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The impacts of stress on sodium metabolism and copper accumulation in a freshwater fish

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ABSTRACT

In freshwater fish, stress can often result in significant modifications to Na^+ metabolism and may be an important aspect to consider in conservation efforts; as maintaining ion balance is critical to survival and ion transport is also a key determinant of metal toxicity. In order to better quantify the response of stress, Na^+ influx, Na^+ efflux, and copper accumulation were measured as a result of handling stress in inanga (*Galaxias maculatus*). This species is a culturally and economically important fish in New Zealand as one of the major species in the local 'whitebait' fishery. Na^+ influx rates in inanga were found to be 2–3 times greater after handling than in 'recovered' fish, and Na^+ efflux rates increased in the range of 5–6 times. Both influx and efflux rates quickly returned to resting levels within 24 h. Increases in Na^+ efflux were strongly correlated with opercular beat frequency. This suggests an increas in ventilation, and subsequent enhanced diffusive loss of Na^+, as the mechanism of increased Na^+ efflux. Total body copper levels were also measured under similar treatments. Fish had significantly higher levels of copper directly after handling than following a 24 h recovery; likely due to a shared Na^+/copper uptake pathway. As accumulation is linked to toxicity, fish exposed to elevated copper levels in stressful environments will consequently be more at risk to metal toxicity. In a natural environment, stress can come from many different sources; among which, anthropogenic disturbances can often be a cause. Given that inanga must migrate through metal-contaminated coastal regions to reach breeding habitats, they will be exposed to toxicants under conditions where perfusion and ventilation of the gill is increased. As such, ion loss would be exacerbated, leading to an enhanced compensatory ion uptake and an increase in accumulation of ion-mimicking toxicants such as copper, exacerbating toxicity. This is a concern as conservation efforts in more disruptive environments may not be adequately protected.

1. Introduction

Freshwater fish are continuously faced with the task of maintaining internal ion levels higher than those of their surroundings. Because of this, the active uptake of ions (e.g. Na^+, Cl^-) against their osmotic gradients is fundamental to survival. The major site for this uptake is the gills; however, this is also an important locus for the toxic effects of dissolved metal ions that can interfere with ion absorption through mimicry or inhibition of key ion transporters (Wood, 2012). This disruption of ion uptake, when severe enough, will eventually lead to death. Factors that alter gill function not only impact the ability of the fish to maintain ion homeostasis, but could also influence their overall susceptibility to waterborne toxicants.

Stress has been shown to result in the inhibition of reproduction, growth, and the immune system in multiple fish species (McDonald and Milligan, 1997; Postlethwaite and McDonald, 1995). The hormones cortisol and adrenaline are released in response to a stressful event, leading to a host of physiological effects; including increases in heart rate and the permeability of the gills (Eddy and Handy, 2012; Wendelaar Bonga, 1997). Specific responses, however, will vary according to species and the magnitude/duration of the applied stress (Barton, 2002). In terms of ion homeostasis, stress is thought to increase the functional surface area of the gills, and subsequently increase the rates of diffusional ion loss (Gonzalez and McDonald, 1992; Randall et al., 1972). Specifically, handling stress has been shown to result in large Na^+ losses, leading to a decrease in whole body Na^+ (Postlethwaite and McDonald, 1995). Ventilation frequency has also been shown to increase in response to stress, with fish from low-predation areas having a lower frequency under normal conditions than those from high-predation areas (Brown et al., 2005). This response, combined with elevated gill permeability, exacerbates exchange between the external environment and the blood, resulting in elevated ion transport rates (Cairns et al., 1982; Hughes and Roberts, 1970). Na^+ transport rates are also thought to scale with size, with smaller fish showing higher influx rates owing to the larger surface area

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to body mass ratio (Grosell et al., 2002). Because of this, small fish may exhibit a more exaggerated efflux in response to stress (McDonald and Milligan, 1997).

Inanga (*Galaxias maculatus*) is a small freshwater fish species widely distributed in coastal streams throughout the Southern Hemisphere (McDowall, 1989). They are members of the order Osmeriformes (the smelts), and although they belong to different orders, the Galaxiidae family is considered a close relative of the salmonids (family Salmoniformes; McDowall, 2002). In New Zealand, inanga are the major species in the local 'whitebait' fishery; and as such is a culturally and economically important fish. Over the past century, fishery catches have declined as a result of changes in land use and the introduction of invasive salmonids (Rowe et al., 1999). Laboratory studies have shown that inanga are capable of rapidly adjusting their physiology to maintain ion homeostasis (Chessman and Williams, 1975; Urbina et al., 2013). Elevated levels of metal contaminants have been found in many of the migratory streams used by inanga along New Zealand's West Coast (Greig et al., 2010). As a consequence, inanga will be exposed to toxicants under conditions where gill perfusion and ventilation is increased due to greater energy demands during migration, possibly resulting in the increased uptake of ion-mimicking toxicants such as copper.

Na^+ absorption is essential in balancing diffusive Na^+ loss in freshwater fish. Copper is thought to compete with Na^+ for absorption at the apical surface of gill epithelium (Grosell and Wood, 2002), although the exact nature of the transporter (i.e. epithelial Na^+ channel, Na^+/H^+ exchanger, and/or Rhesus protein metabolon) remains controversial. At high concentrations, copper is thought to displace calcium in tight junctions leading to an increase in gill permeability and Na^+ efflux (Grosell and Wood, 2002). In addition, copper is also thought to impair Na^+/K^+-ATPase (NKA) activity on the basolateral membrane of the gills, resulting in an impaired ability to absorb Na^+; owing to the proposed role of this enzyme in generating electrochemical gradients that drive Na^+ absorption (Grosell et al., 2007). Hence, Na^+ balance may be more difficult to restore in the presence of copper. Understanding how stress can influence ion transport and resulting contaminant uptake could provide important information regarding the environmental susceptibility of inanga and fish with similar physiology and behaviour.

