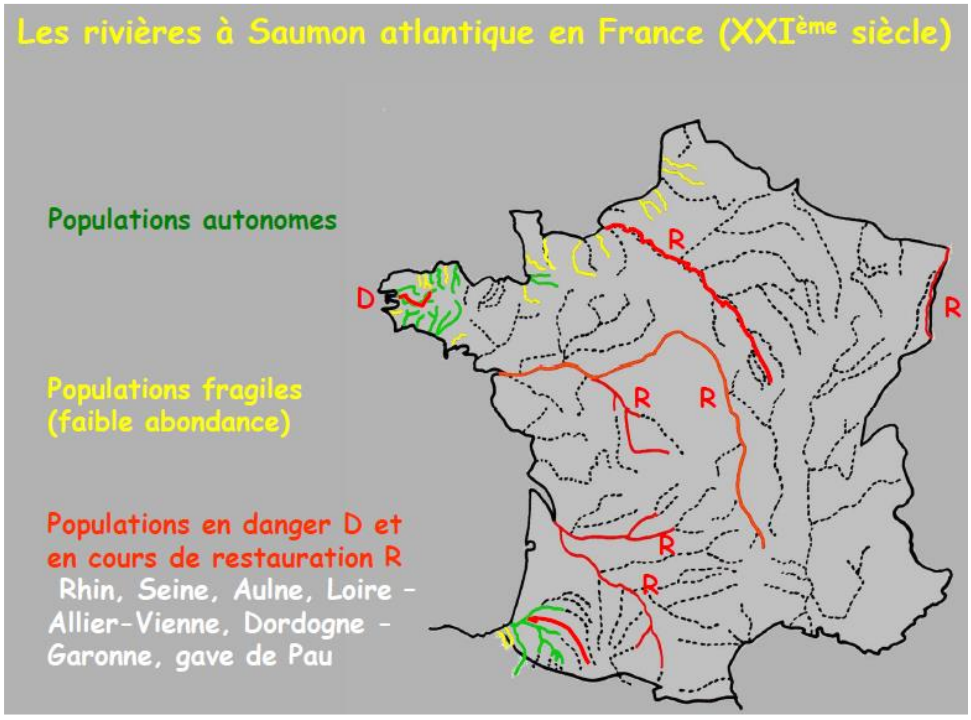


Figure 3. Salmon rivers in France – Where and what is being done. Adapted from Baglinière, 2013

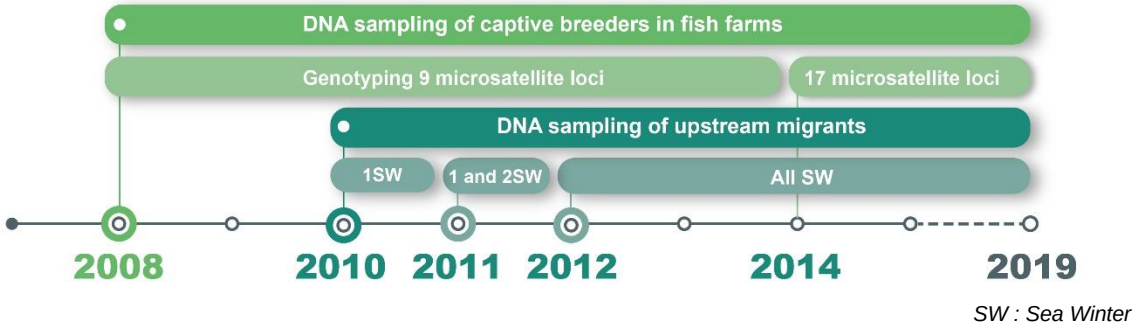
In 2006, the genetic practices of MIGADO association were audited by SYSAAF in the GENESALM national project. GENESALM was managed by the French fish farmer association Comité Interprofessionnel des Produits de l'Aquaculture (CIPA) and the Club de la Chartre des Salmonidés de Repeuplement (CCSR) with INRA, CNRS, SYSAAF and Labogena as partners. This project analyzed breeding practices of the main French restocking programs of Atlantic salmon (Rhein, Dordogne-Garonne, Brittany, Adour-Gaves, Loire-Allier) and

brown trout based on wild parents. Several aspects were considered (Haffray et al., 2008) to minimize creation inbreeding by captivity such as reproduction practices (use of extender for gamete conservation and fertilization, sperm/ova ratio, success of incubation) mating design, generation interval, number of parents, sex-ratio, sanitary practices, traceability, staff training, external expertise or capture of innovations as sperm cryopreservation. For the MIGADO restoration project several actions were proposed: the use of DNA parentage assignment to evaluate the impact of breeding practices and monitor its activity, the limitation of the number of years in captivity of F0 wild parents, the increase in number of wild parents, an improved balance of the wild parents sex-ratio, the maximization of the number of families created by systematic partial factorial mating design, secured data collection by using an adapted database system, systematic and automatic individual tagging of F0 and F1 broodstock by electronic transponder, systematic collection of fin samples in alcohol in baredcoded tubes for extensive genotyping with the microsatellite panel developed and automated in GENESALM by Labogena genotyping laboratory for parentage assignment, adapted incubation and rearing practices in order to be able to trace back origins of the parents to evaluate the efficiency of different restocking practices (sites, stage at restocking, headcounts, year, age of the parents, …).

The objectives of the present study are: 1) the evaluation of the efficiency of restocking through the improvements proposed by GENESALM project and implemented by MIGADO, 2) the assessment of the genetic impact of hatchery born fish on the wild born population, 3) the estimation of the level of natural spawning, and 4) the establishment of guidelines and advice for the future.

2. Materials and Methods

This study is based on the Atlantic salmon population of the Garonne-Dordogne basin that is currently being restored. The restoration program is operated by MIGADO. It is based on salmon breeders caught in the wild, called F0 broodstock and their enclosed offspring F1 used also as breeders. The study workflow is presented in Figure 4. The data date back to the salmon spawning season of winter 2007-2008 following SYSAAF audit in the GENESALM project. Since 2010, all salmon migrants that are trapped at the control stations during their upstream migration are genotyped. Those fish can be either released offspring of previously hatchery reared fish of the program or wild salmon stemming from wild reproduction. Parentage assignment with 9 microsatellite loci has been initially used to determine the origin of all genotyped migrants (see details later).



SW : Sea Winter

Figure 4: Study workflow since 2008 (Source: MIGADO)

2.1 MIGADO PRODUCTION SCHEME

2.1.1 Population monitoring

Since 1995, to follow the evolution and the distribution of the salmon population in the Garonne-Dordogne basin, monitoring stations have been settled in fish passes all along the rivers. The most downstream stations are at the Tuilières dam (Saint-Capraise-de-Lalinde, France), 200 km away from the ocean and 10 km upstream to Bergerac (France) for the Dordogne river and on the Golfech (France) nuclear power station dam 270 km away from the ocean and 20 km upstream to Agen (France) for the Garonne River. These stations are equipped with fish video counter working permanently. They provide an exact number of migrants that return above each station. The individuals that pass through the control stations can colonize the upstream spawning areas. The video systems are coupled with an image analysis software that allows to determine the total length (+- 2 cm) (TL) of each individual which is correlated to the number of winter at sea of the fish (Gardner, 1976; Hutchings & Jones, 1998). Therefore, we are able to estimate for each salmon its numbers of winter at sea. The following size-classes have been used:

- If TL <= 65 cm: 1SW
- If 65 < TL < 75 cm and passing Golfech or Tuilières before 1st June: 2SW
- If 65 < TL < 75 cm and passing Golfech or Tuilières after 1st June: 1SW
- If 75 < TL < 85 cm: 2SW
- If TL > 85 cm: 3SW

2.1.2 Fish trapping

Monitoring stations are also equipped with trapping devices used to catch salmon during their upstream migration. Trapped fish have migrated at sea and have thus gone through some natural selection pressure. Trapping takes place at the counting stations of Tuilières (Dordogne) and Golfech (Garonne). Once captured and anesthetized, a piece of fins (caudal or pectoral) was sampled by individuals in Eppendorf tubes, containing ethanol (96%). All samples were genotyped by LABOGENA-DNA laboratory (Jouy-en-Josas, France). A part of the captured and sampled fish is sent to the F0 breeding center in Bergerac. The other part is released directly upstream the monitoring stations. The Bergerac breeding center can hold a broodstock of 150 F0 breeders. The proportion of captured and kept fish vary among years between 10% and 25% of the video monitored population for the Dordogne River and between 17% and 80% for the Garonne River. Since 2014, only in the Garonne River, a maximum of monitored fish are trapped and sampled. Fish from the Garonne River that are not kept as F0 broodstock for the stocking program are directly brought by truck, 200 km upstream Golfech to spawning areas in the Ariège River. Therefore, those fish have a facilitated access to the spawning areas in the river and a greater chance to spawn naturally.

2.1.3 Breeding plan creation

The MIGADO production scheme (*Figure 5*) uses a mix of F1 and F2 fish derived from the Bergerac F0 broodstock.

Creation of the F1 generation

Wild fish that are caught each year in both rivers and reared in captivity to Bergerac breeding center are called the “F0 generation”. The yearly turnover rate of the F0 broodstock in Bergerac is around 40% which can vary following the number of trapped fish in the rivers on a given year. The broodstock can produce a maximum of 750 000 eggs by year. These eggs are used for two main goals: About 3000 eggs, are sent to the 3 F1 multiplication centers of

Castels (24), Cauterêts (65) and Pont-Crouzet (31) that produce F2 eyed eggs from the "F1 generation" breeders. They are called F1 captive breeders because they reach maturity in the river-based fish farms in captivity, without any wild and marine phase in their life cycle. The remaining F1 eyed eggs produced (> 99%) are released at different life stages in the rivers for direct stocking.

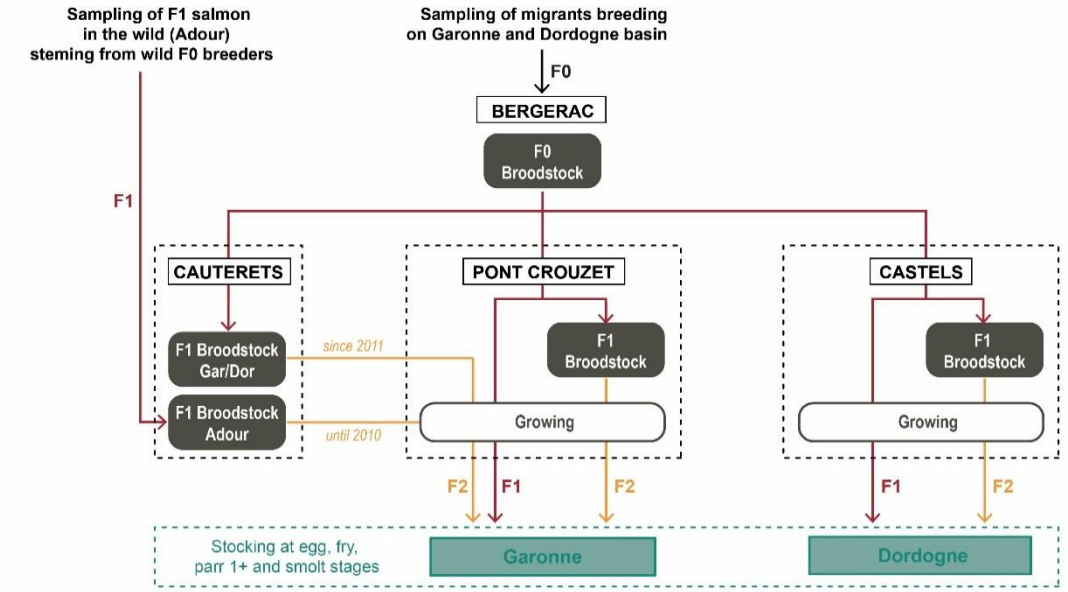


Figure 5: MIGADO production scheme (Source: MIGADO)

The following protocol is used to produce F1 eyed eggs at Bergerac breeding center: Each F0 individual is identified through an electronic transponder at its first spawn. Its electronic identifier is collected in the SYSAAF INFAQUA database system by electronic tag reader connected to the computer holding the MIGADO data base. This guarantees traceability in recording the different fish used in the different mating plans throughout their life in the farm. Indeed, each fish can spawn a maximum of five times in the F0 breeding center, the spawn of one female is fertilized with a group of males in a partial factorial design. Each spawn is subdivided in approximately 1000 eggs batch for a separate fertilization by different males. In average, the ova of one female are fertilized by 12 males. The composition of the male group varies for each female. Each day of spawning, up to 9 females are fertilized. The spawn of each female is collected in separated batches. Traceability of each incubator is recorded in the MIGADO database. They will be released in the wild at spring and early summer or kept to create enclosed breeders.

Creation of the F2 generation

The three F1 multiplication centers broodstock from F1 Bergerac eggs. They use those enclosed F1 breeders to produce the major part of the individuals of the stocking program: the F2 generation. The F1 broodstock of each multiplication center comprises between 800 and 1 200 individuals, of which between 30% and 40% is renewed each year. An individual can spawn a maximum of 7 years. In total, nearly 2 million F2 eggs are produced each year. In F1 hatcheries each F1 individual is also identified following the same protocol as for F0 and its electronic identifier is recorded in the MIGADO data base. F1 enclosed breeders are also genotyped with the same protocol as described for their F0 trapped parents. The mating protocol differs between the creation of F1 and F2 generations. In the F1 multiplication hatcheries the spawns of 12 to 18 dams are pooled and then sub-divided in three containers. Each container is

fertilized with the pooled milt of two different males, in order to secure the quantitative production of eggs, even if differential of fertilization success. Thus, each F2 batch originates from 12 to 18 are fertilized with 6 males in a partial factorial mating design. It is important to notice that it is difficult to anticipate the daily breeding plan (F0 and F1) due to asynchronicity between individuals.

