Szent István University

REPRODUCTION BIOLOGY PROPERTIES OF JUNDIÁ (*RHAMDIA QUELEN*), A PROMISING SPECIES FOR AQUACULTURE PRODUCTION

PhD thesis

István Ittzés

GÖDÖLLŐ

2018

The Doctoral School

name: Doctoral School for Animal Husbandry

science: Agriculture

sub-program: Fish Biology and Aquaculture

leader: Dr. Miklós Mézes
Professor, Member of the Hungarian. Academy of Sciences
Department of Animal Nutrition
Institute for Basic Animal Sciences
SZIU, Faculty of Agricult. and Environm. Sciences

supervisor: Dr. Béla Urbányi, D.Sc.
Professor
Department of Aquaculture
Institute of Aquaculture and Environmental Safety
SZIU, Faculty of Agricult. and Environm. Sciences

co-supervisor: Dr. Zoltán Bokor, Ph.D.
Senior scientist
Department of Aquaculture
Institute of Aquaculture and Environmental Safety
SZIU, Faculty of Agricult. and Environm. Sciences

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approval of the leader of the Doctoral School

approval of the Supervisor

.....

approval of the Co-supervisor

1. THE HISTORY OF WORK, OBJECTIVES

1.1. Antecedents of work

The jundiá (*Rhamdia quelen*) is an endemic species to South America from the *Siluriformes* order. The idea for my work was that this species can be reared at a good economic value due to its excellent breeding value. Therefore it is suitable for the diversification of fish-farming in Brazilian subtropical areas. The production volume of Brazilian aquaculture has increased 14.2% yearly since 2004 (primarily due to tilapia (*Oreochromis niloticus*). I have carried out my work in the southernmost state of Brazil, Rio Grande do Sul (RS). RS, similarly to the majority of the country has abundant precipitation, large freshwater and brackish lakes. Brazil is known to have a very rich fish fauna that provides a large supply for the selection of economically feasible species. The rearing of some of the species within Brazilian fauna are accompanied by increased interest due to their fast growth rate and excellent meat quality. The continuous physiological examination of these species are essential for the further economic development.

1.2. Objectives

I have formed my objectives in the following five bullet points:

- Tracking the blood plasma concentration of hormones affecting the reproductive state of females by monthly sampling
- Tracking the blood plasma concentration of hormones affecting the reproductive state of males by monthly sampling
- Examination of blood plasma concentration of cortisol and glucose due to technological stress
- Recording the reproduction biology data necessary for the induced propagation and factory-level rearing of fry:
    - Determination of pseudo-gonadosomatic index (PSGI%)

    - Determination of the time necessary for the ripening of females
    - Determination of the number of egg in 1 unit weight of dry egg
    - The examination of the development stages and speed of the embryo and larvae depending on the water temperature
    - Tracking the PSGI during the spawning and propagation season
    - Examination of the propagation frequency ability of the same individuals within one breeding season
- Recording the changes in the propagation measures of jundiá due to different, practically applied hormonal treatments

2. MATERIALS AND METHODS

I have carried out my experiments in Brazil, in Rio Grande do Sul (RS) state, at the education and research centre of the University of Passo Fundo (PF) between July 1998 and July 1999 and in October 2013, at 687 m altitude (2815' S/5224" W).

2.1. Climatic conditions

Maximum and minimum water and air temperatures were recorded daily. The natural day length was calculated from sunrise to sunset in Passo Fundo.

2.2. Fish used in the experiment

I have used 6 months old males and females. The body weight of the fish varied between 165 g and 1330 g. I have kept the fish in 100 m^2 ponds, with 1 m average water depth. Water flow was 6 l/min, the dissolved oxygen concentration varied between 5.0 and 7.0 mg/l. The water pH varied between 7.0 and 7.2. Examination of the steroid hormone concentrations in cultured male and female jundiá in their first reproductive cycle.

2.3. Examination of the steroid hormone concentrations in cultured male and female jundiá in their first reproductive cycle

2.3.1. Sampling schedule and procedures

Six males and six females were sampled in every month. 1-2 ml blood samples were taken from the caudal vessel of the fully anesthetized fish. The ovaries of the females were removed, and its weight was recorded (±0.001 g). The GSI of the fish were calculated based on the known bodyweight and the weight of the ovaries and testicles.

