

*Ex situ Crocodylus intermedius* (Caimán Llanero) at Roberto Franco Tropical Biology Station (EBTRF), Colombia

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Federico Medem (1912-1984) dedicated special attention to the study of the Crocodylia species diversity in Colombia and in south America (Medem, 1981, 1983) and he established the first *ex situ* individuals nucleus *Crocodylus intermedius* at the "Roberto Franco" Tropical Biology Station (EBTRF) in 1970. Those animals reproduced for the first time in 1991 (Ramírez-Perilla J. 1991a; Lugo M, 1995).

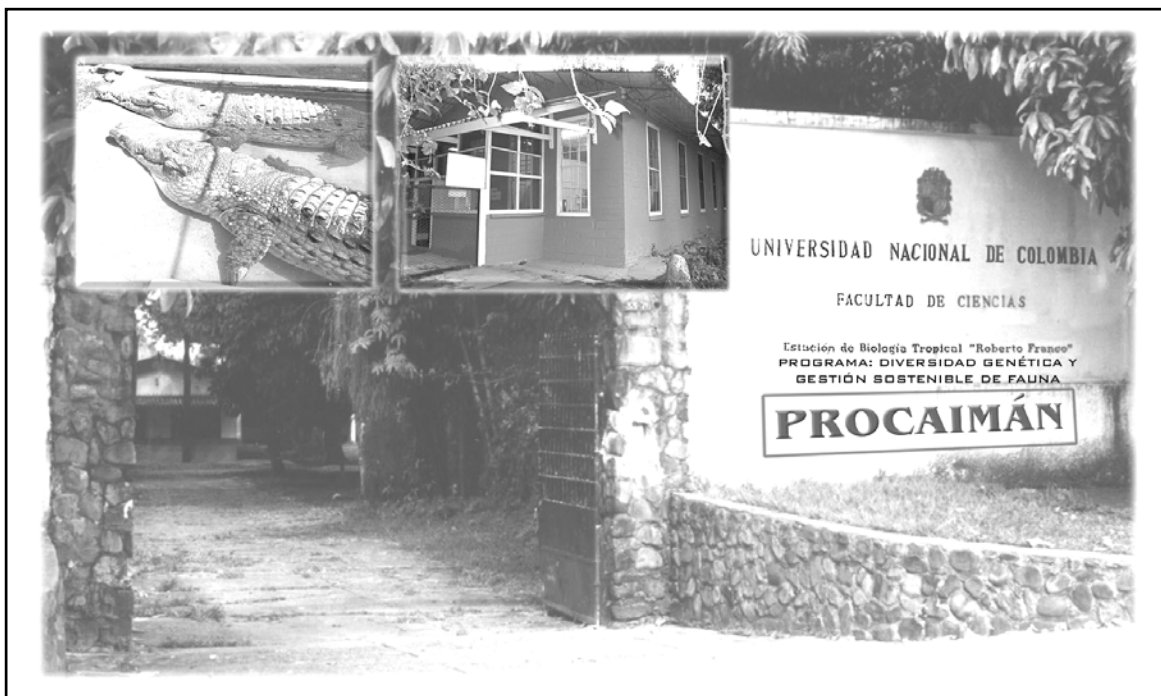


Fig. 1. "Roberto Franco" Tropical Biology Station, EBTRF, (Sciences Faculty, National University of Colombia), Villavicencio, Colombia; 4°09' N, 73°39' W; 430 m.s.n.m., 25.8°C; annual precipitation of 3967 mm; 1820.5 hours light day per year.

The first censuses of *C. intermedius* in wild life (Medem, WWF, 1974, 1976) noticed the presence of 280 individuals in the Colombian orinoquense region and the most important cause in the reduction of population was due to the excess of the commercial hunt happened since 1920 at 1948 years (Medem 1981, op. cit., p. 177).

Next censuses occurred in 1993 to 1996 years and were sponsored by the Wildlife Conservation Society (WCS), COLCIENCIAS and EBTRF (National University of Colombia), establishing the presence of only 34 individuals in the whole region (Barahona & Bonilla 1996, 1999; Lugo, 1998a; Ardila-Robayo *et al.* 1998; Rodríguez, M., 2000; Barahona *et al.*, 2001, not yet published).

*C. intermedius* was declared in Appendix I of CITES in 1975; in critical danger for the UICN in 1984 (Thorbjarnarson and Arteaga, 1995) and in extinction danger in Colombia by Resolution number 0676 of July 21 (1977) of the Environment Ministry. That is the reason why a National Program for the Conservation of the Caimán llanero, *Crocodylus intermedius*, (PROCAIMAN) was formulated by the Environment Ministry, Von Humboldt Institute and National University of Colombia, in 1998 and given to know to the CSG/IUCN organization in Venezuela and Colombia countries.

Ex situ studies about breeding, handling and growth of *C. Intermedius* in captivity were made when the animals reproduced for the first time at the EBTRF, in 1991 (Ramírez-Perilla J. 1991b; Cárdenas D, 1994; Lugo M, 1995; Ardila-Robayo *et al*, 1999a, 1999b, 1999c).

Another study intended to capture the public conscience about the Conservation Program PROCAIMAN (Ramírez-Perilla, 2000) sponsored by Spanish Cooperation.

At the same way, it made up a molecular genetic characterization using Amplified Fragments Polymorphism (AFLP's) (Bejarano C, 2001) and microsateletal DNA (Burbano C, 2001, com. pers.) financed by Holland Government.

No births occurred since 1997 up today.

One parental male was sick and died in 1998. Other couple laid eggs presenting a high percentage of shell rupture with membrane exhibition. Most eggs were already damaged when collecting them or during *ex situ* hatching process.

At the EBTRF there are 113 crocodiles (included confiscated animals) to distribute them to public or private institutions with the purpose of giving total opening to the PROCAIMAN program, and the same time, to impel its Action Plan and to reactive the crocodile reproduction.

#### **THE ORIGIN OF THE *C. intermedius* COLONY AT THE EBTRF**

At the EBTRF had occurred 199 hatchlings of *C. intermedius* and died, for different reasons 119 animals, so the mortality rate was about 59.8%.

Actually, there are 80 alive crocodiles, 65 are female and 15 are male. Beside that at the station exist 33 individuals (10 females and 23 males) from different origin places; finally there are 113 animals (38 males and 75 females), *ex – situ*, in the whole PROCAIMAN program.

Seventy three crocodiles have been marked with electronic chipping; 53 individuals was born in the station and the other 20 in different places. The rest of *C intermedius* has been marked with a code settled down by the cut of double and simple crests of the tail (tail notching).

Actually, the EBTRF has distributed in 9 ponds the 81 individuals that handled. The parental couples are in ponds numbers 15, and 44-45 (Fig. 2).

Each pond, for parental couples, has a total area of 94 m<sup>2</sup> (0.021 ind/m<sup>2</sup>) and 83.1 m<sup>2</sup> (0.024 ind/m<sup>2</sup>) with a density of 0.04 ind/m<sup>2</sup> and 0.05 ind/m<sup>2</sup>, respectively, calculated with base in water mirror.

CHART 1. ORIGIN AND CURRENT LOCATION OF *C. intermedius* HANDLED BY EBTRF, 2001.

CHART 1 A. Number of <i>C. intermedius</i> for individual origin.				CHART 1 B. Current location of <i>ex-situ</i> individuals			
Origin	M*	F*	Total	Location	M*	F*	Total
Diverse origins (See charts 3 and 4)	23	10*	33	EBTRF <sup>(1)</sup>	23	58	81
				YAMATO <sup>(2)</sup>	13	14	27
Alive individuals born in EBTRF (see chart 5)	15	65	80	Maní <sup>(3)</sup>	1	1	2
				Granja Picón <sup>(4)</sup>	1	2	3
<b>Total</b>	<b>38</b>	<b>75</b>	<b>113</b>	<b>Total</b>	<b>38</b>	<b>75</b>	<b>113</b>

\*M= male, \* F = female; \*\* two females were probably born in the EBTRF and send them to Yopal city; <sup>(1)</sup> Villavicencio, Meta; <sup>(2)</sup> Sn. Miguel, Puerto Gaitán, Meta; <sup>(3)</sup> Maní, Casanare; <sup>(4)</sup> Granja El Picón, Yopal, Casanare.

The other individuals are distributed in 7 cement ponds. In the pond number 14 there are 22 crocodiles about 7, 8, 9 and 10 years old and 5 of unknown ages.