2.1.4 Stocking strategy

The stocking strategy is based on the release of large numbers of Atlantic salmon offspring from the two generations different produced (25% F1 and 75% F2). The stocking areas are showed on the map in *Figure 6a*. For the Garonne basin, three areas are used for stocking:

- Garonne River upstream Pointis (31)
- Neste River upstream Pointis (31)
- Ariège River downstream Pamiers (09)

For the Dordogne Basin, three areas are used for stocking:

- Dordogne River upstream Carennac (46)
- Corrèze River upstream Brive (19)
- Vézère River

The stocked fish are released at different stages released:

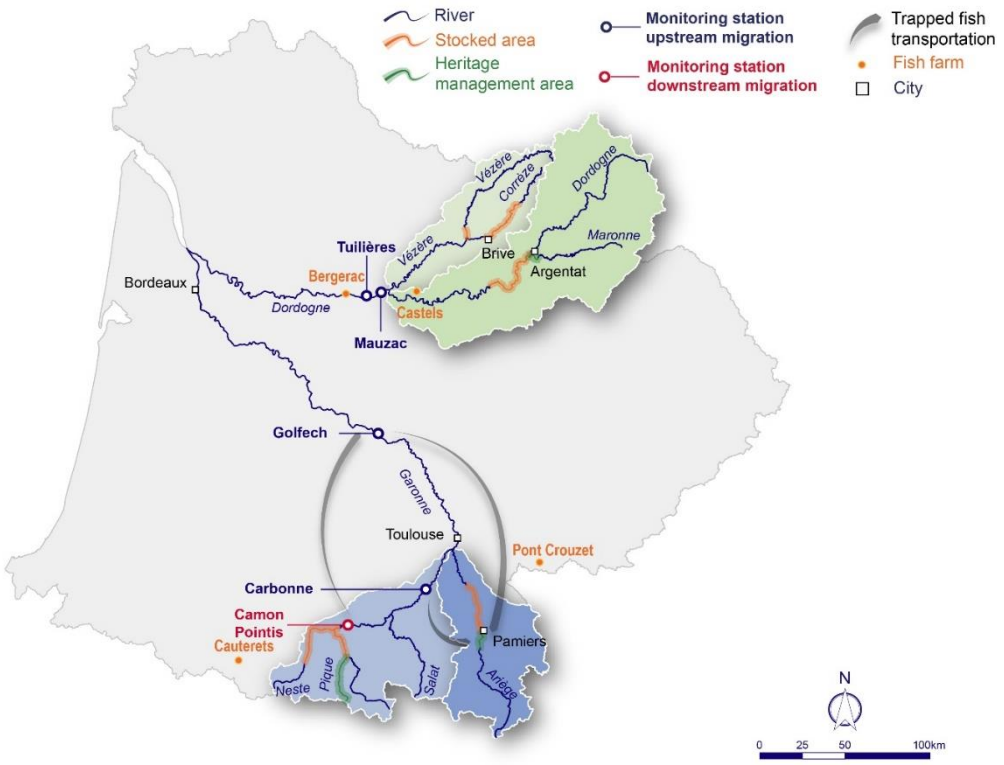
Eyed egg: advantageous on economical (low production costs) and biological (individual facing environmental conditions at birth) aspects. This stage is more difficult to stock (needs more time and people) than the other ones.

Fry: Limited intensity of domestication and low breeding cost (feeding between 1 and 3 months). Quantitatively, the most important released stage.

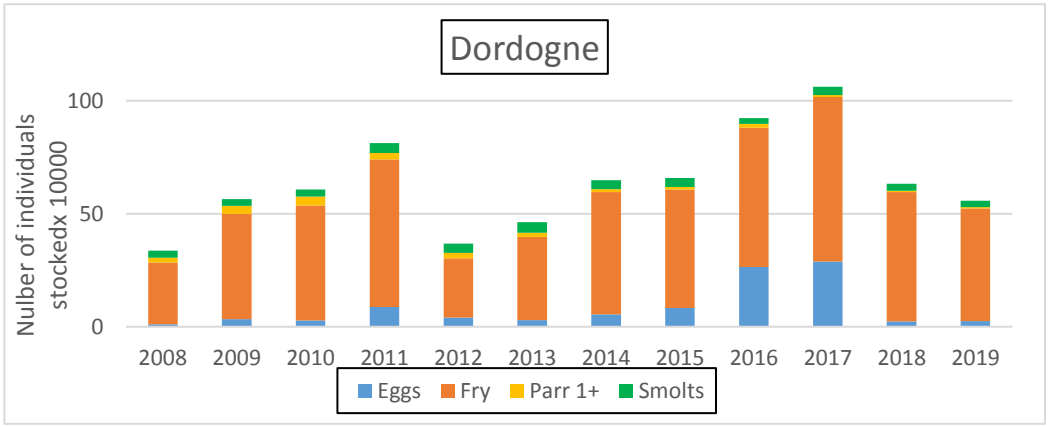
Parr 1+: One-year old salmon that did not smoltify. They are released downstream the fry stocking area.

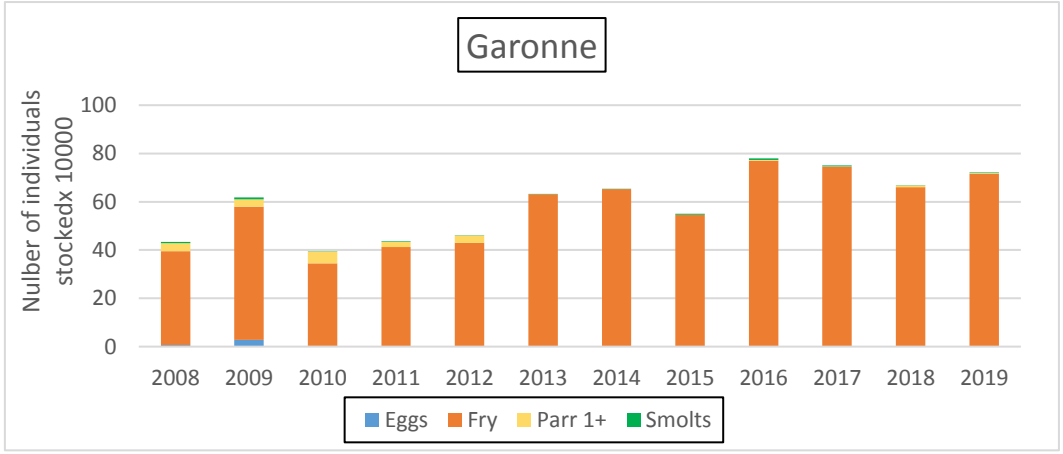
Smolt: High rearing cost but minimized mortality rate during the freshwater phase. They are released in the most downstream areas after the last hydroelectric development.

Figure 6b shows the yearly numbers stocked by stages for both basins since the beginning of the current study in 2008. In the Garonne basin, the stocking strategy has been directed towards fry that represent in average 96% of the stocked individuals. Eggs, parr and smolts are barely used, respectively 0.5%, 2.5% and 0.5% of stocked individuals. In the Dordogne basin, fry is also the most important stocked stage but the egg stage represents in average 13% of the individuals. Smolt and parr are also more frequently stocked in the Dordogne with respectively 6% and 3% of stocked individuals.



a)





b)

Figure 6: Maps showing stocking area (a). Number of stocked fish (b)

2.2 PARENTAGE ASSIGNMENT AS A MONITORING TOOL

2.2.1 Principles of parentage assignment

Parentage assignment is expected to identify fish pedigree. As all breeding plans are registered in the database and all F0 and F1 broodstock are genotyped, we should be able to identify the parents (and hence the origin) of any fish trapped in the river that comes from the restocking program. In total, 1014 migrants between 2010 and 2019 were genotyped with a panel of 9 microsatellite loci developed and tested in GENESALM project on experimental families produced by MIGADO based on Paterson et al (2004) . The assignment has been carried out by LABOGENA-DNA with the software AccurAssign based on a maximum likelihood approach (Boichard et al, 2014).

The AccurAssign gives five different types of results:

Type 1: Individuals considered non-compliant (NC) for which the quality of the DNA sample only allows genotyping less than 7 markers out of 9. Those individuals were removed from the dataset and all following analyses. Some potential parents can also be non-compliant because of some issue with their DNA sample. All those parents have been identified to be able to estimate the percentage of offspring considered as non-assigned while they could be offspring of non-compliant parents.

Type 2: Individuals assigned in the breeding plan (ABP) for which a single pair of compatible breeders has been found, which is recorder in a hatchery breeding plan (F0 or F1). These individuals necessarily come from the stocking program.

Type 3: Individuals that are not assigned (NA) for which no compatible parent pair has been found. They necessarily come from natural spawning.

Type 4 and 5: AccurAssign can also assign an individual to a parent pair that is not in the breeding plan (ANBP, Type 4) or to multiple possible parent pairs (MABP, Type 5). According to simulation conducted before this work (Marc Vandeputte, personal communication) in 2016 with the genotypes of 3 500 females and 2 000 males from Bergerac, Castels, Pont-Crouzet and Cauterets fishfarms, Type 4 and 5 fish should be considered as originating from wild spawnings (Unpublished data).

Therefore, only fish assigned in the breeding plan are (ABP, Type 1) considered as stocked fish.

2.2.2 Assignment power of the microsatellite panel

To assess the power of assignment with 9 microsatellites loci at a larger scale than in the GENESALM, we applied APIS software (Griot et al., 2020), as AccurAssign was not available, on F1 enclosed breeders originating from F0 wild parents. Individuals from both generations were tagged and genotyped. As every mating was recorded in breeding plans, it was possible to estimate the power of the panel of microsatellite between 2008 and 2016.

2.2.3 Identification of wild-born individuals from known parents

Since 2014, in the Garonne River a maximum number of individuals are captured and genotyped with the formerly described protocol. Part of those animals are directly sent to spawning areas in the Ariège River, a tributary of the Garonne River. As those animals are also genotyped, we should be able to assign their potential offspring to them if they mate together in the wild. Therefore, we simulated a mating scheme in the wild with all genotyped individuals (n = 145) sent to the upper spawning areas between 2014 and 2016 to test this hypothesis. The

sex of those parents were not known and all animals could then be either male or female in the mating scheme Then, we try to assign to them salmon that came back after 2016 that were considered as wild salmon. Indeed, the first conveyed salmons in 2014 must have spawned in 2014. Therefore, their offspring migrated downstream end of 2015, and came back at the earliest in 2017 after having spent at least one winter at sea. We also conducted an assignment analysis on wild parrs caught by electric fishing performed by MIGADO in Ariège River in 2015, 2017 and 2018 in areas where no stocking of juveniles occurs. The aim of those analysis was to evidence that the population is capable of reproduction in the wild. Assignment tests were conducted with APIS software, as AccurAssign require the sex of each individuals. If a parr or a wild-considered migrant were assigned to a non-possible couple (meaning two adults of different conveyed years), this salmon was considered as non-assigned in the wild mating scheme. This assignment analysis was conducted on a total number of 132migrants and 63 parrs.

2.3 DATA ANALYSIS

2.3.1 Sampling analysis

As only part of the population is trapped, it was important to know if the sampled population was representative of the video monitored population in terms of age at sea. We analyzed the sampling strategy by testing the difference between the number of 1 Sea Winter (1SW), 2 Sea Winters (2SW) and 3 Sea Winters (3SW) in the monitored and sampled population in both basins. The proportion of the monitored and sampled population for each week were compared by cumulating years from 2010 to 2019.

2.3.2 Stocking analysis

To conduct analysis on stocking efficiency by stages, from a hatchery point of view, we transformed the number of eggs, smolts and parr stocked to fry equivalents. These equivalents have been calculated with the following average recorded survival rates in the hatchery: 75% from egg to fry and 83% from fry to smolt or parr. We gathered smolts and parr stages in the category salmon 1+ as both stages spend nearly the same time in the fish farm, only a part of them smoltifying. Thus, an egg accounts for 0.75 fry, and a smolt or a parr to 1.20 fry. Then we compared the number of fish stocked in fry equivalent and the number of migrants that came back for each category.

To compare the relative efficiency of each stage, we calculated a “capacity for coming back in the river” based on the ratio between the number of migrants that have returned for each stage and the number of individuals stocked for each stage (not converted to fry equivalent). All further analyses were then conducted only with fish stocked as fry to avoid introducing bias with the other stages. We compared stocking efficiency between, basins, rivers, generations (F1 or F2) and cohorts (corresponds to one year of stocking). Therefore, we were able to identify the points that presented issues in the stocking strategy.

2.3.3 Straying rate analysis

As hatchery-salmon are generally more susceptible to stray than wild ones (Ford et al., 2015), we evaluated the straying rate in this population (i.e. the proportion of salmon released in one basin [Garonne or Dordogne] and returning in the other one at the adult stage). As the stocked basin is known for most of the stocked juveniles (each batch of stocked juveniles has its stocking area recorded in the database), we were able to compare the straying rates between basins, generations and the number of sea winters to assess the most impactful parameters.

2.3.4 Sea-winter phenotype analysis

Sea winter phenotype (number of winters at sea) has some heritable components in Atlantic salmon (Gjerde et al., 1994; Wild et al., 1994). It was therefore interesting to get some information on this hypothesis. When a salmon is caught and sent to Bergerac center, its sea winter phenotype (age at sea) is known thanks to the classification showed in 2.1.1. As each hatchery-reared F1 or F2 migrant was assigned to a couple of parents, we knew the average parent age at sea phenotype for each F1 migrant and the average grandparent age at sea phenotype for each F2 migrants (as F1 parents are enclosed breeders that do not migrate at sea). For this analysis, the sea winter phenotype was classified as either 1SW or MSW (Multiple Sea-Winter). We compared the mean F0 parental sea age between 1SW F1 and MSW F1. The same was done with F2 migrants (with grand-parental age at sea).

