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Catchment-derived stressors and School Prawn productivity

Matthew D. Taylor

Catherine McLuckie

Angela Russell

R. Hugh Dunstan

Natalie A. Moltschaniwskyj

Geoffrey MacFarlane

Marcus Crompton

Neil R. Loneragan

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Researcher Contact Details

Name:	Matthew D. Taylor
Address:	Port Stephens Fisheries Institute New South Wales Department of Primary Industries, Taylors Beach Rd, Taylors Beach, New South Wales, Australia
Phone:	02 4916 3900
Fax:	02 4982 2265
Email:	matt.taylor@dpi.nsw.gov.au

FRDC Contact Details

Address:	25 Geils Court Deakin ACT 2600
Phone:	02 6285 0400
Fax:	02 6285 0499
Email:	frdc@frdc.com.au
Web:	www.frdc.com.au

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The Principal Investigator wishes to note that much of the text contained in *Hypoxia exposure experiment* and *Salinity exposure experiment* (and associated areas of Discussion) are taken, with permission, from the draft PhD thesis of Catherine McLuckie, University of Newcastle (who is also an author on this report) and associated publications.

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Executive Summary

Concept

New South Wales Department of Primary Industries (NSW DPI) presents new information exploring the effect of catchment-derived stressors on Eastern School Prawn. Declines in School Prawn productivity over decadal time scales have been reported anecdotally across many estuaries in New South Wales, and are evident in the catch statistics in some locations. This has included reports that indicate that prawn landings have become decoupled from freshwater flows, which generally enhance catches in estuarine and inshore fisheries. To date, no research has been conducted into the direct effects of environmental conditions within nursery habitats that may be contributing to these changes in productivity. This project commenced this investigation using the Camden Haven estuary as a case study, and through a combination of high-resolution logger data, aquarium experiments, habitat mapping, extensive field sampling, and analysis of commercial catch statistics, provide evidence to link catchment-derived stressors with changes in productivity of School Prawn. We use this evidence to propose recommendations for targeted repair in the Camden Haven estuary catchment, as well as other New South Wales estuaries supporting School Prawn harvest.

Background

Estuaries support multiple ecological and economic functions with one of the most significant being a nursery and adult habitat for exploited species, and thus provisioning of fisheries productivity is a major ecosystem service derived from estuarine ecosystems. Variability in estuarine systems can have a large impact on fisheries productivity, influencing reproductive and recruitment cycles, juvenile growth, survival and distribution, or stimulating certain adult behaviours (such as aggregation) which are exploited for fisheries harvest. Estuaries are inextricably linked to the catchments that they drain, and much of the variability in estuarine ecosystems is driven by land-based natural processes and anthropogenic activities throughout these watersheds.

Penaeid prawns are an important group of exploited crustaceans distributed in estuarine and coastal habitats around the world, and thus their productivity is influenced by estuarine conditions and the various stressors derived from the adjacent catchments. Importantly, freshwater inflow into the estuary can enhance recruitment, growth and fisheries harvest of some species, but land-based activities can also moderate potentially positive effects of freshwater inflow, particularly where catchment clearing has occurred or overall catchment condition is poor. This can lead to poor water quality in estuaries such as hypoxia, acidification, heavy metal contamination, sedimentation and nutrient loading, potentially in an interactive or multiplicative fashion.

Camden Haven Estuary, on the north coast of NSW, is subjected to multiple catchment-derived stressors. Anecdotal information from commercial fishers in this system suggest that productivity bottlenecks periodically occur, especially with Eastern School Prawn (*Metapenaeus macleayi*) but also with Mud Crab (*Scylla serrata*) and Blue Swimmer Crab (*Portunus armatus*). This recently culminated in 2006/2007, when productivity of School Prawn in the estuary abruptly declined. This project sought to investigate the presence and potential causes of productivity bottlenecks for School Prawn in this estuary, by examining the lethal and sublethal effects of a suite of catchment-derived stressors common in south-eastern Australian floodplain estuaries.

Objectives

Specifically, the objectives of this project were to:

1) Examine school prawn recruitment to different areas within the Camden Haven estuary, to determine if recruitment limitation in certain areas is likely

2) Evaluate whether post-recruitment processes in Camden Haven estuary may be adversely affecting school prawn growth and survival

3) Synthesise research findings to provide recommendations to catchment, habitat and fishery managers regarding restoration of School Prawn productivity

Methodology

A combination of high-resolution data logging, aquarium experiments, mapping, field sampling, and analysis of commercial catch statistics was employed to provide a comprehensive picture of how various catchment-derived stressors may impact productivity of School Prawn fisheries. Dissolved oxygen, temperature, conductivity, and estuary inflow were logged in Camden Haven Estuary for a period of up to 2.5 years. School Prawn and aluminium concentrations were also sampled at up to 18 sites across the estuary every 1-2 months during this period, and the current and historic extent of dominant estuarine habitats was mapped from aerial imagery (with ground truthing). Conditions measured in the estuary were simulated through a series of laboratory experiments to establish lethal and sublethal effects of these stressors on School Prawn. Acute toxicity and sublethal effects of aluminium were tested under normal (pH 8) and acidic (pH 5) conditions, and histological examination conducted to determine pathological effects. The acute effect of hypoxia on mortality was tested in the presence (0.5 mg L^{-1}) and absence of aluminium, and the effect of salinity on growth and mortality was tested over a period of 60 days. The organism metabolome (a complement of metabolites in the organism), was analysed in the latter two experiments, to further elucidate putative sublethal effects of these stressors. School Prawn abundance and condition derived from field sampling was analysed alongside water quality data, and commercial catch statistics were also related to estuary inflow to the main nursery area in the estuary.

Key findings

The various data streams and experiments employed allowed the compilation of a diverse data set that identified several lethal and sublethal impacts of catchment-derived stressors on School Prawn. Logged dissolved oxygen data series indicated a moderate frequency of hypoxia throughout the Camden Haven Estuary, with occasional periods of anoxia. Dissolved aluminium concentrations remained above the relevant marine water quality guideline for the majority of the study period, and concentrations tended to correlate with estuarine inflow.

Laboratory experiments indicated that School Prawn were not particularly sensitive to acute exposure to aluminium under normal conditions, but mortality and tissue bioaccumulation of aluminium was greater under acidic conditions, suggesting an enhanced response where multiple stressors are involved. Histological studies revealed sublethal effects of aluminium including structural abnormalities in the gills and hepatopancreas, and evidence of viral infection and an immune response, particularly at lower pH and higher aluminium concentrations.

There was a significant increase in survival with increasing dissolved oxygen and the LC_{50} was ~0.9 mg L^{-1} regardless of aluminium exposure, although survival decreased at higher DO concentrations in the presence of aluminium. Metabolomic analyses indicated that total fatty acid concentrations and total amino acid concentrations were significantly greater in the muscle tissue from School Prawn subjected to hypoxia, but this was unaffected by aluminium. Docosahexaenoic acid, stearic acid, palmitic acid and behenic acid, and leucine, isoleucine, phenylalanine, and asparagine, contributed to dissimilarity in fatty and amino acid profiles (respectively) among treatments.

Survival consistently exceeded 70% for salinity treatments other than the lowest treatment (salinity of 0.2). Survival was 0% in the lowest salinity treatment, and all prawns had died within 3 days of achieving the endpoint salinity. Salinity did not impact relative growth across the range of conditions examined. In contrast, relative somatic condition appeared to be greater at lower salinities, and was significantly negatively related to salinity, suggesting that lower salinities promote enhanced condition in School Prawn. There was no linear relationship between total fatty acid concentration and salinity, or condition

index. However, total fatty acid concentration was significantly and positively related to total amino acid concentrations. Quantitative profiling of fatty acids and amino acids showed some changes in response to salinity. These complex patterns point to several shifts in cellular chemistry throughout the salinity range investigated.

Field sampling of School Prawn in Camden Haven Estuary revealed that Watson-Taylor Lake was the main nursery area for School Prawn in this system. In the main nursery area, hypoxia led to depressed prawn abundance, and both hypoxia and high estuary inflow led to decreased somatic condition in prawns across the estuary. Long-term commercial catch negatively correlated with estuary inflow (both direct, and one-month lagged correlations), which was the opposite of the expected pattern for the species. A significant amount of habitat has been lost from the Camden Haven Estuary over decadal time scales, with total seagrass cover decreasing from 960 ha in 2004 to 602 ha in 2015, with most of the loss occurring in the main nursery area (Watson-Taylor Lake).

Implications for relevant stakeholders

This study highlights the potential cumulative impacts of a complex array of habitat changes (physico-chemical and biological [i.e. aquatic vegetation]) and catchment-derived stressors, particularly low dissolved oxygen, runoff, and elevated aluminium, on an important exploited penaeid species. The densities of School Prawn in some parts of the system were reasonably high, particularly in Watson-Taylor Lake, indicating that prawns were recruiting to the system during the study period, but a major reduction of seagrass from the main nursery area (Watson-Taylor Lake) could contribute to a productivity bottleneck. Acid sulfate soil (ASS) risk maps indicate a high probability for ASS around the main tributaries to Watson-Taylor Lake, and these represent a potential source of the stressors observed in the main nursery area. Further investigation and consultation highlighted two ASS priority areas in this region: 1) Rossglen ASS Priority Area (adjacent to the Camden Haven River); and 2) Stewarts River ASS Priority Area

With respect to ASS, while the data collected here point to a potential problem they are not yet sufficient to provide specific recommendations regarding targeted remediation strategies, and require more fine-scale information to pin-point exactly which parts of the catchment contribute most to the issue. This will involve generating a robust time series of pH concentrations around western Watson-Taylor Lake and identified ASS priority areas (and associated drains), followed by targeted remediation. This should be followed by additional monitoring to establish the effectiveness of the remediation in terms of the water quality and the productivity of the fishery.

Keywords

Penaeidae; School Prawn; Acid-sulphate soils; aluminium; hypoxia; somatic condition; growth; survival

Scientific literature arising from this report at the time of publication

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Please note that some of the information contained in this report will appear in the PhD Thesis of Catherine McLuckie, University of Newcastle, and her associated publications.

Introduction

Estuaries are dynamic ecosystems, representing the interface between terrestrial and marine environments (Attrill and Rundle, 2002). These systems support multiple ecological and economic functions, with one of the most significant being a nursery habitat for transitory or resident aquatic species (Elliott et al., 2007). Estuarine ecosystems are supported by productivity derived from aquatic micro- and macrophyte producers, which proliferate in shallow and sheltered habitats within these systems (Raoult et al., 2018). Many aquatic macrophytes also create structural habitats and refuges for fauna, and the combination of habitat function (structural and physicochemical) and productivity supports refuge and growth for the early life history stages of many species.

A diverse range of exploited fish and crustacean species are supported by estuaries during juvenile and adult stages of their lifecycle (e.g. Boesch and Turner, 1984; Potter et al., 2016). Thus, provisioning of fisheries productivity is a major ecosystem service derived from estuarine ecosystems (Creighton et al., 2015; Barbier et al., 2011). A substantial level of fisheries harvest often occurs within estuaries themselves, or adjacent coastal ecosystems to which they are linked (Pease, 1999). It follows that natural variability in estuarine systems can have a large impact on fisheries productivity, influencing reproductive and recruitment cycles, juvenile growth, survival and distribution, or stimulating certain adult behaviours (such as aggregation) which are exploited for fisheries harvest. However, as estuaries are inextricably linked to the catchments that they drain, much of this variability is derived from natural and anthropogenic processes in adjacent catchments (Loneragan and Bunn, 1999; Caddy, 2000; Gillanders and Kingsford, 2002).

Penaeid prawns (=shrimp) are an important group of exploited crustaceans distributed in estuarine and coastal habitats around the world (Dall et al., 1990). These fecund, fast-growing and productive crustaceans also represent important prey for a range of higher trophic level species, and support extensive fisheries (e.g. Turner, 1977; Taylor et al., 2018a; Blaber et al., 1990). Estuarine and coastal inshore ecosystems are obligate habitats for many species (Dall et al., 1990), and can also represent areas where fishing effort and harvest is concentrated (Vance et al., 1998; Loneragan et al., 2005; Taylor et al., 2017a; Loneragan et al., 2013). Thus, this group of exploited species is particularly susceptible to the variability that occurs within estuaries, and potential stressors derived from adjacent catchments (Loneragan and Bunn, 1999).

Many factors can affect growth, survival, reproduction, and fisheries productivity of prawns within estuaries. Saltmarsh, mangrove and seagrass habitats have all been clearly linked with prawn productivity (e.g. Taylor et al., 2017b; Manson et al., 2005; Sheaves et al., 2007; Loneragan et al., 2013; Loneragan et al., 2005). In certain species (e.g. the Eastern School Prawn, *Metapeaneus macleayi* and Banana Prawn, *Penaeus merguinesis*), freshwater inflow into the estuary can enhance recruitment, growth and fisheries harvest (see Ruello, 1973b; Vance et al., 1985) whereas these conditions can adversely affect other co-occurring penaeid species (Tyler et al., 2017). Land-based activities can also moderate potentially positive effects of freshwater inflow, particularly where catchment clearing has occurred or overall catchment condition is poor. This can lead to poor water quality in estuaries such as hypoxia (e.g. Tweedley et al., 2016), acidification (e.g. Sammut et al., 1996; Wilson et al., 1999), heavy metal contamination (e.g. Wilson and Hyne, 1997), sedimentation (e.g. Newcombe and Jensen, 1996) and nutrient loading (e.g. Hagy et al., 2004). These factors can adversely affect both the aquatic vegetation in estuarine ecosystems as well as prawns and the fisheries they support. In addition, these factors may act interactively, and be amplified in microtidal (tidal range <2 m) estuarine systems (Warwick et al., 2018). Consequently, quantifying relationships between catchment-derived stressors and estuarine and coastal fisheries is essential for both identifying productivity bottlenecks and designing management measures to address them.

Using the Eastern School Prawn (*Metapenaeus macleayi*, hereafter referred to as School Prawn) as a case study, the overall aim of this research was to examine the lethal and sublethal effects of a suite of catchment-derived stressors common in south-eastern Australian floodplain estuaries. We combine

high-resolution data logging, habitat mapping, and sampling of free-ranging prawn populations, with laboratory experiments, histology and analysis of changes in the prawn metabolome, to provide a comprehensive picture of how various catchment-derived stressors may impact productivity of School Prawn fisheries. Camden Haven Estuary was employed as a focal estuary for this case study, due to the presence of a broad range of stressors (Ryder et al., 2012), and recent evidence of declining productivity (outlined below).

Catchment-derived stressors

Of the catchment derived stressors outlined above, several are particularly relevant to floodplain estuaries of the northern New South Wales coast. Acid sulphate soils are common throughout south-eastern Australia, and contribute to a broad range of issues for estuaries that drain modified coastal floodplains in this area (e.g. Sammut et al., 1995; Sammut et al., 1994). Dissolved oxygen (DO) levels in estuaries can decrease due to the breakdown of vegetation by bacteria in stagnant pockets of water (such as salty water present in deeper holes, or floodplain drains), increases in temperature, or as byproduct of acid sulfate soil oxidation.

The pyritic sediments of catchments that suffer from acid sulfate soils are naturally occurring. It is when these sediments are exposed to air through anthropogenic interactions (including lowering of the water table) that problems occur. Exposure of these soils leads to their oxidation, and a range of chemical processes follow which lead to the synthesis of sulfuric acid. Subsequent inundation with water, either through runoff or tidal exchange, transports this acid into aquatic systems. The associated changes to estuarine water quality are known to lead to the displacement or death of estuarine fish (Sammut et al., 1995), or other potentially lethal or sublethal effects (Sammut, 2002; Callinan et al., 1993).

One of the indirect effects of acid sulphate soils is the mobilisation of metals naturally present in floodplain sediments and soils (Johnston et al., 2003). Decreases in pH can lead to the release of metal ions from clay minerals, particularly aluminium (Al), iron, sodium, potassium and magnesium (Sammut, 2002). Aluminium solubility in freshwater increases with decreasing pH, and the resultant leachate can drain into estuarine systems, often after heavy rainfall. This is of concern since Al in both soluble and particulate forms is toxic to a broad range of aquatic organisms, impacting the gill surface and contributing to ionoregulatory, osmoregulatory and respiratory dysfunction (e.g. Exley et al., 1991; Poléo et al., 1994; Sharma, 2003). However, existing work deals primarily with freshwater organisms, and very little is known about Al toxicity in estuarine or marine species, particularly crustaceans. This may be due to complexity of Al solubility in seawater, which was recently highlighted in Golding et al. (2015). Importantly, Al solubility tends to decrease with increasing salinity. Solubility for Al in full-strength seawater is usually limited to ~0.5 mg L^{-1}, but dynamic solution chemistry means that dissolved Al concentrations may persist above this limit (Golding et al., 2015). A threshold water quality guideline for 95% species protection was estimated as 0.024 mg Al L^{-1} (Golding et al., 2015), but this study also noted that most fish species were not sensitive at concentrations between 0.01–10 mg total Al L^{-1}. The widespread distribution of acid sulphate soils in coastal floodplains of eastern Australia (Callinan et al., 1993), and the relative lack of knowledge on how Al may affect estuarine fish and crustaceans (particularly exploited species), mean further work in this area is necessary.

Most estuarine and marine organisms require oxic conditions to survive, and intermittent or chronic hypoxia, or anoxia can have detrimental effects on organism growth and survival. Hypoxic or anoxic water can be mobilized during periods of rain and lead to hypoxic or anoxic conditions in other areas of the estuary, which in turn affects organism respiration. Heavy metal contaminants that impede respiration, such as Al, may further exacerbate the effects of hypoxic water. Anoxia, hypoxia, and Al concentrations exceeding screening criteria (Golding et al., 2015), have previously been detected in

important estuarine nursery areas in south-eastern Australia (e.g. Ryder et al., 2017; Santos et al., 2011). While anoxic conditions will invariably lead to death, hypoxic conditions may lead to sublethal effects on exposed animals. The effects of catchment-derived stressors are not always evident in the gross biometrics or condition indices often calculated for aquatic species (e.g. length, growth, or indices of condition), especially after acute exposure. Consequently, other more sensitive indicators may be required to detect these sublethal effects.

Environmental metabolomics

Metabolomics is the study of naturally occurring organic metabolites within the tissues of organisms. Environmental metabolomics, or ecometabolomics, is the application of metabolomics to characterise the interactions between organisms and their environment (Bundy et al., 2008). Ecometabolomics is a relatively novel area of investigation (Sardans et al., 2011), particularly as a direct measure of the response of an organism to change in aquatic environments. The approach measures concentrations of different molecules that vary as a result of alterations to biochemical and cellular processes, and thus provides data which reflects the biological function of regulation within an organism (Lin et al., 2006). Metabolic changes that occur in response to changes in environmental conditions generally reflect the rearrangement of an entire metabolic network. Thus, changes in metabolite concentrations need not be linked in a direct fashion to the environmental variable that has changed, but may ultimately lead to the generation of hypotheses surrounding these metabolic changes (Bundy et al., 2008). Ultimately, ecometabolomics can provide biochemical insights into the mechanisms of stress of animals exposed to different environmental conditions, including metabolic responses that may be associated with secondary effects of these conditions (such as viral infection during periods of immune suppression associated with stress, Liu et al., 2015). Characterisation of responses to different stressors, however, ultimately requires exposure under controlled conditions followed by measurement of metabolites of interest.

Fatty acids and amino acids form part of the suite of metabolites studied in ecometabolomics, and are useful for establishing trophic linkages or alterations in metabolic processes arising through exposure to abiotic changes (Sardans et al., 2011). While fatty acids have been used extensively as trophic markers in marine environments (Dalsgaard et al., 2003), they have rarely been used to study potential responses to environmental change in estuarine species. In aquatic species, total fatty acid composition is generally determined by dietary fatty acids (Colvin, 1976; Shewbart and Mies, 1973), especially for essential fatty acids that are not readily synthesised by marine and estuarine fish and crustaceans (Kanazawa et al., 1979). However, lipids are also important components of cell membranes, and changes will also provide an indication of the dynamic physiology of these structures in response to environmental change. Amino acids have rarely been examined in the ecometabolomics of aquatic animals (see summary in Sardans et al., 2011), but free amino acids are indicative of resources available to fuel metabolism and biosynthesis (Finn and Fyhn, 2010; Samuelsson and Larsson, 2008). The few studies available provide evidence for substantial changes in the amino acid pool for aquatic species exposed to oxygen stress (Podrabsky et al., 2007) and heavy metal contaminants (Samuelsson and Larsson, 2008). Together, fatty acids and amino acids can provide evidence of changes in a range of metabolic processes in estuarine species, which may occur in response to numerous catchment-derived stressors.

School Prawn

School Prawn is an exploited species of penaeid prawn common to coastal regions of south-eastern Australia, between south-east Queensland and eastern Victoria. School Prawn is a valuable species, and while it does not normally yield a market price as high as other penaeid species harvested in south-eastern Australia (for example, Eastern King Prawn), it is a high volume species yielding annual catches between 650-1400 tonnes per annum harvested (Taylor et al., 2016a). In New South Wales, the species is harvested in most commercially fished estuaries within its range, and adjacent inshore coastal areas (Taylor et al., 2016b) after large rainfall events (Glaister, 1978), through the Estuary Prawn Trawl Fishery, the Estuary General Fishery and the Ocean Trawl Fishery. Fresh and cooked product supports a strong consumption market, and a large proportion of harvested product is also sold into the recreational bait market which can yield retail prices equivalent to product sold for consumption. School Prawn display a Type-II penaeid life cycle (Dall et al., 1990), including both a juvenile nursery phase and an inshore coastal adult stage. During the estuarine phase, the species usually distributes through the middle and upper estuary (Taylor et al., 2017a), with strong recruitment and high growth occurring over the warmer months of the year (Racek, 1959). School Prawn usually reside in estuaries for most of their life, but move to inshore coastal areas to spawn (with maturation and spawning migration enhanced by freshwater inflow, Racek, 1959). Following spawning, larvae remain inshore and disperse over relatively short distances (Ruello, 1977). They often recruit into the same estuary from which their parents came, or exhibit limited dispersal into adjacent estuaries.

The relationship between freshwater flow into estuaries and commercial catch of School prawn was first outlined by Racek (1959). Racek (1959) outlined both regular and irregular migrations undertaken by the species from estuaries as part of their life-cycle; the former relating to predictable runs to the sea in the months of December through March around the last quarter of the moon, and the latter relating to downstream movements of prawns (and egression from the estuary) in response to rainfall and freshwater inflow to the estuary. Ruello (1973b) later studied these irregular movements in more detail, and made several conclusions surrounding the lagged (i.e. lagged by year, pre-recruitment impacts) relationship between freshwater inflow and prawn catch (studying post-recruited size classes though). Firstly, the action of freshwater inflow pushing (or stimulating the movement of) adults down the front and out of the estuary produces a “density” or “aggregative” effect that enhances reproductive potential (and also their susceptibility to catch). Secondly, freshwater inflow alters the salinity regime and enhances the areal brackish extent of the estuary, thus increasing the lower salinity habitats available to support school prawns (and excluding competing *Penaeus plebejus* juveniles from these areas). Thirdly, in addition to the enhanced abundance of young prawns derived from increased reproduction, the hydrodynamics of the estuary under conditions of elevated inflow stimulate the transport of coastally spawned postlarvae further upriver. Finally, runoff and inundation of high marsh and mangrove stands stimulates nutrient and food availability in the estuary, which thus enhances growth and survival. The latter two remain hypotheses, which have never been directly examined.

Glaister (1978) further explored the direct (i.e. lagged by day/week/month, post-recruitment impacts) relationship between freshwater inflow and commercial prawn landings and catch rates (also studying post-recruited size classes). Several direct correlations were detected between estuarine inflow to the Clarence River and both estuarine and oceanic production (when considered separately); which indicated that freshwater inflow had a distinct aggregative effect on adolescent and adult prawns that manifested in enhanced catch shortly after the elevated flow came into the estuary. Interestingly, in contrast to Ruello (1973b), he found no lagged (annual) relationship present in the Clarence River.

Considering the above work, freshwater inflow to the estuary is probably the most influential variable arising from climate variability affecting School Prawn populations. This general conclusion was supported by Montgomery (1990), and the later work by Ives et al. (2009). Despite the proposed positive influences of freshwater on both catchability and settlement/ recruitment of School Prawn, depending on the condition of the catchment, freshwater flow can result in severe and adverse conditions in estuaries through the mechanisms outlined above that manifest in hypoxia, acidic run-off from acid sulphate soils, and/or heavy metal mobilisation. Sublethal effects of these conditions have

yet to be examined with School Prawn; however, previous research indicates that a behavioural response is possible (Kroon, 2005). Similarly, Pinto and Maheshwari (2012) highlight a relationship between dissolved oxygen, pH, temperature and School prawn landings in the Hawkesbury River, but again fail to provide any empirical data to relate these variables with any sublethal effects.

Camden Haven estuary

Camden Haven Estuary is an wave-dominated barrier estuary on the mid-north coast of New South Wales (Roy et al., 2001). The estuary has a trained, permanently open entrance, covers a waterway area of 32 km^2 and has a catchment area of 589 km^2. Catchment land use is primarily agricultural, state forest, and national park, but the lower estuary includes a small residential settlement (~17,000 people). The estuary supports a wild harvest fishery dominated by crustaceans (School Prawn, Mud Crab *Scylla serrata*, Blue Swimmer Crab *Portunus armatus*) and Sea Mullet *Mugil cephalus*, and also has extensive aquaculture lease areas where Sydney Rock Oyster (*Saccostrea glomerata*) are cultured. Two large, shallow (< 1 m depth) lakes form prominent features of the estuary, comprising much of the available waterway area (Figure 1). Queens Lake in the north is fed by Herons Creek, and is connected to the main channel of the estuary (Camden Haven Inlet) by Stingray Creek. Watson-Taylor Lake lies on the main tributary (Camden Haven River), but is also fed by Stewarts River from the south-west. These tributaries are typically much deeper than the shallow lake systems.

Anecdotal information from commercial fishers in the Camden Haven Estuary suggest that productivity bottlenecks periodically occur for commercially fished species in this system, especially with School Prawn but also with Mud Crab and Blue Swimmer Crab. This recently culminated in 2006/2007, when fishers noticed that School Prawn were smaller than usual, and then that School Prawn were present in significantly smaller numbers the following year. This was followed by a sustained period of depressed annual landings from 2006-2013 (~5 tonnes per annum) relative to landings from 1998-2006 (~17 tonnes per annum). Similar anecdotes have been reported during this period from other estuaries in south-eastern Australia that support School Prawn fisheries (e.g. Clarence River, M.D. Taylor, pers. comm.).

Previous monitoring work conducted in the Camden Haven Estuary and associated tributaries indicated that several catchment-derived stressors may impact estuarine water quality (Ryder et al., 2012) and macrophyte habitat (Creighton, 1982), and could have concomitant impacts on School Prawn. These included low levels of dissolved oxygen, high levels of Al, freshwater inflow, and sedimentation. Low dissolved oxygen is thought to occur due to the build-up and decomposition of terrestrially-derived organic matter in the deeper bathymetry of the tributaries to Watson-Taylor and Queens Lake, which suffer from poor tidal exchange. Aluminium is a by-product of acid sulfate soils (ASS) present in the catchment (Ryder et al., 2012), and can have deleterious effects on aquatic biota (e.g. Corfield, 2000; Hyne and Wilson, 1997; Sammut et al., 1995). Brackish water is also thought to provide essential juvenile habitat for School Prawn (Ruello, 1973b; Taylor et al., 2017a; McLuckie et al., 201X), but freshwater discharge into the estuary usually takes place over a short time period, so brackish conditions quickly dissipate (Creighton, 1982). Finally, sedimentation in the lake systems may contribute to changes in macrophyte distribution and species composition (Creighton, 1982), which may affect School Prawn abundance and distribution

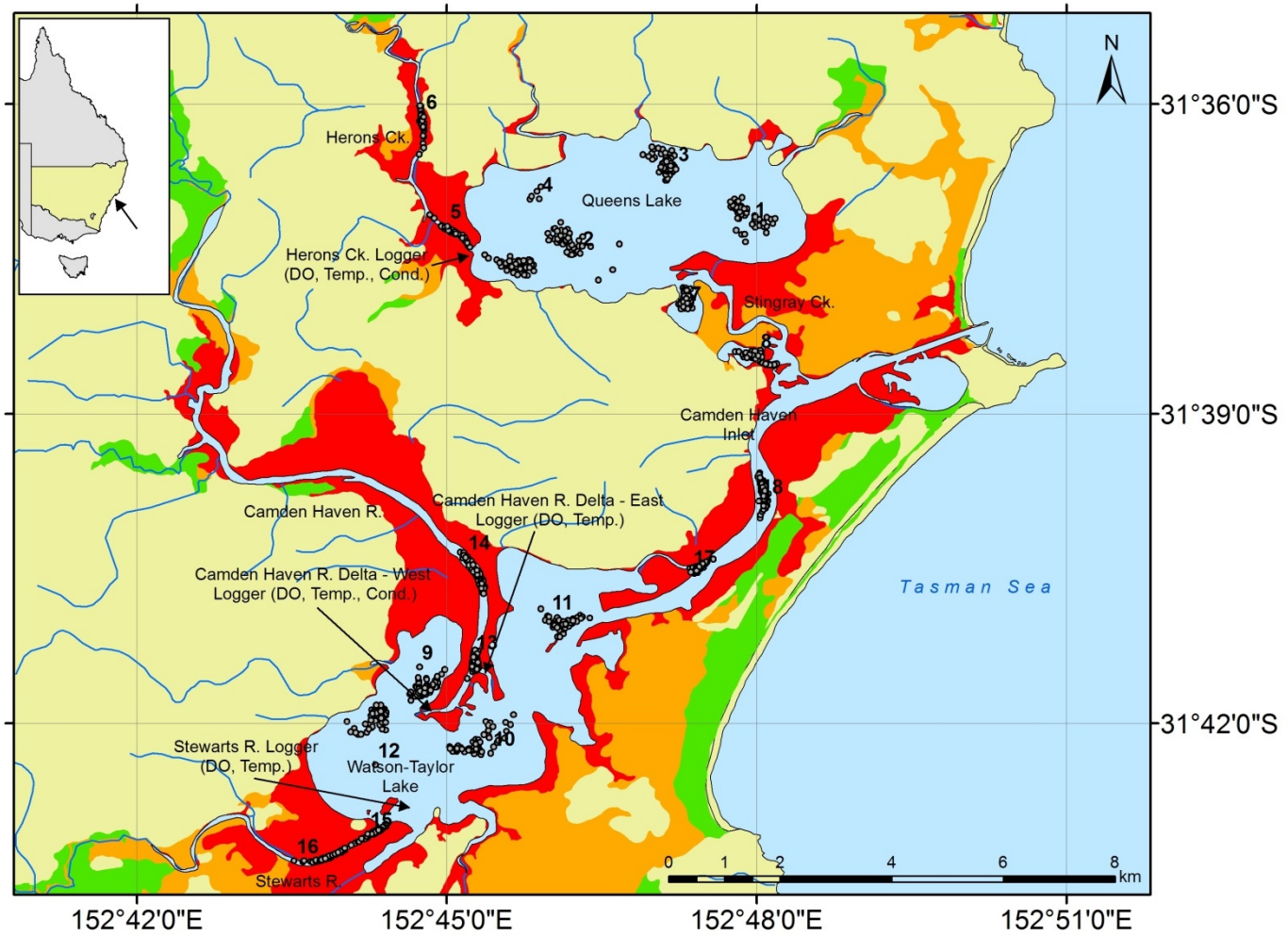

Figure 1 Map of Camden Haven Estuary showing the two main lakes (Queens Lake and Watson-Taylor Lake), site names, and the main tributaries into each of these lake systems. Trawl samples are indicated as grey circles, and the position of the four logger stations is indicated. Acid sulfate soil probability is indicated as red (high probability), orange (medium probability) and green polygons (no known occurrence). The location of the study estuary on the eastern Australian seaboard is indicated in the upper left panel.