The density is about 0.37 individuals/m<sup>2</sup> of water mirror (0.18 ind/m<sup>2</sup> total area). The sizes of these individuals fluctuate between 217.8 and 300.8 cm. The maximum density is about 1.7 ind/m<sup>2</sup>, corresponding to the pond number 30, in which there are 14 animals of different sizes and growing stages.

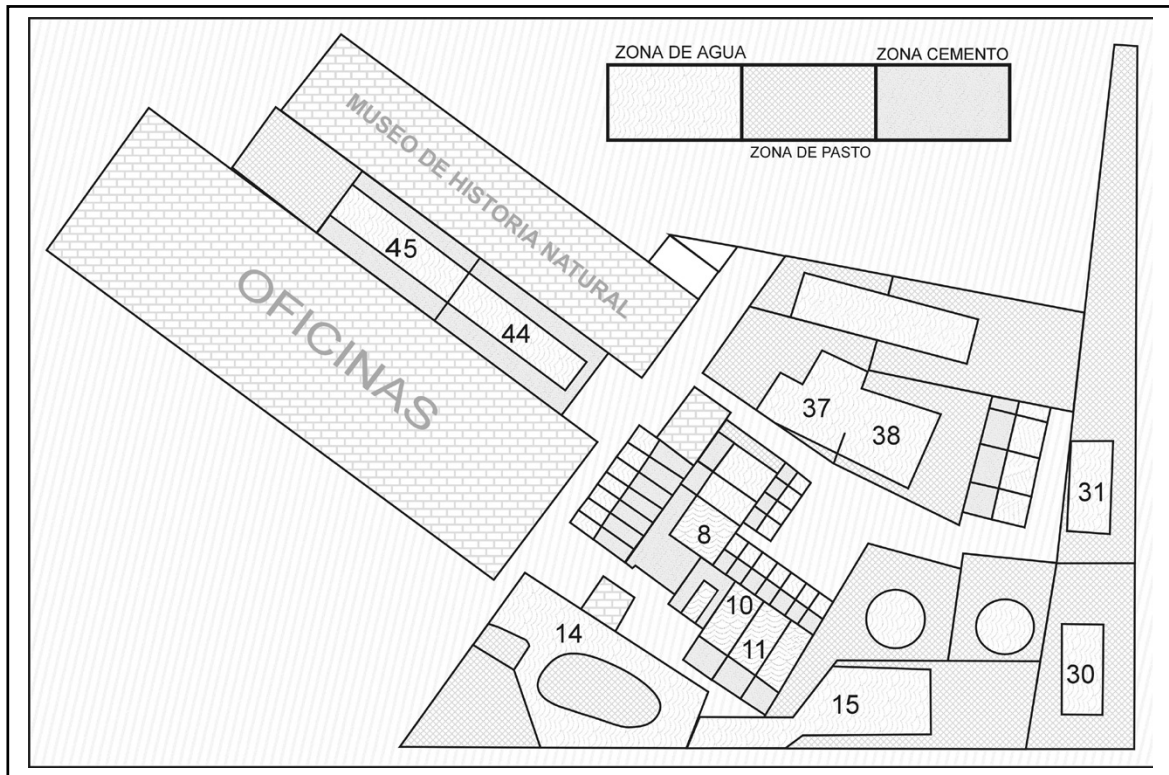


Fig 2. EBTRF Ponds.

According to the class intervals for sizes suggested and applied by Rodríguez M (2000) in censuses of *Crocodyles* in Colombia, the size structures of 79 individuals born at the EBTRF is this way (Fig 3A): 53 females (81.3%) have mature size (Class IV and V) and 12 females (18.7%) are near to the mature stage (class II); 14 males are mature (class V). Out of 26 crocodiles came from different places (Fig 3B) all the females (6, 100%) have mature size; only 3 males (15%) have adults' size (class V), 10 animals (50%) are near to the mature size (class IV) and 7 crocodiles (35%) have juveniles size (Class III). All crocodiles at the EBTRF are probably of similar ages but not well determined.

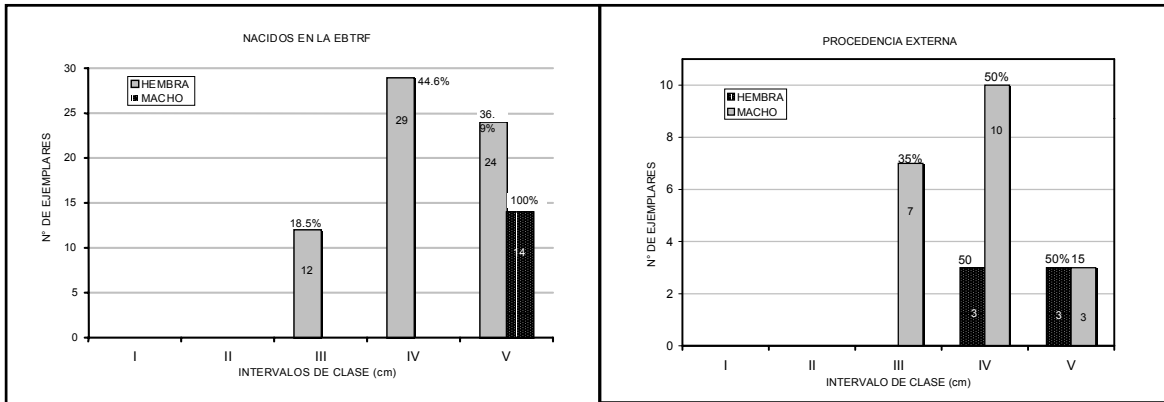


Fig 3. Population's structure of *C. intermedius* born in the EBTRF (Fig. 3 A) or of different origin (Fig. 3B) (I, II, III, IV, V to see in the text)

The animals born at the EBTRF come from two parental couples ( $F_0$ ) that were formed by 6 animals getting for the station between 1970 and 1976 years (Medem 1981, op. cit., pp. 168 and 243-248). See chart 2.

CHART 2 . PARENTAL FOUNDERS OF <i>C. intermedius</i> AT THE EBTRF.				
Register	Entrance Date	Sex*	Initial Total Longitude	Origin Place
1	17 – I - 1970	F	72.6 (cm)	Meta River, Puerto López region, Meta **
2	16 – VI- 1970	M	178 (cm)	Puerto Alicia, Meta River, up of Puerto López***
3	28 – VI – 1974	?	39.1 (cm)	Born in March – April de 1974 (origin ?)
4	14 – II – 1975	M	183 (cm)	San Carlos de Guaroa, Metica River, Meta.
5	16 – V – 1976	?	32.6 (cm)	Born in April of 1976 (origin?)
6	19 – XI – 1976	F	116 (cm)	Humea River (Meta)

M = Male and F = female \*\* parental individuals  $F_0$  (shady of same intensity indicate couples); \*\*\* it died in 1998 and was replaced by one crocodile captured in the Cusiana river, Maní, Casanare, in 1980

The reproductive history in the EBTRF is shown in the Chart 3. The first birth happened in 1991 from two parental couples (Dabeiba-Polo and Lizeth-Custodio, female-male, respectively). Dabeiba and Lizeth (females) were about 24 and 22 years old, respectively; while Polo and Custodio (males) were 30 and 26 years old.

