Veterinary management of european pond turtle reintroductions

Benoit Quintard, Jean-Yves Georges

[Quintard B, Georges JY (2021) Veterinary management of European pond turtle reintroductions. Miller - Fowler's Zoo and Wild Animal Medicine Current Therapy, Vol. 10, Chapter 75]

VETERINARY MANAGEMENT OF EUROPEAN POND TURTLE REINTRODUCTIONS

BENOIT QUINTARD, DVM, DECZM (ZHM)[1], Jean-Yves GEORGES, PHD[2,3]

[1]Parc Zoologique et Botanique de Mulhouse, 51 rue du Jardin Zoologique, 68100 Mulhouse, France; benoit.quintard@mulhouse-alsace.fr

[2]Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France ; jean-yves.georges@iphc.cnrs.fr

[3]Research Station Petite Camargue Alsacienne, Rue de la Pisciculture, 68300 Saint-Louis, France

Abstract

Reintroductions are considered as operational measures to limit present biodiversity erosion. They consist in releasing captive bred and raised individuals of a species at a place it used to live. Its success relies on the quality of the release site, but also on the quantity and quality of released individuals. The later depends on the management of conservatory captive facilities usually dedicated to produce numerous healthy individuals. Veterinarians can contribute to the success of such projects through zootechnician and sanitarian surveys, population management and scientific expertise throughout every step of the process. This study presents the case of the European pond turtle (*Emys orbicularis*) reintroduced in Alsace, north east of France, since 2013. Approved husbandry methods are detailed here: egg harvesting, artificial incubation, population management (feeding, genetic survey), pre- and post-release individual monitoring. These methods are key points to prevent natural mortality when individuals are maintained in captivity. Rare, yet sometimes dramatic, diseases (ie. astigmatic mite infestation, *Citrobacter* infections) are also reported in captive individuals and should be monitored and treated. Veterinarian expertise is also required after release in the wild, to

support fundamental and operational research but also decision making by land managers and stakeholders. Reintroduction projects are more prompt to success when they benefit from coordinated contribution from interdisciplinary experts, veterinarian acting at all level of their implementation.

Introduction and history of the project

In the present context of the 6th crisis of mass extinction, mostly due to natural habitat destruction (IPBES 2019), reintroductions of threatened species have become numerous, since they are considered as an operational strategy for limiting biodiversity loss (Sarrazin & Barbault 1996, IUCN 2013). Zoological parks massively contribute to this effort. EAZA mentioning 240 reintroduction or translocation projects for 156 species in Europe (Gilbert et al. 2017). In France, reintroductions can be associated with national action plans (NAP) labelled by the Ministry in charge of the Environment. A case study is the European pond turtle (*Emys orbicularis*, hereafter referred as EPT), whose life traits are listed in **Table 74-1**. This small-sized freshwater turtle occurs in wetlands from southern Europe to West Asia and North Africa. The species has suffered the most dramatic decline in any reptile in Europe, where natural wetlands have collapsed by 95% since the 18th century (Hu et al. 2017). In France, its current distribution is much more restricted than the historical range, and EPT have even become extinct from some regions like Alsace, in the north east of France, most likely due to the channelization of the Rhine River in the 19th century.

In France, the EPT benefits long term conservation efforts through two successive National Action Plans (2011-2015 and 2020-2029). The NAP is implemented locally through regional projects. In the Alsace region, the local council (Conseil Départemental Bas-Rhin) initiated the project “European pond turtle without border”, with its German counterpart Landkreiss Germersheim, funded by European funds for restoring wetlands suitable for reintroducing a population of 500 individuals raised in dedicated captive breeding facilities. Here we present

the roles a veterinarian can play as zoo-technician, sanitarian veterinarian, population manager or scientific advisor in such a reintroduction project.

