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SUMARiS

Sustainable management of rays and skates

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WP 1 Fishery Knowledge

A1.2 Gaining knowledge on key life traits (growth, maturity)

D1.2.4 Report on fecundity and post-natal survival in captivity

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D1.2.4 Report on fecundity and post-natal survival in captivity

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Summary/Abstract

As part of the project SUMARiS, the National Sea Centre, NAUSICAA carried out a study between 2019 and 2020 to estimate the fecundity of the Thornback Ray (*Raja clavata*) and the post-natal survival of its eggs in captivity. To do this, data was collected from two breeding groups, one having lived in NAUSICAA's aquariums for several years and the second captured from the natural habitat for the SUMARIS project, shortly before the start of the study. The percentage of capsules in which developing embryos were observed was found to be 43.5% for the NAUSICAA group and 95.4% for the SUMARIS group. At the end of the period of incubation and embryonic development, the survival rate at hatching (the number of juveniles hatching/the number of capsules with embryos) was found to be 33.1% for the NAUSICAA group and 86.7% for the SUMARiS group. All the individuals hatching alive subsequently developed normally. This equates to a productivity over the study period of 1.09 hatchling Rays per Kg of mature female biomass for the NAUSICAA group, and of 2.88 for the SUMARIS group. The monitoring of rearing conditions during the study provides evidence that supports the hypothesis that both temperature and luminosity influence the Thornback Ray's reproductive cycle.

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I. Introduction :

The SUMARiS project

More than forty species of Ray live in the English Channel and North Sea. Among these, a number are regularly caught and commercialised by fishing businesses in surrounding countries, in Belgium, France, the Netherlands and the United Kingdom.

In the European Union, comprehensive regulation has been in place for all species belonging to the Genus Raja spp (defining quotas, minimum capture size, etc.) for a number of years. In 2013, Europe updated its common fisheries policy (Regulation (EU) No 1380/2013). One of the key changes was an obligation to land all catches (the landing obligation). The EU decided that, for species subject to quotas, individuals that were previously discarded (under-sized, damaged fish, lack of commercial value, etc.) must be landed. This new requirement also applied to Rays. It was in force up to 2019, at which time the fishing industry obtained a "survivability exemption" for Rays captured in the Channel (Regulation (EU) 2018/2034) and in the North Sea (Regulation (EU) 2019/2238). These exemptions allow fishermen to return any living Rays they do not want, to the sea.

To keep this ‘survivability exemption’ and continue to improve fisheries stewardship, it has become necessary to improve our understanding of Rays. The spatial distribution of their different species and the study of fisheries data are important topics for the fishing industry. However, scientific information on Ray biology, which can optimize fisheries resource modelling, should not be overlooked.

In this context, the INTERREG 2-Seas SUMARiS project (Sustainable Management of Rays and Skates) started in 2017 to run over a three-year period (from July 2017 to October 2020). Its goal is to propose a common transnational 2 seas strategy around the English Channel and the North Sea, setting out proposals for concrete measures to improve how Rays are fished from a stewardship perspective. One of its objectives is to gather all the currently available scientific data on these species in these areas, with a view to adding to it. A year-long study was therefore undertaken by NAUSICAÁ - National Sea Centre, to shed further light on the fecundity and survival rates of the eggs of Channel and North Sea Rays laid in an Aquarium setting.
Early in the SUMARiS project, the partners opted to study the Thornback Ray (Raja clavata), which is the most fished species in the region.

Case study: the Thornback Ray (Raja clavata)

The Thornback Ray (Raja clavata) (Figure 1) is one of the most fished species in the Channel and North Sea. It belongs to the subclass elasmobranchii and specifically to the Rajidae (Ray) family. This includes

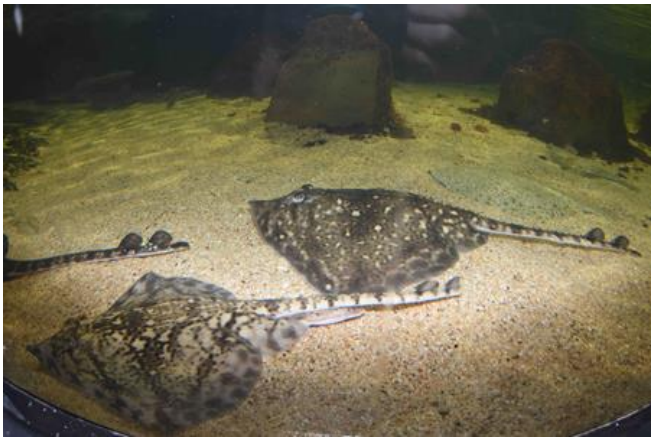

Figure 1 : Mature Thornback Rays (©NAUSICAA)

oviparous species, which fertilize their eggs internally and lay the fertilized eggs inside protective egg capsules made of keratin. According to several sources, females reach sexual maturity at the age of 7.5 years and males at 5.8 years, with females reaching a total length of 784mm and males 676mm (from the tip of their snout to the tip of their tail) (Serra-Pereira et al., 2011). In British waters, the literature indicates that the egg-laying season begins in February and ends in September, with a peak in June (Holden, 1975). Reproduction is through internal fertilization; the male stores its gametes in spermatophores, which it deposits in the female's reproductive tract using its 'claspers' and which release at the optimum moment to fecundate the female's ovocytes (Holden, 1975). After mating, females exhibit characteristic 'mating marks', which can remain for several weeks (Figure 2). In 1995, Ellis and Shackley determined that female Raja clavata are able to store the male's sperm for 15 weeks. Females can produce between 48 and 150 eggs per year (Ellis and Shackley, 1995; Serra-Pereira et al., 2011). The ability to use the male gametes stored for a long period post copulation enables Rays to lay eggs on a regular basis over these extended periods, without needing to seek a male (Quignard and Bruslé, 2018). In the natural habitat, once the capsules have been laid, on the sea bed or anchored using the capsule's horns, the eggs will incubate in them for 4 to 5 months (Ellis and Shackley, 1995). According to Chevolot, the eggs in a single laying may have multiple paternities (Chevolot, 2006).

Figure 2 : Female with mating marks (©NAUSICAA)

While much is already known about the number of eggs laid by the genus Raja, and about the incubation periods of their eggs, only a few journal articles have been published on the relationship between the number of eggs laid, egg survival and successful hatching. Ellis and Shackley proposed a hatching rate of 72.9% in their 1995 article. In a study published in 2005, Koop presented the results of their study to monitor the egg-laying of 5 species of Ray, including the Thornback Ray, at the Dolfinarium Harderwijk. This study, which ran from 1993 to 2003, found a hatching rate of 3%. The large gap between these two Figures suggests it would be useful to investigate this question further. In another study by Lécu, Herbert et al. dated 2018, the authors show that in some other species of Ray, hatchlings could die shortly after hatching, often due to an inability to feed themselves, and sometimes their exterior appearance would be different to that of their parents (Appendix 3).

The main purpose of the present study was therefore to estimate the general proportion, and proportion to biomass, of adult females and the proportion of productive eggs in the total quantity of eggs laid by Raja clavata. By "productive" we mean eggs that produce juveniles which are able to feed themselves and which exhibit a standard phenotype. In parallel, to enable a comparison with previous studies and discussion of the results, variations in the physico-chemical parameters of the hatching/rearing environment during the study, were monitored.

II. Materials and methods

The legal and ethical framework

Under its status as a ‘Centre National de la Mer’, NAUSICAA is fully licensed to accommodate and keep marine animals. These authorisations are based on the transposition into French Law of European Directives on the environment, and include:

- A License granted to NAUSICAA’s Director of Aquariums, Stéphane HÉNARD, by the Secretary of State for the Environment to the Prime Minister on 20 March, 1989: this License certifies the capacity to rear and display a number of species of wild animals, including all species of Ray in the genus Raja. (Appendix 1).
- A Permit to open, granted to NAUSICAA’s Managing Director, Philippe VALLETTE, by the Prefect of the Pas de Calais Department on 18 January 2017: this permit authorises NAUSICAA to keep animals and sets down the conditions for their care (Appendix 2).

This legal framework allowed NAUSICAA’s aquarology teams to lead this study.

Timetable

This SUMARIS project was carried out from 1st February 2019 (start of the collection of eggs laid) to 31 June 2020 (the first intake of food by the final animals to hatch).

Step one: the breeding groups

Origins of the breeding groups

The specimens, which were divided into two different groups, were Thornback Rays of the species Raja clavata, all captured in areas in the east of the Channel (code FAO VIId) and in the south of the North Sea (code FAO Ivc). These groups were made up exclusively of individuals considered to be mature, meaning that their total length was greater or equal to 784 mm for females, and 676 mm for males. The ‘NAUSICAÁ’ group contained specimens captured from January 2011 to December 2014, in the course of research undertaken by IFREMER or during trips on commercial fishing vessels equipped for bottom trawling. This group was made up of 6 males and 10 females (Figure 3).

The SUMARIS group was captured between July 2018 and January 2019, either with trammel nets or using a bottom trawl during trips on commercial fishing vessels out of Boulogne-sur-Mer. These specimens were captured as part of a study on the survival rate of Rays led by ILVO - INSTITUUT VOOR LANDBOUW-, VISSERIJ-EN VOEDINGSONDERZOEK for the SUMARiS project. All the females in this group exhibited mating marks at their time of capture. This group contained 4 females and three males, all considered to be mature (Figure 3).

Figure 3 : The NAUSICAA Breeding group (left) and the SUMARIS Breeding group (right) (©NAUSICAA)

Water management (LSS): common to all of the aquariums used in the study

The water in all of the tanks used in this study was recycled through a water treatment system (LSS) composed of a gravity settling tank, where the water from all the aquariums’ overflows mixes, a pressurized sand filter for mechanical filtration down to 50 microns, biological filtration (aerobic phases of the nitrogen and phosphorous cycles), a titanium plate heat exchanger to maintain temperature, a UV sterilizer providing 20mj/cm^2 with each pass, and circulation and filtration pumps (Figure 4). The filtration system replaced the water at a slow rate of 1 to 3 % per day with ‘new’

water extracted directly from the sea (from the Boulogne-sur-mer beach), which is filtered down to 10 microns.

The volume of water entering and leaving the LSS in each tank varied between 50 % and 70 % of its total volume per hour. In light of this water renewal, and the small quantity of biomass present in both the tanks for the entire duration of the study period, we believe that the physiochemical parameters of the water were identical in each aquarium throughout the entire duration of the study.

The physiochemical parameters of the water (temperature, dissolved oxygen, pH, ORP, and conductivity), were measured daily by NAUSICAA' s laboratory. The levels of dissolved ammonium, nitrites, and nitrates were measured weekly. An analysis of the total composition of the sea water in the LSS, including micronutrients, pollutants, and the principal elements, was also carried out by an external laboratory.

Figure 4 : The water treatment system or LSS (© NAUSICAA)

The choice of temperature level for the LSS and lighting for the aquariums

According to Holden, two parameters appear to influence Ray reproduction: water temperature and luminosity (Holden, 1975).

Our initial intention was to vary the temperature in the aquariums used in the study in order to mirror the known variations in the Ray's natural habitat (Figure 5). Such variations can however disrupt the biological filtration of the LSS and lead to variations in the concentrations of dissolved nitrites, which could harm the Rays. It was therefore finally decided to maintain the temperature for the breeding group tanks, the eggs, and the juveniles at a constant 10° C with a 1° C fluctuation.
The lighting of the study's aquariums was systematically provided by natural light 24h/24. It was however necessary to provide some additional artificial lighting in order to facilitate aquarium maintenance and to ensure good conditions for observing the Rays. This additional lighting was used at fixed times during the course of the study. This led to different observed lighting values for the different aquariums, as discussed in the following paragraphs.

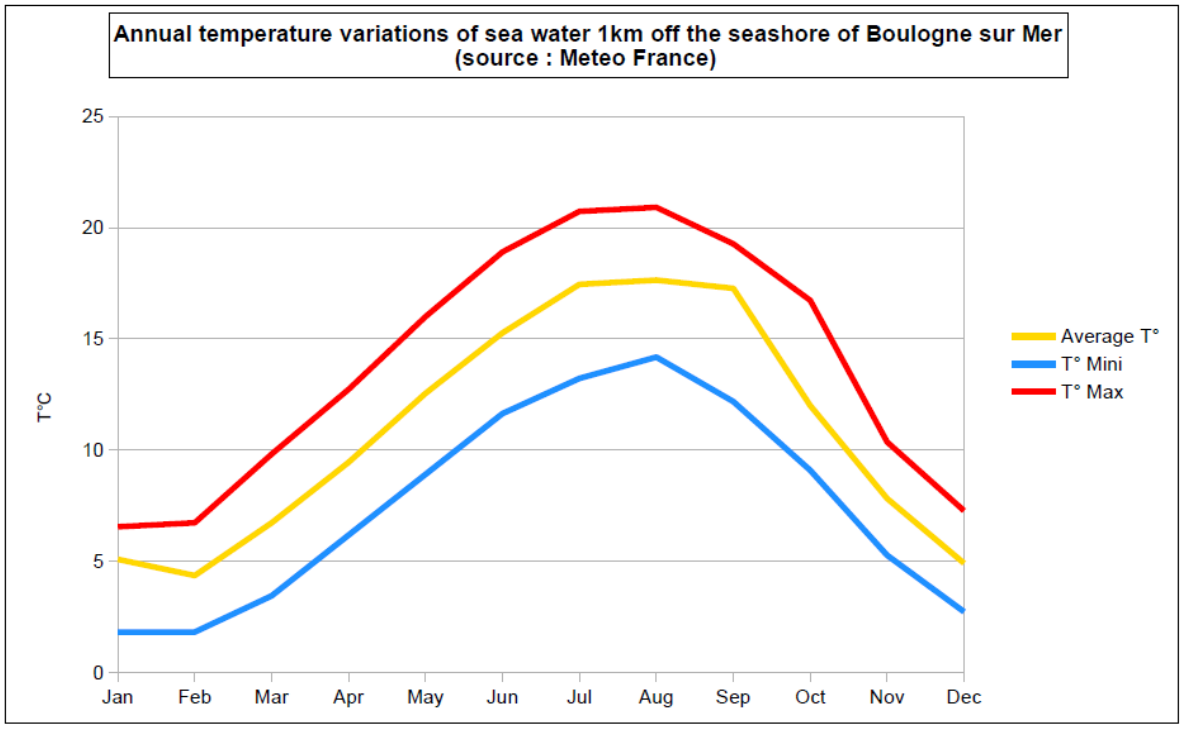

Figure 5 : Annual temperature variations in the Ray's natural habitat (Source: Météo France)

Characteristics of the breeding group tanks

Throughout the duration of the study, which lasted for more than a year, the two breeding groups were kept in two distinct tanks/areas.

