



# Dioxins in fish and prawns in Homebush Bay and Parramatta River, Australia

## Results from the Phase 2 study

Prepared by the New South Wales Office of Environment and Heritage, Environment Protection Science Branch and NSW Department of Primary Industries - Fisheries

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## Executive summary

Homebush Bay has an extensive history of industrial activities, including the production of chlorinated pesticides. This has resulted in the sediments in Homebush Bay being contaminated with polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs). In 2005/06, prior to the remediation of the area, a sampling program (referred to as the Phase 1 study) was undertaken to measure concentrations of dioxins (PCDDs, PCDFs and dl-PCBs) in fish (Yellowfin Bream and Sea Mullet) and prawns (School Prawn and King Prawn). The study focussed on four zones in the Parramatta River: Upper Parramatta River, Homebush Bay, Middle Parramatta River and Lower Parramatta River. The results showed that concentrations of dioxins were above a level considered safe for human consumption of 6 pg TEQ/g (pg dioxin toxicity equivalence per g).

During 2015/16, a follow-up study (referred to as the Phase 2 study) was conducted using the same experimental design, to determine if concentrations of dioxins in the same species had decreased following the remediation of Homebush Bay. When the results from the two phases of the study were compared, there was no overall change in the concentrations of dioxins in both of the fish species (Yellowfin Bream and Sea Mullet). This may be due to the presence of residual contamination in Homebush Bay/Parramatta River or indicate that insufficient time had passed between the two phases of the study to result in measurable changes for these species. The results also showed that the dioxin concentrations in fish and prawns from Homebush Bay were either equal to or higher than all the other zones.

In contrast to the fish, the dioxin concentrations in both of the prawn species showed a decrease when data were considered on a fresh weight basis. For the prawns, however, the lipid content in the Phase 2 study was considerably lower than in Phase 1. As dioxins are known to accumulate in the lipid of organisms, it was assumed that the decrease in lipid content may have a limiting effect on the capacity of the prawns to accumulate dioxins. Due to this, the dioxin concentrations on a lipid normalised basis were also considered. When the lipid normalised data were compared, overall there was no change in the concentration between the two phases of the project. This result suggests that the decrease in dioxin concentration on a fresh weight basis may have been driven by the observed drop in lipid content in the Phase 2 study. In future, if the lipid content of the organisms increases, for example in a change of season, the overall fresh weight concentration may also increase as their capacity to accumulate dioxins will increase.

Dioxin concentrations in all of the fish samples analysed during Phase 2 were considerably higher than the limit for human consumption of 6 pg TEQ/g. In contrast, all of the prawn samples were marginally lower than this limit, with the exception of School Prawn from Homebush Bay. It should be noted that decisions relating to the suitability of fish and prawns for human consumption were outside the scope of this project and it is recommended that the current commercial fishing closure and recreational fishing advisories in the area remain.

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# 1 Project background

Homebush Bay was used as a site for many industrial activities until the 1990s, including the state brickworks, an abattoir, a naval armaments depot and the manufacture of pesticides (e.g. 2,4-D, 2,4,5-T and DDT) and other chemicals such as paints, plastics and industrial alcohols. As a result of these activities, the sediments within Homebush Bay became considerably contaminated with a range of chemicals, including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) (Roach, 2009). The presence of dioxins was primarily linked to the production of chlorinated pesticides, which can contain PCDDs, PCDFs and dl-PCBs, at the former Union Carbide site (Figure 1). The remediation of the site was funded by the State Government and included the removal of about 8 ha of sediment to a depth of about 0.5 m, which equated to about 10% of the sediments in Homebush Bay (Figure 1). The remediation work commenced in 2006 and was completed in 2010 with the aim of removing the most contaminated area along the shore line to allow the area to be redeveloped for residential use.

Prior to the commencement of the Homebush Bay remediation work, in 2005-2006 a fish and prawn sampling program was undertaken within Homebush Bay and the Parramatta River to measure dioxin concentrations in the tissue of fish (Yellowfin Bream and Sea Mullet) and prawns (School Prawn and King Prawn). These species were chosen as they represent organisms at different trophic levels with different feeding habits. The results from this study (referred to as the Phase 1 study) showed concentrations of total dioxins (PCDDs, PCDFs and dl-PCBs) that were above acceptable levels and therefore considered not safe for human consumption (FSANZ, 2006). This study triggered a more extensive investigation into dioxin concentrations in seafood throughout Parramatta River and Sydney Harbour and as a result of these studies, a ban on commercial fishing was implemented in the area and an advisory was implemented for recreational fishers that no fish or prawns caught west of the Harbour Bridge should be consumed.

This report presents a follow-up study (referred to as the Phase 2 study) that used the same sampling design, species and sample preparation as the Phase 1 study. The objective was to determine if there had been any change in dioxin concentrations in fish and prawn tissue from Homebush Bay and the Parramatta River following remediation. Completion of the Phase 2 study was a requirement of the Environmental Management Plan (EMP) (Thiess, 2013) for the Homebush Bay site. The timing of the Phase 2 study was planned to allow new cohorts of the four species to be established following remediation of Homebush Bay (5-6 years).

