

**EFFECTS OF SEDIMENT-ASSOCIATED TRI-N-ORGANOTIN  
COMPOUNDS ON THE OSMOREGULATION  
OF FRESHWATER-ADAPTED 0-GROUP  
EUROPEAN FLOUNDER, *PLATICHTHYS FLESUS* (L.)**

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**Abstract**

Chronic (5 weeks) exposure of freshwater-adapted European flounder, *Platichthys flesus* (L.), to environmental concentrations of sediment-associated tri-*n*-butyltin chloride (TBTCl) and tri-*n*-phenyltin chloride (TPhTCl) caused significant changes to hydromineral fluxes and membrane permeability, mechanisms that maintain osmotic homeostasis. The half-time of exchange of tritiated water (THO) in TBTCl- and TPhTCl-exposed fish was significantly increased during the first 2 weeks of the experiment and then decreased steadily, eventually reaching the level that the control group had constantly maintained throughout the experiment.

This change in apparent water permeability was accompanied by a significant decrease in diffusional water flux across the membranes. Passive Na<sup>+</sup>-efflux across the gills was increased significantly but effluxes in the control group were near constant over the same time span. Drinking rates in the organotin groups increased significantly while the rate of urine production did not change. This led to an increased net water balance in the organotin groups and consequently to a significant reduction of the blood osmolality of both organotin groups when compared to a control. There would appear to be a metabolic cost attached to the changes produced by exposure to organotin compounds which are manifested as a minimal increase in body length compared to the controls.

## Introduction

Following the partial ban on the use of organotin-based anti-fouling paints on boats and maritime equipment in most industrialised countries, water concentrations of organotin compounds have dropped dramatically, albeit with hotspots remaining in areas of intense shipping activity (Waite *et al.*, 1996). However, there is an increasing amount of evidence to show that organotin compounds are persistent in marine and freshwater sediments, which can act as reservoirs and sources for the secondary introduction of organotins to the environment (Waldock *et al.*, 1990; Langston & Burt, 1991; Watanabe *et al.*, 1995; Harris *et al.*, 1996).

Despite this wealth of data there are few studies on the effects on benthic organisms exposed to environmental concentrations of organotin compounds in sediments; the most recent of these being the studies by Krone *et al.* (1996), Rouleau *et al.* (1998) and Werner *et al.* (1998). Although *in vivo* studies by Chliamovitch & Kuhn (1977) and Pinkney *et al.* (1989) have shown that high concentrations of organotins in aqueous suspension disrupted osmoregulation in euryhaline fish, the present study is the first to investigate the effect of environmental concentrations of sediment-bound organotin compounds on a benthic euryhaline fish.

The aim of this study was to detect and quantify any significant effects of chronic exposure of 0-group flounders to sediment-associated Tri-*n*-butyltin chloride (TBTCI) and tri-*n*-phenyltin chloride (TPhTCI) on osmoregulation that might affect the viability of such juvenile fish in terms of their ability to fully exploit a euryhaline environment. In addition to the physiological measures of the effects of organotin exposure, at the level of the whole organism, the metabolic cost was investigated by following the length increase of experimental and control groups.

## Material and Methods

0-group flounders (0.4 - 1.8g) were caught at Woodmill, River Itchen, Southampton (Hutchinson & Hawkins, 1993) and kept in a 3,500 litre glass-fibre fish-farming tank in the hatchery at the Southampton Oceanography Centre, that was shielded from direct sunlight and rain by a roof, but exposed to natural temperature fluctuations and light/dark cycles. Prior to experiments, the fish were sampled from the stock populations and acclimated to tap water at a temperature of 15°C and a light/dark regime of 12 hours on and 12 hours off for at least 2 weeks.

Fish were fed *ad libitum* on live *Artemia sp.* during acclimation and the 6 week experimental period but starved for 24 h prior to sampling (once a week). TBTCI and TPhTCI exposure experiments were performed in 25 litre polyethylene buckets (Carter, *et al.*, 1989) containing silver sand with a nominal TBTCI or TPhTCI concentration of 150 ng g<sup>-1</sup> dry weight. To achieve this concentration the method described by Waldock *et al.* (1989) was modified in that TBTCI or TPhTCI in glacial acetic acid was adhered to approximately 20 g of fine deep-sea mud collected from the Porcupine Abyssal Plain, north-east Atlantic (TBT & TPhT concentration < 1 ng g<sup>-1</sup>) and then mixed into 2 kg of clean sand. Preliminary experiments showed that the concentration of TBTCI and TPhTCI in these preparations decreased to mean values of 121 ng g<sup>-1</sup> and 115 ng g<sup>-1</sup> dry weight after five weeks, respectively.

