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Control of reproduction in juvenile *Oreochromis niloticus* using *Nelumbo nucifera* (Nelumbonaceae) powder

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Abstract

Context: Aquaculture represents a significant share of the Cameroonian economy, with an annual production of 5,000 tons of Nile tilapia (MINEPIA 2020). However, challenges related to fish reproduction and growth still limit the potential of this industry. This study aims to evaluate the effect of adding Nelumbo nucifera leaf powder to the diet of juvenile Nile tilapia on their growth and reproductive parameters.

Methodology: The study was conducted at the Agropastoral FishCam farm, located in the Central Region of Cameroon. A total of 240 juvenile *Oreochromis niloticus* with an average weight of 12.3 ± 2.82 g were divided into four groups of 60 juveniles each, housed in 1m x 1m x 1m happas within a 400 m^2 pond. Each group was further divided into three subgroups of 20 fish, corresponding to the group's repetitions. The fish were fed an isoproteic pelleted feed made from local ingredients for 42 days. Fish in Group 0 received feed without *Nelumbo nucifera* powder, while those in Groups 1, 2, and 3 were fed diets containing 0.03%, 0.06%, and 0.09% of *Nelumbo nucifera* leaf powder, respectively.

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After 42 days, 30 fish were randomly selected per treatment and sacrificed to assess reproductive parameters. **Results:** The results show that treatment T1 (0.03% of *Nelumbo nucifera* powder) recorded the highest values for average weight gain, average daily weight gain, and specific growth rate. The highest survival rate was recorded in animals from group 2. The T1 diet also significantly induced the highest indices (Gonadosomatic Index, Hepatosomatic Index). **Conclusion:** In light of the results, we recommend using *Nelumbo nucifera* at 0.03% in the diet of *Oreochromis niloticus* to improve reproductive and growth parameters. This study provides valuable information for fish farmers seeking to optimize Nile tilapia production.

Context: The expansion of aquaculture over the past decades has driven the overall growth of aquatic animal production in inland waters, which increased from 12% of total production in the late 1980s to 37% in 2020 (FAO 2022). This production (178 million tonnes) experienced a slight decline from the all-time record reached in 2018, with 179 million tonnes. Capture fisheries contributed 90 million tonnes (51%) to this figure, while aquaculture contributed 88 million tonnes (49%). Of the total produced, 63% (112 million tonnes) came from marine waters (70% from capture fisheries and 30% from aquaculture) and 37% (66 million tonnes) from inland waters (83% from aquaculture and 17% from capture fisheries). The total value of global production at the first sale is estimated at USD 406 billion, including USD 141 billion from capture fisheries and USD 265 billion from aquaculture.

In addition to the production of aquatic animals, 36 million tonnes (fresh weight) of seaweed were produced in 2020, 97% of which was from aquaculture, primarily mariculture (FAO, 2022). In Sub-Saharan Africa, aquaculture is expected to grow by 28% by 2030 (OECD-FAO Agricultural 2021-2023). More broadly, the positive outlook for aquaculture in Sub-Saharan Africa aligns with a global context in which the sector has experienced strong growth in recent years, becoming the primary source of fish for human consumption, surpassing capture fisheries, whose production has remained stable over the past decades (OECD-FAO Agricultural 2021-2030).

Aquaculture thus appears to be an asset in addressing food insecurity and natural resource challenges faced by most low-income populations in Africa in general and Cameroon in particular. The national demand for fish in Cameroon is approximately 500,000 tonnes per year, while national supply is estimated at 230,000 tonnes per year, including 150,000 tonnes from aquaculture (MINEPIA 2019). Therefore, there is a production deficit of 270,000 tonnes, which is compensated by imports. To address this deficit, the Cameroonian government spends approximately one hundred (100) billion CFA francs each year on fish imports (MINEPIA, 2019).

Through the implementation of a strategic development plan, the country aims to ensure food self-sufficiency and conquer the markets of the Central African Economic Community and the West African

Economic Community. Achieving this goal necessarily involves aligning with agro-industrial production, modernizing agriculture, livestock farming, fishing, and aquaculture, and marketing agro-industrial production on the international market.