Conservatory captive facilities

The project "European pond turtle without border" relies on 3 captive breeding facilities, including 2 in France: one at the research station of the National Natural Reserve Petite Camargue Alsacienne (PCA, Saint-Louis) and another at the Mulhouse zoo (ZOO). At PCA, the captive breeding facility consists of an outdoor enclosure (120 m2) including an artificially dug semi-natural pond (max depth: 2m) connected to table water, surrounded by a belt of grass with sandy banks, including an egg-laying mound. The enclosure is surrounded by a 60 cm high vertical metallic panel to prevent turtles escaping while limiting the entry of predators. At the zoo, turtles are housed in a fully artificial 60m2 densely planted concrete pond, with access to a sandy bank and an egg-laying mound. This enclosure is covered with a 2 m high aviary with 4 cm diameter mesh. At both PCA and ZOO, captive facilities are restricted areas, without any access for visitors. In addition to natural food they may find by themselves, adult EPT are fed for 6 months, starting 2 weeks after they leave wintering (March-April) until mid-late October (when water temperature < 15°C). This complementary feeding occurs once to twice a week (depending on the quantity of leftovers) with various dead freshwater fish species, mostly common roach (*Rutilus rutilus)* and brown trout (*Salmo trutta)*.

All breeding individuals (PCA: 15 females, 8 males; ZOO: 5 females, 3 males) were captured as adults in 2005 and 2006 in the wild in La Brenne, Center of France, where the largest natural populations of EPT occur. Genetic analyses confirmed they all were haplotype IIa (naturally occurring in this part of Europe), so these 31 individuals could be considered as the founder population of the reintroduction program. All adults are marked with a passive

transponder (RFID). In addition, every year, adults are captured (using traps, nets and/or by hand) about two weeks after they leave wintering for medical examination (physical examination, biometry, weighing) and paint marking on the shell. External marking permits remote identification without capture, for behavioral observations such as social interactions or oviposition. In Alsace, egg-laying season lasts from mid-May to mid-July: from 6pm to 10pm, observers are placed close to the nesting mounts in order to identify females coming ashore and locate their nest. At PCA, females are captured after oviposition and before they return to water, in order to be weighed, before their release close to water. At ZOO, to alleviate night observations constraints, a system of antennas placed on the ground permits automatic detection of the RFID of females exiting water to reach the nesting mount. Each detection activates a phone message sent to the person in charge of collecting eggs the day after.

Incubation and hatching

All eggs produced at PCA and ZOO are placed in artificial incubators to maximize yearly hatchling production (**Table 74-2**). Eggs are collected after nest excavation by hand using soft spoons and brushes. Nest excavation is performed just after oviposition at PCA and early next morning at ZOO. Immediately after being collected, eggs are carefully cleaned of substrate with a dry soft paintbrush and individually marked with a pencil using the mother's ID and an individual egg number. Eggs are measured (length, width, mass) and placed, as they stood in the nest, in plastic boxes half-filled with humidified coarse vermiculite, with a maximum of 6 eggs per box, and without mixing different clutches. All eggs are placed in artificial incubators (n=4, Jaeger FB80, Jaeger, 63607 Wächtersbach, Germany) at the hatchery house of the ZOO. For all four incubators, humidity is set to 90% and maximal temperature is set to either 28°C (most favorable to males) or 30°C (most favorable to

females) as temperature dependent sex determination occurs between the 30th and 42nd days of incubation. Eggs are distributed amongst incubators in order to produce 2/3 of females to improve population growth in the releasing site. Large clutches are split in 2 to 3 plastic boxes placed in different incubators to produce female and male siblings. Temperature is set to drop by 6°C at night to mimic natural circadian cooling. Incubators are opened daily to ventilate, remove condensation drops on the cover, and check each egg, comparing each with the diagram of normal development of EPT during incubation (**Figure 74-1**).

Artificial incubation lasts about 60 days and hatching usually lasts 3 days. Hatchlings' umbilicus is cleaned with a chlorexidine solution (0.5%, Hibitane, MSD Santé animale, 49071 Beaucouze, France) and they are placed from day 1 to 10 in a dry individual small container until the umbilicus is completely dry and clean. To limit yearling mortality, naturally high in the wild due to predation and wintering, hatchlings are kept indoors for one year in small heated aquaria (50L, filled with 5cm water at 25°C, with plants and cork for hiding and basking). They are fed *ad lib* with pellet diet (Aquatic Turtle Monster diet) and live insects (fruit flies, pea weevils, and locust hatchlings). All aquaria are equipped with biological filters to prevent water pollution and with UV light (DL 12:12, Reptisun 5.0 UVB, Zoomed, 93401 St Louis, California). The relatively warm water maintained during the first year also ensures significantly faster growth compared to natural winter conditions.

