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A Novel Biopsy Technique for Monitoring Environmental Contaminants in Fish

David C. Moy[1] and M. C. L. Dredge[2]

[1]Biochemistry Branch, Queensland Department of Primary Industries, Animal Research Institute, 665 Fairfield Road, Yeerongpilly, Queensland, Australia, 4105,
[2]Fisheries Branch, Queensland Department of Primary Industries, Queensland

One of the major problems in detecting and monitoring pollutant chemicals in living organisms is the normally destructive nature of the sampling and testing. In controlled experiments, a large number of animals is required to meet the statistical validation requirements. There is an urgent need for non-destructive methods of sampling and analysis.

Biopsy techniques have long been used in animal studies (FISLER and DRENICK 1972, TREVINO et al 1973) and in various medical applications in humans (EDWARDS 1973). The methods do not appear to have been successfully applied in environmental samples involving small animals, birds and fish. This seems to be attributable in part to the size of core sample extractable with the biopsy instruments normally available. However, with the rapid advancement of analytical techniques which permit of the reliable analysis of smaller and smaller samples, it seems timely to report on the successful use of a biopsy sampling program during a large scale tagging of sea mullet (Mugil cephalus Linnaeus) from a polluted estuarine region.

METHODS

The tagging-biopsy procedure was performed in the field with one operator standing at the head of the fish and placing the operculum tag with pliers held in one hand, while holding the upper part of the fish with the other hand. At the same time, a second operator steadied the lower portion of the fish and extracted a small piece of musculature for subsequent chemical analysis. This biopsy was performed with a 16 gauge stainless steel needle of 15° taper attached by a Luer fitting to a 20 mℓ disposable plastic syringe. The needle, taper up, was inserted behind a scale on the upper side of the fish, level with the rear dorsal fin. With the needle lying at an angle of ca 30° to the skin of the fish, penetration could normally be achieved quickly without scale loss or apparent damage to the fish. Suction was then applied by partial withdrawal of the syringe piston - a somewhat difficult one-handed operation. The needle was then pushed in 2 to 3 cm, rotated 180° and

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withdrawn so that the portion of tissue held within the needle was cut from the fish. Just before the bevel of the needle emerged, now face down, the pressure on the piston was released. This prevented the sample being pushed into the syringe barrel by atmospheric pressure on withdrawal. Each needle was used until insertion became difficult. Blunt needles cause unnecessary skin tearing and trauma. After the fish had been released (in the same area from which it had been captured), the syringe needle was passed through a covering sheet of aluminium foil into one of a series of cavities (6 mm diameter, 8 mm deep) drilled into a brass plate. The sample was then ejected into this cavity and frozen for subsequent storage and analysis. Rapid tissue freezing was accomplished by resting the brass plate on a tray filled with salt-ice packs or dry ice (solid carbon dioxide). The tray was strapped at right angles to the operator's body. The cavities in the brass plate were precoded to permit identification of tissue samples from individual tagged fish.

The small sample size makes quantitative analysis difficult but improved techniques should overcome this problem. In the present work, all samples were scanned for the presence of mineral hydrocarbons (kerosene) using a Carle Thermal Evolution Analyzer operated at 130°C. Samples were taken directly from storage and placed in a small aluminium boat which was then inserted into the oven. The liberated gases were monitored using a Flame Ionization Detector.

In a separate experiment about 30 sea mullet, preconditioned in aquaria, were tagged and biopsied as in the field work. A breakdown of pumping facilities after four days prevented a prolonged examination.

DISCUSSION

Two people could measure, tag and remove a tissue sample from a fish in less than thirty seconds while a third person recorded the relevant information. As the biopsy was performed in the same interval as used for tagging, no additional handling time was required and the trauma of the operation was minimized. Experienced operators attained better than a 90 per cent success with sampling. The size of sample ranged from ca 1 mg to 3 mg. Provided adequate analytical techniques are available, the sample can be used for detection and monitoring a variety of chemicals.

Fish biopsied ranged in length from ca 20 cm to ca 50 cm. Out of 3,500 fish treated only one was observed to be immediately affected by the biopsy. This fish, which on release swam in a circle round the release point, was thought to have been biopsied too

near the lateral line. No mortalities up to day 4 were observed during the aquarium trial on 30 fish.

Further, careful examination of skin and underlying tissue from more than 100 fish returned up to 2 years after tagging revealed evidence of the biopsy on only one fish. No lesions were observed and the fish, in every case, compared favourably both in size and appearance with other members of the schools in which they were caught. It should be noted that disinfectants and similar medications were not used for biopsy.

Problems arise if storage is prolonged as the small sample size facilitates dehydration so that quantitation on a wet weight basis is difficult. Autolysis and oxidation also appear to occur more rapidly and the possibility of contaminant migration and subsequent evaporation can not be overlooked (LAMBERT et al 1973). Rapid freezing on dry ice and analysis without thawing and related drip losses help overcome the problems.

The results obtained in the present work verified the value of the technique for screening large numbers of fish for the presence of mineral hydrocarbon so as to measure accurately the proportion of a population contaminated. Uptake and elimination measurements can also be obtained though difficulties arise with variation in the bodily distribution of the pollutants so that the sampling site has to be restricted for repeated measurements. Work is continuing on the analytical methodology necessary to use the biopsy samples for pesticide monitoring.

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