Samples for organotin analysis in the subsequent exposure experiments were taken immediately after the addition of TBTCI or TPhTCI and again at the end of the experiment (five weeks) and stored at -20°C. Organotin analysis was performed by GC-FPD according to the method of Waldock *et al.* (1989). Control groups were placed on the same mix of sediments but without the addition of organotin. The water in all buckets was continuously aerated and changed once a week.

The apparent water permeability, drinking and urine production rates of 0-group flounders were determined, enabling an estimation of the net water balance. Measurements were carried out at the beginning of the experiment (t<sub>0</sub>) and in weekly intervals for five weeks. The apparent water permeability was determined by measuring the half-time of exchange of tritiated water (THO) as described by Lockwood *et al.* (1973) and adapted for flounders by Hutchinson (1984). The diffusional water flux was calculated from the unidirectional flux (Hutchinson, 1984) and normalised to the fish wet weight (µl g<sup>-1</sup> h<sup>-1</sup>). The drinking rates were determined by placing the animals into a loading medium containing <sup>51</sup>Cr-EDTA and measuring the activity of the imbibed water (Hutchinson & Hawkins, 1990). The urine production was determined from the clearance rate of injected <sup>51</sup>Cr-EDTA from the blood (Babiker, 1975; Hutchinson & Hawkins, 1990) and the net water balance was determined by subtracting the drinking rate from the urine production rate (Smith, 1932).

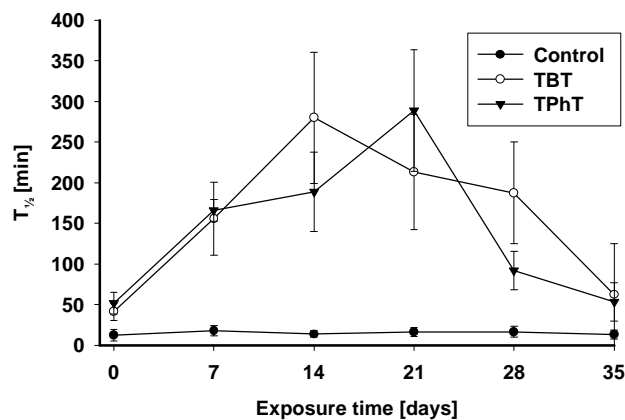
The osmolality of blood samples and the surrounding medium were measured by the cryoscopic method of Ramsay & Brown (1955). Na<sup>+</sup>-fluxes were measured using <sup>22</sup>NaCl (Hutchinson, 1984), modified from Shaw (1959) and normalised to gill area (nmol mm<sup>-2</sup> h<sup>-1</sup>). Gill area was estimated using  $y = ax^b$ , where y is the total gill area of a given fish [mm<sup>2</sup>], x is the fish wet weight [g] and the constants a = 239.02 and b =

0.723 (Hartl, *et al.* (in press)). At weekly intervals, the fork lengths were recorded to the nearest decimal place with a pair of vernier callipers.

Apart from net water balance data, that first required a square root transformation, all data sets were parametric. Comparisons between TBTCI, TPhTCI and control groups were analysed by a repeated measurement one-way analysis of variance (RM-ANOVA), followed by a Student-Newman-Keuls multiple comparison procedure. Maximum increases or decreases within each treatment group were compared to the respective initial values ( $t_0$ ) using a paired *t*-test (Fry, 1993).

## Results

Chronic exposure of 0-group flounders to sediment containing  $150 \text{ ng g}^{-1}$  TBT and TPhT caused a significant reduction of the half-time of exchange ( $T_{1/2}$ ) of tritiated water



**Fig. 1.** Halftime of exchange of THO during chronic exposure to  $150 \text{ ng g}^{-1}$  sediment-associated TBTCI or TPhTCI (minutes;  $n = 15 \pm \text{S. D.}$ )

(THO), compared to that of a control group (Fig. 1).