2.3.2. Radioimmunoassay of steroids

Plasma T, 11-KT, 17,20β-P and 17-P plasma concentration was examined in male individuals. Furthermore, I have examined the *in vitro* production of T and 11-KT. I have also examined the plasma concentration of T, 11-KT, 17,20β-P and 17-P, and E_2 in the case of females.

2.4. The change of cortisol and glucose concentration in the blood plasma due to the emerging stress due to rearing technology interventions in jundiá

I have used one-year old jundiá males and females for sampling, their body weight was 400 ± 50 g. One week before the experiment I have caught 8 males and 8 females form the fish pond for the measurement of the basic cortisol level (as a control group). I have tested the males and females immediately after capture and 4, 12, and 24 hours later as well. I have used unextracted plasma samples for the determination of the amount of cortisol. I have used [125 I] DPC- cortisol RIA test (Coat-H-Count®, DPC Los Angeles, CA) for the experiment. The obtained results were validated with the standard curve of the kit.

2.5. Recording the reproduction biology data for the hormone treatment induced propagation and plant-level nursing of fry in jundiá

2.5.1. Pseudo-gonadosomatic index (PGSI%)

I have measured the gamete production of 109 2-4 years old females for the determination of PGSI. I have sorted the females into groups, using a scale of 100 g difference in bodyweight, and I have calculated the average of PSGI% in each group. I have calculated the average bodyweight (g) and the average volume of the gametes (ml).

2.5.2. Determination of the ovulation time of jundiá

I have determined the ovulation time of jundiá in hour-degree, which means the time passed between the hormone treatment and the first appearance of the eggs. 109 females were tested in this experiment. One part of the females received the carp pituitary injection dose in one treatment, the other part divided into two treatments. I have carried out my experiment on 19-26 °C temperature range. I have calculated the ovulation time in hour-degree based on the results and compared the difference in the ovulation time between the groups treated in one treatment and in two treatments.

2.5.3. Number of eggs in 1 kg dry eggs

I have weighed 1 g of dry eggs on analytical scale after stripping. I have counted the gamete product of 10 fish and I have taken 3 samples from each fish. The bodyweight of the females varied between 300 g and 800 g. I have fertilised the egg samples, and after 10 minutes of hydration I have counted them in Petri-dishes and projected their mean to 1 kg egg weight.

2.5.4. Embryo and larvae development

I have measured the egg phase of embryo development in this experiment. The water temperature varied between 17 and 26 °C during my measurements. I have examined the embryo development at 23-24 °C. I have put the jundiá larvae in 30 cm deep polypropylene tanks with 20000 pcs / 100 l density after hatching.

2.5.5. Comparison of the pseudo-gonadosomatic index (PGSI%) between the different months of the spawning season

In these experiments, I have calculated the mean of the PGSI% values of the propagations monthly between September 1996 and February 1997.

2.5.6. Possible maximum propagation frequency of jundiá

In this experiment I have investigated how many times can one female individual be hormonally induced to ovulate within one spawning season. I have carried out 4 propagations with 6-weeek long breaks in the spring and summer months and one in April, the second month of autumn. I have formed three experimental groups. Each group contained 10 female individuals. I have used three calculated values for the evaluation of propagation success: ovulation ratio (number of ovulated females/numbers injected), fertilised egg ratio and hatched larvae ratio (hatched larvae/number of fertilised eggs) in percent.

2.6. Propagation measures of jundiá at the different hormone treatments

I have used two different hormones (sGnRHa and carp pituitary) and two DA antagonists (domperidone and metoclopramide). I have formed five different experimental groups with the following combinations: the control group received only Saline vehicle, one test group was treated only with sGnRHa, 2 groups were treated with the 2 DA antagonists combined with the same sGnRH analogue for DA inhibition testing.

