webs of the feet being spread to their fullest extent. 8. The whole operation lasted 2 h. Wager² described the nest building of the South and Central African species C. xerampelina in which only a single male assisted the female. Duff-McKay informs me that he has observed the construction of nests by the East African species C.petersii in which, again, only one male participates.

The co-operation of several males in this activity seems most unusual in the vertebrate kingdom. The construction of a foam nest is by no means an unusual adaptation among the amphibia for rendering their eggs independent of water during the early stages of their development. It seems possible that the advantage of using more than one male may be that a larger nest can be constructed and thus a larger number of eggs can be reared. In addition, because this activity leaves the female exhausted, the assistance afforded by extra males may render her less susceptible to predation at the end of the operation.

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¹ Schiotz, A., Vedensk. Medd. Dansk. Naturh. Foren., Kbh., **127**, 19 (1964). ² Wager, V. A., Trans. Roy. Soc. S. Afr., **163** (1926).

³ Wager, V. A., Afr. Wild Life, 11, 29 (1957).

A Method of Marking Fish Eggs and Larvae

An entirely satisfactory method of marking very young fish has not yet been found, but an experiment carried out at the Windermere laboratory of the Freshwater Biological Association has yielded promising results.

The method is based on two observations; first, that sudan black can be used to dye adult living carp¹, and, second, that in trout the fat soluble carotenoids apparently pass unchanged from a female parent trout into the egg and from there into the young fish². These observations suggest that if a fat soluble substance is fed or injected into an adult female parent fish it may pass into the egg and young fish in detectable quantities.

Female brown trout Salmo trutta L. were fed with dry hatchery pellets that had been mixed with a small quantity of dry sudan black powder. The brand of pellets used contained 4 per cent fat and the dye was quickly taken up. These pellets were readily eaten and within 2 weeks the whole fish took on a blue-black appearance that was particularly marked around the jaw and inside the mouth. When one of the fish was dissected it was found that the blue colour was present throughout the flesh, gut and all internal organs, particularly in the fat around the alimentary canal. The surface mucus from this fish was also distinctly blue. Later the live fish were stripped and yielded dark blue eggs which were fertilized with sperm from an undyed male. The eggs were treated in the normal hatchery manner and were kept in running Lake Windermere water, the temperature of which fluctuated naturally from $4 \cdot 2^{\circ}$ to $10 \cdot 2^{\circ}$ C. After 10 weeks the eggs hatched to yield dark alevins with dark blue yolk sacs. The late stage alevins were given artificial trout-fry food and after feeding commenced it was noted that the blue colour became less noticeable. It persisted, however, to a useful extent for more than 6 weeks after feeding commenced. The faeces of the blue fry were noticeably darker than those of normal trout of the same age fed the same food. The colour lasted longest in the region of the lower jaw and in the brain. The fry appeared to be completely unaffected by the dye and deaths throughout the rearing period were low.

Although this method of marking has some disadvantages, it does allow the eggs from particular parents to be used in population experiments in the field and makes it possible to follow the fry as a group to the stage when they are free feeding individuals. The method is clearly capable of further refinements, for example, it may prove possible to inject the dye into the parent fish so that different-coloured individuals can be kept together, or to use a more persistent colourless substance that can be identified chemically in trace amounts.

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 ¹ Loeb, H. A., Kelly, W. H., and Stafford, K. F., N.Y. Fish. Game J., 8, 151 (1961).
² Steven, D. M., J. Exp. Biol., 26, 295 (1949).

Area Treatment to Combat Mosquitoes

FEMALE mosquitoes may become "aware" of a nearby host by a rise in the prevailing concentration of carbon dioxide which causes them to fly off and begin a generally up-wind search^{1,2}. Once activation has taken place the "attack programme" uses multiple clues to target location of which the most important are convection currents from the body of the host, and, during the day, visual stimuli. If the insects fail to detect these emanations from the host, and if the raised concentration of carbon dioxide is maintained, they soon come to rest again, presumably because they adapt to the new concentration of carbon dioxide².

If the mosquitoes were adapted by previous exposure to a very low concentration of some chemical other than carbon dioxide, it is possible that their ability to seek out a host would become impaired. An area treatment with such a chemical may be a useful supplement to the usual protective measures, especially in regions where resistance to insecticides has developed. The object would not be to expel the mosquitoes from the treated **area** but to render them less capable of responding to emanations from the persons or animals that share it with them.

The normal response of *Aedes aegypti* and of *Anopheles quadrimaculatus* to a rise in the concentration of carbon dioxide can be inhibited in just this way by continuous exposure to the vapour of 2-ethylhexanediol-1,3 ('Rutgers 612').

The experiments were made in an apparatus that passes a pre-conditioned air stream (or pair of streams) upward through a chamber containing the test insects³. Using a single air stream and female mosquitoes of mixed ages and demonstrated avidity, and with the air stream drawn from outdoors and conditioned to 27° C and about 50 per cent relative humidity, the number of mosquitoes actually flying was counted at intervals of 1 min to establish the basic activity. The content of carbon dioxide in the air stream was then raised to 0.1 per cent by volume and the counting continued.

Fig. 1 shows typical results for the two species. Excitation by carbon dioxide is rapid with both, but A. aegypti becomes adapted more quickly than An. quadrimaculatus. The first effect of the repellent vapour is to excite the mosquitoes, but the effect lasts only a short time and once adaptation has occurred the addition of 0.1 per cent of carbon dioxide has none of its previous effect. The concentration of '612' vapour was determined by gas chromatography and was generally between 0.5 and 5 p.p.m. This is well below saturation at the prevailing temperature.

In view of the other ways that repellents interfere with the attack programme, it is likely that area treatments with '612' or other chemicals could produce a substantial reduction in the number of bites, and thus in the amount of irritation or disease transmission. The chemical might be absorbed in a porous material, such as expanded vermiculite, and scattered broadcast, or it could be sprayed