2.3.5 Broodstock and breeding plan evaluation

For both generations, we calculated for each year between 2010 and 2019, the sex ratio, the number of reconditioning and the mean time spent for a fish in the hatchery. We also quantified the number of families created each year and we compared that to the number of crosses recorded in the breeding plans archived in the database. Therefore, we were able to evaluate the proportion of crosses made more than one time through years of spawning. Finally, we evaluated genetic variability each year by calculating genetic diversity indices: allelic richness (AR), expected heterozygosity (He), observed heterozygosity (Ho). The inbreeding coefficient (FIS) was also evaluated for each spawning cohort in each hatchery. Those analyses were used to estimate the evolution of the genetic diversity in the broodstock of all hatcheries and in the breeding plan strategy to advice the next evolution.

2.3.6 Evaluation of genetic diversity loss between generations

To evaluate the potential loss of genetic diversity between F0 and F1 generation and F1 and F2 we calculated first the effective population size of each year for the F0 broostock and for the F1 broodstock (Ne) with the following formulae (Chevassus, 1989) :

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$$\frac{4}{Ne} = \frac{\left(Km + \frac{Vm}{Km}\right) + \left(Kf + \frac{Vf}{Kf}\right) - 2}{N - 2}$$

$$With\ Km/f\ :\ mean\ of\ offspring\ number\ per\ males/females$$
$$Vm/Vf\ :\ variance\ of\ offspring\ number\ per\ males/females$$
$$N\ :\ total\ number\ of\ offspring$$

Then, the loss of genetic variability each year between F0 broodstock and F1 broodstock as well as between F1 broodstock and F2 migrants was evaluated with the following formulae:

$$Loss\ of\ genetic\ diversity = 1 - \left(\frac{1}{2Ne}\right)$$

2.3.7 Genetic analysis of the population in the basin

As the main goal of the program is to restore a self-sustainable population in the basin, we evaluated the proportion of the non-hatchery-reared origin fish in both basins through years and we quantified the evolution of this proportion. Moreover, as migrants stemming from wild spawning are genotyped, we compared their genetic diversity indices (H_E, A_R, H_O, F_{IS}) with those of hatchery-reared migrants.

2.3.8 Software used

Allele number and allelic richness were obtained using Fstat 2.9.3.2 (Goudet 1995). Allelic richness was calculated for the smallest number of individuals in a sample. Expected heterozygosity, He (Nei 1978), observed heterozygosity, Ho and inbreeding coefficient (Fis) were calculated with GENETIX 4.05.2 (Belkhir et al., 1996). Significance of Fis coefficient was tested with 1000 bootstraps on all loci with GENETIX. All statistical analysis, chisquare and Mann-Whitney tests and regression were conducted with R software. Chisquare test were used to assess significant differences between the monitored and the sampled population but also to evaluate differences between the quantity of stocked fish and the number of migrants that came in the basin. Mann-Whitney tests were used to assess significant differences between groups of fish for genetic diversity indices. Graphics and Figures have been made using Excel.

3. Results

3.1 STUDY VALIDATION

3.1.1 Validation of the parentage assignment panel

To validate the microsatellite panel, we assigned F1 hatchery-reared salmon of multiplication hatcheries to their F0 parents from the Bergerac breeding center, each with APIS software. Assignment rates are reported in Table 1 for the different cohorts produced from 2008 to 2016.

The assignment rates were high, from 92.9% in 2012 to100% in 2008, 2009, 2010 and 2011. A lower assignment rate (91.0% in 2016) can be explained by the proportion of non-compliant parents (individuals genotyped for less than 7 markers), in that year class.

Table 1: Assignment rate for F1 offspring (NC = non-compliant fish with less than 7 markers genotyped)

Year of birth	Number of F1	NC Offspring	Assignment %	NC Parents
2008	601	5	100.0%	0%
2009	1154	17	100.0%	0%
2010	1296	8	100.0%	0%
2011	831	3	100.0%	0%
2012	414	4	92.9%	0%
2013	1922	12	94.1%	0%
2014	809	5	96.4%	0%
2015	849	5	95.9%	0%
2016	794	9	91.0%	2%

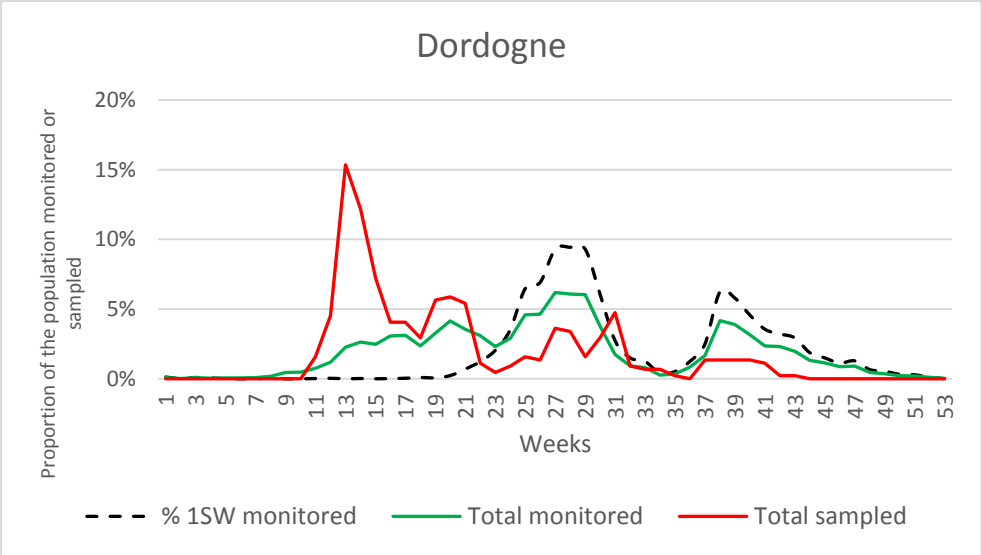
3.1.2 Sampling validation in terms of age at sea structure

Table 2: Distribution of sea winters (SW) in the sampled (Sam.) and monitored (Mon.) Atlantic salmon population in the Garonne Dordogne basin from 2012 to 2019

	Garonne						Dordogne					
	1SW		2SW		3SW		1SW		2SW		3SW	
Year	Mon.	Sam.	Mon.	Sam.	Mon.	Sam.	Mon.	Sam.	Mon.	Sam.	Mon.	Sam.
2012	27	7	93	14	13	4	88	6	220	29	44	4
2013	2	0	40	8	9	1	123	21	48	13	33	8
2014	8	0	123	50	11	2	42	0	263	80	30	4
2015	10	9	188	81	21	9	91	2	524	76	59	4
2016	53	13	92	44	4	4	261	15	276	39	33	7
2017	1	0	82	32	3	3	71	2	155	19	17	3
2018	36	5	39	11	2	0	57	6	421	52	17	1
2019	12	2	113	96	16	11	72	9	156	15	28	3
Total	**149**	**36**	**770**	**336**	**79**	**34**	**805**	**61**	**2063**	**323**	**261**	**34**
Mean%	**14,9%**	**8,9%**	**77,2%**	**82,7%**	***7,9%***	**8,4%**	***25,7%***	**14,6%**	***66%***	**77,3%**	***8,3%***	**8,1%**
	Mon. Tot	**998**	**Sam. Tot**	**406**			**Mon. Tot**	**3129**	**Sam. Tot**	**418**		

We checked if the sea winter number varied among year classes and rivers and if the population sea age structure of the sampled fish was similar to that of the whole population. Table 2 shows the number of fish sampled and monitored according to their number of year in the sea (SW) by basin and year class.

For Garonne and Dordogne Rivers, there were significant differences between the monitored and the sampled population in terms of sea winter (Garonne : $X^2 = 9.28$, df = 2, p-value = 0.0097 and Dordogne : $X^2 = 25.791$, df = 2, p-value = 7.098e-07). We note an under-sampling of 1SW salmon for both rivers, which must be taken in account for the subsequent analysis. The Figure 7 shows that one-year at sea salmons begin their upstream migration in early summer which correspond to an under-sampling period.



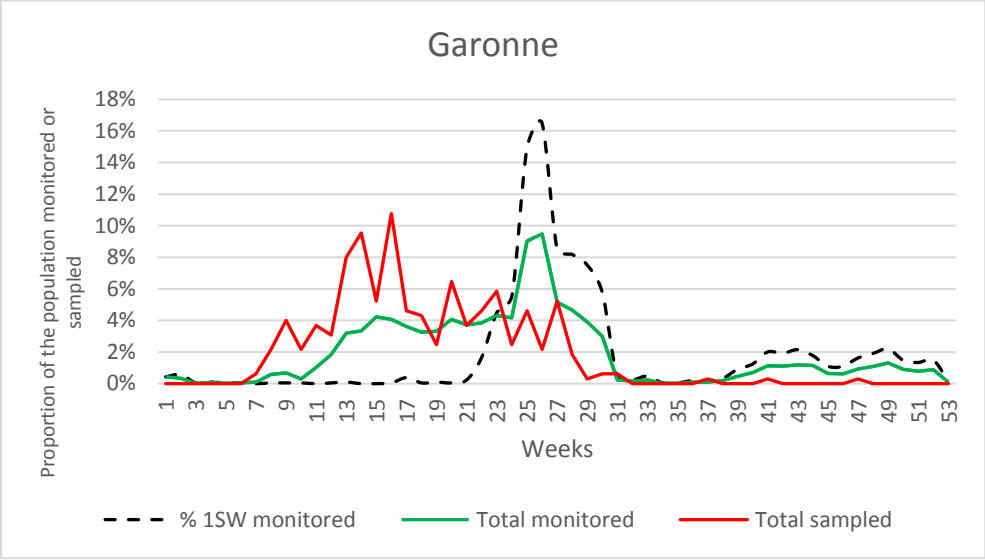


Figure 7: Monitored and sampled population –Average for years 2012 to 2019

3.2 ANALYSES OF THE STOCKED POPULATION

This part of the analysis has been conducted on the sampled fish identified as hatchery-born returning adult. In total, the 8 cohorts stocked between 2008 and 2015 represent 559 sampled returning adults identified as hatchery born and almost 9 million restocked egg, fry, parr and smolt.

3.2.1 What does influence the return rate?

3.2.1.1 Influence of stocking stages:

In this program, stocking occurs at different stages. In a given year, the same parents provide offspring that are stocked at a single stage (egg, fry or salmon 1+ (parr and smolt)). The estimated return rate for each stage is presented in Table 3. It shows that fry stage is the most efficient stocking stage. Smolts stage has also a return rate but parr stage has the lowest.

Considering the survival rates in the hatchery, any stage can be converted to fry equivalents, in order to compare the most efficient strategy from the manager's point of view (*Table 3*). The fry stage is the most used stages representing 90% of the stocking effort. There was significant heterogeneity between the proportions of fry equivalent stocked and the proportion of returning migrants from each stage ($X^2 = 21.57$, df = 2, p-value = 2.54e-05). The fry stage was the most efficient stage to stock as the proportion of migrants is more important than the proportion of fish stocked at this stage. In contrast, egg stage and one-year salmon, either parr or smolt, are less efficient with proportionally a lower proportion of fish returning than stocked.

Table 3: Assigned migrants and headcount stocked between 2008 and 2015.

	Egg	Fry	Salmon 1+	
Assigned migrants	9	534	16	
Number stocked	460908	8001827	485105	
Fry equivalent*	345681	8001827	584464	
% Assigned migrant	**1,61%**	**95,53%**	**2,86%**	
% Fry equivalent stocked	**3,87%**	**89,59%**	**6,54%**	
	Egg	Fry	Smolts	Parr1+
Estimated return rate**	1,96E-05	6,67E-05	4,61E-05	1,10E-05

*based on survival in hatchery (1 egg = 0,75 fry and 1 salmon 1+ = 1,23 fry)
**ratio between assigned migrants and the number of individual stocked

3.2.1.2 Influence of generation, basin and cohorts

To avoid introducing bias and because it is the most important stage in terms of stocking and migrants, all further analyses have been conducted only on migrants stocked at the fry stage which represents 534 returning adults (95.5% of the returning adults) and 8 001 827 stocked fry (89.4% of the stocked individuals).

Stocking of F1 fry (from F0 parents) and stocking of F2 fry (from captive F1 hatchery parents) had the same efficiency (X^2=1.99, df = 1, p-value = 1). Indeed, 25.3% of the returning migrants are F1s and 25.2% of the stocked fish were F1s. Therefore, there is no difference in return capacity between F1 and F2 fish stocked at this stage.

Analyses were also conducted to know if some cohorts were more efficient. Chisquare test showed an inhomogeneity in data (X^2 = 229.5, df =7, p.value < 2.2e-16) with three years that gave more migrants than stocked fish in terms of proportion (2008, 2011 and 2012), and four years where we noticed less productivity (2009, 2010, 2014 and 2015). An important parameter to test was the basin and the river of stocking to know if the stocking in Garonne and Dordogne had the same efficiency. However, a less important number of fish was available for this analysis due to traceability issues at the beginning of the program. All batches of stocked fish and their corresponding migrants with uncertain stocking places have been removed for this analysis. Therefore, it has been conducted on 445 migrants from 7 million stocked fish at fry stage. Results are shown in Figure 8. A migrant is considered as a Garonne migrant if it has been stocked in the Garonne basin. Straying effect will be treated afterwards.