The touch pool, which is a public exhibit in a NAUSICAA display area, housed the 'NAUSICAA' breeding group. It contains 35 m^3 of water, in dimensions of 1500 X 400 X 110 cm (Figure 6). While the touch pool allows contact between the public and the Rays, the Rays were able to retreat at any time away from the reach of visitors. The touch pool's lighting was provided through its transparent and unobscured glass, which allows sunlight and moonlight to pass through. In the day, this provided between 13 % and 60 % of the light received and followed the seasonal nycthemeral variation of latitude 50.71. Supplementary artificial lighting was provided by 6000K LED projectors, which in the day provided between 40 % and 87% of the light received, following a regular schedule of 11h30/day, from 7h to 18h30. This figure could rise to 100 % in winter, when sunset and sunrise occurred within the hours of artificial lighting. From 18h30 to 7h, the light received was 100 % natural lighting (sun and moon). The lighting values at the water's surface varied in the summer between 475 lux (overcast conditions) and 672 lux (clear conditions).

The SUMARiS group was housed in a technical area not open to the visiting public, called 'cold sea reserves' (réserves mer froide). This technical area, which is equipped with several dozen aquariums, is used for animal reception, quarantine, and the reproduction and rearing of the cold sea-dwelling fish and invertebrates displayed at NAUSICAA.

The tank housing the SUMARIS group had a water volume of 5 m^3 in dimensions of 300 X 170 X 110 cm (Figure 6). This tank's lighting was provided through its transparent and unobscured glass, which allows through both sunlight and moonlight, which in the day provided between 40% and 75 % of the light received and followed the seasonal nycthemeral variation of latitude 50.71. Supplementary artificial lighting was provided by T8 4000K fluorescent tubes, which in the day provided between 25 % and 60% of the light received, following a regular schedule of 11h30/day, from 7h to 18h30. This figure could rise to 100 % in winter, when sunset and sunrise occurred within the hours of artificial lighting. From 18h30 to 7h, the light received was 100 % natural lighting (sun and moon). The lighting values at the water's surface varied in the summer at midday between 169 lux (overcast conditions) and 480 lux (clear conditions).

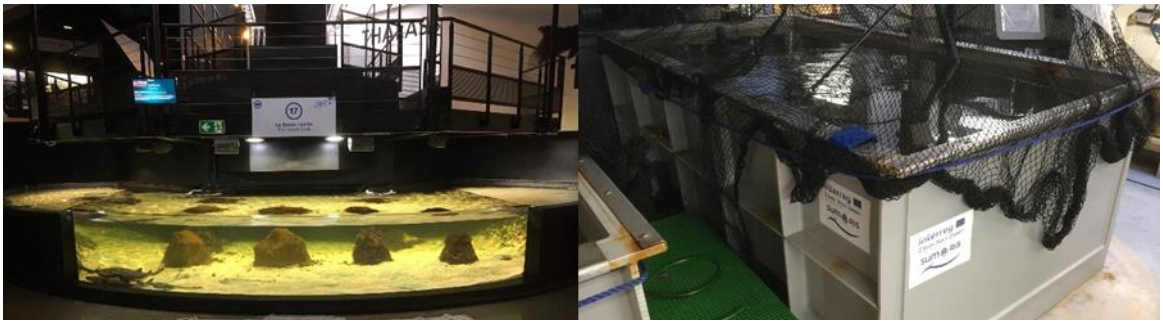

Figure 6 : The breeding group aquariums, showing group NAUSICAA (on the left) and group SUMARiS (on the right) (© NAUSICAA)

The Breeding Group Protocol

One of NAUSICAA's animal carers followed the same protocol on a daily basis for both tanks, that is, for both breeder groups:

- FEEDING: the breeding groups were fed once per day, 6 days per week, with a mixture of frozen seafood products (40 % herring, 30 % sprat, 30 % cooked prawns), cut into pieces of 2 to 4 cm. The quantity of food provided per day was equivalent to 2 to 4 % of the biomass of the Rays in the tank.
- CLEANING - the tanks used for the breeding groups were cleaned once per day, surplus food and waste were removed.
- COLLECTING EGGS - the eggs were collected with a net from each tank once per day, without taking them out of the water.

Step two: the eggs and the juveniles

Incubation

The carer transferred the eggs to different aquariums according to the date when they were laid and the origin of their breeding group. The aquariums were located in NAUSICAA's cold sea reserve room and had a capacity of 45 litres in dimensions of 50 X 30 X 30 cm. The eggs remained there until they hatched (Figure 7). The tanks' lighting was provided through transparent and unobscured glass walls, which allowed both sunlight and moonlight to pass through. In the day, this provided between 75 % and 90 % of the light received and followed the seasonal nycthemeral variation of latitude 50.71. Supplementary artificial lighting was provided by T8 4000K fluorescent tubes, which in the day provided between 10 % and 25 % of the light received, following a regular schedule of 11h30/day, from 7h to 18h30. This figure could rise to 100 % in winter, when sunset and sunrise occurred within the hours of artificial lighting. From 18h30 to 7h, the light received was 100 % natural lighting (sun and

Figure 7 : Aquariums used to incubate the eggs (left) and close up of the capsules (right) (©NAUSICAA)

moon). The lighting values at the water's surface varied in the summer between 319 lux (overcast conditions) and 579 lux (clear conditions).

The eggs were suspended 2-3 cm apart by their horns using plastic cable ties. The concave anterior margin visible on the egg where the animal exits the capsule at hatching was placed downward. This method of suspension ensured an optimal circulation of water between the capsules.

The Monitoring Protocol

The eggs' development was checked twice per week using the 'candeling' method (mirage in French) (Figure 8). Using a light source placed behind the egg, the aquariology technician checked, making use of the capsule's transparency, for the presence or not of the yolk sac and then checked the development of the embryo within the capsule. Capsules determined to be unfertilized (i.e. having no yolk sac or no embryo movement) were discarded following a final check using a binocular magnifier. Perished eggs displaying signs of decomposition were removed daily. To carry out these operations, the eggs were raised out of the water for less than 3 minutes, and any air bubbles trapped in the capsule were removed when the capsules were returned to the aquarium.

Figure 8 : The candeling procedure (left) and close up (right) (©NAUSICAA)

The newly hatched juveniles were kept in the incubation tank containing the eggs and were offered food on a daily basis. Immediately upon taking food for the first time, individuals were transferred to aquariums accommodating only the young Rays involved in the present study. These aquariums contained 400 litres of water, in dimensions of 200 X 50 X 40 cm and had no sand substrate (Figure 9). Tank lighting was provided through transparent and unobscured glass, which allowed both sunlight and moonlight to pass through. In the day, this provided between 75 % and 90 % of the light received and followed the seasonal nycthemeral variation of latitude 50.71. Supplementary artificial lighting was provided by T8 4000K fluorescent tubes, which in the day provided between 10 % and 25 % of the light received, following a regular schedule of 11h30 per day, from 7h to 18h30. This figure could rise to 100 % in winter, when sunset and sunrise occurred within the hours of artificial lighting. From 18h30 to 7h, the light received was 100 % natural lighting (sun and moon). The lighting values at the water'

s surface varied in the summer at midday between 319 lux (overcast conditions) and 579 lux (clear conditions). The juveniles were individually weighed, measured, and their gender determined.

The young Rays were fed up to three times per day, with a mixture of thawed and chopped fish meat (50 % salmon, 50 % sprat), corresponding to between 4 and 6 % of the total biomass of the juvenile Rays present in the aquarium per day. The size of the chopped food varied between 2 and 5 mm. The food was chopped using a knife, by hand, up to April 2020. After this date, the food was chopped using mechanical food chopper. The good health status of the young Rays was checked on a daily basis on the occasion of the first feeding session of the day.

Figure 9: Aquariums accommodating the juvenile Raja Clavata (©NAUSICAA)

III. Results and discussion

Physico-chemical monitoring

The analysis of the levels of metals and micronutrients in the sea water sampled from the Life Support System (LSS) (Table 3) reveals that all the values were within the limit values set down in the European Directives 2006/44/EC and 2006/113/EC.

The basic parameter values of the water in the LSS (see figures 10 and 15) remained stable within the limit values set down by the European Directives 2006/44/EC and 2006/113/EC. It should be noted that the temperature, which should have remained between 9 and 11° C, peaked at 13° C in March and again in July at 12° C (see Figure 10). This was due to a fault in the LSS. These peaks lasted only a few hours. The temperature in the sea off Boulogne can vary over the course of a year, between 2° C and 21° C, and on average varies between 4.5 and 17° C (see figure 5). Raja clavata congregate throughout the year in this region, which indicates that the species has a preference for a thermal range of at least 4.5 to 17° C. It is therefore highly unlikely that these two very brief temperature

peaks will have had any significant effect on the experimental conditions. However, a repeat study would eliminate any doubt on this question.

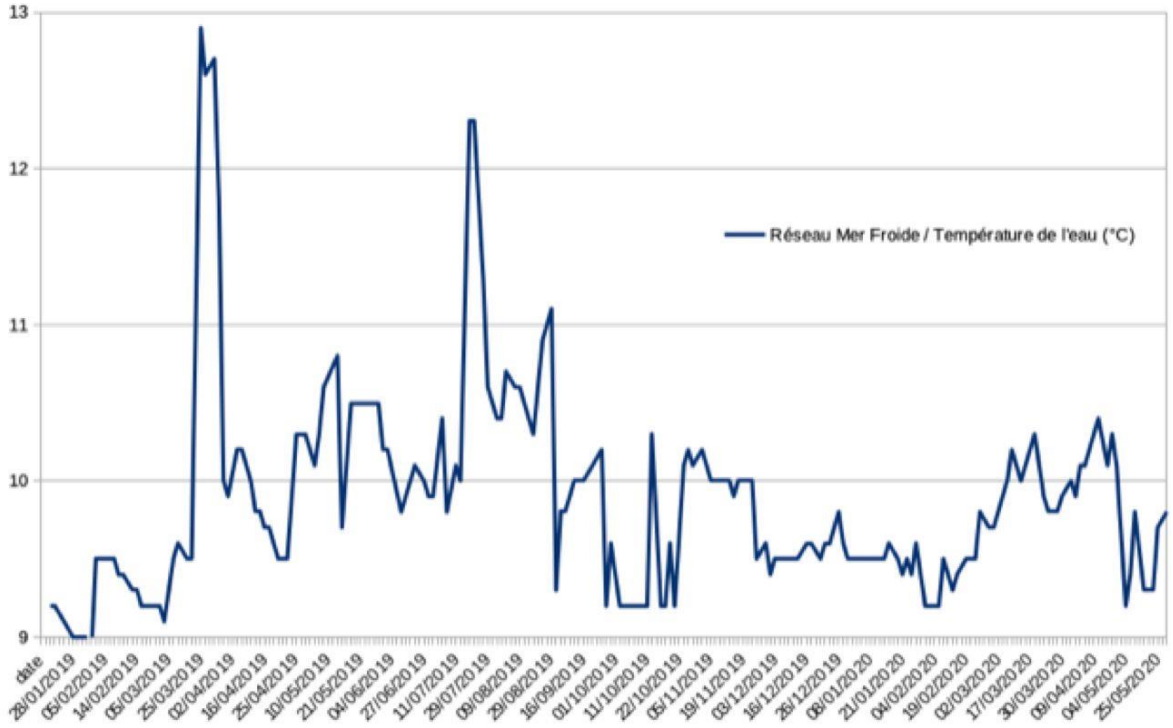

Figure 10 : Temperature levels in the aquariums

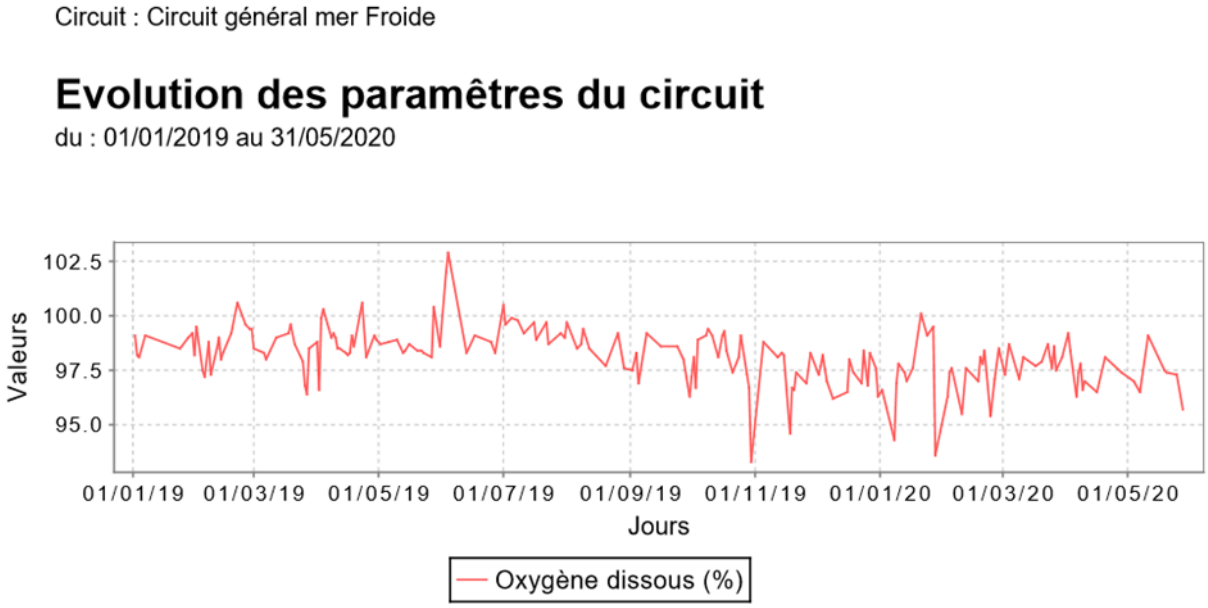

Figure 11 : Dissolved oxygen levels in the aquariums

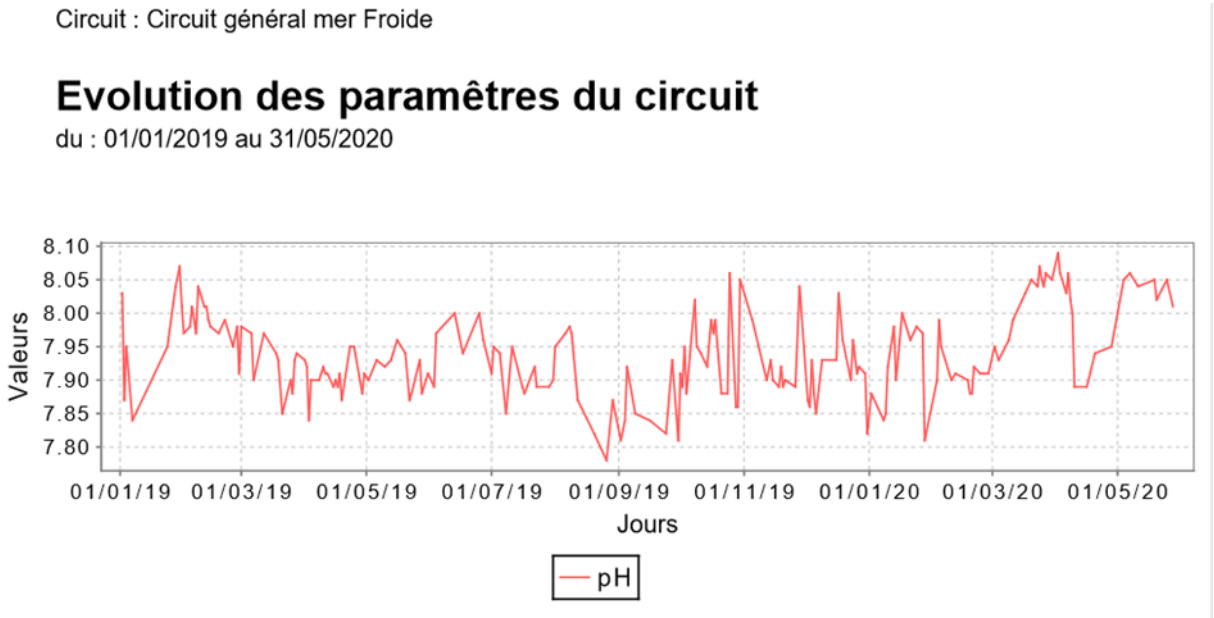

Figure 12 : PH levels in the aquariums

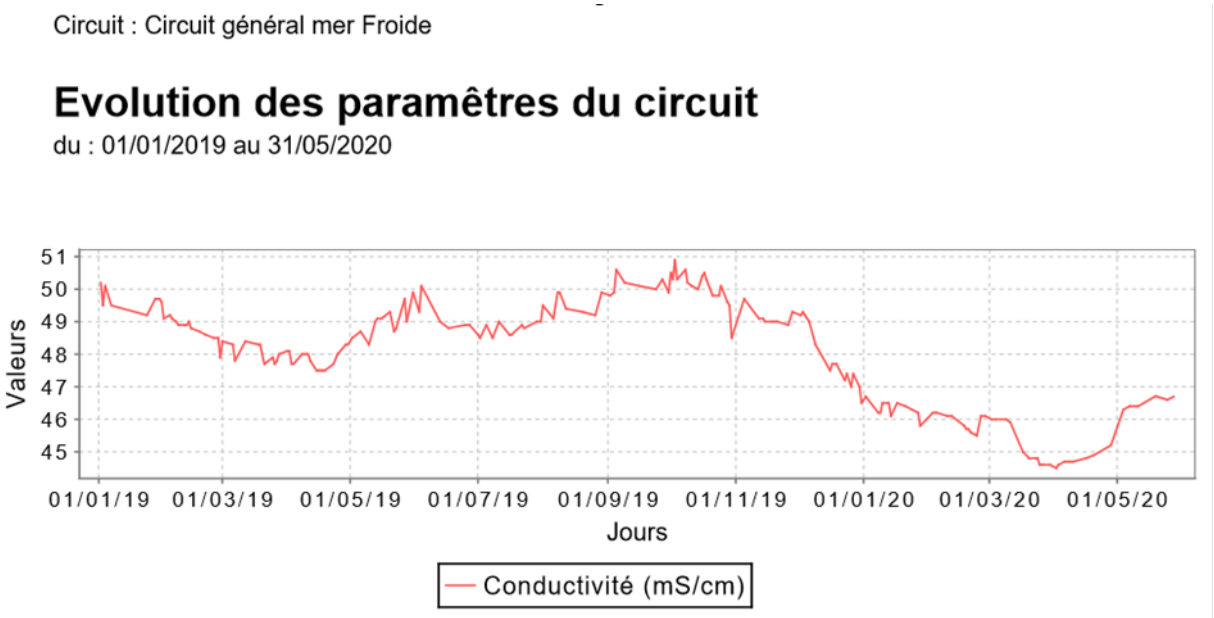

Figure 13 : Conductivity in the aquariums

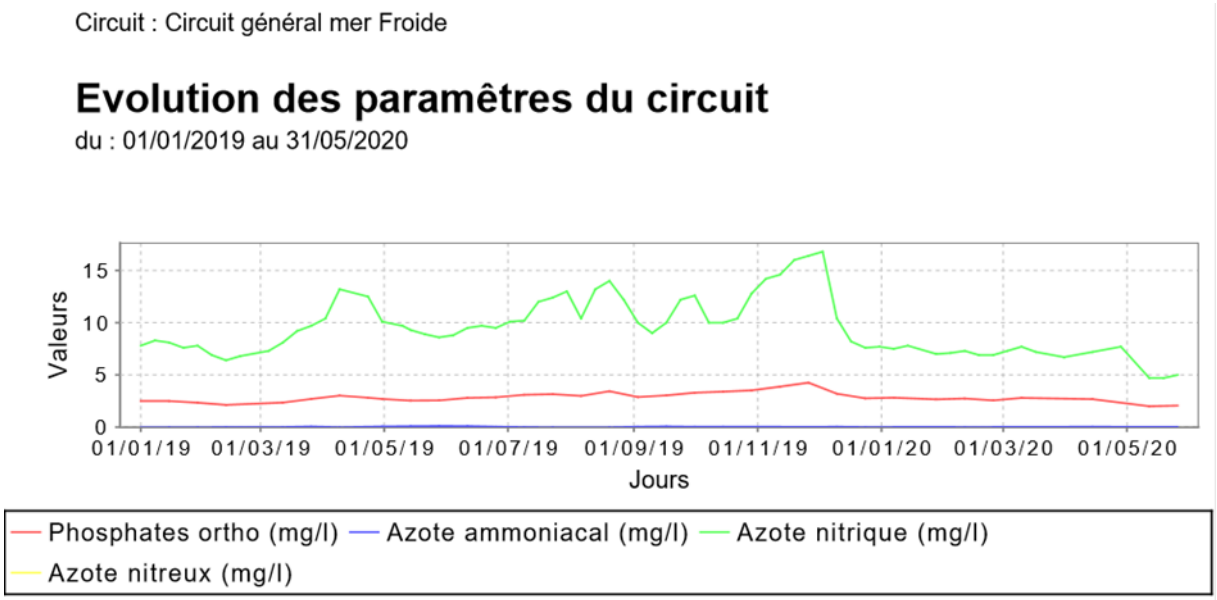

Figure 14 : Nitrogen and phosphorous levels in the aquariums

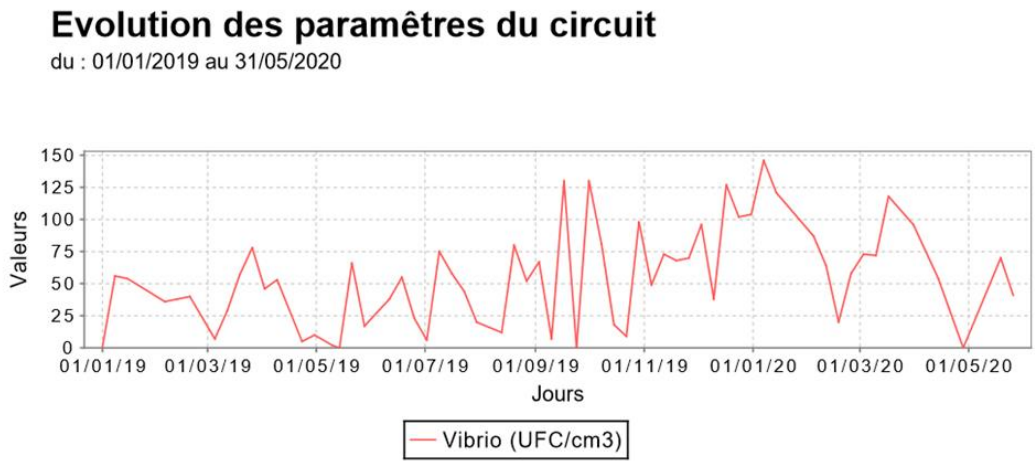

Figure 15 : Vibrios in the aquariums (cultures grown on a TCBS medium)

Differences between the breeding groups

The compositions of the two breeding groups differed in certain respects (Table 1). The NAUSICAA group was composed of 10 mature females (with total lengths greater than 784 mm) and 6 mature males (total length greater than 676 mm). These individuals were kept together for more than two years, at a constant temperature of 10° C. It is therefore at this temperature that mating and fertilization took place. The SUMARiS group was composed of 4 mature females and 3 mature males captured from the wild shortly before the start of the study. These animals had therefore experienced seasonal temperature variations. It should be noted that the females in the SUMARiS group all exhibited external marks at the time of capture, indicating that mating with possible internal fertilization had occurred in the wild.

In both groups, the ratio of males to females was less than the 1:1 ratio found in the wild (Ellis and Shackley, 1995):

- the NAUSICAA breeding group had a sex ratio of 1 male to 1.7 females
- the SUMARiS breeding group had a sex ratio of 1 male to 1.4 females.

It can be hypothesized that this situation reduces the likelihood of multiple paternities, as proposed by Chevolot.

The sizes of the females are very similar in the two groups and this was also true for the males.

Results of egg laying and egg development

The egg collection period was from the beginning of February to the end of September. These dates were selected on the basis of Holden's 1975 findings. It was however observed that the females in the NAUSICAA group laid eggs all year round, but that fewer eggs were laid from October to January. One possible hypothesis for this is that the females' reproductive cycles were disrupted by the artificial lighting, which may have modified their perception of the length of day, but without completely suppressing the influence of the natural light.

The eggs were collected from the 1st of February 2019 with the first eggs being laid on the 15 February, 2019. Between this date and 30 September, 602 eggs were collected at an average of 51.5 eggs per female from the NAUSICAA group and 21.7 eggs per female from the SUMARiS group (Table 2). Both of these figures are lower than those observed by Koop (117) and Holden (140). Koop's data on the size of the breeders and the sex ratio are insufficient for comparative purposes, however, Holden's data set is complete. In light of these figures, one plausible hypothesis is that the drop in winter temperatures is favourable to this species' reproductive activity, and also that differences in the in-aquarium breeding protocol, and specifically: disturbance to the diurnal light cycle perceived by the animals due to the use of supplementary artificial lighting; a different sex ratio compared to the ratio

in the wild; and a non-seasonalized diet, which was possibly different to the diets of the animals studied by Koop and Holden (the data provided in their study make any comparison impossible).

In both groups, the graphs showing the number of eggs laid per Kg of adult female biomass (Figure 16) follow the same trend, with a peak in March, a second peak in June/July and a declining trend through to September. Given that the water temperature remained constant, we could advance the hypothesis that the natural light perceived by the animals in the aquariums was influential and appears sufficient to have synchronized the egg laying trends of the two groups, which is consistent with Holden's hypotheses (1975). The females in the SUMARiS group stopped laying at the end of August. With the exception of March 2019, the number of eggs laid per Kg of female biomass in the NAUSICAA group was two to four times higher. It is not possible to compare these data with the studies in the bibliography, as their calculation methodologies were different (they were based on the number of eggs laid per female, irrespective of biomass).