Objectives

The broad objectives of this project were to:

1) Examine school prawn recruitment to different areas within the Camden Haven estuary, to determine if recruitment limitation in certain areas is likely

2) Evaluate whether post-recruitment processes in Camden Haven estuary may be adversely affecting school prawn growth and survival

3) Synthesise research findings to provide recommendations to catchment, habitat and fishery managers regarding restoration of school prawn productivity

These objectives were evaluated through an extensive field survey which occurred alongside a series of comprehensive laboratory experiments. These laboratory experiments were designed to simulate the stressors observed in the field data under controlled conditions, to evaluate lethal and sublethal effects on School Prawn. The outcomes of the various components of the field survey, and laboratory experiments, are described separately in subsequent sections

Water quality monitoring and habitat mapping

As noted in the introduction, Creighton (1982) and Ryder et al. (2012) outline a suite of catchment-derived stressors that periodically occur with the Camden Haven Estuary. These include periodic anoxia and hypoxia, elevated concentrations of aluminium, heavy pulses of freshwater inflow to estuaries, and declines in seagrass habitat. Both these studies, however, were limited in the regularity with which samples were collected. Consequently, extensive sampling of these variables was undertaken during the course of the field survey, which was used to both define parameters for laboratory experiments, and as supporting covariates in the School Prawn abundance and condition data.

Methodology

Four logger stations were deployed within the Camden Haven Estuary, with one at the mouth of Herons Creek, two on the delta of the Camden Haven River, and one at the mouth of Stewarts River (Figure 1). Hobo U26-001 Dissolved Oxygen (DO) loggers (Onset Corporation, Bourne, MA) were deployed at each station from November 2015 until January 2018, which logged dissolved oxygen and temperature at 15 minute intervals. Loggers were equipped with a U26-GUARD-2 antifouling protective cap, and DO sensor caps (U26-RDOB-1) were replaced every ~5-6 months. Odyssey Conductivity and Temperature loggers (Dataflow Systems, Christchurch, New Zealand) were later added to one logger station at the mouth of Herons Creek and one at the Camden Haven River mouth to record conductivity and temperature at 15 minute intervals. Daily average flow data was obtained for gauging stations 207009 (Camden Haven River) and 207008 (Stewarts River) from the NSW Government Water Data Portal (see https://realtimedata.waternsw.com.au/water.stm).

Benthic water samples were collected at each site (Figure 1) on every second month, using a submersible pump apparatus. The pump was deployed ~20 cm above the sediment (to prevent contamination of water samples with sediment, and water was flushed through the pump and associated piping for ~2 minutes. After flushing, water was collected into a polypropylene tube, which was immediately placed on ice, and shipped directly to the Environmental Analysis Laboratory (EAL) at Southern Cross University for analysis of Al using Inductively Coupled Plasma Mass Spectrometry (ICPMS).

Existing spatial imagery held in the NSW Department of Primary Industries Fisheries Spatial Database (see https://www.dpi.nsw.gov.au/about-us/science-and-research/spatial-data-portal) were used to quantify the cover of major macrophyte habitats in the estuary, and changes in macrophyte cover over time. Three time points were evaluated, and abundance of habitat in the years 2004, 2009 and 2015 was determined.

Results

Logger data provided an almost continuous time-series of dissolved oxygen and temperature (Figure 2). Broad-scale variation in temperature followed seasonal cycles but with some short-term variability. Temperatures ranged from 12 – 30°C, and were typically ~25°C from November until March. The

dissolved oxygen data series indicated a moderate frequency of hypoxia events (<4 mg L^{-1}) at all logger stations, and occasional brief and extended periods of severe hypoxia or anoxia (designated as Critical, see above, Figure 2). This was most common in Stewarts River, but also occurred in Herons Creek, and these events were up to two weeks in duration. Conductivity varied throughout the study period, although logger failure meant there were substantial gaps in the time series (Figure 3). The conductivity in Herons Creek was much more variable than that in Camden Haven River with frequent rapid fluctuations between 10 and 60 ms cm^{-1} (e.g. between June and December 2016, Fig. 3), while Camden Haven River experienced a maximum conductivity of ~40 ms cm^{-1} and tended to take longer to increase following freshwater events (Figure 3). Periods of hypoxia or anoxia did not appear to consistently coincide with periods of low conductivity. Periodic spot measurements of water quality variables during sampling generally reflected the logger data (at the closest station – data not shown). Spot measurements revealed that pH fell as low as 5, but generally remained relatively stable during the study (8.1 ± 0.7; mean ± sd).

Dissolved Al concentrations were also very variable across the study period (Figure 3). There was a notable peak in Al in mid-March 2017 (up to 6.8 mg L^{-1}, Fig. 3), which coincided with a large peak in flow at the same time (Figure 3). At all locations, Al concentrations remained above the marine trigger level of 0.024 mg L^{-1} (Golding et al., 2015) for the majority of the study period. Generally, trends in Al concentration tended to follow trends in estuary inflow (i.e. they were greatest during times of greatest flow, Figure 3).

The abundance and distribution of vegetated habitat changed markedly from 2004 until 2015 (Figure 4, Table 1). Total seagrass cover was greatest in 2004 (960 ha), and then steadily declined in 2009 (784 ha) and again in 2015 (602 ha), representing a decline in cover from 30% to 18.8% of the total waterway area during this 12 year period. The assemblage composition of seagrass also changed markedly, with mixed *Zostera* and *Halophila* beds becoming dominated by *Zostera* (Table 1). Given the increase in *Zostera* in 2015, it would appear likely that the majority of the lost seagrass during the 12 years was *Halophila* (Table 1). *Ruppia* was only recorded in 2009, with 73 ha of cover recorded (plus cover in mixed *Zostera/Halophila/Ruppia* beds, Table 1). There was only minor variation in the abundance of mangrove and saltmarsh habitats over this time period, although saltmarsh habitat decreased by approximately 10%. When the spatial coverage was examined, *Zostera* and *Zostera*/*Halophila* beds were almost completely lost from Watson-Taylor Lake between 2004 and 2015 (Figure 4). While a gradual retreat of the main seagrass bed was also evident in Queens Lake, there was also a shift from mixed *Zostera*/*Halophila*, and *Ruppia* vegetation (2009 only) to almost complete dominance by *Zostera* in 2015 (Figure 4).

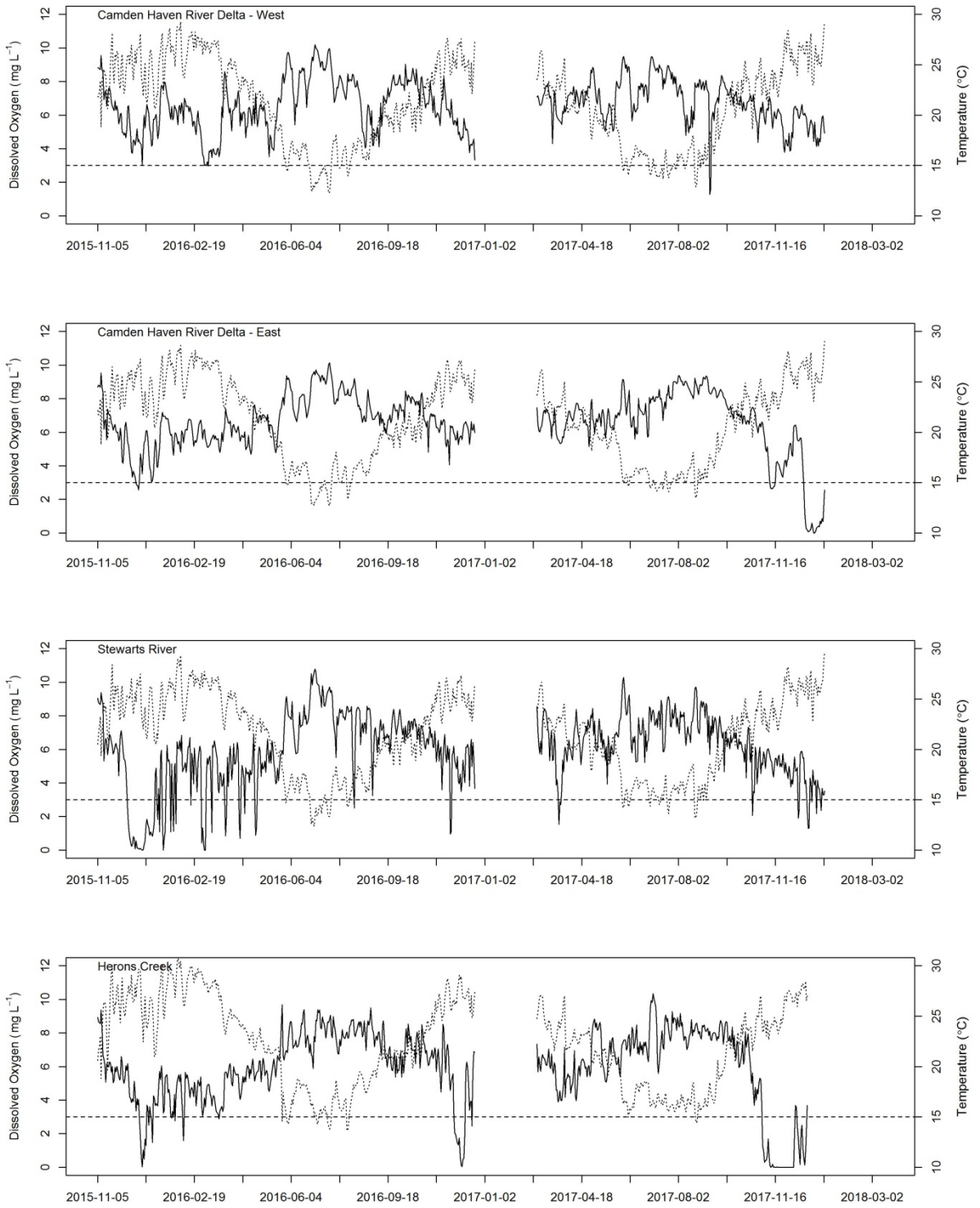

Figure 2 Dissolved oxygen (solid line) and temperature (dotted line) data collected on four fixed position logger stations over the course of the study (indicated in Figure 1). The horizontal dashed line indicates the critical dissolved oxygen threshold used in the analysis. Some gaps in the data series appear due to logger loss or logger failure.

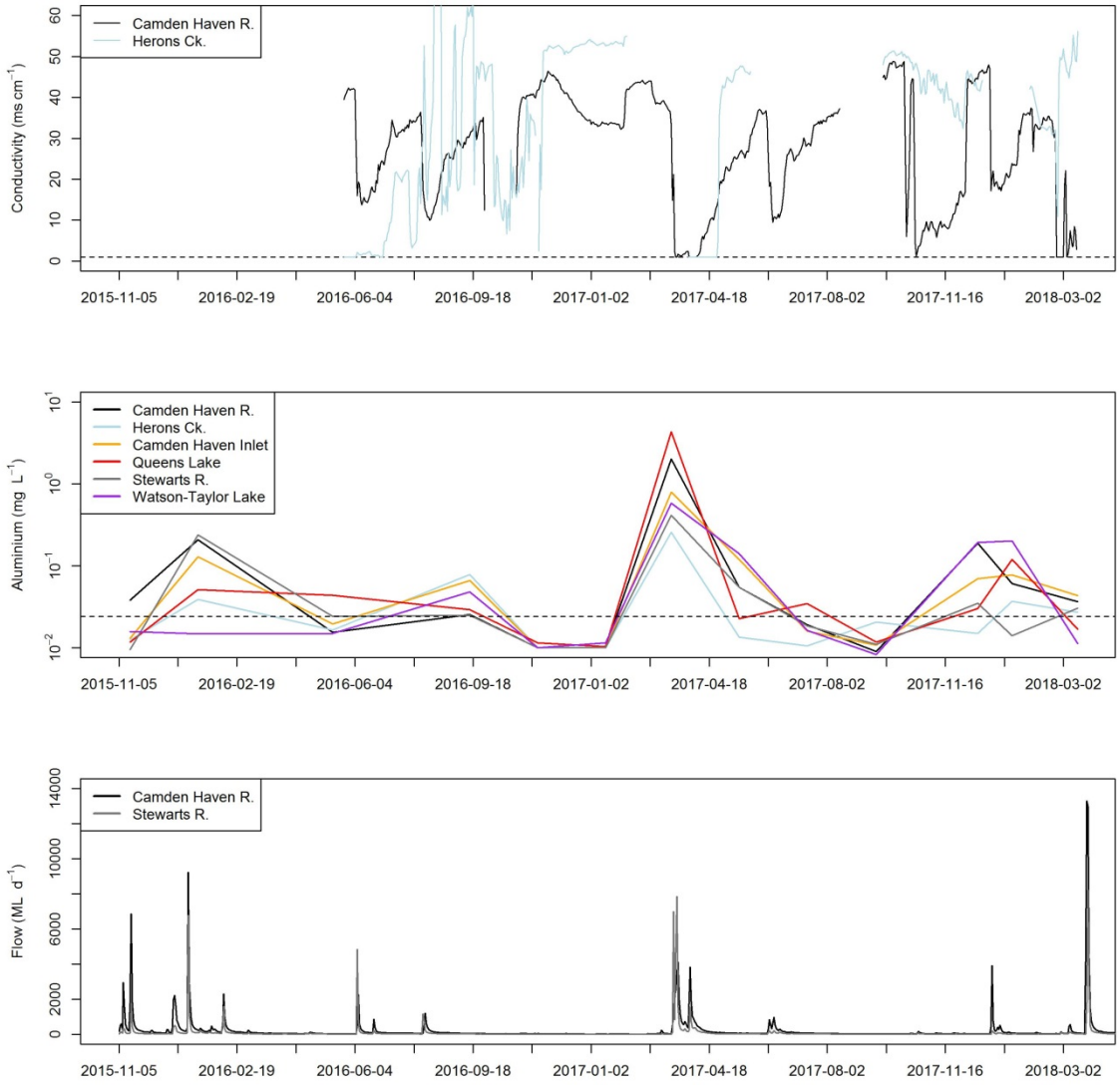

Figure 3 Conductivity data (upper panel) from Camden Haven River (black line) and Herons Creek (light blue line), average Al concentrations (middle panel) measured throughout the study period at various locations across the Camden Haven Estuary, and estuary inflow measured in Camden Haven River and Stewarts River (lower panel). Location names correspond to those indicated in Figure 1. The horizontal dashed line indicates the calibration limit for the conductivity logger in the upper panel, and the trigger value for Al reported in Golding et al. (2015) in the middle panel. Some gaps in the data series appear due to logger loss or logger failure.

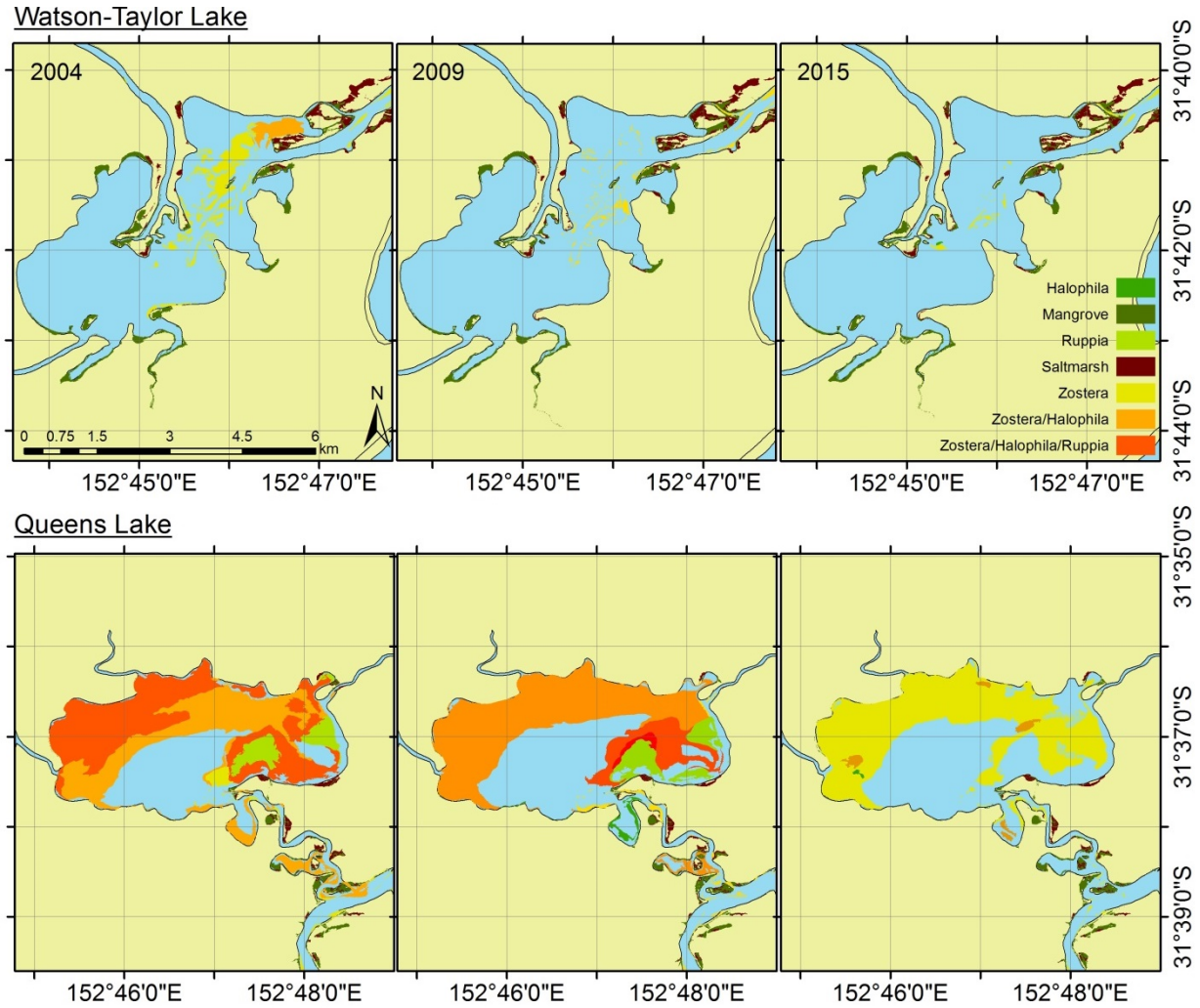

Figure 4 Map showing the change in the extent of seagrass extent over time in Watson-Taylor Lake (top panels) and Queens Lake (lower panels). Time points include 2004 (left panels), 2009 (centre panels) and 2015 (right panels), and actual areas are presented in Table 1. Macrophyte habitats are coloured as per the figure legend.

Table 1 Temporal change in the spatial extent of macrophyte cover over time in Camden Haven Estuary.

Macrophyte	2004 (ha)	2009 (ha)	2015 (ha)
Zostera sp.	134	71	575 (264)*
Zostera/*Halophila* spp. complex	826	513	25
Zostera/*Ruppia* spp. complex		11	
Zostera/*Halophila*/*Ruppia* spp. complex		103	
Halophila sp.		13	2
Ruppia sp.		73	
Total seagrass	960	784	602
Mangrove	141	146	150
Saltmarsh	77	75	70

Aluminium and pH exposure experiment

This experiment investigated lethal and sublethal effects of Al exposure on juvenile School Prawn. Specifically, the study sought to 1) assess the lethality of Al exposure for juvenile School Prawn at pH 5 and 8; 2) evaluate the bioaccumulation of Al in the whole body tissues of juvenile School Prawn; and 3) qualitatively evaluate sublethal effects of Al exposure through histological examination of gill and hepatopancreas tissue in exposed prawns.

Methodology

School Prawn collection and husbandry

Juvenile School Prawn (carapace length [CL] 5 – 10 mm) were collected from the Hunter River estuary (-32.853, 151.765) using a benthic sled net (1 × 0.4 m mouth, 4 m length, 26 mm diamond mesh body and 6 mm octagonal mesh cod-end, Taylor et al., 2017a). Shots were generally a few minutes in duration, towed at 0.5 knots, to ensure live prawns were captured in good condition. Prawns were sorted and identified immediately after landing and School Prawn were placed in an onboard aerated holding tank for transport to the Port Stephens Fisheries Institute (PSFI) Research Aquarium. At PSFI, animals were acclimated to environmental conditions at the aquarium over 48 h (32 salinity, 22°C, pH 8.2) and held in a 5,000 L aerated polyethylene tank with a 25% exchange of water each day. Animals were held under these conditions for 8 days prior to testing, and fed *ad libitum* with a dry feed (Otohime Hirame® by Aquasonic) every evening.

Aluminium exposure

Experimental treatments were undertaken in 6 L aquaria tanks, maintained in a static aerated system within a temperature (22°C) and light (12-h light/dark) controlled room. School Prawn used in the experiments were 5.99 ± 0.03 mm CL (mean ± SE) and 0.28 ± 0.03 g. Prawns were unfed for 24 hours prior to testing, and unfed during the experimental period. Experimental treatments involved 96 h assays conducted under normal (pH 8) and acidic (pH 5) conditions, which exposed prawns to target Al concentrations between ambient (concentration in fresh, uncontaminated seawater) and a target upper concentration of ~128 mg Al L^{-1}, at roughly twofold increments (nominal concentrations corresponded to 0, 2, 4, 8, 16, 32, 64, 128 mg Al L^{-1}, with 3 tanks per concentration). For each trial, treatment conditions were randomly assigned among tanks, and individuals were randomly assigned among experimental treatments with ten animals per treatment tank. We note that the upper concentrations used were much higher than those usually encountered in estuarine systems. These higher concentrations were included to provide data in support of an LC_{50} for Al to be calculated for School Prawn, if Al contributed to mortality in the species.

A 4.6 g L^{-1} Al stock solution was prepared by dissolving Al sulphate ($Al_2(SO_4)$, Chem-Supply, Gillman, South Australia) into estuary water. Aliquots of this stock solution were slowly added to each treatment tank to achieve the target concentrations. Following the addition of Al the pH of the water was adjusted in small increments using strong sulphuric acid or 8M sodium hydroxide/estuary water solutions. A 20 mL water sample was collected from each treatment tank for analytical testing. Tanks were observed for mortalities during the first 0.5, 2, 4 and 8 h intervals, and then every 12 h thereafter, with mortalities recorded and removed at each time interval. During the experiment, water exchanges with “new” water prepared to match the experimental treatments occurred every 24 h. Water quality

measurements (temperature, pH, dissolved oxygen and salinity) were collected at each exchange from the old and new water to monitor stability in conditions.

Water samples were tested to determine the exact exposure concentrations at the Environmental Analysis Laboratory (EAL), Southern Cross University, New South Wales. To determine dissolved Al content, samples were passed through a 0.45 μm cellulose acetate filter and then acidified with nitric acid. The total recoverable Al was obtained through a hotblock digest using nitric acid and then analysed using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS), and reported against recovery (96%) of a reference standard (DORM 4 Fish Protein)

Sample preparation and histopathology

At the conclusion of each experiment, all surviving animals were euthanised in an ice slurry, gently blotted with tissue paper, and measured for CL and weight. Two individuals from each replicate in each treatment were placed in Davidson's fixative for 48 hours and then transferred into a 2 × 5 cm histology-embedding cassette and submerged in specimen jars filled with 70 % ethanol. All histology samples were sent to the University of Tasmania where samples were processed using a Leica TP1050 tissue processor. Samples were dehydrated in an alcohol series and embedded in paraffin using a Shandon Histocentre 3 embedding centre (Thermo Electron Corporation, Massachusetts, USA). The resulting paraffin blocks were transversely and longitudinally sectioned at 5 μm using a Microm HM340 rotary microtome (Microm International GmbH, Germany), stained with haemotoxylin and eosin using a Shandon Linistain GLX automatic stainer (Thermo Scientific, USA) and mounted onto microscope slides with DPX for cover slipping. All slides were qualitatively analysed for microscopic changes to the gill and hepatopancreas using an Olympus CX41 compound microscope coupled with a Jenoptix ProgRes® C5 Lasor Optik System (Germany) and ProgRes Capture Pro 2.9.01 software system to capture images. While examination was primarily qualitative, a grading system was used to estimate changes in haemocyte density in the structures according to the criteria: Grade 1 = <10 % cover; Grade 2 = 10 – 50 % cover; and Grade 3 = >50% cover.

Aluminium tissue testing

At the conclusion of the experiment, juvenile School Prawn were retained and tested for tissue Al concentrations (mg kg^{-1}). For each treatment combination, ~0.1–0.15 g sub-samples of muscle were composited (yielding a composite mass >0.4 g), immediately frozen, and shipped to EAL for analysis. Samples were hotblock digested with nitric acid and analysed using ICP-MS with results reported against recovery (96%) of a reference standard (DORM 4 Fish Protein).

Data analysis

An analysis-of-covariance was used to analyse the interactive effect of pH (fixed; 2-levels; pH 5 and pH 8), and $\log_{10}$(total Al), on $\log_{10}$(dissolved Al), survival and bioaccumulated tissue Al concentration. Further ANCOVA's were conducted to assess the interactive effect of pH and $\log_{10}$(dissolved Al), on survival and $\log_{10}$(tissue Al). All analyses were undertaken using R v.3.2.0 (R Core Team, 2016).

Results

Speciation of Aluminium across the treatments

Aluminium was more soluble at lower exposure concentrations but began to form precipitate as Al exposure concentrations increased. Despite this, concentrations of dissolved Al increased with total Al ($F_{1,48}$ = 56.42, P < 0.01) and there was an effect of pH, with lower pH treatments (pH 5) exhibiting higher dissolved Al concentrations ($F_{1,48}$ = 11.72, P < 0.01). There was no interaction between pH and total Al ($F_{1,48}$ = 0.99, P =0.33).

Mortality and bioaccumulation following exposure to aluminium

There was minimal mortality observed following exposure to Al across the ranges tested (Figure 5), with the majority of pH 8 treatments showing 90% survival or greater (98.9 ± 0.6%; mean ± SE, averaged across all concentrations). Survival was lower in the pH 5 treatment (75.2 ± 4.1%; mean ± SE, averaged across all concentrations), but did not appear to form a consistent relationship with the concentration of total Al (Figure 5). These relationships were confirmed by the statistical analysis, where pH had a significant effect on survival ($F_{1,50}$ = 31.92, P << 0.01), but there was no significant effect of total Al ($F_{1,50}$ = 0.49, P = 0.49), or interaction between Al and pH ($F_{1,50}$ = 0.59, P = 0.45). Similar patterns were obtained when examining survival with dissolved Al, where lower survival was observed at pH 5 ($F_{1,50}$ = 31.27, P << 0.01), but with no significant effect of dissolved Al ($F_{1,50}$ = 0.04, P = 0.84) nor a significant interaction ($F_{1,50}$ = 0.01, P = 0.95).

Patterns in bioaccumulation with total Al were similar to those observed for survival, with significantly higher bioaccumulation at pH 5 ($F_{1,12}$ = 13.98, P < 0.01) but no significant effect of total Al ($F_{1,12}$ = 1.15, P = 0.31), or interaction between Al and pH ($F_{1,12}$ = 1.12, P = 0.31, Figure 6). Although there appeared to be a positive relationship between dissolved Al and bioaccumulation (Figure 6), this was not significant ($F_{1,12}$ = 0.18, P = 0.67) as the patterns were likely masked by increased precipitation at higher Al concentrations. There was, however, a significant effect of pH ($F_{1,12}$ = 12.78, P < 0.01), but no interaction between pH and dissolved Al ($F_{1,12}$ = 0.85, P = 0.38).

Histology of the gills of School Prawn

The structural integrity of the dendrobranchiate gills of control animals from both pH 8 and pH 5 treatments appeared intact, with uniform arrangement of the lamellae (Figure 7). The inter-lamellar spaces were consistent and the hemocaelic space, with immune-responsive hemocytes in the circulatory hemolymph, was unremarkable (see Bell and Lightner, 1988).

Various changes were observed following exposures to concentrations ≥ 2 mg Al L^{-1}. Cuticle separation and Grade 2 haemocyte aggregations were observed at both pH levels (Figure 7), which increased to Grade 3 in pH 5 treatments of Al concentrations ≥ 8 mg Al L^{-1} (Figure 7). The density of haemocytes appeared to increase in pH 8 treatments with ≥ 4 mg Al L^{-1}, and there also appeared to be debris in the inter-lamellar spaces (ILS) in both pH treatments, which is typically seen where reduced activity decreases gill flushing, and particles such as algae cells or heavy metal salts become trapped between the filaments (Untersteiner et al., 2003).

Melanisation of the distal gill filaments occurred alongside cuticle separation in prawns exposed to ≥ 4 mg Al L^{-1} at pH 8 (Figure 8) and ≥ 2 mg Al L^{-1} at pH 5 (Figure 8), indicating that melanisation commenced at lower concentrations under more acidic conditions. Melanisation tended to increase with increasing Al concentrations, with cuticle separation and haemolymph coverage increasing with higher concentrations. In pH 5 treatments, melanisation was more severe and coverage appeared to be more extensive (Grade 2 + 10% cover), especially at ≥ 16 mg Al L^{-1} (> 50% cover). Infiltration of

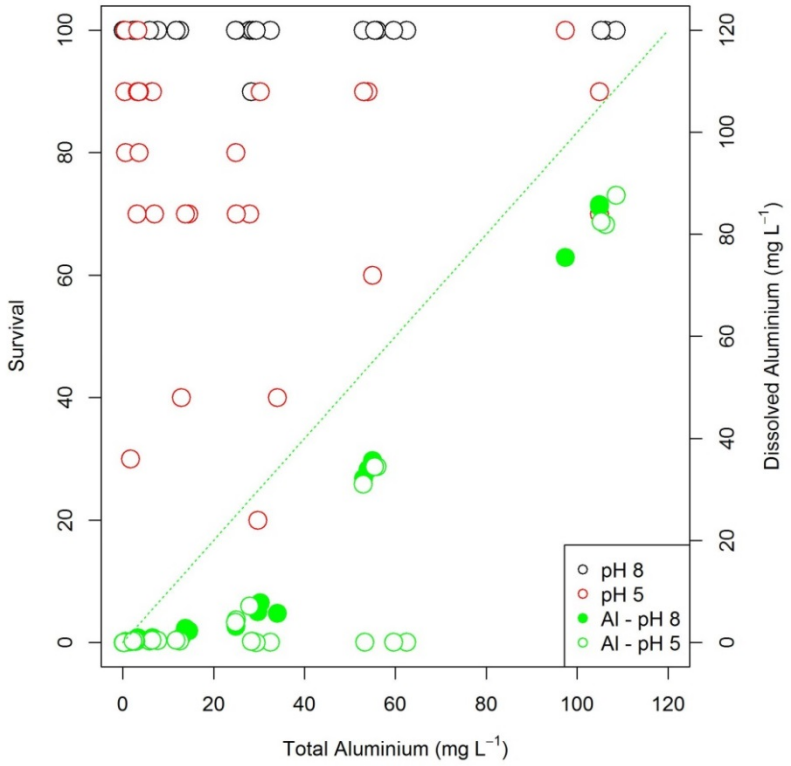

Figure 5 Survival of School Prawn (in each tank) over a range of different Al concentrations tested at pH 8 (black circles) and pH 5 (red circles). Also shown are the laboratory results from Al testing, showing total and dissolved Al (indicated on secondary *y*-axis) for each replicate (green circles; for pH 8 [closed] and pH 5 [open]). A line of parity between total and dissolved Al is provided as a reference (green dashed line).