The first test group was treated with dried carp pituitary glands, 4.0 mg per kg^{-1} body weight. The hormone was dissolved in 0.7% Saline vehicle (NaCl) and introduced to the test individuals. The next test group was injected with Ovaprim. Ovaprim contains 20 μg sGnRHa and 10 mg domperidone per 1 ml in liquid form. This test group individuals received 0.5 ml Ovaprim solution per kg^{-1}. The following two test groups received 10 μg per kg^{-1} sGnRHa treatment. From these two groups, one has received an additional 20 mg kg-1 dopamine receptor antagonist metoclopramide with the sGnRHa, the other did not receive the antagonist. The fifth group was the control group, the females of this group received only Saline vehicle which was the vehicle for the different hormone preparations in the test groups.

The riping water temperature was 26.0±1.0 °C from injection till ovulation. I have used two calculated values for the evaluation of the success of ovulation: ovulation ratio (number of ovulated females per number of females injected), the other was PSGI. Fertilisation rates were determined 24 hours after fertilisation.

3. RESULTS

3.1. Steroid hormone concentrations in cultured male and female jundiá in their first reproductive cycle

3.1.1. Weather conditions

The water temperature is the coldest in winter time (July, 8 °C), and the warmest in summer time (January, 31°C). The longest day was measured on 13th December (13 hours and 45 minutes), the shortest in July (10 hours and 14 minutes). I have continually tracked spontaneous spawning during the one-year experimental period (July 1998-July 1999) in a pond where I kept males and females in an equal ratio. These fish was in the same age group as the sample fish. I have observed two spawning seasons, the first in November (one day before the planned sampling), the other in January (7 days after the due sampling).

3.1.2. Gonadosomatic index (GSI)

The GSI values were low until the beginning of vitelogenesis (July 1998). I have observed a fast growth in early spring (September) and GSI values reached their maximum in mid-spring (October), with a value of 12.28±0.76%. The weight of the ovaries reduced, but it was followed by growth again. This growth stopped in summer (December) at a maximum value of 9.1±1.22 % and showed high values for 2 months (December and January, 7.48±0.61%). The GSI% decrease in February and March was followed by another, third peak in April (8.63±2.12%), but there was no spontaneous spawning this time. After this peak, the weight of the ovary drastically decreased, and the GSI% dropped to 2.13± 0.55% in May.

3.1.3. Changes in the plasma sex steroid concentrations in females

E_2 concentration of the plasma was low (0.15±0.056 ng/ml) in July 1998, at the beginning of the experiment, but it increased intensively until November. After the peak in November (9.1±1.21 ng/ml) the E_2 started to decrease from December, and stayed low from January till July 1999. The concentration of T remained below 15 ng/l between July and September 1998, but drastically increased in October to 53.5±0.8 ng/ml (spring). The concentration of 17-P increased between July and September. We could observe two peaks. The first was in October at a value of 0.97±0.13 ng/ml, the second in January at the value of 0.94±0.22 ng/ml.

The 17,20β-P concentration of the plasma started to increase in August, the last month of winter from a very low value (0.2 ng/ml) I have measured already 0.7±0.2 ng/ml concentration in September, and the second peak was reached in the next month, October (1.29±0.18 ng/ml) which overlapped with the first observed spontaneous spawning. There was only a slight increase observed in the 17,20β-P concentration of the plasma in January 1999, at the time of the second spontaneous spawning season. The 20β-S showed a similar curve to 17,20β-P, but its concentration was higher during both the first (October 1998) and the second (January 1999) spontaneous spawning seasons. The 11-KT concentration of the plasma did not reach a value detectable with the applied method in August (1998), but after a drastic increase it reached its first peak (91.2±5.61 ng/ml). The second peak was observed in January 1999. The value of 11-KT in the plasma was 94.37±22.9 ng/ml. The concentration of this steroid dropped to a hardly measurable level in February and stayed at this level for the rest of the reproduction cycle.

3.2. Plasma steroid concentration of males during the reproduction cycle

3.2.1. Gonadosomatic index (GSI%)

GSI% was low in winter time (July and August). The size of the gonads started to increase in early spring (September), and reached its first peak in mid-spring (October, 8.03±0.64%), the second peak 3 months later, in summer (January, 8.25±0.82%). GSI% decreased in February and March, and reached its minimum in May (0.81±0.34 %). Gonads slowly grew in June and July, 1.18±0.34 % and 1.6±0.25 % respectively.