CHART 3. REPRODUCTIVE HISTORY OF PARENTAL COUPLES OF *C. intermedius* AT THE EBTRF, 1986-2001

Laying Year	Female Name	Eggs Number	Fertility		Birth Date	Births		Hatch T. °C	Hatch H.R. %	Hatch. Days.	Hatchlings mortality		alive 2001	Total Mortality	
			No	%H		No	%H				No	%		No	%
1986	Lizeth	?	1	?	-	-	-	-	-	-	-	-	-	-	-
	Dabeiba	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1987	Lizeth	20	6	30	-	-	-	-	-	-	-	-	-	-	-
	Dabeiba	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1988	Lizeth	0	0	0	1989	-	-	Env..	-	-	-	-	-	-	-
	Dabeiba	0	-	-	-	-	-	Env.	-	-	-	-	-	-	-
1989	Lizeth	14	2	14,3	1990	0	0	Env.	-	-	-	-	-	-	-
	Dabeiba	35	7	20	-	0	0	Lab.	-	-	-	-	-	-	-
1990	Lizeth	16	11	68,7	?	7	63,6	32-33	90	85	2	28,57 1	0	7	100
	Dabeiba	38	30	78,9	30/03/91	28	93,3	32-33	90	90	3	10,71	13	15	53,57
1991	Lizeth	31	24	77,4		0	0	30-31	90		-	-	0	-	-
	Dabeiba	42	36	85,7	1/04/92	32	88,8	30-31	90	94	2	6,25	23	9	28,13
1992	Lizeth	26	20	76,9	?	5	25	*	90	122	5	100	0	5	100
	Dabeiba	43	38	88,3	? - 93	17	44,7	*	90	108	11	64,71	3	14	82,35
1993	Lizeth	24	14	58,3	30/03/94	14	85,7	29-30	90	104	3	21,43	6	8	57,14
	Dabeiba	46	37	80,4	24/03/94	30	81	29-30	90	100	3	10	21	9	30
1994	Lizeth	?	55	-	8-12/4/95			-	-	-			3	-	-
	Dabeiba	?	*	-	8-12/4/95	52	*	-	-	-	7	13,5	4	-	-
1995	Lizeth	37	27	72,9	12/04/96	14	51,8	32-34	90-96	106	2	14,29	6	8	57,14
	Dabeiba	41	-	-		-	-	-	-	-	-	-	0	-	-
1996	Lizeth	42	?**	?	1997	-	-	-	-	-	-	-	-	-	-
	Dabeiba	44	0	0		-	-	--	-	-	-	-	-	-	-
1997	Lizeth	42	?	?	1998	-	-	-	-	-	-	-	-	-	-
	Dabeiba	44	0	0		-	-	-	-	-	-	-	-	-	-
1998	Lizeth	50	44	?	1999	-	-	-	-	-	-	-	-	-	-
	Dabeiba	45	0	0		-	-	-	-	-	-	-	-	-	-
1999	Lizeth	39	?	?	2000	-	-	-	-	-	-	-	-	-	-
	Dabeiba	35	0	0	-	-	-	-	-	-	-	-	-	-	-
2000	Lizeth	45	?	?	2001	-	-	-	-	-	-	-	-	-	-
	Dabeiba	33	0	0	-	-	-	-	-	-	-	-	-	-	-

\* fertile eggs 55, births 52, damaged eggs 3 , dead hatchlings at birth 2 and y dead one week later 5. It has not been given the information about the number of the eggs for each female in 1994. \*\* Eggs hatched in situ were pick up from the nest after 30 to 45 hatching days; and delivery to station for artificial incubation, but they have been damaged.

Lizeth laid eggs for the first time in 1986 at 17 year-old. The eggs were picked up of the water. One egg had embryo and no registrations are available about how many eggs corresponding to the first laying posture.

Dabeiba laid 35 eggs for the first time in 1989 when she was 22 years old, seven of those were fertile (20%). Dabeiba laid 38 eggs in 1990 and the fecundity was 78.9% (30 fertile eggs). The maximum fertility for that female was 88.3% in 1992 and the maximum fecundity (46 eggs) in 1993.

Dabeiba was on heat for the first time in 1974, when she was 13 years old and 265 cm total longitude (Medem, op. cit. 1981); 17 years later (when she was 30 year-old), in 1991, she had the first hatchlings. Dabeiba's partner was Polo died on December 7 of 1998 and was replaced by Juancho.

From 1997 up to today, at the EBTRF have no been births and the animal feeding was been made exclusively with fresh water fish, without supplement of vitamins neither minerals. It is well known that in alligators, a monodiet of marine fish may cause a gradual decline of the clutch size and also reduce the fertility and fecundity percentages, and no laying occur in some individuals (Joanen, T. and McNease L., 1987)

The other female laid eggs with severe shells fractures with membrane exposure. The eggs had always been collecting 30 and 45 days after laying and most of them were already damage when pick them up, these means that is possible that some eggs were fertile. Lizeth laid 50 eggs on December, 1998. Forty four eggs presented opaque band but were damaged during the hatching.

### **THE FIRST BIRTH AT THE EBTRF**

On March 17, 1991, seven individuals of *C. intermedius* were born from a clutch of 16 eggs. Those eggs were laid by Lizeth in the dawn of the day December 20, 1990.

Those eggs were put into incubation inside a recipient with humidified sand and inside a room tempered 33°C and 92% H.R. The hatchling time was of 87 days. Then when adults all were sexed (males); today, no one is alive.

Another clutch of 38 eggs was laid on January 23, 1991, by Dabeiba in the last week of December of 1990. Thirty three eggs presented opaque band (86.8% of fertility) and 5 (13%) were infertile; two of the fertile eggs (5.2%) presented shell fracture. After 90 days of incubation, were born 28 hatchlings. Today 14 of those are still alive and they are already 10 years old.

### **Incubation System**

The incubation system was made in a room of 3 x 2 x 2.2 m with regulated temperature of 32°C ± 1°C, with R.H. of 85-95%. It had a big plate of wide mouth (50 diameter cm) and depth of 28 cm that provided when filling contain enough reserves of water to produce vapor of hot water by means of a vaporizer of willing pediatric clinical use on an icopor ring that floated on the water. The vaporizer was connected to a 1000 W resistance that in turn was willing on the mesh metallic protector of the fan crosses with willing horizontal axis on an expandable support to a height of 1.6 m. The thermal sensor of the termoregulator registered the temperature of the incubation room ceiling and when this arrived at 32°C, it faded the resistance together with the vaporizer. One minute later the system was activated; hot vapor and relative humidity were dispersed homogeneously in the room by effect of the front fan that rotated an horizontal angle of displacement approximately in way of 120°C.

The temperature maximum limit was 33°C and the minimum was 31°C with H.R. between 85 to 95%. In spite of having these conditions, the eggs were put down in plastic boxes of 31x23 x 10 cm. The eggs were covered with humid sand substrate and protect them with a plastic to avoid a probable dryness motivated by an unforeseeable court of the electric power supply. The plastic allowed the water vapor

condensation, in such a way that stayed the humidity of the sand but the fact was never over-saturation presented. The sand surface became moist using a water manual sprayer in two opportunities.

The first *C. intermedius* individuals were born under those conditions at the EBTRF. The next hatchlings did without of the vaporization devised system and it was only enough to maintaining the termorregulated room as well the sand humid substrate inside the plastic vessels.

### Previous Nutritional Management to the First Birth

The feeding of *C. intermedius* was occasionally made by supplying bovine viscera, red meats or waste chicken in the EBTRF since 1970 to 1987 years. The reproductive efficiency was null due to the poor nutritional conditions.

In 1988 and 1989, a nutritional strategy was thought to improve the reproductive status of the two parental couples, in such a way, that the laying, fecundity and fertility rate were incremented each year.

The reproductive season ration contains Lysine (130 mg), Methionine (65 mg) and Carosen ® multivitamins by using the feeding for laying hens (we thought to improve the vitamin E contribution). The females were fed with that diet weekly in an equivalent quantity to 9% of corporal weight (5 Kg/week, assuming a weight average of 56 Kg) and males were fed with 6% of corporal weight (10.8 Kg/week assuming a weight average of 180 Kg). With that feeding management, both female laid 14 and 35 eggs (fecundity). For each clutch the fertility percentage was 14.3% and 20% in 1989.

In 1990, the diet was improving by giving more quantity of energy (Staton *et al.*, 1990a, 1990b). and vitamin E (Dierenfeld, E.S., 1989, Lance, 1987). With that nutritional support, males were fed with 10 Kg and females with 7 Kg weekly, according to the following formulation:

Nutritional formulation for Parental of <i>C. intermedius</i>			
		Male ration 10.000 g	Female ration 10.000 g
Animal fat	10%	1000	700
Fish	80%	8000	5600
Minerals	3%	300	210
Multivitamins	2%	200	140
Vitamin E	0.5%	50	35
Lysine	0.25%	25	19.6
Methionine	0.125%	12.5	8.75
Glucose	4.12 %	412.5	288.75
Oxitetraciline		1.5	1.05

In that year (1990), Lizeth laid 16 eggs, the same as the previous one but improving the fertility from 14.3% to 68.7%, while Dabeiba laid 38 eggs, its fertility was 78.9% compared with 20% of the previous year. The eggs was hatched and the first hatchling occur on March 17, 1991, as it was already been expressed.

Alligators were fed with supply to maximal ration of 1% of its corporal weight in the decade of 1980 (Joanen, T and McNease, L., 1987). Around of 1990, in Colombia several ration formulation were experienced that include fish flour (37.5%), feathers flour (19.5%), rice flour (18.0%), whole meat flour (12%), extrude soy cake (8.5%), corn gluten (4.0%), Rovimix crocodylia (Roche) (0.2%), Endox (0.035%), Microcub (0.1%) (Rodríguez, M., com. written, 1998).

## **GROWTH AND DEVELOPMENT OF *C. intermedius* AT THE EBTRF.**

### **Growth Standard**

The first growth registrations of *C. intermedius* in the EBTRF were made by Medem (1981) with 6 individual (4 of those are the two even historical parentals of the EBTRF) that entered in 1970 and they were pursuit each year up to 1979.

