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# Fish gill damage by harmful microalgae newly explored by microelectrode ion flux estimation techniques

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ABSTRACT

Harmful algal blooms (HAB) are responsible for massive mortalities of wild and aquacultured fish due to noticeable gill damage, but the precise fish-killing mechanisms remain poorly understood. A non-invasive microelectrode ion flux estimation (MIFE) technique was successfully applied to assess changes in membrane-transport processes in a model fish gill cell line exposed to harmful microplankton. Net $Ca^{2+}$, $H^+$, $K^+$ ion fluxes in the rainbow trout cell line RTgill-W1 were monitored before and after addition of lysed cells of this Paralytic Shellfish Toxins (PST) producer along with purified endocellular dinoflagellate PST. It was demonstrated that PST alone do not play a role in fish gill damage during *A. catenella* outbreaks as previously thought, but that other ichthyotoxic metabolites from lysed algal cells (i.e. lipid peroxidation products or other unknown metabolites) result in net $K^+$ efflux from fish gill cells and thereby gill cell death.

## 1. Introduction

Harmful algal blooms (HABs) have caused multi-million dollar economic losses to the global fish farming industry (Hoagland et al., 2002; Kim, 2010; Park et al., 2013). Fish mortality in Japan resulted in losses of ~US$352 M between 1972 and 2012 (Itakura and Imai, 2014) and a massive HAB event in 2016 caused ~US$800 M in economic losses to the Chilean fishing industry (León-Muñoz et al., 2018). Fish pathology following HAB events may include alteration in ion balance and severe gill damage with morphological anomalies of cellular hypertrophy and lysis of epithelial chloride cells, epithelial necrosis and loss of secondary lamellae (Hiroishi et al., 2005; Deeds et al., 2006). This affects gill functions such as mucus excretion and vasodilatation (Marshall et al., 2003), changes in the cardiorespiratory system (Lee et al., 2003), induction or decrease of enzymatic activities and osmotic distress caused by an augmentation of branchial chloride cells (Endo et al., 1985; Tang et al., 2007), which all are likely to lead to fish death. Gills are multifunctional organs involved in osmoregulation, gas exchange, metabolic waste excretion, as well as ion uptake or extrusion, and therefore an ideal target to assess the effect of toxicants or pathogens in aquatic toxicology studies (Evans et al., 2005). Studies on gill function conventionally are carried out *in vivo* using whole experimental animals (Lee et al., 2009), but which require special animal rearing facilities and ethics approvals. A growing body of literature now recognizes the value of gill cell cultures as an alternate model system for fish gills (Wood et al., 2002; Leguen et al., 2007; Lee et al., 2009). These cell lines derive from a complex gill epithelium that comprises a number of different cell types including pavement, mitochondria-rich and mucous (goblet) cells (Wood, 2001). RTgill-W1 is an epithelial cell line originating from the gill explant of adult rainbow trout (*Oncorhynchus mykiss*) (Bols et al., 1994) which has been widely used in a broad-spectrum of chemical toxicity testing (Bury et al., 2014) and more recently successfully applied for the study of harmful microalgae (Dorantes-Aranda et al., 2011, 2015).

Several mechanisms have been proposed to explain fish-kills by harmful microalgae (Dorantes-Aranda et al., 2015), including a proposed role of well-studied Paralytic Shellfish Toxins (PSTs) (Montoya et al., 1996). The specific effect of PST on excitable cell membranes is mediated by the interaction between the positively charged guanidinium groups of saxitoxin (STX) with negatively charged carboxyl groups at site 1 of the sodium ($Na^+$) channel, thereby blocking the pore (Catterall et al., 1979; Catterall, 1980; Cestèle and Catterall, 2000). Blockage of voltage-gated sodium channels can result in death by cardiovascular shock and respiratory arrest in mammals (Lagos and