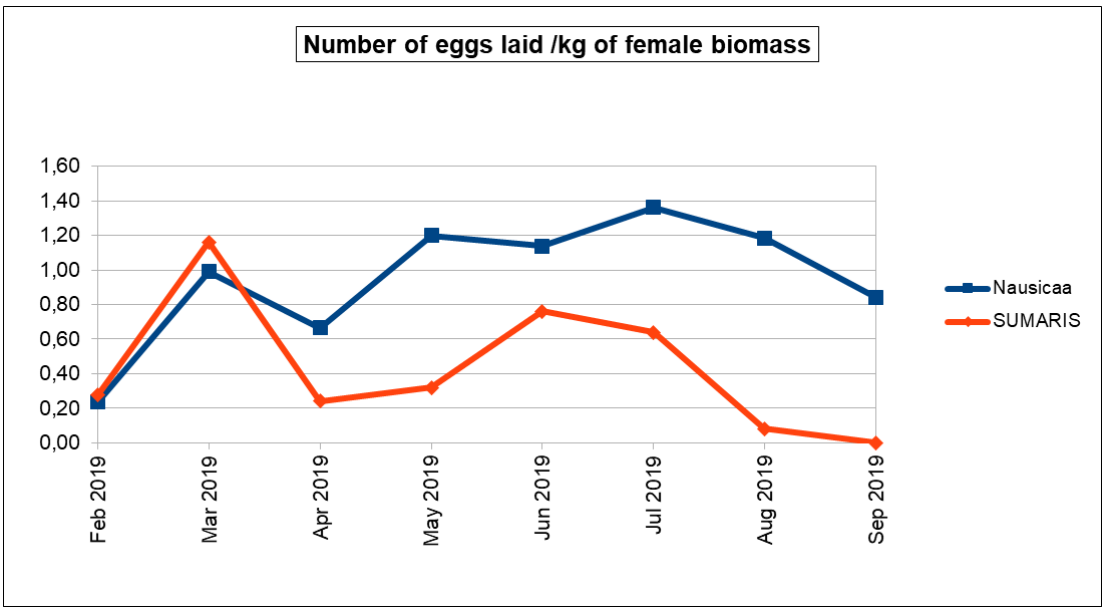

Figure 16 : Number of eggs laid / kg female biomass

The fertility rate (the relationship between the number of eggs laid and the number of eggs producing embryos observed to be developing) varied between the groups (Figure 17). The 4 females in the SUMARiS group laid 87 capsules containing 95.4% of fertilized eggs. Of the 515 eggs laid by the 10 females in the NAUSICAA group, only 224, or 43.5%, were found to be fertilized. It should be recalled that the SUMARiS females exhibited mating marks at the time of their capture. It seems reasonable to assume that they benefited from better conditions for fertilization due to the 1:1 sex ratio observed in the wild, as such conditions facilitate mating and favour multiple paternities. We can also hypothesise that the absence of fluctuation in water temperature influenced the results of the NAUSICAA group, that is, the absence of a winter temperature drop or a summer peak, led to eggs being laid at a more even rate over time. In addition, mating occurred at a more constant rate, but at a lower frequency.

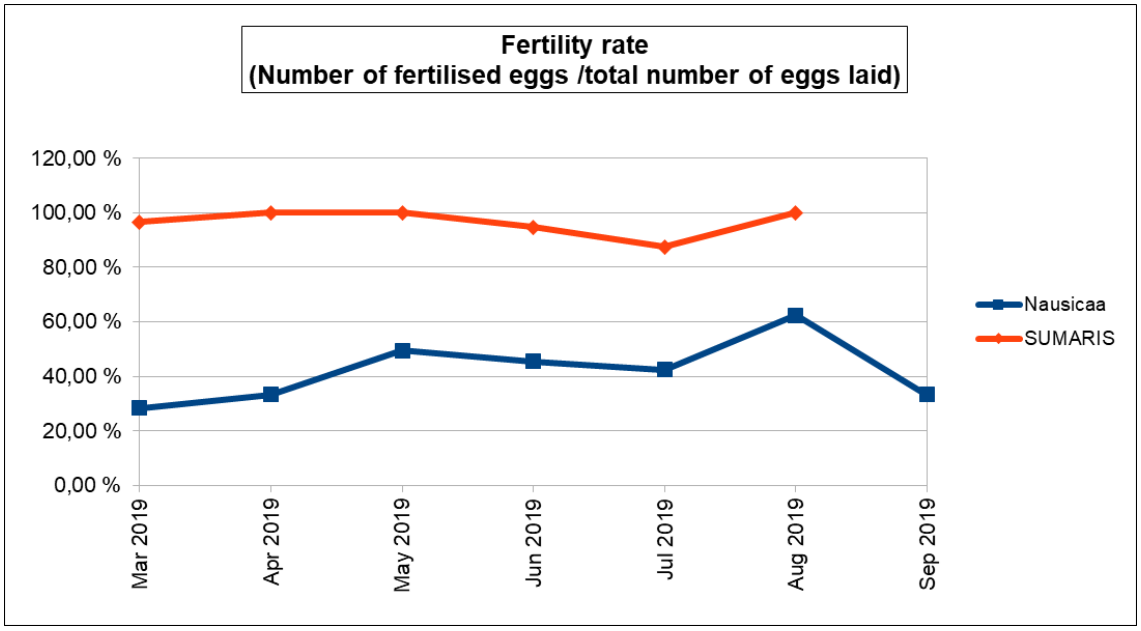

Figure 17 : Fertility rate

At the constant temperature of 10° C, the observed incubation time was on average 308 days (10 months) for the NAUSICAA group and 317 days (10.5 months) for the SUMARiS group (Table 4 and Figure 18). In contrast, in the wild, with normal seasonal temperature variations, the observed

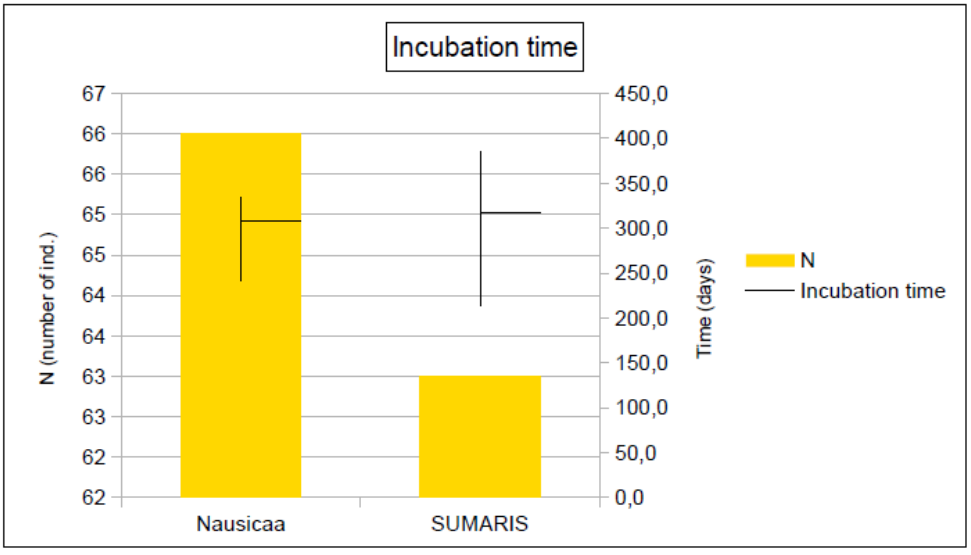

Figure 18 : Incubation period

incubation period would be 4-5 months (Ellis and Shackley, 1995). However, in 1922, Clarks found that the incubation period for elasmobranch eggs varies with temperature.

Clarks observed that, in 1920, when the water temperature was 1° C lower than in 1921, the incubation period increased by two months for certain species of elasmobranch. In the present study, with the temperature having been kept at 10° C throughout the eggs' whole incubation period, the

test groups were deprived of any benefit from the increase of 5° C or more which would occur in the natural habitat. This factor could explain the lengthened incubation period, which is in a proportion consistent with Clarks' observations. Koop's observations (also) support this hypothesis. He observed incubation periods of 4.8 to 7 months with a constant average water temperature of 15.5° C. The calculation of the incubation period expressed as degree-days for the two groups (Figure 19) gives directly proportional values, as all the eggs were incubated at the same temperature.

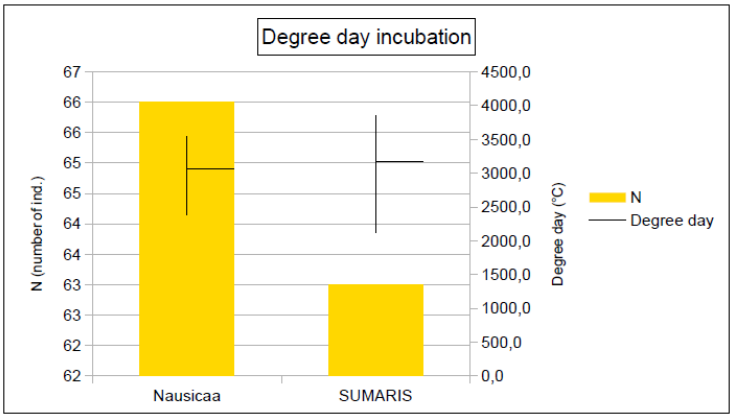

Figure 19 : Degree-day incubation periods

Regarding the short delay in hatching of the capsules laid by the females in the SUMARiS group, we can hypothesise that the lower sea temperature, compared with the aquariums, at the moment the SUMARiS Rays were captured, was influential. Embryo development begins before the eggs are laid, meaning that the eggs will have been affected by the temperature of the water. And, as already noted, the females in the SUMARiS group all exhibited mating marks at the time of their capture. The first eggs to be laid are very likely to have been fertilized before capture. In addition, female Rays have the evolved ability of storage and extended maintenance of the male sperm (gametes) received through mating, which enables them to use the sperm at any time according to their needs (Quignard, Bruslé, 2018).

The proportions of eggs not producing an embryo and of eggs producing viable Rays varied between the groups (Figure 20). The females in the NAUSICAA group laid more eggs, but a smaller proportion of them – 10 times fewer – hatched. These results can be compared directly, but also:

- for the SUMARIS group, with the results of Ellis and Shackley (1995) which, using eggs laid by freshly captured females, found a proportion of eggs producing young Rays of 73 %, compared with 82.3 % for the SUMARIS group,
- and for the NAUSICAA group, with the results of Koop (2005) which, using eggs laid by females having lived for several years in an aquarium, found a proportion of eggs producing young Rays of 3 %, compared with 14.4 % for the NAUSICAA group.

The Ellis and Shackley results and those of the SUMARIS group differ only slightly. This difference is very likely explained in part by the difference in the make-up of the samples in terms of the individual

animals. The conditions of capture from the natural habitat and in incubation conditions are insufficiently detailed to allow a comparison, but these conditions very likely differ as aquarological techniques have evolved significantly over the last 25 years.

The difference between Koop's results and those of the NAUSICAA group is larger. Here, also, differences in individuals will have played some role in these differences. Data on the size of the females and how long they had lived in an aquarium before the study was carried out by Koop are insufficient to base a comparison on.

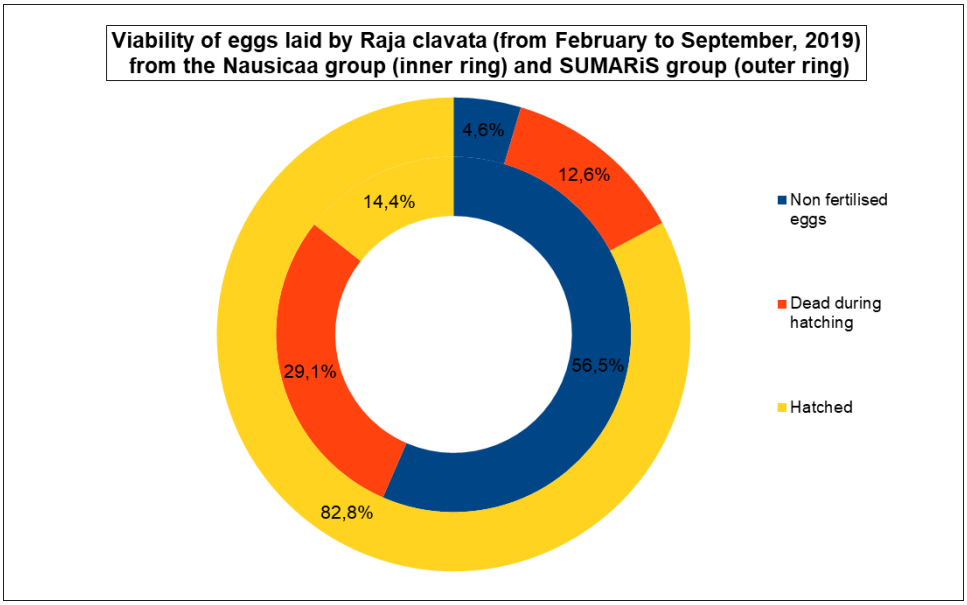

Figure 20 : Viability of eggs from the NAUSICAA group (inner ring) and the SUMARiS group (outer ring)

In order to compare results from the NAUSICAA and SUMARiS groups, given the differences discussed above, we decided to relate the number of eggs that hatch to adult female biomass (see Figure 21). The average values of this relationship were 0.41 for the SUMARiS group and 0.14 for the NAUSICAA group. To explain this difference (a difference not only previously observed but also found to be of greater magnitude when we compare with the studies carried out by Koop and by Ellis and Shackley), we can put forward the hypothesis that the sex-ratio, the seasonal water temperature variations, and the duration of daylight, all influence the number of viable individuals produced by each female. In the aquarium, where these variations are attenuated, this figure seems to fall and the egg laying peaks flatten out. We can also hypothesise that, while the Raja clavata's current aquarium diet supports its rapid growth, it does not necessarily support a fecundity equivalent to that observed in the wild.