During the first 14 days in the TBTCI group and 21 days in the TPhT group the  $T_{1/2}$  had increased from initially 41 to 279 minutes ( $P < 0.05$ ) and 62 to 288 minutes ( $P < 0.05$ ). This in turn was reflected in a decrease in diffusional water flux, falling from  $6.19$  to  $0.58 \text{ } \mu\text{l g}^{-1} \text{ h}^{-1}$  ( $P < 0.05$ ) in the TBT group and  $6.6$  to  $0.3 \text{ } \mu\text{l g}^{-1} \text{ h}^{-1}$  ( $P < 0.05$ ) in the TPhT group (Fig. 2).

After two weeks of exposure,  $T_{1/2}$  began to decrease steadily, eventually reaching the level that the control group had constantly maintained throughout the experiment. It must be stressed that during most of the experiment, the THO flux across the membranes of both organotin groups was significantly lower than that of the control group ( $P < 0.001$ ). There was no significant difference between the organotin groups. In the TBT and TPhT groups, drinking rates increased in the first three weeks and the first two weeks, respectively, from  $0.45$  to  $0.90 \mu\text{g}^{-1} \text{h}^{-1}$  ( $P < 0.001$ ) and from  $0.4$  to  $0.89 \mu\text{g}^{-1} \text{h}^{-1}$  ( $P < 0.05$ ) and then towards the end of the experiment, slowly decreased to  $0.64$  and  $0.73 \mu\text{g}^{-1} \text{h}^{-1}$  (Fig 3). The drinking rates of the control group did not change significantly ( $P > 0.05$ ). The urine production rate in both organotin groups decreased slightly but was generally

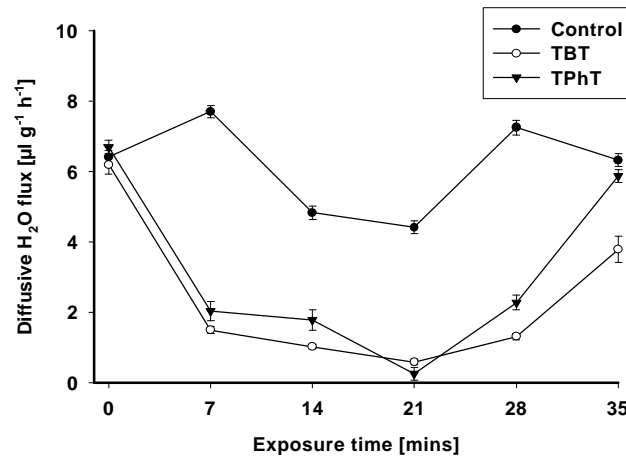


Fig. 2. Diffusive H<sub>2</sub>O flux during chronic exposure to 150 ng g<sup>-1</sup> sediment-associated organotins ( $\mu\text{l g}^{-1} \text{h}^{-1}$ ;  $n = 15 \pm \text{S. D.}$ ).

never significantly ( $P > 0.05$ ) different from the values at the start of the experiment (Fig. 4). During the first three weeks of the experiment there was no significant difference ( $P > 0.05$ ) between the net water balance of the TBT and control groups. However, during week four the net water balance in the TBT group increased from  $0.33$  to  $0.74$  % body weight ( $P < 0.001$ ) and differed significantly ( $P < 0.001$ ) from the control values that were maintained at a stable positive level during the entire experiment.

During the first three weeks the net water balance of the TPhT group peaked at 0.73 % body weight. During week four and five the net water balance returned to the initial value (Fig. 5). Passive Na<sup>+</sup>-efflux rates in the control group remained unchanged during the experiment ( $P > 0.05$ ). In the TBT and TPhT groups, however, passive Na<sup>+</sup>-efflux increased from 32.21 to 88.33 nmol mm<sup>-2</sup> h<sup>-1</sup> ( $P < 0.05$ ) and 38.1 to 70 nmol mm<sup>-2</sup> h<sup>-1</sup> ( $P < 0.05$ ), respectively, over the first three weeks of exposure and towards the end of the experiment decreased to 60.3 and 66.6 nmol mm<sup>-2</sup> h<sup>-1</sup>, respectively, and were generally significantly higher ( $P < 0.05$ ) than the values of the control group. (Fig.6).

