Organotins and Hydromineral Homeostasis in Aquatic Animals

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Abstract. Enzymes, non-enzymatic proteins and other organic molecules are vital components in living cells. Their respective function depends on specific spatial configurations which are linked to intracellular conditions. Any fluctuation of these conditions, beyond certain threshold values, such as a disruption of ionic regulatory mechanisms, can lead to the destabilisation of a finely balanced intracellular dynamic physiological equilibrium or homeostasis. Hydromineral homeostasis in aquatic organisms is maintained by a complex endocrine controlled array of specialised cross-membrane ion transport systems and the regulation of membrane water permeability. Depending on how aquatic organisms maintain hydromineral homeostasis, they can be roughly divided into two groups: osmoconformers and osmoregulators; the former are mostly invertebrates with high water permeability, the latter include some invertebrates and most fish species, whose permeable external epithelia are usually restricted to the gills. Other important organs involved in hydromineral regulation include the intestine and the various phyla-specific organisational types of renal systems. Environmental concentrations of organotin compounds, such as tributyltin and triphenyltin, have been shown to interfere with the maintenance of hydromineral homeostasis by inhibiting ATPases and affecting membrane permeability for water. The present chapter reviews the impact of organotin exposure on fresh- and seawater organisms of various phyla by examining the histopathological, physiological and molecular interactions of organotin compounds with relevant enzymes, membranes, the endocrine system, and the consequential ramifications for individuals, populations and community structure in aquatic ecosystems.

Keywords: Hydromineral regulation - gills - membranes- osmosis - ionic flux - organotins – ATPase.

INTRODUCTION

The vast majority of the Earth’s water is found in the oceans, but also exists on landmasses as glacial ice, groundwater, freshwater and saline lakes, and as vapour in the atmosphere. Despite the very dynamic nature of water, giving rise to a complex hydrological cycle, discrete hydrological domains can be distinguished: seawater, freshwater and, where the two meet and mix, brackish water. These domains have very distinct characteristics in terms of ionic composition and pH. The most important anions in freshwater are CO$_3^{2-}$ and HCO$_3^-$; SO$_4^{2-}$, Cl$^-$ and NO$_3^-$ are of lesser importance.

Among the cations Ca$^{2+}$ dominates, followed by Mg$^{2+}$, Na$^+$ and K$^+$. Over eons, the easily soluble salts (e.g. NaCl and Na$_2$CO$_3$) have been washed out and are now in the oceans, leaving behind the poorly soluble salts, such as CaCO$_3$. In seawater, the most important ions are the anions Cl$^-$, SO$_4^{2-}$, HCO$_3^-$, Br$^-$ and the cations Na$^+$, Mg$^{2+}$, Ca$^{2+}$, K$^+$ and Sr$^{2+}$. Together these ions constitute 99% of the dissolved salts in seawater and, because they maintain a nearly constant proportionality, independent of the salinity, are often referred to as the “conserved” elements, as opposed to the variable or “non-conserved” elements, such as nutrients and dissolved gases (Table 1).

The water content of living organisms approximately ranges between 40 and 99%. The physico-chemical characteristics of water convey a remarkable capacity as a solvent of both inorganic and organic substances, and provide the medium for the chemical reactions associated with life. Intraorganismal water contains solutes involved in cell metabolism and structural components, such as proteins, the solubility of which can be influenced by the presence of particular ions, while certain metals may act as co-factors in enzymatic reactions. Solutes may also provide chemical gradients used as potential energy stores and signal transduction, as well as in the regulation of the osmotic mobility of water.

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Cells are the basic units of all living beings and consist of a water-insoluble phospholipid structure, the plasma membrane that encloses a space separating it from the surrounding media. The plasma membrane is an approximately 75Å thick complex, highly organised and dynamic structure made of lipid and protein molecules that are in constant motion (see Chapter 5). The plasma membrane and its associated proteins allow for the maintenance of a nearly constant internal (intracellular) environment essential for many life-supporting biologically important processes, by regulating the passage of water and material in and out of the cell. In addition, most tissue and blood cells are bathed in fluids that serve as osmotic buffers for cells, preventing potentially harmful fluctuations to the intracellular milieu. Some of the materials transported across the plasma membrane possess electrical charges and are selectively accumulated in either the intracellular or extracellular fluids, thus affecting a charge or potential difference across the membrane. Such charges may aid or prevent the passive movement of charged materials, such as ions, across the membrane. Whereas passive transport of ions occurs mainly through ion co-transporters, active transport against either electrical or concentration gradients, is an energy-consuming process facilitated by specialised membrane bound ion pumps, mainly ATPases [1]. As will be shown later, many of these processes are under endocrine control.

Triorganotin compounds, such as tributyltin (TBT) and triphenyltin (TPhT) are highly toxic man-made biologically active substances [2]. In an aquatic context, they have been widely used as active ingredients in self-polishing copolymer antifouling paint formulations and as such, represent one of the most toxic substances intentionally introduced into the aquatic environment[3]. TBT and TPhT have been shown to interfere with the hydromineral regulation of aquatic organisms through endocrine disruption, ATPase inhibition and plasma membrane destabilisation. The present chapter reviews the impact of triorganotin exposure on the hydromineral regulation of fresh- and seawater organisms of various
phyla, by examining the histopathological, physiological and molecular changes induced by interactions of TBT and TPhT compounds with relevant enzymes, membranes, the endocrine system, and the consequential ramifications for individuals, populations and community structure in aquatic ecosystems.

HYDROMINERAL HOMEOSTASIS

In fresh water the blood of most fish species is hyperosmotic with respect to the external medium, causing an increase in water influx and a loss of electrolytes. In seawater the blood is hyposmotic with respect to the external medium inducing the loss of water and a diffusional influx of electrolytes through the intestinal epithelium.

Osmoregulating organisms, as opposed to osmoconforming organisms, employ a variety of mechanisms to regulate osmotic balance and body fluid volume, thereby maintaining constant intracellular conditions enabling efficient cellular function. Such mechanisms include, as well as strategies for ionic regulation, the control of drinking rates, urine production and membrane permeability [4-6].

The normal osmolarity of the body fluids of aquatic species can range between ~20 mOsmol L\(^{-1}\) in freshwater cnidarians to 1,100 mOsmol L\(^{-1}\) in seawater molluscs [24, 25] (Table 1). Thus, in freshwater, all organisms are hypertonic in relation to the osmolarity of the surrounding medium and maintain this condition through hyperosmoregulation. In seawater, in terms of osmolarity, two conditions can be distinguished: hypoosmotic osmoregulators, whose body fluid osmolarity is hypotonic in relation to the surrounding medium, and osmoconformers, whose body fluids are isosmotic towards seawater. Some species have developed considerable tolerance to fluctuating external osmotic conditions, usually encountered during changes to salinity, such as those seen in estuaries over a tidal cycle, and are referred to as euryhaline; conversely, stenohaline species require relatively static osmotic conditions and are therefore unlikely to be found in environments that are prone to frequent and often wide changes in salinity, such as estuaries and tide pools.