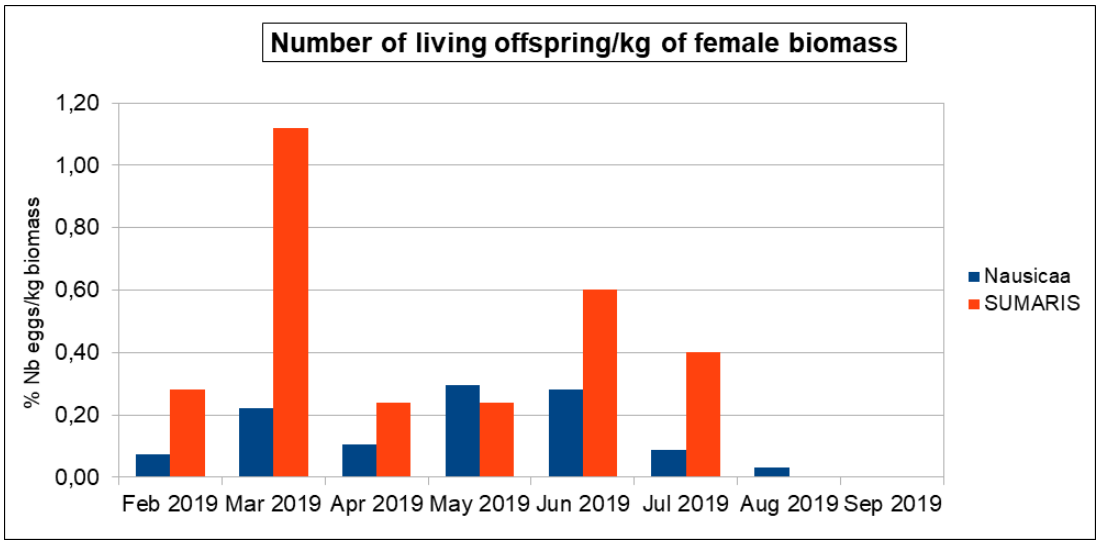

Figure 21 : Number of eggs hatching / kg of female biomass

Results: juveniles

The NAUSICAÁ group produced 74 juveniles and the SUMARiS group produced 72 (Table 4). All the individuals that hatched survived and were able to feed without assistance in the days after their hatching. This result differs from the findings of Lécu, Herbert et al. for the species Glaucostegus cemiculus (see Appendix 3). These differences can be explained by the fact that G. cemiculus is ovoviviparous, unlike R. clavata, which is oviparous. Ellis and Shackley observed a sex ratio (number of males/number of females) of 1:1 in wild populations. The sex ratio observed among the juveniles hatched from the NAUSICAA group was 0.85; it was 1.58 for the SUMARiS group. At this point, it is impossible to say if this difference is significant. A longitudinal study over several consecutive years on a larger sample would help make this determination. The juveniles from the NAUSICAÁ group had an average size of 131.5 mm [114160] and weight of 12.0 gr [9.51-14.68]. The juveniles from the SUMARiS group had an average size of 133.7 mm [122-149] and weight of 13 gr [11.1-15.4] (Figure 27). These values do not appear to us to be significantly different. We can hypothesise that the living conditions in the aquarium did not influence the size of the young Rays when they hatched.

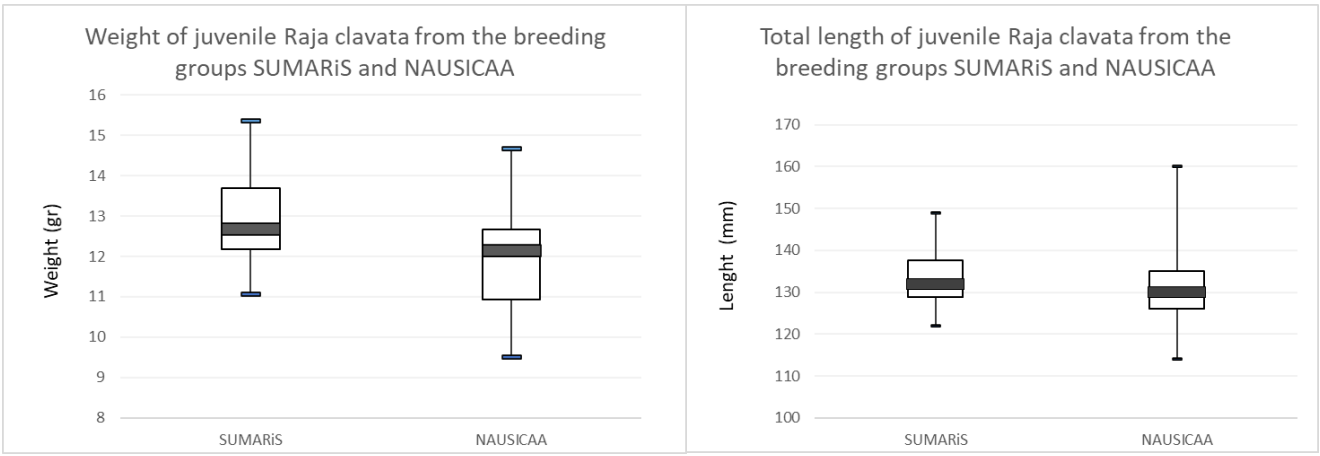

Figure 22 : Weights and lengths of juveniles

IV. Conclusions

The ultimate goal of the SUMARiS project is to propose stewardship measures that are better adapted to the prevailing status/conditions of the stocks of the species of Ray present in the Channel and North Sea. To do this, it is necessary to understand the status of the resources in question. This includes understanding the resource and fishery dynamics, but also the biology of the individual animals that constitute these resources. The present study highlights the importance of having access to estimates of fecundity rates and post-natal survival rates for oviparous elasmobranchs. This information makes it possible, using data on the number of mature females, to estimate the number of viable capsules and therefore the recruitment situation. Once incorporated into statistical models, it can generate supplementary information that will provide a more concrete understanding of the real status of each stock.

For the Thornback Ray, this study, which was undertaken in a protected and partially controlled environment, confirmed that even in the absence of natural predation, not all the eggs laid by the female Raja clavata will hatch or produce viable young. Its results are similar to those published in 1995 by Ellis and Shackney, and suggest that only 70 % to 80 % of the eggs laid by females in the wild will hatch and release young Rays able to feed themselves. To have a more accurate idea of recruitment, it is also important, in our view, to consider the impact of the environment. It would be useful to couple these results with the predation rate, estimated on the basis of counting the number of capsules pierced or ripped by predators and found on the coastline. The present study also reveals the gaps that remain between the results for reproduction obtained using individuals freshly captured from the natural habitat – and having experienced the seasonal cycles and their consequences on the quality and availability of food – compared with results obtained using individuals having spent several years in the protected environment of an aquarium, where observation is far easier, where growth is facilitated, but where the influence of the seasons is still largely suppressed. The results indicate such an environment can have a dampening effect on reproduction. Therefore, improving aquarological techniques using new technologies to support animal reproduction and repeating studies like the one described here are important and will ultimately make it possible to establish data correction factors that would improve the usefulness of data obtained from observation in aquariums, to the benefit of research in the field of fisheries resources management (stewardship).

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VI. Tables

Table 1 : Composition of breeding groups

Broodstock structure

Females are considered mature at TL> 784 mm
Males are considered mature at TL>676 mm

♂ Nausicaa			♀ Nausicaa			♂ SUMARIS			♀ SUMARIS		
BW (kg)	TL (cm)	WS (cm)	BW (kg)	TL (cm)	WS (cm)	BW (kg)	TL (cm)	WS (cm)	BW (kg)	TL (cm)	WS (cm)
2,7	69	46	6,5	91	63	3,7	80	55	4,1	90	60
6	85	55	6,3	88	60	3,3	73	47	5,6	90	63
2,6	72	51	6,9	87	62	2,5	73	46	7,8	92	65
2,6	72	46	7,6	91	64				7,5	100	67
2,8	70	47	6,3	88	59						
3,9	77	52	7,8	99	64						
			8,3	101	71						
			7,4	88	65						
			3,9	78	53						
			6,6	88	59						

Weight interval (kg)	♂ Nausicaa	♀ Nausicaa	♂ SUMARIS	♀ SUMARIS
N = Size of the group (ind.)	6	10	3	4
Mini	2,6	3,6	2,5	4,1
Maxi	6	8,3	3,7	7,8
Average	3,43	6,76	3,17	6,25

Table 2 : Egg laying data record

	Nausicaa								
	Eggs laid	Non fertilised eggs	Fertilised eggs	Dead during hatching	Hatched	Fertility rate	Broodstock mature ♀ total biomass (kg)	Laying rate	Number of living offspring/kg biomass
Feb 2019	16	9	7	2	5	43,8%	67,6	0,24	0,07
Mar 2019	67	48	19	4	15	28,4%	67,6	0,99	0,22
Apr 2019	45	30	15	8	7	33,3%	67,6	0,67	0,10
May 2019	81	41	40	20	20	49,4%	67,6	1,20	0,30
Jun 2019	77	42	35	16	19	45,5%	67,6	1,14	0,28
Jul 2019	92	53	39	33	6	42,4%	67,6	1,36	0,09
Aug 2019	80	30	50	48	2	62,5%	67,6	1,18	0,03
Sep 2019	57	38	19	19	0	33,3%	67,6	0,84	0,00
Total	**515**	**291**	**224**	**150**	**74**	**43,5%**	**67,6**	**0,95**	**0,14**
	100,0%	56,5%	43,5%	29,1%	14,4%				

	SUMARIS								
	Eggs laid	Non fertilised eggs	Fertilised eggs	Dead during hatching	Hatched	Fertility rate	Broodstock mature ♀ total biomass (kg)	Laying rate	Number of living offspring/kg biomass
Feb 2019	7	0	7	0	7	100,0%	25,0	0,28	0,28
Mar 2019	29	1	28	0	28	96,6%	25,0	1,16	1,12
Apr 2019	6	0	6	0	6	100,0%	25,0	0,24	0,24
May 2019	8	0	8	2	6	100,0%	25,0	0,32	0,24
Jun 2019	19	1	18	3	15	94,7%	25,0	0,76	0,60
Jul 2019	16	2	14	4	10	87,5%	25,0	0,64	0,40
Aug 2019	2	0	2	2	0	100,0%	25,0	0,08	0,00
Sep 2019	0	0	0	0	0		25,0		
Total	**87**	**4**	**83**	**11**	**72**	**95,4%**	**25,0**	**0,50**	**0,41**
	100,0%	4,6%	95,4%	12,6%	82,8%				

Table 3 : Metals and micronutrient monitoring data

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PROFESSIONAL WATER TESTS

Test results. Saltwater sample

Sample nr.:	067971	Finish date:	2019-11-28
Tank name:	Mer Froide	Lab remarks:	-

Micronutrients

Element	Result mg/l (ppm)	Reccomended levels mg/l (ppm)
Li	0.1620 mg/l	0.15 - 0.20 mg/l
Si	0.2260 mg/l	0.02 - 2.90 mg/l
I	0.1680 mg/l	0.055 - 0.07 mg/l
Ba	0.0199 mg/l	0.00 - 0.1 mg/l
Mo	0.0100 mg/l	0.0045 - 0.012 mg/l
Ni	0.0000 mg/l	0.00 - 0.01 mg/l
Mn	0.0000 mg/l	0.00 - 0.0022 mg/l
Be	0.0000 mg/l	0.00 mg/l
Cr	0.0000 mg/l	0.00 - 0.0004 mg/l
Co	0.0000 mg/l	0.00 - 0.0006 mg/l
Fe	0.0002 mg/l	0.002 - 0.006 mg/l
V	0.0000 mg/l	0.00 - 0.0025 mg/l
Zn	0.0030 mg/l	0.00 - 0.007 mg/l

Pollutants

Element	Result mg/l (ppm)	Reccomended levels mg/l (ppm)
Hg	0.0000 mg/l	0.00 mg/l
Se	0.0000 mg/l	0.00 - 0.0015 mg/l
Cd	0.0000 mg/l	0.00 - 0.0002 mg/l
Sn	0.0000 mg/l	0.00 - 0.001 mg/l
Sb	0.0000 mg/l	0.00 - 0.0005 mg/l
As	0.0000 mg/l	0.00 - 0.003 mg/l
Al	0.0022 mg/l	0.00 - 0.01 mg/l
Pb	0.0000 mg/l	0.00 mg/l
Ti	0.0000 mg/l	0.00 - 0.01 mg/l
Cu	0.0000 mg/l	0.00 - 0.005 mg/l
La	0.0000 mg/l	0.00 mg/l
Sc	0.0000 mg/l	0.00 mg/l
W	0.0000 mg/l	0.00 mg/l

Main elements

Element	Result mg/l (ppm)	Reccomended levels mg/l (ppm)
Na	10085 mg/l	9720 - 11880 mg/l
Ca	405 mg/l	380 - 460 mg/l
Mg	1175 mg/l	1188 - 1460 mg/l
K	345 mg/l	360 - 420 mg/l
Br	53.8 mg/l	55.00 - 74.00 mg/l
B	3.51 mg/l	4.05 - 5.00 mg/l
Sr	7.20 mg/l	6.00 - 10.00 mg/l
S	814 mg/l	700 - 990 mg/l

Nutrients

Element	Result mg/l (ppm)	Reccomended levels mg/l (ppm)
P	0.9220 mg/l	0.00 - 0.007 mg/l
PO_4	2.8250 mg/l	0.00 - 0.02 mg/l

Additional parameters	Result	Reccomended levels
Salinity:	32 ppt	33 - 36 ppt
KH:	6.7 dKH	6.5 - 8.5 dKH

Client remarks:

-

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Table 4 : Incubation and hatching data