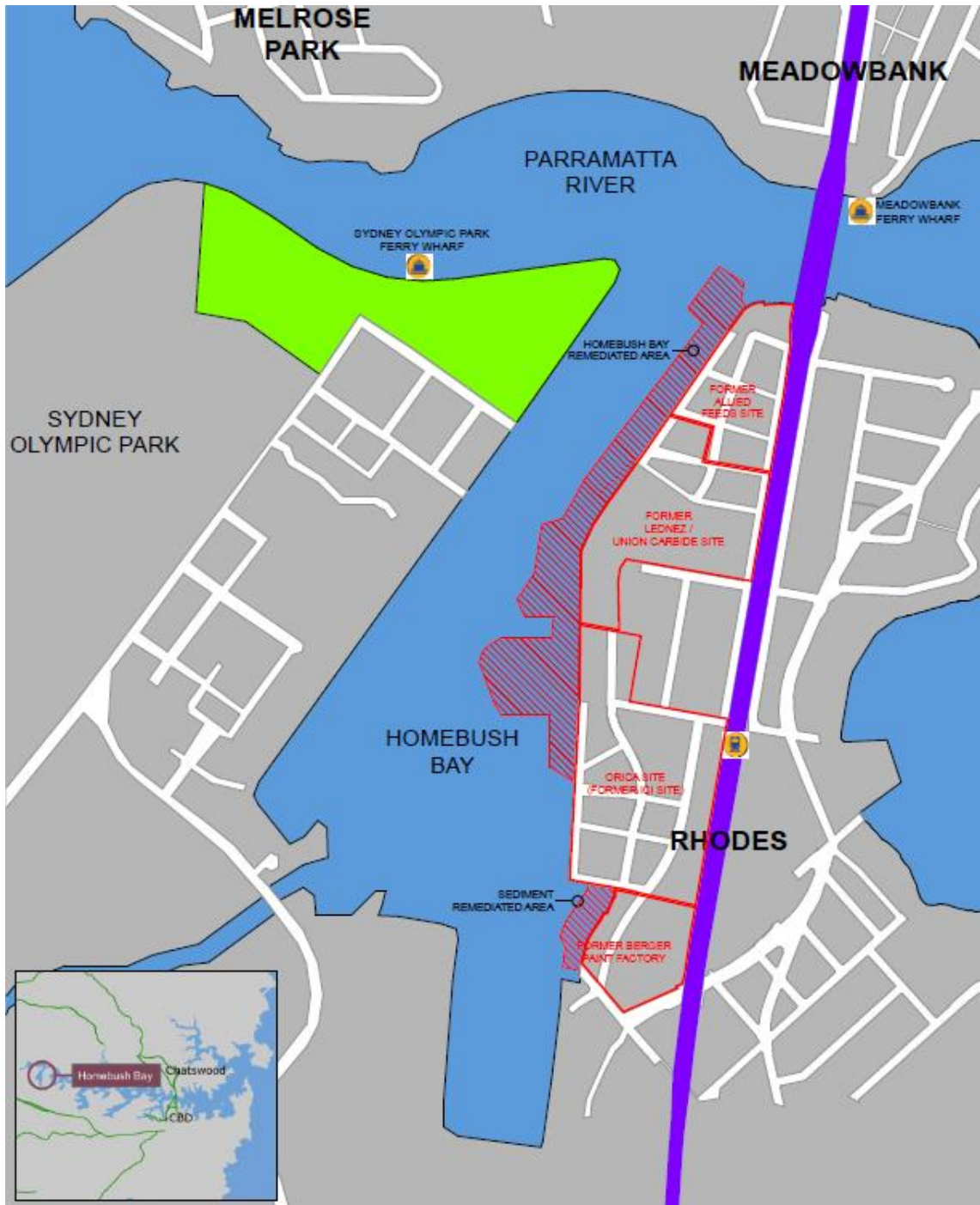


Figure 1: Diagram of Homebush Bay and the former industrial sites. The red dashed area in the Bay indicates the locations that were remediated from 2006 to 2010. This diagram was sourced from the Environmental Management Plan for the site prepared by Thiess (Thiess, 2013).

## 1.1 Dioxins and dioxin toxicity

PCDDs and PCDFs are two groups of planar tricyclic compounds that have very similar structures and properties (Figure 2). They are mainly formed as by-products during the industrial production of other chemicals. They can contain between one and eight chlorine atoms which results in a total of 75 possible congeners of PCDDs and 135 possible congeners of PCDFs. Of these there are seven toxic PCDD and ten toxic PCDF isomers. The most toxic compound of these is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

In contrast to PCDD and PCDF, which are mainly formed as by-products, PCBs have been produced historically for their use in a range of products. Their production and use has largely been phased out due to concerns with persistence and toxicity. There are 209 PCB congeners possible depending on the number and location of chlorine atoms on the structure (Figure 2). Twelve of these congeners are coplanar and therefore can exert toxicity in a similar way to the PCDD and PCDF compounds. Because of this, these 12 PCBs are referred to as dioxin-like (dl-PCBs).