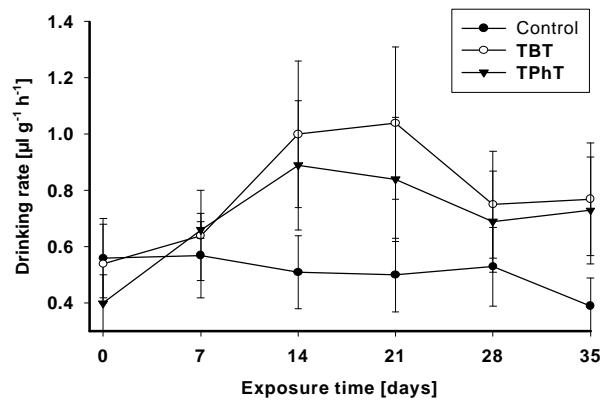


Fig. 3. Drinking rates during chronic exposure to 150 ng g<sup>-1</sup> sediment-associated organotins (µl g<sup>-1</sup> h<sup>-1</sup>; n = 15 ± S. D.)

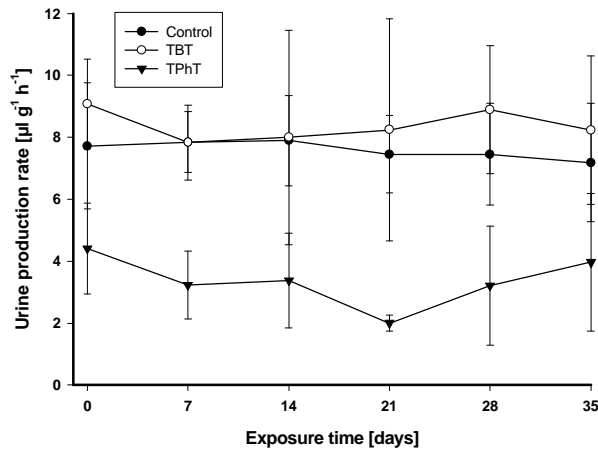


Fig. 4. Rate of urine production during chronic exposure to 150  $\text{ng g}^{-1}$  sediment-associated organotins ( $\mu\text{l g}^{-1} \text{h}^{-1}$ ;  $n = 15 \pm \text{S. D.}$ ).

After groups averaged  $287 \pm 13$  and  $286 \pm 7 \text{ mOsmol kg}^{-1}$  and was significantly lower than the values determined in the control group ( $309.28 \pm 14 \text{ mOsmol kg}^{-1}$ ; Fig. 7;  $P$

$< 0.05$ ). The control group showed a 12 % increase in length during the first week and was subsequently reduced and stabilised at 7% per week during the rest of the experiment. While the TPhT group only increased in length by 2 % throughout the experiment, eventually decrease in length group.

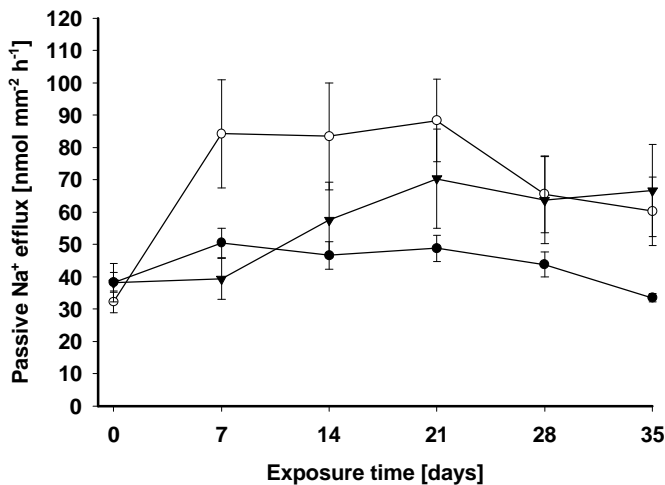
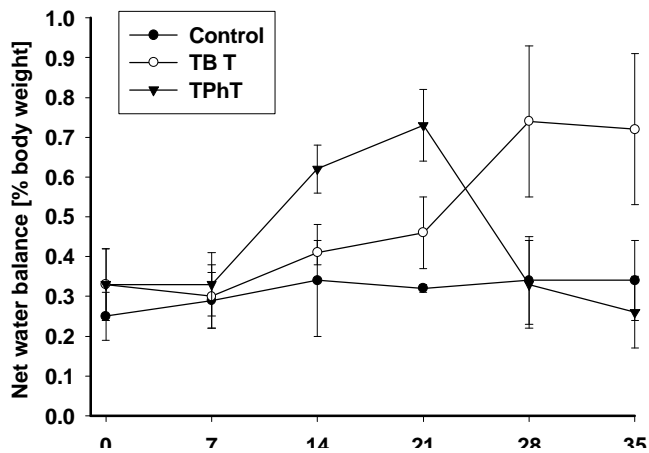