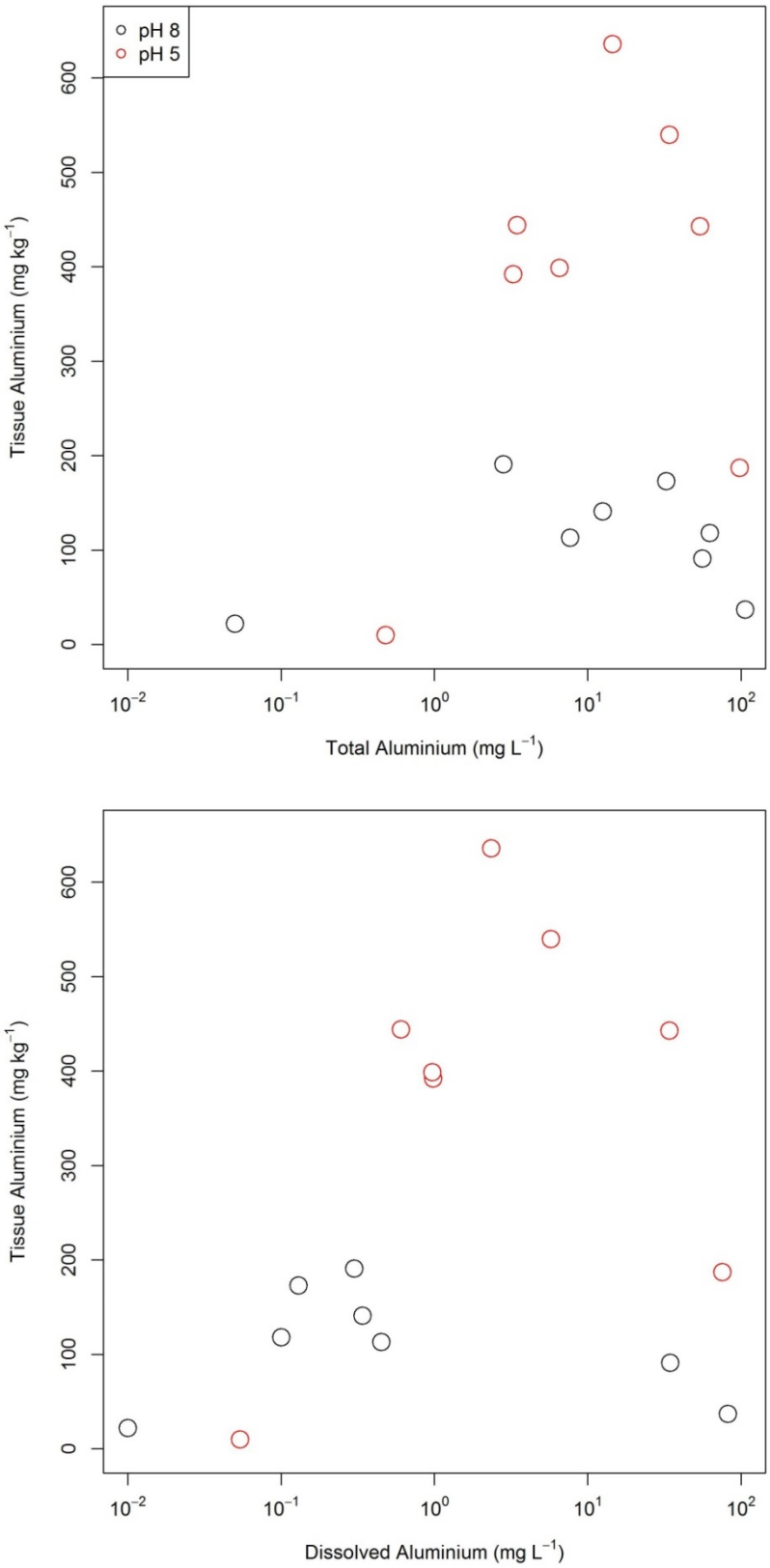

Figure 6 Bioaccumulation of Al in School Prawn muscle tissue composites over a range of different Al concentrations. Data shown is for total Al (upper panel) and dissolved Al (low panel) at pH 8 (black circles) and pH 5 (red circles).

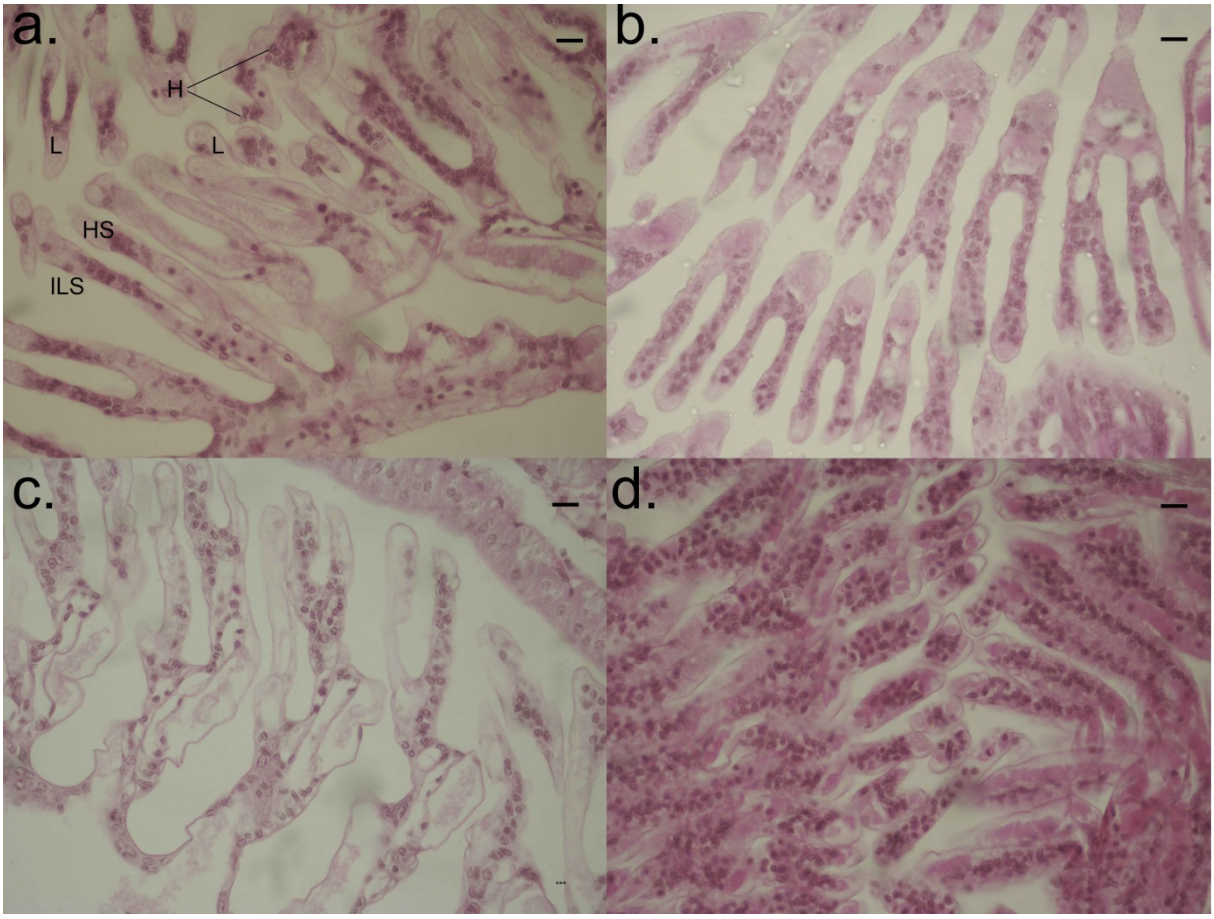

Figure 7 Structure of dendrobrachiate gills in School Prawn (hematoxylin and eosin, 40x magnification, scale bar 50 µm in all panels) exposed to pH 8 (a) and pH 5 (b) in the absence of Al. The lamellae (L), inter-lamellar spaces (ILS), haemocaelic space (HS) and haemocytes (H) are indicated in (a) for reference. Cuticle separation at the edge of the lamellae and increased haemocyte aggregation was evident in gills following exposure to ≥ 2 mg Al L^{-1}, as shown for concentrations 2 mg Al L^{-1} at pH 8 (c) and 8 mg Al L^{-1} at pH 5 (d).

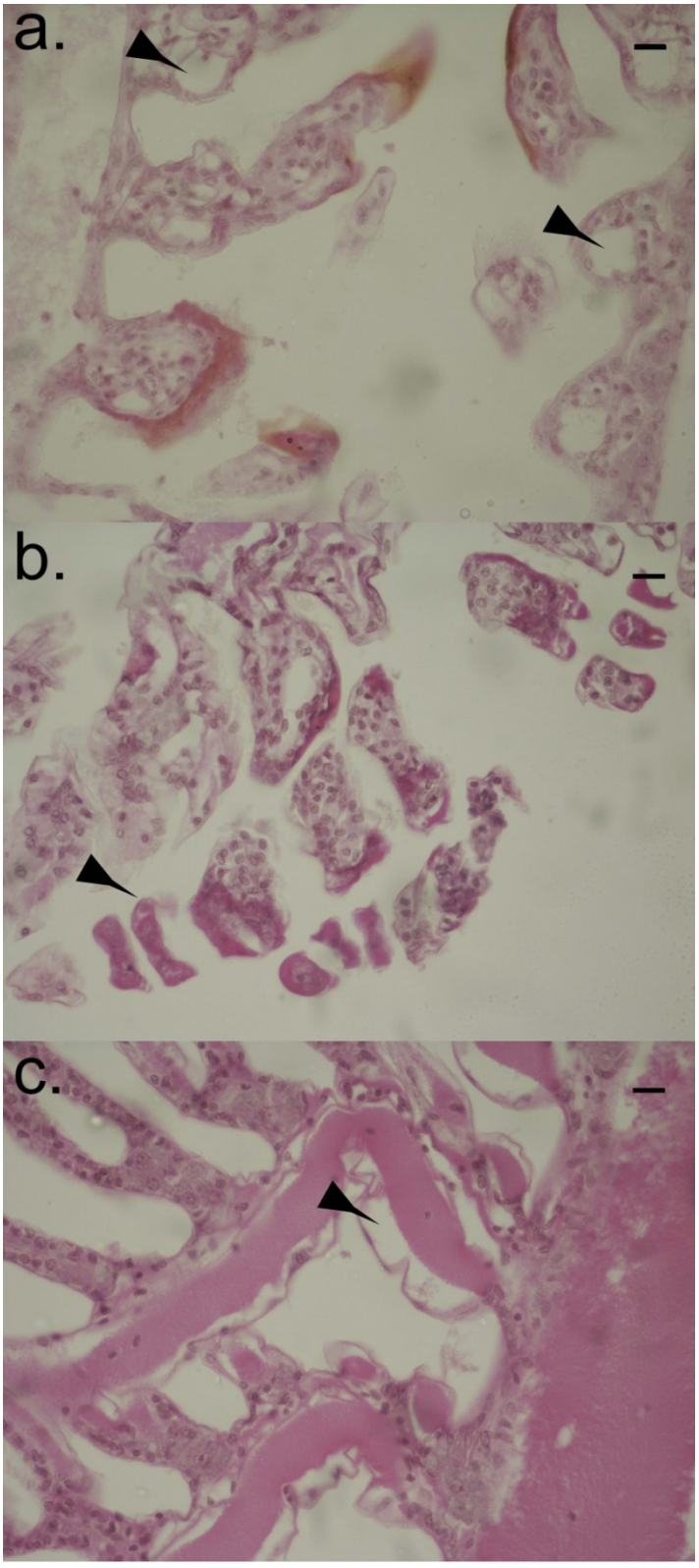

Figure 8 Melanisation of gill tips in School Prawn (hematoxylin and eosin, 40x magnification, scale bar 50 μm in all panels) exposed to 4 mg Al L^{-1} at pH 8 (a), 16 mg Al L^{-1} at pH 5 (b), and 128 mg Al L^{-1} at pH 5. The dense, melanised areas of the gills tips are evident in (a) and (b), while infiltration of the haemolymph (seen as bright pink areas) is evident in (c). Arrows indicate oedema between the epithelial pillar cell processes in (a), melanised gill tips in (b), and cuticle separation and infiltration of haemolymph (bright pink areas) in (c).

haemolymph was evident in gill sections of prawns exposed to ≥ 128 mg Al L^{-1} at pH 5 (Figure 8), but was not detected in specimens exposed to the same Al concentrations at pH 8. Protozoans also proliferated on the gill filaments in prawns exposed to > 2 mg Al L^{-1} at pH 5 (not shown), most likely due to a compromised host immune defence. Protozoans were not observed in any prawns held at pH 8, irrespective of Al exposure. Ciliate parasites were also observed between gill filaments in prawns exposed to ≥ 16 mg Al L^{-1} and ≥ 4 mg Al L^{-1} and at pH 8 and pH 5 respectively, but not in prawns exposed to ambient conditions (pH 8, 0 mg Al L^{-1}). These observations suggest that acidic conditions may make School Prawn more vulnerable to parasitic invasion.

Histology of the hepatopancreas of School Prawn

The hepatopancreas from prawns in the control group at pH 8 showed closely aligned tubules separated by the haemal sinus (Figure 9). The star-shaped lumen of the inner digestive tubules were regular in form, and the large, distinctive vacuole of the secretary (Blasenzellen) or B-cells, was as expected (with a nucleus attached to the inner wall). The structural integrity of the organ appeared to be intact and no abnormalities were detected (Figure 9). In contrast, prawns from the pH 5 control group appeared to have structural changes (Figure 9), where the interstitial spaces between the tubules were abnormally wide and there were more numerous haemocytes (~10 % increase). There also appeared to be haemolymph infiltration into the lumen (not shown). In all prawns from the pH 5 treatment, regardless of Al concentration, the nuclei of the B-cells were enlarged and contained viral occlusion bodies characteristic of some baculoviruses (Figure 9).

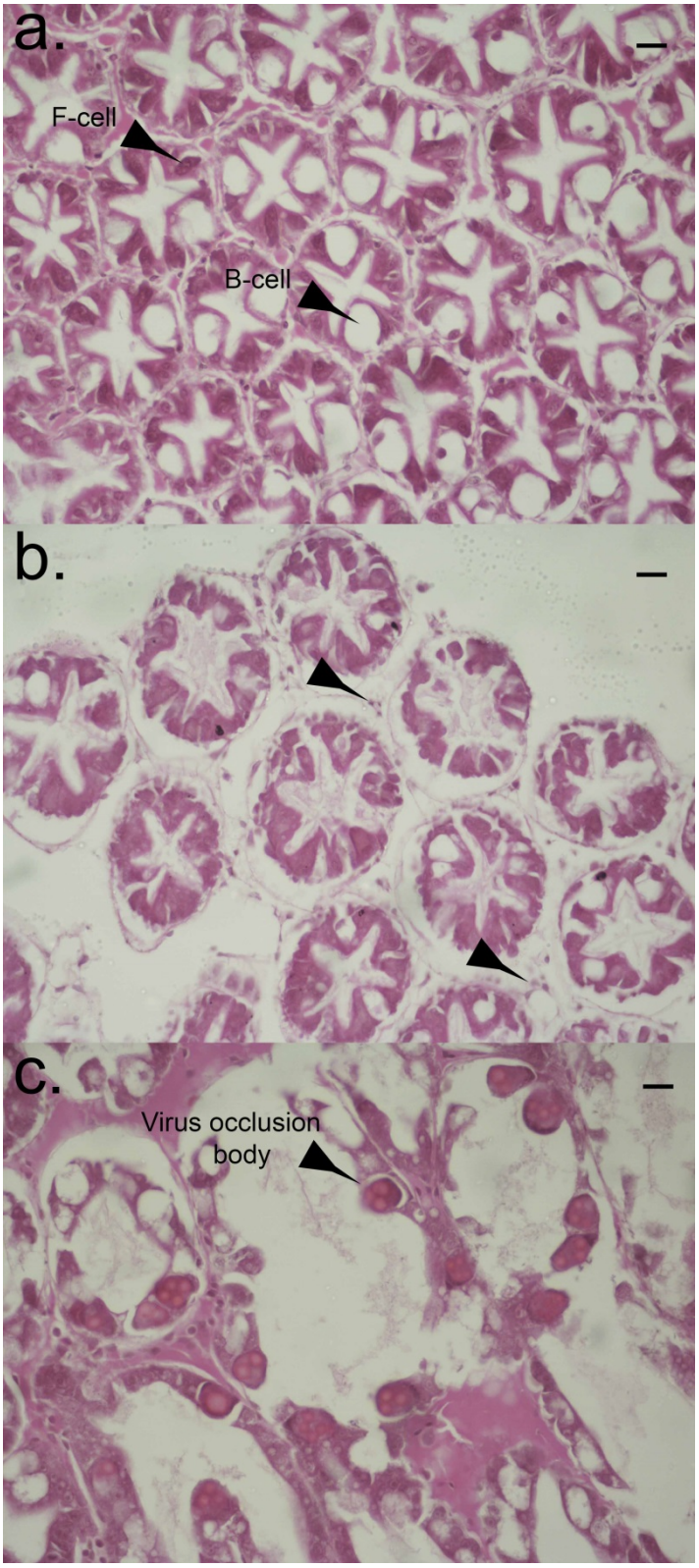

Figure 9 Transverse section of hepatopancreas in School Prawn (hematoxylin and eosin, 40x magnification, scale bar 50 μm in all panels) from control treatments at pH 8 (a), control treatments at pH 5 (b), and prawns exposed to 2 mg Al L^{-1} at pH 5 (c). Intact structural integrity is evident in (a), with closely aligned tubules, normal haemal sinus, normal secretary B-cells, and fibrous F-cells surrounded by absorptive and storage cells. At pH 5 (b), sinus haemolymph was reduced with haemocytes occurring within the lumen (indicated with arrows), and evidence of infection with baculovirus (viral occlusion bodies) evident in B cells (c).

Hypoxia exposure experiment

As noted in the introduction, both hypoxia, and Al concentrations exceeding screening criteria (Golding et al., 2015) have previously been detected in important School Prawn nursery areas in south-eastern Australia, including Camden Haven (Figure 2 and Figure 3). This study sought to examine the potential interactive effect of these catchment-derived stressors on School Prawn productivity, by examining the combined effect of Al and hypoxia on the survivorship and physiology of juvenile School Prawn. Specifically, we aimed to: 1) determine the lethal and sublethal effect of hypoxia on School Prawn; and 2) to evaluate whether contamination with dissolved Al exacerbates the effect of hypoxia in School Prawn. Impacts on prawn physiology were also assessed through metabolomics analysis, including the measurement of changes in fatty acid and cytoplasmic amino acid compositions in muscle tissue following the various treatments.

Methodology

The effect of low DO was evaluated over a range of concentrations, in the presence and absence of Al, in three 96-hour experiments. Wild juvenile School Prawn were caught from the Hunter River, New South Wales, Australia (32º52.022`S, 151º41.517`E) on 14 June, 5 July and 10 August 2017. Prawns were collected using a knotless drag net (26 mm mesh size for the body and 5 mm for the cod end) over numerous short trawls (~2 min, to decrease the possibility of damage to the prawns during collection). Net contents were emptied into a water filled sorting tray and prawns transferred to 20 L aerated containers for transport to the aquarium. Prawns were gradually acclimated to conditions at Port Stephens Fisheries Institute over a period of 48 h, and then placed in poly-plastic holding tanks at a density of ~50 prawns per tank. Each holding tank was supplied with a continuous flow of filtered sea water, and contained a thin (50 mm) layer of sand with aeration provided through a 5 m length of micro-water-weeper soaker hose submerged in the substrate. Prawns were kept at ~21ºC (±1 ºC) and at pH ~8.5 with a 14:10-hour light-dark regime, and fed "NutraXtreme C2" aquarium diet pellets (www.aquasonic.com.au) *ad libitum* every evening. Prawns were held for eight days prior to commencement of the experiments to acclimate to aquarium conditions, and remained in good condition throughout this period (98% survival through acclimation period).

Prior to each experiment, prawns were relocated into a single tank. In this tank dissolved oxygen was progressively lowered at a rate of ~0.65 mg L^{-1} every hour, taking approximately twelve hours to reach the lower DO concentrations. This rate-of-decrease was based on observations of natural conditions (Figure 2), and was achieved by the intermittent gassing of compressed nitrogen into the tank. When the tank water reached the appropriate DO concentration, six litres of water and ten prawns were randomly chosen and placed into the experimental containers. Six litre food-grade plastic containers were used as experimental chambers, and contained bubble-wrap (wrapping plastic) on the surface of the water (to prevent gas exchange) and an air-tight lid fitted (air bubbles trapped under the plastic were carefully removed).

The DO concentrations averaged between 0.5 mg L^{-1} and 8.3 mg L^{-1} (ambient) over the 96 hour experimental period. Dissolved oxygen was monitored twice daily using a 'Fibox 4' Single Channel Oxygen Meter, Sensor type PSt3, and non-invasive oxygen sensors (PreSens GmbH). Sea water remained static in the chambers, without aeration, and the prawns were not fed over the period of the experiments. A daily water exchange of 50% chamber volume was maintained to help avoid build-up of ammonium and to maintain stability in dissolved oxygen concentrations, with 3 L of water siphoned from the experimental containers and replaced with 3 L of fresh filtered sea water at the relevant DO and Al (see below paragraph) concentration.

An Al stock solution of 1000 mg Al L^{-1} was prepared by adding 0.447 g of Aluminium Chloride Hexahydrate ($AlCl_3.6H_20$) to 50 mL of 0.11 M Sodium Hydroxide (NaOH). The solution was shaken vigorously for one minute (Angel et al., 2016). Two Al treatments were tested; no Al and 0.5 mg L^{-1} (an environmentally relevant dissolved aluminium concentration exceeding the screening criteria outlined in Golding et al., 2015); over the range of DO concentrations. The treatment concentrations of 0.5 mg Al L^{-1} were prepared by adding 3 mL of the Al stock solution to the 6 L experimental containers, and 3 mL of NaOH were added to the other tanks.

During the experiment, observations were carried out and mortalities recorded at the 12, 24, 48, 72 and 96 hour water changes. Mortalities were determined by lack of movement or response to touch. At the 96-hour mark any surviving animals were euthanised in an ice slurry. Each prawn was blotted with paper towel, weighed (g) and the carapace length (CL, mm) measured. Four individuals from each of two high and low DO concentration treatments, with and without Al (32 prawns in total) were prepared for measurements of fatty acid and amino acid compositions. This involved removing the heads, legs, tail, vein and exoskeleton. Each individual was then placed into a separate, labelled Eppendorf tube and frozen before transportation to the University of Newcastle for analyses.

Fatty Acid analysis of prawn muscle and gas chromatography mass spectroscopy (GCMS)

Fatty acids were extracted by adding 2 mL of methanol/toluene solution (4:1 v/v) to 20 mg (wet weight) lyophilised prawn muscle in glass tubes. 5-α-cholestane was added as the internal standard (21.875 ng) and the glass tubes were sealed with Teflon. The extraction tubes were held chilled in ice/water and 200 µL of acetyl chloride was delivered via a glass syringe piercing the Teflon to initiate transesterification. The Teflon was replaced, the tubes capped and vortexed for 15 seconds. The tubes were then placed in a dry heating block at 100°C for 30 minutes, vortexed again, and then heated at 100°C for another 30 minutes. The tubes were then cooled to room temperature and 5 mL of potassium carbonate (6% w/w K_2CO_3) were added. The tubes were then inverted 6 times prior to centrifugation at 2,500 × g at 23°C to separate the phases. The fatty acid methyl ester (FAME) products in the upper toluene phase (100 µL) were transferred to an autosampler insert (modified method of Lepage and Roy, 1986). Free cholesterol liberated in the reaction did not form methyl esters and thus 20 µL of BSTFA were then added to form the trimethylsilyl derivative of cholesterol. The sample was then analysed through GC-MS, comprising a Hewlett-Packard 6890 series gas chromatograph coupled with a Hewlett-Packard 5973 Mass Selective Detector as described previously (Crompton and Dunstan, 2018).

Amino acid analyses in tissue samples

The amino acids were extracted from the lyophilised prawn muscle samples through the use of an acetonitrile/methanol/water solvent extraction (Rabinowitz and Kimball, 2007). Approximately 5 mg of wet prawn tissue was weighed out into a 1.5 ml Eppendorf tube and subsequently freeze dried. 500 µL of acetonitrile/methanol/water solution (40/40/20) was then added along with 20 nmoles of norvaline as the internal standard. The samples were stored at -20°C for 20 minutes and then centrifuged at 16,000 × g for 5 minutes. The solvent was collected with a glass Pasteur pipette and transferred to a clean borosilicate glass tube. The prawn sample was washed with a further 200 µL of solvent and centrifuged at 16,000 × g a further two times with the solvent washings collected and added to the borosilicate glass tube. The solvent extracts were then dried under vacuum using a centrifugal vacuum concentrator. A 200 µL volume of 0.1 M HCl was then added to each sample in preparation for analysis. The amino acids in the cellular extracts were initially prepared by using the

EZ:Faast™ derivatisation kit and analysed using a gas chromatograph coupled with a flame ionisation detector (as described by Evans et al., 2008).

Data treatment and statistical analyses

All data analysis was conducted in R v. 3.2.1 (R Core Team, 2016). The relationship between dissolved oxygen and mortality was modelled separately for the aluminum and no-aluminum treatments. Initially, modelling was conducted using a non-linear least-squares model, which represented survival as mortality. The model specified mortality as a function of dissolved oxygen concentration according to the formula, $M = a \cdot e^{-\beta \cdot DO}$, where M is mortality, DO is the dissolved oxygen concentration, and β and a are the slope and intercept respectively. A 2-part regression was also conducted to verify the non-linear relationship between dissolved oxygen and survival, and to estimate the breakpoint where mortality dramatically increased, using the segmented function in the regression package in R. For hypoxic treatments (<2 mg L^{-1}), the relationship between mortality and time was assessed using a logarithmic model, and the time taken to reach 50% mortality derived for each of these groups.

For metabolite analysis, samples were split between treatments that experienced hypoxia (<2 mg L^{-1}), and non-hypoxic concentrations (>2 mg L^{-1}), and in the presence and absence of Al. Total fatty acids and total amino acids were compared among these groups using a 2-factor analysis of variance. Each metabolite dataset was centered and scaled using the preprocess function in the caret package (Kuhn, 2008), and differences in metabolite composition among these groups were evaluated using a discriminant function analysis of fatty acids and amino acids using the lda function in the MASS package (Venables and Ripley, 2002). Differences in metabolite composition were also analysed using the adonis function in the vegan package (Oksanen et al., 2017).

Results

Mortality

Overall, there was variable survival among the experimental conditions tested. School Prawn appeared to be relatively resilient to moderate sub-hypoxic conditions (2-5 mg L^{-1}) and generally survived well at dissolved oxygen (DO) concentrations >3 mg L^{-1}, but mortality increased at hypoxic concentrations (Figure 10). Both statistical analyses confirmed these relationships. The non-linear least squares model fit indicated a significant increase in survival with increasing DO for both aluminium (β = 1.388, t = 3.070, P = 0.004) and no aluminium (β = 2.471, t = 4.007, P < 0.001) treatments. Aluminium did not appear to greatly exacerbate the effect of hypoxia on overall survival, with dissolved oxygen concentrations at which 50% mortality occurred (LC_{50}) ~0.9 mg L^{-1} for both aluminium and no aluminium treatments. However, some impact was evident in slight differences between the two fitted non-linear models, with survival beginning to decrease at higher DO concentrations in the presence of aluminium (Figure 10).