This study aimed to evaluate the effects of handling stress on ventilation and Na^+ metabolism in inanga. Specifically, Na^+ influx and efflux were evaluated directly after handling and subsequently following a 24 h recovery period. The effect of handling stress on copper absorption (using a concentration of 500 $\mu g\,Cu\,L^{-1}$) was also evaluated by measuring total body copper levels following similar handling treatments. Quantifying the effect of stress is important in establishing acclimation periods necessary for laboratory studies examining branchial physiology; given that processes such as ion transport, toxicant uptake, waste excretion, and oxygen consumption are all located at the gills, and are all likely to be modified by ventilation rates. Furthermore, the impact of stress on gill ventilation and perfusion may alter metal handling, possibly leading to increased susceptibility to waterborne contaminants taken up by the branchial epithelium. This has been previously observed for organic toxicants such as ethynyloestradiol in swimming killifish (Blewett et al., 2013). Social stress has also been shown to lead to an elevated uptake of copper in rainbow trout (Sloman et al., 2002).

The type of stress used to initiate a response often results in different physiological consequences (Wendelaar Bonga, 1997). Handling and confinement stressors have frequently been used in elucidating the stress response in fish (Artigas et al., 2005; Papoutsoglou et al., 1999; Wendelaar Bonga, 1997). Both stressors have been shown to result in an increase in cortisol (Sumpter et al., 1986), which induces the energetically costly physiological responses to stress (Wendelaar Bonga, 1997). Increases in cortisol are also seen in fish during exposure to stressors in their natural environment (Barton, 2002; Schreck and Lorz, 1978). For example, salmon that migrate experience an increase in plasma cortisol (Carruth et al., 2002). As an amphidromous species, inanga migrate through potentially contaminated coastal freshwater streams in order to reach adult freshwater habitats (McDowall et al., 1994). Because of this, the resulting increase in contaminant uptake due to handling stress in this study, is likely to be relevant to stressors in their natural habitat and thus may be an important consideration in terms of risk assessment, conservation, and maintenance of a sustainable inanga fishery.

2. Methods

Adult inanga (*G. maculatus*), in the size range 0.4–3.2 g, were collected by seine net from streams in the Canterbury region of New Zealand. Fish were transported under constant aeration to the University of Canterbury's aquarium facility, and then transferred to 50-L plastic aquaria, receiving flow-through freshwater (pH 6.7, [Na] 375 $\mu mol\,L^{-1}$) at a density no greater than 2.5 $kg\,m^{-3}$. Water temperature was maintained at 11–15 °C, with a photoperiod of 12L:12D. Fish were fed ad libitum with commercial flake food (NutrafinMax, USA) daily, and allowed to acclimate to aquarium settings for at least one month before any experimental manipulations. Fish were not fed during any of the trials. All animal manipulations were approved by the Animal Ethics Committee of the University of Canterbury.

2.1. Na^+ influx

Inanga were exposed to handling stress at the start of each experiment through capture by a mesh net (dimensions: 1.5 cm × 2 cm × 17 cm), transport in a 5-L (19 cm × 15 cm) rectangular plastic bucket (containing aquarium water; transport occurred for no more than 5 min); followed by re-capture and handling during setup. All procedures were performed in a temperature controlled room at 15 °C. Na^+ influx rates were assessed using methods similar to Glover et al. (2012). Individual inanga ($n = 6$, mass ranging from 0.4 to 1.2 g) were placed in separate 250 mL round plastic containers (diameter: 8.5 cm, height: 5 cm) of constantly aerated Na^+-free experimental water consisting of 1 mM Ca^{2+} (added as calcium acetate) and pH adjusted to 7 by adding either KOH or HCl (Glover et al., 2012). This water chemistry was chosen to match that used in efflux experiments (see below). In order to determine the impact of water chemistry on influx, an identical trial was also conducted using constantly aerated aquarium water (ionic composition ($\mu mol\,L^{-1}$): 375 Na^+, 574 Ca^{2+}, 0.022 Cu^+, 119 Mg^+, 29 K^+, 310 Cl^-; total hardness: 70 $mg\,L^{-1}$; ammonia: 1.5 $\mu mol\,L^{-1}$; total alkalinity: 52 $mg\,L^1$; pH: 6.7). To each container, 10 μL of ^{22}Na isotope (~5 $\mu C_i\,L^{-1}$; Perkin Elmer) was added. Triplicate 1 mL initial water samples were taken for measurement of total water Na^+ and radioactivity levels. After 1 h, final triplicate 1-mL water samples were taken, fish were washed in a high Na^+ rinse (1 M NaCl) with a lethal dose of 3-aminobenzoic acid ethylester (MS-222, 1 $g\,L^{-1}$). The high Na^+ rinse was used to remove any isotope adsorbed to the body surface of the fish. This rinse was followed by a series of two distilled water rinses. Following spinal cord transection to ensure complete euthanasia, fish were blotted dry and weighed. Whole fish and water samples were measured for radiolabelled Na^+ incorporation via gamma counting (Wallac Wizard 1470; Perkin Elmer). Water samples were analysed for Na^+ via flame spectrophotometer (Sherwood Instruments). These values were used to calculate specific activity ($cpm\,\mu M^{-1}$) before