Nausicaa									SUMARIS								
Hatching date	sex	BW	TL	PL	WS	Month of egg-laying	Incubation time	degree day	Hatching date	sex	BW	TL	PL	WS	Month of egg-laying	Incubation time	degree day
		g	mm	mm	mm		days				g	mm	mm	mm		days	
11/19/19	F					02/15/19	277	2804,35	11/19/19	M					02/15/19	277	2804,35
01/05/20	F					02/15/19	324	3547,3	11/19/19	F					02/15/19	277	2804,35
01/05/20	F					03/15/19	296	2993,2	11/29/19	M					02/15/19	287	2901,6
01/05/20	F					03/15/19	296	2993,2	12/04/19	M					02/15/19	292	2948,9
01/27/20	F					03/15/19	318	3201,85	01/05/20	F					03/15/19	296	2993,2
01/27/20	F					03/15/19	318	3201,85	01/05/20	F					03/15/19	296	2993,2
01/27/20	F					03/15/19	318	3201,85	01/05/20	F					03/15/19	296	2993,2
01/27/20	F					03/15/19	318	3201,85	01/27/20	M					02/15/19	346	3463,1
01/27/20	F					03/15/19	318	3201,85	01/27/20	M					03/15/19	318	3201,85
01/27/20	F					03/15/19	318	3201,85	01/27/20	M					03/15/19	318	3201,85
01/27/20	M					03/15/19	318	3201,85	01/27/20	M					03/15/19	318	3201,85
01/27/20	M					03/15/19	318	3201,85	01/27/20	M					03/15/19	318	3201,85
01/27/20	M					03/15/19	318	3201,85	01/27/20	M					03/15/19	318	3201,85
02/11/20	M					06/15/19	241	2391,25	01/27/20	M					03/15/19	318	3201,85
02/11/20	M					06/15/19	241	2391,25	01/27/20	F					03/15/19	318	3201,85
02/11/20	F					06/15/19	241	2391,25	01/27/20	F					03/15/19	318	3201,85
02/11/20	F					06/15/19	241	2391,25	01/27/20	M					02/15/19	346	3463,1
02/14/20	M	14,68	142	72	92	03/15/19	336	3079,45	02/10/20	F		130	70	85	02/15/19	360	3594,2
02/14/20	M	10,49	131	65	80	03/15/19	336	3370,95	02/10/20	M		131	70	90	06/15/19	240	2381,75
02/18/20	M	12,25	131	62	81	04/15/19	309	3079,45	02/10/20	M		134	69	89	06/15/19	240	2381,75
02/18/20	F	11,68	137	64	86	03/15/19	340	3079,45	02/10/20	M		132	68	90	06/15/19	240	2381,75
02/22/20	M	12,3	120	55	75	04/15/19	313	3117,45	02/10/20	M		135	64	89	03/15/19	332	3332,95
02/22/20	M	10,46	115	55	70	04/15/19	313	3117,55	02/10/20	M		125	64	84	03/15/19	332	3332,95
02/22/20	F	12,56	114	58	79	04/15/19	313	3117,55	02/10/20	M		125	63	80	03/15/19	332	3332,95
03/06/20	F	12,67	130	61	83	05/15/19	296	2942,35	02/10/20	F		131	69	88	03/15/19	332	3332,95
03/06/20	M	9,51	130	60	78	05/15/19	296	2942,35	02/10/20	M		125	67	80	03/15/19	332	3332,95
03/06/20	F	10,85	130	62	80	05/15/19	296	2942,35	02/10/20	F		137	68	85	05/15/19	271	2700,55
03/06/20	M	12,4	129	61	75	05/15/19	296	2942,35	02/10/20	F		127	62	77	04/15/19	301	3003,45
03/06/20	F	12,87	128	65	79	05/15/19	296	2942,35	02/10/20	M		139	70	90	04/15/19	301	3003,45
03/06/20	M	11,87	135	70	88	04/15/19	326	3245,25	02/10/20	F		132	60	80	04/15/19	301	3003,45
03/06/20	F	10,45	160	62	77	04/15/19	326	3245,25	02/10/20	M		135	65	80	04/15/19	301	3003,45
03/14/20	M	13	135	70	88	04/15/19	334	3325,85	02/10/20	M		131	65	82	04/15/19	301	3003,45
03/14/20	M	12,87	129	65	80	05/15/19	304	3022,95	02/11/20	M					03/15/19	333	3342,45
03/26/20	M	11,67	140	75	96	05/15/19	316	3143,95	02/11/20	M					03/15/19	333	3342,45
03/26/20	F	12,29	146	75	95	05/15/19	316	3143,95	02/11/20	M					03/15/19	333	3342,45
03/26/20	M	11,45	141	77	93	05/15/19	316	3143,95	02/11/20	M					03/15/19	333	3342,45
03/26/20	F	10,24	135	73	90	05/15/19	316	3143,95	02/11/20	F					03/15/19	333	3342,45
03/26/20	M	12,48	135	78	98	05/15/19	316	3143,95	02/11/20	F					03/15/19	333	3342,45
03/26/20	M	11,85	140	75	93	05/15/19	316	3143,95	02/14/20	M	12,8	129	70	87	07/15/19	214	2119,45
03/26/20	F	13,48	140	79	104	05/15/19	316	3143,95	03/14/20	F	12,7	128	65	79	03/15/19	365	3655,35
03/26/20	F	11,93	142	76	94	05/15/19	316	3143,95	03/14/20	M	11,5	131	66	81	03/15/19	365	3655,35
04/03/20	F	10,25	120	60	77	06/15/19	293	2904,35	03/14/20	F	12	131	66	77	03/15/19	365	3655,35
04/03/20	F	10,98	130	60	78	05/15/19	324	3223,15	03/14/20	M	12,6	128	66	87	03/15/19	365	3655,35
04/03/20	F	12,15	126	65	81	05/15/19	324	3223,15	03/26/20	M	12,2	130	67	81	03/15/19	377	3776,35
04/10/20	M	13,7	135	70	92	05/15/19	331	3294,15	04/03/20	M	12,4	128	67	80	03/15/19	385	3855,55
04/10/20	M	12,08	130	65	85	05/15/19	331	3294,15	04/03/20	M	12,4	130	65	78	04/15/19	354	3526,05
04/10/20	M	10,64	130	65	81	05/15/19	331	3294,15	04/10/20	M	11,5	125	62	84	05/15/19	331	3294,15
04/10/20	F	12,65	120	67	75	05/15/19	331	3294,15	04/13/20	F	11,9	135	65	84	06/15/19	303	3006,35
04/10/20	M	10,94	120	67	75	06/15/19	300	2975,35	04/28/20	F	11,1	124	65	80	06/15/19	318	3159,15
04/13/20	F	12,55	132	65	84	06/15/19	303	3006,35	04/28/20	M	12,2	122	66	85	06/15/19	318	3159,15
04/13/20	M	11,95	130	62	80	06/15/19	303	3006,35	04/28/20	M	13	135	70	85	06/15/19	318	3159,15
04/13/20	F	11,86	133	65	83	06/15/19	303	3006,35	05/14/20	M	14,3	148	80	94	06/15/19	334	3312,75
04/28/20	M	10,51	126	60	75	07/15/19	288	2858,85	05/14/20	F	15,4	145	79	100	06/15/19	334	3312,75
04/28/20	F	12	130	61	85	06/15/19	318	3159,15	05/14/20	F	14,4	146	80	94	06/15/19	334	3312,75
04/28/20	M	10,79	125	64	80	06/15/19	318	3159,15	05/22/20	M	13,6	135	70	88	07/15/19	312	3088,5
04/28/20	F	12,15	125	63	80	06/15/19	318	3159,15	05/22/20	M	13,6	132	70	88	07/15/19	312	3088,5
04/28/20	F	14,18	120	65	81	06/15/19	318	3159,15	05/22/20	F	14,1	135	72	90	07/15/19	312	3088,5
05/06/20	M	10,78	140	93	94	06/15/19	326	3235,35	05/27/20	F	12,7	142	76	98	07/15/19	317	3135,55
05/06/20	F	13,89	135	76	104	06/15/19	326	3235,35	05/27/20	F	14	145	78	98	07/15/19	317	3135,55
05/06/20	F	12,53	132	73	93	07/15/19	296	2935,05	05/27/20	F	13,6	146	78	98	07/15/19	317	3135,55
05/14/20	I	12,24	140	75	95	07/15/19	304	3012,45	06/08/20	M	12,7	149	75	93	07/15/19	329	3244,4
05/15/20	M	12,92	142	76	95	07/15/19	305	2983,95	06/08/20	M	13,7	140	76	95	07/15/19	329	3244,4
05/22/20	F	12,73	130	65	85	08/15/19	281	2762,1	06/08/20	F	15	140	80	98	07/15/19	329	3244,4
05/22/20	M	12,89	126	67	86	08/15/19	281	2762,1									
05/22/20	M	13,85	125	66	89	07/15/19	312	3088,5									
05/22/20	F	10,79	126	66	83	07/15/19	312	3088,5									
Average							308,39	3068,25								317,86	3177,41

VII. Appendices

Appendix 1 : Scientific Paper by Lécu, Herbert et al - 2018

HUSBANDRY AND BREEDING MANAGEMENT OF *Rhinobatos cemiculus*, GEOFFROY SAINT HILAIRE,1817

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Abstract

Eight juvenile guitarfish, *Rhinobatos cemiculus,* were acquired by Nausicaá in 2006. Within 4 years these elasmobranchs became sexually mature and a first birth was recorded in 2009. Since then, three female guitarfish gave birth to babies every year, which brought the total number of offspring up to 45 by the end of 2012. Husbandry techniques, biometry on adults and juveniles, ultrasonography and regular blood sampling are now included in the regular husbandry routine in order to evaluate reproductive physiology of this species, that is not common in aquariums.

Introduction

In 2006, Nausicaà opened a new exhibit named "Cap au Sud". It takes the visitor on a trip along the east coast of the Atlantic Ocean from North Norway to Cape Town in South Africa. During this trip, visitor discovers various biotopes and impacts of human activities on this environment. During a stopover in Lagos, Nigeria, visitors are introduced to the blackchin guitarfish (*Rhinobatos cemiculus*, Geoffroy Saint-Hilaire, 1817). They become aware of the issues with water pollution from plastics that over time breakdown into fine particles that can be ingested by fishes, making fish sterile.

This exhibit is an opportunity for Nausicaà to introduce a new species into its elasmobranch collection: the blackchin guitarfish. These captive animals have been given special attention in order to optimize breeding. Monitoring of growth is preformed through regular biometric measurements. After a few years, guitarfish reach sexual maturity and begin to reproduce on an annual basis. Regular monitoring of broodstock is occurs using ultrasonography and blood sampling for hormonal changes. Juveniles also are followed through development (ie., growth, first feeding, feeding rate, malformations, etc).

1- Characteristics of the species

The blackchin guitarfish (*Rhinobatos cemiculus*) is a demersal species living on sandy-muddy soft bottom of the coastal zone to about 100 m depth. It is found along the eastern Atlantic coast, North of Portugal to Angola, and in the Mediterranean Sea. It feeds primarily on benthic invertebrates (60% crustaceans) and occasionally small fishes, especially as adults. It is considered as an endangered specie by the IUCN (2012) due to overfishing for finning.

This species rarely occurs in aquariums. In a study conducted among European aquariums in 2011, only two aquariums were reported having this species in their collection: the aquarium of Hanover in Germany and Nausicaà in France. However, the juvenile stage of this species can easily be confused with common guitarfish: *Rhinobatos rhinobatos,* Linaeus, 1758, with a similar distribution area as *R. cemiculus*. Once adult, *R. cemiculus* is the only species to reach 265 cm in length, while *R. rhinobatos* remains small and does not exceed 100 cm. To identify juveniles, other criteria must be used, rather than general morphology of the two species, as described by Fischer et. al. (1987). However, these criteria are subjective and sometimes difficult to understand if the two species cannot be compared.

The criteria to differentiate *R. cemiculus* of *R. rhinobatos* are mainly:

- A triangular nose with a closed angle: 60-65° against 65° in *R. rhinobatos.*
- A narrow rostral cartilage.
- The rostral ridges very close.
- A preorbital length less than or equal to the distance between the orbit posterior edge and the posterior insertion of pectoral fin.
- A preorbital length equal to 6-8 times the diameter of the eye (against 5 times in *R. rhinobatos*).
- A black pigmentation at the ventral tip of the rostrum.
- A beige to brown color (khaki brown in *R. rhinobatos*).
- A small anterior nasal lobe of the nostril.

It seems that even fishermen and aquarium suppliers have trouble distinguishing these two species. Indeed, individuals provided to Nausicaà in 2006, were sold as *R. rhinobatos.* Proper identification was made with certainty after checking morphological criteria specific to each of the two species. The identification was further confirmed once they matured.

2- Maintenance conditions

Eight individuals are held at Nausicaà with a sex ratio of 1.1. Wild-caught, they were provided by "Fauna Marina" a Spanish company based in Cadiz specialized in the capture and fish supply for aquariums. On their arrival in Boulogne-sur-Mer, the animals weighed 3.5 to 4 kg and measured approximately one meter in length.

The stock was then divided into two groups. One half was placed in the exhibit while the other half was maintained in quarantine. In both situations, the LSS is similar. It is a closed system with a turnover of about one hour, with mechanical and biological filtration, an UV sterilizer, and a heat exchanger. The seawater renewal varies between 1-3%/day. Seasonal temperature variation of a few degrees Celsius was observed. Water in the exhibition is slightly colder by about 2 to 3°C than that in quarantine (Figure 1). Nevertheless, temperature curves parallel each other and are subject to similar variations. Observed water parameters for both systems are presented in Table 1. In the wild, temperature ranges are similar, but with greater amplitude.

The quarantine tank is a 70 m^3 circular tank in black GRP, with a floor area of 50 m^2. The exhibition tank is a shallow rectangular pool (30 cm), giving animals a floor area of 12 m^2, with a volume of 3.6 m^3. The available bottom area is more important, than the shape or the volume, for this animal.

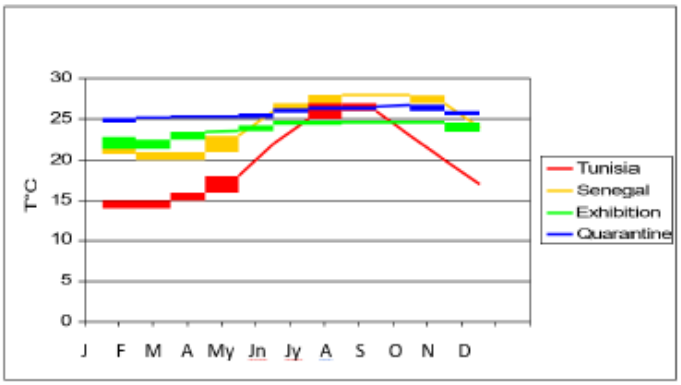

Figure 1. Annual temperature profile in the different rearing tank and in the wild.

In both holding situations, animals were exposed to artificial lighting with a photoperiod of 12:12; however, the photoperiods were reversed on the two systems. Quarantine lighting consists of six fluorescent tubes and 400 watts HQI spotlight that operates during the day. This tank is dark during the night. In exhibition five 400 watt HQI spotlights illuminate the tank overnight. During the day it is kept dark.