3.2.2. Changes of sex steroid levels in males

The T concentration of the plasma started to increase in the last winter month (August) and has reached its peak by late spring (November, 55.7±7.0 ng/ml, $P<0.01$). Later the concentration of T continually decreased till Autumn. In the second cycle the level of T concentration of plasma reached a higher value by September, but the peak was observed in October (58.8±5.7 ng/ml, Spring). The concentration of this hormone started to decrease in November but showed a slight increase in April 2000 (Autumn). The 11-KT concentration of the plasma was low in July, August and September 1998, but they started to increase in October (second month of Spring) and reached a particularly high value in December (1998, 1243.42±337.32 ng/ml). The production of 11-KT varied around 100-250 ng/ml in the second reproduction cycle. The highest production of T in the testis was reached in November. However, the plasma concentration of this hormone was also the highest at the same time. T concentration was still high in December but it steadily decrease in the following months. The peak of tissue 11-KT in the incubation medium was measured in December (0.48±0.19 ng/ml), that, similarly to T, overlapped with the peak of the concentration of 11-KT in the plasma. The values of 11-KT have

remained low both before and after the peak both in the plasma and the tissue. The concentration of 17-P remained high during the whole spermatogenesis, but I could not observe a clear peak. After spermiation, the concentration of 17-P dropped to 0.2 ng/ml. The concentration of 17,20β-P did not reach a value detectable with the used method in winter (August 1998, <0.2 ng/ml). The concentration of this hormone was 0.31±0.07 ng/ml in September (Spring), the peak was observed in October (1998, 0.81±0.14 ng/ml) in the first reproductive cycle. The levels of this hormone were low in the second cycle unlike the first cycle, however, the highest value was observed also in October.

3.3. Examination of blood plasma concentration of cortisol and glucose in jundiá due to technological stress

The cortisol concentration level of both males and females was the highest at the one-hour post treatment examination, then the level of cortisol started to decrease but still remained higher than the base sample after 24 hours. The base value of cortisol in the plasma was 15.8±3.12 ng/ml for males and 29.6±5.45 ng/ml for females. The plasma glucose concentration showed a curve similar to the curve of the sample of the one-hour post treatment for both the males and the females, just like in the case of cortisol. The base bloodsugar value was 61.3±5.8 mg/dl for males and 68.7±9.3 mg/dl for females. The glycemia of the males decreased drastically 4 hours post-treatment, and almost reached the original, pre-treatment plasma glucose concentration. Females preserved a higher plasma glucose concentration for a longer time. The blood cortisol level of males were always lower than that of the females at all sampling times. Glucose levels had similar pattern for the two sexes.

3.4. Recording the reproduction biology parameters for the induced propagation and rearing of fries for jundiá

3.4.1. Pseudo-gonadosomatic index (PGSI%)

The average body weight of the measured females were 654.87 g (max. 1330 g, min. 165 g). The average volume of gamete production was 76.12 g (max. 205 g, min 10 g). The average PGSI% is 11.552% (max. 20.095%, min 4.872%). The average gamete production volume of the males were 21.512 ml (max. 42 ml, min. 10 ml) in the case of the 41 males, and their average body weight was 372.097 g (max. 655 g, min 148 g). The males can be stripped again 1 hour after the first stripping and they provide approximately half of the volume mentioned above.

3.4.2. Determination of the ovulation time of jundiá

Ovulation time of the pre-treated females at 19-26 °C was between 285 and 198 hour-degree, for the females treated only once, it was between 330 and 192 hour degrees.

3.4.3. Number of eggs in 1 kg dry eggs

Dry egg volume in 1 g portions was 810 466 pcs on average (max. 937 000, min. 758 000), therefore the number of eggs in 1 kg dry eggs were 810 000 on average.

3.4.4. Embryo and larvae development

The embryo development happened in 1-4 days depending on the temperature, in the 17-26 °C temperature range. The water temperature was mostly 24 °C in the propagation season. At this temperature, 30 hours, therefore 720 hour-degree is necessary for the hatching of the larvae. First feeding of the larvae happened approximately 2 days post hatching at 23-24 °C temperature.

3.4.5. Ripening of the gamete products of the females

Similar propagation indicator values were measured in all three test groups after the 6 weeks regeneration period than in the first cycle. The ripening ratio of the different groups did not vary significantly (Chi^2-test, $p < 0.05$). Fertilisation ratio and hatching ratio was also similar.