As indicated above aquatic organisms are subject to constant osmotic exchanges with the environment, which are driven by physico-chemical gradients between the body fluids and the surrounding medium, and affect the movement of water and solutes (nutrients, metabolites, ions) in and out of the body. Many of these exchange processes are obligatory and, left unchecked, may lead to the disruption of internal homeostasis: freshwater organisms tend to accumulate water and lose ions, while the opposite occurs in seawater organisms. Consequently, aquatic organisms need to be able to control these processes in order to develop a dynamic steady state between internal and external fluxes, thus facilitating the maintenance of internal homeostasis. The degree of intervention required will depend on the osmotic anatomy and will vary with species. Generally, osmotic exchange occurs across those surfaces exposed to the external medium, the integument, and is related to surface area and its permeability for water and solutes. The forces facilitating the exchanges of water and solutes across the integument of aquatic organisms include diffusion, osmosis, facilitated diffusion and active transport, the manipulation of which forms the basis for the regulation of hydromineral homeostasis in aquatic organisms.

Ionic Regulation

Ionic regulation in fish has been extensively reviewed [26-30]. Under hypoosmotic conditions (freshwater) aquatic organisms may lose ions to and gain ions under hyperosmotic conditions (seawater) from the external medium. The net passive ion exchange occurs as a consequence of physico-chemical gradients across the integument and through the process of eating and drinking. The European flounder, *Platichthys flesus* is a highly euryhaline catadromic fish that can tolerate rapid fluctuations in external salinity over a wide range. Although juveniles will tend to avoid freshwater where possible, because of the increased permeability of their integument [31] and the need to channel as much energy into growth rather than ionic regulation, adults spend much of their time in estuaries, often way beyond the tidal limit, in what is essentially freshwater, but can also be found in seawater where they spawn [32, 33]. Consequently, ionic regulation has been extensively studied in *Platichthys flesus* [34-36] and other euryhaline fish species, such as *Fundulus heteroclitus* [37-39] and invertebrates such as *Tigriopus brevicornis* [40].

Integument permeability can vary greatly between phyla and even between species within phyla and during species ontogeny [31, 32, 41]. Whilst the regulation of integument permeability plays a major role in volume regulation of euryhaline osmoconforming invertebrates [42-49], the intact skin of most adult fish species is a complex layered structure
containing numerous mucus glands, often covered with scales, and is considered to be largely impermeable [31, 50]. The majority of research points towards gills of fish, rather than the skin, as the principle pathway for hydromineral exchange [51-54]. Fish gills consist of delicate, highly vascularised internal epithelia, with the primary function of gas exchange [55-57]. Three distinct elements make up the gill structure: the skeletal elements of gill arch and filaments that support the epithelia, and the gill epithelium itself whose surface area is greatly increased by folds branching out either side of each filament, the so-called lamellae. The secondary epithelium is a two layered structure, characterised by highly differentiated pavement cells, that rests on the basal laminar of pillar cells which stabilise the epithelial folds, forming the lamellae. The large surface area and relative thinness contributes to the maximised permeability of the secondary epithelium for gases and at the same time less permeable for ions, particularly in seawater species. The primary epithelium is multi-layered and surrounds all the filaments and the interlamellar spaces [58]. Interspersed within the primary epithelium are various functionally distinct cell types whose frequency and distribution may change with species and external conditions, such as mucus secreting goblet cells, non-differentiated cells and mitochondria-rich cells, the latter constituting the basic element of branchial ionic regulation. Mitochondria-rich cells, first described by Keys and Wilmer [59] as chloride secreting cells (henceforth referred to as chloride cells) occupy less than 15% of the total area of epithelial cells exposed to the environment [60], although under osmotic challenge, the gas exchange may be compromised by the proliferation of chloride cells and subsequent increase of blood-to-water diffusion distance in favour of osmoregulatory adjustment [60, 61]. Chloride cells are characterised by an abundance of mitochondria associated with a densely branched tubular system that communicates with the basolateral membrane, an array of sub-apical vesicles and a large ovoid nucleus [62-65]. The apical membrane of each cell is characterised by the presence of microvilli and is firmly connected to neighbouring pavement cells forming a tight junctional complex. In seawater, the apical chloride cell membrane is buried in a crypt that appear as holes in the pavement cell layer. Furthermore, in seawater-adapted species, chloride cells develop an association with accessory cells, which develop rapidly during seawater adaptation, forming chloride cell complexes [66-68]. The membranes of chloride and accessory cells form a much weaker junctional apparatus than the tight junctions found in freshwater adapted species and form the leaky junctions of the paracellular pathway [68]. In euryhaline species, such as European flounder [69, 70], tilapia [71], guppy [72] and Fundulus heteroclitus [73], chloride cells undergo morphological and physiological changes and the differentiation of various subtypes has been observed [72]. Whereas in seawater adapted fish, large chloride cells are found at the base of the lamellae, in some species, freshwater adaptation is accompanied by a reduction in size of these interlamellar chloride cells and the development of chloride cells within the lamellar epithelium [69, 70, 74, 75]. There is an increasing amount of evidence pointing towards the hormonal control [68; 76-78] of chloride cell dynamics occurring during seawater or freshwater adaptation: cortisol [79-81] and growth hormone [79, 80] play a role in the differentiation (proliferation) of seawater chloride cells and prolactin (PRL) [82] that inhibits the proliferation of seawater chloride cells and promotes the differentiation of freshwater chloride cells. This notion may be complicated by the fact that the degree of responsiveness to hormonal control may vary in different chloride cell types within the same species, possibly due to differential upregulation of cortisol receptors [78, 83]. More recent work has shown that cortisol also has a regulatory effect on V-type H+-ATPase (see below) and may therefore also be an important endocrine component of ion uptake in smolting salmon [84] and other freshwater-adapted species [85].

In seawater, the driving force for NaCl secretion by seawater teleost gills is provided by a basolateral Na⁺/K⁺-ATPase [86], a basolateral Na⁺, K⁺, 2Cl⁻ co-transporter enabling Cl⁻ entry across the basolateral and an anion channel in the apical membrane permitting Cl⁻ to flow down its electrochemical gradient out in to seawater. Na⁺ follows that of Cl⁻ passively down its electrochemical gradient via a cation-selective paracellular pathway located in the tight junctions between mature chloride cell complexes formed by chloride cells and accessory cells [87]. The rapid increase of Na⁺/K⁺-ATPase activity following transfer of juvenile Platichthys flesus to seawater [88, 89] suggests that a post-transcriptional activation process may be involved as has been observed in killifish [90, 91].

In freshwater, the application of bafilomycin, a specific inhibitor of V-type H⁺-ATPase proton pumps [92], to the exterior medium reversibly inhibited the activity of V-type H⁺-ATPase and Na⁺ uptake in carp [93], thus confirming V-type H⁺-ATPase as the driving force for NaCl uptake in freshwater teleosts [94, 95]. There is also evidence that the enzyme is involved in acid-base regulation [26]. V-type H⁺-ATPase has been immunolocalised to the apical areas of gill lamellae, in particular chloride and pavement cell membranes [96, 97], although the precise apical cellular location appears to be species-specific [26, 98]. Sodium is then driven through an epithelial sodium channel down its electrochemical gradient, provided by the V-type H⁺-ATPase into the cell. Sodium uptake across the basolateral membrane of the chloride cell is then facilitated by Na⁺/K⁺-ATPase [97].
Divalent ions, such as calcium, play important roles in metabolic homeostasis in both freshwater and seawater organisms [26, 60, 99-101]. Much like terrestrial vertebrates, teleost fish require calcium, which is deposited in bones and scales, to grow. Calcium ions can be taken up directly from water through the gills or through the intestine by drinking water or absorbed from food [99]. In seawater fish, up to 30% of Ca\textsuperscript{2+} uptake can occur through the intestinal epithelia, especially in females during reproduction [102]. In freshwater species that do not drink under often hypocalcic conditions, Ca\textsuperscript{2+} is actively taken up across the gill epithelia in a multistep process, involving passive entry of Ca\textsuperscript{2+} through apical chloride cell membrane channels, followed by the energy-dependent movement across basolateral membrane via a high-affinity Ca\textsuperscript{2+}-ATPase or a lower-affinity Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger [60]. Any surplus Ca\textsuperscript{2+} taken up by seawater adapted species is excreted through the extrabranchial routes of the renal system and/or the intestinal tract [99, 103].