To this end, the country plans to develop priority agro-industrial sectors (including cotton, cocoa-coffee, palm oil, sugar, rubber, rice, maize, plantain, fish, milk, and meat), which will be subject to specific development plans (SND30 2020-2030). However, concerning aquaculture, these programs aim to promote the sector by providing financial support to producers. However, these programs do not emphasize research, which remains one of the pillars of development.

Several tilapia species are found in Cameroon, including the Nile tilapia (*Oreochromis niloticus*), which is the most valuable species for fish farming (Efole, 2011). It is one of the most widely farmed fish species in the world.

Methods

The study was conducted from May 13 to July 7, 2023, at the FishCam agropastoral complex. Its objective was to contribute to the improvement of tilapia aquaculture production by controlling the growth and reproduction of *O. niloticus* juveniles in ponds through the use of *Nelumbo nucifera* powder as a reproductive potential inhibitor.

To achieve this, a total of 240 *Oreochromis niloticus* juveniles with an average weight of 12.3±2.82g were divided into four groups of 60 juveniles each, placed in 1m x 1m x 1m happas within a 400m^2 pond, corresponding to four treatments in a completely randomized design. Each group was subdivided into three subgroups of 20 fish, corresponding to the group replicates.

For 56 days, the fish were fed twice daily (7 a.m. and 5 p.m.) with an isoproteic pelleted diet made from local ingredients. The fish in Group 1 received a diet containing 0% *Nelumbo nucifera* powder, while those in Groups 2, 3, and 4 received diets containing 0.03%, 0.06%, and 0.09% of *Nelumbo nucifera* leaf powder, respectively, over the same period and by the same method. The concentrations of 0.03%, 0.06%, and 0.09% of *Nelumbo nucifera* leaf powder were selected based on preliminary results and previous studies showing beneficial effects at these doses.

Control fishing was conducted every two weeks to assess the growth progression of the fish. After 56 days, 30 fish were randomly selected per treatment and sacrificed to evaluate the reproductive parameters.

Experimental setup

The experimental setup consisted of 12 hapas, each 1m^3 in size, made of netting with 0.2mm mesh, arranged in triplicate, each containing 20 juvenile *O. niloticus* individuals. These hapas were positioned with markers in the form of squares, proportionally sized to the hapas' dimensions, within a 400m^2 pond.

Materials used

• Animal materials

240 juvenile *O. niloticus* with an average weight and size of 12.3±2.82g were selected from the farm ponds. The sex parameter was not considered. These juveniles were acclimated for three days before the start of the experiment and were fasted for 24 hours (on the last day of acclimation) to empty their stomach contents before the study began.

• Vegetal materials

The vegetal material consisted of powder from the leaves of *Nelumbo nucifera*, harvested from a river in the western region and dried in an electric dryer for four days at a temperature of 38°C. Once dried, the material was crushed in a grinder to obtain powder.

Four isoproteic rations (38%) were formulated using *Nelumbo nucifera* powder (Figure 6). The ingredients were purchased from a feedstore in Yaoundé, with three rations containing 0.03%, 0.06%, and 0.09% of *Nelumbo nucifera* powder (for treatments T1, T2, and T3) and one control ration with no powder (treatment T0). The purchased ingredients are listed in Table I below.

Results

• Average weight gain

Figure 1 illustrates the daily evolution of the average weight gain of *Oreochromis niloticus* juveniles in relation to time and the level of substitution of *Nelumbo nucifera* powder.

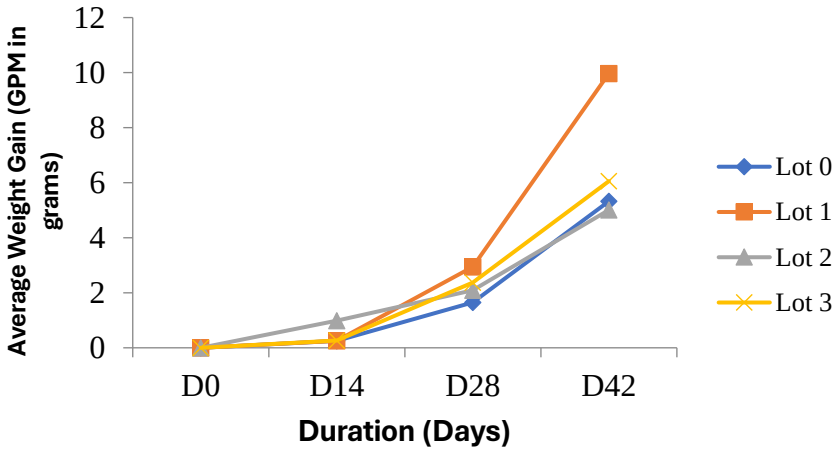

Figure 1: Evolution of the average weight gain over time.