In 8 years and 8 months, a male (Polo) with initial total longitude (TL) of 178 cm on June 16 of 1970, increased 122 cm up to February 15 of 1979; which is equal to a daily increment average of 0.378 mm during 3225 days. A female called Dabeiba, with initial TL of 72.6 cm, increased 109.1 cm, 0.329 mm/day, between January 17 of 1970 and February 15 of 1979 (3315 days). Another male (Custodio), with initial TL of 183 cm, in February 14 of 1975, increased 117 cm in 4 years, that is to say an average of 0.8 mm/day. The other female (Lizeth), with initial TL of 116 cm in 1976, increased 72.3 cm in 2 years 8 months, that means, 0.74 mm/day.

The initial males longitudes are comparable, however Polo grew so much (122 cm) in 9 years like Custodio (117 cm) in 4 years. It is quite probable that the captivity place of each animal impacted over their growth. Polo received very little solar light per day while Custodio had more open place to the solar light the whole day.

### **Eggs and Newly Born Size**

The *C. intermedius* eggs vary between 8.4 x 5.0 and 7.2 x 5.0 cm in big and small diameters, respectively. Their weight were of 118.6 - 110.3 g (Medem, 1983; pp. 65) for normal sizes; however, smaller eggs were found with measures of 67 x 30 mm and 54 x 33 mm in maximum and minimum diameters with maximum weight of 103 g and minimum of 86 g (n=34) (Goshalk, R. 1978).

Recently information about eggs (n=181) of *C. intermedius*, in the EBTRF, laid for females with on average of 30 years old had shown the following size parameters: bigger diameter, 80 mm (88.8 – 60 mm range); smaller diameter, 51.01 mm (57 - 42 mm range); weight, 124.4 g (224.53 - 81 g range); volume, 115.48 ml (134.32 - 94.74 ml range); density, 1.08 ml/g (1.93 - 0.60 range). The volume was determined by using picnometrical technique (Ramírez-Perilla, J. 1999, see Fig. 4).

Some eggs were laid into the water (excess of weight). The eggs recollected at the laying day had an average weight (n=16) of 112.56 g (118.4 - 106.5 range) in 1991. They were no different from the others laid 25 days latter (112.8 ± 5.6, 103.3 - 124.2 range). Eggs had an average weight of 132 g (n=14) at the time of hatchling, higher to the whole clutch average 126.7 g (n=36), in 1996. (Ardila-Robayo *et al* 1999b).



Fig. 4 Picnometer to determine volume of eggs (Ramírez-Perilla J, 1999)

It is difficult to find scientific literature about volume, density or specific graveness indexes (G.E.) of eggs like quality indicators of the same ones applied to wild species (Evans, R., 1969; Preston, F., 1968; Ramírez-Perilla, J. 1999).

### Neonates and Breeding Growth

The hatchling weight average was 86.26 g (99.80 - 67.2 g range) in 1995 (Ardila-Robayo *et al*, 1999b), while the it was  $76.8 \pm 3.0$  (80.5 - 75.7 range) in 1991. In this case, the difference of neonates weight was not related with the eggs weight. (Ardila-Robayo 1999b).

Thirty males hatchlings of *C. intermedius* born at the EBTRF in 1991 (Lugo, 1995), presented an annual average growth of 275.0, 480.0 and 573.0 mm in total longitude (TL) and of 604, 4550 and 13560 g in weight for the first one, second and third years, respectively.

For comparison, the annually averages growth of 14 females hatchlings were 623.2, 3387.89 and 4716 g in weight and 267.96, 460.46 and 332 mm in total longitude for the first one, second and third years, consecutively (Ardila-Robayo *et al*, 1999b).

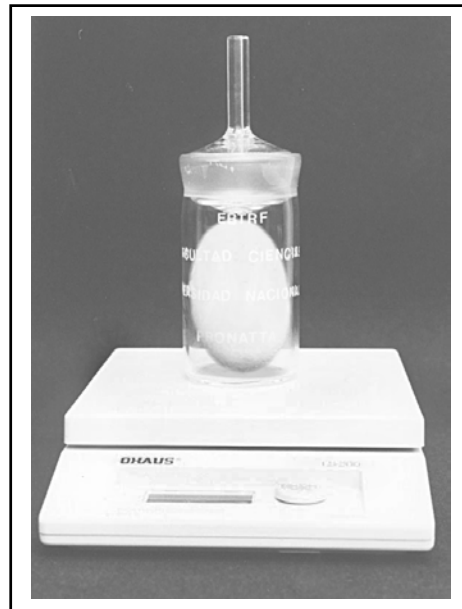
In this last case the hatchlings were in aquariums until 213 days with growth rates of hardly 0.78 g/day. When they moved to bigger ponds, the animal's recovery was very significant, with daily increments up to 4.59 g (Ardila-Robayo *et al*, 1999b).

Recently information (1998) said that the accumulated absolute maximum weight in a 3 year- old female was 13 750 g; at the same way, another female, with similar age, presented an absolute minimum weight of 2200 g. The group average was 7153.8 g (n=13) and the average weight increment was 3053.8 g (n=13) for second and third years.

It is possible a daily growth of 1.2 mm of TL in *C. acutus* hatchlings and juveniles when they are feeding with an weekly equivalent rations of 30% corporal biomass in five portions (one per day, 6% with relationship to body weight for portion) (Rodríguez & Rodríguez, 1991).

In the EBTRF, it had observed similar growth, 1.24 mm/day and 27.7 g/day (Ramírez-Perilla, 1991) or 1.12 mm/day in males hatchlings of *C. intermedius*, but with 10% of biomass, three times per week (Lugo, 1995).

In such a way, the pondered average growth (weight and total longitude) was of 52.48 g/month, 1.72 g/day and 0.74 mm/day, 22.56 mm/month for 361 days (Ardila-Robayo *et al*, 1999a). Those data were compared with 15 months old *C. acutus* that grew from 0.5 to 1.17 cm/month (Stone *et al*, 1996-1997) or 14.2 to 20.7 mm/day (Seijas *et al*, 1990). The weight increment of *C. porosus* in function of its size (Webb *et al*, 1998) in individuals of 310 - 320 mm was 0.5 g/day; for animals with a longitude of 360 to 410 mm was 0.7 g/day and for those having 500 mm was 1.6 g/day.



*C. intermedius* was feeding with more frequency and with higher rations in the EBTRF, during the rainy season ("winter"), corresponding to May up to November months (Medem, 1981,op. cit.). This behavior repeats cyclically year by year and it expressed a direct relationship with the environment conditions as well the reproductive cycle of *C. intermedius*.

The sexual activity retinue begins in July and could be prolonged until December. This sexual behavior coincides with a second rains cycle during the last semester and corresponding to the coldest month of the year (July).

The *C. intermedius* growth curves in the EBTRF presented as follow, express all the factors and they also constitute our institutional standard. For that purpose, we handled animals that born in 1991 (males) and others hatchlings from these year up to 1996 (females), that is to say that we are dealing with individuals of 5 to 10 years old range up today (2001).

These annual data correspond for a period between 1998 to 2001. During that period of time, the animals born in 1996 were two years old; those born in 1995 were three years old and those born in 1992 were 6 years old, when the registrations forms began (1998).

This way, the growth curves are built averaging the parameters (v. g. weight) to measure according to the chronological age with the determinations corresponding to other animals with the same chronological age, although they had been born in different year.

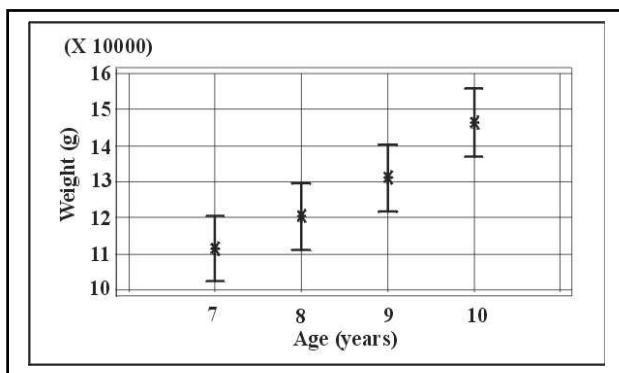
### Males Growth

The weight growth curve for males of 7 and 10 years old is shown in the Fig. 5. The Tuckey averages difference test establishes significant statistical differences between the 10 year-old ages and the other ones. The averages weight are similar between 7 and 8 years old; and also between 8 and 9 years old; but they are different between 7, 9 and 10 years old. The seven years old weight was 111.46 kg (53.8 - 145 kg range); instead of the 10 years old average weight was about 146.46 kg. (167.75 - 115.75 kg range).