**Water Balance**

As described above, with respect to their external environment, hyperosmotic organisms tend to take up water and hypoosmotic organisms will lose water through diffusion via permeable membranes. Water flux between organisms and their environment is tightly regulated by the balance between membrane permeability, drinking and urine production rates and are under endocrine control [78, 104]. According to their speed of action, osmoregulatory hormones can be divided into two groups: fast-acting hormones, secreted immediately following a change in external salinity, have rapid onset effects and half-lives of only minutes and regulate channels and target transport epithelia through phosphorylation or dephosphorulylation. These include amongst others catecholamines, natriuretic peptides, urotensins, arginine vasotocin (AVT) and angiotensin II (ANG II). ANG II in particular is active in the renin-angiotensin system (RAS) that acts as a powerful vasoconstrictor increasing arterial blood pressure through activation of the sympathetic nervous system [105], and relates to water balance, playing an important role in inducing spontaneous drinking behaviour in fish [106, 107]. AVT on the other hand reduces renal water loss in teleost fish by reducing the number of filtering glomeruli which leads to a reduction in urine production during adaptation to seawater [108]. The second group are slow or long-term active hormones whose level gradually increases and remains elevated for extended periods of time following a change in external salinity and include steroid hormones, such as cortisol, and protein hormones, including growth hormone (GH) and prolactin (PRL). PRL regulates the number of channels in transport epithelia and also the morphological transformation of osmoregulatory tissues during adaptation to a changed osmotic environment. PRL is associated with freshwater adaptation, by decreasing the osmotic permeability to water and increase Na\textsuperscript{+} and Cl\textsuperscript{-} uptake (see above) across transport epithelia in euryhaline fish species and is particularly enhanced in stenohaline freshwater fish.

Gills, as the main permeable epithelia exposed to external environment, are particularly important in regulating water balance in aquatic organisms [109]. As outlined above, the outer-most layer of gill epithelia is constituted by the plasma membranes of pavement cells. According to the fluid mosaic model [110], plasma membranes consist of a fluid lipid bilayer of phospholipid molecules in which the proteins are embedded or otherwise associated, the position of which changes constantly. The fluidity of the membrane depends on the types of lipids present: a higher proportion of saturated lipids will create a more rigid membrane, whereas an increase in unsaturated and polyunsaturated lipids results in a more fluid membrane. An important constituent in the regulation of the membrane fluidity is cholesterol, a slightly amphiphatic largely hydrophobic steroid. Owing to the presence of a single hydroxyl group, cholesterol is able to associate with the hydrophilic heads of the phospholipids with the hydrophobic remainder of the molecule fitting between the lipid hydrocarbon chains. By acting as a spacer between the lipid hydrocarbon chains, cholesterol’s condensing effect contributes to plasma membrane viscosity [111, 112]. The phenomenon of membrane viscosity and its opposite, membrane fluidity, is of great significance in determining the water permeability of plasma membranes, because the degree to which a phospholipid membrane is permeable for water and other small hydrophilic molecules is directly proportional to its fluidity. In addition, aquaporins, a specialised group of membrane-integrated proteins that let water and small solutes pass through the phospholipid membranes of transport epithelia [113-115], can account for a large amount of observed water permeability [113], and in teleosts their expression has been shown to be regulated by cortisol [116].

**ORGANOTINS**

Organotins are organometallic compounds where Sn (in the majority of cases Sn\textsuperscript{+4}) is covalently bound to one or more carbon atoms of any aryl or alkyl group (R), an inorganic derivative, such as a halide or carboxylate, and are represented by the chemical structures RSnX\textsubscript{3}, R\textsubscript{2}SnX\textsubscript{2}, R\textsubscript{3}SnX and R\textsubscript{4}Sn. Organotin compounds have three main uses: as catalysts to stabilize PVC polymers, antifungal agents and as biocides to protect plants from insects and immersed structures from fouling [2, 117]. Triorganotin compounds consist of a Sn (IV) atom covalently bound to three organic moieties and an
associated anion. They are bioactive at very low concentrations [118] and therefore were marketed in the early 1970s as the perfect biocides, finding heavy application in the maritime industry [117] (see Chapter 2). The impact of triorganotins, in particular tributyltin (TBT) and triphenyltin (TPhT), on non-target organisms in the marine environment, such as imposex in female dogwhelks (*Nucella lapillus*) [119] and shell-thickening in Pacific oysters *Crassostrea gigas* [120-122], led initially to an International Marine Organization (IMO)-initiated partial ban at the end of the 1990s and a recently internationally ratified total ban on the use of these compounds as active ingredients in antifouling paint formulations1 (see Chapters 1 & 5).

TBT consists of three \( n \)-butyl chains covalently bound to a single Sn (IV) via Sn-C bonds and a moiety conveying a univalent positive charge. The main commercial formulations are bis(tributyltin) oxide (TBTO), tributyltin-acetate (TBTAc), tributyltin halides, namely fluoride (TBTF) or chloride(TBTC), and as a co-polymer with methymethacrylate (TBT-M) [123]. Similarly, TPhT contains three covalently bound Sn-C phenyl groups, with the main industrial TPhT derivatives being acetate (TPhTA), chloride (TPhTC) and hydroxide (TPhTH) [124, 125].

**Organotin Compounds in Aqueous Environments**

The potential environmental risk of a substance to organisms is a function of exposure and toxicity. Underpinning these factors, however, are bioavailability, bioconcentration and accumulation, pathways of exposure and persistence. The persistence of any xenobiotic substance is a function of the sum of removal mechanisms acting upon them, biotic: adsorption, uptake and transformation by biota [126-128]; physical: volatilization, freezing and adsorption to suspended particulate matter [129, 130]; chemical: chemical and photochemical reactions [131]. The speed of degradation and removal can be influenced by a number of factors, such as the density of suspended particulate, dissolved organic and colloidal matter, pH, CO₂, salinity and hydrological conditions [132, 133]. The fate of organotin compounds in particular in the natural environment is complicated by the large number of possible species [134, 135]. Of the species found in aqueous solution, the oxide, chloride and carbonate forms are of particular significance in the aquatic environment [123]. The solubility of the various species in water is very different and plays an important role in determining their fate [136], with the oxide species being generally more water soluble than the chlorides [137]. The occurrence, persistence and general fate of TBT and TPhT in freshwater [132, 138-140], seawater [130, 133, 141-143] and estuaries [144-146] has been extensively reviewed elsewhere.