Segmented regression estimated similar breakpoint concentrations for both the aluminium (0.97 ± 0.18 mg L^{-1}) and no aluminium treatments (1.05 ± 0.09 mg L^{-1}) (Figure 10). The slope for the first regression segment (dissolved oxygen concentrations less than the breakpoint concentration) showed a significant increase in survival with increasing dissolved oxygen for the aluminium treatment (β = 1.920, t = 3.094, P = 0.006). However the first regression segment for the no aluminium treatment was not statistically significant (β = 1.805, t = 1.867, P = 0.073), which probably reflects low mortality in this treatment at DO concentrations greater than the breakpoint. Critical oxygen levels (that is, the intercept of the fitted models with the x-axis which reflected the dissolved oxygen concentration at

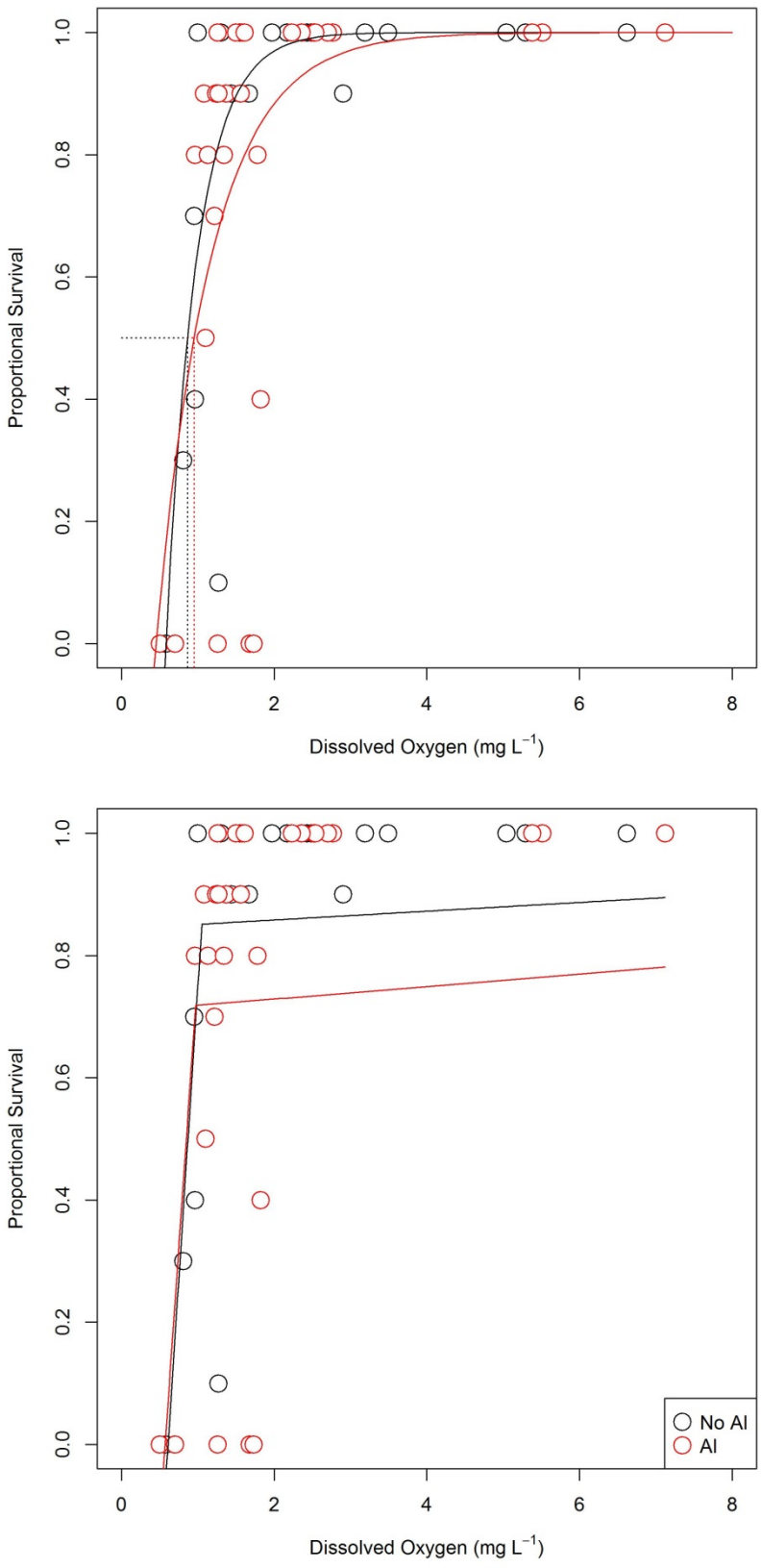

Figure 10 The relationship between proportional School Prawn survival and dissolved oxygen concentrations without aluminium (black) and in the presence of aluminium (red). The upper panel presents the outcomes of the non-linear least squares regression model, and the lower panel presents the outcomes of the segmented regression model. In the upper panel, dotted lines indicate the estimated dissolved oxygen at which 50% mortality had occurred for both experimental groups

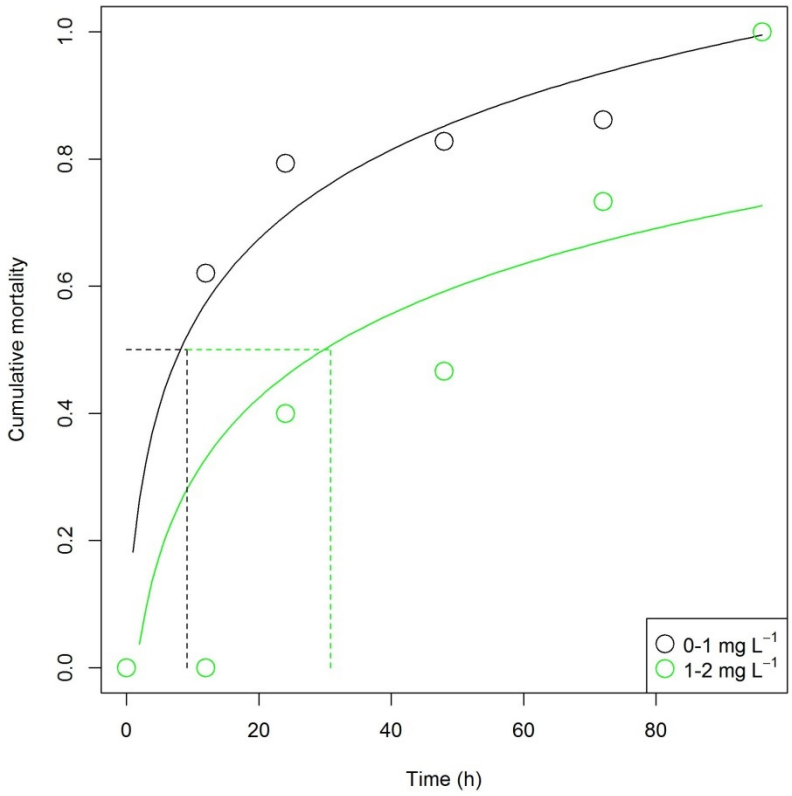

Figure 11 Proportional survival of School Prawn in hypoxic treatments over time (see legend). Dotted lines indicate LT_{50} for both groups

which 100% mortality occurred) barely differed between aluminium and no-aluminium treatments for both the non-linear model or 2-part regression (Figure 10). For low DO concentrations, mortality formed a significant non-linear relationship with time for treatments both below ($\beta = 0.209$, $t = 12.341$, $P < 0.001$, Figure 11) and above ($\beta = 0.198$, $t = 3.045$, $P = 0.038$, Figure 11) the breakpoint. These relationships indicated that the lethal time for 50% mortality (LT_{50}) was 9.1 hours for DO concentrations of <1 mg L^{-1}, compared to 30.8 hours for DO concentrations of 1-2 mg L^{-1} (Figure 11).

Metabolite analysis

Total fatty acid concentrations were significantly greater in the muscle tissue from School Prawn subjected to hypoxia ($F_{1,29} = 7.351$, $P = 0.011$), however, there were no significant differences in total fatty acids between aluminium exposure ($F_{1,29} = 0.541$, $P = 0.467$) or evidence of an interactive effect of DO and aluminium ($F_{1,29} = 1.202$, $P = 0.281$, Figure 12). The profiles of fatty acid composition are shown for each treatment in Table 2, where it is evident that there were generally higher levels of most fatty acid components per mg of tissue in School Prawn subjected to hypoxia, and within both DO treatments exposure to aluminium appeared to lead to slightly greater fatty acid concentrations for most molecules. Discriminant function analysis of the lipid dataset revealed fatty acid profiles of each group were different. The first LD axis explained 72% of the variance in group centroids (Figure 13), and DHA, stearic acid, palmitic acid and behenic acid were among the highest contributing variables to the observed separation (Figure 13). School Prawn that were exposed to neither hypoxia nor aluminium, formed a distinct grouping relative to the other groups, and those exposed to aluminium grouped separately from those groups without aluminium exposure (Figure 13). Similarly to the results for total fatty acids, PERMANOVA indicated that the fatty acid profile was significantly different between DO treatments ($F_{1,29} = 7.360$, $P = 0.014$), but there were no significant differences in the fatty acid profile between aluminium exposure ($F_{1,29} = 0.345$, $P = 0.672$) or evidence of an interactive effect of DO and aluminium ($F_{1,29} = 1.299$, $P = 0.283$).

Total amino acid concentrations were also significantly greater in School Prawn subjected to hypoxia ($F_{1,29} = 7.725$, $P = 0.009$), but there were no significant differences between aluminium exposure ($F_{1,29} = 0.005$, $P = 0.942$) or evidence of an interactive effect of DO and aluminium ($F_{1,29} = 1.791$, $P = 0.191$, Figure 14). Amino acid profiles are shown for each treatment in Table 3. Similarly to fatty acids it is evident that there were generally higher concentrations per mg of tissue in School Prawn subjected to hypoxia, and within both DO treatments exposure to aluminium appeared to lead to slightly greater concentrations. Discriminant function analysis revealed differences among the amino acid profiles of each grouping. For amino acids, the first LD axis explained 89% of the variance in group centroids, and leucine, isoleucine, phenylalanine, and asparagine were among the highest contributing variables to the observed separation (Figure 14). School Prawn that were exposed to neither hypoxia nor aluminium also formed a distinct grouping relative to the other groups, and those exposed to aluminium grouped separately from those groups without aluminium exposure (Figure 14). PERMANOVA indicated that the amino acid profile was significantly different between DO treatments ($F_{1,29} = 9.132$, $P = 0.005$), but there were no significant differences in the amino acid profile between aluminium exposure ($F_{1,29} = 0.739$, $P = 0.503$) or evidence of an interactive effect of DO and aluminium ($F_{1,29} = 2.143$, $P = 0.060$).

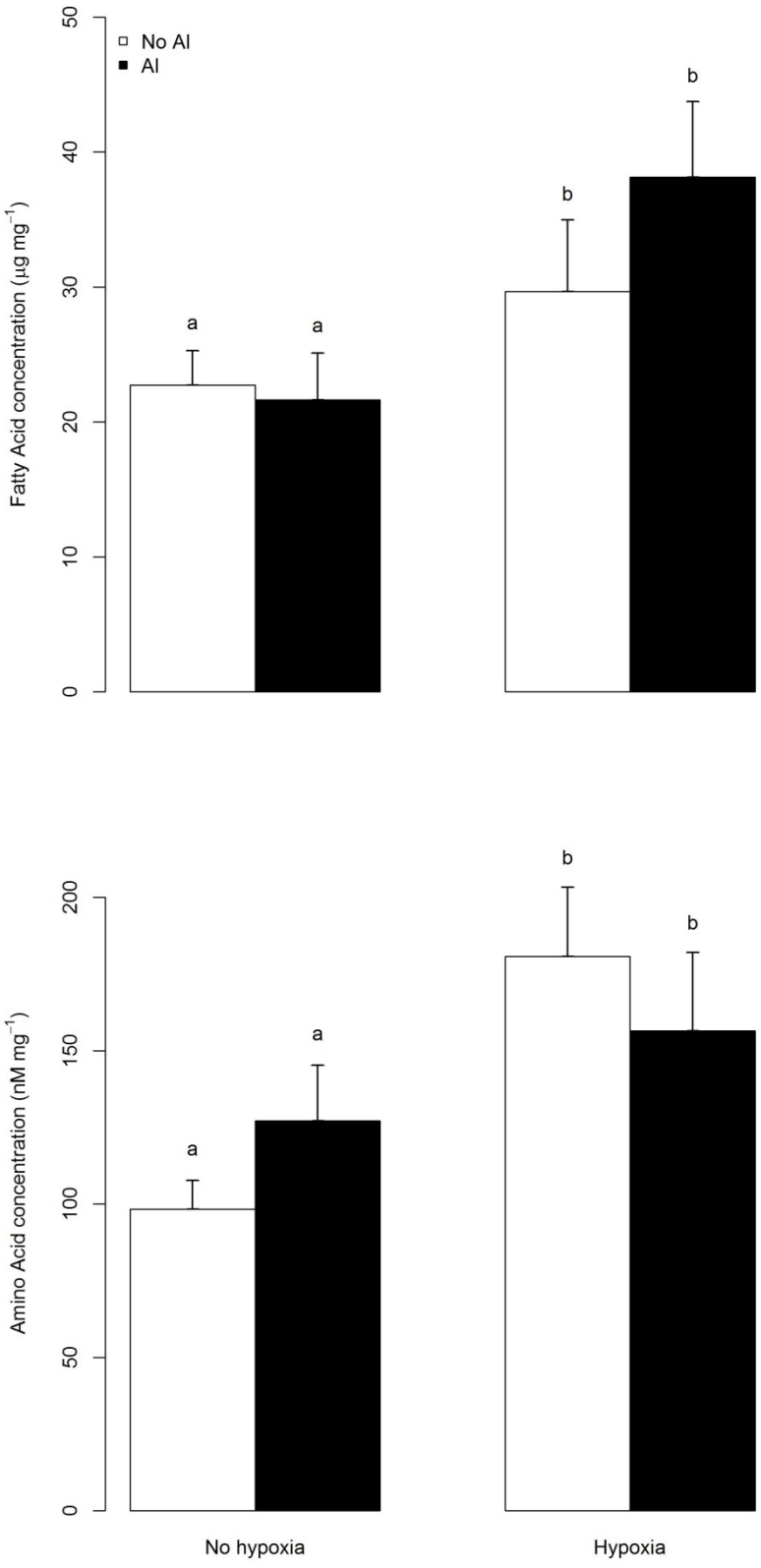

Figure 12 Total fatty acid (upper panel) and amino acid (lower panel) concentrations measured in muscle tissue after School Prawn were exposed to non-hypoxic (>2 mg L^{-1}) and hypoxic (<2 mg L^{-1}) dissolved oxygen conditions in non-aluminium (unfilled bars) and aluminium (filled bars) treatments (see figure legend). Different letters denote significant differences between groups (see text for statistical analysis)

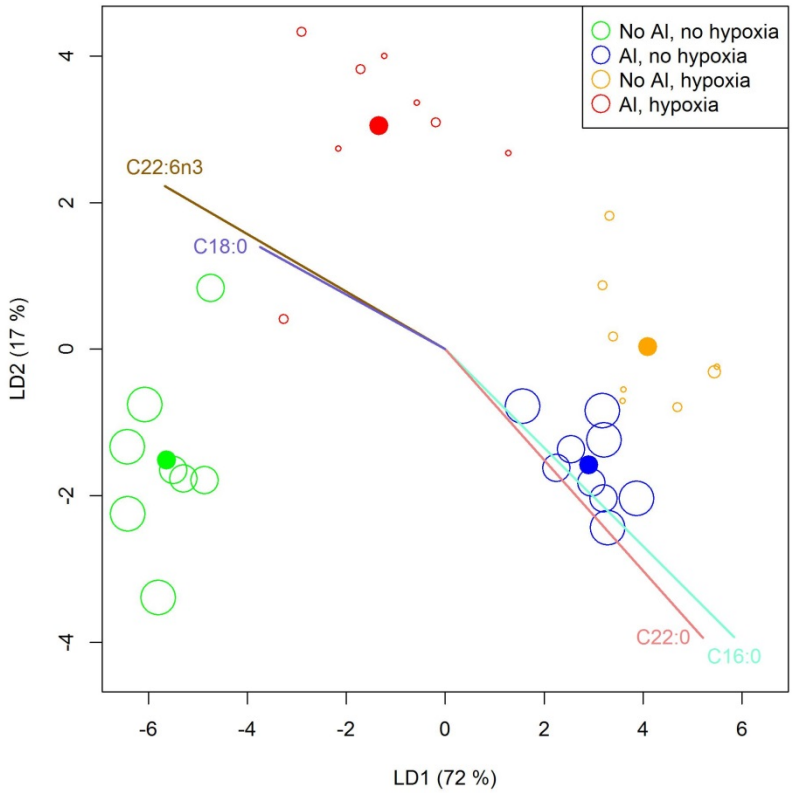

Figure 13 Results of linear discriminant function analysis of fatty acid concentrations measured in muscle tissues from School Prawn in non-hypoxic (>2 mg L^{-1}) and hypoxic (<2 mg L^{-1}) treatments, alongside exposure to aluminium (see figure legend). Group centroids are shown as filled circles, and the total variance explained by the each linear discriminant is given in brackets. Vectors indicate relative loading of linear coefficients for important fatty acids, and symbol size provides a relative indication of dissolved oxygen concentration

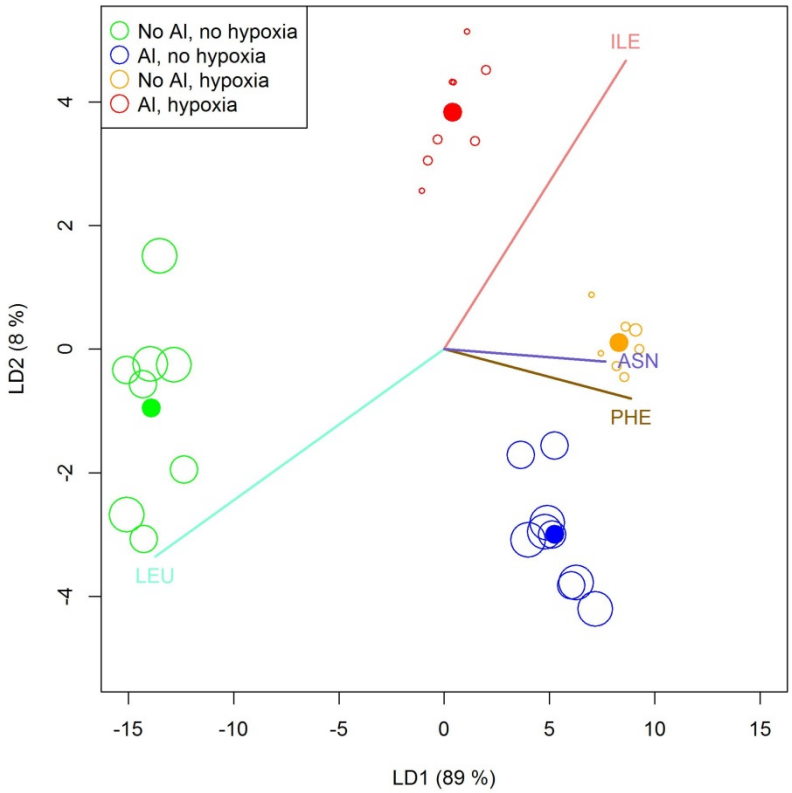

Figure 14 Results of linear discriminant function analysis of amino acid concentration measured in muscle tissues from for School Prawn in non-hypoxic (>2 mg L^{-1}) and hypoxic (<2 mg L^{-1}) treatments, alongside exposure to aluminium (see figure legend). Group centroids are shown as filled circles, and the total variance explained by the each linear discriminant is given in brackets. Vectors indicate relative loading of linear coefficients for important amino acids, and symbol size provides a relative indication of dissolved oxygen concentration.

Table 2 Fatty acid concentrations (μg mg^{-1}, mean [SE]) measured in the muscle tissue across experimental groupings

Notation	Formula	Common name	No hypoxia		Hypoxia	
			No Al	Al	No Al	Al
C14:0	$C_{14}H_{28}O_2$	Myristic acid	0.15 (0.02)	0.14 (0.03)	0.21 (0.04)	0.27 (0.04)
C15:0	$C_{15}H_{30}O_2$	Pentadecanoic acid	0.29 (0.03)	0.27 (0.06)	0.39 (0.07)	0.46 (0.07)
C16:1	$C_{16}H_{30}O_2$	Palmitoleic acid	0.54 (0.07)	0.49 (0.08)	0.69 (0.15)	0.90 (0.13)
C16:0	$C_{16}H_{32}O_2$	Palmitic acid*	6.88 (0.79)	6.27 (0.99)	8.03 (1.25)	10.14 (1.41)
iC17:0	$C_{17}H_{34}O_2$	Iso-Margaric acid	0.15 (0.01)	0.14 (0.03)	0.19 (0.03)	0.25 (0.04)
aiC17:0	$C_{17}H_{34}O_2$	Anteiso-Margaric acid	0.09 (0.01)	0.08 (0.02)	0.11 (0.02)	0.16 (0.03)
C17:1	$C_{17}H_{34}O_2$	Heptadecenoic acid	0.26 (0.02)	0.24 (0.05)	0.37 (0.08)	0.41 (0.07)
C17:0	$C_{17}H_{34}O_2$	Margaric acid	0.74 (0.07)	0.69 (0.13)	0.98 (0.18)	1.21 (0.18)
C18:2n6	$C_{18}H_{32}O_2$	Linoleic acid	0.72 (0.09)	0.63 (0.12)	0.97 (0.16)	1.28 (0.19)
C18:1n7	$C_{18}H_{34}O_2$	Octadecenoic acid	1.24 (0.16)	1.14 (0.17)	1.53 (0.26)	2.03 (0.3)
C18:1n9	$C_{18}H_{34}O_2$	Oleic acid	0.88 (0.09)	0.85 (0.15)	1.09 (0.18)	1.44 (0.23)
C18:0	$C_{18}H_{36}O_2$	Stearic acid*	2.77 (0.3)	2.43 (0.33)	3.28 (0.59)	4.15 (0.60)
C19:1	$C_{19}H_{36}O_2$	Nonadecenoic acid	0.09 (0.01)	0.11 (0.02)	0.17 (0.04)	0.14 (0.04)
C19:0	$C_{19}H_{38}O_2$	Nonadecanoic acid	0.16 (0.02)	0.17 (0.03)	0.30 (0.08)	0.34 (0.05)
C20:4n6	$C_{20}H_{32}O_2$	Arachidonic acid	1.12 (0.13)	1.06 (0.17)	1.55 (0.31)	2.12 (0.34)
C20:5n3	$C_{20}H_{30}O_2$	Eicosapentaenoic acid (EPA)	3.24 (0.36)	3.14 (0.46)	4.31 (0.83)	5.64 (0.84)
C20:2	$C_{20}H_{36}O_2$	Eicosadienoic acid	0.15 (0.02)	0.17 (0.04)	0.26 (0.05)	0.31 (0.07)
C20:1	$C_{20}H_{38}O_2$	Eicosenoic acid	0.15 (0.03)	0.15 (0.04)	0.22 (0.05)	0.31 (0.06)
C20:0	$C_{20}H_{40}O_2$	Arachidic acid	0.29 (0.03)	0.26 (0.04)	0.36 (0.07)	0.41 (0.06)
C21:0	$C_{21}H_{42}O_2$	Heneicosanoic acid	0.04 (0.01)	0.04 (0.01)	0.06 (0.01)	0.08 (0.01)
C22:6n3	$C_{22}H_{32}O_2$	Docosahexaenoic acid (DHA)*	2.09 (0.31)	2.47 (0.46)	3.59 (0.68)	4.88 (0.79)
C22:5n3	$C_{22}H_{34}O_2$	Docosapentaenoic acid (DPA)	0.23 (0.03)	0.18 (0.03)	0.27 (0.06)	0.33 (0.05)
C22:0	$C_{22}H_{44}O_2$	Behenic acid*	0.31 (0.04)	0.31 (0.05)	0.41 (0.09)	0.52 (0.07)
C23:0	$C_{23}H_{46}O_2$	Tricosanoic acid	0.07 (0.01)	0.08 (0.02)	0.12 (0.03)	0.16 (0.03)
C24:0	$C_{24}H_{48}O_2$	Lignoceric acid	0.10 (0.02)	0.11 (0.01)	0.18 (0.05)	0.20 (0.02)

* denotes fatty acids that were important in discrimination among groups

Table 3 Amino acid concentrations (nmol mg^{-1}, mean [SE]) measured in the muscle tissue across experiment groupings

Notation	Formula	Common name	No hypoxia		Hypoxia	
			No Al	Al	No Al	Al
AAA	$C_6H_{11}NO_4$	Alpha-aminoadipic acid	3.79 (0.46)	4.35 (0.82)	5.08 (0.90)	2.99 (0.21)
ALA	$C_3H_7NO_2$	Alanine	3.91 (1.00)	7.05 (1.29)	13.04 (2.22)	9.1 (1.79)
ASN	$C_4H_8N_2O_3$	Asparagine*	1.38 (0.11)	1.96 (0.18)	3.61 (0.49)	3.37 (0.73)
ASP	$C_4H_7NO_4$	Aspartic acid	2.60 (0.38)	3.47 (0.79)	4.69 (1.06)	3.7 (0.82)
BAIB	$C_4H_9NO_2$	Beta-aminoisobutyric acid	0.15 (0.04)	0.18 (0.06)	0.48 (0.08)	0.34 (0.07)
CTH	$C_7H_{14}N_2O_4S$	Cystathionine	0.01 (0.00)	0.03 (0.01)	0.07 (0.02)	0.06 (0.01)
GLN	$C_5H_{10}N_2O_3$	Glutamine	2.87 (0.29)	3.77 (0.27)	6.74 (0.94)	6.06 (1.31)
GLU	$C_5H_9NO_4$	Glutamic acid	2.85 (0.36)	3.69 (0.48)	5.47 (0.70)	5.25 (0.99)
GLY	$C_2H_5NO_2$	Glycine	71.6 (6.86)	88.62 (14.95)	111.55 (15.69)	96.95 (18.21)
GPR	$C_7H_{12}N_2O_3$	Glycine-proline dipeptide	0.07 (0.01)	0.12 (0.03)	0.23 (0.04)	0.16 (0.03)
HIS	$C_6H_9N_3O_2$	Histidine	0.33 (0.03)	0.41 (0.05)	0.82 (0.10)	0.98 (0.22)
HYL	$C_6H_{14}N_2O_3$	Hydroxylysine	0.01 (0.01)	0.06 (0.02)	0.05 (0.03)	0.05 (0.03)
HYP	$C_5H_9NO_3$	Hydroxyproline	0.02 (0.01)	0.12 (0.03)	0.38 (0.07)	0.25 (0.12)
ILE	$C_6H_{13}NO_2$	Isoleucine*	0.40 (0.05)	0.55 (0.09)	0.84 (0.11)	1.28 (0.36)
LEU	$C_6H_{13}NO_2$	Leucine*	0.79 (0.08)	1.15 (0.18)	1.67 (0.25)	2.32 (0.63)
LYS	$C_6H_{14}N_2O_2$	Lysine	0.17 (0.01)	0.38 (0.11)	0.39 (0.06)	0.57 (0.13)
MET	$C_5H_{11}NO_2S$	Methionine	0.34 (0.05)	0.55 (0.09)	1.17 (0.18)	1.19 (0.30)
ORN	$C_5H_{12}N_2O_2$	Ornithine	0.24 (0.05)	0.18 (0.02)	0.24 (0.04)	0.23 (0.06)
PHE	$C_9H_{11}NO_2$	Phenylalanine*	0.29 (0.03)	0.52 (0.08)	0.71 (0.10)	0.86 (0.21)
PRO	$C_5H_9NO_2$	Proline	2.39 (0.44)	4.80 (0.74)	13.93 (2.99)	9.88 (2.36)
SAR	$C_3H_7NO_2$	Sarcosine	0.26 (0.19)	0.09 (0.07)	0.15 (0.07)	0.29 (0.15)
SER	$C_3H_7NO_3$	Serine	1.79 (0.16)	2.02 (0.32)	3.49 (0.72)	3.77 (0.77)
THR	$C_4H_9NO_3$	Threonine	0.68 (0.05)	1.05 (0.15)	2.19 (0.39)	2.19 (0.57)
TPR	$C_4H_7NO_2S$	Thioproline	0.08 (0.02)	0.07 (0.02)	0.13 (0.04)	0.13 (0.05)
TRP	$C_{11}H_{12}N_2O_2$	Tryptophan	0.03 (0.00)	0.09 (0.02)	0.07 (0.02)	0.06 (0.01)
TYR	$C_9H_{11}NO_3$	Tyrosine	0.32 (0.03)	0.53 (0.09)	0.89 (0.17)	0.99 (0.21)

Notation	Formula	Common name	No hypoxia		Hypoxia	
			No Al	Al	No Al	Al
VAL	$C_5H_{11}NO_2$	Valine	0.95 (0.11)	1.34 (0.27)	2.56 (0.38)	3.44 (1.06)

* denotes amino acids that were important in discrimination among groups

Salinity exposure experiment

As identified above, juvenile School Prawn can generally be found across a wide-range of salinities (Ruello, 1973a), however seaward emigration is often stimulated in response to a freshwater pulse (Glaister, 1978; Ruello, 1973b). Ruello (1973b) suggested that rainfall and freshwater inflow have some effect on the growth of School Prawn, but despite the apparent euryhaline tolerance of this species and the large influence of salinity during the species life history no published studies have examined the potential effect of salinity on respiration, growth, or mortality. This information is necessary to establish how estuary inflow and salinity patterns may affect productivity of the species during their juvenile phase. Consequently, this experiment sought to 1) assess the effect of salinity on mortality of juvenile School Prawn; 2) identify sublethal effects of salinity on growth; and 3) measure changes in metabolite profiles following exposure to different salinities.

Methodology

Collection of juvenile School Prawn and husbandry

School Prawn were collected from the juvenile nursery area within the Hexham Wetland, Hunter River estuary, New South Wales (Hart et al., 2018, 32.867°S 151.069°E). Prawns were captured using a sled net constructed from 26 mm knotless mesh in the body, and 5 mm knotless mesh in the cod-end. The sled was towed slowly for short (2 min) intervals to ensure prawns were not damaged or overly stressed during collection. Following landing, prawns were immediately identified and sorted, and placed in an on-board, aerated tank filled with estuary water (19.9ºC and 28.9 salinity).

Prawns were transported by road (45 mins) to the Port Stephens Fisheries Institute Research Aquarium, and gradually (48 h) acclimated to ambient conditions in the aquarium system (20.4ºC and 30.0 salinity). Initially, prawns were held in 4 x 200 L tanks which were continuously supplied with estuary water, and each had a thin layer of sand at the bottom so that the prawns could bury. Aeration was achieved using a mat of perforated hose, placed on the bottom of the tank beneath the sediment. This aerated both the water in the tank, and also ensured that the sediment did not become anoxic. The aquarium temperature was held at 23.0ºC with a controlled 14:10 hr light:dark photo-period. Following initial acclimation, prawns were allowed to acclimate to the aquarium conditions for a further 6 days, before tagging and manipulation of salinity at the commencement of the experiment (described below). Prawns were fed "NutraXtreme C2" aquarium diet pellets (www.aquasonic.com.au) *ad libitum* every evening during this period. Water quality measurements (salinity, temperature, dissolved oxygen and pH) were recorded daily, and the ammonia levels tested.

Study design and experimental setup

The effect of salinity on mortality, growth, and metabolite profiles was evaluated across a salinity gradient ranging from 0.2 – 36 in 18 x 200 L tanks. Treatment conditions were randomly assigned to tanks within the aquarium, and prawns were randomly assigned among treatments. All prawns were individually tagged and their lengths and weights measured at the commencement of the experiment (9.2 ± 0.3 mm mean SE CL), such that individual growth trajectories could be calculated. Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc.) were used, using different elastomer colours to allow identification of individuals, and placed in experimental tanks supplied with the same ambient seawater (i.e. conditions were identical between tanks). Prawns were held in these tanks for an additional 8 days to ensure they survived handling and tagging.

Salinity was adjusted at a rate of 4 units d^{-1} by removing water and adding full strength sea water (salinity of 36) and rain water (salinity of 0), which were mixed in the appropriate quantities until the desired salinity was reached in each tank. The salinity change of 4 units d^{-1} was lower than the rate of 5 units d^{-1} generally acknowledged as low risk for mortalities for prawn species (Rosas et al., 1997). No animals were lost during the salinity adjustment or the post-tagging acclimation period. Water in the treatment tanks was exchanged (50%) with new water of equivalent salinity every 2-3 days.