Fig. 6. Passive  $\text{Na}^+$  efflux across the gills during chronic exposure to 150  $\text{ng g}^{-1}$  sediment-associated TBT and TPhT ( $\text{nmol mm}^{-2} \text{h}^{-1}$ ).

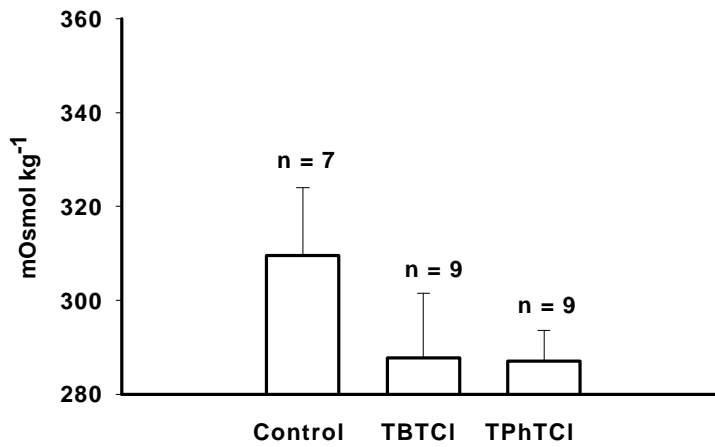


Fig. 7. Blood osmolality after five weeks of chronic exposure to 150 ng g<sup>-1</sup> sediment-associated organotin compounds (mOsmol kg<sup>-1</sup>; mean ± S. D.).

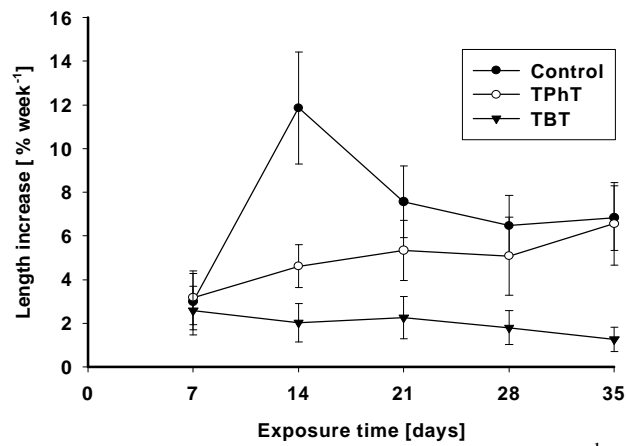


Fig. 8. Length increase during chronic exposure to 150 ng g<sup>-1</sup> sediment-associated TBTCI or TPhTCI expressed as % increase week<sup>-1</sup>; n = 10 ± S. D.).

## Discussion

Freshwater adapted euryhaline fish are hyper-osmotic in respect to the external medium. They therefore have not only to compensate for ion loss but also for an osmotic water influx across the gill membrane by adjusting the membrane permeability, drinking and urine production rates (Evans, 1969). The control values in the present study for these measures of osmoregulation are comparable with those reported by Hutchinson & Hawkins (1990). The increased  $T_{1/2}$  values in the TBT group (Fig. 1) suggest that TBT interaction with the gills decreased the diffusional flux of THO across the membrane (Fig. 2), indicating a decrease in the apparent membrane permeability.