From this figure, it appears that the average weight increased over time regardless of the treatment considered. However, from the first to the second and a half week, the curve for the fish in Lot 2 (0.06%

Nelumbo nucifera leaf powder) was above that of the other lots. From the second and a half week onward, the curve for the fish in Lot 1 (0.03% *Nelumbo nucifera* leaf powder) remained above that of the other lots.

Average daily gain

Figure 2 illustrates the evolution of the average daily gain of *Oreochromis niloticus* juveniles in relation to time and the level of substitution of *Nelumbo nucifera* powder in the feed.

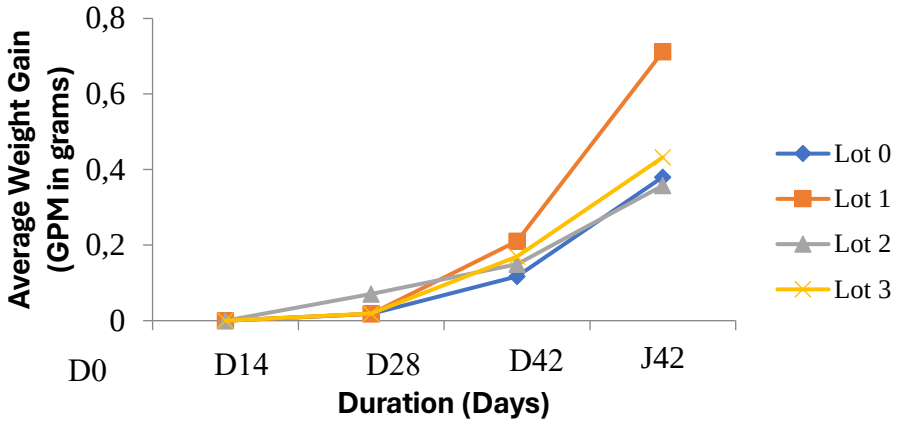

Figure 2: Evolution of the Average Daily Gain over Time

It should be noted that the average daily gain increased over the period, regardless of the type of treatment considered. However, from the first to the second and a half week, the curve for the fish in Lot 2 (0.06% *Nelumbo nucifera* leaf powder) was above that of the other lots. From the second and a half week onward, the curve for the fish in Lot 1 (0.03% *Nelumbo nucifera* leaf powder) remained above that of the other lots. A higher average daily gain was observed in T1 (0.49±0.2g), followed by T3 (0.43±0.6g), and the lowest was observed in T0 (0.33±0.1g).

Specific growth rate

Figure 3 illustrates the variation in the specific growth rate of *Oreochromis niloticus* juveniles in relation to time and the level of substitution of *Nelumbo nucifera* powder in the feed.

