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# THE ACCUMULATION OF ARSENIC BY THE PLAICE AND THORNBACK RAY: SOME PRELIMINARY OBSERVATIONS

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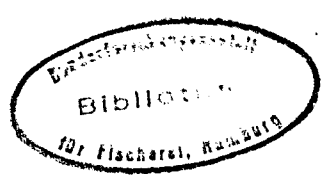
## ABSTRACT

The accumulation of $^{74}As$ - as sodium arsenate - from sea water by plaice eggs, larvae and I-group plaice and rays has been studied, and comparisons made with the accumulation of arsenic from labelled food. Accumulation direct from sea water was slow and resulted in a fairly uniform labelling of all internal organs. In contrast, retention of $^{74}As$ from labelled food was high for the ray (85%) but low (10%) for the plaice. Both species contained the largest fraction of the $^{74}As$ body burden in muscle, although rays also had very high concentrations in the kidney.

## INTRODUCTION

Marine organisms are unique in their exemption from the UK regulations (MAFF, 1966) for the concentrations of arsenic in food: all other foodstuffs must not contain more than 1 µg As/g. The edible portions of a number of marine fauna contain much higher concentrations of arsenic; concentrations which are presumed to derive from the natural accumulation of the element at normal environmental levels. Recent analyses of arsenic in marine fish muscle have shown a large variation in concentrations both within, and between, species. For example Bohn (1975) has reported a range of 17 to 290 µg As/g dry weight for the American plaice (*Hippoglossoides platessoides*), and an overall range of 11 to 512 µg As/g dry weight for a number of different species taken west of Greenland. There has also been a marked trend towards higher levels of arsenic being recorded for elasmobranch than for teleost fish (Windom *et al*, 1973; Le Blanc and Jackson, 1973).



Arsenic is also of particular interest because, like mercury, it exists in the aquatic environment in a number of inorganic and organic forms, the characterization and cycling of which are imperfectly understood (Wood, 1973; Penrose, 1974). Analyses have shown that fish contain a high proportion of organo-arsenic forms of arsenic (Lunde, 1969; Edmonds and Francesconi, 1977) which, fortunately, are less toxic to man than the inorganic forms (Lu, Baker and Henley, 1973). There are, however, few data on the rates of accumulation of arsenic by aquatic organisms, and data for marine fish appear to be lacking.

MATERIAL AND METHODS

Locally caught plaice (*Pleuronectes platessa*) and thornback rays (*Raja clavata*), each within a weight range of 35 to 70 g, were kept at 10°C in filtered sea water. The general procedure of experiments was similar to those described previously for other elements, such as zinc (Pentreath, 1973, 1976 a). To study the accumulation of arsenic direct from sea water, $^{74}As$ (half-life 17.7 days) was added, as sodium arsenate, such as to give ≈ 1 μCi/l, and the concentration was maintained against a standard by daily additions. Sea water contains, typically, 1 to 3 μg As/l; the principal species is believed to be H As $O_4^{2-}$ (Sturm and Brauner, 1975). The addition of the tracer added < 3 ng As/l.

Artificially fertilized plaice eggs were placed in labelled sea water after gastrulation. Live fish were whole-body counted between two 7.6 cm x 7.6 cm NaI crystals, and dissected organs analyzed by auto-gamma spectrometry. The radionuclide content of all samples was counted against suitable standards and corrections made for different counting geometries and for decay. Counting accuracy was frequently not better than ± 20%.

To calculate percentage distributions of the $^{74}As$ in the fish, it has been assumed that for plaice, muscle, skin and bone constitute 55%, 9% and 15% respectively of the whole-body weight: for rays the values used were 44%, 13% and 16% respectively.

RESULTS

The very low concentration factors ($^{74}As$/g wet of organism divided by $^{74}As$/g of sea water) of the plaice eggs, and more rapid accumulation by the larvae, can be seen in Figure 1. The data for the larvae, up to day 8, gave a good ($P < 0.01$) fit to the linear expression

$C_t = It \pm C_o$

where $C_t$ is the concentration factor at t, I the input/g/t in equivalent mls of sea water, t is time in days and $C_o$ the concentration factor at $t_o$. The value obtained was

$C_t = 0.23\ t + 0.20$

and thus at 2 µg As/l the larvae had a net uptake of 0.46 ng As/g/day.

