SURVEY OF THE REPRODUCTIVE CAPACITIES OF THREE SEPARATE AGE GROUPS OF WILD-TYPE AMBYSTOMA MEXICANUM (DUTCH STRAIN).

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At the Hubrecht Laboratory Axolotl eggs and (very) young embryos are used for developmental biological research. It is therefore important that during the breeding season (December-August) many batches of high quality are available.

The following method is used to stimulate reproduction. The Axolotls are kept in groups of ten animals, males and females separately. The water temperature is about  $17^{\circ}\text{C}$ . Ten days before a batch is required a male and a female are transferred to separate tanks at a water temperature of  $23^{\circ}\text{C}$ , in which they are kept for seven days. Then the couple is put together; the water temperature is maintained at  $15^{\circ}\text{C}$  and the animals are kept in the dark. The next day spermatophores are deposited on the bottom of the tank and the day after that egg deposition follows.

During many years data concerning the reproductive capacity of Axolotls have been collected and screened in order to gain more insight into the optimal age for breeding and the optimal period during the breeding season. Three items have been taken into account:

- (1) the percentage of batches produced;
- (2) the percentage of unfertilized batches;
- (3) the percentage of normal larval development (scored at st. 35-36 Harrison).

In good-quality batches items 1 and 3 score high and item 2 scores low. The percentages of batches produced and the average percentages of normal development of batches produced by three age groups during six breeding seasons are given in Graph I. An age group is a group of siblings reared in a given year, and is indicated by a numeral (75-80).

The percentages of unfertilized batches (item 2) are so low (between 0-2%) that they could be left out of consideration. This is in itself an important fact.

In the graph the line at 65% separates the good batches (above) from the bad ones (below). As soon as both percentages drop substantially below this line the age group has to be replaced by a younger one.

The graph shows that an age group can be used for four seasons. The quality of the batches is highest during the first season. It is still good during the second and third seasons but gets low during the fourth. This is a general phenomenon, which has also been found in other age groups not represented in the graph. In particular the percentages of batches produced decline substantially with increasing age. In the 1980 age group it is already too low during the second season, but this is an exception.

These results mean that age has an influence on the reproductive capacities of Axolotls. When a continuous supply of good-quality batches is required a group of siblings should be reared at least every three years.

Another aspect is the possible influence of the time of year on batch quality. Graph II shows the average percentages of normal development of age group 1977 during four subsequent breeding seasons. The corresponding percentages of batches produced are given in Graph III. It is clear that the results in June and July are worse than those during the first part of the season. This holds for each season. The only exception is the high percentage of normal development in July of the first season; however, the corresponding percentage of batches produced is low, so that after all the total result is bad. From the second half of April onwards the couples then put together have already produced a batch earlier in the season (about four months ago). This also might be a factor influencing the reproductive performances. When no batch is produced this is most often due to failure to deposit spermatophores. Females very rarely fail to react to spermatophore deposition.

The conclusion to be drawn is that couples put together for the second time in a season and at the end of the breeding season are often unable to stimulate one another enough to produce a batch. This holds especially for older animals. Experiments in which Axolotl eggs and (young) embryos are used should therefore be carried out preferentially between December and April.

In order to reduce the risk of obtaining low-quality batches or no batches at all, during each breeding season we use an older and a younger age group simultaneously.

## LEGENDS TO GRAPHS

## Graph I

```
I -75 = season 1976-1977
II -75 = season 1977-1978
III-75 = season 1978-1979
IV -75 = season 1979-1980
V -75 = season 1980-1981

I -77 = season 1978-1979
II -77 = season 1979-1980
III-77 = season 1979-1980
IV -77 = season 1980-1981
IV -77 = season 1980-1981
IV -77 = season 1981-1982
II -80 = season 1981-1982
II -80 = season 1982-1983

★ = percentage of normal development (average)

★ = percentage of normal development (average)

★ = percentage of normal development (average)

★ = percentage of normal development (average)
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At the head of each column are indicated the numbers of couples in the three age groups.

## Graphs II and III

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△ = first season 1978-1979

○ = second season 1979-1980

■ = third season 1980-1981

▲ = fourth season 1981-1982
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Vertical lines: start of second-time use of couples (see text).



