

## A new rapid method for the relaxation and killing of slugs

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Anyone interested in the systematics of slugs faces the problems of killing and preservation. For a reliable identification dissection is usually necessary. Material preserved without special care is often not very suitable for dissection. This may be either because the specimens are shrunk and hardened after having been thrown into the preservative without any previous treatment, or because the internal organs are macerated. The latter situation is most often encountered in large slugs, especially *Arion* (*Arion*) species, which have been drowned in water in order to relax, prior to preservation. The water absorbed by the animal causes dilution of the preservation fluid, usually ethanol, which makes it less effective. Another disadvantage of relaxation of slugs by means of drowning is the large variation in duration of the killing process. During cold weather this may take over 24 hours, whereas during very hot weather a few hours may be sufficient to start rotting.

Last, but not least, the long lasting dying process is nowadays by some people considered to be no longer ethically acceptable.

Van der Schalie (1953), Runham et al. (1965), and Meier-Brook (1976) recommended the use of barbiturates like nembutal and pentobarbital for the relaxation of different groups of molluscs. Apart from difficulties in obtaining these chemicals, my experiences in slugs are not too good. Nembutal often causes the protrusion of parts of the genitalia, which complicates the study of their morphology.

I have obtained excellent results by putting slugs into about 25% ethanol or methylated spirits. The main advantages are the following:

- The animals stretch themselves almost to the extent obtained by drowning, without noticeable deformation of the internal organs; especially in medium-sized and large arionids deformation of the body occurs less frequently.

- The animals are killed within a few minutes; moreover, the ethanol is likely to have an anaesthetic effect.

- The animals can be kept in the fluid for a considerable time, without the deterioration of the internal organs (at least 12 hours, but probably longer).

- The final preservative needs not to be changed several times, at least with regard to its preservation properties, especially if it is a little stronger than usual, e.g. 80% instead of 70% alcohol. This is a great advantage during field work, when ethanol cannot be carried in unlimited quantities.

- Spermatophores, especially of arionids, very rapidly desintegrate in the bursa copulatrix by means of enzymes. This process continues during drowning in water, which is one of the main reasons that spermatophores are relatively rarely encountered in collections. The use of diluted ethanol as a relaxation agent slows down this process and maybe stops it altogether.

— The animals excrete less abundant mucus. The mucus is less sticky and can be more easily wiped off by means of a tissue or absorbent toilet paper. This is necessary in large slugs, to facilitate the penetration of the final preservative.

The concentration of the relaxation fluid is somewhat dependent on the size of the animal and the thickness of its skin. Agriolimacids react more strongly to a solution which is satisfactory in e.g. arionids. I have carried out a few experiments in order to get some idea about optimal concentrations. Agriolimacids are best killed in 15% ethanol. However, even in 30%, the animals are excellent for dissection, as no deformation of the genitalia occurs, compared to the drowning method. In larger species (*Malacolimax*, probably all Arionidae, *Limax*) 25-30% ethanol is satisfactory. If the animal needs to be stretched out to its fullest extent, i.e. including the extrusion of the tentacles, 10% ethanol is recommended, but the preservation properties of this solution are much less, which nullifies some of the advantages described above. Nevertheless, the animal is immobile within a few minutes, and can be preserved after about an hour.

In my limited experience this method is of little use in shelled land pulmonates. Even a solution as low as 10% caused *Cepaea nemoralis* as well as *Trichia hispida* to withdraw permanently into their shells.

#### REFERENCES

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- MEIER-BROOK, C., 1976. An improved relaxation technique for molluscs using pentobarbital. — *Malac. Rev.* 9: 115-117.
- VAN DER SCHALIE, H., 1953. Nembutal as a relaxing agent for molluscs. — *Am. Midl. Nat.* 50: 511-512.

#### SAMENVATTING

Als methode om (land)naaktslakken snel en humaan te strekken wordt aanbevolen deze in 25-30% alcohol (ethanol) te plaatsen, waarna de dieren binnen enkele minuten dood en bovendien gestrekt zijn. Ze kunnen tenminste 12 uur hierin blijven zonder gevaar voor aantasting van de inwendige organen. De voordelen van deze methode boven de klassieke verdrinkingsmethode worden besproken. De voorgestelde methode lijkt ongeschikt te zijn voor huisjesslakken.