**IMPACT OF ORGANOTIN ON HYDROMINERAL REGULATION**

Biochemical activity of various triorganotin compounds varies depending on organ system and structure under exposure, but generally, TBT and TPhT are the most potent [147] (see also Chapter 2 of this volume). The general mechanistic considerations of TBT and TPhT toxicity have been widely reviewed in the literature: endocrine disruption [122, 148-150], immunotoxicology [151-155], cytotoxicity [156-159], genotoxicity [160, 161] and enzyme inhibition [122, 148-150, 162, 163] (see Chapters 1, 5, 7). Many of these modes of toxicity can interfere with the physiology of hydromineral regulation, whereby the main routes of exposure are the interaction of organotin compounds with gill membranes and intestinal mucosa. Furthermore, cell shrinkage observed in many tissues following organotin exposure is an important effect of TBT as it is likely to cause the indirect inhibition of many cellular membrane-bound ion pumps (ATPases) [164].

**Ionic Regulation**

As outlined above, membrane bound transport enzymes, such as ATPases and membrane ion channels are key elements in ionic regulation. The toxicity of triorganotin compounds to ion translocating ATPases has been known for many years [165]. In fact, trialkyltin compounds have been instrumental in the unravelling of the biochemistry of oxidative phosphorylation as they inhibit electron transport phosphorylation in mitochondria [166, 167] (see Chapter 8). For membrane-bound ATPases the binding site for alkyltins has been shown to be on the membrane-bound components leading to an inhibition of the electron flow through these components [166, 147]. The V-type H⁺-ATPase, that couples ATP hydrolysis to transmembrane proton translocation through a rotating proton carrier subunit, provides the driving force for NaCl uptake in freshwater teleosts, is organotin susceptible. *In vitro* studies on subcellular components from various species have demonstrated the inhibitory capacity of triorganotin compounds on V-type H⁺-ATPase (Table 2) [168, 169].

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Table 2: *In vitro* effects of organotin compounds on ATPase activities.

<table>
<thead>
<tr>
<th>Species &amp; Localization</th>
<th>Cell type</th>
<th>Structure</th>
<th>Enzyme</th>
<th>Organotin species</th>
<th>Concentration</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Gill, mantle</td>
<td>subunit a</td>
<td>Na(^+)ATPase; Na(^+)/K(^+)ATPase</td>
<td>TBTC, TBT, DBT, MBT, TeET</td>
<td>200 nM, 0-34 µM</td>
<td>↓</td>
<td>[192, 193]</td>
</tr>
<tr>
<td><em>Tapes philippinarum</em></td>
<td>Gill, mantle</td>
<td>mitochondria</td>
<td>Mg(^{2+})ATPase</td>
<td>TBTC</td>
<td>0–34 µM</td>
<td>↓</td>
<td>[194]</td>
</tr>
<tr>
<td><em>Mytilus galloprovincialis</em></td>
<td>Gill, mantle</td>
<td>cytoskeleton</td>
<td>Ca(^{2+})ATPase</td>
<td>Triorganotin</td>
<td>1.2 nMol mg(^{-1}) protein</td>
<td>↓</td>
<td>[197]</td>
</tr>
<tr>
<td><em>Botryllus schlosseri</em></td>
<td>Heart</td>
<td>sub-mitochondrial particles</td>
<td>oligomycin-sensitive ATPase F(<em>{0})F(</em>{1})-ATPase</td>
<td>DBTCl</td>
<td>2 nMol g(^{-1}) protein, 82 nMol g(^{-1}) protein</td>
<td>↔</td>
<td>[198]</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adrenal gland</td>
<td>adrenal chromaffin granules</td>
<td>V-type H(^+)-ATPase F(<em>{0})F(</em>{1})-ATPase</td>
<td>DBT</td>
<td>0-20 µM</td>
<td>↓↔</td>
<td>[168]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Thymus</td>
<td>thymocyte membrane</td>
<td>Ca(^{2+}) ATPase</td>
<td>TBT</td>
<td>100 nM-1mM</td>
<td>↓</td>
<td>[199]</td>
</tr>
<tr>
<td>Brain</td>
<td>synaptosomes</td>
<td>Na(^+)/K(^+)ATPase</td>
<td>TMT, DMT, MMT, TET, TPT, TBT, TPhT</td>
<td>1-10 µM</td>
<td>↓</td>
<td>[200]</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Muscle</td>
<td>sarcoplasmatic reticulum</td>
<td>Ca(^{2+}) ATPase</td>
<td>TPhT</td>
<td>0.5-10 µM</td>
<td>↓</td>
<td>[201]</td>
</tr>
<tr>
<td>Rat</td>
<td>Heart</td>
<td>sarcoplasmatic reticulum</td>
<td>Na(^+)/K(^+)ATPase; Mg(^{2+})-ATPase</td>
<td>TBT</td>
<td>0.6 µM</td>
<td>↓</td>
<td>[202]</td>
</tr>
<tr>
<td>Liver</td>
<td>Ca(^{2+}) ATPase</td>
<td>F(<em>{0})F(</em>{1})-ATPase</td>
<td>DBTBr</td>
<td>0.4 µM</td>
<td>↓</td>
<td>[203]</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>homogenates</td>
<td>Na(^+)/K(^+)ATPase</td>
<td>TBT</td>
<td>0.9-56 µM</td>
<td>↓</td>
<td>[204, 205]</td>
<td></td>
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<tr>
<td>Erythrocytes</td>
<td></td>
<td></td>
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<tr>
<td><em>Squalus acanthias</em></td>
<td>Epithelial cells</td>
<td>isolated rectal gland</td>
<td>Na(^+)/K(^+) ATPase; H(^+) ATPase</td>
<td>TBT</td>
<td>10-100 nM</td>
<td>↓</td>
<td>[207]</td>
</tr>
<tr>
<td><em>Morone saxatilis</em></td>
<td>Gill</td>
<td></td>
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<tr>
<td><em>Fundulus heteroclitus</em></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

TBT: tributyltin; TBTC: tributyltin chloride; DBT: dibutyltin; MBT: monobutyltin; TeET: tetraethyltin; TBTBr: tributyltin bromide; BDTBr: dibutyltin bromide; DBTC: dibutyltin dichloride; DBTSCN: dibutyltin isothiocyanate; TMT: trimethyltin; TET: triethyltin; TPhT: triphenyltin; ↓: inhibition; ↔: unchanged; ↑: inhibited & stimulation;
Although there are a number of in vivo studies involving the inhibitory effects of metals and other organic xenobiotics on V-type H⁺-ATPase [168-170], to my knowledge, there is no available published data on the effects of organotin compounds on V-type H⁺-ATPase in aquatic organisms following in vivo exposure. The mechanism by which organotins inhibit V-type H⁺-ATPases involves a decoupling of the ATPase component from the membrane-bound H⁺-porter components [147, 168]. More recent investigations have revealed that organotins arrest the elementary steps for rotary catalysis of a V-type ATP-driven rotary motor by reducing the rotational rate of the peripheral subunit (V1), without influencing the binding affinity for ATP [171]. The consequence of this decoupling process is the secession of ATP hydrolysis by the ATPase component that cannot function unless protons are pumped through the membrane. Proton movement, on the other hand is restricted unless it is driven by the ATPase reaction. Furthermore, in vitro studies on rainbow trout, O. mykiss, erythrocyte membranes demonstrated a sensitivity of adrenergically activated Na⁺/H⁺ exchanger to 0.1 – 1 µM TBT [172]. As the Na⁺/H⁺ exchanger of the basal pavement cell membrane is an integral constituent of the Na⁺ uptake process in freshwater fish [26], inhibition of this ion exchanger is likely to affect the ionic regulatory capacity of exposed fish.