Prawns were retained in treatment tanks for a period of 60 days following tagging, with survival examined daily, and animals were fed to satiation. At the conclusion of the experiment, surviving animals were euthanised in an ice slurry, were weighed, and carapace length measured. Five individuals from each tank (90 prawns in total) were prepared for metabolomic profiling by removing the heads, legs, tail and exoskeleton. These prawns were placed into a labelled Eppendorf tube, frozen and held at -80°C until analysis.

Fatty Acid and amino acid analysis and gas chromatography mass spectroscopy (GCMS)

Fatty acid (Table 4) and amino acid (Table 5) composition was analysed as described previously for the *Hypoxia exposure experiment*.

Data handling and statistical analyses

All analyses were conducted using R v. 3.2.1 (R Core Team, 2016). Survival was expressed as a proportional value. Carapace length was used to calculate individual growth trajectories for surviving prawns, and these were expressed as a proportional change in carapace length (CL [mm]) over the experiment. A length-weight relationship ($\log(\mathrm{Weight}) = \log(\mathrm{CL})$) was fitted to CL and weight (g) data for surviving prawns using ordinary least squares regression. This relationship was used to derive a condition index, by calculating the residuals for each data point (after Moltschaniwskyj and Semmens, 2000b) and averaging across individuals in each tank. Following inspection of the data, survival was modelled against salinity according to $Survival = \beta + \beta_1 \log(Salinity)$. The effect of salinity on proportional growth, and the condition index, were analysed using simple linear regression.

Total fatty acid and amino acid concentrations were modelled against salinity using simple linear regression, and the linear relationship between total fatty acids and total amino acids was also assessed. To evaluate metabolite profiles, salinity was converted into six groups of salinity <6, 8-12, 14-18, 20-24, 26-30, and >32. Metabolite datasets were centered and scaled using the `preprocess` function in the caret package (Kuhn, 2008), and differences in metabolite profiles among salinity groups were evaluated using a discriminant function analysis (lda function in the MASS package, Venables and Ripley, 2002).

Results

Survival and growth

Survival exceeded 70% over the course of the experiment for salinity treatments other than the lowest treatment. Survival was 0% in the lowest salinity treatment (0.2), and all prawns had died within 3 d

Table 4 Fatty acid concentrations (µg 100 mg^{-1}, mean [SE]) in School Prawn exposed to different salinities. The molecules with the five largest parameter coefficients for the two LD axes shown in Figure 20 are indicated as superscripts (i.e. *LD1*, *LD2*) in the notation column.

Notation	Formula	Common name	Salinity <6	Salinity 8-12	Salinity 14-18	Salinity 20-24	Salinity 26-30	Salinity >32
Total Fatty Acids			1548.7 (112.4)	1552.2 (120.2)	1500.5 (122.2)	1268.5 (123.2)	1717.2 (149.1)	1260.2 (104.6)
C14:0	$C_{14}H_{28}O_2$	Myristic acid	12.5 (1.1)	14.2 (1.5)	11.9 (1.1)	11.5 (1.8)	13.9 (2.0)	9.9 (0.6)
C15:0	$C_{15}H_{30}O_2$	Pentadecanoic acid	9.3 (0.6)	10.9 (1.3)	11.1 (1.2)	8.9 (1.1)	11.4 (1.3)	9.8 (1.3)
C16:1	$C_{16}H_{30}O_2$	Palmitoleic acid	34.5 (3.3)	35.6 (3.8)	32.7 (2.8)	30.0 (4.3)	36.5 (3.4)	27 (2.3)
C16:0$^{LD1, LD2}$	$C_{16}H_{32}O_2$	Palmitic acid	521.0 (40.8)	500.1 (42.6)	477.6 (36.2)	409.1 (40.7)	558.5 (51.1)	402.1 (36.7)
iC17:0^{LD1}	$C_{17}H_{34}O_2$	Iso-margaric acid	4.2 (0.3)	4.4 (0.3)	3.9 (0.4)	3.4 (0.4)	4.8 (0.7)	3.5 (0.3)
aiC17:0^{LD1}	$C_{17}H_{34}O_2$	Anteiso-margaric acid	2.6 (0.2)	2.8 (0.2)	2.6 (0.2)	2.3 (0.2)	3.0 (0.4)	2.6 (0.2)
C17:1	$C_{17}H_{34}O_2$	Heptadecenoic acid	6.8 (0.5)	7.9 (1.0)	7.4 (0.7)	6.0 (0.7)	7.9 (0.8)	6.6 (0.9)
C17:0	$C_{17}H_{34}O_2$	Margaric acid	28.9 (1.8)	28.9 (2.2)	28.2 (2.4)	23.5 (2.2)	33.6 (3.4)	26.4 (2.2)
C18:2n6	$C_{18}H_{32}O_2$	Linoleic acid	68.1 (5.5)	70.0 (6.7)	64.2 (5.6)	58 (6.8)	76.1 (7.7)	51.1 (5.2)
C18:1n7	$C_{18}H_{34}O_2$	Octadecenoic acid	119.0 (10.0)	138.7 (13.3)	126.5 (10.4)	107.4 (10.2)	157.4 (17.9)	101.4 (8.9)
C18:1n9	$C_{18}H_{34}O_2$	Oleic acid	47.2 (3.8)	47.5 (4.2)	46.9 (4.1)	38.8 (3.8)	52.7 (5.0)	39.7 (3.6)
C18:0$^{LD1, LD2}$	$C_{18}H_{36}O_2$	Stearic acid	179.9 (13.3)	174.7 (13.6)	172.2 (13.9)	147.3 (11.8)	198.8 (15.2)	151.5 (13.1)
C19:1	$C_{19}H_{36}O_2$	Nonadecenoic acid	11.6 (2.7)	14.2 (3.0)	10.5 (1.5)	7.7 (2.9)	14.7 (3.2)	9.1 (3.5)
C19:0^{LD2}	$C_{19}H_{38}O_2$	Nonadecanoic acid	9.3 (0.6)	10.1 (1.0)	9.5 (0.7)	7.5 (0.5)	9.5 (0.8)	9.0 (1.0)
C20:4n6	$C_{20}H_{32}O_2$	Arachidonic acid	42.8 (3.6)	44.8 (3.6)	40.9 (3.3)	35.2 (3.5)	44.1 (3.5)	38.5 (3.6)
C20:5n3	$C_{20}H_{30}O_2$	Eicosapentaenoic acid (EPA)	209.8 (15.6)	185.8 (11.0)	195.4 (16.3)	161.5 (15.4)	221.3 (19.3)	166.6 (13.3)
C20:2^{LD2}	$C_{20}H_{36}O_2$	Eicosadienoic acid	9.2 (0.8)	9.6 (0.8)	9.7 (1.0)	7.5 (0.7)	10.0 (1.1)	7.6 (0.8)

Notation	Formula	Common name	Salinity <6	Salinity 8-12	Salinity 14-18	Salinity 20-24	Salinity 26-30	Salinity >32
C20:1^{LD1}	$C_{20}H_{38}O_2$	Eicosenoic acid	16.6 (1.4)	18.8 (1.8)	18.1 (2.1)	12.9 (1.6)	18.6 (2.2)	13.0 (1.8)
C20:0	$C_{20}H_{40}O_2$	Arachidic acid	14.1 (1.1)	18.6 (1.6)	17.2 (1.2)	13.2 (0.6)	17.3 (1.5)	15.6 (0.9)
C21:0	$C_{21}H_{42}O_2$	Heneicosanoic acid	2.2 (0.2)	2.6 (0.3)	2.3 (0.2)	1.9 (0.1)	2.1 (0.3)	2.4 (0.3)
C22:6n3	$C_{22}H_{32}O_2$	Docosahexaenoic acid (DHA)	163.2 (20.1)	167.7 (13.5)	172.1 (20.8)	141.4 (18.8)	185.7 (21.6)	131.0 (16.0)
C22:5n3^{LD2}	$C_{22}H_{34}O_2$	Docosapentaenoic acid (DPA)	12.8 (1.1)	13.1 (1.0)	13.5 (1.0)	13.1 (1.4)	14.5 (1.7)	12.8 (1.3)
C22:0	$C_{22}H_{44}O_2$	Behenic acid	15.3 (1.0)	19.8 (1.5)	17.5 (1.5)	13.9 (0.9)	17.3 (1.7)	15.7 (1.2)
C24:1n9	$C_{23}H_{46}O_2$	Tricosanoic acid	4.2 (0.4)	6.5 (0.7)	4.9 (0.8)	3.5 (0.4)	4.7 (1.1)	3.6 (0.6)
C24:1	$C_{24}H_{48}O_2$	Lignoceric acid	3.3 (0.4)	4.7 (0.6)	3.6 (0.5)	3.2 (0.2)	2.8 (0.6)	3.7 (0.5)
C24:0	$C_{14}H_{28}O_2$	Myristic acid	4.6 (0.5)	6.7 (0.7)	5.5 (0.5)	4.0 (0.3)	6.1 (1.0)	5.2 (0.5)

Table 5 Amino acid concentrations (nM mg^{-1}, mean [SE]) in School Prawn exposed to different salinities. The molecules with the five largest parameter coefficients for the two LD axes shown in Figure 21 are indicated as superscripts (i.e. *LD1*, *LD2*) in the notation column.

Notation	Formula	Common name	Salinity <6	Salinity 8-12	Salinity 14-18	Salinity 20-24	Salinity 26-30	Salinity >32
Total Amino Acids			153 (13.4)	157.9 (21.0)	142.9 (7.1)	164.1 (12.2)	169.9 (15.5)	159.5 (20.4)
AAA$^{LD1, LD2}$	$C_6H_{11}NO_4$	Alpha-aminoadipic acid	1.8 (0.3)	2.2 (0.5)	2.6 (0.3)	2.8 (0.5)	2.9 (0.6)	3.3 (1.1)
ALA	$C_3H_7NO_2$	Alanine	12.0 (1.5)	11.9 (1.7)	10.2 (1.1)	10.0 (1.2)	13.2 (1.4)	10.5 (1.4)
ASN	$C_4H_8N_2O_3$	Asparagine	4 (0.8)	2.5 (0.6)	3.4 (0.4)	5.4 (0.6)	4.5 (0.5)	3.9 (0.7)
ASP	$C_4H_7NO_4$	Aspartic acid	0.7 (0.3)	0.6 (0.3)	1.1 (0.4)	1.6 (0.4)	1.3 (0.4)	2.2 (1.0)
BAIB	$C_4H_9NO_2$	Beta-aminoisobutyric acid	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	-	0.1 (0.1)	0.2 (0.1)
CTH	$C_7H_{14}N_2O_4S$	Cystathionine	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
GLN	$C_5H_{10}N_2O_3$	Glutamine	7.0 (2.0)	4.1 (1.0)	5.3 (1.4)	6.8 (2.1)	6.2 (1.5)	5.9 (1.7)
GLU^{LD1}	$C_5H_9NO_4$	Glutamic acid	4.9 (0.8)	4.7 (0.7)	5.5 (0.7)	9.1 (1.1)	6.5 (1.4)	6.4 (0.7)
GLY^{LD1}	$C_2H_5NO_2$	Glycine	77.4 (6.4)	99.7 (15.3)	86.9 (4.2)	87.1 (7.3)	95.1 (10.8)	92.8 (16.7)
GPR	$C_7H_{12}N_2O_3$	Glycine-proline dipeptide	0.3 (0.1)	0.1 (<0.1)	0.2 (<0.1)	0.2 (<0.1)	0.2 (<0.1)	0.2 (<0.1)
HIS^{LD1}	$C_6H_9N_3O_2$	Histidine	0.7 (0.1)	0.6 (0.1)	0.5 (0.1)	0.7 (0.1)	0.6 (0.1)	0.5 (0.1)
HYL	$C_6H_{14}N_2O_3$	Hydroxylysine	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
HYP	$C_5H_9NO_3$	Hydroxyproline	0.2 (0.1)	0.1 (0.1)	0.1 (<0.1)	0.5 (0.3)	0.1 (<0.1)	0.3 (0.2)
ILE^{LD2}	$C_6H_{13}NO_2$	Isoleucine	1.1 (0.2)	0.8 (0.1)	0.8 (0.2)	1.6 (0.4)	0.9 (0.1)	1.1 (0.3)
LEU$^{LD1, LD2}$	$C_6H_{13}NO_2$	Leucine	1.8 (0.3)	1.2 (0.2)	1.2 (0.2)	2.1 (0.4)	1.2 (0.2)	1.7 (0.4)
LYS	$C_6H_{14}N_2O_2$	Lysine	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)	1.0 (0.3)	0.3 (0.1)	0.4 (0.1)
MET	$C_5H_{11}NO_2S$	Methionine	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.4 (0.1)

Notation	Formula	Common name	Salinity <6	Salinity 8-12	Salinity 14-18	Salinity 20-24	Salinity 26-30	Salinity >32
ORN	$C_5H_{12}N_2O_2$	Ornithine	<0.1 (<0.1)	0.2 (0.1)	0.1 (<0.1)	<0.1 (<0.1)	0.1 (0.1)	0.1 (<0.1)
PHE^{LD2}	$C_9H_{11}NO_2$	Phenylalanine	0.4 (0)	0.4 (0.1)	0.3 (0.1)	0.5 (0.1)	0.4 (<0.1)	0.4 (0.1)
PRO	$C_5H_9NO_2$	Proline	25.2 (4.0)	13.6 (2.8)	13.6 (3.1)	17.6 (3.7)	24.1 (3.7)	16.4 (3.5)
SAR	$C_3H_7NO_2$	Sarcosine	0.3 (0.3)	0.1 (0.1)	0.2 (0.2)	0.3 (0.2)	0.1 (0.1)	0.2 (0.2)
SER	$C_3H_7NO_3$	Serine	5.2 (1.9)	7.7 (1.8)	3.6 (0.8)	5 (1.1)	3.7 (0.8)	3.7 (1.0)
THR	$C_4H_9NO_3$	Threonine	3.8 (0.8)	2.7 (0.5)	3 (0.5)	5.1 (0.9)	3.5 (0.4)	4.3 (0.8)
TPR	$C_4H_7NO_2S$	Thioproline	0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	0.1 (<0.1)	<0.1 (<0.1)	0.1 (<0.1)
TRP	$C_{11}H_{12}N_2O_2$	Tryptophan	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
TYR	$C_9H_{11}NO_3$	Tyrosine	0.5 (0.1)	0.5 (0.1)	0.4 (0.1)	0.5 (0.1)	0.4 (<0.1)	0.5 (0.1)
VAL^{LD2}	$C_5H_{11}NO_2$	Valine	4.4 (0.7)	3.1 (0.5)	3.0 (0.5)	5.6 (1.2)	3.9 (0.5)	3.9 (0.9)

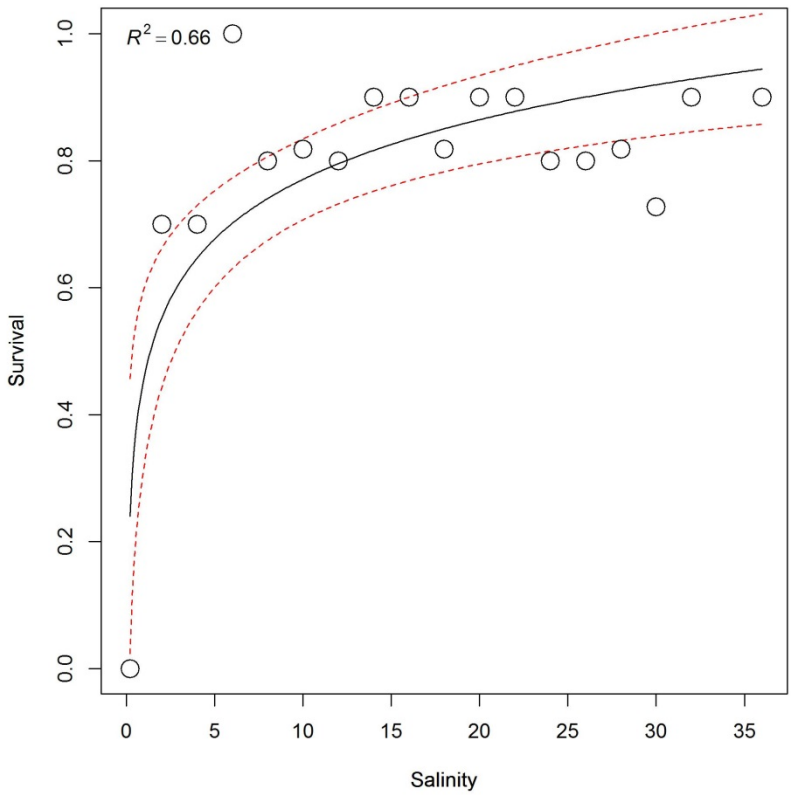

Figure 15 Relationship between survival of School Prawn and salinity following 45 days of exposure to a gradient of salinities from 0.2 – 36. The fitted model is presented as a black line, and the 5% and 95% confidence intervals are presented as dashed red lines.

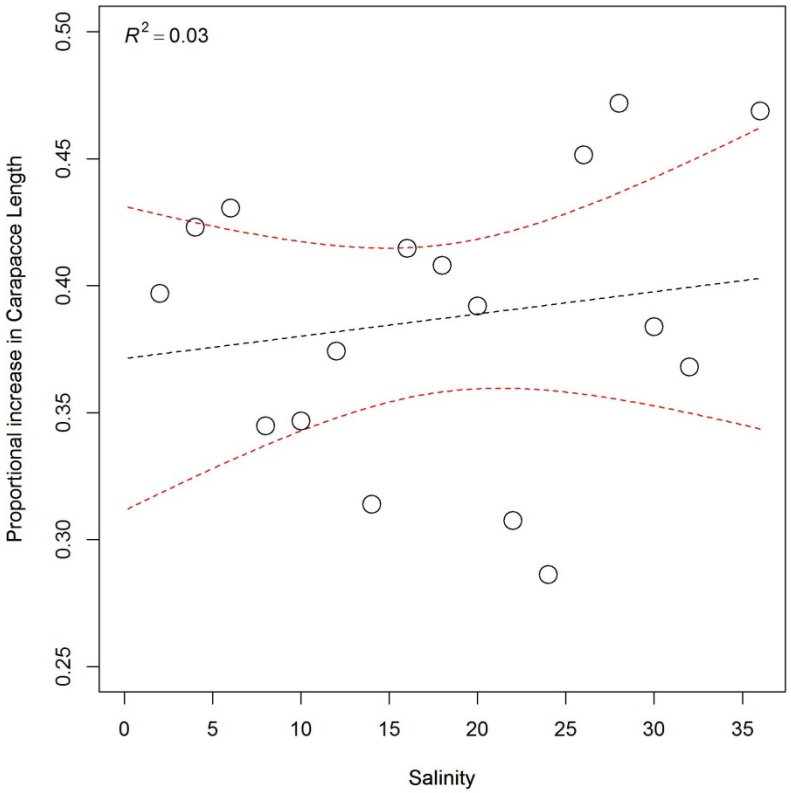

Figure 16 Relationship between proportional increase in carapace length (CL) of School Prawn and salinity following 45 days of exposure to a gradient of salinities from 0.2 – 36, presented as the proportional change in carapace length averaged across each tank. The fitted model was not significant and is presented as a dashed black line, and the 5% and 95% confidence intervals are presented as dashed red lines.

days of achieving the endpoint salinity. Survival formed a significant relationship with the natural logarithm of salinity ($F_{1,16}$ = 31.41, P << 0.001, Figure 15), which confirmed these patterns. Salinity did not appear to impact relative growth across the range of conditions examined (Figure 16), and this was confirmed by linear modelling ($F_{1,15}$ = 0.42, P = 0.529). In contrast, relative somatic condition as expressed by the condition index appeared to be greater at lower salinities, and formed a significant negative relationship with salinity ($F_{1,15}$ = 8.85, P = 0.009, Figure 17).

Metabolite analysis

Total fatty acid concentrations in School Prawn ranged between 6.95 and 28.05 μg mg^{-1} of muscle tissue. There was no relationship between total fatty acid concentration ($F_{1,15}$ = 0.52, P = 0.483) or total amino acid concentration ($F_{1,15}$ = 0.42, P = 0.528) and salinity (Figure 18). Total fatty acid concentration also had a significant positive relationship with total amino acid concentrations ($F_{1,15}$ = 6.16, P = 0.025), with fatty acid concentration explaining ~30% of the variation in amino acid concentration (Figure 19).

There was no consistent pattern in the fatty acid profile with groups of increasing salinity (Table 4), and this was evident in the outcomes of the discriminant function analysis (Figure 20). The first two linear discriminant axes explained 49% (LD1) and 18% (LD2) of the variation among groups. While there was some differentiation between groupings, there was substantial within group variability and centroids did not follow a consistent consecutive pattern with increasing salinity (Figure 20). Overall, prawns exposed to salinity <19 tended to be negatively correlated with LD1 and positively correlated with LD2, with the opposite relationship true for School Prawn exposed to salinity >19. Total fatty acid concentrations were relatively consistent for School Prawn exposed to <19, but substantially decreased in School Prawn exposed to 20-24, before reaching a maxima at salinity 26-30 and a minima for salinity >32 (Table 4). In general, this pattern was followed by most fatty acid molecules, including those identified as important in driving separation of School Prawn held at different salinities (Table 4, identified by superscript).

Total amino acid concentrations in School Prawn ranged between 44.69 and 363.06 nmole mg^{-1} of muscle tissue, and there was no clear pattern in the amino acid profile across groups of increasing salinity (Table 5), but variation in the concentration of the suite of amino acids within these groupings was slightly lower for amino acids than fatty acids (Figure 21). The first two linear discriminant axes described 68% of the variation among groups. There was reasonable separation between School Prawns that experienced salinity <19 and >19 along LD1, except for of salinity group 26-30 which was loaded separately from all other groups along LD2 (Figure 21). As for fatty acids, this grouping (26-30) had the greatest total amino acid concentration, driven by large concentrations of proline and alanine. The minima occurred for salinity group 14-18, and the total amino acid concentrations for the other groups were similar (Table 5). Concentrations of amino acids identified as important in driving separation of the groups (Table 5, identified by superscript) were largely inconsistent with the pattern for total amino acids. For example, alpha-aminoadipic acid increased across salinity groups, whereas salinity group 20-24 displayed the maximum average concentrations of valine, phenylalanine, leucine, isoleucine, and glutamic acid. Glycine (the most abundant amino acid) concentrations were largest in School Prawns held at salinities 8-12 (Table 5).

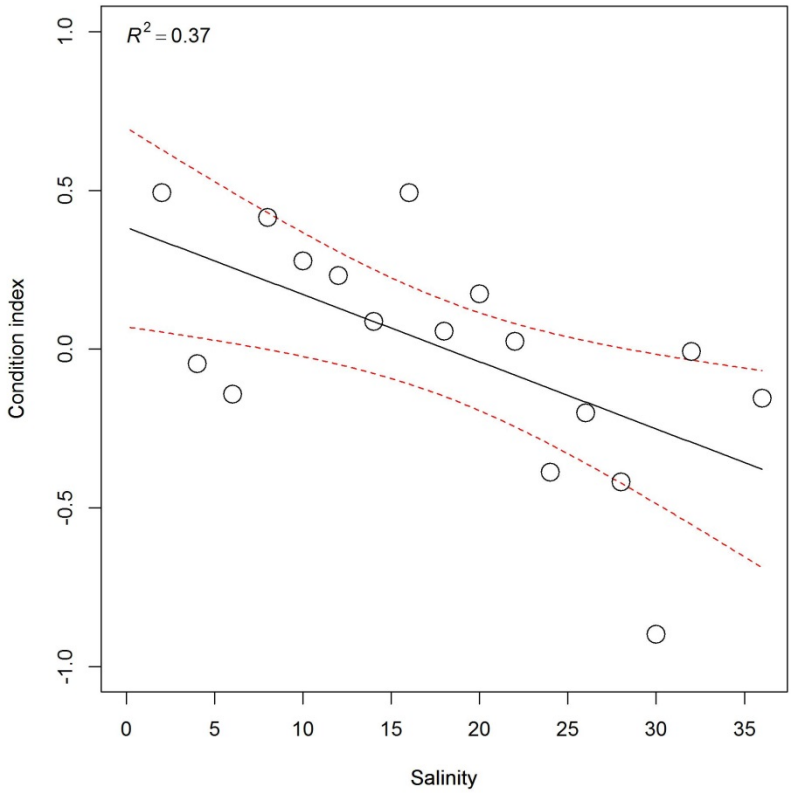

Figure 17 Relationship between condition index calculated for School Prawn and salinity following 45 days of exposure to a gradient of salinities from 0.2 – 36. The fitted model is presented as a black line, and the 5% and 95% confidence intervals are presented as dashed red lines.

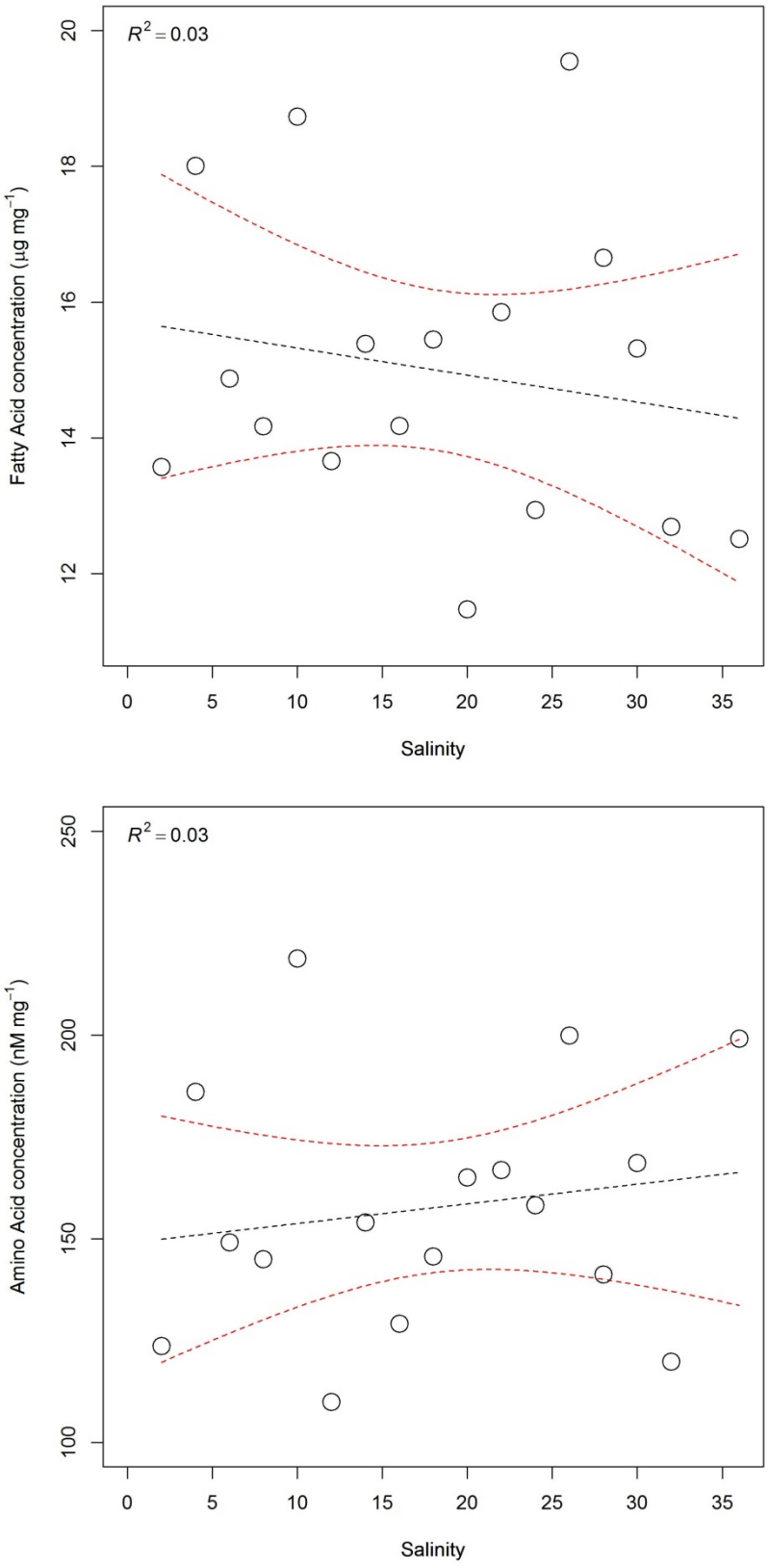

Figure 18 Relationship between salinity and total fatty acid concentration (upper panel) and amino acid concentration (lower panel) in School Prawn, following 45 days of exposure to a gradient of salinities from 0.2 – 36. The fitted model was not significant for either fatty acids or amino acids, and is presented as a dashed black line. The 5% and 95% confidence intervals are presented as dashed red lines.

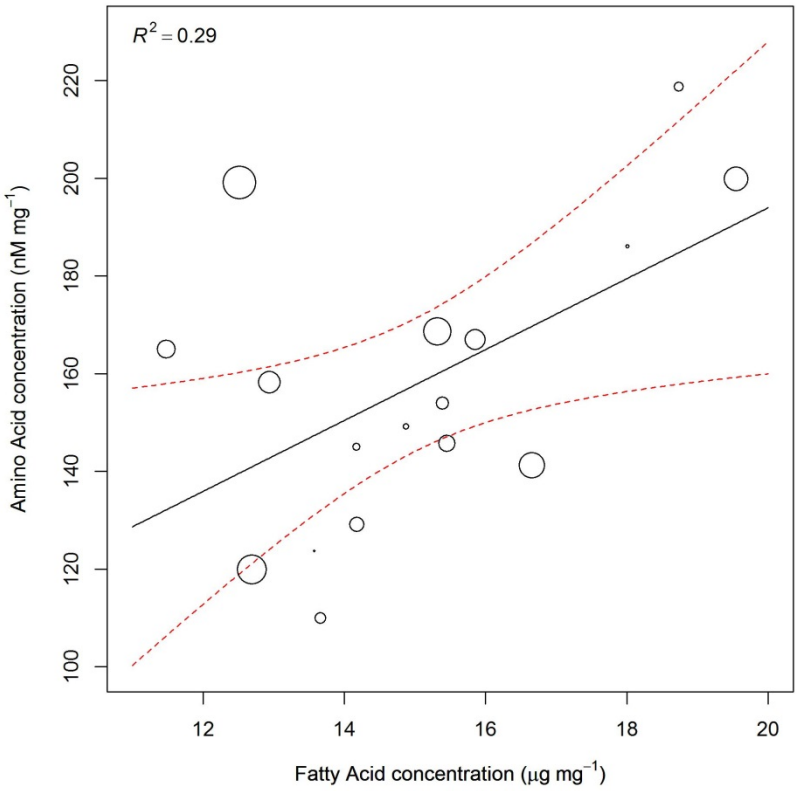

Figure 19 Relationship between total fatty acid and total amino acid concentration for School Prawn, following 45 days of exposure to a gradient of salinities from 0.2 – 36. Symbols are sized to provide a relative indication of the salinity in which prawns were held, with larger symbols reflecting higher salinities. The fitted model is presented as a black line, and the 5% and 95% confidence intervals are presented as dashed red lines.