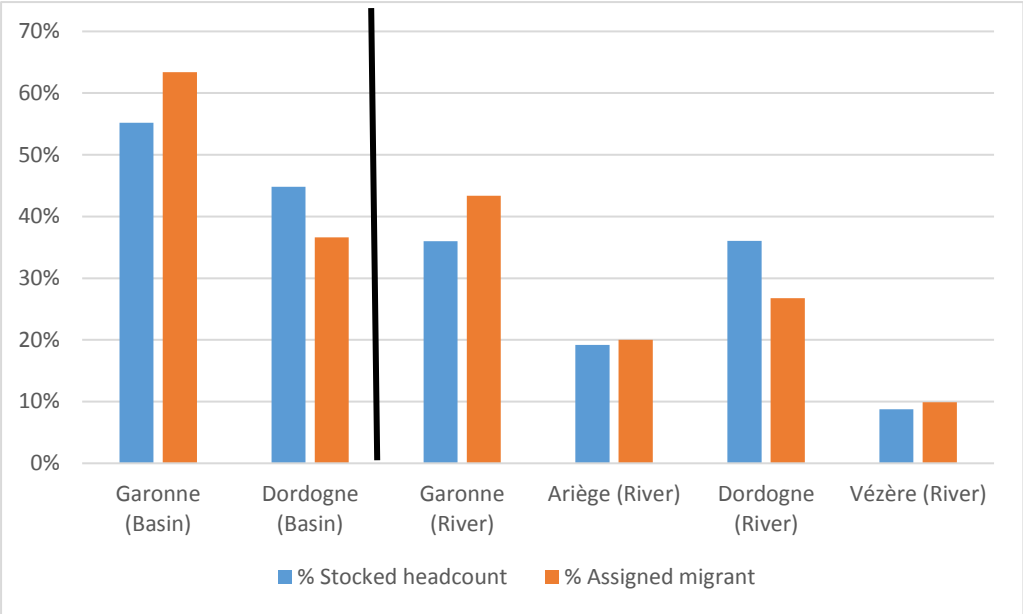


Figure 8: Comparison between stocked salmons and their assigned migrants in the basins and the rivers of the study between 2008 and 2015

There were significant differences between Garonne and Dordogne (X^2 = 11.68, df= 1, p-value = 0.00063) with a clearly lower efficiency of return rate for the fish stocked in the Dordogne basin. They represent 44.8% of the stocked fry, but migrants that came back to this basin were only 36.6% of the total number of migrants. To be more specific and because there are two rivers for stocking in both basins, we also tested the river effect. There were again significant differences (X^2 = 18.23, df = 3, p.value = 0.0004) between stocked rivers. The lower efficiency observed for the Dordogne basin was clearly specific to the Dordogne River, while the higher efficiency in the Garonne basin was specific to the Garonne river. Stocking in Vézère (Dordogne basin) and Ariège (Garonne basin) had an average efficiency.

3.2.2 What does influence straying rate?

In this part, we considered all the migrants (n=455), whatever their stocking stage, that came back from a known stocking river. A fish is considered as a straying salmon when its upstream migration river is different from the river where it was stocked. In total we observed a global straying rate of 11%, which corresponds to 52 strayers. We tested different effects on straying. Salmon from F1 and F2 generation have different straying rates (respectively 18,9% and 10,2%), although this difference is not significant (X^2 = 2.6941, df = 1, p.value = 0.100). We found a significant difference between Garonne salmon straying rate (16%) and Dordogne salmon straying rate (4%) (X^2 = 13.12, df = 1, p-value = 0.0003). We also tested the influence of the number of sea winters with a binomial regression as a Chi-Square test was unsuitable. 2SW salmon have significant lower odds to stray (Estimate = -1.23, z-value = 1.27E-05) than 1SW salmons. No significant impact for 3SW was found (Estimate = -0.74, z-value = 0.205) compared to 1SW. Figure 9 shows the differences between homed and strayed salmon. There was not enough data to test the effect of the upstream migration year. However high variation of straying rate has been observed through years.

3.2.3 Sea winter population structure.

The population sampled showed a bias in terms of population structure with an over-sampling of 2SW salmon and an under-sampling of 1SW. We tested whether the sea-winter structure of the stocked fish was influenced by some parameters. In this part we used the 559 fish from 2008 to 2015 cohorts (year of stocking), except when we tested the effect of the stocking basin, where we used 454 fishes for which the true stocking location was known.

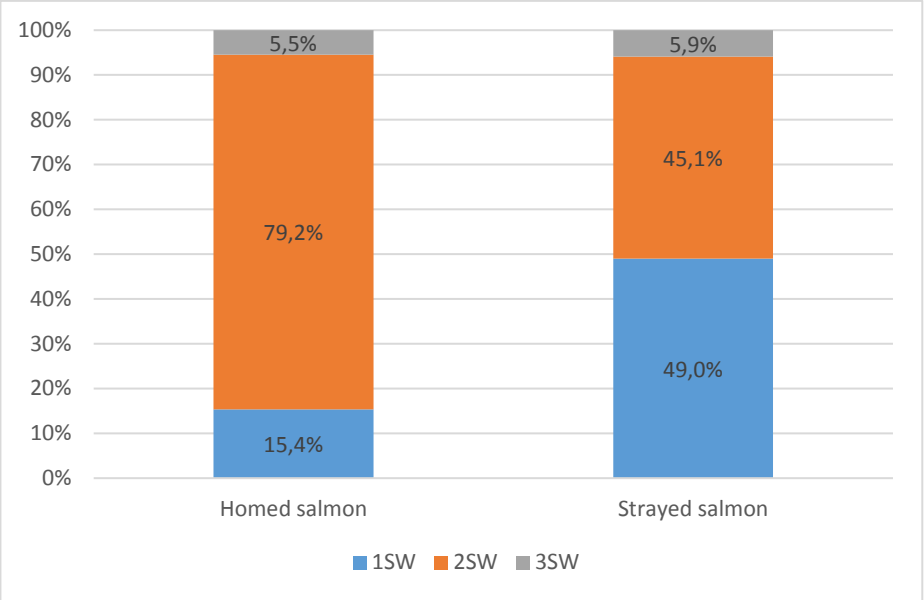
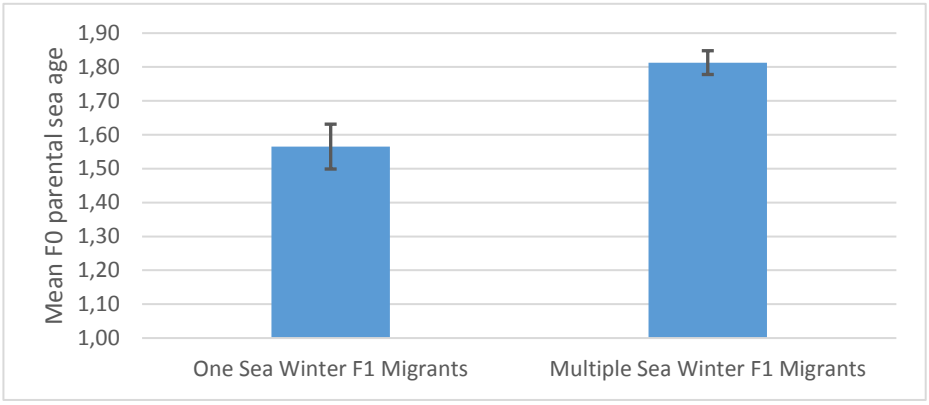


Figure 9: Sea-winter population structure comparison between homed and strayed salmon – Year 2008-2015

The sea winter structure did not differ between the two basins ($X^2 = 2.37$, df =2, p-value = 0.31) with a global structure of 19.2% of 1SW, 75.3% of 2SW and 5.5% of 3SW. No significant differences were found between F1s and F2s ($X^2= 4.88$, df =2, p-value = 0.087). The impact of stocking stages, cohorts and year of upstream migration could not be tested, but some cohorts seemed to have different age structure.

We tested whether we could observe a link between the sea-winter phenotypes of the migrants and their parents. First, we tested if F1 offspring sea winter phenotype was correlated with the sea winter phenotype of its parents. Figure 10 (a) points out that the average parent's sea age of 1SW F1 offspring was lower (1.57) than that of multiple sea winters (MSW) F1 offspring (1.81). Figure 10 (b) also shows this link through the regression between the average sea age of all F1 offspring of a cohort and the average sea age of their parents. Apart from the 2009 cohort (see BR09 on the graph), the relation between offspring and parental phenotypes is clear. We can also point out a temporal effect as average sea winter phenotype seems to increase since the beginning of the study. The same effect has been tested on F2 migrants on their grandparent's phenotype as their parents are captive breeders, with no sea-winter phenotype. The difference between 1SW grand-parental phenotype (1.60) and MSW grand-parental phenotype (1.67) is not as high for F2 as for F1 generation but still present a significant difference.



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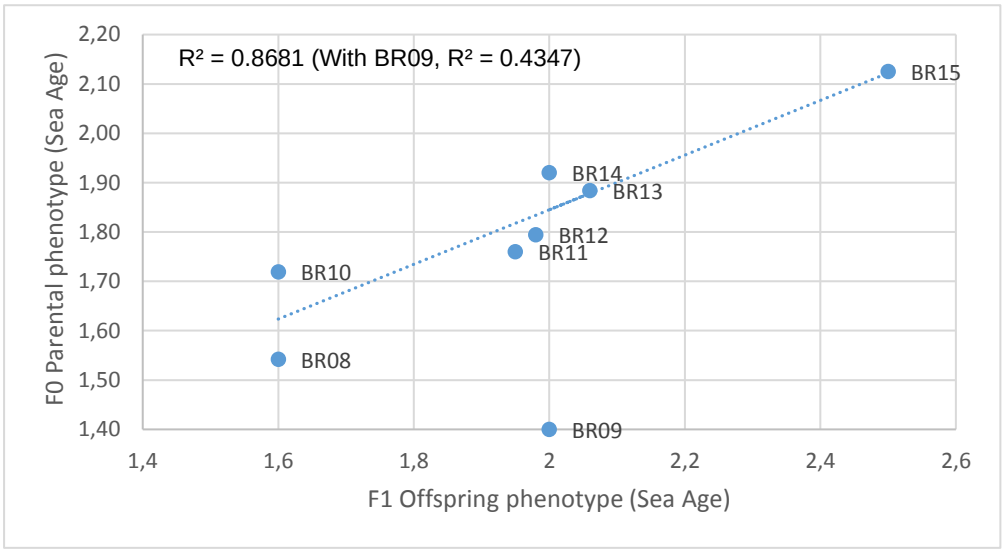

b)

Figure 10: Relation between F1 migrants sea-age phenotype and F0 sea-age phenotype

3.3 IMPACT OF BREEDING STRATEGIES ON GENETIC VARIABILITY.

All eggs, fry, parr 1+ and smolt that are stocked stem from hatchery-reared broodstock that are limited in number, especially the Bergerac F0 population. In this study, we analyzed all breeding plan made since 2008, to assess 1) the composition and genetic variability of the broodstocks and 2) the consequences of breeding plan practices on F1 and F2 generation.

3.3.1 F0 breeding plan and outcomes

F0 crosses take place at Bergerac center according to the protocol described in Materials and Methods. For each spawning season in Bergerac center, we calculated average number of year spent by the broodstock in the center (e.g : in 2010, on average, males that spawn had spent on average 2.4 years in the fish farm and females 2.8 years). The evolution of this parameter is presented in Figure 11. We noticed a clear decrease in the number of years spent by breeders in the hatchery after 2011. We also calculated the evolution of the number of use of the same fish in mating plans throughout the different spawning seasons, called “reconditioning” (Meaning that a fish is used in year n, n+1, etc..). This number of reconditioning has decreased drastically since the beginning of the program, from 3.5 times for fish caught in 2007 to 1.5 times for fish caught in 2015. This means that the renewal rate is now more important between each spawning season.

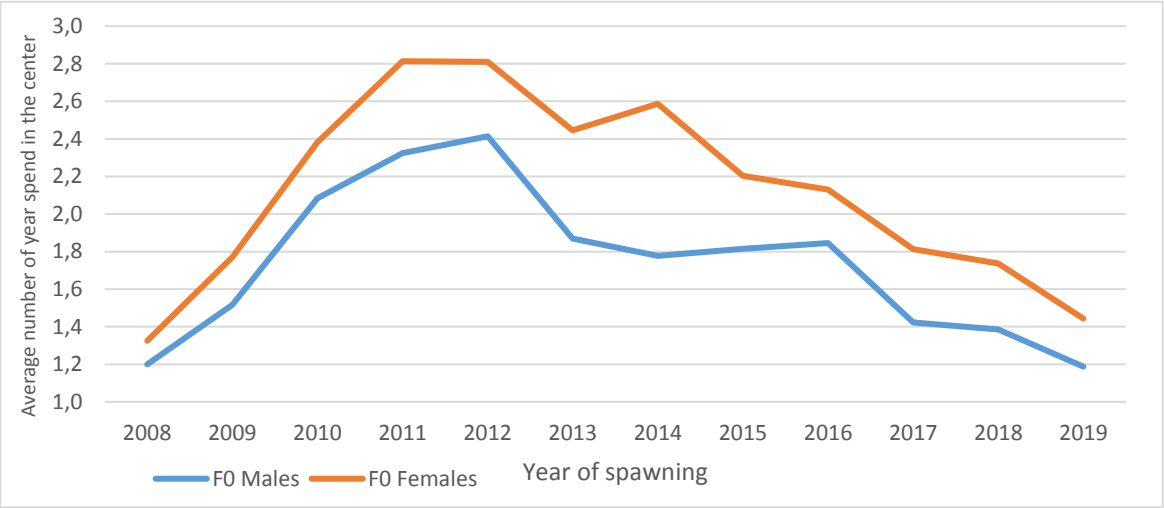

Figure 11: Number of years spent in the F0 Bergerac center in average for each spawning year

In addition, we found that the age of a female in hatchery (Number of use) had no impact on migrant's efficiency by comparing the number of spawns made by female at different age and their number of produced migrants (X^2 = 2,07, df = 4, p.value = 0,72) (*Figure 12*). Therefore, the capacity of a migrant to come back is not linked with the number of female reconditioning at Bergerac center.