The daily weight average increment was 26.8 g but exceptionally there were individuals that shown 69.2 g/day.

There was an animal in the group loosing weight (-23.0 g/day). Animals with 7 and 8 years old presented a weight increment about 8871 g; other ones having 8 and 9 years old shown 10500 g; and the last one with 9 and 10 years old increment 15914.3 g; that means that they are growing (positive growth rate).

Fig. 5 Weight growth curve (g) in *C. intermedius* males.



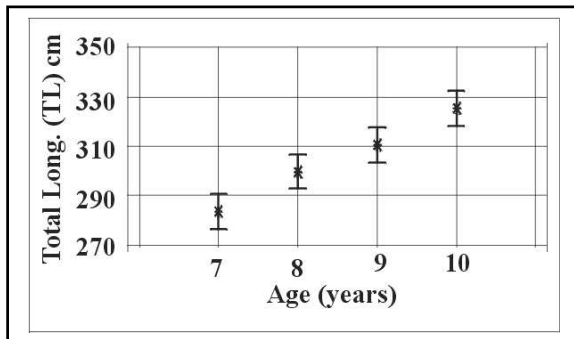
The regression analysis is of lineal type and corresponding to the follow model:  $Weight = 29217.1 + 11540.5 * Age$ ,  $R^2 = 34.1\%$ ; correlation coefficient = 0.584,  $p < 0.01$ . That indicates a highly significant relationship between the age and the weight (99% of trust).

The males ventral total longitude (Fig. 6) evidences significant differences in growth between all ages ( $p < 0.05$ , Tuckey test). Average total longitude for 7 years old crocodiles ( $n=14$ ) was 283.64 cm (308.3 - 240.8 cm range); for those of 8 years old was 299.67 cm (320 - 256 cm range); for animals with 9

years old was 310.45 cm (335 - 280 cm range); and for the last individuals of 10 years old was 325.246 cm (344.8 - 295.3 cm range).

The annual increment of total longitude for animals of 7 and 8 years old was 15.1 cm; for those of 8 and 9 years old was 12.04; and for individuals of 9 and 10 years old was 13.45 cm. This is equal to daily growth, for the whole year, about 0.4 mm for 8 years old, 0.35 mm for 9 years old and 0.357 mm for 10 years old. The weight variation coefficient correspond to 7 years was 23,12% but when animals were 10 years old, was 12,4%; Those expression explains why some smaller growth rates were presented and why there were few differences between individuals with higher age.

Fig. 6. Ventral Total Longitude Growth Curves (TL) of *C. intermedius* males



The lineal correlation pattern for TL vs. Age of *C. intermedius* males explains 55.5877% ( $R^2$ ) of the data variability, with a correlation coefficient of 0.74;  $TL=189.537 + 13.5546*Age$ .

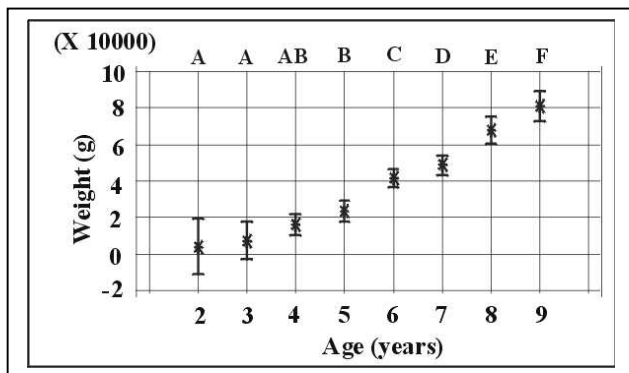
*Females Growth (n = 65): Total weigh*

The averages growth profile of weight vs. age in *C. intermedius* females is shown in the Fig. 7.

The weight average of *C. intermedius* females were 2 years old was 4100 g (6250 -2200 g; C.V.=33%). and representing 5% of 9 years old weight (81206.8 g). The individual weight variations are high between ages by looking the percentage variation coefficients (C.V.). They increase between 2 and 6 years old (33.04%, 39.2%, 42.79%, 42.75 and 51.49%, respectively) and the same way, they tend to reduce between 7 and 9 years old (43.85%, 29.16%, 26.19%). Those data suggest a maximum variability between ages of quick growth (4, 5 and 6 years old) whose averages weight were  $15930 \pm 6817.6$  g (38000 - 5300 g);  $23230.23 \pm 9931.18$  g (55500 - 8300 g) and  $41808.47 \pm 21528.02$  g (92000 - 13500 g), respectively.

No significant differences exist between the average weight at 2, 3 and 4 years old. The same happens with the average weight between 4 and 5 years old, but at beginning of 5 years old, the average weight differ between ages ( $p < 0.05$ , Tuckey Test), whose their annual average weight increments between 3 and 9 years old were 3053.84 g, 8776.16 g, 7300.2 g, 18578.47 g, 6861.7 g, 19589.8 g and 12946.8 g, respectively.

Fig. 7. Weight growth curves in *C. intermedius* females



The linear regression pattern  $Weight=-33202.6 + 12241.9 * age$ , is not adjusted to the data reality, in spite of the correlation coefficient = 0.769 and the  $R^2 = 59.2 \%$

At the 7 years old, the males weighed 2.25 times more than the females (109807.14 g / 18670.19 g). When they were 8 years old weighted 1.73 times more (118678.57 g/68260 g) and finally when they reached 9 years old weighted 1.59 times more (129178.57 g/81206.81 g).

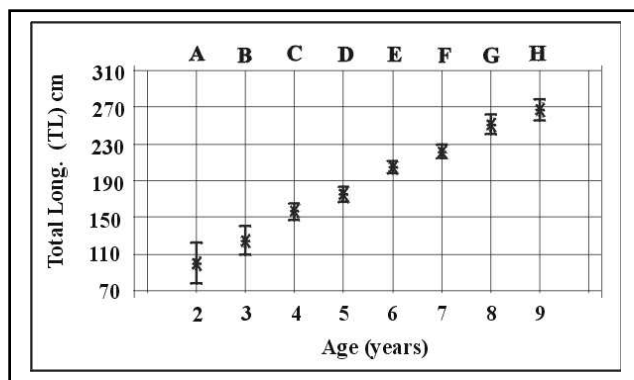
Ardila-Robayo (1999a) registered the weight in *C.*

*intermedius* females during the first three years of age as follow: 623.2 g, 4011.09 g and 8727.09 g; while for males of the same age the weight were: 604 g, 5154 g and 13316 g (Lugo, 1995). This means that males at 2 years old grow in weight 1.28 times more rapidly than females, and 1.52 times more in the third year.

### Females Total longitude (TL)

Between the TL and the age of the females exists a direct relationship (Fig. 8) that explains by the lineal regression pattern  $TL = 58.5259 + 23.7151 * age$ ,  $R^2 = 72.4566\%$  and correlation coefficient = 0.8512 ( $p < 0.0001$ ). When females were 2 years old, its size was 37% (99.33 cm) of the corporal size when they were 9 years old (268.036 cm) and also exist significant differences about average TL between all ages ( $p < 0.05$  Tuckey Test)

Fig. 8 . Total longitude Growth (TL, cm) of *C. intermedius* females.



When females were 2 years old had 99.33 cm (115 cm - 82 cm range); at 3 years of age had 124.73 cm (151 - 87 cm); when they were 6 years old had 204.66 cm (258 cm - 147.99 range) and when they were 8 years old had 250.29 cm (280 - 172 cm). Ardila-Robayo (1999b) registered growths of 72.84 cm and 106.04 cm for females of 2 and 3 years old respectively. In males hatchlings with the same age (2 and 3 years old) the TL was of 75.5 cm and 132.8 cm (Lugo, 1995).

### Relative Head Growth in males and females

The head longitude (HL) in relation with the total longitude (HL/TL) and also in function of TL to different females and males ages is shown in Fig. 9.22. That figure indicates that females at 2 years old, the HL represents 17.8% of the total longitude (TL) being increased until 18.51%, 18.65% and 18.61% when the animal's age was about 7, 8 and 9 years old respectively. For comparison, males have a relation between the head and TL bigger than females at the same age as follow: 19.15% at 7 years old, 19.18% at 8 years old and 18.7% at 10 years old. At 8 years old, the proportion between HL/TL vs. TL for both sexes spreads to be smaller.