Veterinary follow-up

Adult EPT's are very tough animals with very few medical conditions. The main issue concerns gravid females brought to the zoo with a few cases of post ovulatory egg retention (eggs being palpable through the pre-femoral fossa skin). Placement in a calm environment with suitable parameters (20°C, natural lighting, access to UV and sand) usually leads to egg-laying. Alternatively, medical treatment has been used as follows: a dorso-ventral x-ray

(45kV, 20mAs) to estimate the number of eggs, 10 UI/kg oxytocin (Ocytovem, Ceva Santé Animale, Libourne, France) are injected IM. The female is then placed in the dark, in a plastic container with a 10 cm layer of sand. Injections can be repeated every 90 minutes until all eggs are laid.

Young individuals are more susceptible to various diseases and predation in the wild (Martinez-Silvestre 2017, Mitrus 2004). Main issues encountered in the wild are dermatitis with bacterial or fungal infection leading to necrosis of the shell or the plastron. These affections are known to be linked to water pollution (Aleksic-Kovacevic, 2013). However, this is avoided in captivity by strict adherence to the recommended diet and environmental parameters (Boyer 2006). Over the last 10 years, PCA and ZOO had one outbreak of heavy flagellate infestation, and another of *Citrobacter* infection (**Figure 74-2**). Deworming (fenbendazole 100mg/kg PO, once, Panacur, Intervet, Angers, France and metronidazole 25mg/kg PO q24, 5days; Flagyl, Sanofi-Aventis, Paris, France) have been used to treat parasitic infestation. Non-specific symptoms for diseased young turtles include apathy, anorexia and soft shells. As they are more sensitive than adults to high nitrogen and ammonia levels in the water, and are more prone to dehydration, diseased young animals should be placed in fresh, clean water. Young individuals are also prone to stress and aquaria should provide enough cover for retreat and should only be translucent on one side.

In cases of post hatching death, necropsies are systematically performed, even if rapid autolysis due to heated facilities makes it very difficult to isolate specific pathogens postmortem. In 2018, liver biopsies of freshly dead weak animals showed *Aeromonas hydrophila* and *Aeromonas caviae*: treatment with trimethoprime sulfadiazine (Adjusol TMP sulfamide, 20 mg/kg, PO, sid; Virbac, Carros, France) was used on animals losing weight or apathetic ones with good therapeutic success (no more death after treatment was initiated).

Death in egg occurs every year varying between sporadic cases to >50% (see table 2). Several reasons have been identified: malfunctioning of the incubators is rare (yet great care should be paid to humidity), whereas maternal identity, health and stress levels appear to be key elements in hatchling success. Further, Hennig (2020) reported that stress and female's loss of fertility may be related to harassing courting males in EPT.

Rotten eggs need to be removed from incubators without delay in order to avoid any dispersal of fungal, bacterial or parasitic (mites) disease (**Figure 74-3**) through the whole incubator. The smell of rotten eggs could also attract insects like phorids flies whose larvae can penetrate through the eggshell.

Before release in the wild, individuals are placed for one month in quarantine in order to screen for pathogens and to implement appropriate treatment if required. Quarantine thus prevents sanitary risks for the native fauna on the releasing site. Individuals are placed in large plastic tanks (150x100 cm) offering basking places. Water level (5 to 30 cm) is controlled using tap water at ambient temperature. On the first day of quarantine, animals are sampled: blood samples (from either jugular vein or subcarapacial sinus, depending on the size of individuals) are sent for virology (ranavirus, herpesvirus) and feces for bacteriology (*Salmonella, Klebsiella, Pseudomonas, Proteus, Pasteurella, Citrobacter, Escherichia*) and parasitology. Blood samples can also be used for genetic analyses.

Managing populations

The captive population consists of 31 founders formerly captured in the wild (see "captive facilities") and their annual offspring, raised with a goal of 500 immature and sub-adult turtles released to the wild. For maximum genetic diversity and minimum inbreeding in the released cohorts, a follow-up of the mean kinship of the population has to be implemented. Thanks to the daily monitoring of marked gravid females, maternity is known as females are

seen laying their eggs. Alternatively, genetic analysis of blood stored on Nucleocards (Macherey-Nagel, Düren, Germany, **Figure 74-4**) permits assessment of filiation of any offspring. Sampling on Nucleocards allows a result to be obtained with very limited amounts of blood (0.05ml, 1 drop), making it possible to assess young animals via the subcarapacial sinus (Mans 2008). Paternity tests have been implemented to identify males' contribution to annual offspring production and to ensure all founder males have offspring in most, if not all, cohorts released in the wild. Similar genetic analysis run on individuals born in the wild allows assessment of relatedness of the entire population.

