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A Kerosene-like Taint in the Sea Mullet *Mugil cephalus* (Linneaus) II. Some Aspects of the Deposition and Metabolism of Hydrocarbons in Muscle Tissue

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INTRODUCTION

A kerosene-like taint has caused significant economic damage to the sea mullet, *Mugil cephalus*, fishery based in Queensland, Australia. The taint has been shown to be due to a kerosene-like mixture of hydrocarbons (Connell 1974*; Sidhu *et al* 1970. Although n-alkanes ranging from n-nonane to n-tridecane and several aromatic hydrocarbons were identified by gas chromatography coupled with mass spectrometry the specific compounds within this complex mixture which cause the taint were not identified. It is possible that it may be due to minor constituents, which normally occur in kerosene fractions of petroleum, but are not hydrocarbons. The results obtained indicate that fish absorb the hydrocarbons from contaminated sediments, food or water in the Brisbane River, the site of the major urban and industrial complex in the region.

Previously Sidhu *et al* (1970) found that the presence of kerosene-like hydrocarbons in sea mullet leads to enhanced accumulation of fat in the liver and other tissues. This is believed to be related to hydrocarbon detoxifying mechanisms which also give an elevated proportion of the C18:1 fatty acid in the liver fats. In addition Sidhu *et al* (1970) found that, in aquaria containing water in which trace kerosene was dissolved (4-7 p.p.m.), mullet would become tainted. More recently Gieszler *et al* (1977) have shown that n-alkanes taken up via the digestive tract in mullet *(Mugil cephalus)* are readily metabolised whereas more limited metabolism was observed when n-alkanes are taken up via the gills.

Overall a limited understanding has been obtained of the uptake, metabolism and deposition of petroleum hydrocarbons in higher marine organisms. This present investigation was undertaken into some aspects of this problem as specifically related to the mullet. Other studies, not reported here, are underway on the behavior of petroleum hydrocarbons in marine food webs.

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EXPERIMENTAL

Determination of Lipid and Hydrocarbon Content of Muscle Tissue: A total of 21 tainted sea mullet were obtained from the Queensland Fish Board. The fish were classified subjectively as "heavily tainted" and were 28-33cm long. The muscle tissue of each fish was dissected according to the scheme shown in Fig. 1. To reduce the number of samples for analysis the corresponding segments from randomly selected sets of three fish were bulked to give a total of seven sets of complete segments. The lipid content was determined by blending the weighed sample with an equal volume of water, vigorous shaking of the product with highly pure diethyl ether (50ml) followed by centrifugation and removal of the ether extract. This procedure was repeated three further times, the ether extracts bulked, dried with anhydrous sodium sulphate, concentrated at 100° to remove the ether solvent and the remaining substance weighed to obtain lipid content. The lipid extract was now steam distilled using a 10ml. Dean and Stark distillation trap and the steam volatile material removed by solution in ether. The solution was carefully concentrated at 100° initially and finally at room temperature and weighed to obtain the kerosene hydrocarbon content. Experiments indicated that hydrocarbon content estimated by this technique were approximately ± 20% accurate.

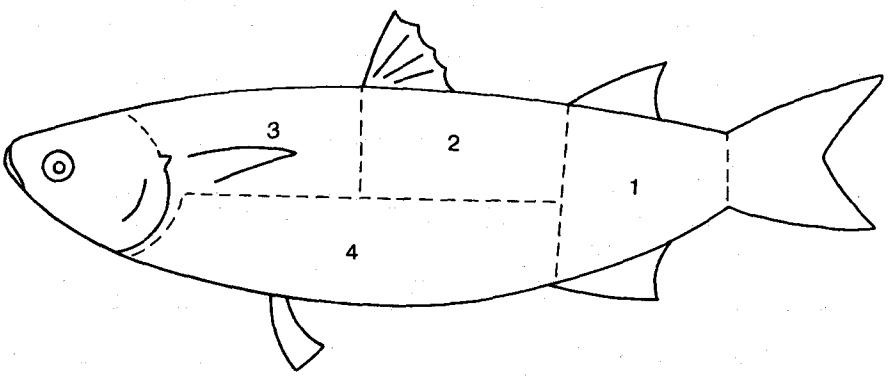

Fig. 1 - Dissection Scheme for Muscle Tissue (Segments 1 to 4 ca. 2 m.m. thick; Segment 5 ca. 5 m.m. thick of remaining muscle; Segment 6 all muscle tissue remaining after removal of other segments).