As outlined above, many substances have been demonstrated to inhibit the Na⁺/K⁺-ATPase: Pb [173-175], NaNO₃ [176], Cd [177], Cu [178, 179]; Hg [36, 180]; Cr [181]; Ag [19]; Zn [180]; NH₃ [180], including triorganotins [70, 88, 89, 182, 183]. Ouabain, a steroid glycoside, specifically inhibits Na⁺/K⁺-ATPase activity by binding to specific isoforms of the E3P (membrane open/cytoplasm closed state) α-subunit, and is consequently used in Na⁺/K⁺-ATPase activity assays [184-187]. The ion gradients maintained by this protein are responsible for actively pumping (active transport) Na⁺ and K⁺ ions across cell membranes, driven by the hydrolysis of the terminal phosphate of ATP [188]. It is one of the most studied P-type ATPases, mainly because it contains core sequences that are conserved throughout most higher eukaryotes and has consequently been extensively reviewed [184].

Briefly, Na⁺/K⁺-ATPase is a hetero-dimeric protein consisting of an α and β subunit present in 1:1 stoichiometry. The α-subunit with approximately 1000 amino acids and a molecular mass of about 110 kDa is the catalytically active subunit as the binding sites for cations are primarily located here [188; 189]. It has 10 transmembrane segments with 5 extracellular loops, one of which, between M7 and M8 provides the important contact region with the β-subunit [189]. In contrast to the α-subunit, β subunits have only been described in Na⁺/K⁺-ATPase and intestinal H⁺/K⁺-ATPase [184; 189]. The β subunit is a 55 kDa glycoprotein consisting of 330 amino acids and is thought to be involved in the structural stabilisation of the Na⁺/K⁺-ATPase in associated membranes [189]. Na⁺/K⁺-ATPase with previously bound ATP and Mg²⁺ works by binding 3 intracellular Na⁺ ions and hydrolysing ATP which leads to the phosphorylation of the Na⁺/K⁺-pump at a highly conserved aspartate residue on the α-subunit and subsequent release of ADP [184]. A conformational change in the Na⁺/K⁺-pump exposes the Na⁺ ions to the extracellular milieu where they are released as the phosphorylated form of the Na⁺/K⁺-pump has a low affinity for Na⁺ ions. The Na⁺/K⁺-ATPase then binds 2 extracellular K⁺ ions, which results in the dephosphorylation of the ATPase, thereby returning it to its original conformational state, and transporting the K⁺ ions into the cell. The unphosphorylated form of the pump has a higher affinity for Na⁺ ions than K⁺ ions, so the two bound K⁺ ions are released. ATP binds, and the process starts again [190, 191]. The relative ineffectiveness of triethyltin and trimethyltin compounds at inhibiting Na⁺/K⁺-ATPase suggests that the hydrophobic properties of these compounds are very important in the interaction with the enzyme. Moreover, inhibition does not occur instantaneously, but is rather a gradual process taking several minutes. Indeed, it has been suggested that it may take up to 60 TPhT moieties to inhibit Na⁺/K⁺-ATPase in vitro, although the actual number may be less, as some may be bound to inactive subunits or non-specific sites or in fact dissolve in membrane lipids [147].

The bulk of the available published data concerning the underlying mechanics of the inhibitory effects of organotin compounds on the activity of Na⁺/K⁺-ATPase involves in vitro studies on primary cell extracts and cell lines from a variety of species, including mammals (Table 2). TBTC has been shown to inhibit the ATP hydrolysis by the Na⁺-translocating ATP synthase of the bacteria Ilyobacter tartaricus and the H⁺-translocating counterpart of Escherichia coli and also the binding of Na⁺ to the α-subunit of Na⁺/K⁺-ATPase, suggesting that the α-subunit ion channel may be the site of inhibition [192]. In the search for human analogues, much research on Na⁺/K⁺-ATPase-triorganotin interaction has been carried out on mammals. In rats, the Na⁺/K⁺-ATPase in cardiac membrane fraction, prepared from heart ventricles, was inhibited by 0.6 µM TBT [202]; in brain homogenates and erythrocyte preparations, the Na⁺/K⁺-ATPase was inhibited by 0.9-56 µM and 1.2-66 µM TBT, respectively [205]; in brain synaptic membranes the Na⁺/K⁺-ATPase was significantly inhibited by 64-252 µM dibenzyltin dichloride and 132 - 399 µM dibenzyltin diisothiocyanate [206]. In vitro studies into the impact of organotin compounds on the Na⁺/K⁺-ATPase in aquatic species have yielded more relevant data. In bivalves...
As outlined above, maintenance of divalent ion concentrations, such as Ca$^{2+}$ and Mg$^{2+}$, are important for growth, muscle contraction, oxidative phosphorylation and therefore general metabolic homeostasis. Organotin compounds may disrupt calcium homeostasis by several means: TBTC has been shown to interact with calmodulin (CaM), a calcium modulating protein, by non-covalent hydrophobic interaction between the aliphatic chains of TBTC and the hydrophobic regions of Ca$^{2+}$-activated CaM. The consequence of this is an inhibition of CaM-dependent Ca$^{2+}$-ATPase at TBTC concentrations ranging from 0.1 to 1 mM, leading to a detrimental increase in cytosolic Ca$^{2+}$ concentration in vitro [151, 216]. TBTC is also known to inhibit sarcoplasmic-endoplasmatic reticulum Ca$^{2+}$-ATPase (SERCA), thus triggering the release of Ca$^{2+}$ stores from the endoplasmic reticulum and the activation of the store-dependent Ca$^{2+}$ influx. The massive accumulation of Ca$^{2+}$ observed is a result of the activation of the Ca$^{2+}$-influx pathway, especially following prolonged inhibition of SERCA [217]. Similar results have been reported for isolated mouse SERCA exposed to 2 μM TBT, 63 μM TBT and 280 μM TMT [203], as well as isolated rabbit SERCA exposed to 0.5-10 mM TPhT [201]. Experiments with mouse thymocytes showed that 1 μM TBT increased the membrane permeability of the intracellular organelles for Ca$^{2+}$ and decreased the membrane Ca$^{2+}$ pump activity, again resulting in a sustained increase in the intracellular Ca$^{2+}$ concentration [199]. TBT has been shown to cause a rapid depletion of thiol, suggesting that organotin compounds may be interacting directly with Ca$^{2+}$ pumps by binding to its thiol groups, especially as TBT induced effects can be prevented by various thiol reducing agents [218]. Interestingly, DBT appears to be a more potent inhibitor of Ca$^{2+}$-ATPase than TBT, possibly because of the hydrophobicity of TBT facilitates its insertion into the membrane and retention within lipidic bilayers [219], leaving less free TBT available in the cytoplasm to interact directly with Ca$^{2+}$-ATPase [220].
Table 3: In vivo exposure to organotin compounds and their effects on ATPases.

<table>
<thead>
<tr>
<th>Species</th>
<th>Developmental stage</th>
<th>Duration</th>
<th>Tissue</th>
<th>Enzyme</th>
<th>Organotin species</th>
<th>Route of Exposure</th>
<th>Concentration</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>neonatal/adult</td>
<td>?</td>
<td>Brain/liver mitochondria</td>
<td>Na⁺/K⁺ATPase</td>
<td>TET</td>
<td>?</td>
<td>1-260 µM</td>
<td>↓</td>
<td>[209]</td>
</tr>
<tr>
<td>DET, MET, TMT</td>
<td></td>
<td></td>
<td>Liver</td>
<td>DBTCl</td>
<td>intraperitoneally</td>
<td>10 or 30 mg kg⁻¹d⁻¹</td>
<td>↔</td>
<td>[210]</td>
<td></td>
</tr>
<tr>
<td>Artemia salina</td>
<td>Nauplii</td>
<td>?</td>
<td>?</td>
<td>fenbutatin oxide³</td>
<td>Aqueous</td>
<td>0.02 µg L⁻¹</td>
<td>↑</td>
<td>[214]</td>
<td></td>
</tr>
<tr>
<td>Penaeus japonicus</td>
<td>adult</td>
<td></td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTC, TPhTC</td>
<td>sediment-associated</td>
<td>150 ng g⁻¹</td>
<td>↓</td>
<td>[88; 183; 89; 70]</td>
</tr>
<tr>
<td>Morone saxatilis</td>
<td>juvenile</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>0-1.09 µg L⁻¹</td>
<td>↓</td>
<td>[208]</td>
<td></td>
</tr>
<tr>
<td>juvenile</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>0-1.09 µg L⁻¹</td>
<td>↑</td>
<td>[208]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oreochromis niloticus¹</td>
<td>juvenile</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>0.1-10 µg L⁻¹</td>
<td>↓</td>
<td>[211]</td>
<td></td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td>adult 3-96h</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>10-90 µg L⁻¹</td>
<td>↓</td>
<td>[212]</td>
<td></td>
</tr>
<tr>
<td>adult 7-28d</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>4.1 µg L⁻¹</td>
<td>↓</td>
<td>[212]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult 3-96h</td>
<td>Gill</td>
<td>Mg²⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>10-90 µg L⁻¹</td>
<td>↓</td>
<td>[212]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult 7-28d</td>
<td>Gill</td>
<td>Mg²⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>4.1 µg L⁻¹</td>
<td>↓</td>
<td>[212]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Tilapia²</td>
<td>?</td>
<td>?</td>
<td>Gill</td>
<td>Na⁺/K⁺ATPase</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>5 µg L⁻¹</td>
<td>↓</td>
<td>[213]</td>
</tr>
</tbody>
</table>

¹) Tilapia niloticus; ²) hybrid of several other Oreochromis species; ³) bis[tris(2-methyl-2-phenylpropyl)tin] oxide; TBTC: tributyltin chloride; TBT: tributyltin oxide; DBTC: dibutyltin chloride; MBT: monobutyltin; TMT: trimethyltin; TET: triethyltin; DET: Diethyltin; MET: Monoethyltin; TPhTC: triphenyltin chloride; ↓: inhibition; ↔: unchanged; ↑: inhibited & stimulation;

Mg²⁺ balance which is important for oxidative phosphorylation and anion transport has been shown to be compromised by organotin compounds. In vivo exposure of F. heteroclitus to 5-50 µg L⁻¹ TBT yielded significant inhibition of gill Mg²⁺-ATPase, whereas, Mg²⁺-ATPase activity was unchanged in the gills of M. saxatilis exposed to the far lower concentration of 1.09 µg L⁻¹ TBT, although the same concentration was enough to significantly stimulate the Na⁺/K⁺-ATPase activity. The latter probably reflected the increased metabolic activity due to osmotic stress as a result of organotin exposure [208].

Water Balance

Being able to control the integumental water fluxes, especially across delicate permeable membranes, such as those of gill and intestinal epithelia, is of paramount importance for the regulation of the water balance in aquatic, particularly estuarine, species. As described earlier, one way of doing this is to regulate the fluidity of the membrane by adjusting the fatty acid composition and cholesterol concentration [221, 222]. In fact gills, often the first target of water-borne contaminants, can experience a significant change in lipid composition in epithelial membranes following exposure [223, 221]. Interaction of organotin with membranes leads to membrane-associated tin-containing aggregates, which appear intercalated between the inner and outer membranes of human erythrocytes, and may cause physical alterations to the state of membrane proteins and lipids, including membrane fluidity[224] (Table 4). Triorganotin compounds and their degradation products interact with phospholipid membranes according to their hydrophobicity and also steric structure [225-227], whereby the membrane surface charge appears to play an important role [219]. It has been observed that hydrophobic butyl and phenyl moieties of TBT and TPhT align themselves with the prevailing directions...
of the phospholipid acyl chains [228]. This is likely to disrupt the packing of the membrane, thereby increasing its fluidity and permeability [229]. The all important permeability of biological membranes has been found to be highly sensitive to TBT [230-233] and TPhT [234]. Indeed, changes to membrane fluidity can affect the permeability of the membrane for water and solutes. In in vitro preparations, the permeability of model membranes for dimethylarsinic acid (DMA) was found to increase following exposure to TBT but decreased after treatment with DBT, which may be a reflection of the hydrophobicity and steric structure of the two compounds and their subsequently different mechanics of interaction with phospholipid membranes [231]. Similar observations have been made for TPhT that associates with the headgroup region of the lipid bilayer vesicles, whereas DPhT caused changes to the hydrophobic regions[233]. The perturbations of model phosphatidyl choline membranes following exposure to 10-100 nM TBTC and TPhTC has caused the depolarisation of these membranes [235]. TBTC and TPhTC, albeit at the much higher exposure concentration range of 30-200mM, are also known to significantly increase the fluidity of model membranes [228, 236, 237], which is likely to be the underlying cause of changes to membrane permeability (see Chapter 4).

A change of membrane fluidity and permeability has demonstrably increased hemolysis of erythrocytes exposed in vitro to TBTC [238] and TPhTC [233, 239]. Haemolytic potency appears to depend on the location of the compound in the bilayer, as TPhT adsorbed on the surface in one study caused a much higher degree of hemolysis than DPhT that had penetrated much deeper [233]. As the perturbations of the membranes by butyl groups disrupt the packing of the lipid alkyl chains, the shape and flexibility of erythrocytes can be affected, caused by an increase in motion and disorder of the erythrocyte membrane [240].

Table 4: Organotin-induced membrane perturbations in vivo, in vitro and in model membranes

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ/Type</th>
<th>cell Effect</th>
<th>Organotin species</th>
<th>Route of Exposure</th>
<th>Duration</th>
<th>Concentration</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platichthys flesus</td>
<td>gills</td>
<td>reduced water permeability</td>
<td>TBTC, TPhTC</td>
<td>sediment-associated</td>
<td>35d</td>
<td>150 ng g⁻¹</td>
<td>[88]</td>
</tr>
<tr>
<td>Salmo irideus</td>
<td>erythrocytes</td>
<td>increased membrane fluidity</td>
<td>TBTC</td>
<td>in vitro</td>
<td>-</td>
<td>20 µM</td>
<td>[229]</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>hemolysis</td>
<td></td>
<td>TBTC; DBTC</td>
<td>in vitro</td>
<td>-</td>
<td>10-90 µM</td>
<td>[238]</td>
</tr>
<tr>
<td>Porcine</td>
<td>hemolysis</td>
<td></td>
<td>TBTC; TPhTC</td>
<td>in vitro</td>
<td>-</td>
<td>2-10 µM</td>
<td>[239]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TPhTC; DPhTC</td>
<td>-</td>
<td>0-4h</td>
<td>100 µM</td>
<td>[233]</td>
</tr>
</tbody>
</table>

