EXPERIMENTAL INDUCTION OF THE PRODUCTION OF EPHIPPIA
BY DAPHNIA MAGNA STRAUS (CLADOCERA)

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INTRODUCTION

The ability of natural and laboratory populations of the cladoceran genus Daphnia to reproduce either parthenogenetically or by sexual means, the latter accompanied by the formation of dormant eggs enclosed within a thickened, pigmented portion of the brood chamber, the ephippium, has long been recognized, and the identity of the factors, extrinsic and/or intrinsic, responsible for the shift from one form of reproduction to the other has been the source of much speculation (see reviews of Banta, 1939, and Berg, 1934). However it is only recently, and largely from the studies of Stross (Stross, 1969a, b; Stross & Hill, 1965, 1968), that the effects of any of these factors have been measured quantitatively. Thus, for example, interactions between photoperiod and population density provide the stimulus for induction of sexual reproduction in D. pulex (L., 1758) (cf. Stross & Hill, 1965) and an arctic population of D. middendorffiana Fischer, 1851, (cf. Stross, 1969a), the critical photoperiod for the latter (L22: D2 at 12° C) being greater than that for D. pulex (L12.75: D11.25 at 12° C), a finding in accord with the latitudinal separation of the two species.

Stross & Hill (1968) also reported that, under inductive conditions, D. pulex would produce sexual broods predominantly if not exclusively and for the duration of the experiments. Some observations by the present authors using D. magna, suggested that, although reproduction in this species might also be to some extent controlled by interactions between photoperiod, population density, and other environmental parameters, the response to inductive conditions did not seem to be of long duration, the animals reverting more or less rapidly to parthenogenesis while maintained under the same conditions that initially stimulated them to produce sexual broods. In view of this apparent difference between D. magna and D. pulex the following experiments were conducted.

MATERIALS AND METHODS

Stock cultures of Daphnia magna and D. pulex of supply house origin were maintained in 10 liter glass battery jars containing filtered lake water, and fed as necessary on a commercial preparation of dried yeast. Subcultures were established at regular intervals. The stock cultures were held at temperatures between 18° C and 21° C and were illuminated by daylight except when more precise
control of temperature and photoperiod was required. In the latter instances the stock cultures were transferred to light-tight water baths in which the temperature could be held within 0.5°C of that desired. Illumination for these baths was provided by "cool white" fluorescent lights such that the intensity of light falling on the surface of the baths was measured, by photoelectric cell, as being 5920 lux. Photoperiod was controlled by automatic time switches.

These water baths were also used to examine the responses of experimental groups to various environmental conditions. The groups were kept in 30 ml Nalgene beakers containing filtered lake water and placed in 20 cm diameter specimen dishes. The latter were supported within the water baths by sheets of wire mesh and contained water to a depth matching the level in the beakers.

The *D. magna* individuals used in the experiments were selected as follows: Ten to fourteen days before the start of an experiment all of the adults and most of the older immature animals were netted from one or two stock cultures. The remaining immature animals were then used to start a new culture. Shortly before the ecdysis terminating the adolescent instar (the last juvenile instar) the ovaries become visible as two dark strips, one on either side of the digestive tract. All animals with ovaries in this condition were removed from the culture at a time when the culture contained many individuals approaching sexual maturity. The females selected were checked every 30 minutes until a minimum of 96 animals that had molted within 3 hours of each other had been obtained. These were then used to begin an experiment.

For those experiments in which it was necessary to use animals born and raised at particular photoperiods, complete stock cultures were transferred to water baths providing the required temperature and photoperiod. Any young produced were removed daily, and raised to maturity under the same conditions in other battery jars. Upon reaching maturity they were treated as described above.

Experimental populations of *D. pulex* were obtained by collecting the offspring of 20 to 30 adults produced over a two day period. When young born and raised under test conditions were required, 50 to 60 adults were kept under the appropriate conditions and their offspring collected at 3 day intervals. On some occasions the young produced by these adults during the course of one day were collected late in the afternoon of that day.

The experimental populations of *D. magna* were separated into 10 test groups and 6 to 14 reserve groups. Each group usually contained 6 individuals though some experiments used groups containing from 1 to 8 animals per 25 ml water. Each morning the beakers holding the animals were examined and ephippia, cast exoskeletons, progeny, and dead animals were removed. Dead animals were replaced by others taken from the reserve beakers maintained under the same conditions. Occasionally some beakers were examined more than once per day if, for example, it seemed probable that liberation of young and/or ecdysis would occur during the day.

During the daily examination the following data were recorded for each group: