Sediment-associated tri-\textit{n}-butyltin chloride and its effects on osmoregulation of freshwater-adapted 0-group European flounder, \textit{Platichthys flesus} (L.)

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Abstract

The disruption of osmoregulatory processes was examined in European flounders exposed to environmental concentrations (150 ng TBTCI g$^{-1}$ dry weight sediment) of sediment-associated tri-\textit{n}-butyltin chloride (TBTCI), by using radiotracers to measure changes in hydromineral fluxes and water balance. The water permeabilities of TBTCI-exposed fish varied during the course of the experiment and were significantly lower than those of the corresponding controls that did not change significantly with time. It was found that the maximum decrease in water permeability of TBTCI-exposed fish occurred after 14 days; thereafter there was an increase towards control values. However, there was a differential reduction of the diffusional ($P_d$) and osmotic ($P_{os}$) permeability coefficients, where the former decreased more rapidly than the latter, reflecting the reduction of diffusional membrane permeability and the increasing importance of osmotic permeability.

In fish exposed to TBTCI sodium efflux and drinking rates were significantly increased but Na$^+$/K$^+\text{-ATPase}$ activities and urine production rates were not affected. The effects of TBTCI exposure are also manifested at the level of the whole organism by a reduction in the increase of the body length of exposed fish, when compared to controls. It was concluded that tributyltin-\textit{n}-chloride in sediments is capable of significantly disrupting the osmoregulatory functions of a benthic estuarine fish, at concentrations found in the sediments of Southampton Water and the River Itchen. © 2001 Elsevier Science B.V. All rights reserved.

\textit{Keywords:} Tri-\textit{n}-butyltin; Organotin; Osmoregulation; Water balance; Sodium efflux; \textit{ATPase}

1. 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 tri-\textit{n}-butyltin (TBT) have dropped dramatically, albeit with “hotspots” remaining in areas of intense shipping activity.
However, there is an increasing amount of evidence to show that organotin compounds are persistent in marine and freshwater sediments, that can act as reservoirs and sources for the secondary introduction of organotins to the environment (Waldock et al., 1990; Langston and 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), Werner et al. (1998), Hartl et al. (2000a). In vivo studies by Chliamovitch and Kuhn (1977), Pinkney et al. (1989) have shown that high concentrations of organotins in aqueous suspension disrupted osmoregulation in euryhaline fish and Hartl et al. (2000a) demonstrated a significant reduction of blood osmolality in 0-group flounders exposed to environmental concentrations of sediment-bound organotin compounds.

The juveniles of Platichthys flesus will be particularly vulnerable to xenobiotic disruption of osmoregulation because of their exploitation of estuaries as nursery grounds (Summers, 1979; Kerstan, 1991; Hutchinson and Hawkins, 1993; Jager, 1998; Bos, 1999), where they are exposed to tidally cycling salinity regimes. The aim of this study was to detect and quantify the effects of chronic exposure of 0-group flounders to sediment-associated TBT on selected osmoregulatory mechanisms, that may have led to the previously observed changes in osmolality.

2. Materials and methods

2.1. Fish husbandry

0-group flounder (0.06–1.8 g) were caught at Woodmill, River Itchen, Southampton at low tide (freshwater; salinity $S <$ 2) and kept in a 3500 l glass-fibre fish-farming tank that was shielded from direct sunlight and rain by a roof, but exposed to natural temperature fluctuations and light/dark cycles. Prior to experimentation, five fish for each treatment group and respective control groups were sampled from the stock populations and acclimated to tap water (15°C; salinity $S <$ 2; ionic composition (mmol l$^{-1}$): Na: 0.2; Cl: 0.11; Ca: 0.62; NH$_4$: 0.027; Mg: 0.21; K: 0.03; pH 6.5) with a light/dark regime of 12 h on and 12 h off for at least 2 weeks. Fish were fed ad libitum on live Artemia sp. during acclimation and the 5-week experimental period but starved for 24 h prior to sampling (once a week). Prior to the injection of $^{51}$Cr-EDTA for the determination of urine production rates, the fish were anaesthetised using MS-222 (0.1 mg l$^{-1}$). With the exception of Na$^+$/K$^+$-ATPase activity assays, that required the removal of the gills, all methods used were non-destructive so that individual fish could be followed throughout the entire course of each experiment.

2.2. Sediment preparation and analysis

Tributyltin chloride (TBTCl) exposure experiments were performed in 25 l polyethylene tanks (Carter et al., 1989) containing silver sand with a nominal TBTCl concentration of 150 ng g$^{-1}$ dry weight. To achieve this concentration sediments were prepared as described in Hartl et al. (2000a). Briefly, TBTCl in glacial acetic acid was adsorbed to approximately 20 g of dry, fine deep-sea mud collected from the Porcupine Abyssal Plain, north-east Atlantic (TBT concentrations < 1 ng g$^{-1}$) and then mixed into 2 kg of clean horticultural ‘silver sand’ (grain size < 1 mm). Preliminary experiments showed that the concentration of TBTCl in these preparations decreased to a mean value of 121 ng g$^{-1}$ dry weight after 5 weeks. Samples for organotin analysis in the subsequent exposure experiments were taken immediately after the addition of TBTCl and again at the end of the experiment (5 weeks) and stored at $-20^\circ$C. Organotin analysis was performed, following hydride generation, by gas chromatography with flame photometric detection (Waldock et al., 1989). Control groups were placed on the same mix of sediments but without the addition of TBTCl. The water in all tanks was continuously aerated and changed once a week.
2.3. Radiotracer methods

Measurements of all parameters were carried out at the beginning of the experiment ($t_0$, before the addition of TBTCl) and at weekly intervals for 5 weeks after the addition of TBTCl. The apparent water permeability was expressed as the half-time of exchange ($T_{1/2}$) of tritiated water (THO). From $T_{1/2}$, unidirectional diffusional water fluxes were calculated using the methods described by Lockwood et al. (1973) as adapted for flounders by Hutchinson and Hawkins (1990). THO efflux was measured after loading each animal for 5 h in 300 ml of a THO-spiked loading medium (activity: 1 MBq ml$^{-1}$). The animals were rinsed and transferred to 300 ml of unloading medium. Both loading and unloading media were of the same salinity and temperature as the acclimation medium. All containers were kept sealed, in order to avoid evaporation, except briefly for taking water samples during unloading (250 μl), which were taken at $t_1$ (exactly 5 min) and $t_2$ (5 h), added to 5 ml of scintillation cocktail (‘Optiphase HiSafe 3’) and counted in a ‘Wallac’ 1409/11 Liquid scintillation counter.

Drinking rates were measured using the procedures described by Hutchinson and Hawkins (1990). Individual animals were placed in 50 ml of loading medium containing $^{51}$Cr-EDTA (774 Bq ml$^{-1}$) for 2 h, after which the animals were rinsed in order to remove any activity from the body surface and the buccal cavity. The activity of the imbibed water in each animal was measured by placing the whole animal in a water-filled crystallising dish where whole body counts were performed with a ‘Panax’ NaI well γ-scintillation counter.

The measurement of the clearance rate of injected $^{51}$Cr-EDTA (2 μl; activity 3.7 kBq μl$^{-1}$) from the blood enables the estimation of the glomerular filtration rate, from which the urine production rate was calculated (Babiker and Rankin, 1975; Hutchinson and Hawkins, 1990).

Osmotic ($P_{o}$) and diffusional ($P_{d}$) permeability coefficients were calculated from the above measurements using the formulae given by Motais et al. (1969).

$Na^+$-efflux was measured using a method modified from Shaw (1959). Flounders acclimated to freshwater were weighed, their lengths measured and then loaded for 5 h in a freshwater loading medium containing $^{22}$Na$^+$ (activity 106 kBq ml$^{-1}$; $Na^+$ concentration of the loading medium: 2 mmol). The fish were rinsed to remove any $^{22}$Na$^+$ from the body surface, placed in a small crystallising dish filled with water of the same temperature and salinity as in the acclimating and loading media and whole body counts performed using a ‘Panax’ NaI well γ-scintillation counter. Measurements of fish activity were made in triplicate and corrected for background counts. The fish were then transferred to a large volume of unloading medium, with the same temperature and salinity as the loading medium, and left for 2 h, after which the fish were recounted. It was assumed that constant circulation of the large volume (5 l) of unloading medium prevented any significant backflux of $^{22}$Na$^+$ into the fish. The efflux was calculated from the half-time of exchange of $^{22}$Na$^+$ and from the total body Na$^+$ content. The exchange is described by the equation for an exponential loss.

$$A_0 = A_i e^{-k t_{unload}}.$$  

The rate constant $k$ is calculated as

$$k = \frac{1}{t_{unload}} \ln \frac{A_0}{A_f},$$

where $t_{unload}$ is the unloading time (h), $A_0$ are the counts at the end of the loading period and $A_f$ are the counts at time $t_{unload}$. Using the rate constant ($k$), the half-time of exchange ($T_{1/2}$) can be determined.

$$T_{1/2} = \frac{\ln 2}{k}.$$  

$Na^+$-efflux is thus calculated using

$$Na^+ - efflux = 1000 \frac{\ln[2][Na^+]}{T_{1/2} A} \text{ nmol mm}^{-2} \text{ h}^{-1},$$

where $T_{1/2}$ is the half-time of exchange (h) and $[Na^+]$ is the total body sodium concentration of freshwater-acclimated 0-group flounders (μmol g$^{-1}$) and $A$ is the gill area (mm$^2$ g$^{-1}$).
Total body sodium concentrations were measured from animals ashed in a muffle furnace at 550°C for 4 h. The ash was dispersed in 2 ml of 0.2 M hydrochloric acid using an ultrasonic bath and 5 μl samples from animals and standards were placed in 10 ml of deionised water. The Na⁺ concentration was determined by flame emission spectrophotometry using a ‘Pye Unicam’ SP9.

2.4. Na⁺/K⁺-ATPase activity

The Na⁺/K⁺-ATPase activity was determined by a method modified from Mayer-Gostan and Lemaire (1991). All eight gill arches were excised, wrapped in ‘Clingfilm’, dipped in liquid nitrogen and stored at −70°C until required. The frozen gills were thawed on ice and placed in ice-cold homogenising buffer at pH 7.4, that consisted of a hypertonic saline solution containing 25 mmol NaCl, 1 mmol dithiothreitol and 1 mmol HEPES–Tris. The gill tissues were separated from the underlying cartilage by homogenisation using an ‘Ultra Turrax’ homogeniser and the homogenate centrifuged at 2212g for 10 min in a ‘MSE Coolspin’ centrifuge. The pellet was discarded, the supernatant drawn off and centrifuged again at 2212g for 10 min. The supernatant was drawn off and this time discarded. The resulting pellet was rinsed twice in ice-cold isosmotic sucrose solution and re-suspended in ice-cold buffered reaction medium, that consisted of 100 mmol NaCl, 0.1 mmol H₂-EDTA, 5 mmol MgCl₂ and 20 mmol HEPES–Tris (pH 7.4). This assay is based on the rate of orthophosphate (Pₒ) accumulation in the reaction medium as the product of the enzymatic hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate + Pₒ. The Pₒ concentration is an indirect measure for ATPase activity that is expressed as μmol Pₒ mg⁻¹ protein h⁻¹. The protein concentration in the reaction medium was determined according to the method of Bradford (1976) and the Na⁺/K⁺-ATPase activity was determined by subtracting the activity in the presence of 1 mmol ouabain from the activity in the absence of ouabain (total ATPase).

2.5. Morphometrics

The total lamellar surface area of the gills was estimated using \( y = ax^b \), where \( y \) is the total gill area of a given fish (mm²), \( x \) is the fish wet weight (g) and the constants \( a = 239.02 \) and \( b = 0.723 \) (Hartl et al., 2000b). Total lengths were recorded at weekly intervals by measuring from the tip of the lower jaw to the end of the caudal fin to the nearest decimal place with a pair of vernier calipers. Fish were blotted dry with tissue paper and weighed to the third decimal place on a ‘Sartorius’ electronic analytical balance.

2.6. Statistical analysis

Apart from net water balance data, that first required a square root transformation, all data sets were normally distributed. Comparisons between TBTCl and control groups were analysed by one-way ANOVA or repeated measurement one-way ANOVA (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 \((t₀)\) using a paired t-test (Fry, 1993).

3. Results

Chronic exposure of 0-group flounders to sediment containing 150 ng g⁻¹ TBTCl caused a significant reduction of the half-time of exchange \((Tₜ/₂)\) of THO, compared to that of a control group (Fig. 1). During the first 14 days of the experiment, mean \( Tₜ/₂ \) in the TBTCl group increased by 595%, from \( 41 ± 7 \) to \( 279 ± 94 \) min \((P < 0.001)\). This in turn was reflected in a significant decrease in mean diffusional water flux \((P < 0.001)\), that fell by 90% from \( 6.19 ± 0.29 \) to \( 0.58 ± 0.08 \) µl g⁻¹ h⁻¹ (Fig. 2). After 2 weeks of exposure, mean \( Tₜ/₂ \) began to decrease steadily, eventually reaching the level that the control group had maintained throughout the experiment. During most of the experiment, the mean THO flux of the TBTCl group was significantly lower than that of the control group \((P < 0.001)\).
In the TBTCl group mean drinking rates were markedly increased in the first 3 weeks by 100%, from 0.45 ± 0.12 to 0.90 ± 0.23 μl g⁻¹ h⁻¹ (P < 0.001) and then, towards the end of the experiment, slowly decreased to 0.64 ± 0.17 μl g⁻¹ h⁻¹, 42% above the initial values (Fig. 3); the drinking rates of the control group averaged 0.42 ± 0.1 μl g⁻¹ h⁻¹ and remained unchanged (P > 0.05). The mean urine production rates in the TBTCl group (9.08 ± 1.44 μl g⁻¹ h⁻¹) were not significantly different (P > 0.05) from the values at the start of the experiment (Fig. 4) and were also not significantly different (P > 0.05) from those of the control group (7.72 ± 2.04 μl g⁻¹ h⁻¹). During the first 3 weeks of the experiment there was no
significant difference ($P > 0.05$) between the net water balances of the TBTCl and control groups (Fig. 5). However, during week 4 the net water balances in the TBTCl group increased by 123%, from 0.33% body weight to 0.74% body weight ($P < 0.001$) and were significantly higher ($P < 0.001$) than the control values that were at a stable positive level during the entire experiment. The results of $P_{os}/P_d$ after chronic exposure to sediment-associated TBTCl are presented in Table 1. The control values averaged at 0.04 ± 0.008. After chronic exposure to sediment-associated TBTCI $P_{os}/P_d$ was significantly increased and averaged 0.47 ± 0.2 ($P < 0.05$).

Mean Na⁺-efflux rates in the control group were 55.23 ± 16.03 nmol mm⁻² h⁻¹ and did not significantly change throughout the experiment ($P > 0.05$). In the TBTCI group however Na⁺-efflux showed a 175% increase, from 32.21 ± 8.32 to 88.33 ± 22.81 nmol mm⁻² h⁻¹ ($P < 0.001$) over the first 3 weeks of exposure and decreased towards the end of the experiment to 60.27 ± 15.56 nmol mm⁻² h⁻¹, 88% above the initial value at $t_0$ and was significantly higher ($P < 0.05$) than the values of the control group (Fig. 6).

Following 6 days of exposure of 0-group flounders to sediment-associated TBTCI, the Na⁺/K⁺-ATPase activity in gill preparations was lower but not significantly different ($P > 0.05$) from that of the control group (Fig. 7).

The control group showed a 12% increase in length during the first week that was subsequently reduced to 7% per week for the rest of the experiment. The TBTCI group reached a maximum length increase of 3% per week throughout the experiment and overall growth rates were significantly lower ($P < 0.001$) than those of the control group (Fig. 8).

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>$P_{os}$ (cm s⁻¹ × 10⁶)</th>
<th>$P_d$ (cm s⁻¹ × 10⁶)</th>
<th>$P_{os}/P_d$</th>
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<tr>
<td>Control</td>
<td>0.21 ± 0.02</td>
<td>5.95 ± 0.87</td>
<td>0.04 ± 0.008</td>
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<tr>
<td>TBT</td>
<td>0.17 ± 0.07</td>
<td>0.36 ± 0.06</td>
<td>0.47 ± 0.280</td>
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<td>$P &gt; 0.05$</td>
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*Mean calculated values ± S.D. derived from individual fish of mean wet weight of 0.56 ± 0.04 g and mean gill area of 143.13 ± 7 mm²; $n = 15$. 

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Fig. 5. The net water balances (% body weight) of freshwater-adapted 0-group flounder, *P. flesus*, during chronic exposure to 150 ng g⁻¹ sediment-associated TBTCl; mean ± S.D., $n = 15$, mean fish weight 0.09 ± 0.02 g (○) TBT, (●) control, (†) indicates significant difference from control $P < 0.001$; (*) indicates significant difference from initial ($t_0$) values $P < 0.05$.

Fig. 6. Na⁺ effluxes (nmol mm⁻² h⁻¹) across the gills of freshwater-adapted 0-group flounder, *P. flesus*, during chronic exposure to 150 ng g⁻¹ sediment-associated TBTCl; mean ± S.D., $n = 15$, mean fish weight 0.19 ± 0.05 g; gill area 59.08 mm² (○) TBT, (●) control, (†) indicates significant difference from control $P < 0.001$; (*) indicates significant difference from initial ($t_0$) values $P < 0.05$. 

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Fig. 7. Na\(^+\)/K\(^+\)-ATPase activities (\(\mu\)mol \(P_i\) mg\(^{-1}\) protein h\(^{-1}\)) in the gills of freshwater-adapted 0-group flounder, *P. flesus*, during the first 6 days of exposure to 150 ng g\(^{-1}\) sediment-associated TBTCl; mean ± S.D., \(n = 6\).

4. Discussion

Freshwater-adapted euryhaline fish are hyperosmotic in respect to the external medium and so, not only have to compensate for ion loss but also for an osmotic water influx across the gills by adjusting the membrane permeability, drinking and urine production rates (Evans, 1993; Kar naky, 1998).

The diffusional water flux values for the control group averaged 6.15 ± 1.18 \(\mu\)l g\(^{-1}\) h\(^{-1}\) and are comparable with the data previously reported for the same species (9.3 \(\mu\)l g\(^{-1}\) h\(^{-1}\) (Hutchinson and Hawkins, 1990) and 4.5 to 3.7 \(\mu\)l g\(^{-1}\) h\(^{-1}\) (Evans, 1969)). The increased \(T_i\) values in the TBTCl group suggest that TBTCl interaction with the gills decreased the diffusional flux of THO across the gill epithelium, indicating a decrease in the apparent membrane permeability. A caveat must be attached to such flux data as some authors have suggested, on mainly theoretical grounds, that the apparent flux rates may be underestimates of the true values; these arguments were summarised by Maetz (1974).

The nominal organotin concentrations used in this study were based on sediment concentrations found in the River Itchen (Langston et al., 1994). In previous studies (Heywood et al., 1989; Falcioni et al., 1996; Cullen et al., 1997), the interaction of organotin compounds with model membranes were examined. These authors found that organotins locate themselves in the hydrophobic core of model membranes, causing membrane disruption and changes to membrane ‘fluidity’. However, the concentrations used were several orders of magnitude higher than those applied in this study. This may explain the membrane disruption observed in previous studies. A reduction in membrane ‘fluidity’ has also been shown to be caused by other lipophilic compounds, such as cholesterol (Houslay and Stanley, 1982) and \(\alpha\)-tocopherol (Fukuzawa et al., 1979). Morris et al. (1982, 1987) found plasticisers and petroleum hydrocarbons, lipophilic pollutants, in the gill membranes of the amphipod *Gammarus duebeni*, that caused alterations to the fatty acid composition of the gill phospholipids which may also have lead to changes in membrane permeability.

The significantly increased \(P_{os}/P_d\) ratios observed following the 5-week exposure to TBTCl supports the above interpretation of the THO flux data. These data indicate that there was differential reduction in diffusional and osmotic permeabilities where the former decreased more rapidly than the latter. The \(P_{os}/P_d\) ratios for the control
group are much lower than the values reported previously for adult freshwater-adapted fish (Potts et al., 1967; Evans, 1969; Motais et al., 1969). In the present study it was observed that juvenile flounders did not develop scales until they were 75 mm in length and are therefore likely to be more permeable than the adult fish used by previous authors. The $P_{os}/P_d$ ratios in this study are also lower than those reported by Hutchinson and Hawkins (1990) and this is entirely attributable to differences in the estimations of gill area that were used to calculate $P_{os}$ and $P_d$. The gill area estimates used in this study were derived from measurements performed on 0-group flounders (0.008–2.860 g) using digital image analysis as described by Hartl et al. (2000b). The previous calculations of $P_{os}/P_d$ ratios for 0-group flounders were, of necessity, extrapolated from published data based on the gill area of adult flounders.

Accurate estimates of gill area are essential since the gills account for 90% of the diffusional water flux (Evans, 1969; Motais et al., 1969). Also, a reduction in gill permeability and subsequent reduction of diffusive water flux will alter water balance and offset factors tending to reduce blood osmolality. In an osmoregulator, such as $P$. flesus, drinking rates and urine production are adjusted in order to maintain body fluid osmolality. A healthy freshwater acclimated flounder will drink occasionally and produce large volumes of dilute urine, in order to keep the net water influx and the ion loss at a minimum and therefore the blood osmolality within a narrow range (Evans, 1979). These processes were observed in the control group. The drinking rates at $t_0$ (before the addition of TBTCI) for freshwater-adapted flounders from both TBTCI and control groups measured in this study averaged $0.42 \pm 0.11 \text{ ml g}^{-1} \text{ h}^{-1}$ or $0.56 \pm 0.14\%$ body weight h$^{-1}$. These values fall within the range previously reported for freshwater-adapted 0-group flounders ($0.38 \pm 0.06\%$ body weight g$^{-1} \text{ h}^{-1}$, Hutchinson and Hawkins, 1990), but are higher than the values measured for freshwater-adapted adult flounders ($0.3\%$ body weight h$^{-1}$, Balment and Carrick, 1985; 0.04\% body weight h$^{-1}$, Perrott et al., 1992). The significant increase in drinking rates in the TBTCI group, when combined with the increased sodium efflux and the lack of a compensatory increase in urine production (seen below) would cause a decrease in blood osmolality. The TBTCI-induced increase in drinking rate observed here explains the decrease in the blood osmolality of TBTCI-exposed flounders described by Hartl et al. (2000a).

TBTCI exposure had no effect on the urine production rates and the values did not differ significantly from those of the control group, that averaged $7.72 \pm 2.4 \text{ ml g}^{-1} \text{ h}^{-1}$ or $0.35 \pm 0.09\%$ body weight h$^{-1}$. These values are within the range previously reported for freshwater-adapted 0-group flounders ($0.48 \pm 0.06\%$ body weight h$^{-1}$, Hutchinson and Hawkins, 1990). Again, the values for adult flounders are much lower than for 0-group flounders ($1.78 \text{ ml g}^{-1} \text{ h}^{-1}$, Lahlou, 1967; 2.87 ml g$^{-1}$ h$^{-1}$, Motais et al., 1969). As with the higher drinking rates in 0-group flounders, the elevated urine production rates are an effect of size rather than handling stress. Lahlou (1967) did not observe stress-induced laboratory diuresis in $P$. flesus and Fletcher (1992) found stress-induced diuresis to be insignificant in experiments conducted with plaice ($P$. platessa).

Published data indicates that approximately 62% of the water swallowed by a variety of freshwater-adapted fish is absorbed by the intestine (Smith, 1930; Hickman, 1968; Oide and Utida, 1968; Shehadeh and Gordon, 1969). If this holds true for freshwater-adapted 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-exposed fish. This predicted shift was found in the present study.

The mean $\text{Na}^{+}$-efflux rate in freshwater-adapted 0-group flounders, before exposure to TBTCI, was $55.23 \pm 16.03 \text{ nmol mm}^{-2} \text{ h}^{-1}$ (equivalent to $16.31 \pm 4.71 \text{ mmol g}^{-1} \text{ h}^{-1}$) and is comparable to values $(18 \pm 2 \text{ mmol g}^{-1} \text{ h}^{-1})$ found by Hutchinson (1984) for the same age group. The importance of taking into account size when comparing flux measurements is demonstrated by comparing the present data with those given by Motais et al. (1966) who reported a sodium flux of $0.22 \text{ mmol g}^{-1} \text{ h}^{-1}$ for flounders weighing 60–200 g.
In the present study, sodium flux was significantly increased in the TBTCl group \((P < 0.001)\) but remained unchanged in the control group. In a healthy freshwater-adapted euryhaline fish, ion efflux across the membrane is balanced by enzyme-mediated active influx (Lin and Randall, 1995; Evans et al., 1999). Active sodium absorption from freshwater is mediated by coupled \(\text{Na}^+ / \text{H}^+\) (Maetz, 1971) and/or \(\text{Na}^+ / \text{NH}_4^+\) exchanges (Kerstetter et al., 1970; Wood and Randall, 1973; Kerstetter and Keeler, 1976; Payan, 1978; Heisler, 1984). It is widely assumed that basal-lateral membrane-bound \(\text{Na}^+/\text{K}^+\)-dependent ATPase (\(\text{Na}^+/\text{K}^+\)-ATPase) of the chloride cell, is involved in this coupled \(\text{Na}^+\)-transport in freshwater-adapted fish (Avella et al., 1987; Laurent and Perry, 1990; Perry et al., 1992; Uchida et al., 1996), although there is some evidence that suggests, that pavement cells may also be involved in this process (Karnaky, 1998; Perry, personal communication). Studies of \(\text{Na}^+/\text{K}^+\)-ATPase activity in freshwater-adapted flounders exposed to sediment-associated TBTCl may offer additional clues to the understanding of the changes in ionic fluxes observed in this study. In vitro experiments have shown that organotins significantly inhibit the activity of \(\text{Na}^+/\text{K}^+\)-ATPase (Aldridge, 1976; Pinkney et al., 1989) and also the intracellular ATP-driven sequestration of \(\text{Ca}^{2+}\), as shown by Girard et al. (1997) with sea urchin eggs. However, Pinkney et al. (1989) failed to find any significant changes in \(\text{Na}^+/\text{K}^+\)-ATPase activity, in vivo, during exposure of striped bass (\emph{Morone saxatilis}) to an aqueous suspension of TBT. The \(\text{Na}^+/\text{K}^+\)-ATPase activity measured in this study for freshwater-adapted flounders exposed to TBTCl for 6 days produced values that were not significantly different from those of the control group. Organotin compounds can also affect ionic fluxes across biological membranes by acting as ionophores (Selwyn, 1976; Wieth and Tosteson, 1979). The observation of unchanged \(\text{Na}^+/\text{K}^+\)-ATPase activities, despite the increased \(\text{Na}^+\)-efflux, suggests that TBTCl may be acting as an ionophore, as any adaptive changes in ATPase activity should have been recorded within hours of exposure to organotin (Girard et al., 2000). It is therefore likely that the lack of change in ATPase activity has had an effect by not changing active \(\text{Na}^+\)-influx; this may explain the inability of flounders to counteract the increased passive \(\text{Na}^+\)-efflux during TBTCl exposure.

There would appear to be a metabolic cost attached to the changes produced by exposure to TBTCl that are manifested as a minimal increase in body length compared to the controls. 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 TBTCl.

The results presented here indicate that benthic fish in contact with contaminated sediments are more likely to suffer adverse effects to their osmoregulatory system than pelagic species. Furthermore, this source of exposure may be a more important factor than organotin in the water column. This seems all the more likely as fish exposed to sediment-associated organotins showed a reduced blood osmolality (Hartl et al., 2000a) but no effect on osmolality was observed in fish exposed to far higher concentrations in aqueous suspension, as shown in previous studies by Chlamavitch and Kuhn (1977), Pinkney et al. (1989).

5. Conclusions

We conclude from the results presented here that TBTCl in sediments is capable of significantly disrupting the osmoregulatory functions of a benthic estuarine fish, at concentrations found in the sediments of Southampton Water and the River Itchen.

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