Five plaice and eight rays were maintained in separate tanks of labelled sea water for six weeks, and repeatedly whole-body counted. Accumulation of the $^{74}As$ was slow for both species (Figure 2) and again a linear equation was applied:

Plaice: $C_t = 0.006\ t + 0.17$ $(P < 0.01)$

Ray: $C_t = 0.004\ t + 0.10$ $(P < 0.01)$

Thus, again at 2 µg As/l, these represent accumulation rates of 0.012 and 0.008 ng As/g/day for plaice and rays respectively.

After six weeks four of each species were dissected and their organ concentration factors calculated (Table 1). The $^{74}As$ was fairly evenly distributed in both species, and the scatter of the data was such as to preclude any conclusion as to specific organ preference.

To study the retention of $^{74}As$ from food, live *Nereis* were maintained in labelled sea water for seven days, immersed in non-labelled sea water for one day, and then fed to the fish. Three fed plaice and five fed rays were repeatedly whole-body counted for five to six weeks - during which they were fed daily on non-labelled *Nereis* - and then dissected. A further three plaice were fed and dissected five days later following complete defecation of the *Nereis*. The organ concentrations of $^{74}As$ obtained are given in Table 2.

The initial three fed plaice each retained 6, 12 and 13% of the $^{74}As$ after defecation, and these fractions were excreted with biological half-times of 22, 32 and 46 days respectively. None of the fits were better than $P < 0.05$, and the best is shown in Figure 3. At the time of defecation, as determined from dissecting the second set of three plaice, the highest concentration of $^{74}As$ was that of the liver, which accounted for some 40% of the whole body-burden value.

After a subsequent thirty days, however, during which half the $^{74}As$ body-burden was lost, the highest concentration observed was that of the muscle which accounted for most of the whole body-burden.

The results obtained with the five rays were markedly different. In contrast to the plaice (Figure 3), the amount of $^{74}As$ remaining after defecation was high, and a mean of 85% ± 6% (SD) was obtained. Longer biological half-times were also obtained, giving a mean of 61 ± 29 days. After 45 days the highest $^{74}As$ concentration was that of the kidney in each fish, but again the largest fraction of the whole body-burden was that of the muscle. The cartilage of the ray accumulated relatively less $^{74}As$ than the bone of the plaice.

DISCUSSION

The data obtained are few but serve to show that different rates of uptake of arsenic from food and water are likely to obtain in the environment. Accumulation direct from sea water would appear to be relatively unimportant for both plaice and rays, although the importance of the food pathway cannot be estimated without concomitant stable element analyses and these have not, as yet, been made.

Previous studies with young plaice have shown that the muscle usually contains one of the lowest organ concentrations for a number of metals (Zn, Mn, Co, Fe, Ag) although, in view of its large fraction of body weight, it may nevertheless contain up to a third of the body-burden (Pentreath, 1977). An important exception is the organic form(s) of mercury (Pentreath, 1976 b, c). It is of interest, therefore, to compare the results obtained with $^{74}As$ labelled food with those obtained with $^{203}Hg$. With the latter, the inorganic form, accumulated by *Nereis* which were subsequently fed to plaice, was poorly absorbed across the gut and only a small fraction appeared in the muscle. No evidence was found for the methylation of the mercury. When fish were fed the methylated form, however, it was readily absorbed and over 80% eventually appeared in the muscle. In the present study with $^{74}As$ the largest fraction of the body-burden of both species eventually appears in the muscle even though it was initially introduced, to the *Nereis*, in an inorganic form. Unfortunately, because samples were taken only for radioassay in order to ascertain the body distribution, no attempt was made at this stage to determine the form of $^{74}As$ in the fish. It is of interest, therefore, that in

studies with the brown trout Penrose (1975), having fed fish with inorganic $^{74}As$, determined that after 72 hours a large fraction of the $^{74}As$ in the gut contents, and most of that in the liver, was "organic". Lunde (1972) had also shown that rainbow trout could synthesise both lipid soluble and water soluble arseno-organic compounds when fed inorganic arsenic. The conversion was considered by Penrose (1975) to be mediated by the intestinal flora.

It is also not clear as to whether the $^{74}As$ which accrues within the liver of the plaice after defecation is that which is lost during the subsequent thirty days, and what fraction of it may, or may not, be transferred to the muscle. A second feature of interest is the marked difference between the intestinal absorption of arsenic by the two species, and the high accumulation of arsenic by the kidney of the ray. In view of the relatively high concentrations of arsenic in marine fish, its high toxicity, and its apparent existence in a number of different chemical forms, further studies would clearly be of interest.