The effect of organotin compounds on membrane permeability has been widely studied using model membranes. For example, Cullen *et al.* (1997) reported a decrease in membrane 'fluidity', following the addition of TBTCI to the extraliposomal compartment of an egg phosphatidylcholine liposome preparation, that lead to a decreased efflux of encapsulated dimethylarsenic acid by passive diffusion. Heywood *et al.* (1989) recorded changes of membrane structure, such as lysis, caused by tributyltin compounds and suggested that this could lead to an increased permeability. Experiments with fluorescent probes have indicated that TBTCI locates itself in the hydrophobic core of erythrocyte membranes causing haemolysis (Falcioni *et al.*, 1996). They suggested that the oxygen radicals produced during this process could cause structural defects to the membrane by increasing the number of double bonds in the hydrocarbon chains, leading to modifications of membrane permeability.

In all of the above cases model membranes were used in conjunction with organotin concentrations that were several orders of magnitude higher than the sediment concentrations found in the River Itchen, which may explain the observed membrane disruption in those experiments. The gills account for 90 % of the diffusional water flux (Evans, 1969, Motais *et al.*, 1969), so a reduction in gill permeability and subsequent reduction of diffusive water influx will alter water balance, so as to cause an increase in blood osmolality. In an osmoregulator such as *P. flesus* drinking rates and urine production are adjusted in order to offset any elevation of osmolality. A healthy freshwater acclimated flounder would be expected to drink occasionally and to produce large volumes of dilute urine, in order to keep the net water influx and the ion loss at an absolute minimum and therefore the blood osmolality within a narrow range (Evans, 1979). This behaviour was observed in the control group (Figs. 3 & 4) with the exception of a slight increase of urine production towards the end of the

experiment, which may be an artefact caused by handling stress (Eddy, 1981; McDonald & Milligan, 1997). However, Lahlou (1967) found stress-induced diuresis not to be a significant factor in laboratory experiments conducted with *P. flesus*. Seawater-adapted flounder exhibit an increase in drinking rates and a reduction in urine production in order to compensate for increasing blood osmolality. This behaviour was observed in both organotin groups (Figs. 3 & 4) although the fish were kept in freshwater throughout the entire experiment.

This suggests that the fish is compensating for increasing blood osmolality, caused by the reduction of membrane permeability for water. This process can also be observed in freshwater-acclimated fish when subjected to osmotic stress as reported by Lahlou *et al.* (1969) for the goldfish *Carassius auratus*. The data available in the literature suggests that between 62% and 80% of the water swallowed by fish is actually absorbed by the intestine (Smith, 1930; Hickman, 1968; Oide & Utida, 1968; Shehadeh & Gordon, 1969). If this is also true for flounders, then the shift in the osmotic water influx, caused by the enhanced drinking rates, should be reflected by a shift in the net water balance of TBTCI- and TPhTCI-exposed fish as shown in Fig. 5.

A further effect of triorganotins on the gill membranes observed in this study involves the passive efflux of  $\text{Na}^+$ , that was significantly increased ( $P < 0.05$ ; Fig 6) and may be contributing further to the changes in blood osmolality observed in the organotin groups (Fig. 7). There would appear to be a metabolic cost attached to the changes produced by exposure to TBTCI and TPhTCI that are manifested as a minimal increase in body length compared to the controls, as shown in Fig. 8. This observation is consistent with the findings of a study by Seinen *et al.* (1981), who observed significant growth retardation and weight loss in rainbow trout yolk sac fry during chronic exposure to 1 ppb TBTCI. Thus the reduced blood osmolality in the organotin groups in this study could be a reflection of the increased osmotic water influx rates caused by stress-induced increase in drinking as a consequence of permeability changes to the gill membranes following the interaction with re-mobilised sediment-associated TBTCI and TPhTCI.

The results presented here also suggest that benthic fish that are in contact with contaminated sediments are more likely to suffer adverse effects to their osmoregulatory system than pelagic species. This suggests further that this source of exposure may be a more important factor than organotin in the water column, especially as far higher concentrations in water seemed to have little effect on blood osmolality as shown by previous studies (Chliamovitch & Kuhn, 1977; Pinkney *et al.*,

1989).

We conclude from the results presented here that TBTCI and TPhTCI in sediments is capable of significantly disrupting the osmoregulatory functions of an estuarine fish, at concentrations currently found in local sediments.

### **Acknowledgements**

The authors would like to gratefully acknowledge the assistance of Sylvia Blake from the CEFAS Laboratory in Burnham-on-Crouch with the organotin analysis. MGJH was supported by a University of Southampton research studentship.

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