Table 1. Water quality

Parameter	Unit	Quarantine		Exhibition	
		Average	Range	Average	Range
Temperature	°C	25,2	22,6 – 27,4	23	19,3 – 25,7
Conductivity	mS/cm	45,7	43,3 - 49,9	48,2	45,2 - 50,7
pH		7,94	7,67 - 8,17	7,91	7,55 - 8,15
TAC	French degree	14,1	12,2 - 15,5	14,0	12,5 - 19,5
N-NH4	mg/L	0,037	0 - 0,165	0,048	0 - 0,121
N-NO2	mg/L	0,002	4,3 - 14,8	3,63	0,92 - 6,32
N-NO3	mg/L	9,49	0 - 0,012	0,007	0,001 - 0,08
PO4	mg/L	4,23	2,57 - 5,41	2,67	1,16 - 8,78
Vibrio spp.	CFU	65	0 - 260	90	0 – 560

In Nausicaà, both sexes occur in the same tank. Sometimes in the quarantine area, they are mixed with other species such as humphead wrasse (*Cheilinus ondulatus*, Rüppel, 1835), zebra shark (*Stegostoma fasciatum*, Hermann, 1783), Blacktip reef shark (*Carcharhinus melanopterus*, Quoy & Gaimard, 1824) and/or juvenile sandbar shark (*Carcharhinus plumbeus*, Nardo, 1827).

3- Feeding and growth of adult animals

Broodstock were fed three times a week. Feeding rate is 2 to 2.5% BW / day. Diet consisted mainly of various fishes (Figure 2) between 50 to 100 grams. Half of the species had high lipid content.

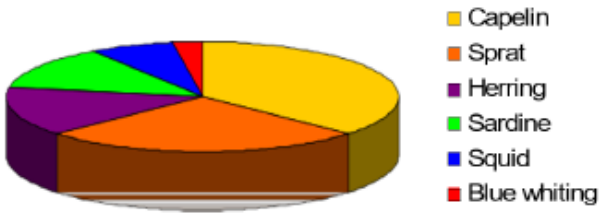

Figure 2. Diet items provided to adult blackchin guitarfish by relative percentage.

There is a weight gain during pregnancy, resulting from development of the embryo. However, this gain does not seem to be proportional to the number of embryos. In fact, the female with the largest litter usually weighs the least. Once parturition takes place, there is a sudden net weight loss, which is reflected in Figure 3.

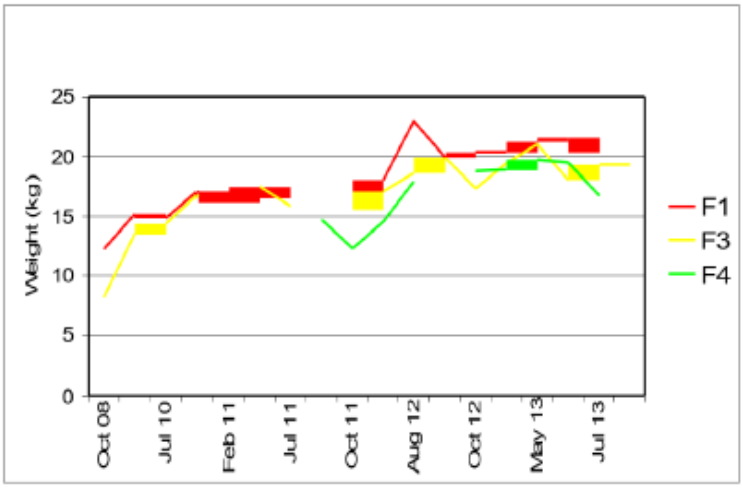

Figure 3. Growth of the 3 adult females blackchin guitarfish in kilograms from 2008 to 2013.

4- Reproduction

4-1- Sexual cycle

4-1-1- Sexual dimorphism

In *R. cemiculus*, there is little sexual dimorphism. Besides the presence of claspers in males, the only dimorphism observed is the difference in size between the two genders, which is common in elasmobranchs. Females are generally larger than males.

4-1-2- Sexual maturity

The first birth was observed in 2009. Since then, three females gave birth annually, resulting in 11 litters. The females have reached a total length of 144 to 150 cm and an average weight of 12 kg. Seck et al (2004) estimated that females reach 163 cm and 14.4 kg in length at maturity for those caught off the coast of. However, Capapé & Zaouali (1981) fixed maturity at 110 cm, in a study in Tunisia.

For males the size of first reproduction has been observed between 125 and 140 cm and a weight of more than 7.5 kg. For females, Seck et al. (2004) describes juveniles being less than 152 cm.

The age of sexual maturity is difficult to determine. The age of our animals at Nausicaa upon arrival in 2006 was not known. However, given the total length of the animals, their age could be estimated between 2 and 3 years old. These animals bred after three years of captivity, bringing their age of maturity between 5 and 6 years.

4-1-3- Folliculogenesis

In the wild, *R. cemiculus* occurs annually. Only one of the ovaries is productive, follicles from other waves undergo atresia. In the wild, vitellogenesis takes place from April to August. Oocytes reached a maximum size of more than 40mm in the stroma, ready to be released. Gestation runs parallel to vitellogenesis. According to Seck et al (1991), newly formed eggs can be found encapsulated in the uterus from December to March. Embryos can be found from April to August reaching a size greater than 20 cm. These processes are equally distributed between both uteri. Parturition usually takes place in August, to coincide with the age of maturity for the new oocytes. Gestation lasts a duration of 5 to 8 months (Lessa & Lahaye, 1982; Capapé & Zaouali, 1994; Seck et al, 2004).

Embryonic diapause could be environmentally influenced. It has not been described in *R. rhinobatos* nor *R. cemiculus* in the Tunisian warm waters, but could be possible for the same species in waters with more variable temperature such as on the coast of Senegal (Capapé & Zaouali, 1994; Seck et al, 2004). In his study, Seck et al encountered relatively homogeneous values between 20 and 22 eggs in both ovaries. In Tunisian waters , Capapé estimates ovocytes to be between six and 16 (Capapé & Zaouali, 1994; Seck et al, 2004). Similarly, uterine fecundity counts the number of elements in the two uteri, eggs or embryos. Seck et al's results did not statistically differ from those of ovarian fertility and were between 16 and 24 elements, whereas in the Capapé study, uterine fecundity was statistically different witha mean of 7.52 versus 9.16 (Capapé & Zaouali, 1994; Seck et al, 2004).

4-1-5- Mating and fertilization

A female may mate with several males, resulting in sperm competition. A copulatory plug is usually produced by alkaline gland to prevent futher introduction of sperm. Fertilization occurs before encapsulation. No evidence suggests that sperm storage occurs *R. cemiculus* (Hamlett, 1999a; Carrier et al, 2004).

At present, no mating has ever been observed in captivity. The only observable coupling clues are bite marks on the edges of the pectoral fins. The animals of both sexes have bite marks. It was found that the coupling takes place in the days following parturition, when the two sexes occur together.

4-1-6- Pregnancy

In the Rhinobatidae, the common way of gestation is aplacental viviparity by maternotrophy. *Rhinobatos cemiculus* is no exception to the rule, the pregnancy begins with embryos consuming yolk reserves. Halfway through gestation, embryos become free in the uterus and receive, in addition to the yolk, provision of " uterine milk" secreted into the lumen of the uterus (Dulvy & Reynolds, 1997). Uterine epithelium develops villi on the entire surface, that can measure several centimetres in length. These are called trophonemata. Villi are narrow to their base, wide at their distal end and wrap the fetus closely. Surface secretion is increased allowing a large supply of nutrients. This partly explains why the species with that type of gestation produce juveniles that are proportionately larger. The secreted fluid contains proteins, lipids, and mucus. Fetuses absorb secretions either by ingestion or gill absorption. By three quarters of the way through gestation, the yolk sac is almost depleted and will begin to resorb as the cord vitellin (Wood-Mason & Alcock, 1891; Bearden, 1959; Hamlett & al, 1985; Hamlett, 1999b).

About 2 months before parturition, the dorsal part of the animal begins to widen on each side of the rachis between the gills and the pelvic girdle. This area expands more and more throughout gestation. Ventrally, although difficult to observe, there is a swelling in the same area.

Shortly before parturition, the females are captured and isolated in a circular tank of smaller size (4 m diameter - 12 m^3) to reduce the risk of predation on juveniles at birth. Isolation is estimated based on the date, the size of the females, and the observations made by ultrasonography. One week before parturition, a swelling of cloacal papilla can be observed.

4-1-7- Parturition

Little is known about parturition in *R. cemiculus*. From birth pups are autonomous and appear as miniature adults with a ventral scar, where the external yolk sac was located. At necropsy, we observed the presence of an internal yolk vesicle.

In most cases, parturition occurs during the months of July and August as has been observed in the wild. However, the birth of three litters in 2012 occurred between September and November. The same temporal shift occurred in 2013.

Although we have been able to observe the birth, it has not been possible to observe the expulsion of neonates. The female stays almost motionless on the bottom after a few minutes juveniles appear one by one from the female underside and begin to swim immediately. Pups are expelled one by one. The time interval between the birth of two individuals is ranges from two to five minutes. Staff quickly isolate young in a $12m^3$ lack circular pool.

Within 24 to 48 hours after birth, the female is captured, anesthetized with eugenol (30 ppm), weighed and a blood sample is collected. The female is then placed in the presence of the male in the main culture tank.

When birth happened in 2011, three females were isolated for several months after parturition, which is the only case where the females were not placed immediately with males. The delay in placing the two sexes together may explain the shift in the time of birth of 2012 (born September-November 2012 instead of July-August in other years).

4-2-Reproduction monitoring

4-2-1- Population growth

All the broodstock (3 males and 3 females) are marked with microchip transponders, for the purpose of individual monitoring of each animal. The usual transponder size is 2 mm diameter x 12 mm long. Young animals are marked one month after birth with smaller transponders with the idea to follow each individual's growth and also to separate brothers from sisters, avoiding inbreeding when they will be able to reproduce. Liter demographics (Table 2, Figure 5) and population growth (Figure 4) are presented below.

4-2-2- Identification method

On some animals, an external plastic tag was placed through one of the dorsal fins. However, these tags stand out and must be renewed regularly. Over time, the passage of the tag through the fin often generates an inflammatory reaction.

Table 2. Reproduction of female *R. cemiculus* in Nausicaâ since 2009 (litter composition and mortality)

Lot	Birth	Progenitor ID	Litter size	Sex ratio (F/M)	Stillborn	% survival
2009	10/08/2009	?	1	1/0	1	0
2010-F3	09/07/2010	F3	2	1/1	1	0
2010-F1	13/07/2010	F1	7	¾	2	0
2010-F4	17/08/2010	F4	2	1/1	0	100
2011-F1	02/07/2011	F1	4	1/3	0	100
2011-F3	28/07/2011	F3	2	1/1	1	50
2011-F4	22/08/2011	F4	3	½	1	33,3
2012-F1	01-02/09/12	F1	9	2/1	0	88,9
2012-F3	29/10/2012	F3	12	5/7	8	33,3
2012-F4	11/11/2012	F4	4	1/1	0	75
2013-F1	27/06/13	F1	1	1/0	1	0
2013-F4	15/07/13	F1	4	1/1	0	50
2013-F3	07/10/13	F3	4	1/3	0	100

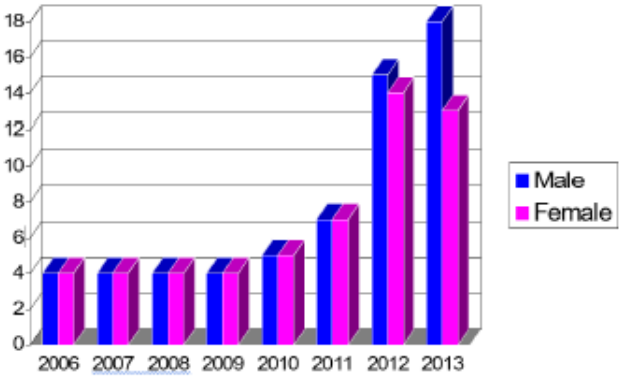

Figure 4. Growth of the blackchin guitarfish population (in number of individuals) at Nausicaä between 2006 and 2013.

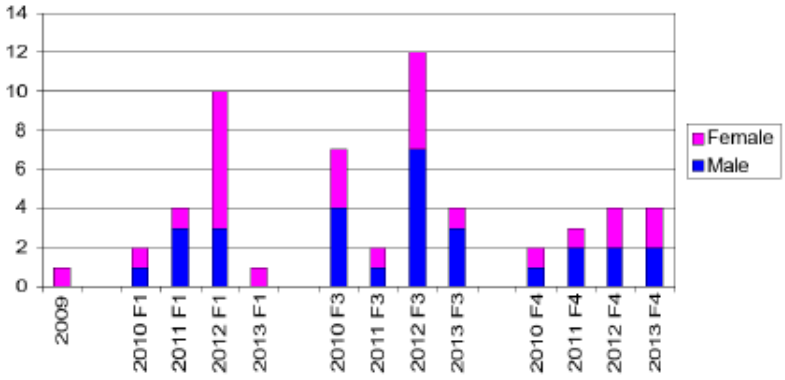

Figure 5. Gender composition of each litter in number of individuals.

4-2-3- Pregnancy monitoring method

Monitoring to follow pregnancy is implemented by ultrasonography and blood sampling. For this examination, individuals are anesthetized using a bath of 35 ppm eugenol (99%) diluted in ethanol in a 1/10 proportion. Prior to anesthesia, the oxygen saturation in the water bath is raised to 200%, in order to increase the partial pressure of oxygen in the blood of the animal. About 15 minutes is required to reach stage III of anesthesia. The ray can then be taken out of the water for

monitoring. Examination takes about 20 minutes outside of water. Recovery takes around 10 minutes. Ultrasonography allows one to follow development of ovary, uterus and embryos, but also allows measurement of the heartbeat of adult and fetus.

Blood samples are collected via a caudal puncture for:

- Smears.
- Cell blood counts (collected in 7 cc tube with EDTA in which we add 0,5 cc heparin).
- Biochemistry (usual parameters like BUN, Total Protein).
- Hormone assay in serum (blood is collected in 7 cc tube with clot activator.
- Plasma is collected passively after 12h and conserved in a dry tube at -10°C. The deep frozen tube with plasma is then sent in a carbo-ice by carrier to the lab which will perform the analysis.

Three steroid hormones (estradiol (E2), progesterone (P) and testosterone (T)) are assayed every month with the aim to establish the annual hormonal cycle for this species and correlate it with the ultrasonographic observations.