3.5. Ovulation indicators of jundiá for different hormone treatments

I have compared the effect of dried carp pituitary glands and different sGnRHa treatments on the ovulation of jundiá. The saline vehicle treatment and the other sGnRHa treated group did not ovulate. 7 out of 8 induced jundiá ovulated (87.5% ovulation ratio) in the groups treated with carp pituitary and Ovaprim treated groups. The effectiveness of sGnRHa + MET induction was 37.5%.

3.6. New scientific results

I form the following new scientific results and statements based on the experiments carried out in my research work presented in my doctoral thesis:

- I have developed a practically applicable induced propagation and larvae rearing technology that can be spread in fish farming in Rio Grande do Sul state of south-Brazil.
- I have characterised the sex ripening of the jundiá population (through the measurement of sex hormones and gonads), its pattern and the possible propagation frequency in subtropical environment, exposed to natural temperatures and photoperiods.
- I have determined the stress reaction of the different technology steps of fish farming (fishing, landing nets, short distance transport within the facility) for both sexes through measuring the cortisol levels.
- I have examined the ovulation measures of jundiá due to traditional hormone treatments, generally applied in fish farming, and I have determined the effective methods. Ovaprim treatment proved to be the most effective.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Change in the steroid hormone concentration in the first reproduction cycle of farmed jundiá

I introduce that at least four artificial propagations can be carried out within one reproduction cycle with carp pituitary treatment, providing good quality eggs. I have observed that the mixed sex fish stock that is kept in an earthen pond has two spawning periods during their spawning season. Spawning always happens in groups. Usually two months pass between two spawnings. This species can belong to the group of species spawning more than one in their spawning season based on their maximum GSI value too. At the beginning of the spawning the GSI value of female jundiá is 12.3.±0.8. A quite rapid growth of GSI can be observed in females before the spawning season. I have observed that the plasma E_2 concentration has significantly increased in the months before the spawning season, reaching 2.6±0.6 ng/ml, which was ten times more than in July or August. It is interesting that the E2 level reached its peak concentration in the plasma (9.1±1.2 ng/ml) in November, on the day after the first spontaneous spawning. On the other hand, E_2 is responsible for starting the vitellogenesis after ovulation, in which a new oocyte stock reaches a status suitable for ovulation for the second spawning in January. The positive correlation (data not shown) between T and GSI suggests that T is a strong indicator of the stage of oocyte development. The role of these hormones decreases during the development of oocytes, and then the production of progesterone was significant during ripening. The maximum value of 17-P was parallel with the two spontaneous spawnings. Comparing the 17,20β-P concentration with the spawnings, we must think that this hormone is responsible for the final ripening of the oocytes. The low plasma 17,20β-P concentration in jundiá (1.5 ng/ml) is typical for multiple spawning species in contrast with salmonids (500 ng/ml). 20β-S can also be detected in mature

jundiá blood plasma. This is supported by the varying level of 20β-S observed in my experiment. The plasma 11-KT value is very high in females during spawning episodes, it reaches 95 ng/ml. The fact that 11-KT is synthetized only during spawning strongly suggests that this hormone affects the oocyte maturation in jundiá. As a final conclusion, E_2, T, 17-P, 17,20β-P, 20β-S participate in the control of the different stages of the reproduction cycle in females, similarly to the previously tested bony fish. The role of extremely high 11-KT synthesis in immature jundiá females is yet to be determined.

4.2. The change in steroid hormone concentrations in cultured male jundiá in their first reproductive cycle

The weight of the testis makes up a relatively large percentage of the bodyweight before propagation in males. It reaches 8.25±0.82% of the total bodyweight in mature males. The size of the genitals varies significantly during the reproductive cycle, similarly to other seasonal spawning bony fish. The genitals develop rapidly in the Spring months and preserve their developed status during almost the whole summer. The blood plasma T concentration increased in parallel with the increase of GSI, and reached its peak at the beginning of spermiation, in the second half of spring. Afterwards its synthesis rapidly decreased in spite of the production of sperm still increasing. My experiment results show that the in vitro T concentration (produced by the testis) peak coincides with the plasma T concentration peak, and afterwards it starts to decrease. The peak of T production could be measured one month before the peak of 11-KT. The extremely high 11-KT concentration (1.0 µg/ml) in the plasma of jundiá suggests that the testicles of this species are capable of very high 11-KT biosynthesis. The level of 11-KT was significantly higher in the first reproduction cycle (so in the first mature year) than in the second. This supports the supposition that this hormone plays a significant role in maturation. Therefore T, 11-KT, 7-P and 17,20β-P participate similarly in controlling the

different stages of the reproduction cycle than it has already been described in other bony fish species.