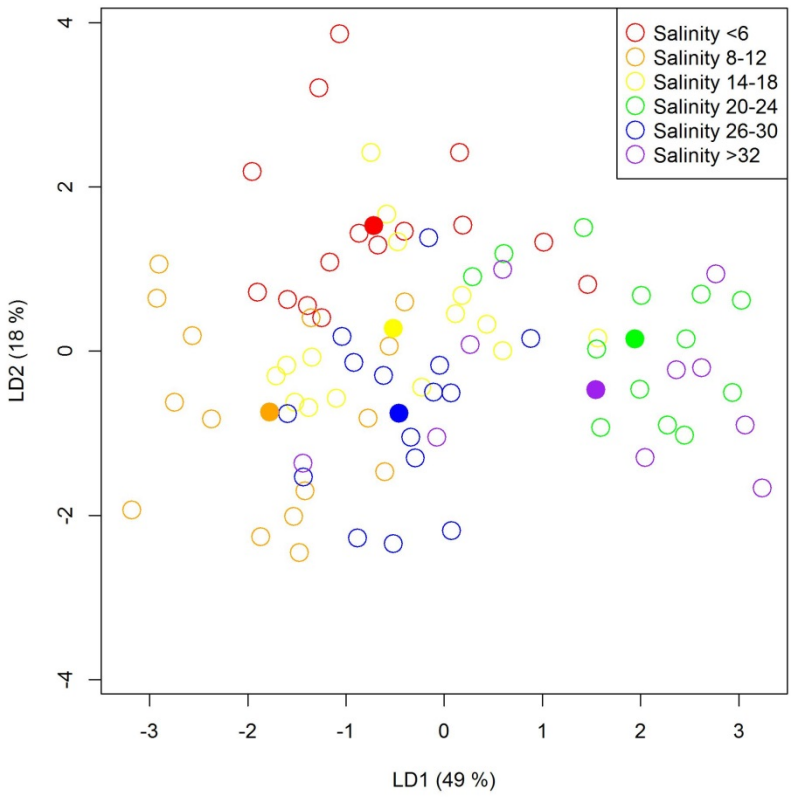

Figure 20 Results of linear discriminant function analysis of fatty acid concentration data for School Prawn following 45 days of exposure to a range of salinities. Group centroids are shown as filled circles, and the total variance explained by the each linear discriminant is given in brackets.

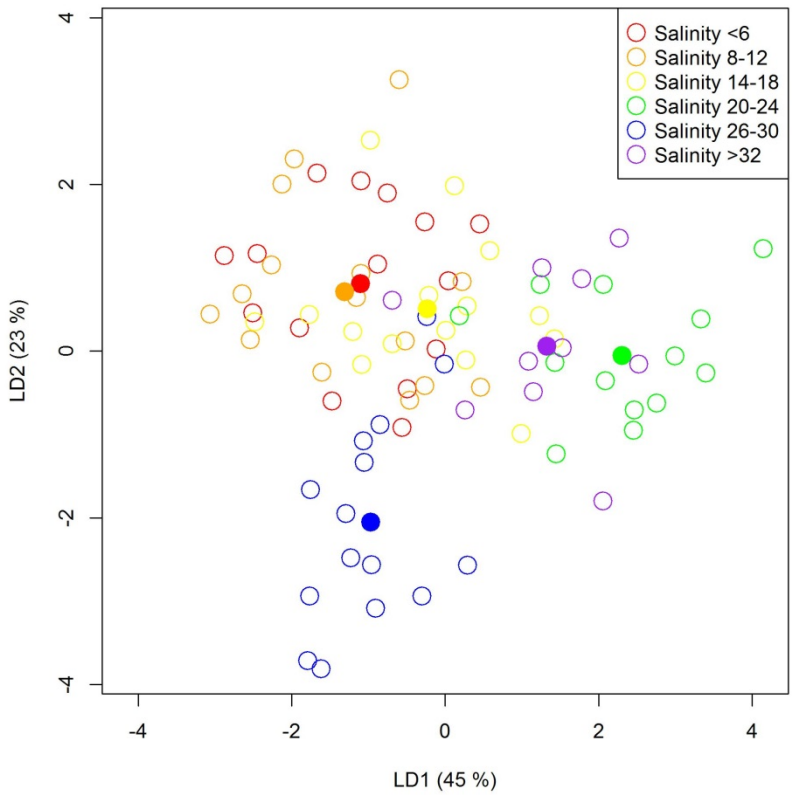

Figure 21 Results of linear discriminant function analysis of amino acid concentration data for School Prawn following 45 days of exposure to a range of salinities. Group centroids are shown as filled circles, and the total variance explained by the each linear discriminant is given in brackets.

Field survey of prawn recruitment, and catch statistics

The experiments outlined in this report highlight the various lethal and sublethal effects of a range of catchment derived stressors on School Prawn, under controlled laboratory conditions. This chapter seeks to evaluate the impact of these stressors on the School Prawn population and the School Prawn fishery, in the Camden Haven Estuary. Specifically, two broad aims were addressed: 1) quantify prawn abundance in the estuary as a proxy for juvenile (generally sizes smaller than size-at-maturity occur in NSW estuaries, Racek, 1959) recruitment, and assess the relationship between variability and changes in physico-chemical variables collected throughout the estuary (described above); 2) evaluate patterns of change in fisheries catch, and its relationship with estuary inflow.

Methodology

Sample collection and sorting

Sampling was conducted monthly from spring 2015 to autumn 2018. A full survey of abundance was conducted across all 18 sites in the estuary, including the three tributary rivers, approximately every two months (which sampled 18 sites), and a subset of 4 sites in Watson-Taylor Lake and Queens Lake were surveyed every other month when the full survey was not conducted (Fig. 1). These data were collected as a proxy for recruitment levels, to quantify the distribution of juvenile School Prawn across the estuary, and to provide data to model the potential impact of estuarine water quality on prawn abundance (and recruitment). During the months that the full abundance survey was conducted, comparative data on prawn abundance were also collected from 4 sites in an adjacent estuary (Wallis Lake) of similar geomorphology and habitat, for qualitative comparison of sample numbers.

Prawns were sampled using a benthic sled net with a 0.75 x 0.45 m mouth, a 4-m long 26-mm diamond mesh body and a 1 m 6-mm octagonal mesh cod-end (see Hart et al., 2018). Sampling commenced after dawn (School Prawn are diurnally active), and each tow was of ~5 min duration, covering a distance of ~100 m and an area of ~75 m^2. A GPS waypoint was marked at the start and finish of each tow to calculate the exact tow-length. Depth and spot measurements of benthic water quality (salinity, pH, dissolved oxygen [mg L^{-1}] and temperature [°C]) were recorded at each site during each survey. The concentrations of dissolved aluminium were compared with the marine water quality guideline of 0.024 mg L^{-1} (based on no-observed-effect concentrations from 11 species, Golding et al., 2015). During the full abundance surveys, four tows were normally conducted at each site at each time point. Upon landing, fish and cephalopods were sorted from the sample and returned to the water, prawns were placed into labelled snap-lock bags, and stored on ice for <2 h before being frozen.

Samples were thawed and sorted in the laboratory. Prawns were identified to species and counted, and a random sub-sample of up to 50 prawns was measured (carapace length [CL, mm] and weight [g]). The tow length (m) was calculated using a Euclidean formula, and this variable was used with the gear dimensions and a gear efficiency estimate (0.48 [M.D. Taylor, unpublished data], determined using the depletion approach described in Loneragan et al., 1995) to standardise abundance estimates to prawns-per-hundred-square-metres (ind. 100 m^{-2}).

Data exploration and analyses

Initially, spatial patterns in the abundance of School Prawn in the two lake systems was visualised by conducting Global Polynomial Interpolation in ArcGIS v 10.3 (ESRI). The relative condition of prawns in each of the two lake systems was assessed by calculating the standardised residuals from the relationship between log(Carapace Length) and log(Weight), which provided a size-independent measure of the somatic condition of an individual (Moltschaniwskyj and Semmens, 2000a). To evaluate the potential impact of critical dissolved oxygen levels on condition, standardised condition data were grouped for whether dissolved oxygen (logger) dropped below 3 mg L^{-1} during the month (classified as "critical", or < 3 mg L^{-1}) prior to capture, and compared with other samples (classified as "normal", or ≥ 3 mg L^{-1}) for Watson-Taylor Lake and Queens Lake using a two-factor ANOVA. Relative condition following the top 25% of all estuary inflow events (classified as "high flows) was compared with condition following all other flows (classified as "normal flows") using a *t*-tests, for estuary inflow occurring via both Stewarts River and Camden Haven River (separately). For the main nursery area, the effect of key water quality parameters on School Prawn abundance was analysed using a Generalised Additive Mixed Model (GAMM, Gaussian family with identity link) in R (R Core Team, 2016). To evaluate the relationship between freshwater inflow to the estuary and fisheries catch, monthly commercial stow net landings for September to March were obtained for the years from 2009 until 2017 from the NSW Department of Primary Industries Commercial Fisheries Catch Statistics Database, and regressed against estuary inflow data from Camden Haven River as the independent variable (unlagged, $Catch_t \sim Flow_t$; and slightly lagged, $Catch_t \sim Flow_{t-1}$), using simple linear regression.

Results

Abundance, condition and commercial catch of School Prawn

The abundance and distribution of School Prawn differed spatially and temporally across the estuary (Table 6, Figure 22). The mean abundance in each of the 8 major regions increased in November or January, and decreased after autumn, with the trend being evident across all sites in Camden Haven Estuary, and in the reference location Wallis Lake (Table 6). During times of high abundance, catch rates were much higher in Watson-Taylor Lake, Camden Haven River and Stewarts Creek, particularly between November 2016 and May 2017, when exceptional recruitment was recorded in these regions (with a maximum recorded abundance of 3,260 ind. 100 m^{-2}, Table 6). The abundance of prawns in most regions was greater than in Herons Creek, where very few prawns were caught throughout the study (Table 6). Samples from Wallis Lake indicated similar temporal variation to Camden Haven Estuary, and abundances similar to those found in Queens Lake (Table 6).

Overall, School Prawn were far more abundant in Watson-Taylor Lake than Queens Lake (Figure 22), with the greatest abundance in the western half of the lake. In 2018, there was a peak in School Prawn abundance in the south-western part of Queens Lake, adjacent to Herons Creek. Of the tributary habitats, the greatest abundances were detected in Stewarts River (Table 6, Figure 22). These data indicate that the western half of Watson-Taylor Lake is the most significant nursery for juvenile School Prawn in the Camden Haven Estuary, and data from this location forms the focus of the subsequent analyses of prawn abundance.

Length-frequency distributions in Watson-Taylor Lake were highly variable throughout the study period, but confirmed seasonal progression through the nursery following the strongest recruitment in early summer (Figure 23). There was also evidence of additional recruitment in January and March each year, with several modes evident in the carapace length distributions (Figure 23). Analysis of the relative condition in School Prawn indicated that there was a decrease in condition following critical

Table 6 Average School Prawn densities (ind. 100 m^{-2}, standard error in brackets) from quantitative sampling throughout the study period.

Estuary and Location	2015	2016						2017						2018	
	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar
Camden Haven															
Queens Lake	77 (12)	73 (38)	26 (8)	35 (8)	36 (9)	14 (10)	28 (10)	52 (26)	228 (66)	6 (2)	13 (7)	17 (11)	11 (5)	187 (64)	34 (7)
Herons Creek	9 (6)	0 (0)	6 (4)	0 (0)	5 (2)	1 (1)	1 (0)	1 (1)	0 (0)	3 (2)	0 (0)	0 (0)	2 (1)	4 (2)	2 (2)
Stingray Creek	108 (41)	35 (25)	6 (2)	9 (4)	0 (0)	0 (0)	5 (4)	68 (18)	94 (37)	23 (8)	0 (0)	0 (0)	4 (2)	88 (40)	35 (14)
Watson-Taylor Lake	148 (47)	196 (97)	28 (7)	35 (14)	0 (0)	57 (22)	29 (16)	1508 (441)	472 (139)	180 (32)	156 (34)	46 (18)	36 (13)	390 (94)	37 (9)
Camden Haven River	316 (87)	162 (52)	12 (9)	3 (2)	0 (0)	2 (1)	4 (1)	40 (18)	335 (76)	78 (29)	9 (6)	6 (4)	20 (10)	15 (10)	7 (5)
Stewarts River	31 (29)	284 (67)	57 (21)	29 (8)	0 (0)	2 (1)	9 (2)	998 (386)	281 (77)	122 (25)	20 (13)	20 (9)	155 (119)	19 (9)	49 (19)
Camden Haven Inlet	0 (0)	4 (4)	1 (1)	0 (0)	0 (0)	2 (2)	0 (0)	19 (15)	70 (24)	6 (6)	35 (11)	10 (7)	0 (0)	10 (5)	1 (0)
Wallis Lake	10 (3)	88 (29)	2 (1)	5 (4)	0 (0)	3 (1)	17 (9)	36 (9)	184 (38)	12 (3)	6 (2)	0 (0)	10 (4)	23 (10)	3 (1)

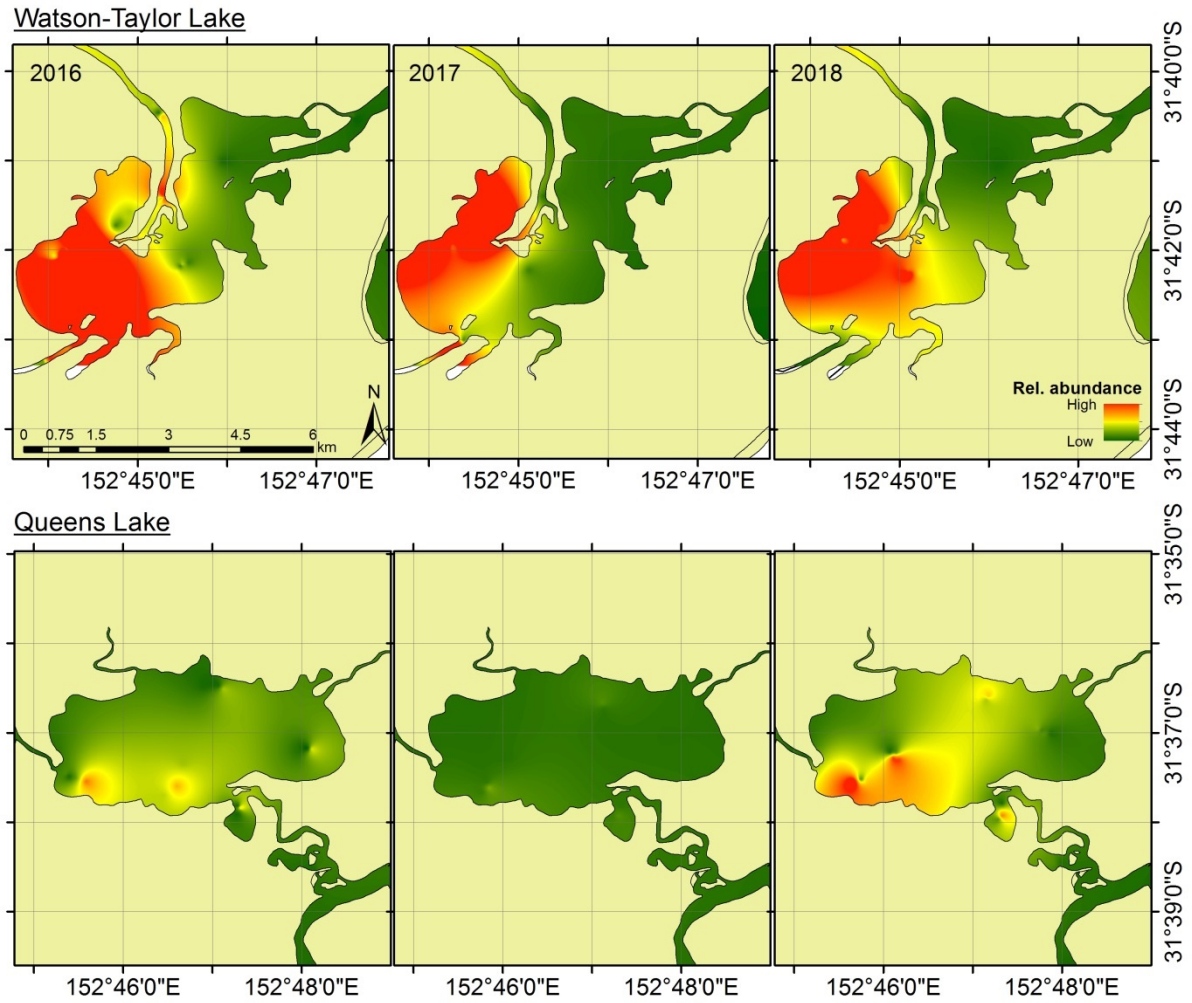

Figure 22 Interpolated surface showing the spatial distribution of relative abundance of School Prawn in Watson-Taylor Lake (top panels) and Queens Lake (lower panels). Time points include 2016 (left panels), 2017 (centre panels) and 2018 (right panels), and reflect samples collected in January of each year (when recruitment was generally greatest). The colourbar (legend) indicates the relative abundance within a year, but colours are not comparable among years (i.e. red in 2018 implies a different relative abundance to red in 2017).

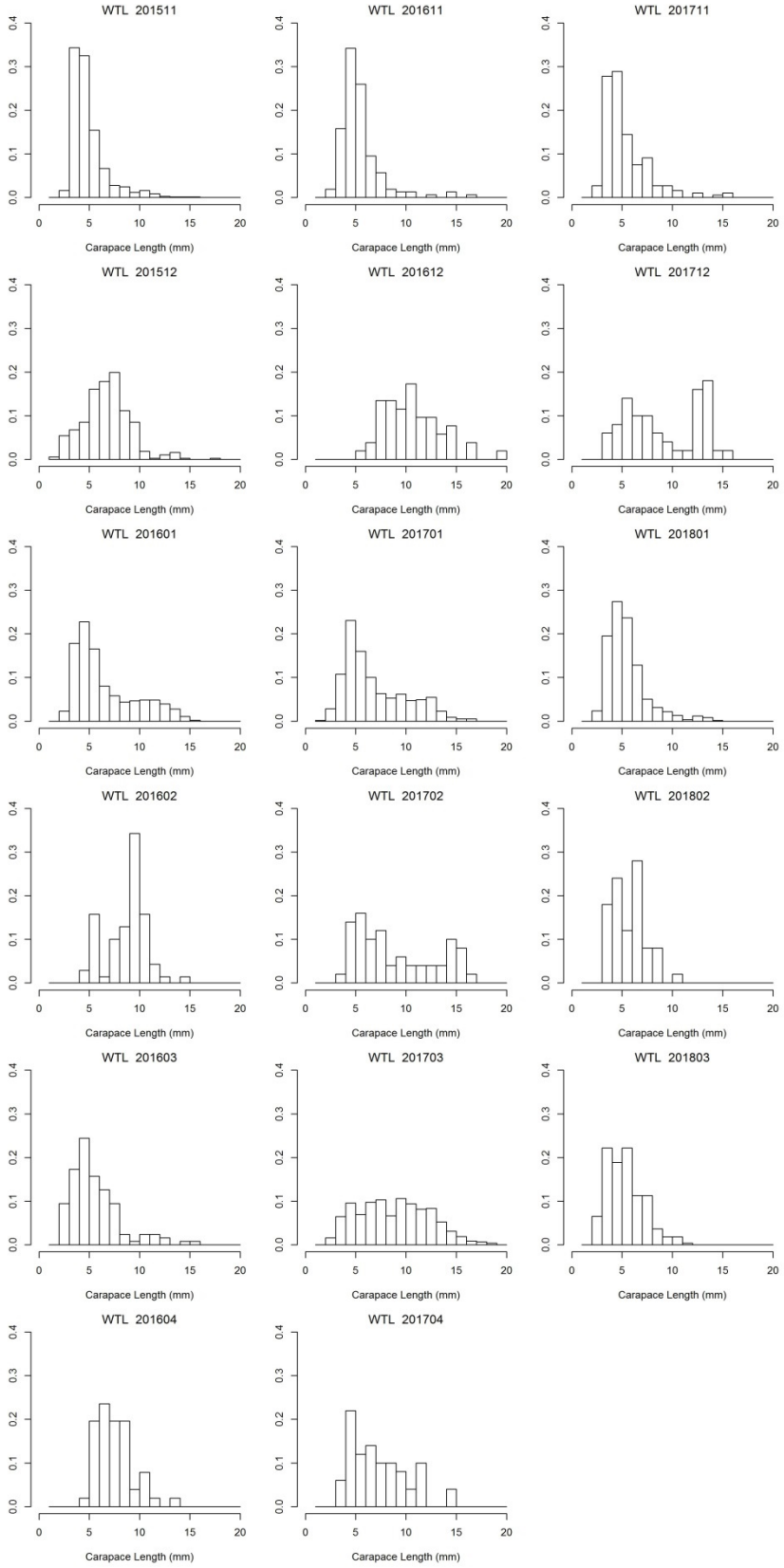

Figure 23 Unweighted length-frequency distributions for School Prawn captured in Watson-Taylor Lake (WTL) during the 2015/16 season (left column, November 2015 – April 2016), the 2016/17 season (middle column, November 2016 – April 2017), and the 2017/18 season (right column, November 2017 – March 2018). For each plot title, the month and year is coded as YYYYMM (e.g. November 2015 is 201511)

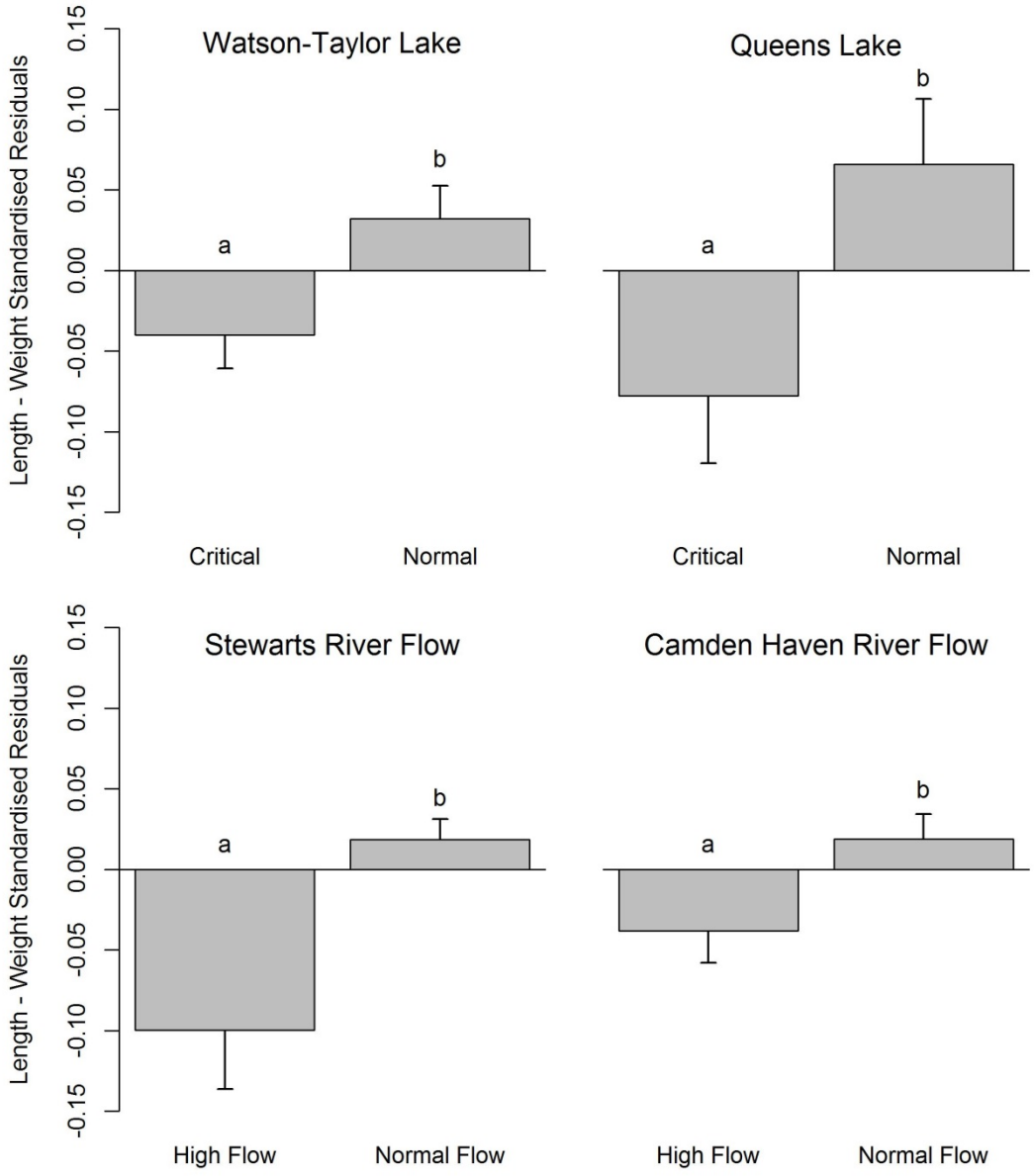

Figure 24 Standardised residuals (mean ± SE) of the length-weight relationship as a measure of relative condition in School Prawn captured from Watson-Taylor Lake and Queens Lake under normal conditions, and following critical dissolved oxygen (DO) events (<3 mg L^{-1}) as detected by loggers (upper panels). Also shown (lower panels) is the relative condition of School Prawn captured in Watson-Taylor Lake following high flows (the top 25% of all estuary inflow events) and under normal estuary inflow conditions (all other flows). Different letters indicate statistically significant differences.

dissolved oxygen events ($F_{1,5896}$ = 10.92, P < 0.001, Figure 24), but there were no differences between lakes, or significant interaction term. In addition, the relative condition of School Prawn in Watson-Taylor Lake decreased following high estuary inflow events (the top 25% of all estuary inflow events) for both Camden Haven River flow ($t_{4826.9}$ = -2.26, P = 0.0032, Figure 24) and Stewarts River flow (t_{1296} = -3.05, P = 0.002, Figure 24).

Generalised additive mixed models (GAMMs), excluding the exceptional recruitment that occurred in January 2017 (Table 6), indicated there were relationships between $\log_{10}$-transformed School Prawn abundance and both dissolved oxygen and salinity. There was a non-linear relationship between dissolved oxygen and abundance (spline equivalent degrees of freedom = 3.8, F = 7.57, P << 0.001, Figure 25), whereby abundance was greatly reduced at dissolved oxygen concentrations less than ~4.5 mg L^{-1} (while the fitted spline closely reflected patterns in the data, it should be interpreted with caution due to the heterogeneous variances). Abundance formed a negative linear relationship with salinity (t_{225} = -6.01, P << 0.001, Figure 25), and indicated that abundance decreased by an order of magnitude over the salinity range from 2 (predicted value ~250 ind. 100 m^{-2}) to 35 (predicted value ~20 ind. 100 m^{-2}, Figure 25). The cluster of samples at salinity < 5 (Figure 25) were collected following minor estuary inflow events and so represented an aggregated effect of prawns pushed down the tributaries to Watson-Taylor Lake, the main nursery area. The effect of salinity was not significant when these data were excluded from the analysis (t_{186} = -0.69, P = 0.491, Figure 25).

Long-term commercial catch data formed significant relationships with several metrics describing estuarine inflow (Figure 26). Direct relationships between log-transformed flow and monthly catch were evaluated using unlagged ($Catch_t \sim Flow_t$), and slightly lagged ($Catch_t \sim Flow_{t-1}$) monthly average flow data. A significant negative relationship was detected for the relationship between catch and flow at the same time (unlagged, $F_{1,94}$ = 10.99, P = 0.001, Figure 26) and catch and flow of the previous month (slightly lagged, $F_{1,93}$ = 18.63, P << 0.001, Figure 26).

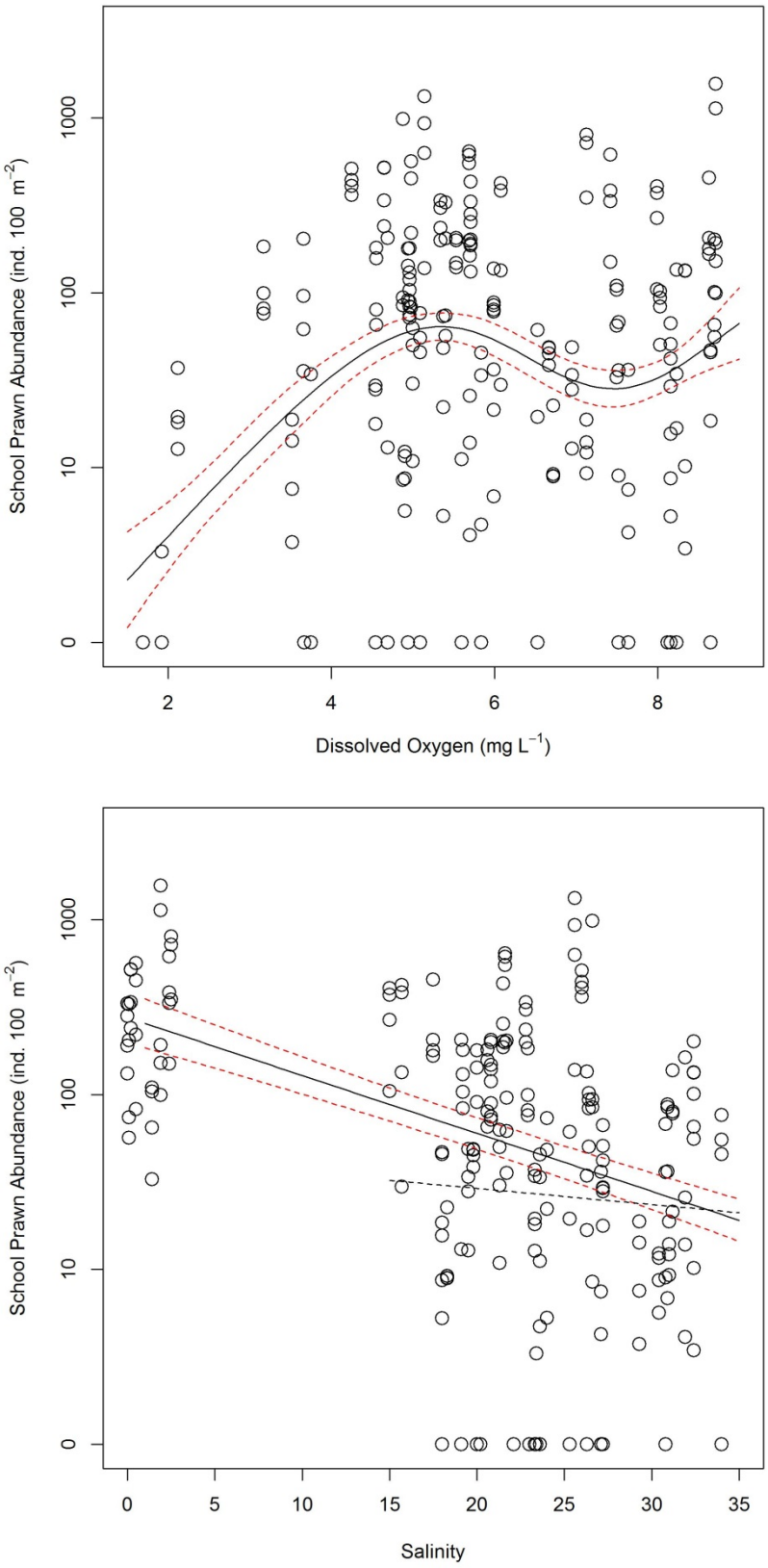

Figure 25 GAMM smoothing curves indicating the partial effects of dissolved oxygen (upper panel), and salinity (lower panel) on the abundance of School Prawn in Watson-Taylor Lake. Red dashed lines indicate 95 % confidence intervals, and the black dashed line on the lower panel indicates the refitted linear relationship following removal of low salinity data.