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TABLE 1 Concentration factors of $^{74}As$ of organs of plaice and rays after accumulation from sea water. Values are mean of 4 ± 1 standard deviation

| Organ | Plaice (Day 42) | Ray (Day 43) |
|---|---|---|
| Blood cells | 0.04 | 0.12 ± 0.18 |
| Blood plasma | 0.12 ± 0.04 | 0.06 ± 0.05 |
| Spleen | 0.18 | 0.08 ± 0.07 |
| Liver | 0.13 ± 0.02 | 0.23 ± 0.09 |
| Kidney | 0.89 ± 0.35 | 0.28 ± 0.18 |
| Stomach | 0.15 ± 0.03 | 0.12 ± 0.09 |
| Intestine | 0.44 ± 0.15 | 1.11 ± 0.87 |
| Gill filament | 0.59 ± 0.52 | 0.22 ± 0.13 |
| Skin | 0.47 ± 0.23 | 0.21 ± 0.05 |
| Muscle | 0.36 ± 0.21 | 0.02 ± 0.02 |
| Bone | 0.65 ± 0.23 | - |
| Cartilage | - | 0.08 ± 0.08 |

TABLE 2 Mean concentrations (counts $min^{-1}$ $g^{-1}$) and approximate % of body-burden of $^{74}As$ in selected organs of plaice and rays fed $^{74}As$-labelled *Hereis*

| Organ | Plaice (Day 5) | | Plaice* (Day 35) | | Rays (Day 45) | |
|---|---|---|---|---|---|---|
| | Counts $min^{-1}$ $g^{-1}$ | % | Counts $min^{-1}$ $g^{-1}$ | % | Counts $min^{-1}$ $g^{-1}$ | % |
| Blood cells | 29 | <1 | <5 | <1 | <5 | <1 |
| Blood plasma | 28 | <1 | <5 | <1 | <5 | <1 |
| Liver | 2267 | 40 | 18 | 1 | 142 | 1 |
| Kidney | 186 | 1 | 18 | <1 | 1100 | 1 |
| Stomach | 65 | <1 | 18 | 1 | 25 | <1 |
| Intestine | 88 | 2 | 5 | 1 | 177 | <1 |
| Gill filaments | 52 | 1 | 6 | <1 | 26 | <1 |
| Skin | 38 | 6 | 5 | 3 | 18 | 6 |
| Muscle | 29 | 27 | 23 | 94 | 70 | 75 |
| Bone | 22 | 6 | 10 | 11 | - | - |
| Cartilage | - | - | - | - | 11 | 4 |
| Gut contents | 21 | <1 | - | - | - | - |