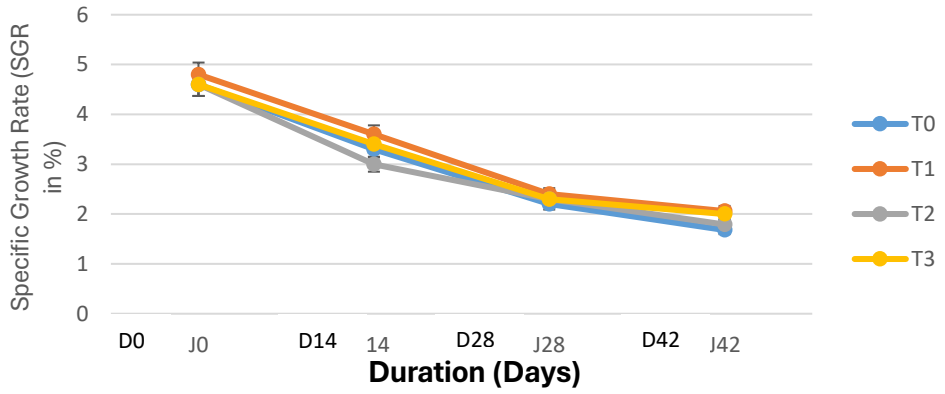

Figure 3: Evolution of the Specific Growth Rate over Time

From this figure, it can be observed that the specific growth rate decreased over the period, regardless of the substitution level in the feed. However, the curve for the specific growth rate of fish in Lot 1 remained above that of the other lots. At the end of the trial, the highest specific growth rate value was recorded for treatment T3 (2.00±0.6ab). Caractéristiques de reproduction chez *Oreochromis niloticus* en fonction de l'utilisation de la poudre de *Nelumbo nucifera*.

Values of Gonado-Somatic Index (GSI)

The following Table 1 presents the values of Gonado-Somatic Index (GSI) and Hepato-Somatic Index (HSI) for *O. niloticus* juveniles based on the ration and sex after 42 days of feeding.

Table 1: Values of Gonado-Somatic Index (GSI) and Hepato-Somatic Index (HSI) for O. niloticus juveniles based on rations and sex at the end of the trial.

Indices		**Treatments**				
	Sexes	T0(n=30)	T1(n=30)	T2(n=30)	T3(n=30)	P
IGS	Male	0,35±0.1^{a}	0,9±0.2^{a}	0,85±0.1^{a}	0,65±0.5^{a}	0,09
	Female	1,20±0.2 ^{a}	1,08±0.4 ^{ab}	0,92±0.2^{b}	0,62±0.2 ^{b}	0,02
IHS	Male	0,44±0.3 ^{b}	0,95±0.3^{a}	0,88±0.5 ^{ab}	0,70±0.3 ^{b}	0,04
	Female	0,39±0.2 ^{b}	0,76±0.2 ^{a}	0,62±0.1 ^{ab}	0,53±0.1 ^{b}	0,05

n = number of fish; T0: Control treatment, T1, T2, T3: Experimental treatments containing 0.03%, 0.06%, and 0.09% *Nelumbo nucifera* leaf powder, respectively; a, b, c: values in the same row with different letters are significantly different ($p < 0.05$).

From this table, it can be observed that no significant difference was found for the GSI in males. However, males from Lot T1 showed a GSI of 0.9±0.1, higher than those observed in the experimental groups T2

(0.85), T3 (0.65), and T0 (0.35). Additionally, the gonado-somatic index in females showed a significant ($p<0.05$) dose-dependent decrease in this characteristic.

The hepato-somatic index increased significantly ($p<0.05$) regardless of sex. The lowest IHS values in both males and females were recorded in T0 compared to the experimental lots (T1, T2, and T3).

The results suggest that the addition of 0.03% *Nelumbo nucifera* powder in the diet of Nile tilapia juveniles significantly improves growth and reproduction parameters, which could have important implications for optimizing aquaculture production.

Discussion

The results of our study show that the values of the physico-chemical parameters of the water recorded throughout the trial in the pond showed little variation. The recorded values remained within the acceptable ranges for the survival, growth, and reproduction of tilapia, as described by Lacroix (2004).

Regarding the survival rate, it is noteworthy that mortality was recorded in the treatments T1 and T3, which contained 0.03% and 0.09% *Nelumbo nucifera* powder, respectively. Since we did not observe any signs of apnea or predation by ichthyophagous birds in these fish during the trial, we can conclude that these mortalities were likely related to stress caused by the various handling during the control fish harvests. However, the treatments T0 (0%) without powder and T2 (0.06%) with *Nelumbo nucifera* powder recorded a survival rate of 100%. This suggests that the *Nelumbo nucifera* powder was not the cause of the mortalities observed during the trial. This assertion is supported by the work of Imana et al. (2013) and Duagjail et al. (2018), who reported that the *Nelumbo nucifera* plant, due to its antimicrobial and antibacterial properties, would rather be beneficial for growth. This is further supported by the results of our study, which showed an increase in growth parameters such as average weight gain, average daily gain, and specific growth rate in fish fed with feed containing 0.03% *Nelumbo nucifera* powder compared to the control lot.