**Model membranes**

| DPPC¹              | depolarization      | TBTC, TPhTC               | in vitro         | -        | 10-100 nM    | [235]|
| phosphatidyl choline| increased membrane fluidity | TBT; TPhT                   | in vitro         | -        | 30-50 mM    | [236]|
| EYL²               | decreased membrane fluidity     | TeMT; TPTC                   | in vitro         | 180h     | 100-400 mM  | [237]|

¹ dipalmitoylphosphatidylcholine; ² Egg yolk lecithin; TBTC: tributyltin chloride; DBTC: dibutyltin chloride; MBT: monobutyltin; TMT: trimethyltin; TeMT: Tetramethyltin; TeET: Tetraethyltin; TPTC: tripropyltin chloride; TPhTC: triphenyltin chloride; DPhTC: diphenyltin dichloride;
Data concerning the in vivo exposure of aquatic organisms to organotin compounds and the effects on membrane permeability are sparse and restricted to euryhaline fish. Freshwater-adapted juvenile European flounder exposed to 150ng g⁻¹ sediment-associated TBTC and TPhTC showed a significantly increased half-time of exchange of tritiated water (THO) across the permeable gill membranes, which is consistent with a reduced gill membrane permeability, in particular a shift from diffusional towards osmotic permeability [88, 183, 241]. A reduction in membrane ‘fluidity’ has also been caused by other lipophilic compounds, such as cholesterol [242] and alpha-tocopherol [243]. In addition, plasticisers and petroleum hydrocarbons, also lipophilic pollutants, have been found in the gill membranes of the amphipod Gammarus duebeni and have caused alterations to the fatty acid composition of the gill phospholipids and changes to membrane permeability [221, 223]. There are also a number of in vivo studies concerning the permeability of gill membranes following exposure to other metals, such as Cd, Cu and Al. For example, freshwater O. mossambicus exposed to 100 and 1000 µg L⁻¹ CdCl₂ experienced a concentration dependent increase in gill permeability [244]. This increased permeability was most likely caused by direct interactions of metals on the gill surface, particularly competitive interactions with Ca²⁺ binding sites on the gill membrane and in tight junctions [245, 246]. These data were supported by the observation of a protective effect of high water Ca²⁺ levels against metal toxicity by competing metals for binding sites on the gill membrane [246].

Many of the above physiological aberrations have observable histopathological origins or consequences, particularly in gill epithelium, blood cell constitution and blood composition of fish (Table 5). Acute exposure of juvenile O. mykiss to 5.85 mg L⁻¹ resulted in significant damage to gills, characterised by a separation of the gill epithelium from the basal membrane and pillar cells. In addition, a swelling of the secondary lamellae and dilation of blood vessels was also observed [182]. Atlantic salmon, Salmo salar, held in TBT-treated nets for up to four weeks showed lamellar hyperplasia and lateral lamellar branching, suggesting interference of TBT in gill epithelial growth and development [247]. 0-group European flounder, Platichthys flesus exposed to 150 ng g⁻¹ sediment-associated TBTC and TPhTC for 35 days showed significant interference in gill lamellar chloride cell dynamics following rapid transfer from freshwater to seawater [89]. The effects of aqueous exposure of freshwater-adapted 0-group Platichthys flesus to 32 µg L⁻¹ for up to 15 days resulted in serious histopathological damage to gill epithelia, including swelling and budding of epithelial cells, epithelial proliferation and fusion of lamellae [248].

Although no changes to the surface morphology of gill epithelia were observed, acute exposure of the mummichog, Fundulus heteroclitus to 17.2 µg L⁻¹ TBT led to hypertrophy of the gill lamellar epithelium and at 35.6 µg L⁻¹ caused 40% reduction in the volume of lamellar blood channels. However, a 42 d exposure to sublethal concentrations (up to 2 µg L⁻¹) showed no histopathological changes to the gill epithelium [249]. During sub-chronic exposure (1-4 months) to much higher concentrations of tributytin hydroxide (TBTH 1-3 mg L⁻¹), the gill epithelia of Oreochromis nilotica had developed hyperplasia, leading to congestion of the lamellar blood vessels and the aneurysmal formation of lamellar capillaries [250]. Acute (12h) and sub-chronic (7-14d) exposure of Chinese rare minnow (Gobioacypris rarus) to 50-5,000 ng L⁻¹ TBTC caused a series of ultrastructural pathological changes to the gills, including fractured nuclei [251].

In addition, histopathological damage has been recorded in other osmoregulatory organs, such as the kidney. O. mykiss yolk sac fry showed hydropic degeneration of the tubule segments of the pronephros following a 10-day exposure to 5 µg L⁻¹ TBTO [252]. The hemopoietic interstitial tissues in the head kidney of juvenile guppies, Poecilia reticulata, exposed for up to 3 months to TBTO (0.01-32 µg L⁻¹) and for 1 month to TBTC (320-3,200 µL⁻¹), developed hyperplasia [253]. At comparable concentrations of TBTH, Oreochromis nilotica, during subchronic exposure (1-3 months) developed hydropic degeneration and accumulation of hyaline droplets in tubular epithelial cells [250]. In embryos and larvae of the minnow, Phoxinus phoxinus, gills were not yet fully developed, but 3 to 10 day exposure to 0.8 - 19.5 µg L⁻¹ TBTC and 1.8 - 15.8 µg L⁻¹ TPhTC, lead to degenerative alterations in renal tissue [254, 255].

Disruption of osmo- and ionic regulatory mechanisms following exposure to organotin compounds has indirect implications for blood constituents and the osmoregulatory capacity of exposed organisms of various phyla (Table 6). The osmoregulatory capacity of the shrimp Penaeus japonicas, was significantly reduced following acute exposure to 50-400 µg L⁻¹ TBTO [215]. Subchronic exposure of Crassostrea virginica to 0.5-2 g L⁻¹ TBTO caused a significant delay in osmotic adaptation of haemolymph in terms of osmotic pressure and chloride ion concentration [256].
Table 5: Histopathological damage caused by exposure to various organotin compounds

