COLLECTING SITES:

The location of the collecting sites is shown in map (II). Specimens of both sexes of both species used in this study, were captured alive in their native habitats surrounding the valley of Srinagar, in the State of Jammu & Kashmir. Majority of *Agama tuberculata* specimens were collected in the foot-hills of Ganderbal, 14 miles North East of Srinagar (Fig. I) at an elevation of 5,800-6,400 feet elevation; although a few collections were made from other areas as well (Baramulla hills, 35 miles North-West of
The ecological habitat: (Ganderbal) from where Agama tuberculata specimens were collected during the investigation.

Study area of Gulmarg - Khilanmarg, from where Lygosoma himalayanum specimens have been collected.
Fig. 3. Dorsal view of *Agama tuberculata* in its breeding pattern (Photograph taken in June).

Fig. 4. Ventral view of *Agama tuberculata* (Photograph taken in June).

Fig. 5. Dorsal view of *Lygosoma himalayanum* in its breeding pattern (Photographs taken in the month of June).

Fig. 6. Ventral view of *Lygosoma himalayanum* (Photograph taken in the month of June).
Srinagar, Acharbal mountains, 40 miles South-East of Srinagar, and Bandipur area 34 miles North-East of Srinagar. Being easily accessible and nearest to the place of investigation, Ganderbal catches were primarily relied upon for compilation of data, although data from other sites (referred earlier) were combined for purposes of comparison. There were no obvious seasonal differences in the data compiled from populations from different localities with the result that data embodied in the text refer to the samples taken from Ganderbal area largely.

On the other hand, both male and female specimens of *Lygosoma himalayum* were collected at an elevation of over 7500 - 8500 feet, from Gulmarg - Khilanmarg continuum about 32 miles West of Srinagar (Map II, Fig. 2), where this species seemed to be apparently very commonly occurring. Although sample collection of the species was extended to other areas like Pahalgam (60 miles East of Srinagar), Sonamarg Thajiwas (62 miles North-East of Srinagar) and Yusmarg (28 miles South-East of Srinagar), these areas did seem to hold these lizards in sizeable numbers. Trips to any of these places turned out invariably futile. The various data pertaining to this species given in the thesis have been compiled from population from Gulmarg - Khilanmarg regions.
HABITAT:

Agama tuberculata is a rock loving lizard, the active period of which extends from late March to end of November. In their active period during day time, when the sun is high, these lizards are normally found basking or feeding over the rocks under which the lizards take cover on slightest disturbance or intrusion. Their normal period of hibernation (brumation of Mayhew, 1965) extends from late November to late March. The lizards appear to prefer rocky thickets with their density decreasing towards the slopes. The conifers and perennial scrub are associated with the residence of this lizard.

Lygosoma himalayamum dwells in the forest and open meadows, where they are seen crawling stealthily under the grass or the scrubs during the day time during the six odd months from late April to October. The period of hibernation begins shortly before the first snowfall in November and extends to around the middle to end of April.

SAMPLING DESIGN:

Collecting of Agama tuberculata was planned to provide a minimum of ten to fifteen lizards monthly from emergence from in late March to entrance into hibernation in late November. The collection trips were
made once two weeks and twice each month from October, 1970 to November, 1972 (period of hibernation excluded). This schedule was adhered to except when inclemency of weather would make it impossible to reach the site. Most lizards were captured by noosing and sink traps of various sizes. Picking by hand was tried quite often but with little success and traps yielded varying success. All collection was done in day time and the total number of 333 lizards of both sexes (Table VI) were captured during the period under report.

The *Lygosoma himalayense* lizards were less difficult to catch and were easily collected by hand picking and noose. The collection of species was done from October, 1970 to October, 1972, save for the months of hibernation. In all 393 individuals of the species were collected, mostly by turning rocks, decaying felled conifers and raking debris of wood piles around. For this species also, the periodicity of collection trips was once two weeks and twice each month. Monthwise sample sizes were shown in Table (VI).

**PROCESSING OF MATERIAL:**

All *Agama tuberculata* lizards collected during a collection trip were immediately sorted out into juveniles and matures. The approximate snout vent length of reproductively mature males and females for
Agama tuberculata, being known (Duda unpublished data), at the beginning of the investigation were arbitrarily employed in assortment of juveniles and matures as given below:

- **Juveniles** up to 69 mm
- **Mature males** 70 mm or above
- **Mature females** 75 mm or above

But in the absence of any such data pertaining to Lygosoma himalayanum, periodic dissections made by the author revealed the following snout vent lengths as near precise to differentiate a juvenile from a mature Lygosoma himalayanum lizard.

- **Juveniles** up to 39 mm
- **Mature males** 40 mm or above
- **Mature females** 40 mm or above

Consequently lizards of both sexes, 70 mm or longer in case of Agama tuberculata and 40 mm or longer in case of Lygosoma himalayanum were, as far as possible, brought alive to the laboratory for subsequent processing. Those lizards which were regarded as juveniles were, however, let off and only few preserved in 4 percent formalin immediately upon capture.
GROSS MEASUREMENTS:

All the measurements, weighings and processing relevant to the present study were done in the laboratory after the animals were chloroformed the following day i.e. within twenty four hours of the catch. The body weights were taken with the help of a simple Kero balance and recorded. The snout vent length was measured from the tip of the snout to the anterior end of the cloacal opening with the help of the plastic ruler (graduated in mm) and recordings made to the nearest mm. These were the only measurements recorded from chloroformed specimens.