Measurement technique is via Radio Immuno Assay (RIA), where shark samples are in competition with mammal serum, using radioactive labeled progesterone. Then radioactivity is assayed and is inversely proportional to the amount of shark hormone. For P measurement, the laboratory uses a progesterone RIA Kit from Beckman Coulter Compagny, and for E2 and T, a Spectria IDS method is used.

4-2-4- Hormone assay observations

When the curves of three hormones overlap, we observe at the time of birth a fall of the oestradiol (Figure 6). Two months after birth, the quantity of oestradiol is always weak but a new peak of progesterone appears, this one lasts 3 months. Finally, 6 months before birth, progesterone drops while the rate of oestradiol increases to > 5.0 nmol / L, as well as in duration of >4mo). This period corresponds to the gestation. The variation of concentration in testosterone seems to follow the oestradiol concentration. The role of testosterone, except for being a precursor of the oestradiol, could not be determined.

There is a:

- seasonal progesterone peak (September, December / January)
- low progesterone concentration observed at delivery time
- high concentration of oestradiol throughout gestation
- punctual decrease of oestradiol at delivery time (could be delivery inductor)
- return to the basal concentration of estradiol, 2 months after delivery

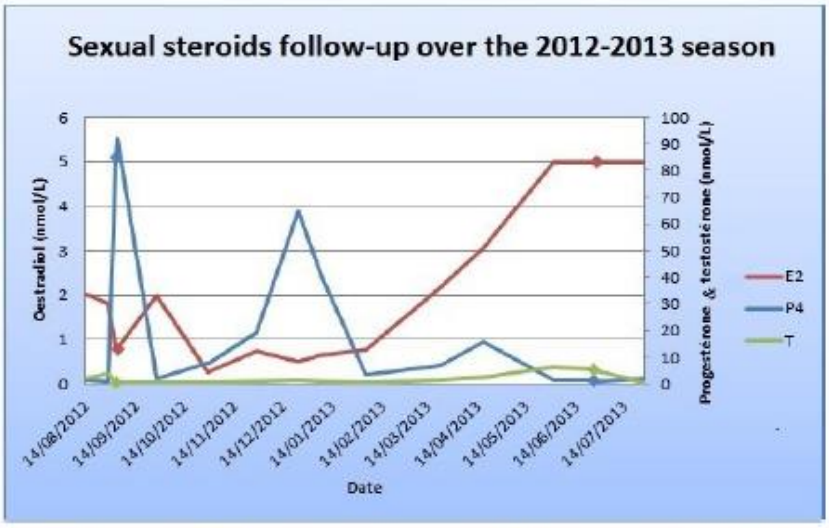

Figure 6: Rate of estradiol, progesterone and testosterone in *R. cemiculus* female during the study (the dots indicate the dates of birth).

4-2-5- Ultrasonography observations

From ultrasonography monitoring, we can observed the following sequences:

- In one female, the observation of an atresic follicle suggests the presence of non-ovulatory follicular waves, 1 to 4 months postpartum.
- Ovulatory follicle wave and fertilization almost six months after parturition (February – April).
- No vitellogenesis observed during gestation: on the contrary to Seck et al (2004) and Capapé and Zaouali (1994) observations, we did not highlight a folliculogenesis during the gestation. Gestation seems to be a low ovarian activity period, and ovulation after delivery appears unlikely. No vitellogenesis observed during gestation.
- The ovulatory follicle wave ends when mature follicles of 30 cm are released.
- Before ovulation, uterus wall thickens (Figure 7).
- The first uterine images confirming gestation are images of segmented uterus. The presence of encapsulated eggs was also described by Seck et al (2004), however he described an encapsulated form during 3 to 4 months: that is much longer than what we observed in our study (1 to 2 months maximum).(Figure 8)
- The thickening of the uterine mucosa, which continues during the first stages of the gestation, will lead to the formation of folds. These are getting organized in individual villi making protrusion in the lumen. It is the appearance of trophonemata that will provide nourishement to the developing embryos in the form of a secretion called histotroph or uterine milk. (Figure 8)

- Trophomata suspended in the uterine fluid not associated with the embryo (Figure 10): The developed trophomata was observed throughout the rest of gestation period. They become spatulate, are in suspension in the uterine liquid, and do not seem to have particular links with the embryos which are very mobile.
- The chronological development of embryos is difficult to determine. The liberation of the egg happens when between 3 to 10 cm(See photo 5).
- We suppose that the limit is situated towards 3 centimeters, because in the size of 10 cm, the foetus is almost autonomous.
- The heart rate is measurable, the breath is active, the nutrition also, and a kind of intra uterine swimming exists. The continuation of the growth seems to be a proportional enlargement of the model existing in 10 cm (Figure 12,13, & 14).
- Gestation lasts approximately 100 days.
- Quick involution of the uterus postpartum (approximately 10 days).

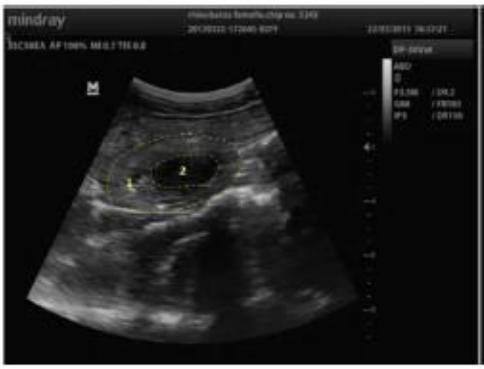

Figure 7. Uterus during thickening in March: female 5245. 1 = uterus mucosa, 2 = uterus lumen

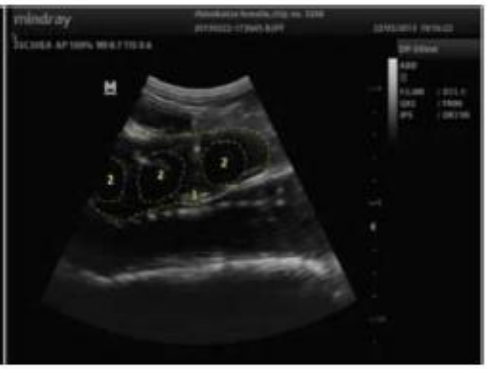

Figure 8. Uterus picture in "lodges" signs of the presence of eggs in the uterus. 1= uterus mucosa, 2 = "lodges" of the egg (diameter = 2.8 cm).

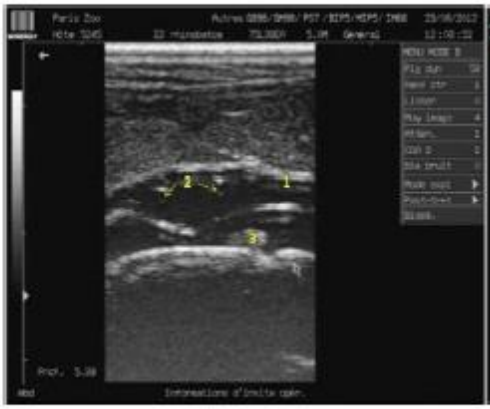

Figure 9. Apparition of trophomata in August 2012 in female 5245. 1 = uterus mucosa, 2 = small trophomata, 3 = embryo

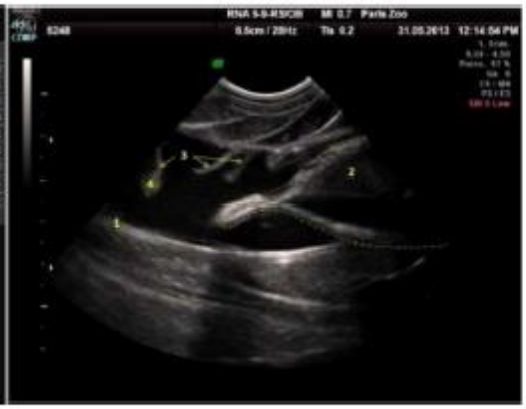

Figure 10. Trophomata developed in female 5248 in May 2013. 1 = uterus mucosa, 2 = fœtus, 3= trophomata, 4 = trophomata extremity in "spatula"

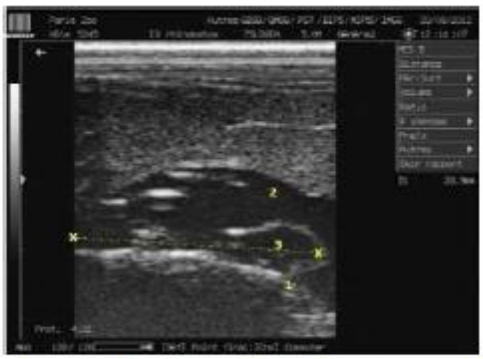

Figure 11. three cm embryo still in the egg in female 5245 in August 2012. 1 = uterus mucosa, 2 = uterus lumen, 3 = embryo.

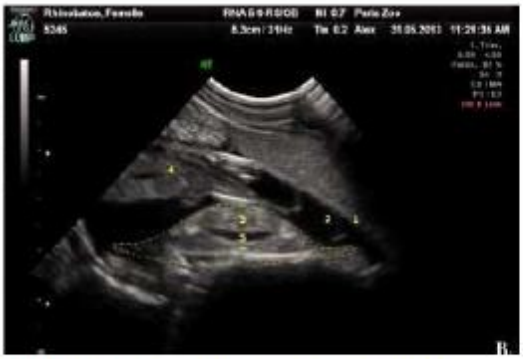

Figure 12. 11cm fetus in transversal section in female 5245 in May 2013. 1 = uterus mucosa, 2 = uterus lumen, 3 = embryo, 4 = second embryo, 5 = pharynx.

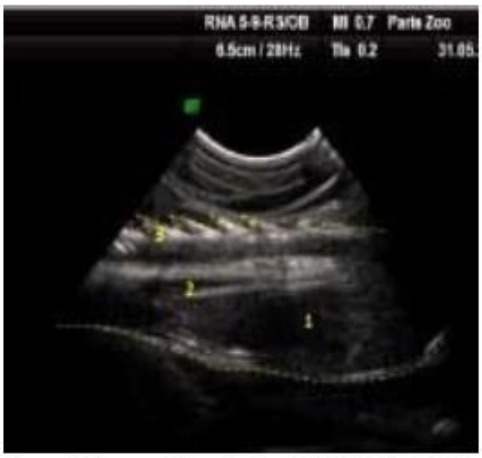

Figure 13. Foetus of 13 cm in female 5248 in May 2013. 1 = abdominal cavity of the foetus, 2 = rachis, 3 = dorsal denticles.

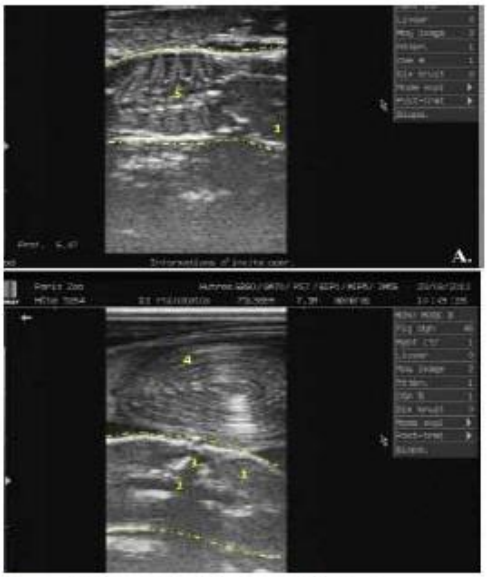

Figure 14. Near to final size foetus [25-30cm]. A: longitudinal section. B: cross section. 1 = foetus, 2 = pharynx, 3 = rachis, 4 = spiral valve, 5 = gill arches.

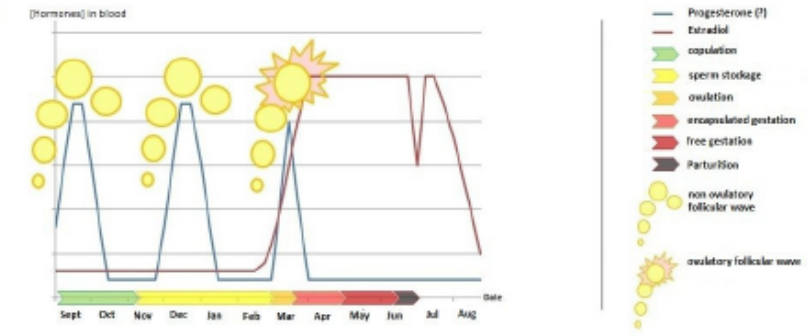

Figure 15. Proposal for a general pattern of annual reproductive cycle observed in captive *Rhinobatos cemiculus* (Boitard, 2013).

4-2-6- Proposal for a general pattern

By considering all of the data a general pattern can be described for the reproductive cycle of *Rhinobatos cemiculus*, even if information does not match perfectly. The main point of difference concerns the ovulatory wave of ovocytes. Indeed, peaks of progesterone were described although not corresponding in ovulatory phases observed by ultrasonography. These peaks seem characteristic of *R.cemiculus* and could be associated with the non-ovulatory follicular waves. On the other hand, ultrasonography placed ovulation for every female, despite the fact that no peak of progesterone resulting from a luteal activity was observed. The variations in progesterone concentrations during ovulation may not be evident if they were too short.

Observations of animals place the mating a short time after the reintroduction of the male, approximately 15 days. But the observations of gestation in the uterus and the estimations of ovulation were done much later (approximately 5 to 6 months): Contrary to Seck, who describes an embryonic diapause to the Senegalese animals, no uterine storage of eggs was observed before the beginning of the gestation. So it seems possible that, like with other species of elasmobranchs, that sperm storage occurs in the female. This would last over winter.

5- Juveniles Feeding

First feeding shortly after delivery is very important. Generally new born babies don't have any internal yolk reserve and need to eat soon after parturition. The best first food is live food such as shrimps which stimulate hunting behavior by their constant motion. They also can be left in self-service in the rearing tank without pollution risk.

Weaning on inert food can be tried after one week. But weight gain must be managed. In some cases, we tried to start with not live food like chopped herring. Although young guitarfish showed a great activity in the presence of this food and seemed to eat it, we have to note that after 3 weeks of this diet without living food, all the animals had lost around 30% of body weight and some individuals died.