In relation with the Body Longitude (BL) to different ages, the proportion of the head (HL/BL) (Fig. 10), in function of BL for females to different ages represents 32.94% to 2 years old, beside that when individuals are 9 years old that relation was about 35.38%.

That growth is fast between 2 and 3 years follow by a plateau corresponding to 3 and 5 years old and also, the positive growth rate was until 9 years old with pick abrupt maximum at 6 years old (34.6%).

The males head was 40.35% of the body longitude at 7 years old. After 8 years old, this proportion was going down to 36.56% with asymptotic progressive tendency until 10 years old of registration.

That difference should be due to a better growth of BL than the HL between 7 and 9 years old because the head longitude growth rate in males only start to diminish at 9 years old.

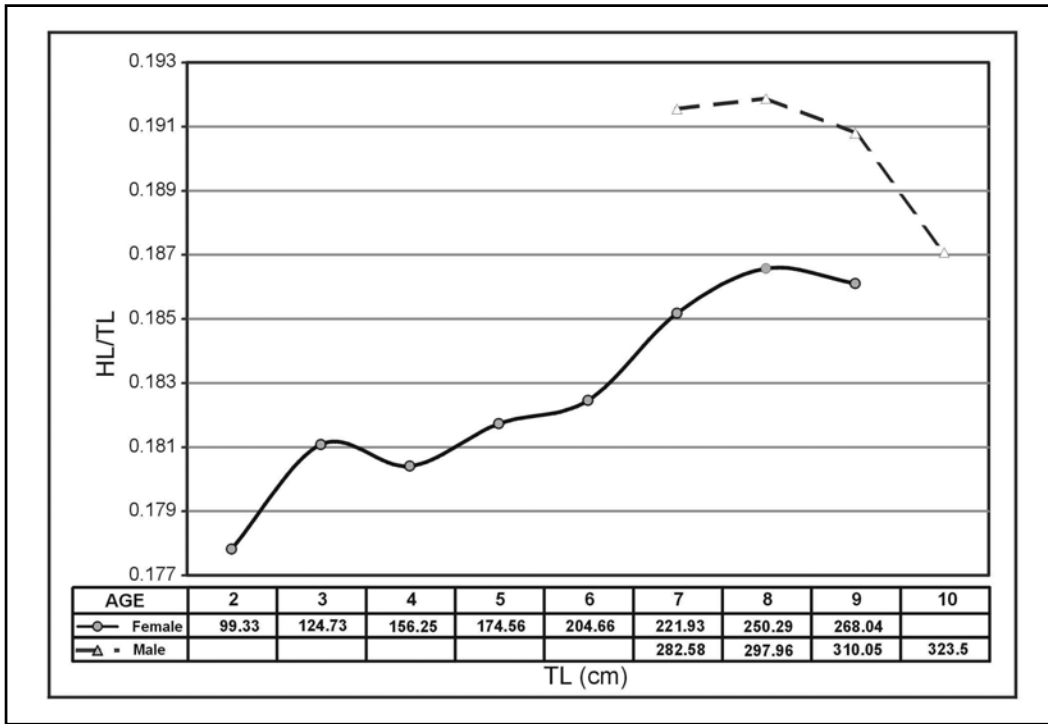


Fig. 9. Growth Proportion of the head longitude (HL/TL) in function of total longitude (TL) of males and females to different ages.

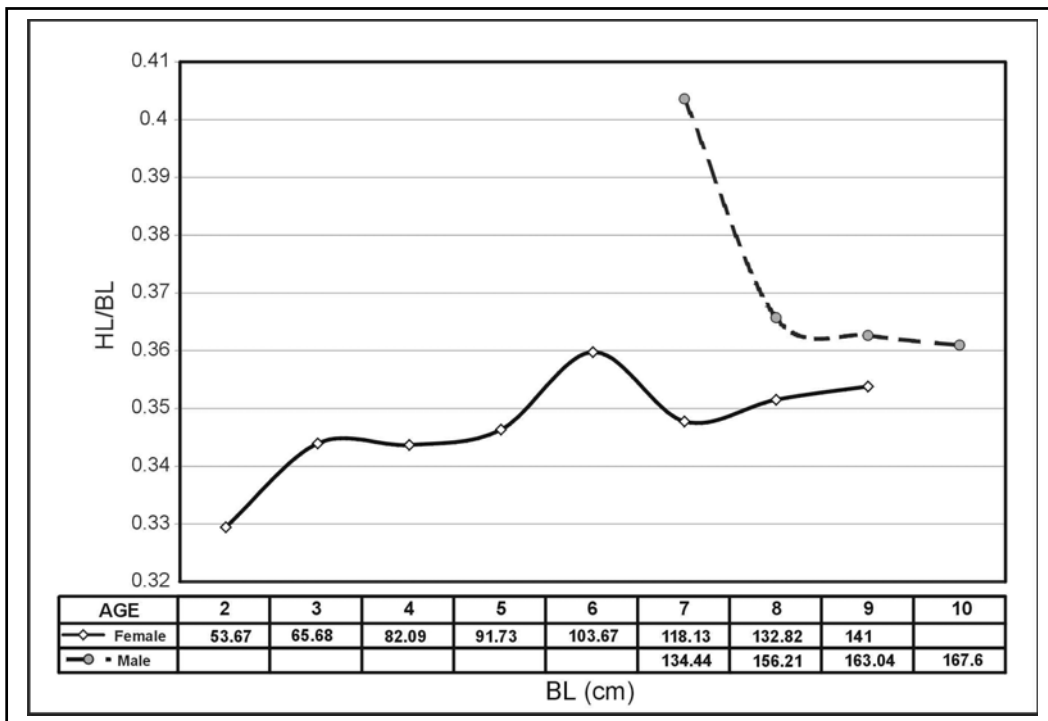


Fig. 10. Growth Proportion of the head longitude (HL/BL) in function of body longitude (BL) of males and females to different ages.

The proportion between the head and the body longitude tends to be similar toward 10 years old in males and females.

In *C. intermedius* juveniles, the relative growth indexes of head Longitude in function of the body longitude (cloacal face longitude, CFL) diminished starting from 160 mm of CFL; but it is important to say that indexes between chart crania longitude (CL) and head longitude (HL), CL/HL, stays at the range of 82 and 90% (Ardila-Robayo *et al.*, 1999b).

## MORPHOMETRIC CRANIAL ANALYSIS

Ardila-Robayo *et al* (1999c) carried out an important pattern study of relative growth in 48 skulls of *C. intermedius* between 6 and 12 months of age (till 60 months) born at the EBTRF.

It was taking 16 dorsal and occipital measures of the skulls following the seven relative growth indexes established by Hall & Portier (1994) according to the measures of more interest (taken of Ardila-Robayo *et al.*, 1999c) that are shown on Fig. 11.

E = Ab, basal face width, through the previous orbital borders.

F = Ai, Interorbital width, minimum width among the orbits.

G = Ao, orbital maximum width among the orbit lateral borders.

H = At, maximum cranial chart width corresponds to the angles posternlaterals of the cranial chart.

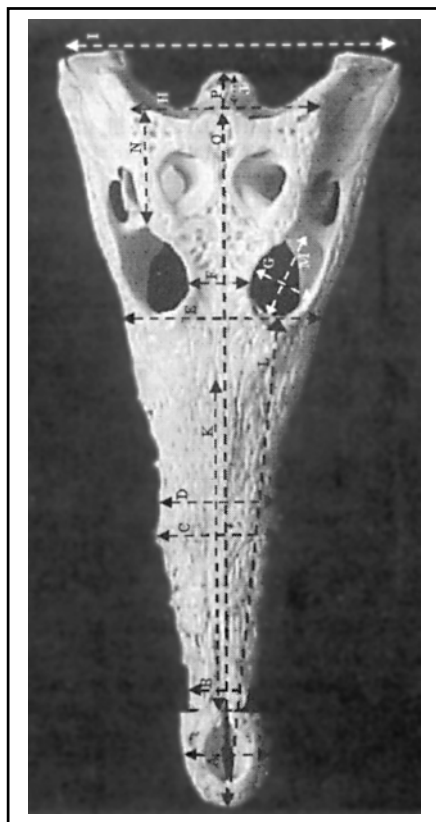
I = Ac, maximum cranial chart width corresponds to the borders

L =Lr, rostral longitude, minimum distance from the point more previous of the suture interpremaxilla until the previous orbital border.

M = Lo, orbital maximum longitude, at level of the previous and later borders of the orbit.

N = Lt, postorbital chart cranial longitude, corresponds to the distances from the orbital later border until the postero-lateral margin of the scaly.

O= Lc, medial skull Longitude from the point more previous of the suture interpremaxilar until the later medial margin of the cranial chart.