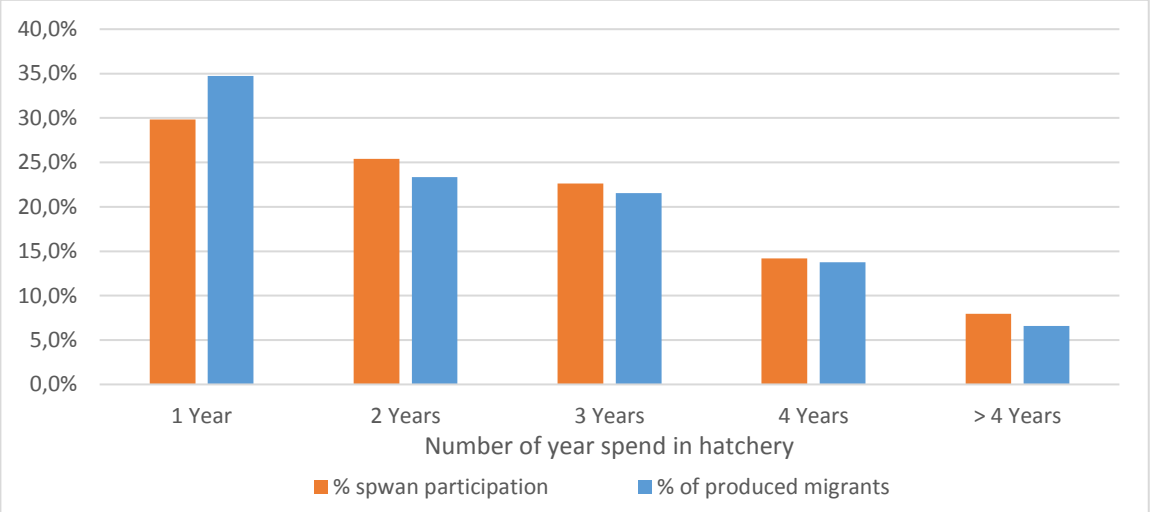

Figure 12: Comparison between the spawn participation and the produced migrants for F0 females, depending their number of years spent in hatchery. 2008-2015

Sex ratio (number of males /number females) was very stable through breeding years, around 0.39. The number of breeders in the broodstock is highly variable through years (min. 51 individuals, max. 108 individuals). As all individuals from the broodstock are not used all together for spawning (it is technically impossible) only a part of the overall available genetic diversity is exploited each year. As such, 11 479 crosses were made, representing a total of 7 779 genetically different families throughout the 2008 to 2019 spawning seasons at Bergerac. As more crosses than families were made, some crosses have been repeated within the same spawn and/ or between years with fish reuse.

3.3.2 Genetic variability of F0 broodstock and impact on F1 enclosed breeders.

Genetic diversity was estimated for all F0 spawning groups since 2008. Data are presented in Table 4. No clear evolution was found on allelic richness (A_R) and expected heterozygosity (H_E), which remained stable through years in the F0 broodstock. However, the inbreeding coefficient (F_{IS}) is significantly higher than 0 for years 2012, 2013 and 2014.

As the Bergerac broodstock is still the main source of restocking for the Garonne-Dordogne salmon population (the percentage of hatchery-reared offspring in the F0 broodstock is around 60% approximately since 2013), a minimal loss of genetic diversity between generations is recommended. The effective size of the F0 broodstock at Bergerac (Ne) and the genetic diversity loss are presented in Figure 13 for each spawning year. The genetic diversity loss seems to diminish since the beginning of the program, meaning a better use of the broodstock, resulting from the increase of the turnover in the broodstock. On average the observed loss of genetic diversity is 1.08% (±0,09%) each year.

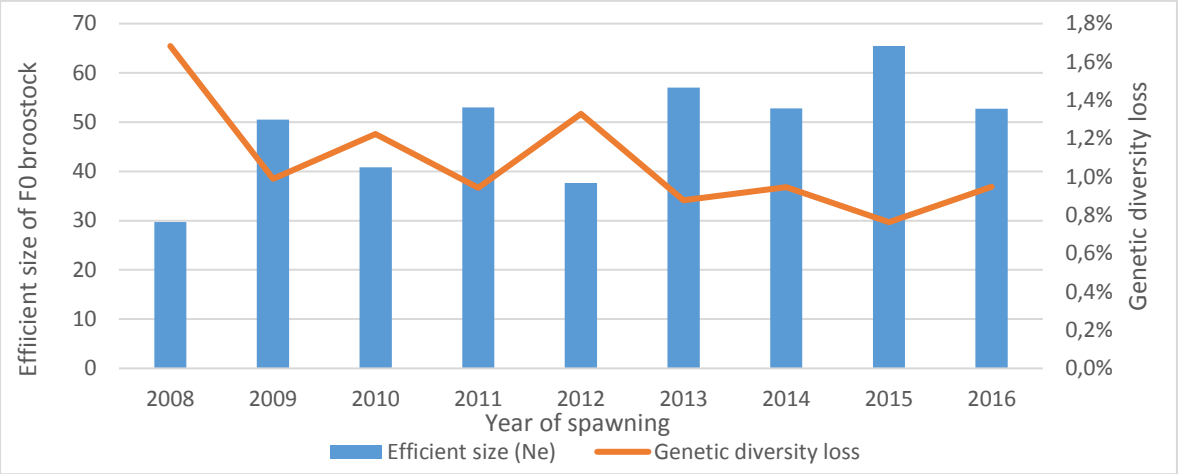

Figure 13: Effective size of the F0 broodstock and genetic diversity loss between F0 and F1

To evaluate if this loss of genetic diversity had an impact on F1 breeders through years, genetic diversity indices were also been calculated for all captive F1 breeders. No significant loss of genetic diversity was found through years, neither on H_E or A_R (calculated on 942 individuals). We also compared expected and observed heterozygosity between F0 and F1 and no significant differences were found.

3.3.3 F1 breeding plan and outcomes.

The same analysis as in 3.3.1 were conducted on all F1 multiplication fish farms. The sex ratio and the number of utilization of fish in the broodstocks did not vary between 2008 and 2019. Number of fish in the broodstocks each is available in Table 4. The main difference with Bergerac broodstock is that the number of breeders is far more important in each farm. Consequences are that almost no crosses are duplicated through spawning seasons and that for a given fish, the number of reuse is less important in those multiplication centers.

3.3.4 Genetic variability of F1 captive broodstock and impact on F2 generation.

Table 4 presents the genetic diversity indices calculated for all captive breeders F1 together. We also calculated these indices within each fish farm. No significant evolution was observed except for Cauterets fish farm where genetic diversity indices showed a clear difference between 2008 to 2010 period and 2011 to 2019 period. Indeed, the composition of the broodstock in the first years of the study (2008-2010) differed slightly from the actual one

(2011-2019). Between 2008 and 2010 fish kept at Cauterêts were fish originated from Adour basin and not Garonne-Dordogne basin. It appears that the initial fish had less genetic diversity (HE from 0.76 to 0.80) than current fish (HE from 0.81 to 0.83) but also an inbreeding coefficient significantly higher than 0. To add more information to the data, Fis was also calculated for each broodstock in each farm. Almost every year for each stock presented an inbreeding coefficient significantly higher than 0 (ranging from -0.003 to 0.059).

Genetic diversity loss between F1 captive breeders and their offspring F2 migrants were calculated the same way as in part 3.3.2. Average loss of diversity was around 0.17% (±0.02%), which is almost 5 times less than between F0 and F1 generation.

3.4 ANALYSES OF THE POPULATION IN THE DORDOGNE-GARONNE BASIN.

In this part we tried to confirm that natural spawning occurs in the basin with parentage assignment of wild born juveniles and we evaluated the return of wild salmons. Genetic variability was also compared between hatchery-origin fish and wild fish.

3.4.1 Assessment of natural spawning level in Ariège

We considered only the fish transported between Golfech and Ariege spawning areas to assess the natural reproduction in this area. 30 parrs out of 74 were assigned to 7 parental pairs from the putative breeding scheme done in the wild, with a range of 1 to 12 offspring per pair, meaning 45% of the tested parr. Concerning the migrants that came back between 2017 and 2019, 3 migrants out of 132 were assigned to one pair, meaning 2.3% of the tested migrants.

3.4.2 Return of wild born salmon

The return of wild born salmons each year can be assessed as the number of salmons trapped and genotyped that are not assigned to hatchery-reared salmons. Those fish have been identified in this study as wild born ones. In total, 305 wild born salmons were identified between 2012 and 2019. Salmon that came back in 2010 and 2011 were removed from further data analyses, as they could either come from wild spawning but also from ancient breeding plans from 2006 and 2007 when breeders were not genotyped or from other basins like the Loire-Allier basin. The number of hatchery-reared origin salmon was calculated per year of upstream migration. Note that the migration basin was considered for all salmons in this analysis and not the stocked basin. Indeed, it was impossible to determine if wild born salmons were strayed fish from other rivers than Dordogne or Garonne basins.

Figure 14 shows the global annual evolution of the wild salmon proportion since 2012 as well as per basin. Between 2012 and 2019, 37% of the sampled salmon were identified as wild born. The ratio of wild born salmons was stable between 2013 and 2017, with 30% on average. A clear increase of this ratio was found for the last two years of the study. Per basin, trends are somehow different, as Dordogne basin showed no evolution in the ratio. In contrast, in the Garonne, the ratio followed the same trend as the global one with a clear increase the last two years, especially for 2019 were 70 wild fish migrated upstream. No significant difference was found in the wild born salmon ratios between basins ($W= 17$, p-value = 0.127).

Table 4: Genetic diversity indices for all groups of salmons

Spawning Samples		Genetic diversity indices				
Population	Sample size	N_A	A_R	H_E	H_O	F_{IS}
F0 spawning 2008	60	13,6	10,5a	0,844	0,855	-0,004
F0 spawning 2009	92	13,6	10,4a	0,84	0,859	-0,016
F0 spawning 2010	92	13,4	10,1a	0,839	0,836	0,009
F0 spawning 2011	109	14,1	10,2a	0,845	0,827	0,027
F0 spawning 2012	108	14,2	10,0a	0,84	0,812	**0,039**
F0 spawning 2013	95	14,1	10,2a	0,847	0,811	**0,048**
F0 spawning 2014	90	14,3	10,2a	0,839	0,804	**0,048**
F0 spawning 2015	106	14,9	10,3a	0,843	0,831	0,019
F0 spawning 2016	95	14,2	10,3a	0,835	0,826	0,016
F0 spawning 2017	85	14,1	10,2a	0,833	0,819	0,023
F0 spawning 2018	51	13,1	10,3a	0,835	0,822	0,026
F0 spawning 2019	68	13,2	10,1a	0,838	0,821	0,027
F1 spawning Castels 2008	541	14,3	9,5a	0,842	0,813	**0,036**
F1 spawning Castels 2009	570	14,6	9,7a	0,847	0,811	**0,044**
F1 spawning Castels 2010	988	14,3	9,1a	0,837	0,815	**0,027**
F1 spawning Castels 2011	846	14,2	9,3a	0,837	0,830	0,010
F1 spawning Castels 2012	858	14,6	9,8a	0,841	0,825	**0,020**
F1 spawning Castels 2013	838	14,4	9,7a	0,831	0,810	**0,027**
F1 spawning Castels 2014	869	14,0	9,8a	0,837	0,808	**0,035**
F1 spawning Castels 2015	643	14,4	9,8a	0,836	0,809	**0,034**
F1 spawning Castels 2016	1184	14,7	9,8a	0,843	0,811	**0,038**
F1 spawning Castels 2017	1212	14,7	9,9a	0,840	0,806	**0,041**
F1 spawning Castels 2018	1066	15,0	10,0a	0,842	0,800	**0,050**
F1 spawning Castels 2019	833	15,0	9,9a	0,838	0,798	**0,049**
F1 spawning Cauterêts 2008	196	10,9	7,2a	0,760	0,739	**0,031**
F1 spawning Cauterêts 2009	201	10,9	7,9a	0,782	0,745	**0,050**
F1 spawning Cauterêts 2010	88	10,2	8,0a	0,800	0,742	**0,078**
F1 spawning Cauterêts 2011	22	9,0	8,9a	0,812	0,836	-0,003
F1 spawning Cauterêts 2012	42	9,7	8,5a	0,814	0,830	-0,006

Migrants Samples		Genetic diversity indices				
Population	Sample size	N_A	A_R	H_E	H_O	F_{IS}
F1 migrants 2010	10	7,4	6,30c	0,781	0,838	-0,017
F1 migrants 2011	10	7,4	6,26c	0,784	0,806	0,028
F1 migrants 2012	14	8,6	6,28c	0,802	0,873	-0,052
F1 migrants 2013	15	9,0	6,34c	0,783	0,798	0,016
F1 migrants 2014	27	10,8	6,50c	0,826	0,841	0,002
F1 migrants 2015	52	11,9	6,49c	0,843	0,812	0,047
F1 migrants 2016	11	8,2	6,37c	0,804	0,838	0,005
F1 migrants 2017	6	5,8	5,88c	0,756	0,796	0,038
F1 migrants 2018	11	7,8	6,35c	0,792	0,761	0,088
F1 migrants 2019	11	7,2	5,71c	0,762	0,783	0,021
F2 migrants 2010	39	11,8	6,85c	0,845	0,798	0,070
F2 migrants 2011	43	12,1	6,73c	0,838	0,828	0,024
F2 migrants 2012	37	11,6	6,65c	0,841	0,783	**0,084**
F2 migrants 2013	19	10,8	6,79c	0,822	0,776	0,084
F2 migrants 2014	64	12,6	6,59c	0,844	0,812	**0,046**
F2 migrants 2015	71	13,1	6,48c	0,848	0,825	**0,034**
F2 migrants 2016	73	12,9	6,57c	0,830	0,785	**0,06**
F2 migrants 2017	33	11,4	6,66c	0,833	0,827	0,024
F2 migrants 2018	25	10,4	6,60c	0,829	0,811	0,043
F2 migrants 2019	45	12,7	6,53c	0,838	0,819	0,035
Wild migrants 2010	60	12,3	6,59c	0,847	0,794	**0,072**
Wild migrants 2011	25	10,3	6,23c	0,813	0,802	0,034
Wild migrants 2012	11	7,8	6,37c	0,801	0,788	0,064
Wild migrants 2013	17	9,6	6,51c	0,812	0,826	0,014
Wild migrants 2014	45	12,4	6,52c	0,833	0,855	-0,015
Wild migrants 2015	57	12,4	6,49c	0,795	0,827	0,035
Wild migrants 2016	36	12,1	6,59c	0,828	0,787	0,064
Wild migrants 2017	18	9,1	6,39c	0,808	0,833	-0,001
Wild migrants 2018	36	11,9	6,64c	0,834	0,823	0,028