The captive facility permits monitoring of all turtles individually from oviposition to hatchling using relatively easy, yet time consuming, procedures. Individual post-hatchling monitoring is more difficult, mainly due to the very small size of the EPT at birth (< 5g) that prevents the immediate use of permanent marking. Both shell notch (commonly used on adults) or suture of nano-pearls on the marginal scales of the carapace are too damaging on a long-term scale since they produce large scars after several years of growth of the carapace. The best option remains the use of various colorful nail polishes on specific scales of the carapace (associated to numbers) until individuals reach 50 grams. An RFID can then be securely implanted in the sub-carapacial fossa. All individuals are additionally marked with coded notches of the marginal scales of the carapace before their release in the wild to allow visual identification.

Released population

Since 2013, 400 captive bred EPT have been reintroduced in 5 runs in Lauterbourg, north of Alsace where dedicated and suitable "turtle-friendly" wetlands have been restored (**Table 2**). Ten ponds (summing 2100 m2) have been dug in the vicinity of a gravel pit (4500m2, exploited until the mid-1990's). Since 2011, ecological succession of these water bodies has

been followed-up by botanists and entomologists to assess that the flora and associated biomass of macroinvertebrates (the main prey of EPT) were suitable for the expected population of 500 EPT. One key issue of the reintroduction, beside limiting mortality in the captive facility, is to ensure an appropriate transition to the natural environment and to follow-up the animals after their release. This can be achieved by building acclimation areas on the release site. An acclimation area is a small outdoor facility, close to the targeted natural space turtles are expected to disperse into, with a pond connected to the water table protected by a metal fence that prevents turtles escaping while limiting predator intrusions. Such design is supposed to permit turtles to acclimatize to local conditions while facilitating veterinary and scientific surveys during the first few months after they have left the captive breeding facilities. After a few months, the fences are opened to permit turtles to disperse by themselves. Alternatively, turtles can be removed from the acclimation areas and brought nearby to the location where they are expected to settle.

Beside the EPT, exotic, potentially competitive species such as the red-eared slider (*Trachemys sp.*) have to be managed on the release site. In Lauterbourg, exotic specimens (mostly *T. scripta scripta*) are regularly removed from the release site in order to improve the success of the reintroduction. The number of individuals removed is not high (from 0 to 3 per year) but regular, including both adult and young individuals, indicating that *Trachemys* species successfully reproduces in the wild in Alsace. Exotic specimens are brought to the ZOO, anesthetized with alfaxolone 20mg/kg, IM (Alfaxan, Dechra veterinary products, 78180 Montigny, France), sexed with cystoscopy, sterilized (females only) with a pre-femoral fossa approach and then exhibited in a pond providing visitor education to prevent further private releases.

Reintroduction follow-up

The monitoring after release has two major aims: assessing the success of the reintroduction, and its potential impacts on the ecosystem and endemic species. Reintroduction success can be assessed through survival, growth, dispersion and effective reproduction using protocols similar to studies in natural populations. Considering the cryptic behavior of the EPT that makes direct observations challenging, survey in the field is based on capture-marking-recapture (CMR) protocols commonly used for demographic monitoring. In Lauterbourg, CMR consist of deploying 70 traps for 5 continuous days every month from April to October (i.e. 2450 trapping days per year) in all water bodies of the release site. CMR analyses are based on the probability of recapturing individuals of known identity, from which age-related survival/mortality can be derived. Every individual captured is carefully examined in order to assess their general health, and measured, to assess structural and ponderal growth. Each trap is numbered and deployed at the same place from one session to the next, allowing evaluation of individual dispersion. Dispersion can also be assessed at a finer scale using telemetry that consists via electronic devices on the carapace. Given the potential impact external devices can have on the behavior, devices cannot exceed 3-5% of the body mass of the equipped individual. Accordingly, young individuals are commonly tracked using small VHF transmitters, but this method is time consuming in the field (active tracking). Larger (> 400g) individuals can be equipped with more sophisticated, but heavier, dataloggers such as GPS (providing precise location every 60 to 20 minutes when emerged) or behavior recorders (providing underwater and emerged behaviors every second, e.g., diving, basking, nesting, accelerometry-derived time budget, in relation to ambient temperature, all recorded onboard). Finally, small unmarked individuals captured in the traps are newly marked and sampled for genetic analyses, confirming effective reproduction in the wild. First evidence of effective reproduction in Lauterboug were observed in 2019 with 2 individuals whose genetics confirmed their filiation with the PCA founder population.