4.3. The change of cortisol and glucose concentration in the blood plasma due to the emerging stress due to rearing technology interventions in jundiá

In my experiment the base value of the cortisol was 15.8±3.12 ng/ml n males, and 29.6±5.45 ng/ml in females. One-hour post capture and transport I have measured the maximum level of cortisol, 158.12 ng/ml for the males and 207.95 ng/ml for the females.

My results suggest that 24 hours are insufficient in case of stressed jundiá for the cortisol level to decrease to the initial level both in males and females.

One possible explanation for the higher cortisol level and glucose concentration 12 hours post capture for females can be that they were in the state of exogene vitellogenesis. The energy needs are very high in this state of vitellogenesis.

We can state that the physiologic reaction of jundiá for the stress due to capture and transport is similar to the other bony fish species.

4.4. Recording the reproduction biology data for the hormone treatment induced propagation and plant-level nursing of fry in jundiá

I have applied the propagation and larvae rearing protocol that I have described in my thesis in the 15 years of industry practice. The presented reproduction biology datas can be practically applied for the production of large amounts of larvae as they facilitate the planning of year-long production, the timing of propagations, and the determination of a suitable female stock. The value of PSGI and the number of eggs in 1 kg dry egg are very good indicators in terms of the planned propagations and the preparation of the ponds.

As I did not carry out the experiment on wild-catched fish, I have no data on how keeping the fish in artificial ponds influences this value. In this present experiment the number of eggs in 1 kg dry egg was 810 000 pcs/kg "dry" egg.

Sperm can be stripped easily and in large volume. This facilitates the work with males during the production. The value of GSI is high in the spring and summer months and preserves its value till the last month of autumn (4-5%). Within this period, it has two peaks with 8% value. Probably this causes the ample amount of gamete product in this species. 21 ml gamete product can be stripped from 400 g males, and it can be repeated one hour post-stripping. This unusually large amount of gamete product in catfish species is due to the storage capacity of the multi lobular testis specific to the *Rhamdia* genus.

The time necessary between the hormonal treatment until ovulation is also a very important parameter in successful artificial propagation. The ovulation time of pre-treated females kept between 19 and 26 °C was between 285 and 198 hour-degree, and between 330 and 192 hour-degree for females treated only once. It is visible that jundiá can be propagated at a wide temperature range, I have received similar results for both groups at 26°C water temperature.

The development of the embryo and the larvae depend on the temperature. I have quantified the development of the embryo with the determination of the hatching time, and the development of the larvae with the determination of the time of first feeding. The water temperature is mostly around 24 °C in the spawning season. 720 hour-degree, 30 hours are necessary for the hatching of the larvae. The larvae start feeding after 2 days at 23-24 °C water temperature. Successful propagations were carried out at 17 °C as the coldest (in August) and 27 °C as the warmest (February) water temperature. Propagation can be carried out in February yet and the embryo development is normal, however the propagation parameters decrease significantly (ripening of females, fertilisation).

The eggs (PGSI) stripped from the females is around 10% monthly (min. 8.43%, max. 12.13%). Therefore we can conclude that the safe and predictable propagation period for jundiá is 5 months (from September until February), that provides an opportunity for the fish famers for production and safety for the accidental loss of production. This conclusion is also supported by my experiment on the possible propagation frequency of jundiá as well. The results of this experiment show that the same individual can be propagated at least four times in one spawning season that I have determined.

4.5 Ovulation indicators of jundiá for different hormone treatments

The high ripening ratio suggests that Ovaprim treatment can be suitable for the artificial propagation of jundiá. If we compare carp pituitary treatment, Ovaprim treatment and sGnRH + metoclopramide treatments, a lower ripening ratio can be observed. The sGnRH treatment on its own was unsuccessful, which can be caused by the low level of gonadotropine which is the result of strong dopaminerg inhibition.