F1 spawning Cauterêts 2013	39	11,4	9,8a	0,829	0,801	0,047
F1 spawning Cauterêts 2014	160	13,0	9,7a	0,834	0,826	0,013
F1 spawning Cauterêts 2015	266	13,1	9,5a	0,830	0,803	**0,034**
F1 spawning Cauterêts 2018	92	13,1	9,7a	0,827	0,770	**0,075**
F1 spawning Cauterêts 2019	131	13,4	9,7a	0,825	0,786	**0,050**
F1 spawning Pont-Crouzet 2008	456	14,3	9,8a	0,845	0,823	**0,027**
F1 spawning Pont-Crouzet 2009	757	14,9	9,8a	0,849	0,801	**0,058**
F1 spawning Pont-Crouzet 2010	579	15,1	9,9a	0,849	0,806	**0,052**
F1 spawning Pont-Crouzet 2011	250	14,3	10,0a	0,851	0,837	0,018
F1 spawning Pont-Crouzet 2012	693	14,3	9,7a	0,836	0,818	**0,022**
F1 spawning Pont-Crouzet 2013	1042	14,4	9,7a	0,834	0,808	**0,033**
F1 spawning Pont-Crouzet 2014	954	14,6	9,8a	0,838	0,816	**0,028**
F1 spawning Pont-Crouzet 2015	1020	15,1	9,9a	0,840	0,810	**0,036**
F1 spawning Pont-Crouzet 2016	1250	14,9	10,0a	0,844	0,809	**0,041**
F1 spawning Pont-Crouzet 2017	1055	15,2	10,0a	0,842	0,801	**0,050**
F1 spawning Pont-Crouzet 2018	987	15,4	10,0a	0,844	0,795	**0,059**
F1 spawning Pont-Crouzet 2019	867	15,2	10,0a	0,841	0,800	**0,050**
F1 spawning 2008	997	15	15,0b	0,8464	0,8178	**0,034**
F1 spawning 2009	1327	15,33	15,3b	0,8503	0,805	**0,054**
F1 spawning 2010	1567	15,44	15,2b	0,845	0,8117	**0,04**
F1 spawning 2011	1118	14,89	14,9b	0,8428	0,8313	**0,014**
F1 spawning 2012	1593	15	14,8b	0,8408	0,8218	**0,023**
F1 spawning 2013	1919	14,89	14,5b	0,8345	0,8083	**0,032**
F1 spawning 2014	1983	14,89	14,5b	0,8389	0,8129	**0,031**
F1 spawning 2015	1929	15,33	15,0b	0,8391	0,8085	**0,037**
F1 spawning 2016	2434	15,11	14,9b	0,8438	0,8101	**0,04**
F1 spawning 2017	2267	15,33	15,1b	0,8416	0,8035	**0,045**
F1 spawning 2018	2145	15,44	15,3b	0,8431	0,7962	**0,056**
F1 spawning 2019	1831	15,56	15,3b	0,8395	0,798	**0,049**
Wild migrants 2019	80	13,2	6,60c	0,837	0,790	**0,063**

LEGEND:
N_A: Number of alleles

A_R: Allelic richness (a: calculated with 18 individuals, b: calculated with 942 individuals, c: calculated with 6 individuals)

H_E: Expected heterozygosity

H_O: Observed heterozygosity

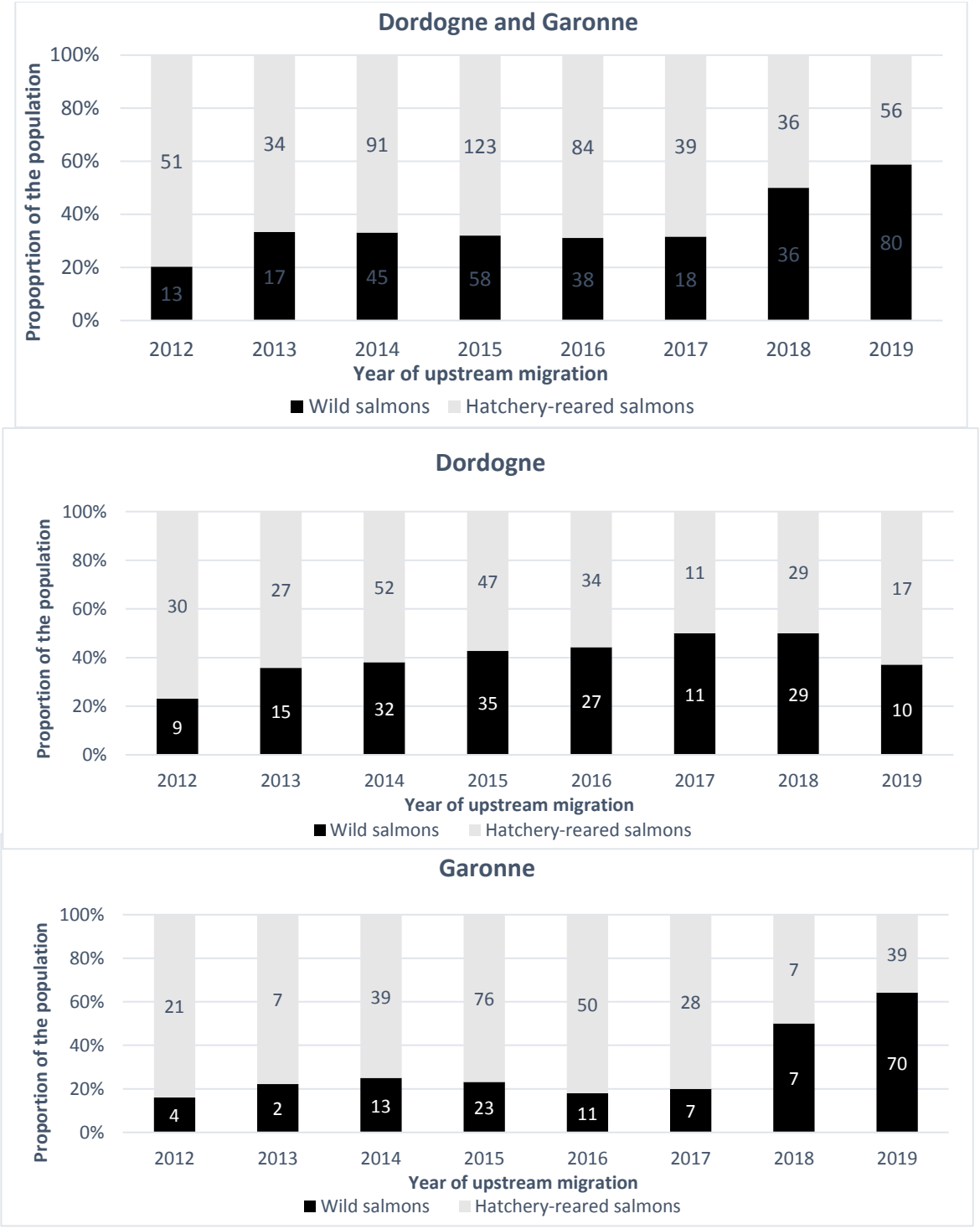

Figure 14: Number and proportion of wild born and hatchery born in the basins

3.4.3 Specific and global genetic variability of the population.

Considering temporal evolution for each group of salmon for allelic richness (A_R) and expected heterozygosity (H_E) no temporal evolution has been found, pointing out no decrease in genetic variability in the population. Then, we compared the genetic diversity indices between groups. All means are presented Table 5. We first tested F_{IS} coefficient and we found

significant differences between F1 salmon and F2 salmon (W = 22, p-value = 0.03749), F_{IS} being higher in F2 salmons. This coincide with significant F_{IS} coefficient found in the captive parental breeders F1. Concerning other tests, no differences were found either between F1 and wild born salmon (W=35.5, p-value = 0.2896) or between F2 and wild born Salmon (W=63, p-value = 0.3441). We also compared Allelic Richness between groups: A_R was significantly higher in F2 salmons than in F1 salmons (W=2, p-value = 4.33E-05) and also than in wild born salmons (W=100,5, p-value = 0.0094). Wild born salmon also showed significantly higher allelic richness than F1 salmons (W=16.5, p-value = 0.0042). Expected heterozygosity were significantly higher for F2 salmons compared to F1 salmons (W=8, p-value = 0.0017) and compared to wild born salmons (W=78.5, p-value = 0.034). Those wild born salmons also presented a significantly higher expected heterozygosity compared to F1 salmons (W=18, p-value = 0.015). Finally, no significant differences were found for observed heterozygosity between all groups.

Table 5: Genetic diversity indices for returning migrants (cohorts 2010 to 2019)

	F1 salmons	F2 salmons	Wild born salmons
F_{IS} mean	0.0176 (±0.0119)	0.0504 (±0.0072)	0.0380 (±0.0091)
A_R mean	6.25 (±0.08)	6.65 (±0.04)	6.49 (±0.04)
H_E mean	0.793 (±0.008)	0.837 (±0.003)	0.821 (±0.005)
H_O mean	0.815 (±0.010)	0.806 (±0.010)	0.812 (±0.010)

Mean indicated ± standard error

4. Discussion

As far as we know, this study is the first study worldwide, whatever the animal species considered, using pedigree information established by DNA fingerprints in a restoration program at such a large scale, here Atlantic salmon in the Dordogne and Garonne rivers. The period covers 11 years of restoration from 2008 to 2019. It considers a very large data set composed of 10290 genotyped individuals at 9 microsatellite markers, the video recording of 6153 migrants and the information provided by restocking of nearly 15 million eggs, fry, parrs and smolts. The access to individual genetic information in this restoration provides a unique opportunity to evaluate the efficiency of management of the genetic variability through one generation of captivity and to propose improvements of the practices to maximize genetic conservation. The use of DNA parentage assignment also provides unambiguous information to evaluate the impact of public support on the conservation of biodiversity.

We will discuss some aspects of the accuracy of parentage assignment with such large number of families, the analysis of factors in relation with the number winters spent at sea before returning in the river, factors involved in the efficiency of restocking practices as the stage of stocking, the river, the straying, the preservation of genetic variability to limit inbreeding based on reproduction practices as the sex-ratio, the rate of renewal of F0 parents, the mating design and the characterization of neutral genetic variation across rivers and generations of captivity.

4.1 A VERY HIGH EFFICIENCY OF THE MICROSATELLITE PANEL

The size of the breeding center and the multiplication hatcheries is limited. It is therefore impossible for technical and financially reasons to rear each family of fish separately. Thus, maintaining pedigree information until fish are large enough to be tagged is one of the difficulties in a stocking program (Norris et al., 2000). A problem was identified during the

work. All captured or captive fish are tagged and genotyped at their first spawning season, meaning one, two or three years after their birth, but some fish have an unknown date of birth, especially those who mature very late. For those fish we have tried to assign them to all others breeding plans and not only the breeding plan of its supposed year of birth. One solution could be to tag and to sample the fish as soon as possible to avoid mixing between years of birth. The use of genomic markers is a technical opportunity to recover the pedigree information and then manage inbreeding by avoiding the mating of too closely related sibs.

We were able to validate the used microsatellite panel in assigning F1 progenies to theirs putative F0 parents for each F1 cohort. Assignment rate was the highest from 2008 to 2011 with 100% but decreased in the recent years to above 90%. This slight decrease in the assignment rate can be explained by several issues : the presence of null alleles can affect the accuracy of microsatellite markers (Castro et al., 2004), some markers are not-enough polymorphic and informative (Gheyas et al., 2009), the number of parents genotyped under 7 markers that make impossible to find their offspring and finally, human mistakes such as fish that can be forgotten in the database. The efficiency of a given marker for parentage assignment is calculated with the exclusion probability. It is the probability of a randomly chosen parent-pair being genetically excluded as parents of a randomly chosen offspring, in case that parental pair did not produce that offspring (Dodds et al., 1996; Villanueva et al., 2002). It is possible to predict the assignment power of the marker set in the population by combining the exclusion probabilities of the different loci in the marker set (Vandeputte, 2012). The assignment power of our marker set was high but using theoretical assignment power when designing a marker set for parentage assignment leads to overly optimistic predictions (Ford & Williamson, 2010; Vandeputte et al., 2011; Yang et al., 2014). Therefore it was previously decided in 2014 to add 8 more microsatellites to expect the highest assignment rates under all circumstances (Vandeputte et al., 2011). As presented in Materials and Method, in 2014 MIGADO began to genotype all fish with 17 microsatellite markers. This increase is expected to enable a higher success in the assignment process in the future. Analysis with those 17 microsatellite markers will practically be fully applied when all fish from all spawning events, either F0 at Bergerac or F1 in multiplication hatcheries, will be genotyped for 17 microsatellite loci. Until now, some parents that still participated in the mating plans were genotyped to 9 microsatellite markers. However, the human factor will still be present and will not allow the possible 100% assignment rate because: the quality of the sample can be limited, therefore the DNA is not good to make the assignment (not enough markers genotyped for example) leading to non-compliant samples, but also because of bad transcription of the data in the system.