After the preliminary weighings and measurements the lizards were dissected for examination of gross morphology of the gonads and their ducts. From the ovaries the number of the yolked eggs and corpora lutea were noted and recorded. The bodies of the lizards were then fixed in 4% formalin and preserved in formol - alcohol (1:1) for subsequent use.

However, a varying number of testes and ovaries along with their ducts destined for sectioning were twice each month taken from males and females respectively of both species and fixed in fluids (given below) appropriate to the various stains tried in the study. The gross measurements of the gonads like weights
and the diameters were obtained prior to the fixation of the gonads. With the help of the sensitive August Sartorius balance, the weights of testes and ovaries were done to the nearest milligrams measure. The diameters of testes and the ovaries were measured to the nearest .01 mms with Vernier callipers. These data were recorded twice each month except for during hibernation months. The number of replicates in each observation was around 6.

MICROTECHNIQUE:

Histological processing

The fixatives used during the study were Zenkers fixative, Bouins fixative, Formalin and Smiths fixative. The sequence of the list does not indicate any order of preference or usage.

Zenkers fluid also proved efficient but for the hindrances during subsequent staining, which needed the section to be pre-treated with Lugol's Iodine to remove crystals of mercuric chloride. Therefore, was not tried after a few initial trials.

Bouin's fixative did not give very satisfactory results. This fixative rendered tissues hard, particularly the testes, which would not remain supple after 24 hours of fixation. Moreover, it is extremely time consuming to remove the fixative out of the tissue.

The use of formalin was not so satisfactory, though
a few trials with this fixative were made. All the same it worked well with Hematoxylin stain.

Of these fixatives Smith's fixative worked the best with any stain used. This fixative wants the tissue to be in it for about 48 hours without producing any hardening effect on the tissue.

After fixation in Smith's fluid, the fixed tissues were washed in tap water and preserved in 70% alcohol, in which fluid the material stayed perfectly fixed for sufficiently long periods till further processing or sectioning would be done.

For sectioning, the usual processing of dehydration through alcoholic grades was carried out. Cleaning of material was done in xylol. From xylol the material was placed in xylol wax at 38°C for about two to three hours. The material was then transferred to pure wax, at its melting point. Mostly, Histomat wax of the melting point of 52 - 54°C was used for impregnation and block preparations during the period of study.

The blocks were sectioned at 6μ to 8μ. The adhesive used was Meyers albumin and spreading done over a hot plate. The slides were then kept for drying in Memmert TV 10 oven at 10°C before staining was undertaken.

The stains used in the present investigations were:
1) Delafields Hematoxylin stain counterstained with Eosin stain.

ii) Heidenhains iron hematoxylin stain.

iii) Harris's hematoxylin - eosin stain.

iv) Mallory's triple stain.

Of these stains Heidenhains hematoxylin and Delafield's hematoxylin Eosin stain worked very satisfactorily for testes. These stains worked far better than Mallory's triple stain in case of testicular cells. However, Mallory's acid fuchsin analine blue and orange-G stain worked more satisfactorily in staining of ovarian tissues. Harris'Hematoxylin proved good for testicular cells, oviducts, ovaries, and epididymis. Harris' Hematoxylin was, therefore, mostly favoured.

**Micrometry:**

All measurements from sections were made using an ocular micrometer. Seminiferous tubules in true or nearly true cross sections were used for diameter measurements. The height of seminiferous epithelium was determined from suitable transverse sections. As the epithelial height of the epididymis varies the measurements were made in the most columnar region to facilitate standardization. The diameters of interstitial cells and their nuclei were compiled from about twenty best showing cells in sections. The ovoid nuclei were
measured in both length and breadth and a mean of the two obtained. Monthly variations in these measurements were recorded and plotted on time.

Photomicrographs were had on Olympus photomicroscope on a DK-3 slow speed documentation film.

**GRAPHIC PLOTTINGS:**

Variations in the testis weight, testis diameter, ovary weight, seminiferous tubule diameters, interstitial cell measurements, epididymal epithelial heights were recorded monthwise, for two years in succession and curves plotted on graph against time.

**EXCAPES:**

An occasional lizard was seen or collected on warm days during early winter or in early spring. A single male specimen of Agama tuberculata was collected on 14th of December, 1970, which would correspond to much later than the beginning of hibernation. Likewise in case of *Lygosoma himalayorum* a male specimen was collected on 10th of November, 1970 and a female specimen on 18th of November, 1971. A solitary female specimen was collected on 20th of April, 1971 and a male specimen on the 18th of April, 1972.

**METEOROLOGICAL DATA:**

The records of the meteorological department of the State of Jammu & Kashmir from October, 1970 to
December, 1972 for Ganderbal area show the maximum air temperature of 31.2°C and minimum temperature of -3.6°C during the period of the study. The temperatures as recorded by the author on the days of the collection trips to Gulmarg and Khilanmarg for *Lygosoma* collection are recorded in Table (1).
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