The potential impacts of reintroduction on the ecosystem and other species are assessed through ecological monitoring of flora and macroinvertebrate populations in water bodies of the release site. Trophic relationships are assessed through eDNA applied on scats and show that EPT operate as a super predator, feeding preferentially on the largest species of macro invertebrates in place (Georges et al. unpublished). Experimental tests also showed that EPT can feed on invasive species such as zebra mussels *Dreissena polymorpha* and crayfish *Orconectes immunis* without contributing to their range expansion (Georges & Quintard, in prep). In addition, sociological surveys in the field, through semi-directive interviews of users and inhabitants, can help to better integrate the conservation efforts in the local context, public acceptance and political strategies for protecting the environment (Philippot & Georges, in prep). Communication and public awareness are strong tools to improve the acceptance and the integration of such projects in the local context.

Conclusion

In the context of the sixth crisis of biodiversity, reintroductions are considered a major forward-looking strategy for sustaining future wildlife populations. All the different phases of such a conservation project require a veterinary input, from egg collection to survey of individuals released in the wild, to ensure success of all aspects of the program. The veterinarian has to be alternately a zoo-technician (husbandry, nutrition and growth, breeding, hygiene), a medical veterinarian (medical, prophylactic measures, quarantine procedures), a population manager (identification, filiation, genetic analysis, invasive species) as well as a scientific advisor (gather data to confirm success or failure of the reintroduction project: basic behavior, fine scale behavior, survival, habitat use, dispersion, nest location, food habit, prey selection).

In such a program, the veterinarian is an appropriate person to be in charge of ensuring animal well-being, health and maintaining the disease risks as low as possible. He/she also should be a scientific advisor for population management, reintroduction monitoring and for the control of invasive species. All this work can only be achieved with a well-organized team benefiting from the expertise of various members ranging from well-trained zookeepers, naturalists, researchers, sociologists, but also land managers, politicians and most importantly the public to ensure the acceptance of such sensitive programs.

Acknowledgments:

The authors would like to thank the Conseil Départemental du Bas-Rhin for initiating this program, Petite Camargue Alsacienne, Mulhouse zoo colleagues and IPHC students for their great implication in egg collecting, breeding and rearing the European pond turtles. Our special gratitude to Kathrin Theissinger for her contribution to genetic approaches. Finally, many thanks to Gabby Drake for the final revision of the manuscript.

References:

Aleksic-Kovacevic S, Ozvegy J, Krstic N, Rusvai M, Jakab C, Stanimirovic Z, Becskei Z. Skin and skeletal system lesions of European pond turtle (*Emys orbicularis*) from natural habitats, Acta Veterinaria Hungarica, 2014; 62:180-193.

Ayres C, Acuna A. Abnormal winter activity and mortality associated in an *Emys orbicularis* population from Northwestern Spain, Symposium on Freshwater Turtles Conservation; Oporto, 2013; 48-49.

Boyer H, Boyer D. Turtles, Tortoises and terrapins in Mader Reptile Medicine and surgery, 2nd ed. St Louis, MI: Saunders Elsevier; 2006.

Cadi A, Joly P. Impact of the introduction of the red-eared slider (Trachemys scripta elegans) on survival rates of the European pond turtle (Emys orbicularis). Biodiversity and Conservation, 2004; 13:2511–2518.

De Haan C. La reproduction en aquaterrarium de la cistude d'Europe (Emys orbicularis). Bulletin de la Société Herpétologique de France, 1981; 19 :17-24.

Gilbert T, Gardner R. Kraaijeveld AR, Riordan P. Contributions of zoos and aquariums to reintroductions: historical reintroduction efforts in the context of changing conservation perspectives. International Zoo Yearbook, 2017; 51(1): 15-31.