The quality of the work and its analysis and conclusions depends strictly on the success and the good pedigree assignment. The parentage assignment between F1 progenies and theirs F0 parents or F2 progenies and their F1 parents was a challenge due to the unusual and very high number of F0 and F1 parents and families produced during the 11 years of reproduction to create the data set. Done by the Labogena genotyping laboratory, it requires also a heavy exchange of well-structured data between MIGADO and Labogena. Improvement and speed up of the process in a secure way by its automation should allow the project to gain in simplificity and security.

A previous advance was the validation by simulations of the significance of the different types of assignment categories: non-assigned, assigned in the mating plans, assigned outside the mating plan. This simulation (M. Vandeputte, INRAE, unpublished results) concluded that only fish assigned in the mating plans can be considered as really originating from the hatchery mating plan. Fish assigned to potential families not in the mating plan but with parents used in the mating plan or non-assigned fish but with acceptable DNA quality have then to be

considered as not being produced by the MIGADO hatchery process. Under these hypotheses the result and analysis presented in this project are considered as non-ambiguous.

4.2 A HIGH CONSERVATION OF GENETIC VARIABILITY THROUGH THE WHOLE REARING PROCESS.

The second important result of the works is to provide first scientifically based information that allow to estimate that the restocking program was not diminishing the genetic variability of the Atlantic salmon in the basin. This is an important information, as a recent study by (Christie et al., 2014) estimated that inbreeding could account at most for a 1-4% reduction in the fitness of hatchery fish relative to wild fish. The use of the microsatellite panel also allows a finer description of the conservation at the different steps of the process. All the following discussion about inbreeding and conservation of genetic has to be considered in the light that, due to generation overlapping, we only analyzed the data by yearly cohorts, even if the genetic unit to be considered in population genetic is the generation interval. For this species and this population, a generation interval of 4 years could be considered in the wild with females and males reproducing for the first time at four years of age (even if in reality some males reproduced at 2 but also at 5 or 6 years and females at 3 and 5 years).

4.2.1 No loss of variability in the F0 broodstock through years

First, no decrease of genetic variability in the F0 broodstock collected from the wild through years was observed. This shows an acceptable management of the stocking program as the F0 broodstock is a representative sample of the population. However, the inbreeding coefficient was significantly higher than 0 for years 2012, 2013 and 2014 (FIS = 0.048). In parallel, our results show that the number of reconditioning (i.e. reuse of spawners) has clearly diminished since the beginning of the study and that all individuals have spent fewer years in the broodstock. This increase of the renewal rate for the broodstock, which is important after 2015, has been incited by the SYSAAF and has probably allowed to limit the increase of inbreeding in the F0 broodstock. In theory, the best genetic variability conservation should be achieved by renewing the F0 broodstock every year. In practice, this is not possible for practical reasons as it depends highly also on the number of F0 fish returning and trapped. We have shown also that most of the F0 broodstock came itself from hatchery-born individuals and the aim is to see that number diminish through years, to limit again inbreeding. According to the results of the present study, the actual renewal practice can be considered as allowing to maintain genetic variability of the F0 broodstock. Limited gain in conservation of genetic variability may be expected by a higher renewal rate but this strategy has to be used preferentially when return rate in the wild are high or above some threshold of effectives to be defined with Authorities.

4.2.2 A limited loss of genetic diversity between generation that could be overestimated

We were able to estimate that the F0 effective population size increased recently. This means a better genetic management, tending to the same number of offspring per male and female. To further increase this Ne, there is two possibilities. Either to increase the number of males and females in the broodstock, which is difficult because the population in the river is not enough important, or to focus on the same number of offspring per males and females.

With this Ne, a limited loss of genetic diversity between F0 and F1 generations was estimated to an average of 1.08% each year. As the broodstock is renewed each year it was difficult to evaluate the true genetic diversity loss among generation (4 years), and this loss is then certainly overestimated when considering the generation interval, a major factor to

consider at population and larger time scale in management and genetic evolution process. A 1% genetic loss each year, seems however to not have altered the F1 broodstock that presents no loss of genetic diversity.

We observed an inbreeding coefficient significantly higher than 0 for almost each year in all multiplication hatcheries. As the number of F1 captive breeder is high for Castels and Pont-Crouzet (from 500 to 1200), all breeders are not fertilized together, the risks that a pair is close in terms of pedigree is lower than in F0 breeding plan. The inbreeding risk is therefore limited. It is however possible that some related fish mate together, as they are not genotyped before spawning and their parents are not known. Here we came back again to the solution proposed before, meaning an earlier identification of the F1 captive breeders to avoid crosses between related fish.

4.2.3 No loss of variability from F1 broodstock to F2 returning migrants

One advantage of the important number of F1 captive breeders is that the effective size of the broodstock is much more important than those of the F0 broodstock. Therefore, the loss of genetic diversity is 5 times lower between F1 and F2 generation than between F0 and F1 generation. This is a real important aspect of the study that provide a quantified estimation of the inbreeding risk to use F1 stock for restocking activity. However, the true loss for a 4 year generation could not be calculated because of reuse of some breeders, this having to be done. Also, the genetic diversity loss has been calculated only with the migrants trapped, that are only a part of the migrants. The real loss is therefore less important.

However, one important thing to note is the presence of some inbreeding in F2 returning adults (from F1 captive breeders), which present an inbreeding coefficient significantly higher than 0 for 4 cohorts out of 10 with an inbreeding coefficient ranging from 0.034 to 0.082. A first thing is that part of the study was done on a limited number of individuals and markers, that may bias results and conclusions. Under these hypotheses, F2 returning adults also presents a significantly higher inbreeding coefficient than F1 returning adult and wild born returning adult. This may be explained by the lower variability of the F1 captive breeders that only represent the genetic variability of their F0 parents. To evaluate if the inbreeding is lower for F2 returning adults than for F1, further analyses based on cohorts and returning years should be performed. Our results pointed out significant differences for the expected heterozygosity (H_E) between the three groups of salmons (F1, F2 and wild born), H_E being higher for F2 salmons. However, the observed heterozygosity (H_O) was not significantly higher for F2 salmons. It is linked to the inbreeding coefficient that is higher for those salmons. As this part of the study have been conducted on few individuals and less markers (8), further analyses need to be conducted in the following years.

As MIGADO does not genotype all individuals coming back in the rivers, the evaluation of the global genetic diversity might be underestimated in the study. It is possible that fish with other alleles or different allele frequencies are present in the unsampled fraction of the population, which in Dordogne accounts for 85% of the population. Another factor to consider is that the Dordogne-Garonne population is not a closed population and it is possible that some strayers from other rivers may come to enrich the genetic diversity of the population.

The study is demonstrating with parentage assignment that the breeding practices applied are not increasing inbreeding through the reproduction of the F1 parents, the main and only and limited genetic loss being reported at the production of the F1 stock from the F0 parents. Attention and improvements of practices to maximize genetic conservation from F0 to F1 generations has to be the major effort to be done

4.3 BREEDING POSSIBLE IMPROVEMENT IN HATCHERIES

One aim of this study was to provide to MIGADO results about the current stocking program but also to identify potential ways to improve it. One way to improve the breeding plan of F0 broodstock can be to avoid duplicating crosses through spawning seasons. Indeed, we calculated that the number of genetically different families (n=7 779) was lower that the number of crosses made (n=11 479). Some crosses are made twice through spawning years and therefore some families are over-represented in the stocked juveniles. The number of reuse of each parents has to be more precisely characterized. If a family is duplicated this increase the chances of producing a different number of juveniles per families. However, and in the same time, the large number of families produced should limit this risk. The accumulation of more data is needed to conclude on the impact of the overrepresentation of some parents and to assess more precisely the level of risk. Avoiding duplicates could also permit to create more families.

In addition, the results demonstrate that the number of reconditioning of a F0 female did not impact the capacity of an offspring to come back, whereas it was hypothesized that older fish produced lower quality offspring. However, this analysis was made with the number of use for each female and not the number of eggs produced. Old females tend to produce more eggs per spawn and therefore more offspring. If we could have calculated the number of stocked offspring produced by each female, old females may still have a lower efficiency. A specific study must be conducted to test this hypothesis. Whatever the conclusions of such study, the number of old fish has clearly diminished since 2008. So, the potential impact of using old female has been reduced. However, as no information on how many egg, fry, smolt or parr are stocked per family, we were not able to evaluate if this effect was due to overstocking or a better survival in the wild for a certain produced family. It is also possible that some offspring batches, stocked at a certain moment of the year or in some specific conditions produced more returning adult. Characterizing and understanding such variability using DNA parentage assignment could contribute to improve the efficiency of the restocking breeding program.

4.4 THE STOCKING PROGRAM, MAY MODIFY SEA-AGE STRUCTURE OF THE POPULATION

In this study, one of the major issues was about sea winter structure of the population, a highly variable traits through rivers and population. First, a bias can arise by the classification (using the total length of an individual) we use for measuring the number of sea winter of a fish. This classification must be updated with new scales reading. The reading of scales allow to define the number of years at sea that a salmon has spent (Baglinière et al., 1985). Following this new scale reading, we will be able to update the classification for a better sampling analysis for this specific context. The results showed an under-sampling of 1SW salmon and an over-sampling of 2 SW salmon. It is difficult to change and improve this sampling because of the inability to sample during the summer, as showed in Figure X, when 1SW come back from ocean migration. Temperatures and water physical conditions are not adapted to trap without causing damage to the salmon. Moreover, there is a limited number of 1 SW salmons in the population, which make them difficult to target. Finally, the summer is also the migrating time of the Allis shad (*Alosa alosa*, Linnaeus 1758) that must not be caught due to a moratorium in place since 2008. Therefore, trapping fish in this period is very difficult for MIGADO.

Can this under-sampling of 1SW salmons change the structure of the stocked populations? In our study, we pointed out again a certain level of inheritance of the sea winter phenotype that has been widely documented in the recent years. Salmonids displays in general moderately high heritability (up to $h^2 = 0.39$) for age at sexual maturation in fish farm and

domesticated lines (Gjerde, 1984; Gjerde et al., 1994; Wild et al., 1994; Gjedrem, 2000). A single locus near the *vgll3* gene was found to be tightly linked to sea-age at maturity in Atlantic salmon (Ayllon et al., 2015). With the selection of 2SW, it is possible that the average sea age increases in the following years in the basin. Such a trend can be supposed in our results (Figure X), where a temporal evolution has been seen for F1 migrants. It is also possible that this selection is natural due to particular climate conditions. For example, that sea-age at maturity of Atlantic salmon decreases with increasing value of the seasonal North Atlantic Oscillation Index (NAOI) from February to April (Jonsson & Jonsson, 2004). Also, the chance of attaining maturity as multi-sea-winter salmon is correlated with high early growth rate at sea (N. Jonsson & Jonsson, 2007). Increase of sea temperature was also identified as an increasing factors of MSW salmons (Martin & Mitchell, 1985). Changes in the age structure of a population can occur in different ways. For example a study reported a clear decline of MSW in the population (Welton et al., 1999), whereas we reported an increase of MSW. It is possible that the sea-age at maturity structure has changed due to multiple environmental factors and was strengthened by the artificial indirect selection in the hatchery. Further analyses with more years have to be conducted in the future to assess if the effect is being confirmed.

4.5 THE STUDY CONFIRMS THAT THE “FRY STAGE IS AN EFFICIENT STAGE FOR RESTOCKING

The results confirmed that the fry stage was the most efficient stage for restocking. Considering costs to breed fry to parr or to smolt stage (Aprahamian et al., 2003), stocking with those older stage is not the best option. For egg stage, the difference between wild and hatchery survival is important (20% survival in the wild and 75% in hatchery), and it can explain this low capacity for coming back. Therefore, it is better to keep eggs and rear them to fry stage for stocking. However, those results must be tempered. The number of stocked eggs, parr 1+ and smolts was very low compared to fry. Also, it was impossible to make an analysis per basin because the number of returning migrants was too low. It could have been interesting, especially in the Dordogne River where a higher diversity of stage is stocked. This combined with the lower results in term of stocking efficiency of the Dordogne River could have influenced the results and the capacity for coming back of eggs, parr and smolt stages.

In addition, the results demonstrated that parr 1+ was the more limited stage in terms of biological interest, as their capacity for coming back is the lowest. Their survival rate is lower than smolts because they spend one more year in the river before their downstream migration. Also, it is known that parr stocked salmon can stay in the river to become satellite males. Such behavior has been demonstrated in some rivers (e.g Hutchings & Myers, 1988) and must be more studied in our context. Moreover, as those fish do not migrate, they are not considered in the population, conducting to an underestimation of the number of males in the overall wild population but also of the existing genetic variability (Perrier et al., 2014).

4.6 STOCKING IN THE DORDOGNE RIVER IS LESS EFFICIENT

The results showed a lower efficiency of stocking for the Dordogne River. This river has a lot of dewatering from dams each year to produce energy with hydroelectric plants (MIGADO, 2018), which is less the case in the Garonne basin. Moreover, those water releases do not take place in the Vézère River, which does not present lower efficiency in term of migrants’ capacity to come back. It is known that the amount of water releases have an impact on fry with the stranding-trapping effect (Halleraker et al., 2003). The fry can be stuck outside the main riverbed and cannot come back to the river. For example, the report presented by

MIGADO in 2018, showed a linear regression with $R^2 = 0.475$ between smolt abundancy in the Dordogne river and the dewatering index. We also showed that some stocked cohorts had a better efficiency for coming back. Each year is characterized with a dewatering index. However, since 2008 no excessive dewatering occurred in the river due to recommendation from MIGADO to EDF. Except in 2009 and 2010, the dewatering index were at its lowest between 2008 and 2015 (MIGADO, 2018). Meaning that other factors interfere to reduce the number of migrants that came back in both basins from the stocking program. Moreover, some dewatering occurs also in the Garonne River but their consequences are unknown. It is also possible that the quality of juvenile habitat is lower in the Dordogne River in comparison to the Garonne River stocking areas. Further analysis must be conducted this question.