*Day-35 plaice received ≈ 3 times the amount of $^{74}As$ as the day-5 plaice

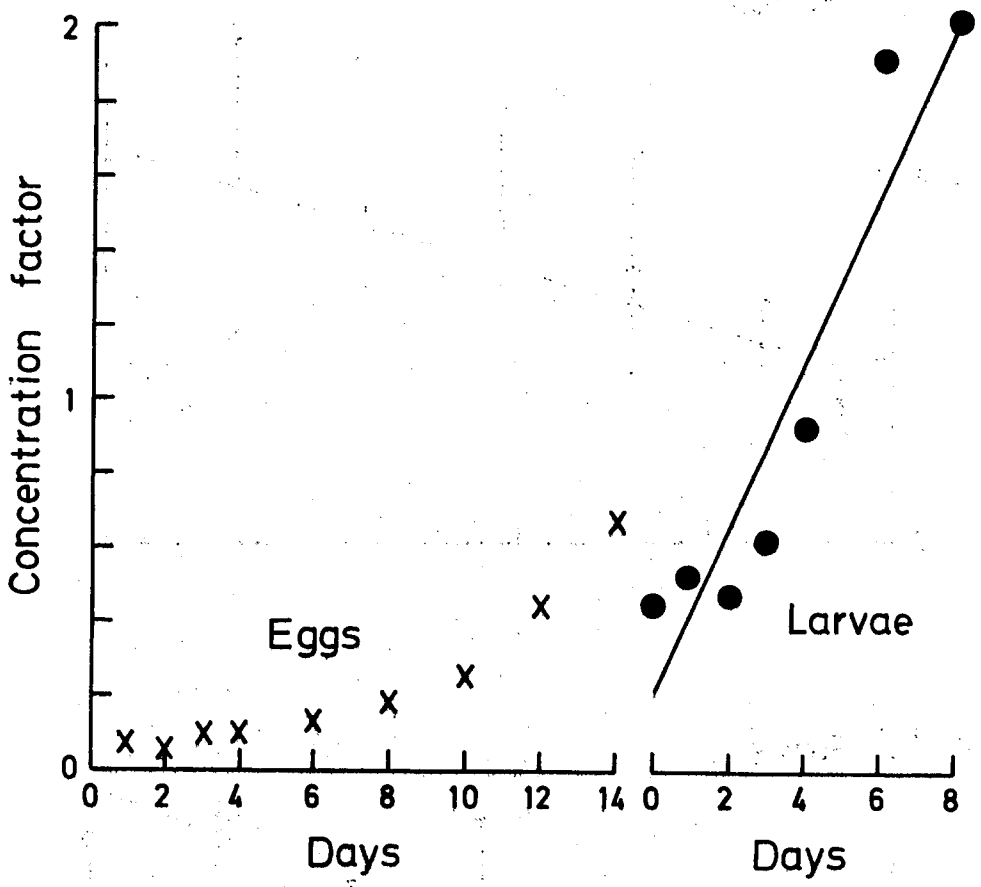


Fig 1. Accumulation of $^{74}As$ by plaice eggs and larvae

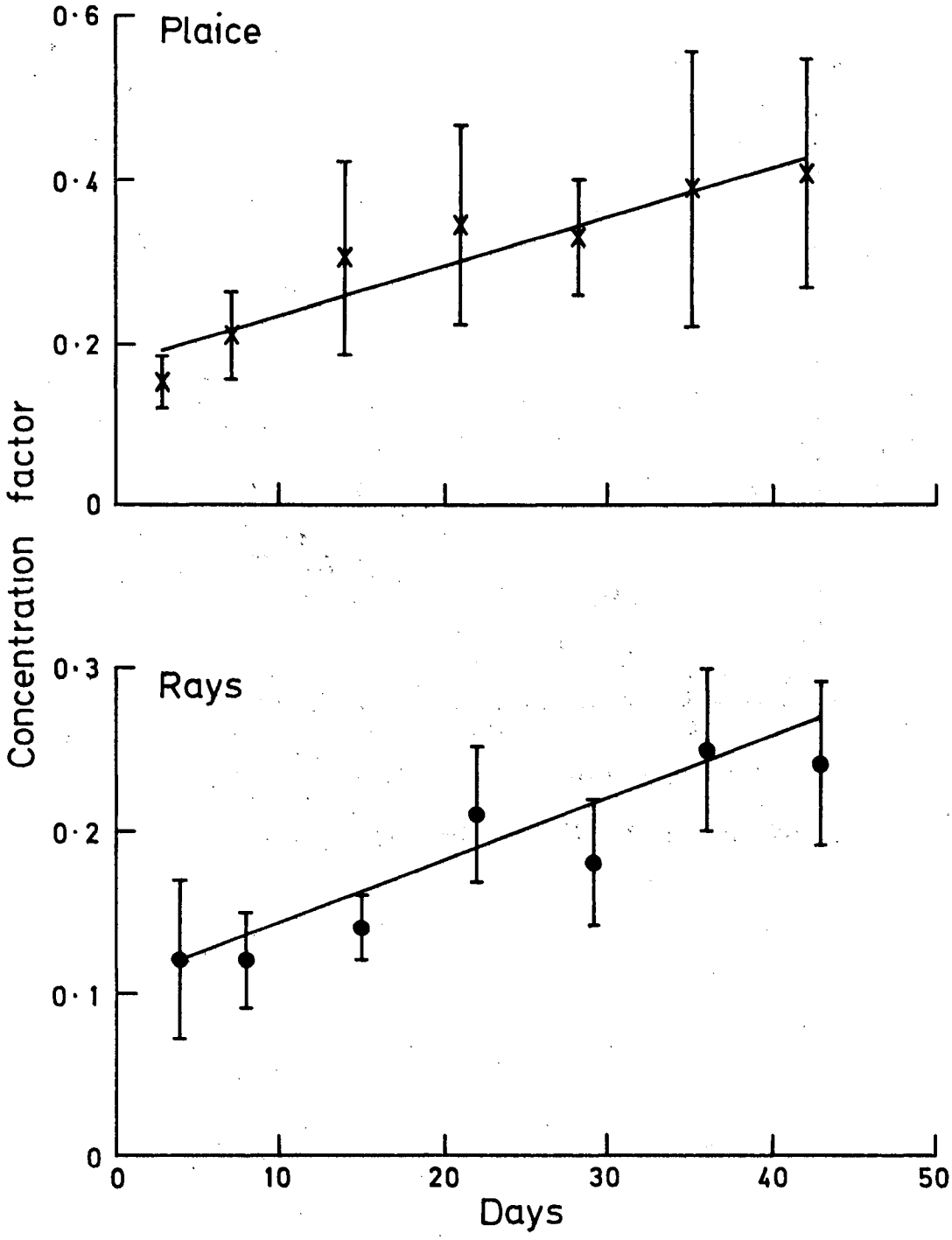


Fig 2. Accumulation of $^{74}As$ by plaice and rays (values are mean ± S.D.)