<table>
<thead>
<tr>
<th>Species</th>
<th>Developmental stage</th>
<th>Tissue</th>
<th>Effect</th>
<th>Organotin species</th>
<th>Route of Exposure</th>
<th>Duration</th>
<th>Concentration</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>0+ group</td>
<td>Gills</td>
<td>swollen mitochondria; disorganised cristae</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>&lt;10d</td>
<td>5.86 mg L$^{-1}$</td>
<td>[182]</td>
</tr>
<tr>
<td><em>Salmo salar</em></td>
<td></td>
<td></td>
<td>lamellar point hyperplasia; secondary branching</td>
<td>TBT</td>
<td>Aqueous</td>
<td>7w</td>
<td>?</td>
<td>[247]</td>
</tr>
<tr>
<td><em>Platichthys flesus</em></td>
<td>0+ group</td>
<td>chloride dynamics</td>
<td>budding of epithelial cells; Epithelial proliferation/ fusion of lamellae</td>
<td>TBTC, TPhTC</td>
<td>sediment-associated</td>
<td>35d</td>
<td>150 ng g$^{-1}$</td>
<td>[88; 183; 89; 70]</td>
</tr>
<tr>
<td></td>
<td>0+ group</td>
<td></td>
<td></td>
<td>TBTO</td>
<td>Aqueous</td>
<td>14d</td>
<td>0-32 µg L$^{-1}$</td>
<td>[248]</td>
</tr>
<tr>
<td><em>Fundulus heteroclitus</em></td>
<td></td>
<td></td>
<td>hynorphy of lamellar epithelium</td>
<td>TBT</td>
<td>Aqueous</td>
<td>96h</td>
<td>17.2 µg L$^{-1}$</td>
<td>[249]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>reduction of lamellar blood channels</td>
<td></td>
<td></td>
<td></td>
<td>36.5 µg L$^{-1}$</td>
<td></td>
</tr>
<tr>
<td><em>Oreochromis nilotica</em></td>
<td>juvenile</td>
<td>gill epithelial hyperplasia; congestion of capillaries; aneurysmal formation of gill lamellar capillaries</td>
<td>TPhTH</td>
<td>Aqueous</td>
<td>1-4m</td>
<td>1-3 mg L$^{-1}$</td>
<td>[250]</td>
<td></td>
</tr>
<tr>
<td><em>Gobiocypris rarus</em></td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>TBTC</td>
<td>?</td>
<td>12h</td>
<td></td>
<td>[251]</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>yolk sac fry</td>
<td>Kidney</td>
<td>pronephros degeneration</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>10d</td>
<td>0.5 µg L$^{-1}$</td>
<td>[252]</td>
</tr>
<tr>
<td><em>Poecilia reticulata</em></td>
<td>juvenile</td>
<td>hyperplasia of hemopoietic interstitial tissue</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>1-3 m</td>
<td>0.01-32 µg L$^{-1}$</td>
<td>[253]</td>
<td></td>
</tr>
<tr>
<td><em>Oreochromis nilotica</em></td>
<td>juvenile</td>
<td></td>
<td>hydropic degeneration and accumulation of hyaline droplets in tubular epithelial cells</td>
<td>TBTC</td>
<td>Aqueous</td>
<td>1 m</td>
<td>320–3200 µg L$^{-1}$</td>
<td>[253]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TPhTH</td>
<td>Aqueous</td>
<td>1-4m</td>
<td>1-3 mg L$^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td><em>Phoxinus phoxinus</em></td>
<td>embryonic/ larval</td>
<td>degenerative alterations</td>
<td></td>
<td>TBTC</td>
<td>Aqueous</td>
<td>3-10d</td>
<td>0.8 - 19.5 µg L$^{-1}$</td>
<td>[254]</td>
</tr>
<tr>
<td></td>
<td>embryonic/ larval</td>
<td></td>
<td></td>
<td>TPhTC</td>
<td>Aqueous</td>
<td>7-9d</td>
<td>1.8 - 15.9 µg L$^{-1}$</td>
<td>[255]</td>
</tr>
</tbody>
</table>

1) *Salmo gairdneri.*
Table 6: Organotin chronic exposure on blood constituents and osmoregulatory capacity of aquatic species

<table>
<thead>
<tr>
<th>Species</th>
<th>Developmental stage</th>
<th>Effect</th>
<th>Organotin species</th>
<th>Route of Exposure</th>
<th>Duration</th>
<th>Concentration</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crassostrea virginica</em></td>
<td>adult</td>
<td>delayed haemolymph adaptation</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>10 days</td>
<td>0.5-2 g L⁻¹</td>
<td>[256]</td>
</tr>
<tr>
<td><em>Penaeus japonicus</em></td>
<td>adult</td>
<td>reduced osmoregulatory capacity</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>6-48h</td>
<td>50-400 µg L⁻¹</td>
<td>[215]</td>
</tr>
<tr>
<td><em>Platichthys flesus</em></td>
<td>0-group</td>
<td>reduced blood osmolality</td>
<td>TBTC, TPhTC</td>
<td>Sediment-associated</td>
<td>35 days</td>
<td>150 ng g⁻¹</td>
<td>[11]</td>
</tr>
<tr>
<td><em>Morone saxatilis</em></td>
<td>juvenile</td>
<td>no changes to plasma Na⁺, K⁺, Ca²⁺, Mg²⁺</td>
<td>TBT</td>
<td>Aqueous</td>
<td>14 days</td>
<td>0-1.09 µg L⁻¹</td>
<td>[208]</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em>¹</td>
<td>0⁺ juvenile</td>
<td>unchanged blood osmolality</td>
<td>TBTO</td>
<td>Aqueous</td>
<td>21h</td>
<td>0.053-1.2 mg L⁻¹</td>
<td>[182]</td>
</tr>
</tbody>
</table>

¹*S.gairdneri*

Freshwater-adapted juvenile 0-group European flounder, *Platichthys flesus*, that usually maintain a blood osmolality of around 310-315 mOsm kg⁻¹, experienced a significant reduction following exposure to 150 ng g⁻¹ sediment-associated TBTC and TPhTC [11]. Under the same conditions, freshwater adapted juvenile flounder, that normally drink very little, if at all, showed significantly increased drinking rates and the urine production was significantly reduced, possibly as a result of osmotic challenge brought about by the exposure to triorganotins [183]. The occurrence and degree of any adverse effects on the osmoregulatory capacity appears to be species-specific and also dependent on a number of factors, including organotin species, external salinity, exposure route and duration. Organotin concentration may play a subordinate role, because, despite exposure to much higher concentrations of TBT (up to 1µg L⁻¹) and fluctuations in gill Na⁺/K⁺-ATPase activity in adult seabass, *M. saxatilis*, no significant changes to plasma Na⁺ levels were observed [208]. Similarly, blood osmolality of *O. mykiss* did not change following acute exposure to 0.053-1.2 mg L⁻¹ TBTO [182].

CONCLUSIONS

The evidence reviewed here demonstrates that organotin compounds, in particular TBTC and TBTO, are capable of significantly disrupting the osmoregulatory functions of euryhaline organisms. Of special concern is the general observation that many of the reported effects were caused *in vivo* at environmentally relevant concentrations. The most sensitive osmoregulatory components appear to be the membrane bound ATPases, thus inhibiting ionic regulation, and the fluidity of epithelial membranes, thus interfering with membrane permeability. Another significant observation is that the expected major re-arrangement of chloride cells in the gill epithelium of freshwater-adapted fish on transfer to seawater, can be significantly inhibited by the chronic exposure to environmental concentrations of TBT. A similar effect following exposure to TPhTC has not been reported, which can be explained by the different modes of membrane interaction of the two organotin species as outlined above.

The main conclusions that can be drawn from the present review are that the effects of organotin exposure are species-specific, both organismic and organotin, and highly dependent on ontogenic stages. There is also a noticeable dearth of information regarding the effects of organotin exposure on volume regulating osmoconformers and macroalgae. Furthermore, there are generally a lot more *in vitro* data available, especially from vertebrate cell culture models involving V-type ATPases. However, *in vitro* studies and artificial membrane models may not necessarily give an accurate account of complex multi-organ processes such as in osmoregulatory physiology. This is particularly apparent for the impact of organotins on the development of major osmoregulatory organs in juvenile fish, such as anadromic salmonids, which is likely to interfere with smoltification and the onset of migratory
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behaviour. In addition the metabolic costs involved in compensating for the effects of organotin exposure has caused reduced growth rates in juveniles and stunted adults with impaired fitness which could potentially lead to population level effects. This illustrates a clear need for whole-organism ecotoxicology studies, particularly where novel materials, such as engineered nanoparticles are involved, in order to avoid the mistakes made in managing the use of organotin compounds and their impact on the marine and aquatic environment.

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