# A Three Year Survey of Reproductive Capacity in the Indiana University Axolotl Colony

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## SPAWNING PURPOSES:

The I.U. Axolotl Colony supplies many laboratories with a wide variety of axolotl material, from embryonic stages up to adult phenotypes. More often than not particular mutants, usually at embryonic stages, are requested by our users. The Axolotl Colony also continually makes test crosses to identify carriers of the genes of interest and thereby maintain important stocks. Those services place restrictions on which adults can be used for various matings, and limits greatly the efficiency of our spawning attempts, since well-identified carriers of a given gene are usually at least two years old. In addition, the genetic load that most (but not all) of our stocks carry also contributes to difficulties in obtaining spawnings.

### MATING ROUTINE:

The breeding adults are kept in individual fishbowls at a constant temperature of 18°C. Since January 1983 the adults have been kept in a room with artificial and time controlled lighting. Prior to 1983 breeding age adults were housed in a room that had windows to the outside and therefore experienced natural lighting conditions, including day-lengthening, etc.

Each selected mating pair is placed in a large enamel dishpan, the bottom of which is covered with large pebbles so that the spermatophores have a substrate to which they can attach. Each evening during the spawning season 9 to 12 pairs are placed in their pans in the 18°C room and left together for 24 hours. A scoopful of ice is generally added to each mating pan before it is covered and left in the dark. After 24 hours each pan is inspected for

spermatophores. Each animal is then returned to its individual container. The females whose mating pans had spermatophore depositions are transferred to a 20°C room, covered and left in darkness until the following day when they should show spawning activity.

Our records show that the spawning season for the I.U. Axolotl Colony runs—in general—from November through May. This past year (1983) we were able to obtain spawnings well into June and July, which is quite exceptional. That might have been due to the fact that we were able to move our adults into a controlled light situation where day—lengthening did not occur. Since our experience is that our spawning efficiency runs to less than 5% in the late summer we no longer attempt to spawn our animals in the month of August.

### RESULTS:

The spawning records available for this report cover <u>only the last three years</u>. Due to extensive remodeling in the Biology Department, many of our older records were placed in storage and, as of this writing, were inaccessible. Table I contains a summary of the available data for the past three years. Two columns are of particular interest: Column C (% fertile spawns per mating attempt); and Column E (% fertile spawns per mating that yielded spermatophores). Note (Column C) that our spawning efficiency is considerably lower than that of the Hubrecht Laboratories due, as mentioned previously, to our need to use older stock and to the mutant background in the majority of our stock. In column E note that we do NOT obtain a spawning every time spermatophores are deposited. It is our experience that only 1 out of 2 or 3 females that have spermatophores in the mating pan will give a successful spawning. It is also of some interest to note that in 1983, after the adults were moved to a light controlled room, spawning efficiency from February through July showed a two-fold or greater improvement over the same

time period in 1981 and 1982.

In an effort to improve our spawning efficiency in the years to come we will be experimenting with (a) new combinations and or amounts of homrone stimulation, and (b) bringing selected pairs to 23°C for a period of time before mating them—as in the Hubrecht report. Several investigators feed the potential mating pairs 2-3 times daily in the week prior to mating and report good success with that method. In our situation the labor and materials cost would be prohibitive to feed all the pairs needed for particular spawnings. Nevertheless, that strategy apparently works well in small colonies.

TABLE I. MATING/SPAWNING RECORDS FOR 3 YEARS

	A) TOTAL # MATINGS			B) TOTAL # FERTILE SPAWNS			C) FERTILE % SPAWN/MATING			D) # MATINGS → SPERMATO- PHORES			E) % FERTILE SPAWNS/SPERMATO- PHORE		
	1981	1982	1983	1981	1982	1983	1981	1982	1983			1983	1981	1982	1983
Jan.	187	289	150	51	41			14.2%	28.0%	93	123	90	54.8%	33.3%	46.6%
Feb.	250	262	99	40	39	38	16%	14.8%	38.3%	110	121	76	36.1%	32.2%	50.0%
Mar.	230	205	109	50	42	49	22%	20.4%	44.9%	118	100	91	42.3%	42.0%	53.8%
Apr.	245	155	171	45	39	49	18.3%	25.1%	28.6%	108	96	113	41.6%	40.6%	43.3%
May	149	87	88	22	12	22	14.7%	13.8%	36.3%	52	41	65	42.3%	29.2%	49.2%
June	120	84	99	13	10	29	10.8%	11.9%	29.2%	46	49	62	28.2%	20.4%	46.7%
July		57	61		2	14		3.5%	22.9%		13	48		15.3%	29.1%
Aug.															
Sept.	86	179	68	12	29	9	13.9%	16.2%	13.2%	38	94	36	31.5%	30.8%	25%
Oct.	162	181	179	19	29	19	11.7%	16.0%	10.6%	75	83	81	25.3%	34.9%	23.4%
Nov.	164	163	221	15	19	12	9.1%	11.6%	5.4%	53	86	80	28.3%	22.1%	15%
Dec.	203	90	110	42	12	9	20.6%	13.3%	8.2%	97	54	48	43.3%	22.2%	18.7%
EE	1855	1768	1355	318	240	302	17.1%	13.5%	22.3%	790	860	790	40.2%	27.9%	38.2%

TABLE II. 3-YEAR TOTALS AND AVERAGES

	TOTAL # MATINGS 1981, 82 & 83	TOTAL # FERTILE SPAWNS	% FERTILE SPAWN/ MATING	# MATINGS → SPER- MATOPHORES	# SPAWN/ SPERMATO- PHORES
Jan.	626	134	21.4	306	43.8
Feb.	611	117	19.1	307	38.1
Mar.	547	127	23.2	309	41.1
Apr.	571	133	23.3	317	41.9
May	324	56	17.3	158	35.4
June	303	52	17.2	157	33.1
July	118	26	22.0	61	42.6
Aug.					
Sept.	333	50	15.0	168	29.7
Oct.	522	67	12.8	239	28.0
Nov.	548	46	8.4	219	21.0
Dec.	403	63	15.6	199	31.6