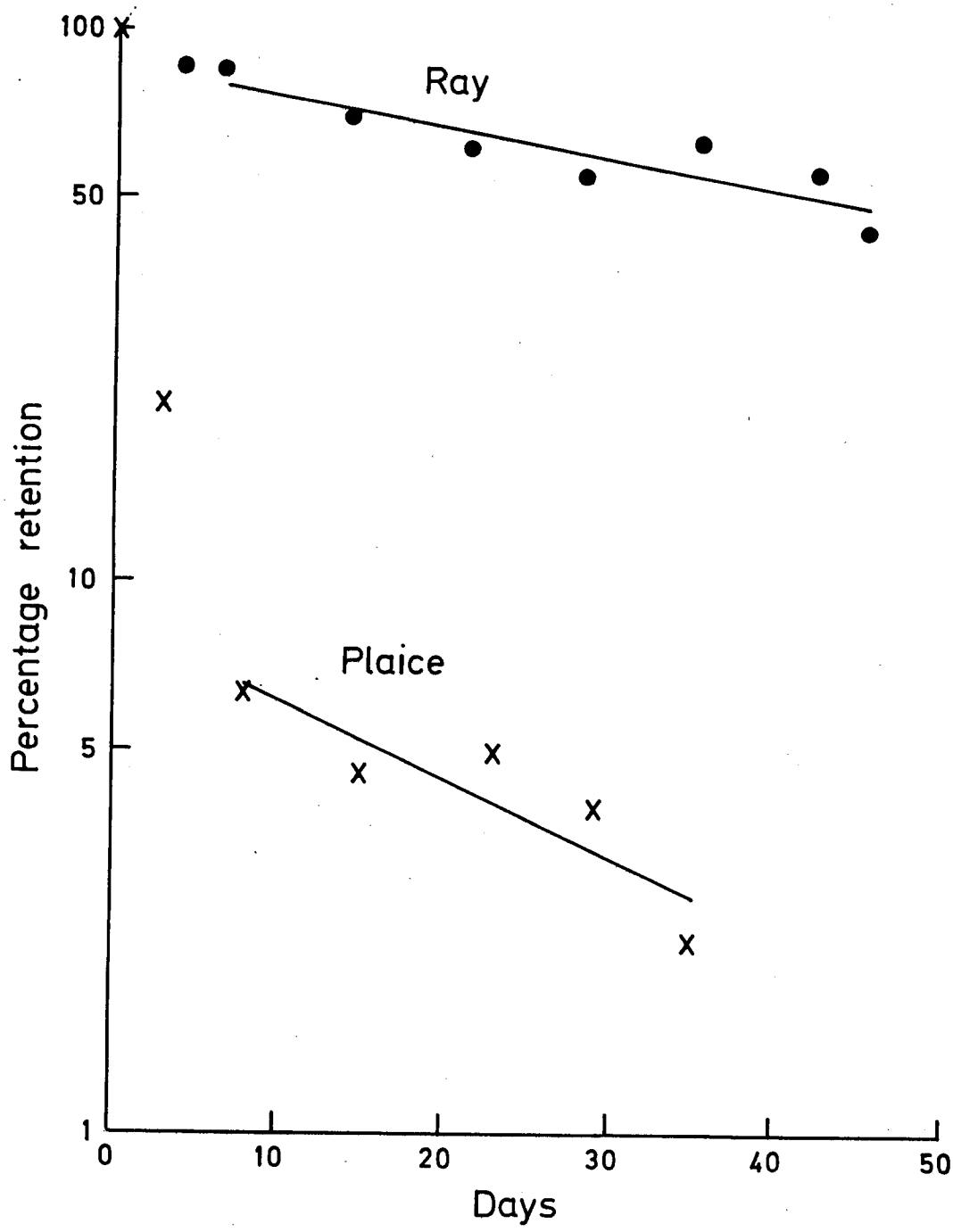


Fig 3. Retention of $^{74}As$ by plaice and ray each fed a single labelled *Nereis* on day 0