The improvement in growth parameters observed in this study could be attributed to the presence of nutrients, such as proteins, found in *Nelumbo nucifera* leaf powder, as shown by the bromatological analysis. Additionally, bioactive compounds such as phenols, saponins, flavonoids, alkaloids, terpenoids, and steroids in *Nelumbo nucifera* leaves, with their antioxidant properties, could have trapped free radicals produced during temperature fluctuations and handling during control fish harvests, thereby providing good health status to the fish. These results align closely with those of Mutlen et al. (2019) and Amira (2021), who obtained similar results with extracts of *Nauclea matifolia*, *Tribulus terrestris*, and *Azadirachta indica* (neem). However, these findings contrast with those of Jegede (2010),

who reported a decrease in weight gain, average daily gain, and specific growth rate in tilapia fed with *Hibiscus rosa-sinensis* leaf meal.

Regarding the feed parameters, the consumption index value of fish fed with the feed containing 0.09% *Nelumbo nucifera* powder (1.15±0.18) was lower than that recorded in treatment T2. The decrease in the value of this characteristic could be explained by an increase in digestibility and palatability due to the bioactive compounds in *Nelumbo nucifera* powder.

Our study on the effect of *Nelumbo nucifera* leaf powder on reproductive parameters shows that the gonado-somatic index (GSI) in males was significantly lower in T0 (control feed without *Nelumbo nucifera* powder) and higher in the lots containing *Nelumbo nucifera* powder. The increase in this characteristic in fish fed with the *Nelumbo nucifera* powder diet could be attributed to the presence of phytohormonal compounds in the powder that would stimulate the development of male gonads.

The GSI in females was significantly lower in fish as the level of *Nelumbo nucifera* powder increased, compared to the control group (T0). The decrease in GSI value in females could be explained by the fact that *Nelumbo nucifera* contains anti-estrogenic compounds, which may reduce the size of female gametes. These results align with those of Mutreja et al. (2008), who reported that the oral administration of an ethanolic extract of *Nelumbo nucifera* (800 mg/kg body weight) in female rats resulted in a significant reduction in the weight of reproductive organs (ovaries, uterus, and vagina).

The results of this study show that the hepato-somatic index (HSI) was significantly higher in the experimental lots, both in males and females, compared to the control group. The low HSI in the control group indicates that the fish were in a reproductive period, as a low HSI means that the animal is using liver reserves to produce yolk. These results are similar to those of Khalil et al. (2013), who fed fish a diet containing papaya seed powder, but they contrast with those obtained by Nyadjeu et al. (2019). This difference may be due to the nature of the plant and the dosage used.

Limitations

To contribute to the improvement of tilapia production by formulating diets based on local phytohormones that would inhibit uncontrolled reproduction in *O. niloticus*, and pending more detailed analyses, we recommend the use of *Nelumbo nucifera* at 0.03% in the diet of *Oreochromis niloticus*. However, the lack of a laboratory for more thorough analysis of our samples hindered a deeper interpretation of our results.

Conclusion

At the end of this study, which focused on controlling the reproduction of *Oreochromis niloticus* (Linnaeus, 1758) using *Nelumbo nucifera* powder as a fertility inhibitor, the objectives were first to verify whether *Nelumbo nucifera* powder would have a positive effect on certain growth parameters of juvenile *Oreochromis niloticus*, and secondly, to evaluate its effect on reproductive parameters. The following conclusions can be drawn:

1. **Growth parameters:** Treatment T1, which contained 0.03% *Nelumbo nucifera* powder, recorded the highest values for average weight gain, average daily gain, and specific growth rate. The highest survival rate was recorded in animals of lot 2.
2. **Reproductive parameters:** The diet containing 0.03% *Nelumbo nucifera* significantly induced the highest values for gonado-somatic and hepato-somatic indices.

This study shows that incorporating *Nelumbo nucifera* powder into the diet of juvenile *Oreochromis niloticus* improves growth performance and slows down gonadal activity in juveniles. We recommend that aquaculturists incorporate 0.03% *Nelumbo nucifera* powder into the diet of Nile tilapia juveniles to improve growth and reproduction. Further studies are needed to assess long-term effects and optimize doses. The use of *Nelumbo nucifera* at 0.03% could not only enhance the profitability of aquaculture farms but also contribute to more sustainable production.