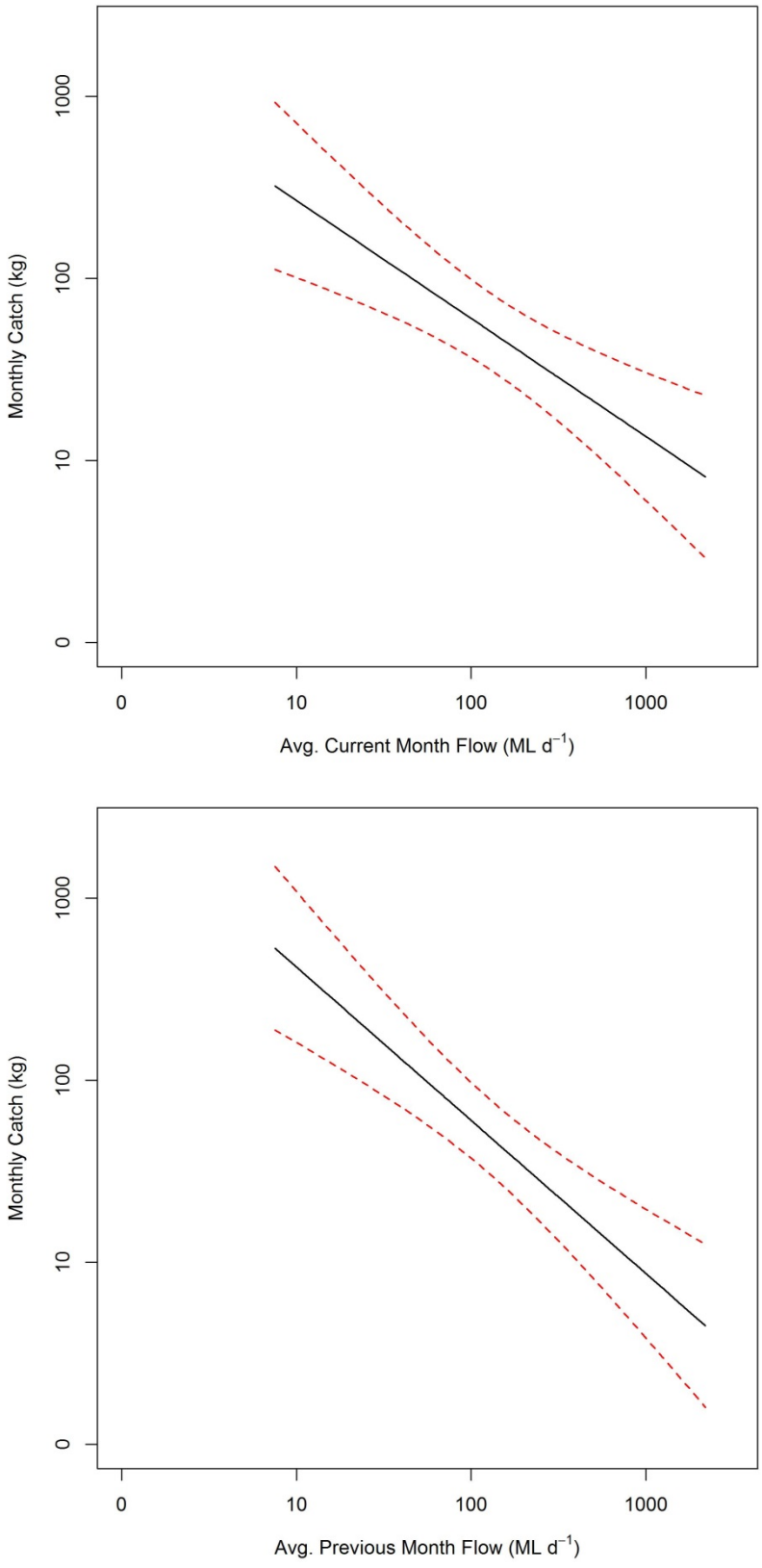

Figure 26 Relationships between monthly School Prawn catch (across summer and autumn) and estuarine inflow (top panel), and estuarine inflow lagged by one month (lower panel) in Watson-Taylor Lake. Note that actual data points cannot be shown to protect the privacy of individual fishing businesses.

Discussion

Effects of Aluminium and pH exposure under laboratory conditions

The results presented here clearly show that Al exposure is not lethally toxic to juvenile School Prawn at an acute level (<106 mg Al L^{-1}), as there was very little mortality evident during the 96 hr trials (even at the highest exposure levels). The lack of relationship between Al concentration and survival precluded the derivation of an estimate of LC_{50} for Al, leading to the conclusion that acute exposure to Al concentrations greater than the threshold marine water quality guideline of 0.024 mg L^{-1} (Gillmore et al., 2016) does not directly result in mortality in School Prawn. Although juvenile School Prawn appeared tolerant to Al, this metal has been shown to have toxic effects in several species of fish (see Wallin et al., 2015; Vuorinen et al., 2003; Slaninova et al., 2014; Poléo et al., 1997; Hyne and Wilson, 1997) and freshwater invertebrates (e.g. Wren and Stephenson, 1991; Quiroz-Vázquez et al., 2010; Redjeki, 2012). There is, however, few studies dealing with the effects of Al on marine benthic crustaceans (Martin and Holdich, 1986) with which to compare our results, which is surprising given the pervasive issue of acid sulphate soils in coastal catchments (Wilson and Hyne, 1997). Examples that are available generally deal with embryonic stages, and show significant mortality occurring at 5–10 mg Al L^{-1} (e.g. Macdonald et al., 1988; Rayburn and Aladdin, 2003).

Speciation of Al in saline waters is very different to that of freshwaters under acidic conditions. Driscoll (1985) reports that in freshwater, Al is fractionated into labile monomeric Al (aqueous Al and inorganic complexes of Al including OH^-, F^-, SO_4^{2-}), nonlabile monomeric Al (organic complexes of Al) and acid soluble Al (colloidal Al), with concentrations of the labile monomeric Al fraction increasing exponentially with decreasing pH. Seawater Al speciation is vastly different however, with low Al solubility and varying Al speciation. For the dissolved phase, at pH of 8 in seawater dissolved inorganic aluminium exists as anionic aluminate ($Al(OH)_4^-$; 68%) and a soluble non-colloidal neutral hydroxide species ($Al(OH)_3^0$; 32%). As the pH becomes more acidic, the proportion of neutral species increases, whereas the anionic aluminate species decreases (Millero et al., 2009). Further, as total Al concentrations increase (above 0.5 mg L^{-1}), precipitation of dissolved Al as $Al(OH)_3$ and to a lesser extent as hydrotalcite ($Mg_6Al_2CO_3(OH)_{16}.4H_2O$) occurs (Angel et al., 2016). Reduced pH, however, can increase metal solubility (Driscoll and Schecher, 1989) which in turn may increase the toxicity of the metal by increasing bioavailability for uptake in solution (Martin and Holdich, 1986). In the experiments presented here, there was direct evidence of increased Al solubility at lower pH. Previous work on penaeid species has shown that toxicity of metals can increase as pH levels decrease (e.g. Das and Sahu, 2005), and we suspected that multiple stressors on School Prawn could act to amplify mortality with increasing Al concentration. This would have been evident in a significant interaction of dissolved Al and pH on survival. While pH did impact mortality (with significantly greater mortality at lower pH), there was no evidence of an interactive effect with Al.

Allan and Maguire (1992) showed that for *Penaeus monodon*, pH was lethal at 3.7 (3.4 – 4.1; 95% confidence intervals), and the minimum acceptable pH was 5.9. This supports the conclusion that reduced pH was a proximal factor leading to mortality in School Prawn. However, the physiological effect of pH on the gills may have enhanced uptake of Al irrespective of the concentration of dissolved Al, and this is evident in elevated bioaccumulation of Al at reduced pH (discussed below). Consequently, the significant effect of pH on mortality could incorporate an effect of Al as well. A factorial experimental design (rather than a bioassay) would be required to confirm this effect; however the sublethal effects of these stressors on School Prawn provide some insight into this possibility.

Bioaccumulation and sublethal effects

There was considerable evidence of sublethal effects of both Al and pH in School Prawn. Low pH led to higher bioaccumulation of Al. As noted above, there was increased solubility of Al at decreased pH. It is likely that elevated bioaccumulation of Al at lower pH was due, in part, to greater Al in the dissolved phase (and thus less precipitate) and the dissolved neutral aluminium hydroxide and especially the anionic aluminate were bioavailable for uptake, presumably through anion membrane channels at the gill surface (Gillmore et al., 2016). However, there was no main effect for dissolved Al and uptake, nor any interaction, suggesting some regulation to Al uptake from the dissolved phase and an effect of pH on the toxicokinetics and uptake dynamics of the contaminant (i.e. the ability to accumulate aluminium across a cellular membrane, Allan and Maguire, 1992) is probable at low pH (see Bryan, 1971).

Immune responses and pathology occurred in specimens exposed to Al in both pH levels, yet were more substantial in individuals from pH 5. Increases in haemocyte density indicated that prawns dealing with multiple stressors (i.e. high Al concentrations under acidic conditions) amplified their haemocyte defence. Structural damage to the gills was substantial in animals exposed to environmentally relevant Al concentrations. Such pathologies are likely to impair respiration, and osmotic and ionic regulations of the gills, which could lead to behavioural changes in School Prawn. In the current experiment, lower activity and responsiveness in Al-exposed prawns provided some evidence of this (A. Russell; pers. obs.), and similar reactions have been previously reported for the freshwater invertebrate *Daphnia magna* following exposure to copper (Untersteiner et al., 2003). Behavioural changes resulting from exposure to metals could affect both feeding and anti-predator responses in prawns, which in turn could affect the survival and growth under natural conditions.

Animals exposed to Al in acidic conditions showed greater haemocytic infiltration in the interstitial sinuses of the hepatopancreas, which is a distinct immune response (Hauton, 2012) and aligns with similar reactions observed in crustaceans exposed to heavy metals (Bhavan and Geraldine, 2009; Tsing et al., 1989; Battistella et al., 1996; Soegianto et al., 1999). Viral occlusion bodies were consistently detected in the hepatopancreas of prawns from pH 5 treatments. This is likely due to baculovirus infection (Family Baculoviridiae), however a positive diagnosis would require molecular identification (Kanjanasopa et al., 2015). Baculovirus virus is prevalent in penaeid aquaculture farms throughout the Indo-Pacific, Asia and Americas (Moss et al., 2012), but is also documented in wild penaeid species in Australia, like *Metapenaeus bennettae* (Spann and Lester, 1996). Infection by this virus does not typically cause mortality, and the virus can be tolerated by penaeid species at low to moderate levels of infection but only when other conditions are optimal (Lightner and Redman, 1981; Fegan et al., 1991). However, presence of the virus can lead to slow growth and associated low productivity when levels of infection are high (Flegel et al., 2004).

Limitations of experimental manipulations and derived data

While our data provide compelling patterns for the experimental conditions tested, it is necessary to place some caveats on our findings. Firstly, while the 96 h bioassay is a common approach in ecotoxicology to evaluate lethal concentrations, it is possible that the exposure duration was not sufficient to produce an effect on mortality. Macdonald et al. (1988) suggested that lethal concentrations established at 96 h were meaningless for crab embryos, because acute toxic thresholds were not attained by that time, and this may be relevant to juvenile prawns as well. However, we did observe considerable bioaccumulation during the 96-hr period, and the exposure period was sufficient for pathological symptoms of exposure to manifest. Furthermore, pulses of Al into an estuary would normally occur through run-off from the catchment following a rainfall event. Short, acute exposure periods for high Al concentrations are probably representative of exposure under natural conditions, as drainage of the catchment and resumption of tidal flushing could be expected to resume within 96-hr of a rainfall event (with the exception of an extreme event, e.g. Tyler et al., 2017). This would improve water quality and dilute any dissolved Al during this timeframe.

The chemistry and toxicity of Al is highly dynamic in saline water (Golding et al., 2015), which was evident in measured water concentrations presented here. The solubility limit (<0.5 mg Al L^{-1}) generally reflects concentrations found under normal (non-flood) conditions in the field, but concentrations as high as 7 mg Al L^{-1} have been detected in estuarine waters in south-eastern Australia (M.D. Taylor; unpubl. data). The complex speciation of Al in saline water includes changing chemistry and increased precipitation with aging of the solution, which means it is likely that dissolved Al exposure concentrations varied over the exposure window, and future sampling of dissolved and total Al should be undertaken at multiple points temporally during the experiment (Angel et al., 2016). Since exposure to Al most likely occurs at lower salinities (following floods) in estuarine systems, it would also be useful for future studies to explore interactions between salinity, pH, and Al concentration. Such complex studies are necessary to fully appreciate the impact of multiple stressors that animals are exposed to under natural conditions.

Effects of hypoxia exposure under laboratory conditions

The experiments demonstrated that School Prawn were generally resilient to sub-hypoxic conditions. In the presence of Al, mortality increased at greater DO concentrations (i.e. survival departed from "1" at greater DO concentrations) than when Al was absent, providing some evidence that Al increased sensitivity to hypoxia in School Prawn. There was evidence that hypoxic conditions changed the total fatty acid and amino acid concentrations and profiles in School Prawn. While mortality is clearly the outcome of hypoxia, sublethal effects are likely to accumulate under sub-hypoxic conditions, which may have further impacts on School Prawn growth and condition.

Lethal effects of hypoxia and multiple stressors

Mortality generally increased at DO concentrations <3 mg L^{-1}, which is consistent with patterns observed for other marine species and the general patterns reported in Vaquer-Sunyer and Duarte (2008), which calculated a cross-species LC_{50} for hypoxia in crustaceans of 2.45 mg L^{-1} and a sublethal concentration threshold (SLC_{50}) of 3.21 mg L^{-1}. The LC_{50} derived for DO in the current study is also consistent with results for juvenile *Penaeus monodon*, which had an LC_{50} of 0.9 mg L^{-1} (Allan and Maguire, 1991), and there are similarities with other marine invertebrate species. For example, the Chinese Scallop *Chlamys farreri* experienced significantly greater mortality at DO concentrations of 2.5 mg L^{-1} (Chen et al., 2007), and similar sensitivity was observed in Abalone *Haliotis diversicolor supertexta* (Cheng et al., 2004). Severe hypoxia also decreased the time-to-death for School Prawn, but our LT_{50} estimates were far smaller than the threshold for crustaceans of 55.5 h reported in Vaquer-Sunyer and Duarte (2008). School Prawn exposed to DO concentrations <1 mg L^{-1} succumbed to mortality ~3x faster than prawns exposed to concentrations of 1-2 mg L^{-1}, which suggests that prawns are more sensitive to event duration under more severe hypoxic conditions. Field data indicated several instances of hypoxia of only a relatively short duration (<2 days, Figure 2); it is thus likely that even these shorts events could result in appreciable mortality for School Prawn.

Multiple studies provide evidence of sublethal impacts of hypoxia in prawn species. Hypoxia significantly increased ammonia toxicity for *Penaeus monodon* (Allan et al., 1990), significantly reduced the growth rates for juvenile *P. vannamei* and *P. monodon* (Seidman and Lawrence, 1985), suppressed glycolysis in *Marsupenaeus japonicas* (Abe et al., 2007), as well as triggering hyperventilation and respiratory alkalosis in *Macrobrachium rosenbergii* (Cheng et al., 2003). Hypoxia also significantly impacted behaviour in the South American white prawn, *Nanmei baiduixia*, with movement speed increasing as DO levels decreased (Yang, 2011). Finally, *Penaeus semisulcatus* were shown to stop moulting during hypoxia (Clark, 1986), which is indicative of potential impacts on

growth. These examples highlight the impact of oxygen availability on prawn physiology and behaviour, which may produce impacts at the population level (Baird et al., 2004).

While hypoxia clearly leads to mortality under controlled laboratory conditions, several factors could ameliorate or exacerbate this effect under natural conditions. Several studies show that fish and prawns can detect and avoid oxygen deficient water, either through lateral or vertical movement (Kramer and McClure, 1982; Renaud, 1986). Assuming prawns can access normoxic habitats, mortality may be avoided through movement away from hypoxic plumes. While such behaviour may support short-term survival, this may however force animals from the juvenile nursery, or force them to exploit sub-optimal habitats to the detriment of growth and survival. In *Penaeus vannamei*, low DO impaired the ability of juvenile prawns to osmoregulate (Charmantier and Soyez, 1994), which suggests that the presence of osmoregulatory stress (e.g. non-optimal salinity) alongside hypoxia may increase mortality. Finally, the stress associated with hypoxia has been shown to increase susceptibility of prawns to infection (Le Moullac et al., 1998), which could lead to secondary mortality associated with hypoxia.

Our results showed that environmentally relevant concentrations of Al did not greatly exacerbate mortality under hypoxic conditions, but there was some evidence for greater sensitivity to sub-hypoxic conditions with School Prawn succumbing to mortality at greater DO concentrations in the presence of Al. As noted above, Al binds to the outer gill surface in prawns and can impair respiration and can lead to structural degradation of the gill surface, but the toxicity is dependent on solubility and speciation (Hyne and Wilson, 1997; Angel et al., 2016). With Al concentrations as high as 6 mg L^{-1} in Camden Haven Estuary (Figure 3), it is possible that Al may reduce resilience to hypoxic conditions in both School Prawn and other species.

Biochemical effects of hypoxia and multiple stressors

The School Prawn metabolome showed significant changes, likely reflecting physiological processes that occurred in response to both DO and Al. The greater concentration of both fatty acids and amino acids in School Prawn exposed to hypoxic conditions seems counterintuitive if these resources are important in allowing prawns to deal with stress. However, this pattern may be explained through the cessation of processes supporting growth under hypoxic conditions. As noted above, *P. semisulcatus* ceased moulting during hypoxia (Clark, 1986), and since moulting is the principle means through which prawns grow, this suggests the cessation of somatic growth. Furthermore decreased growth under hypoxic conditions coincides with an increase in lipid and protein (Seidman and Lawrence, 1985). The duration of our experiment was not long enough to detect a direct effect on somatic growth or condition, however increased fatty acid concentrations following hypoxia may be indicative of slower metabolic rates. This is significant, as it suggests that fatty acid concentrations as a condition index in prawns (e.g. Palacios et al., 2001) may not be reliable.

Concentrations of DHA, oleic acid, palmitic acid, and EPA, which are all important components of phospholipids in crustaceans (Moore, 1976), were greatest in School Prawn subjected to the most stressful conditions of hypoxia and Al. Under hypoxic conditions, penaeid shrimp are oxyconformers (Rosas et al., 1999). Thus the down-regulation of aerobic metabolic processes, especially those relating to somatic growth, is a logical metabolic response to oxygen limited conditions (Seidman and Lawrence, 1985). This is likely to contribute to the accumulation of these substances following exposure to stressors, such as hypoxia and Al. Reduced aerobic metabolism would also be aligned with a reduction of oxidative damage to the fatty acids in the membrane systems that facilitate respiration. Alterations in fatty acid abundance may thus be indicative of unfavourable environmental conditions such as hypoxia (Glencross and Smith, 2001).

The study of the organismal metabolome in the context of environmental variation is a relatively recent arena of investigation, which has strong potential for detecting stress responses in free-ranging animals (Sardans et al., 2011; Bundy et al., 2009). However, the specific implications of the metabolite

changes that are observed are not always clear, especially in aquatic invertebrates (e.g. Spicer, 2014), and depends on the biochemical fraction being measured. The majority of fatty acids increased in concentration with increasing stressors (the only exception being nonadecenoic acid), but key components such as DHA, oleic acid, palmitic acid, and EPA displayed more prominent and characteristic increases providing distinctive fatty acid profiles for each treatment in the discriminant analysis. The amino acid responses were more complex, where some components including the branch chain amino acids (BCAA), isoleucine, leucine, valine, and the essential amino acids lysine, histidine, methionine and phenylalanine displayed prominent increases with increasingly stressful conditions (i.e. Al, and hypoxia). This would be consistent with a shift in the balance in favour of protein catabolism over anabolism under hypoxic conditions, which could lead to an accumulation of certain amino acids in the cytoplasmic pool of the muscle tissue. Others such as alanine, glycine, asparagine, aspartic acid, cystathionine, glutamine and proline concentrations, were greater following exposure to hypoxia without Al exposure. Hydroxylysine, threonine, thioproline and tryptophan, showed no real differences between groups exposed to aluminum in the presence of hypoxia. These treatment-specific alterations in certain amino acids may reflect differential rates of use via specific pathways for certain amino acids to facilitate survival under hypoxia, which is supported by other studies investigating the biochemical effects of hypoxia on crustaceans (e.g. Mente et al., 2003).

It is important to note that the metabolome represents a transitional pool and is essentially a biochemical snapshot at a single point in time. For this reason, we have limited our conclusions following metabolomic analysis to simply suggest a change in homeostasis has occurred following hypoxia, generally reflecting downregulation of somatic growth, and that Al exposure in the presence of hypoxia has led to a further change in homeostasis, as evidenced through specific differences in some cytoplasmic amino acids. Given the complexities associated with multiple interacting biochemical processes, further interpretation of the changes occurring could be supported by other means, such as transcriptome analysis (e.g. Sun et al., 2018). Analysis of metabolomic changes (amino acid composition in particular) appears to provide a useful indicator of stress for potential evaluation of free-ranging animals, and may present a more sensitive sublethal indicator of stress than more traditional analysis of somatic condition.

Implications of results for School Prawn in Camden Haven Estuary

This study highlights the lethal and sublethal impacts of exposure to hypoxia and dissolved Al in School Prawn, and the concentrations of these stressors observed in the Camden Haven Estuary (Figure 2 and Figure 3) exceed the conditions evaluated here. It should be noted that only acute effects were evaluated in our experiment; sublethal effects of hypoxic challenges and secondary factors (e.g. disease and chronic health effects) may accumulate over timeframes greater than the experimental period of 96 h evaluated here, and lead to delayed onset of mortality. Similar experiments evaluating changes and ongrowth following a return to normoxic conditions would help to resolve this.

Effects of salinity exposure under laboratory conditions

The spectrum of salinities tested here revealed several impacts of changing conditions on School Prawn. School Prawn survival was relatively insensitive to salinities >2 and there was no effect on individual growth, fatty acid, or amino acid concentrations. Salinity, however, did impact relative condition, as well as the organism metabolome. The complex patterns observed in metabolite profiles point to several shifts in cellular chemistry throughout the salinity gradient investigated. Together, these patterns shed some light on the physiological tolerances and the impact of physiological challenges for School Prawn.

Salinity, survival and growth

In general, most estuarine species have evolved some capacity to cope with fluctuations in salinity, however even euryhaline animals can suffer osmotic stress at extreme salinities. This can affect reproduction (Young et al., 2018), survival (Crisp et al., 2017), and organism tolerance to other stressors such as hypoxia (Rosas et al., 1997) and ammonia (Kir and Öz, 2015). At salinity concentrations <2, it is likely that School Prawn experience osmoregulatory failure, although this critical salinity level was lower than that detected for other penaeid species, including the euryhaline Greasyback Prawn *Metapenaeus bennettae* (Dall, 1981). Critical salinity for juvenile Giant Tiger Prawn (*Penaeus monodon*) was approximately 5 (Ye et al., 2009), and juvenile Eastern King Prawn (*Melicertus plebejus*) experienced 50% mortality at the same salinity (Tyler et al., 2017).

The lack of impact of salinity on the somatic growth rate of juvenile School Prawn over the experimental period is consistent with other penaeid species. Zeineldin (1963) found no impact of salinity on the growth of *Penaeus setiferus, P. aztecus* and *P. duoarum*, and similarly there was no impact on the growth of *P. monodon* juveniles (although the lowest salinity tested was 15, Allan and Maguire, 1992). Notwithstanding this, other studies have detected an optimum salinity for growth in other penaeid species (Song and Brown, 2006; Staples and Heales, 1991). For example, *Penaeus vannamei* had significantly higher mean final weights between salinities of 5 and 15 relative to higher salinities (Bray et al., 1994), and *P. indicus* juveniles demonstrated fastest growth at a salinity of 15, with growth rates decreasing at higher or lower salinities (Vijayan and Diwan, 1995). A salinity of 30 was found to result in the fastest growth for both juvenile Brown Tiger Prawn (*Penaeus esculentus*) (O'Brien, 1994) and Pink Shrimp (*Farfantepenaeus duorarum*) (Zinc et al., 2017). Inspection of the data presented here indicates no evidence of an optimum growth rate across the salinity range tested for School Prawn. This is not surprising, as School Prawn are likely to be relatively efficient osmoregulators between salinities 2-36. Despite this, the negative relationship between salinity and condition index suggests some metabolic cost for osmoregulation as salinity increases, which concurs with general knowledge on the biology of the species (Ruello, 1973b).

Biochemical effects of salinity

Many organisms exhibit a relationship between growth and metabolism, and salinity has previously been shown to influence metabolism. For example, Sea Bream (*Sparus sarba*) experienced improved growth at a salinity of 15, most likely due to the reduced cost of osmoregulation at that salinity (Woo and Kelly, 1995), and juvenile Turbot (*Scophthalmus maximus*) experienced decreased oxygen consumption in moderate salinities (10 and 19) resulting in improved growth (Gaumet et al., 1995). One study on prawns also found a relationship between growth, metabolism and salinity. The juvenile brown shrimp (*Farfantepenaeus californiensis*) displayed an increased rate of oxygen consumption at high salinities, which resulted in less energy being available for growth (Villarreal et al., 2003). With metabolites responding more rapidly to environmental changes than growth rates, changes in fatty acids and amino acids may be used as an early indication of physiological stress occurring at the cellular level, before the animal responds with reduced growth rates or mortalities.

In our research, quantitative profiling of fatty acids and amino acids showed some changes in response to salinity, despite the fact that all animals were fed the same diet. The major shift in total fatty acids in the salinity group 26-30 may indicate energy in excess to metabolic requirements at this salinity (Zubay, 1998), which may have implications for growth. While many studies report on the benefits of fatty acid enhanced diets in promoting growth and health in young marine organisms, few have also assessed the influence of salinity. However, Mika (2014) detected significant changes in the content and profile of fatty acids in adult Brown Shrimp (*Crangon crangon*) in response to both temperature and salinity. The shrimp recorded the largest amounts of muscle fatty acids in spring, corresponding with the lowest average salinity and the second highest average temperature (Mika et al., 2014).

Likewise, Visudtiphole et al. (2018) found *P. vannamei* post larvae, when fed a diet supplemented with high amounts of long-chain poly-unsaturated fatty acids, showed increased tolerance to low salinity along with better growth and improved swimming strength (Visudtiphole et al., 2018). Future targeted research is clearly required to determine the significance of different fatty acids quantities across different salinities.

The salinity group (26-30) that showed the highest total fatty acid concentration also showed the greatest separation in the discriminant function analysis for amino acids. Few studies have examined amino acids (or proteins) in response to salinity in estuarine organisms. However, Rosas (1999) found that *P. setiferus* juveniles obtained their energy from proteins in low salinities at different DO concentrations, and at high salinities were capable of changing their metabolic substrate from lipids-proteins to proteins as DO concentrations changed (Rosas et al., 1999). Similarly, Shinji (2012) found that the Whiteleg Shrimp *(Litopenaeus vannamei*) metabolised amino acids as an energy source in low salinities for the purposes of hyposmotic adaptation. Our study showed an increase in hydroxylysine, hydroxyproline and proline in the salinity group 20-24. These three amino acids are major components of collagen, and in mammals, hydroxylysine and hydroxyproline are markers of collagen turnover. Collagen is an extracellular matrix protein and a major component of connective tissue (Zubay, 1998). A high rate of collagen turnover may indicate an imbalance between protein synthesis and degradation due to osmoregulation. There was no evidence for linear correlations between individual fatty acids or amino acids and the salinity gradient, with the data being more suggestive of a threshold response. Consequently, we propose that the observed alterations in the profile composition of fatty acids and amino acids provide the desired changes in membrane properties that are required to contend with changes in salinity.

Implications of results for School Prawn in Camden Haven Estuary

A key motivation of this study was to understand potential lethal and non-lethal impacts of freshwater inflow to estuaries, and the influence this may have on fisheries productivity. It is clear that extreme freshwater pulses will ultimately lead to mortality and/or emigration of School Prawn, as salinity decreases below the species physiological tolerances. However, low freshwater inflow will allow the influence of high salinity water to extend further up the estuary, thus limiting the brackish water habitat available for juvenile prawns. While this is unlikely to lead to the death of prawns, our data suggest that this may come at some cost to overall somatic condition in School Prawn. In the controlled conditions of our experiment, foraging resources were held constant between all salinity treatments, so the observed impact is likely driven by physiology. In natural systems, lower estuary inflow will limit the extent of the productive estuary transition zone (e.g. Martineau et al., 2004), and limit the influx of nutrients to support the estuarine food web (Darnaude, 2005). This may further exacerbate the decrease in condition observed at higher salinity for School Prawn in natural systems.

In Camden Haven Estuary, effects such as those described above may well limit the overall productivity of the resource, and its resilience to other adverse sources of environmental variation. However, terrestrial run-off can also lead to deleterious conditions in estuarine habitats. Thus, in the Camden Haven Estuary, it appears that the potential positive impact of freshwater flow on School Prawn, is being mediated by other catchment-derived stressors and contaminants that may simultaneously be transported into the estuary. This is discussed in more detail below. While experiments such as these are useful for teasing apart the impacts of concomitant changes occurring in estuaries across a spectrum of estuary inflow scenarios, it is likely that overall catchment health and maintenance of natural estuary function are paramount to maintaining fisheries productivity.