Hennig A. Incubating chelonian eggs. 1st ed. Frankfurt am Main Edition: Chimaira; 2020.

IUCN/SSC. Guidelines for Reintroductions and Other Conservation Translocations. 2013; Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, 57 pp.

Hu S, Niu Z, Chen Y, Li L, Zhang H. Global wetlands: Potential distribution, wetland loss, and status. Science of The Total Environment, 2017; 586: 319–327.

IPBES. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. S. Díaz et al. (eds.). IPBES secretariat, Bonn, Germany, 2019; 56 pages.

Mans C. Venipuncture techniques in chelonian species, Lab animal, 2008; 37:303-304.

Martinez-Sivestre A, Verdaguer I, Vidal F, Fortuno L, Franch M, Soler J, Verlarde R. High mortality associated with Thyroïd hyperplasia in European pond turtle, *Emys orbicularis* (L., 1758) (Emydidae) in a breeding centre facility at the Ebro delta, NE Spain, Acta Zoologica Bulgarica, 2017; 10:85-89.

Mitrus S, Zemanek M. Body size and survivorship of the European pond turtle *Emys orbicularis* in Central Poland, Biologia, 2004; 59:103-107

Sarrazin F, Barbault R. Reintroduction: challenges and lessons for basic ecology. TREE, 1996; 11(11): 474-478.

Table, boxes :

Figure 74-1: Normal development of *Emys orbicularis* during incubation (view from the top) from De Haan (1981)

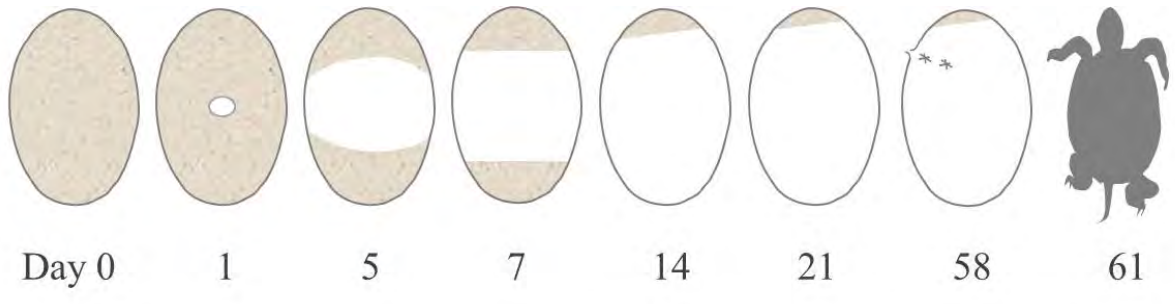

Figure 74-2: *Citrobacter sp.* generalized septicemia (all organs are congested) in a European pond turtle (*Emys orbicularis)* hatchling.

Figure 74-3: Astigmatic Mite (of family Acaridae) infestation in a European pond turle (*Emys orbicularis*) egg (Courtesy: Jacques Guillot, Parasitologie ENVA)

Figure 74-4: Blood draw in the subcarapacial sinus in a European pond turtle (*Emys orbicularis*) hatchling for haplotyping and filiation testing

Table 74-1 : Life history traits of the European pond turtle (Emys orbicularis)

Biological characteristics (means)	Value/Fact
Shell length (adult)	13-20 cm
Shell length (newborn)	22- 26 mm
Weight (adult)	450-1000 g (female > male)
Weight (newborn)	3-6g
Feeding	Opportunistic scavenger (mainly fishes) but also preys on live insects, mollusks, crustaceans.
Sexual maturity	6-8 years
Breeding season timing	Egg laying in May-June
Incubation length	60 days
Eclosion length	3 days
Clutch size	4-12 eggs
Longevity	45-50 years

Table 74-2: Numbers of individuals produced in captivity since 2012 and released in the wild

Year	Nb of eggs collected	Nb hatchlings	Nb of individuals alive after 6 months	Nb of individuals released in the wild
2012	96	67	62	-
2013	83	52	49	-
2014	96	41	40	-
2015	111	64	54	-
2016	67	42	41	43
2017	106	87	81	-
2018	182	161	156	200
2019	173	42	26	119
2020	167	37	22	-
Total	**1081**	**593**	**531**	**362**
% of total production		**55%**	**49%**	**33%**