What is already known on this topic:

Numerous studies have been undertaken on certain plants to evaluate their anti-fertility potential in domestic mammals. For example, extracts of *Nelumbo nucifera* have been used as an anti-fertility agent in female rats by local tribes in Rajasthan. The results of these studies suggest that *Nelumbo nucifera* has anti-estrogenic properties without altering the general physiology of female rats. Mukherjee et al. (2005) demonstrated that *Nelumbo nucifera* rhizome extract has antipyretic activity. Gupta et al. (2009) showed that *Nelumbo nucifera* seed extract has an anti-steroidogenic effect in the testes and ovaries of rats, while Mazumdar et al. (1992) reported an anti-fertility effect in mice.

Kounde (2017), after evaluating the alcoholic extract of *Hibiscus rosa-sinensis* on survival and sex ratio in *Oreochromis niloticus*, reported that *H. rosa-sinensis* has a positive effect on larval survival and acts as a growth stimulator in *O. niloticus*. Mutlen et al. (2019) showed that extracts of *Nauclea latifolia* and *Tribulus terrestris*, at doses of 200 mg/kg and 2.5 g/kg of feed respectively, led to a significant deviation of the sex ratio in favor of males, with 92 ± 2.0 % for *Nauclea latifolia* and 88.33 ± 1.52 % for *Tribulus terrestris*. These sex ratio shifts also corresponded to the best growth performance. Amira (2021) demonstrated that *Azadirachta indica* causes sexual deviation in *Oreochromis niloticus*. Jegede and Fagbenro (2008) showed that the introduction of *Azadirachta indica* at concentrations of 1.5 and 2 g/kg of feed led to disintegration of testicular and ovarian cells in *Tilapia zillii*.

What this study contributes:

In recent years, tilapia has become the predominant species in African commercial aquaculture (FAO, 2014). Among these tilapia species, *Oreochromis niloticus* is the most well-known and widely used, having been the subject of extensive research and dissemination programs in Africa and around the world. This species has long been considered the jewel of African aquaculture due to its high demand in the market, ease of reproduction, cultivation, and particularly its relatively flexible diet. However, the growth of this industry faces a major problem that is paradoxically linked to the species' high reproduction rate (Mutlen et al., 2019). To address this issue, various techniques have been developed to produce monosex male populations in tilapia (Baroiller et al., 2009; Cnaani and Levavi-Sivan, 2009; Lozano et al., 2013), such as the use of synthetic steroids (e.g., 17-alpha-methyltestosterone, androstenedione, or 1-dihydrotestosterone acetate (Mutlen et al., 2019)), thermal shock, hybridization, and manual sexing. However, the use of steroids in aquaculture is undesirable and is avoided in many countries due to its negative environmental effects (Baroiller et al., 2009), health risks for aquaculture workers and consumers, impacts on tilapia aquaculture labeling, and high costs (Kophytigol et al., 2018). Additionally, other methods remain underutilized (Amira et al., 2021). These techniques, which are not mastered by many fish farmers, lead to sexing errors of 2.7 to 10% (Toguyeni, 1996) and a 50% population loss after two to three months of cultivation (Baroiller and Jalabert, 1989). The use of plants with androgenic properties presents a potential alternative worth exploring. Indeed, plant extracts contain various bioactive compounds such as alkaloids, flavonoids, pigments, phenolics, terpenoids, and steroids, which have been reported to promote various activities like growth stimulation and appetite enhancement in fish production (Mutlen et al., 2019). It is within this context that the study on the control of growth and reproduction in juvenile *Oreochromis niloticus* through the inclusion of *Nelumbo nucifera* powder in their diet was conducted.

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Conflicts of Interest and Financial Conflicts

No conflicts of interest.

Contributions of Authors

The principal author conducted all the work.

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- Development of the protocol
- Implementation of the experimental setup
- Monitoring of the experiment
- Data collection
- Data analysis and processing
- Review and correction

MOUKORIEBOA Félix

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- Data analysis

NGALLE Fen Josué Raymond

- Supervision of the work
- Discussions
- Review

ESSENDJE MBELLA Sariette

- Data analysis
- Data processing
- Review

ASSENG Gabriel Arnaud

- Data analysis
- Data processing
- Review

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