Analysis of Hydrocarbon Isolates: A varian-Aerograph Model 1400 gas chromatograph (Injector port and detector-200°, Oven programmed from 100°-170° at 2° per min.) incorporating a flame ionisation detector and modified for fitting capillary columns was used with stainless steel columns (50m by 0.5 m.m. i.d.) and nitrogen as carrier gas (2 ml per min). Apiezon M was used as liquid phase.

RESULTS AND DISCUSSION

Relationship Between Total Lipid Content and Hydrocarbon Deposited in Muscle Tissue: In previous work on mullet Vale *et al* (1970) found that mullet containing a kerosene-like taint had a higher lipid content than untainted fish. This could indicate that fish higher in lipids more readily take up hydrocarbons than those low in lipids or that the metabolism of tainted fish is altered to generate excess lipid materials (Vale *et al*, 1970).

In this present study for each set of three fish the average total hydrocarbon and total lipid content for each full set of muscle tissue segments examined was calculated. This data was taken as a measure of the lipid content and the corresponding quantity of hydrocarbon deposited in the muscle tissue of whole fish. Statistical analysis indicated that there was no significant relationship between lipid content and the quantity of hydrocarbon deposited in the muscle tissue of whole fish. This result does not give a clear indication that such a relationship does not exist since the hydrocarbon content could be expected to vary in individual fish with such factors as time of residence in waters containing hydrocarbons and proportion of food containing hydrocarbons consumed. Further work is in progress on this aspect under controlled laboratory conditions.

Distribution of Hydrocarbons in Muscle Tissue: The factors affecting the deposition and distribution of hydrocarbons in muscle tissues are important aspects to gaining an understanding the uptake and metabolism of petroleum hydrocarbons in higher marine animals.

Considering the lipid soluble nature of petroleum hydrocarbons it could be suggested that the lipid content of the various body tissues would influence the distribution of hydrocarbons within fish. Thus for each set of three fish the percentage of lipid and hydrocarbon in the bulked samples of each segment were calculated from the total present in each full set of muscle tissue segments investigated. The results were plotted out as in Fig. 1.

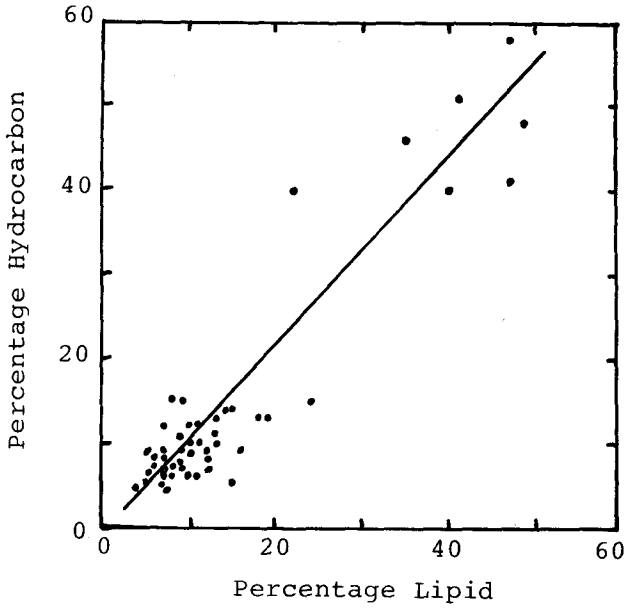

Fig.2. - Plot of Percentage Hydrocarbon against Percentage Lipid for Segments of Fish Muscle Tissue.

Statistical analysis of the data indicated that there was a highly significant correlation between the percentage of hydrocarbon and the percentage of lipid in the muscle tissue segments examined. A Correlation Coefficient = 0.93 was obtained and the calculated line of best fit is shown in Fig. 3. The percentage of hydrocarbon has a Standard Deviation from this line of ± 5.18 while the equation to fit the line is:

$$\left[\% \text{ Hydrocarbon}\right] = 1.086 \left[\% \text{ Lipid}\right] - 1.23$$

Thus with this sample of mullet examined the deposition of hydrocarbons into segments of muscle tissue was strongly influenced by the lipid content.

Metabolism of Kerosene-like Hydrocarbons in Mullet: Recent work by Gieszler (1977) has shown that n-alkanes are readily metabolised by the mullet when taken up through the gut and more slowly when taken up through the gills. It was suggested that micro-organisms in the gut may be responsible for much of this metabolic activity.