We can state as a result of our experiment that DA inhibits the gonadotropine production of GnRHa in jundiá. GnRHa was unable to initiate ovulation when being the only treatment. Ovaprim contains sGnRH and DA receptor antagonist, domperidone, and it induced ovulation in the test animals with a good success rate. Lower ratio of ovulation was reached by the sGnRHa + metoclopramide than with Ovaprim treatment. The amount of sGnRHa treatment was the same in both groups (10 $\mu g/kg^{-1}$). The accompanying concentration of domperidone and metoclopramide was 5 and 20 mg/kg^{-1} respectively. Therefore we can conclude that domperidone (the active ingredient of Ovaprim) strengthens the effect of sGnRHa strongly in the induction of ovulation. The group treated with carp pituitary ovulated 9 hours after treatment. The groups treated with Ovaprim and with GnRHa+metoclopramide ovulated 13-16 and 16-18 hours post-treatment. The longer ovulation time in the case of

Ovaprim treatment and GnRHa+metoclopramide treatment is the result of two consecutive processes (production of gonadotropine from the pituitary of the treated fish, and the reaction of the ovaries for the stimulation of gonadotropine). This reaction is a natural characteristic of the GnRH treatment. The other group, treated with carp pituitary has a shorter ovulation time due to the 1-step ovulation (the ovaries react to the external gonadotropine). A significant difference is that the females treated with pituitary has synchronised ovulation, the females from the groups treated with either Ovaprim or GnRHa+metoclopramide treatment did not ovulate at the same time. Most of the PSGI values were high and similar in the groups treated with carp pituitary, Ovaprim and GnRHa+metoclopramide treatment. The fertilisation ratio was high and showed similar results in the groups treated with carp pituitary, Ovaprim and GnRHa+metoclopramide treatment.

In summary, this experiment shows that both the application of carp pituitary and GnRHa (in the form of Ovaprim) is an efficient method for ovulation induction, noting that this causes not synchronised and protracted ovulation in the treated female stock.

4.6. Recommendations

- I recommend examining the possible propagation frequency with shorter timeframes and determining the minimum preparation time necessary between the propagation period.
- I recommend the clarification of the role of cortisol in the propagation of jundiá.
- 17-P showed the strongest connection with the final ripening and spawning from the three progestogens. The role of the other two progestogens in the reproduction could be clarified with more frequent sampling.
- The presence of 11-KT in females during the spawning season proves that it has a role in the reproduction of jundiá. Its exact function is yet to be determined.
- I recommend the separate examination of the effect of photoperiod and the temperature on the reproduction cycle.
- I recommend the examination of the possibilities of feminisation and masculinisation of jundiá.
- I recommend the determination of the materials of the died and the hatching residue adhesive eggshell and the possible development of its enzymatic removal.

5. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

5.1. Publications in scientific journals

ITTZÉS I., SZABÓ T., KRONBAUER E. C., URBÁNYI B. 2015.: Ovulation induction in jundia (*Rhamdia quelen, Heptapteridae*) using carp pituitary extract or salmon GnRH analogue combined with dopamine receptor antagonists. *Aquaculture Research*, 46, 2924-2928.

BARCELLOS, L.J.G; WOEHL, V.M.; WASSERMANN, G.F.; QUEVEDO, R.M.; **ITTZÉS, I**; KRIEGER, M.H. 2001: Plasma levels of cortisol and glucose in the response to capture and tank transference in *Rhamdia quelen* (Quoy & Gaimard, Pisces, Teleostei), a South American catfish. *Aquaculture Research***,** 32, 121-123.

BARCELLOS, L.J.G.; WASSERMANN GF.; SCOTT,A.P.; WOEHL, V.M.; QUEVED, R.M.; **ITTZÉS, I**.; KRIEGER, M.H.; LULHIER, F.L. 2001: Steroid profiles in cultured female jundiá, the *Siluridae Rhamdia quelen* (Quoy and Gaimard, Pisces Teleostei), during the first reproductive cycle. *General and Comparative Endocrinology* 121, 325-332.