4.7 ONE GENERATION IN CAPTIVITY DOES NOT DECREASE RETURN RATE

One of the original result of the study is that one generation in captivity does not influence the migrant capacity for coming back (F1 and F2 salmons succeeded equally in migrating upwards). This contrasts with other studies that suggested that it was important to avoid the use of F1 offspring (from F0 parents of wild origin) as brood stock for stocking purposes as in rainbow trout in USA (Araki, Cooper, et al., 2007) This result is important in the present conditions as stocked F2 salmon represent 75% of the stocked fish.

However, we could not evaluate the reproductive success of the hatchery born fish in the wild. This may be a key point for the future and DNA-parentage may be an adequate tool to investigate this aspect. If hatchery fish have a good capacity for coming back as mature adults but have less reproductive success, it could alter the efficiency of the stocking program. For example (Jonsson & Jonsson, 2006) reported that the life reproductive success (LRS) of hatchery born fish is 17% of that of the wild born ones. Also, it is possible that F2 fish have a lower reproductive success than F1 fish. It was demonstrated by some studies that salmon release in the wild after one generation of captive breeding, like F2 fish in our context, had their relative reproductive success (RRS) nearly half that of wild-born fish. Hence, it would be important to test F1 and F2 fish have a similar RRS.

4.8 THE RIVER AND THE SINGLE SEA-WINTER AGE MAY INFLUENCE STRAYING RATE

We found that fish stocked in the Garonne River strayed more than salmons from the Dordogne River. The straying rate was also higher for SSW (Single Sea-Winter) than for MSW (Multiple Sea-Winter) salmons. However, no study documented a similar trend and (Jonsson et al., 2003) found the opposite result (i.e. higher dispersal of MSW fish). Some studies pointed out differences between hatchery reared and wild salmon in terms of straying rates with higher straying rates for hatchery-reared salmon (Jonsson et al., 2003; Ford et al., 2015). However, it was impossible in this study to evaluate the straying rate of wild salmon as their birth river was unknown. Also, no difference was found between F1 and F2 generations while some studies pointed out higher straying rate for fishes that spent more time in captivity (Jonsson et al., 2003). In general, the global straying rate in the basin is relatively low for the hatchery-reared salmon as the estuary for the two rivers is the same. It is important to notice that straying is only evaluated with the two basins, Garonne and Dordogne. It is possible that the true straying rate is higher, as some salmons can stray to others river in France.

To explain the differences in straying between the two basins, we must consider multiple

factors: time of upstream migration, water discharge, water temperature and other physical and chemical parameters like silt plug density. One hypothesis was the difference of water discharge between rivers: 650 m3/s for the Garonne River and 450 m3/s for the Dordogne River. However, straying was more important for salmon from the Dordogne river than from the Garonne river, and it is known that salmon are attracted by the strongest water discharge (Pedersen et al., 2007). Therefore, to understand the differences between straying rates, it will be a necessity to evaluate the water flow variability during the salmon migration period. The difficulty is that cannot know at what period a salmon entered one of the two rivers, as they are monitored for the first time at Golfech, 200km away from the estuary or at Tuilières, 150 km away from the ocean. One idea can be to follow the salmons with acoustic telemetry coupled with sensors capable to evaluate all possible parameters. Combining this with parentage assignment could permit to understand why a salmon disperses to another river. Another hypothesis is that strayed salmon follow other schools of salmonids guided by fish odor (Courtenay et al., 2001). Westley et al (2013) showed that straying rate were species specific but that many environmental factors influence the straying rate during the salmon upstream migration.

4.9 RETURN OF WILD BORN SALMON

An important result of the study was to demonstrate that wild spawning occurs at a certain level in the basin. This was made possible with the parentage assignment with two types of studies

First, the project was an opportunity to characterize the efficiency of wild spawning in the Ariège River within the Garonne drainage. However, due to lack of information concerning sex of all individuals, we were not able to assign them with AccurAssign software and we used the recent APIS software for this part of the study. Once again, it must be interesting to know the sex of all individuals to make the parentage assignment easier. A possibility could be to use portable ultrasound equipment at the time of the F0 trapping, recent advance allowing today sexing of 50 g salmons in fish farms. Proof of concept for this improvement can be done in testing ultrasound equipment used for fish trout selective breeding (Haffray et al., 2014) or sturgeon caviar production The issue here is that we found a great part of the parr to be assigned to the fish transported to the spawning area. However, concerning the returning adult we found only three individuals from one parental pair. Interestingly the parental pair that produced those three individuals, had also produced a parr, meaning a certain continuity in the breeding scheme. As the transported individuals, spawn first in 2014, their offspring have begun the upstream migration only in late 2015, or in 2016. It is possible that the low number of fish found is explained by the fact that some fish are not back in the system again. Finally, it is also possible, as all fish were not trapped, that a bigger part of the fish than we thought is not genotyped, and that is why we assigned so few migrants. Parr identified as assigned came from only three spawning areas, whereas the returning adult could come from every spawning areas in the Ariège River. This study has to be conducted in the future with the sex of individuals to be more accurate.

Second, we also observed that the return of wild born salmon increased the last two years but this trend must be confirmed with the analysis of the next years. As the increase was only observed for Garonne River, we could suppose that the fish transported to Ariège produce ‘wild’ returning adults. However, this increase in the Garonne River is relative because in 2018, only 14 fish were trapped. Also, it is important to notice that straying was not taken in account for wild born fish, as their birth river is unknown. Therefore, it is possible that the fish came from Dordogne (or elsewhere) wild spawning. Concerning strayers, it could have been

interesting to know if the wild born fish came from other basins in France. However, due to a lack of systematic DNA collection by the other hatcheries and a common microsatellites panel, we were not able to assign the wild born fish to other rivers. The transfer of MIGADO pilot project to other restocking programs may help to solve this problem if the same panel of marker may be used by managers.

4.10 POTENTIAL INFORMATION FOR OTHER CONSERVATION PROGRAMS

The MIGADO breeding program targets the restauration of new population in the Dordogne-River basin. Important know-how was developed, and the project benefited of transfer of technologies from selective breeding activity of conservation. Improvement of breeding practices and application of DNA parentage assignment was technically advised by SYSAAF during more than 12 years with scientific expertise of INRAE geneticists.

MIGADO during this time performed yearly reproduction, tag the fish electronically, collected electronic tag number (13 digits) and fin samples and bare-coded assay reference in the INFAQUA database system developed for aquaculture breeders. Adaptation of the data base to some MIGADO activity is planned in the coming year. The automation of the tag number and bare coded information archiving was a key aspect and such kind of program cannot be developed without this type of software to avoid traceability errors.

MIGADO develop know-how on the management of the F0 and F1 broodstocks and its staff actively participate to the interpretation of the results. This long-term training in applied genetic and conservation allows to improve the MIGADO capacity to use information created in using parentage assignment to evaluate and propose improvement of the program.

This long work innovates in testing the potential application of DNA-parentage assignment. On the 11 years, an average of 935 F0, F1 and F2 salmons were genotyped each year and provide information to optimize breeding and to evaluate the efficiency of public financial support. The transfer of this pilot study to other restauration projects may contribute to improve their efficiency and also to allow some genetic common approaches that could benefit to the different project, as to identify the origins of potential strayers between basins or to share knowledge and R&D developments in the genetics in conservation.

5. Conclusion

Thanks to a genotyping effort started in 2008, we were able to evaluate the efficiency of the MIGADO stocking program for the recent years. This study has clarified the situation of the Atlantic salmon population in the Garonne-Dordogne basin after years of restocking in the blurred. The good results justify all the work made by MIGADO. The choice to minimize the number of years spent in hatchery has paid off and it is important to not go after the F2 generation for the stocking strategy. The use of the microsatellite markers has allowed to evaluate the proportion of the wild born salmon in the basin, which is the major interest of the program, as its aim is to restore a self-sustainable population. This objective is not yet achieved but some good signs have been observed. It is important to underline that the quality of the re-stocking is not the only factor that influences the recovery of a population. Recent years have been difficult for the migration of salmons and the efficiency of the fish passes is not at its maximum. Finally, like in a lot of other studies, the time of migration at sea stays a black box on which it could be interesting to focus. The results of this study suggest that the management of the restocking program must be continued in the following years.

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ANNEX I – Characteristics of the microsatellite used for the study

Locus	Name	Bank	Primers	TA(°C)	References
1	*SSOSL311*	Z48597	F-TAGATAATGGAGGAACTGCATTCT R-CATGCTTCATAAGAAAAAGATTGT	55	Slettan et al,. 1995
2	*SSOSL85*	Z48596	F-TGTGGATTTTTGTATTATGTTA R-ATACATTTCCTCCTCAATCAGT	55	Slettan et al,. 1996
3	*SSspG7*	AY081813	F-CTTGGTCCCGTTCTTACGACAACC R-TGCACGCTGCTTGGTCCTTG	58	Patterson et al., 2004
4	*SSsp1605*	AY081812	F-CGCAATGGAAGTCAGTGGACTGG R-CTGATTTAGCTTTTTAGTGCCCAATGC	58	Patterson et al., 2004
5	*SSsp2201*	AY081807	F-TTTAGATGGTGGGATACTGGGAGGC R-CGGGAGCCCCATAACCTTACTAATAAC	58	Patterson et al., 2004
6	*SSsp2210*	AY081808	F-AAGTATTCATGCACACACATTCACTGC R-CAAGACCCTTTTTCCAATGGGATTC	58	Patterson et al., 2004
7	*SSsp2213*	AY081809	F-ATGTGGAGGTCAACTAACCAGCGTG R-CATCAATCACAGAGTGAGGCACTCG	58	Patterson et al., 2004
8	*SSsp2215*	AY081810	F-ACTAGCCAGGTGTCCTGCCGGTC R-AGGGTCAGTCAGTCACACCATGCAC	58	Patterson et al., 2004
9	*SSsp2216*	AY081811	F-GGCCCAGACAGATAAACAAACACGC R-GCCAACAGCAGCATCTACACCCAG	58	Patterson et al., 2004

TA : Annealing temperature

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Auteur(s) : Fauchet Louarn Date de naissance* : 20/10/1997	Organisme d'accueil : Association MIGADO Adresse : 18 ter rue de la Garonne 47520 LE PASSAGE D'AGEN
Nb pages : 35 Annexe(s) : 1	
Année de soutenance : 2020	Maître de stage : Anastasia Bestin (SYSAAF)

Titre français : Evaluation de l'impact de la restauration écologique en saumon Atlantique dans le bassin Garonne-Dordogne grâce aux empreintes génétiques

Titre anglais : Evaluation of the impact of Atlantic salmon ecological restoration in the Garonne-Dordogne basin thanks to DNA fingerprints

Résumé : Cette étude est la première mondiale à utiliser l'information établie par les marqueurs génétiques pour un programme de restauration, ici le saumon Atlantique dans le bassin Garonne-Dordogne, à une telle échelle. L'étude couvre 11 ans de restauration de 2008 à 2019 avec le pedigree de 10290 individus génotypé à 9 marqueurs microsatellite ainsi que l'information apportée par près de 15 millions d'individus repeuplés. L'utilisation de l'assignation à parenté donne des informations fiables pour évaluer l'impact du support public dans la conservation de la biodiversité. Les principaux résultats sont : le repeuplement au stade alevins est le plus efficace, une génération de captivité ne diminue le taux de retour, l'augmentation de la consanguinité lors du processus de repeuplement est limitée et due principalement à la création de la génération F1. Etant donné que le cheptel de F0 est composé à plus de 60% de parents issus du repeuplement, il est nécessaire d'adapté le processus pour limiter la consanguinité. Enfin, la récente augmentation de la proportion des individus nés sauvages les deux dernières années oriente vers l'hypothèse d'un programme de restauration ayant un effet significatif pour établir une population durable. Le programme de repeuplement de MIGADO a permis d'accumuler de nombreuses connaissances pour gérer et améliorer d'autres programmes sur des populations en dangers en utilisant l'information génétique.

Abstract: This study is the first study using pedigree information established by DNA fingerprinting in a restoration program, here Atlantic salmon in the Dordogne-Garonne rivers, at a so large scale worldwide. The period covers 11 years of restoration from 2008 to 2019. It considers a very large data set composed of the pedigree of 10290 individuals genotyped at 9 DNA microsatellite markers and the information provided by the restocking of nearly 15 million individuals. The use of DNA parentage assignment provides unambiguous information to evaluate the impact of Public support in the conservation of biodiversity. Main results were that: the stocking strategy at fry stage was the more efficient, one generation in captivity does not decrease return rate, the increase of inbreeding through the rearing process is limited and mostly determined at the creation of the F1 generation. The access to the pedigree allows also to establish that more than 60 % of the F0 parents were originated from the hatchery process, this figure confirming the need to apply adapted breeding practices minimizing the risk of inbreeding. Finally, the recent increase of the proportion of wild born individuals in the last two years is favorable to hypothesize that the restauration program has a significant effect to establish a perennial and sustainable population. The MIGADO stocking program also cumulate expertise and develop specific know-how usable also for other restocking programs to audit, to monitor and to improve restoration of endangered populations or species in using genomic information.

Mots-clés : Saumon Atlantique, Restauration écologique, Diversité génétique, Impact du repeuplement

Key Words: Atlantic salmon, ecological restoration, genetic diversity, impact of stocking