In previous investigations (Connell, 1974) the hydrocarbon contamination of mullet has been shown to be due to kerosene-like hydrocarbons which occur principally in the Brisbane River and are absorbed by fish at this location. Typical gas chromatograms of a hydrocarbon isolate from the Brisbane River and from fish obtained in this present study are shown in Fig. 3.

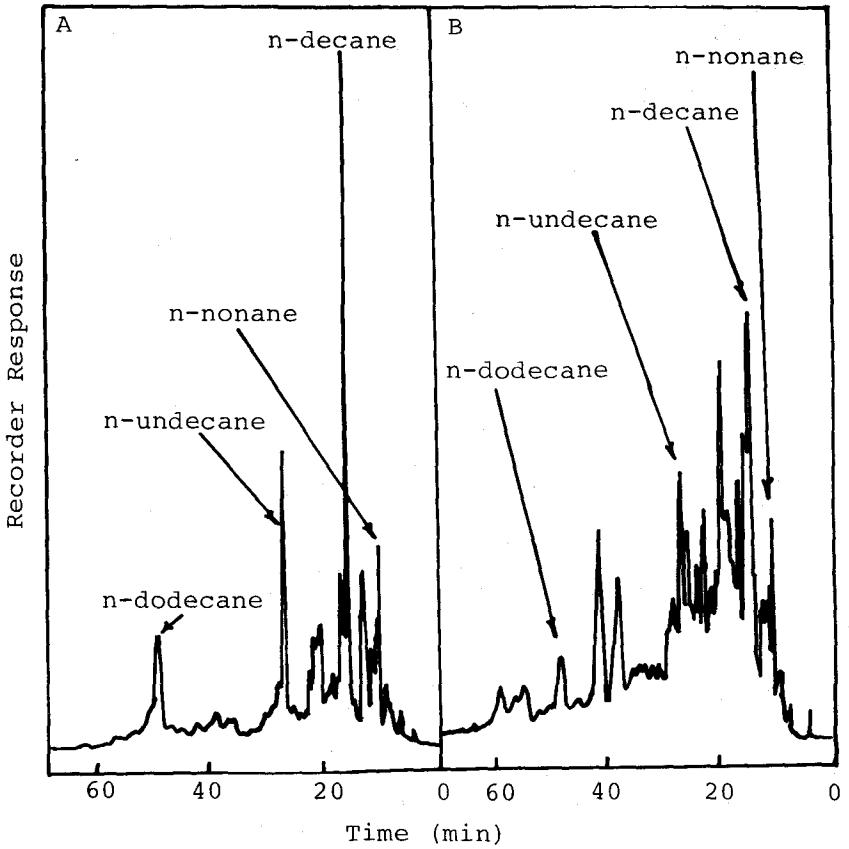

Fig.3. - Gas Chromatograms of a Typical Brisbane River Isolate (A) and a Typical Fish Isolate (B).

In Fig. 3. the large peaks have been identified previously as n-alkanes (Connell, 1974) and from the known composition of kerosene the small peaks in between the n-alkane peaks can be attributed to iso-alkanes, principally, with lesser amounts of cyclo-alkanes and aromatic compounds.

Using gas chromatographic peak areas calculations of the proportions of iso-alkanes and associated compounds in the hydrocarbon isolates were made. Hydrocarbons resembling those from fish and occurring in the Brisbane River contain in the range of 43-65% (Average: 49%) iso-alkanes whereas the contaminated sea mullet examined in this study contained from 63 to 96% (Average: 72%). This supports the results of Gieszler *et al* (1977) that n-alkanes are readily metabolised by mullet and indicates that iso-alkanes are more resistant to metabolic break down. The close similarily in physical and chemical properties of the n- and iso-alkanes would suggest little possibility of differential absorption of these substances from the environment into fish.

SUMMARY

The results obtained indicate that the deposition of absorbed hydrocarbons in different segments of the muscle tissue of the sea mullet is proportional to the lipid content of the segment. However the results give no clear indication as to whether the total lipid content of the muscle tissue of individual fish influences the uptake of hydrocarbons from the environment.

Evidence has been presented for different rates of metabolism of the n-alkanes as compared to the iso-alkanes and related compounds. Metabolic processes in the sea mullet result in the preferential degradation of the n-alkanes leaving a hydrocarbon mixture in the sea mullet enriched with iso-alkanes and related compounds.

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