BARCELLOS, L.J.G; WASSERMANN, G.F.; SCOTT, A.P.; WOEHL, V.M.; QUEVEDO, R.M.; **ITTZÉS, I**; KRIEGER, M.H.; LULHIER, F. 2002: Plasma steroid concentrations in relation to the reproductive cycle of cultured male *Rhamdia quelen*. *Journal of Fish Biology*,61 (3), 751-763.

5.2. Publications published in foreign language journals

ITTZÉS, I.; MEZZALIRA, R.; FIORESE, I. 1997: Jundià: uma espècie nativa com tecnologia dominada. *Revista Panorama da Aquicultura*, Rio de Janeiro, 20-23.

5.3. Foreign language book's chapter

FIOREZE, I.; I. **ITTZÉS, I**.: 2012: Instalações para policultivo. In: LEONARDO JOSÉ GIL BARCELLOS, MICHELE FAGUNDES. *Policultivo de jundiás, tilápias e carpas.* Uma alternativa de produção para a piscicultura rio-grandense Capitulo 3, UPF Editora, 69-94 p.

5.4. Conference proceedings

BARCELLOS, L.J.G.; WASSERMANN, G.F.; PORAWSKI, M.; WOHEL, V.M.; **ITTZÉS, I**; QUEVEDO, R.M.; KRIEGER, M.H. 1999: Determinação do ciclo gonadal e dos esteróides sexuais durante o ciclo reprodutivo do macho do jundiá, *Rhamdia quelen*. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

BARCELLOS, L.J.G.; WASSERMANN, G.F.; WOHEL, V.M.; **ITTZÉS, I**; QUEVEDO, R.M.; KRIEGER, M.H. 1999: Determinação do ciclo gonadal e dos esteróides sexuais durante o ciclo reprodutivo da fęmea do jundiá, *Rhamdia quelen*. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

ITTZÉS, I.; QUEVEDO, R.M.; BARCELLOS, L.J.G.; WOHEL, V.M. 1999: Incubação de ovos de jundiá (*Rhamdia quelen*) em tręs faixas de temperatura. Tempo para a eclosão, viabilidade e tempo para a primeira alimentação das pós-larvas. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

ITTZÉS, I.; QUEVEDO, R.M.; BARCELLOS, L.J.G.; WOHEL, V.M. 1999: Uso de hipófise de carpa húngara (*Cyprinus carpio*) na indução á reprodução de fęmeas e machos de jundiá (*Rhamdia quelen*). Efeito de diferentes faixas de peso. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

QUEVEDO, R.M.; **ITTZÉS, I**.; BARCELLOS, L.J.G.; WOHEL, V.M. 1999: Sobrevivęncia e crescimento de alevinos de jundiá (*Rhamdia quelen*) em alevinagem 1 em tanques escavados, mantido em tręs diferentes densidades. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

QUEVEDO, R.M.; **ITTZÉS, I**; WOHEL, V.M.; BARCELLOS, L.J.G.; WASSERMANN, G.F.; PORAWSKI, M.; KRIEGER, M.H. 1999: Estudo morfológico da gônada masculina de jundiá (*Rhamdia quelen*) durante o ciclo reprodutivo. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

QUEVEDO,R.M.; **ITTZÉS,I**.; BARCELLOS,L.G.; SOUZA,S.M.G. 2003: Effects of artificial saline soluctions on the motility time of jundiá (*Rhamdia quelen*) semen. In IX World Conference On Animal Production, Porto Alegre, vol. 1. pp. 184-184.

WOHEL, V.M.; BARCELLOS, L.J.G.; WASSERMANN, G.F.; **ITTZÉS, I**.; QUEVEDO, R.M.; KRIEGER, M.H. 1999: Estudo morfológico da adeno-hipófise de fęmea de jundiá (*Rhamdia quelen*) no estádio de maturação avançada do ciclo reprodutivo. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom

WOHEL, V.M.; BARCELLOS, L.J.G.; WASSERMANN, G.F.; **ITTZÉS, I**.; QUEVEDO, R.M.; KRIEGER, M.H. 1999: Estudo morfológico da gônada de fęmea de jundiá (*Rhamdia quelen*) durante o ciclo reprodutivo. 51ª Reunião Anual da SBPC, 11 a 16 de julho de 1999. Porto Alegre, RS. CD Rom