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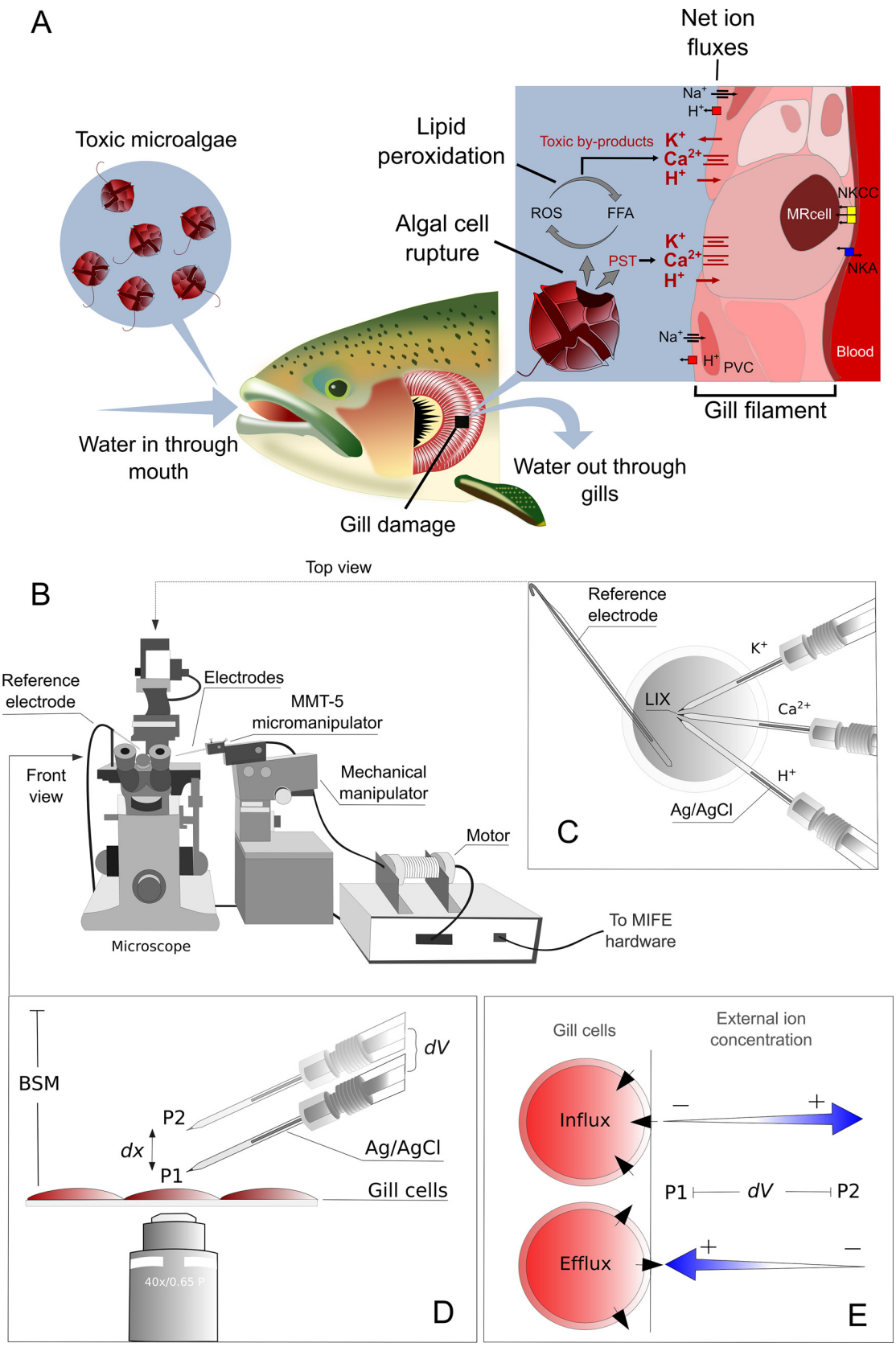


**Fig. 1.** A. Principle of fish gill damage by the toxic microalgae *Alexandrium catenella;* ROS, Reactive Oxygen Species; FFA, Free Fatty Acid; PST, Paralytic Shellfish Toxin; PVC, pavement cell; MRcell, mitochondria-rich cell; NKA, $Na^+/K^+$ ATPase, NKCC, an $Na^+/K^+/2Cl^-$ cotransporter; B. Assembled MIFE system; C. Top view showing reference and ion-selective microelectrode positions within the measuring chamber; D. Front view showing microelectrode movement in a square-wave manner between two positions near (P1) and distant (P2) from the fish gill cell monolayer; and E. If an ion is taken up by a gill cell, its concentration in the proximity of the cell surface is lower than that further away; Vice versa, if the ion is extruded across the plasma membrane, its concentration in the proximity is higher than further away. The electrochemical gradient *dV* is measured between these two positions (P1 at ~15–20 µm and P2 at ~70–100 µm) and converted into concentrations of ions using a calibration curve.