While *C. intermedius* grows, the relative growth indexes (Hall & Portier, 1994) in function of the cranial longitude, Lc, (Ardila-Robayo *et al.*, 1999c) are as follow:

There were no significant differences between the relationship Lc/Lt (medial skull longitude / total longitude) of small individuals' skulls (n=13, 6 - 12 months) and bigger animals skulls (n=9, > 60 months) that is about 0.15. It means that this proportion stays constant independently of sex; that condition did not occur in *A. mississippiensis* in which the relationship stay constant in females but not in males. Lc/Lt is useful to estimate the animal size with more precision measure in wildlife as well in captivity.

Fig. 11. Skull measures in order to establish growth indexes (Ardila-Robayo, *et al.*, 1999c)

· Ai / Lo (orbital width /maximum interorbital longitude) increased of 15% up to 66%. The orbit longitude decreases in relation with the head longitude and the space increases among the orbits.

- The percentage Lo/Lc (maximum orbital longitude / medial longitud of the skull) diminishes from 25% to 12% in connection with the head longitude.
- The face becomes longer in animals of more size. The proportion Lr//Lc (rostral cranial longitude / medial longitud) increase from 54 up to 72%.
- Ac/Lc (maximum medial width / medial longitude of the skull) and Ao/Lo (maximum orbital width / maximum orbital longitude) are relatively constant (41-45% and 77-87%, respectively).
- The cranial chart gets wider lightly in animals of more size. Lt/At, of the maximum posorbital cranial chart longitude /maximum width diminishes, 78%-59% and also the face base in big animals spreads to be narrowed (Ab/Lr, basal width of the orbital maximum rostro/longitud diminishes from 52% to 36%).
- It is necessary to associate the skull measure, the sexual dimorphism in *Crocodylus novaeguineae*, starting the 100 cm of TL, Ao and Lc. They are indicative of sexual dimorphism (Hall & Portier, 1994).

## MOLECULAR GENETIC CHARACTERIZATION

The genetic structure of the *ex situ* population of *Crocodylus intermedius* from Colombia (113 individuals incoming from diverse origin; 38 males, 75 females in the EBTRF) was determined by using Amplified Fragments Longitude Polymorphims (AFLP's) (Bejarano C, 2001).

The AFLP's technique is based on the selective amplification, by PCR, of generated fragments of a genome digested with restriction enzymes. After the obtaining of the genomic DNA, this methodology consists in three main steps: (1) restriction of DNA and binding of adapters; (2) selective amplification of the restriction fragments; (3) fragments electrophoresis amplified in agarose gels or poliacrylamide.

Because of the taxonomic reaches, the low error levels and the high resolution of this technique; the AFLP's is great utility to generate information about the genetic structure of populations of those no having any previous genome knowledge, as happens *with C. intermedius* in Colombia.

With base in fragments obtained in 50 loci, between 100 and 1500 couples of bases in ranges of size, of 78 animals was elaborate a presence-absence womb, where the haplotypic frequencies were determined (haplotype is similar of allele, Nei & Tajima, 1981) for locus (Nei, 1987), and the genetic distances by means of the program PAUP (Swofford, 1999). starting from these data it was carried out a dendrograma for the method UPGMA (Unnweight Pair Group Method With Arithmetic) which shown that *ex situ* population of *C. intermedius* from Colombia is substructured in three (3) genetically distinct groups (GDG).. This group correspond to 55,13% of the total populations. The 44,87% remaining are individuals which are dispersed along the dendrograma.

The genetic diversity was determined by FST statistics, that it is used to compare subpopulations heterocigoty in relationship with the entirety (Travis *et al*, 1996) and the average heterocigoty (HS). The first ones constitutes a very good indicator of genetic differentiation because allows an objective comparison of the effect of the substructured population among different organisms (Hartl and Clark, 1997). On the other hand, the average heterocigosity allows to evaluate the haplotypic diversity (= allelic) and the polymorphism grade inside each group.

These statistical parameters revealed high differentiation levels among the GDS (FST GDG1=0,6306; FST GDG2=0,2234; FST GDG3 = 0,3046), but little variability inside each GDG as well as in the total population (HS: GDG1=0,0605; HS: GDG2=0,1272; HS:GDG3=0,1139, and a total average heterocigosity HT=0,1638.

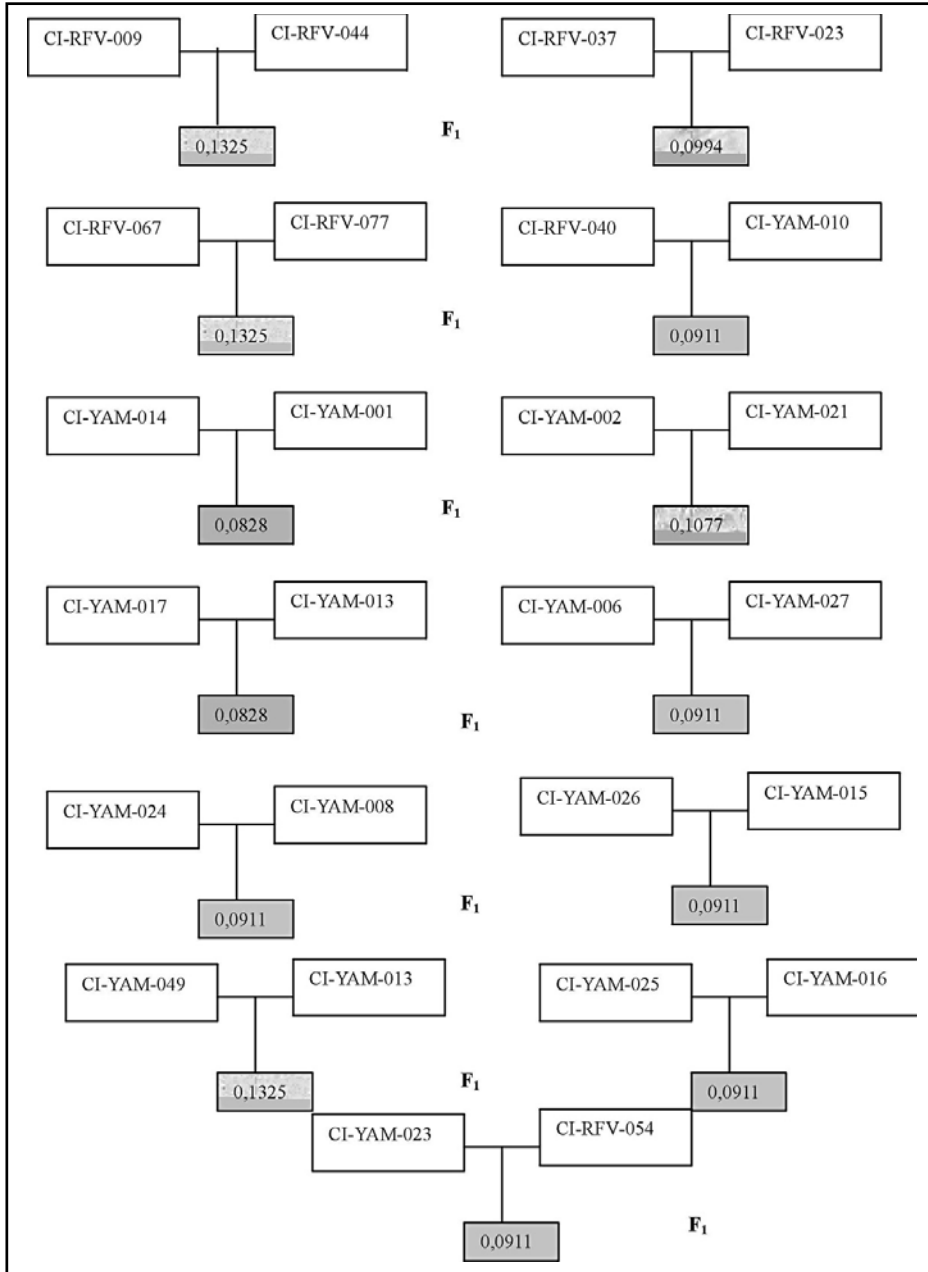


Fig. 12. Expected average heterocigosity for  $F_2$  generation obtained from proposed couples

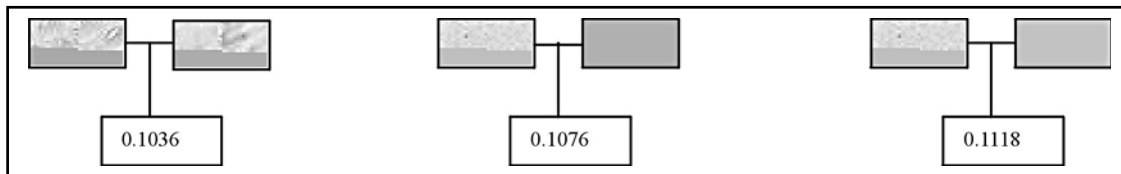


Fig. 13. Expected average heterocigosity for  $F_1$  generation



In order to increase the genetic variability and reduce the high fixation indexes  $F_{ST}$ , we intend the realization of thirteen (13) crosses directed among even of adults (Fig. 12) which will allow to diminish the substructure population as well as to maintain the average heterocigosity among 10% to 11% (Fig. 13) in the  $F_2$  generation. Although the ideal objective is to achieve a average heterocigosity very close to the found total population ( $HT=16,38\%$ ). The proposed crossings will allow to increase the genetic diversity and to modify the HT, so this and the HS be very similar, breaking the substructuring and increasing the variability.