Impact of habitat and catchment-derived stressors on School Prawn abundance and productivity in Camden Haven Estuary

Spatial and temporal patterns in prawn abundance

This data set represents the most comprehensive study of juvenile School Prawn yet reported. Abundance in Queens Lake and Watson-Taylor Lake was usually equivalent to, or greater than, abundance measured in the nearby reference estuary (Wallis Lake). Temporal patterns in the data were consistent with the existing models for School Prawn indicating recruitment occurring in spring and early summer (e.g. Racek, 1959), but there was evidence for multiple cohorts recruiting until March. Prawn abundance was substantially lower over winter, suggesting both a recruitment hiatus over this period and that most individuals had emigrated from the estuary by the end of autumn. Also, there appeared to significant asymmetry in recruitment to juvenile nursery areas across the estuary, with consistent disproportionately high abundance in Watson-Taylor Lake and its tributaries (Stewarts River and Camden Haven River). This is likely a function of two things. Firstly, Watson-Taylor Lake and its tributaries to the east have greater tidal connectivity than Stingray Creek and Queens Lake, which is important for supply of ocean-spawned postlarvae. Secondly, Camden Haven River is a larger tributary than Herons Creek, and appears to have more sustained periods of brackish salinity. This may lead to a stronger recruitment signal from this part of the estuary (Ruello, 1973b), and also provide more sustained brackish water areas for juveniles (see experimental outcomes described under *Salinity exposure experiment*). These impacts, however, may periodically be tempered by the impact of catchment-derived stressors, such lower dissolved oxygen, elevated concentrations of dissolved aluminium, and possibly lower pH, as evidenced in the outcomes of the experiments described above. This is discussed in more detail below.

Habitat-derived productivity bottlenecks

The substantial loss of seagrass in both Watson-Taylor Lake and Queens Lake is likely to partially contribute to a productivity bottleneck in the system. Seagrass has been shown to be critical juvenile habitat for juvenile tiger prawn species *P. esculentus* and *P. semisulcatus* (Loneragan et al., 2013; Haywood et al., 1995; Loneragan et al., 1997; Obrien, 1994), and can be an important source of organic carbon for penaeid shrimp in tropical estuaries (Loneragan et al., 1997). However, *Metapenaeus* spp. in general, are less dependent on seagrass than tiger prawns (e.g. Staples et al., 1985; Taylor et al., 2017a). Saltmarsh has been shown to be a major source of organic carbon for School Prawn in seagrass limited systems (Raoult et al., 2018), and recent work examining these relationships in systems containing both seagrass and saltmarsh indicates that seagrass (*Zostera* spp.) can be the dominant source where saltmarsh is less abundant (Hewitt, 2018). In both these studies, fine benthic organic matter was of minor importance as a carbon source for School Prawn.

Given the low abundance of saltmarsh in Camden Haven Estuary, seagrass could represent an important primary producer supporting School Prawn productivity in this system. Some evidence of this can be seen in the fatty acid composition of School Prawn captured in Camden Haven Estuary (Taylor et al., 2018b), where fatty acids characteristic of marine primary productivity were abundant under non-flood conditions. Despite losses of seagrass habitat in Queens Lake, seagrass remains abundant in this part of the estuary but this area appears to be of lesser importance for juvenile School Prawn. The near complete loss of seagrass from Watson-Taylor Lake is concerning, and since this part of the system represents the main nursery for juvenile School Prawn in the estuary this could constrain productivity of the species. The proximal factor causing the observed loss of vegetation in Camden Haven Estuary is unknown, but has previously been hypothesised to involve sedimentation and increased turbidity in the system (Creighton, 1982).

Catchment-derived impacts on juvenile School Prawn

The logger data collected provided strong evidence of hitherto unknown anoxic and hypoxic events in the Camden Haven Estuary lasting for up to two weeks in different parts of the system. As described earlier, anoxic and hypoxic water in Stewarts River is hypothesised to originate due to stagnant bodies of high salinity water that rest in the deeper bathymetry of the tributary (> 2 m, relative to the depth of ~ 0.4 m where the tributaries enter Watson-Taylor Lake). Tidal magnitude is greatly attenuated at the tributary mouths (Creighton, 1982), and thus detritus and allochthonous organic matter can accumulate in these bodies of high salinity water on the tributary bed with microbial decomposition removing oxygen (stratification, hypoxia, and presence of this leaf litter have been confirmed; M.D. Taylor, pers. obs.). There was no consistent relationship between these events and declines in salinity (indicative of freshwater flow), and the force mobilising this low oxygen water is currently unclear. The international literature suggests poor links between hypoxia and broad-scale declines in fisheries productivity (see review by Breitburg et al., 2009), however consideration of the specific mechanisms at play suggest that such a relationship is possible, particularly for School Prawn.

The experimental outcomes described under *Hypoxia exposure experiment* above, provide a solid basis for interpreting patterns observed in Camden Haven Estuary. In Herons Creek, the main tributary flowing in to Queens Lake, a single window of hypoxia or anoxia was recorded during each summer, but there was a consistent lack of prawns in this tributary (likely due to a connectivity effect as suggested above). Thus, hypoxia in Herons Creek would probably have limited impact on prawn productivity, but these events could affect prawn productivity in the western area of Queens Lake. The lower condition index observed in Queens Lake School Prawn following critical low dissolved oxygen events (< 3 mg L^{-1}) in Herons Creek provides some evidence of this.

Camden Haven River and Stewarts River directly feed into the main School Prawn nursery in the western Watson-Taylor Lake. Critical hypoxic or anoxic events were less common in Camden Haven River (the largest tributary in the estuary), but Stewarts River experienced regular and occasionally protracted hypoxic events. There were fewer critical events during the 2016/17 season, which coincided with the greatest abundances of School Prawn (although there was a gap in data collection in early 2017). Coupled with the modelled relationship demonstrating decreasing mean abundance under hypoxic conditions, and the impact of critical dissolved oxygen events on prawn condition, these data suggest that anoxic/hypoxic water flowing from Stewarts River into western Watson-Taylor Lake (the main nursery area for School Prawn) is adversely affecting juvenile School Prawn.

As noted previously, Al concentrations measured in the estuary consistently exceeded the water quality guideline of 0.024 mg L^{-1} proposed in Golding et al. (2015). Considering the patterns outlined for the *Aluminium and pH exposure experiment* above, with average aluminium concentrations of 0.16 mg L^{-1} and a maximum value of 6.8 mg L^{-1} (Figure 3), it is unlikely that aluminium concentrations led directly to mortality of School Prawn in Camden Haven Estuary. However, chronic exposure to aluminium at these levels means that degradation of the gill surfaces may have occurred, which could further exacerbate the effect of hypoxia and lead to mortality. Aluminium is a known indicator of acid sulfate soils (ASS) in the catchment (Ryder et al., 2012, also see Fig. 1), and the correlation between aluminium and estuary inflow (Figure 3) suggests that run-off from the catchment adjacent to Watson-Taylor Lake carries the byproducts of ASS oxidation (aluminium and acidic water) into the main School Prawn nursery area. The decreased condition of School Prawn in Watson-Taylor Lake following estuary inflow is likely indicative of the sublethal effects of these catchment derived stressors.

Implications for prawn productivity

Links between catchment-derived stressors such as ASS and estuarine species have been proposed previously (Sammut et al., 1995), but there has been little attention given to the potential impact on exploited prawn stocks. Russell et al. (2011) indicated that remediation of ASS-affected tidal wetlands

in a north-eastern Australia led to improvements in Banana Prawn (*Penaeus merguiensis*) abundance, which suggests some impact of ASS on this species. Other than this, there are few examples that have investigated the potential impact of ASS on prawn populations. In pond aquaculture systems, the LC_{50} (lethal concentration) was pH ~4 for the Giant Freshwater Prawn *Macrobrachium rosenbergii*, but EC_{50} (half maximal effective concentration for reduced growth) was as high as pH 6.25 (Chen and Chen, 2003). Allan and Maguire (1992) suggested that the minimum acceptable pH for Black Tiger Prawn *Penaeus monodon* was ~6, and Russell (2017) showed reductions in School Prawn survival (from ~100 to 70%) following acute exposure to pH 5 at a salinity of 32. Furthermore, Kroon (2005) showed that School Prawn actively avoided acidic water in laboratory tests, although it is unclear whether this behaviour is possible under natural conditions. The impact of reduced pH on prawn somatic condition, survival and behaviour suggest that acidic water moving into the estuary following rainfall may be driving the negative relationship between estuary inflow and School Prawn harvest in the Camden Haven Estuary (Figure 26).

As outlined in the *Introduction*, multiple studies have demonstrated positive correlations between School Prawn productivity and freshwater discharge into estuaries (Glaister, 1978; Ruello, 1973b; Gillson et al., 2012; Ives et al., 2009), and prolonged dry weather normally adversely affects School Prawn and results in a smaller population and harvest (Ruello, 1973b). While estuary inflow is generally important for juvenile School Prawn, it is also important in the maturation, emigration and aggregation of sub-adults and adults (Glaister, 1978; Ruello, 1973b). Our data illustrate that despite some aggregative effect increasing abundance in the main nursery area, estuary inflow directly decreased prawn condition and the overall catchment runoff appeared to adversely impact School Prawn catch. Thus, it appears that catchment-derived stressors may be impacting spawning (through effects on adults prawns), recruitment processes, as well as juveniles in the nursery, with the overall impact of reducing productivity of the fishery when moderate to high rainfall levels occur in the catchment.

Project conclusions and recommendations

General conclusions

This study highlights the potential cumulative impacts of a complex array of habitat changes (physico-chemical and vegetated) and catchment-derived stressors, particularly low dissolved oxygen, runoff from ASS and elevated aluminium, on an important exploited penaeid species. The densities of School Prawn in some parts of the system were reasonably high, particularly in Watson-Taylor Lake, indicating that prawns were recruiting to the system during the study period. Extirpation of seagrass from the main nursery area (Watson-Taylor Lake) has potentially contributed to a productivity bottleneck, while anoxia and hypoxia in the nursery reduces survival and somatic condition, and stressors carried in catchment runoff affect the survival, behaviour, and catchability of sub-adults and adults. These factors likely contributed to the observed changes in fishery productivity within Camden Haven Estuary, however anecdotal reports from fishers have indicated similar patterns occurring for School Prawn in other floodplain estuaries in south-eastern Australia (M.D. Taylor, pers. comm.), and hypoxia remains an issue for penaeid fisheries in other parts of the world (e.g. Smith et al., 2014). The current study would have benefited from deployment of pH loggers to provide a continuous time-series of pH in the main juvenile nursery, however this represents a key area for future work and offers the potential to geographically pin-point the source of acidic water and prioritise areas of ASS-affected areas for remediation. This is discussed further in the following section.

Our findings have implications far broader that just School Prawn, with a broad spectrum of species in eastern Australia such as Barramundi *Lates calcarifer,* Banana prawn *Penaeus merguiensis,* and Mud Crab *Scylla serrata* positively linked with estuary inflow (e.g. Meynecke et al., 2006; Meynecke et al., 2010; Gillanders and Kingsford, 2002; Gillson et al., 2009; Staunton-Smith et al., 2004; Robins et al., 2005; Taylor et al., 2014). Further characterisation of direct relationships between catchment–derived stressors and fisheries productivity for additional species will clarify the scope of these issues. Ultimately, such research can also support the estimation of the economic costs of poor catchment and estuary health through reductions in fisheries harvest, and thus provide an economic impetus for remediation.

Acid sulfate soils adjacent to major tributaries in Watson-Taylor Lake

Once the above project findings were identified, further investigation and consultation was undertaken with local and regional land managers regarding these findings and the potential for ASS to impact the south-western part of the Camden Haven Estuary. Acid sulfate soil risk maps (Figure 1) indicate a high probability for ASS around Camden Haven River, and the lower reaches of Stewarts River, and these represent a potential source of stressors to the main nursery area. Some more specific areas of concern for acid sulfate soil are identified in Hastings Council (2002), however Tulau (1999) provides a comprehensive outline for ASS priority areas along the entire Hastings floodplain. This report identifies two priority areas in this region: 1) Rossglen ASS Priority Area; and 2) Stewarts River ASS Priority Area (Figure 27).

According to Tulau (1999), the Rossglen area (Figure 27) is a large backswamp ~350 ha in size separated from the Camden Haven River by a levee, with accompanying drainage works and floodgates. The area has soil profiles indicating pH values <3, and surface waters of pH 3-3.6. As seen in the inset image (Figure 27), the area is bisected by a major highway (Pacific Highway), and the area to the west of the highway was rehabilitated in 2002 including works on tidal gates, weirs and drains, as well as liming and revegetation (Hastings Council, 2006). The area to the east of the highway was not rehabilitated. Tulau (1999) also points out that stratification, poor mixing, and generally poor

water quality is pervasive in the Camden Haven River upstream of Watson-Taylor Lake, and it is likely that periodic expulsion of poor quality water from this tributary into the main nursery area of Watson-Taylor Lake contributes to the patterns observed. Tulau (1999) describes the Stewarts River ASS Priority Area (Figure 27) as ~45 ha in size, where small backswamp wetlands have been isolated from the river by low levees. Several drains transect the area, and pH values in these drains are regularly <3. Some minor rehabilitation works have been undertaken to the west of this area.

With respect to ASS, while the data collected here point to a potential problem, they are not yet sufficient to provide specific recommendations regarding targeted remediation strategies. This is principally due to two factors. Firstly, while both the Al spikes in estuary water following flow events, and the negative relationship between flow and School Prawn catch provide a "smoking gun" which implicates ASS as a potential source for the catchment-derived stressors, no suitable time series of pH data is available to confirm this, either from the current study or previous studies (e.g. Ryder et al., 2012; and comments in Snowy Mountains Engineering Corporation, 1998). Secondly, if ASS are a significant source of stressors to the estuary, the extensive ASS risk across the western catchment suggests that more fine-scale information is likely required to pin-point exactly which parts of the catchment contribute most to the issue and thus propose targeted remediation of these specific locations.

Following the results of this project, data collected to date, and consultation undertaken, the key recommended actions (in sequential order) are as follows:

1. Generate a robust time series of pH concentrations around western Watson-Taylor Lake and identified ASS priority areas (and associated drains), and relate this to co-located conductivity data and estuary inflow data (https://realtimedata.waternsw.com.au/water.stm) – **CURRENTLY UNDERWAY;**
2. Use data generated in 1. to pin-point potential ASS areas for remediation in the Camden Haven floodplain;
3. Undertake remediation of identified areas through regional council's (through their Coastal Management Plans) and the NSW Government (through the Marine Estate Management Strategy);
4. Continue collection of targeted post-remediation water quality time series data, and commercial catch and effort data, to evaluate the impact of remediation efforts;
5. Communicate outcomes of remediation to industry and other stakeholders;

Two recent developments make further quantification of this issue a priority. Firstly, recent reform of marine and coastal management in NSW has led to the *NSW Coastal Management Act 2016* and the *Marine Estate Management Act 2014*. The reform program associated with these new pieces of legislation has underpinned the development and funding of management strategies and management plans for the coast and estuaries throughout NSW, creating an opportunity to action the recommendations above and implement suitable on-ground remediation programs in response to the threats identified in this project. Secondly, 2016/17 was the lowest NSW School Prawn harvest (Taylor et al., 2019) since 1993/94 and the second lowest on record. Preliminary stock assessment modelling indicates that fishing effort and harvest are probably within acceptable limit reference points, which suggests that environmental factors are potentially responsible. Further assessment of this issue is required to establish whether the relationships observed for Camden Haven Estuary represent a pervasive issue that is affecting the productivity of School Prawn (and other) fisheries across northern NSW.

New, low cost pH logger units were recently released (in 2018) by Onset Hobo (MX2501, Onset Corporation, MA, USA). In light of the factors above, two of these units have been purchased and deployed in the Camden Haven Estuary, to further characterise the potential pH issue in relation to the ASS priority areas discussed above. The initial deployment of these units occurred at two locations in Camden Haven River in 21 December 2018 (Figure 28), and this work will continue over the coming season. Alongside the actions recommended above, loggers should be deployed at strategically

selected locations (i.e. surrounding high probability ASS areas) in estuaries supporting more significant School Prawn populations (e.g. Clarence River, Richmond River).

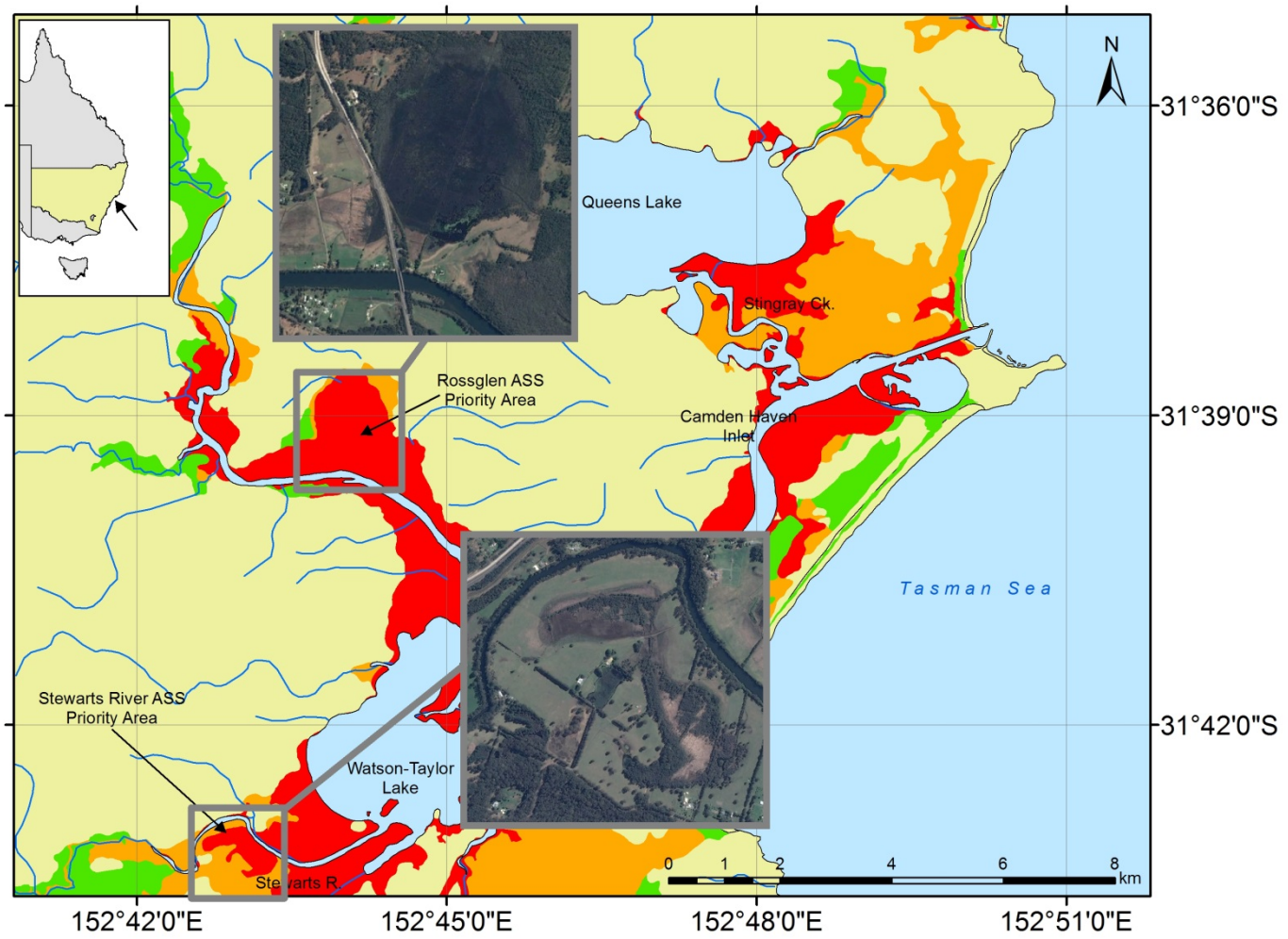

Figure 27 Map of Camden Haven Estuary showing acid sulfate soil probability (red is high probability), and indicating both Rossglen (upper inset image, adjacent to the Camden Haven River) and Stewarts River (lower inset image) ASS Priority Areas from Tulau (1999). The location of the study estuary on the eastern Australian seaboard is indicated in the upper left panel.

Figure 28 Onset Hobo MX2501 data logger (left panel), and a mooring station including an MX2501 co-located with an Odyssey conductivity/temperature logger deployed in Camden Haven River downstream of the Rossglen ASS Priority Area (right panel).

Extension and Adoption

Following the project's commencement, letters were sent to the 306 fishers and 36 oyster-growers recorded as having potential access to the Camden Haven Estuary fishery. The letters provided detail about the project's purpose and methods, as well as outlining ways to get more information and provide further input.

Communication and Extension outputs

Face-to-face meetings and workshops

Several face-to-face meetings were conducted throughout the project, as well as regular communication with the Professional Fisherman's Association and local fisher representative Mr Ross Dobson. Formal project meetings/workshops were conducted during the course of this project. These face-to-face presentations (by the Principal Investigator) provided an opportunity to maintain relationships with management representatives and stakeholders, to discuss project outcomes, provide information on the project's progress, and discuss results:

1. Wallis Lake Estuary Processes and Commercial Seafood Production Meeting, February 2016;
2. Port Macquarie-Hastings Council Coast, Estuary and Floodplain Advisory Sub-Committee, July 2018;
3. Professional Fisherman's Association Annual General Meeting, September 2018;
4. Wallis Lake Estuary Processes and Commercial Seafood Production Meeting, September 2018;

Presentations throughout the project included:

1. McLuckie, C., Moltschaniwskyj, N., Gaston, T., Dunstan, H., Crompton, M., and Taylor, M. D. (2018) Lethal and sub lethal effects of simultaneous exposure to aluminium and hypoxia on juvenile Eastern School Prawn. *Estuarine Coastal and Shelf Association 57: Changing estuaries, coasts and shelf systems*. Perth, Western Australia.
2. Taylor, M. D. (2016) Catchment effects on School Prawn productivity: Camden Haven case study. *Wallis Lake Estuary Processes and Commercial Seafood Production Meeting*. Forster, NSW.
3. Taylor, M. D., McLuckie, C., Russell, A., Moltschaniwskyj, N., Loneragan, N., Dunstan, H., and Macfarlane, G. (2018a) Catchment stressors and fisheries productivity: A tale of the humble school prawn. *Estuarine Coastal and Shelf Association 57: Changing estuaries, coasts and shelf systems*. Perth, Western Australia.
4. Taylor, M. D., McLuckie, M., Russell, A., Moltschaniwskyj, N., Loneragan, N., Dunstan, H., and Macfarlane, G. (2018b) Factors affecting School Prawn productivity: Camden Haven case study. *Port Macquarie-Hastings Council, Coast, Estuary and Floodplain Advisory Sub-Committee*. Port Macquarie, NSW.

5. Taylor, M. D., McLuckie, M., Russell, A., Moltschaniwskyj, N., Loneragan, N., Dunstan, H., and Macfarlane, G. (2018c) Factors affecting School Prawn productivity: Camden Haven case study. *Professional Fisherman's Association Annual General Meeting*. Coffs Harbour, NSW.

6. Taylor, M. D., McLuckie, M., Russell, A., Moltschaniwskyj, N., Loneragan, N., Dunstan, H., and Macfarlane, G. (2018d) Factors affecting School Prawn productivity: Camden Haven case study. *Wallis Lake Estuary Processes and Commercial Seafood Production Meeting*. Forster, NSW.

Dedicated project website

A dedicated project website was produced and provided a portal for project updates with results and key findings:

http://www.dpi.nsw.gov.au/fishing/habitat/publications/pubs/camden-haven-school-prawn-research-project

The website provided:

- detailed background to the project, including aims and objectives
- acknowledgement of funding contributors and project partners
- identification of project staff and their contact details
- access to other resources relevant to school prawn research and information
- a 'useful links' page

Other Publications and products

Several other publications and/or communications have been prepared throughout the project in order to publish preliminary findings, provide research updates, build capacity in the wider community for the project, and maintain communication networks with project partners. Other publications included:

1. Russell, A. (2017) Lethal and sublethal effects of aluminium to juvenile School Prawn (*Metapenaeus macleayi*), Honours Thesis, University of Newcastle, 72 p.
2. Taylor, M.D. (2018) Camden Haven School Prawn study comes to an end. *Professional Fishermen's Association Newsletter*, 4 p.
3. Taylor, M.D., N.A. Moltschaniwskyj, M.J. Crompton, and R.H. Dunstan (2018) Environmentally-driven changes in fatty acid profiles of a commercially important penaeid prawn. *Estuaries and Coasts*, **42**, 528–536, https://doi.org/10.1007/s12237-018-0461-0;
4. Russell, A., G. MacFarlane, B. Nowak, N.A. Moltschaniwskyj, and M.D. Taylor (201X) Lethal and sublethal effects of aluminium on juvenile School Prawn (*Metapenaeus macleayi*). *Thalassas: An International Journal of Marine Science*, **35**: 359-368, https://doi.org/10.1007/s41208-019-00152-4;
5. Taylor, M.D., and N.R. Loneragan (2019) Catchment-derived stressors, recruitment, and fisheries productivity in an exploited penaeid shrimp. *Regional Studies in Marine Science*, **29**, 100628, doi.org/10.1016/j.rsma.2019.100628
6. McLuckie, C., N.A. Moltschaniwskyj, T. Gaston, R.H. Dunstan, M.J. Crompton, and M.D. Taylor (in press) Lethal and sublethal effects of simultaneous exposure to aluminium and hypoxia on juvenile Eastern School Prawn. *Marine and Freshwater Research*, https://doi.org/10.1071/MF18487;
7. McLuckie, C., N.A. Moltschaniwskyj, T. Gaston, R.H. Dunstan, M.J. Crompton, and M.D. Taylor (in review) Survival, growth, and metabolic changes in School Prawn (*Metapenaeus macleay*i) across a spectrum of salinities. *Thalassas: An International Journal of Marine Science*

Please note that much of the information contained in this report will appear in the PhD Thesis of Catherine McLuckie, University of Newcastle, and associated publications.

Project coverage

At the project's inception (September 2015) a media release was developed and provided to a number of news outlets and posted to social media to engage and inform the wider community of the project. The news release was picked up by the outlets listed in Table 7. This media release also led to several radio interviews with the Principal Investigator during the first two weeks of October 2015. One interview ran on Southern Cross Austereo and the other interview was played on both the ABC Rural Report and on ABC Local Radio in Canberra.

Table 7 Summary of uptake of project media release.

Publication	Source	Distribution
DPI media release	http://www.dpi.nsw.gov.au/about-us/media-centre/releases/2015/camden-haven-prawn-study	N/A
Camden Haven Courier	http://www.camdencourier.com.au/story/3405146/where-have-all-the-prawns-gone/	12,495
Northern Star (Lismore)	http://www.northernstar.com.au/news/study-hopes-solve-riddle-falling-prawn-numbers/2809302/	38,000
University of Newcastle - Environmental Science and Management blog	https://uonblogs.newcastle.edu.au/esm/2015/10/22/camden-haven-research-project-to-investigate-school-prawn-productivity/	N/A

Project materials developed

Project materials developed are described in detail in the Extension and Adoption Section (above).

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Appendix 1 – Intellectual Property

Manuscripts outlined above under *Extension and Adoption* are in various stages of submission and publication. These outputs should be cited against the specific components of this work.

No commercially exploitable intellectual property has been generated. Data developed through this project can be accessed through the NSW Department of Primary Industries Information Asset Register.

The Doctoral candidate working on this project, Ms Catherine McLuckie, has provided much of the information contained in this report. As a consequence much of the text presented here is replicated from the PhD Thesis of Ms McLuckie, which will soon be submitted for examination through the University of Newcastle (and associated publications). Ms McLuckie retains ownership of the Intellectual Property associated with these experiments, their write-up, and their interpretation.

Appendix 2 – Project Staff

- Prof Matthew D. Taylor, Principal Research Scientist, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries; and Conjoint Professor, University of Newcastle
- Prof Natalie Moltschaniwskyj, Director – Fisheries Research, New South Wales Department of Primary Industries; and Conjoint Professor, University of Newcastle
- Ms Catherine McLuckie, PhD Candidate, University of Newcastle
- Ms Angela Russell, BSc (Honours) Candidate, University of Newcastle
- Prof R. Hugh Dunstan, Professor, University of Newcastle
- Prof Neil Loneragan, Harry Butler Institute, School of Veterinary and Life Sciences, Murdoch University
- Dr Geoffrey MacFarlane, Senior Lecturer, University of Newcastle
- Mr Marcus Crompton, Research Associate, University of Newcastle
- Dr Troy Gaston, Senior Lecturer, University of Newcastle
- Mr Brad Leach, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Mr Matt Harrison, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Mr Tristan New, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Mr Mitch Burns, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Mr James McLeod, Technical Coordinator, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Ms Emma Mitchell, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Ms Hannah Whitney, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Ms Isabelle Thiebaud, Fisheries Technician, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Ms Kylie Russell, Fisheries Manager – Aquatic Habitat Rehabilitation, Port Stephens Fisheries Institute, New South Wales Department of Primary Industries
- Ms Charlotte Jenkins, Fisheries Manager – Aquatic Habitat Rehabilitation, Wollongbar Agricultural Institute, New South Wales Department of Primary Industries

FRDC FINAL REPORT CHECKLIST

Project Title:	Catchment-derived stressors and School Prawn productivity		
Principal Investigators:	Matthew D. Taylor, Catherine McLuckie, Angela Russell, R. Hugh Dunstan, Natalie A. Moltschaniwskyj, Geoffrey MacFarlane, Marcus Crompton, Neil R. Loneragan		
Project Number:	2014/011		
Description:	New South Wales Department of Primary Industries (NSW DPI) presents new information exploring the effect of catchment-derived stressors on School Prawn. Declines in School Prawn productivity over decadal time scales have been reported anecdotally across many estuaries in New South Wales, and are evident in the catch statistics in some locations. This has included reports that indicate that prawn landings have become decoupled from freshwater flows, which generally enhance catches in estuarine and inshore fisheries. To date, no research has been conducted into the direct effects of environmental conditions within nursery habitats that may be contributing to these changes in productivity. This project commenced this investigation using the Camden Haven estuary as a case study, and through a combination of high-resolution logger data, aquarium experiments, habitat mapping, extensive field sampling, and analysis of commercial catch statistics, provide evidence to link catchment-derived stressors with changes in productivity of School Prawn. We use this evidence to propose recommendations for targeted repair in the Camden Haven estuary catchment, as well as other New South Wales estuaries supporting School Prawn harvest.		
Published Date:	NA	**Year:**	2019
ISBN:	NA	**ISSN:**	NA
Key Words:	Penaeidae; School Prawn; Acid-sulphate soils; aluminium; hypoxia; somatic condition; growth; survival		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)	N	
Acknowledgments	Y	
Abbreviations	N	Not required
Executive Summary	Y	
– **What the report is about**		
– **Background – why project was undertaken**		
– **Aims/objectives – what you wanted to achieve at the beginning**		
– **Methodology – outline how you did the project**		
– **Results/key findings – this should outline what you found or key results**		
– **Implications for relevant stakeholders**		
– **Recommendations**		
Introduction	Y	
Objectives	Y	
Methodology	Y	
Results	Y	
Discussion	Y	

Conclusion	Incorporated in a single section	
Implications		
Recommendations		
Further development		
Extension and Adoption	Y	
Project coverage	N	Covered in E&A
Glossary	N	Not required
Project materials developed	Y	
Appendices	Y	