The couples proposals belong to different GDS or isolated individuals. Additionally they correspond to those whose average heterocigosity waited at the first generation overcomes the opposing ones inside each GDG. The animals proposed in this strategy of crossings came from different regions of the Orinoquia (include EBTRF) and others from INDERENA confiscation.

These results, together with the analyses of microsatelital DNA (in process actually) allow to establish a management strategy for population reintroduction and/or strengthen wild population of *C. intermedius* inside the National Conservation Plan of this specie.

### SOCIAL SENSIBILITY AND PUBLIC KNOWLEDGE

A region convocation was realized in 7 urban centers (Arauca, La Macarena, Maní, Paz de Ariporo, Puerto Carreño, Villavicencio and Yopal) in 2000 and 2001. That convocatory covered the geographical distribution area of *C. intermedius* in Colombia with the following purposes:

1. To give knowing the National Conservation Program of *Crocodylus intermedius*, PROCAIMAN.
2. To promote a minimum organization as support to PROCAIMAN.
3. To value the knowledge grade of a specie in danger and collect very important ideas about how to use or utilize the wild life by a structured inquiry.

More than 300 people attended our call (26.4% women; 73.6% men). But only 261 persons answered to a preinquiry whose results are synthesized in the Chart 5 (Ramírez-Perilla 2000).

CHART 5. KNOWLEDGE AND PUBLIC SENSITIZATION ABOUT PROCAIMAN			
Question	Yes (%)	No (%)	Na/Nk (%)
1. Do you distinguish the caiman llanero from another species?	49	43	8
2. With what another name do you know it? 21 different combination names*			
3. Do they exist in wild life?. 35 different places mentioned.	55	28	17
4. Do they exist in captivity? 11 Different places	32	40	28
5. Did you participate in hunt or in skin trade.	85	12	3
6. Would you be part of an organization to save it from the extinction?	83	12	5
7. Are you accord that your town adopt the flag to save it from the extinction?	87	2	11
8. Do you know what regions would be adopt it as emblem?	75	15	10
9. Do you know something any conservation?	15	65	20
*Crocodile 71 times; Stifle 35 times; cachirre 24 times; three mentions as caiman llanero. Ten combine names as caiman (caiman, cocodrilo, etc.), and 4 names made up with crocodile.			

From those results, it is deduced that exists a confusion about the species identification. There is a relative knowledge about the individuals in wild life or in captivity and very great conservationist sensibility to PROCAIMAN program. Twenty one different common names were identified that include compound nominations (caimán amarillo, corroncho jobo, caimán, cocodrilo, etc.)

### **THE FUTURE OF HANDLING *ex situ* *C. intermedius* IN COLOMBIA: POSSIBLE ACTIONS**

EBTRF has 38 males and 75 females on November 2001 (Chart 1) from those 59 females and 17 males are in immediate reproductive age (Fig. 3). The crossing strategy in order to increase the genetic variability has been advised in this document by molecular genetic characterization (AFLP's) studies, that we expect they will be reconfirmed with the microsatelital DNA study in course. This crosses should be on close attention during next 20 years.

The future of *ex situ* population of *C. intermedius* in Colombia is already included in the strategic objectives of Conservation Program for the Caiman llanero (*C. intermedius*), PROCAIMAN, that are synthesized in three purposes:

- To prevent its extinction
- To promote its recovery in its natural distribution area.
- To integrate the specie to the socioeconomic and cultural systems of the region.

For the first item, the fundamental propose is to protect the scarce population and to incentive its *ex situ* reproduction (Chart 6). This reintroduction plan pretend to increase the wildlife populations up to 2500 individuals in an area of 500 Km<sup>2</sup>.

EBTRF has the support from the Genetic Diversity and Sustainable Managment of Fauna Program for Investigation and Conservation of *C. intermedius*, in study areas like Genetic Conservation, Animal Population (reintroduction and *in-situ* monitoring protocols) and reproduction and growth Ecophysiology.

The immediate future success of the Conservation Program of *C. intermedius* depends on giving so much support and importance to the recovery of the natural populations as well to the commercial opening of the species through a new modality of productive administration based on opinion of social justness in the profit obtaining (small producers associated preferential markets in a conservation context, investigation and economic and cultural productive administration).

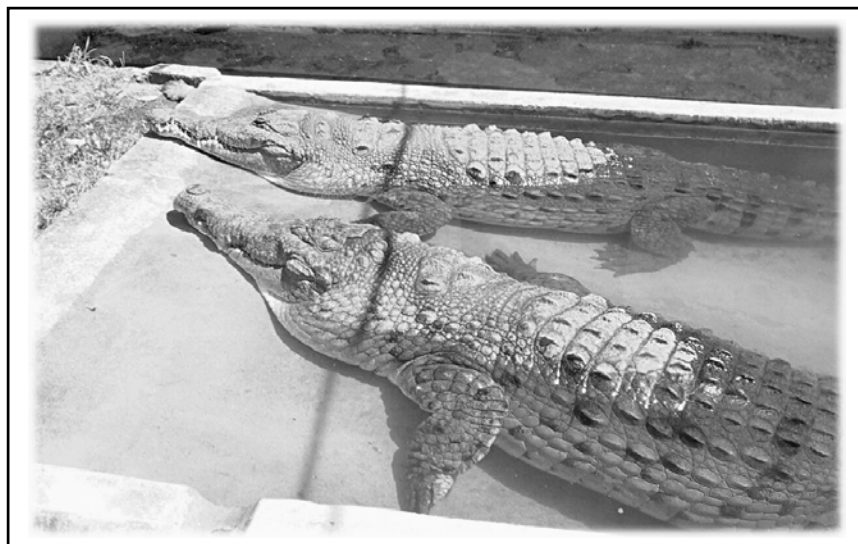


Chart 6. INSTALLED CAPACITY AND POTENTIAL FUTURE OF THE ex situ POPULATION of <i>C. intermedius</i> IN COLOMBIA			
Institutions or natural people	Available area (m <sup>2</sup> )		Observations
	Actual	Potential	
EBTRF	1100	1500	40 ponds. Three reproduction and raise nuclei. More facilities for incubation and experimental laboratories (foreseen) in incubation ecophysiology. Growth test in breeding-get up stage. Animal population laboratory. Behavior and adaptation to the environment. Logical support for genetic conservation studies. Clinical biochemistry and ecophysiology. Nutrition and feeding management. Institutional capacity for environment education (public conscience). Academic National University Programs. EBTRF is support to receive eggs and neonates born in wildlife and to lead a program for small workers associated to PROCAIMAN with the purpose to integrate the species to the economic and cultural processes of the region. Actually, the station has one specialist in animal populations to work in reintroduction an monitoring protocols of in situ populations.
Yamato Foundation	900	5000	Growing up and secondarily reproduction
Casanare Government	900	900	Reproduction and growing up. It has been request a parental nucleus of 5 individual (1 male and 4 females) to EBTRF on October 19, 2001
Natural People	600	Indeterminate	Yopal, Maní, Arauca and Villavicencio manifest interests to join PROCAIMAN program, with purpose of conservation an future commercial production at small scale (reproduction units of 1 male and 3-4 females.
OTHER OPTIONS OUT OF COLOMBIAN ORINOQUIA REGION			
Reptilia City	Indeterminate		There are two hatchery commercial institutes. Reptilia is located in the interandine region (Melgar, Tolima) near Bogotá. And the other one (Pizano S.A) is located in the Colombian Atlantic coast. They both manifested their interest of receiving the ex situ animal colony of EBTRF like support to the Conservation Program
PIZANO S.A.	Indeterminate		

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