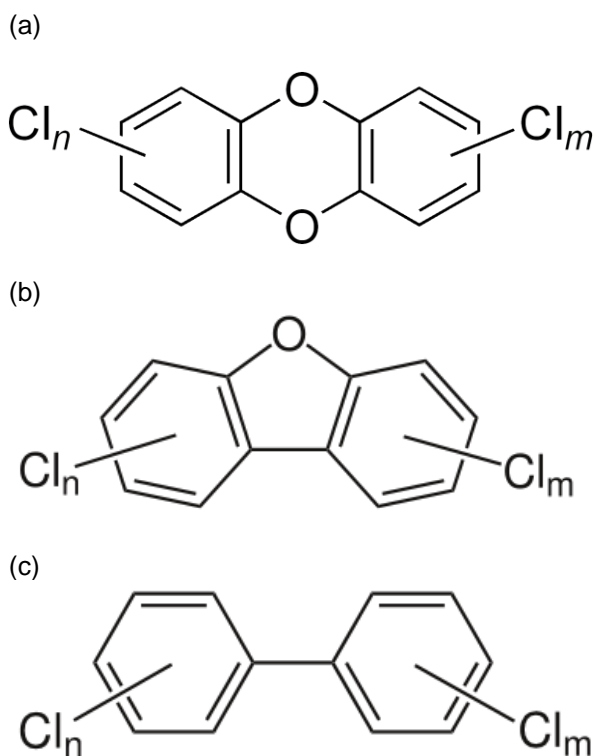


Figure 2: General chemical structure of (a) polychlorinated dibenzo-p-dioxins (PCDDs), (b) polychlorinated dibenzofurans (PCDFs) and (c) dioxin-like polychlorinated biphenyls (dl-PCBs).

Dioxins and dioxin-like compounds are highly persistent in the environment. The half-life of TCDD in sediments has been estimated to be 2-10 years (Chiao et al., 1994). They are also hydrophobic in nature, which means that they partition strongly to solid phases, e.g. soils and sediments. In organisms they accumulate in lipids. They are highly resistant to metabolism and as a result, biomagnification occurs through the food chain. Human exposure is primarily via food but exposure can also occur via incidental ingestion of particles of soil, dust and smoke. Effects that have been observed in humans include systemic, immunological, neurological, developmental, reproductive, genotoxic and carcinogenic effects (Watanabe et al., 1999).

As humans are exposed to a complex mixture of these compounds and they all exert toxicity through the same mode of action, the toxicity equivalence (TEQ) approach is used to summarise the overall concentration of dioxins and dioxin-like compounds (Van den Berg et al., 2006). The total dioxin TEQ for a sample can be calculated using the following equation:

$$TEQ = \sum_{i=1}^n (C_i \times TEF_i)$$

Where  $C_i$  is the concentration of an individual dioxin or dioxin like compound and  $TEF_i$  is the toxicity equivalence factor for that compound. Each of the dioxins or dioxin like compounds have a corresponding TEF value that represents its toxicity relative to TCDD, which is the most toxic of these compounds. The TEF values for the PCDD/PCDF compounds range from 0.0003 to 1.0 and the TEF values for dl-PCBs range from 0.00003 to 0.1 (Van den Berg et al., 2006). TEQ concentrations can be calculated on a total basis (including all PCDDs, PCDFs and dl-PCBs) or independently for each of these groups of compounds. In this report, TEQ refers to the total dioxin TEQ (i.e. all groups of compounds) unless otherwise stated.

The accumulation of dioxins and dioxin-like compounds in seafood is a major pathway for human exposure. Diet, lipid content and species traits are major factors affecting the accumulation of these compounds in seafood (Wan et al., 2010; Ssebugere et al., 2014). Due to the presence of dioxins in seafood, threshold concentrations have been developed throughout the world. Following Phase 1 of the fish and prawn sampling, a human health risk assessment was undertaken to identify risk to the public from consuming fish and seafood from Sydney Harbour and the Parramatta River (FSANZ, 2006). As part of this assessment, a concentration in fish and seafood that was considered to be acceptable and pose a low risk to human health of 6 pg TEQ/g was derived. This value was derived using information on the consumption of seafood as a component of the Australian diet.



## 2 Overview of the Phase 1 study

During 2005/06, prior to the remediation of Homebush Bay, the concentrations of dioxins (PCDD, PCDF and dl-PCBs) in two fish species (Yellowfin Bream (*Acanthopagrus australis*) and Sea Mullet (*Mugil cephalus*)) and two prawn species (School Prawn (*Metapenaeus macleayi*) and King Prawn (*Peneaus plebejus*)) were measured. Fish and prawns were collected from four zones along the Parramatta River. The target was to analyse dioxins in five composites of muscle tissue per species per zone. The zones are presented in Figure 3 and are summarised below:

- Upper Parramatta River (UPR) (near Silverwater)
- Homebush Bay (HB)
- Middle Parramatta River (MPR) (near Breakfast Point)
- Lower Parramatta River (LPR) (near Drummoyne).

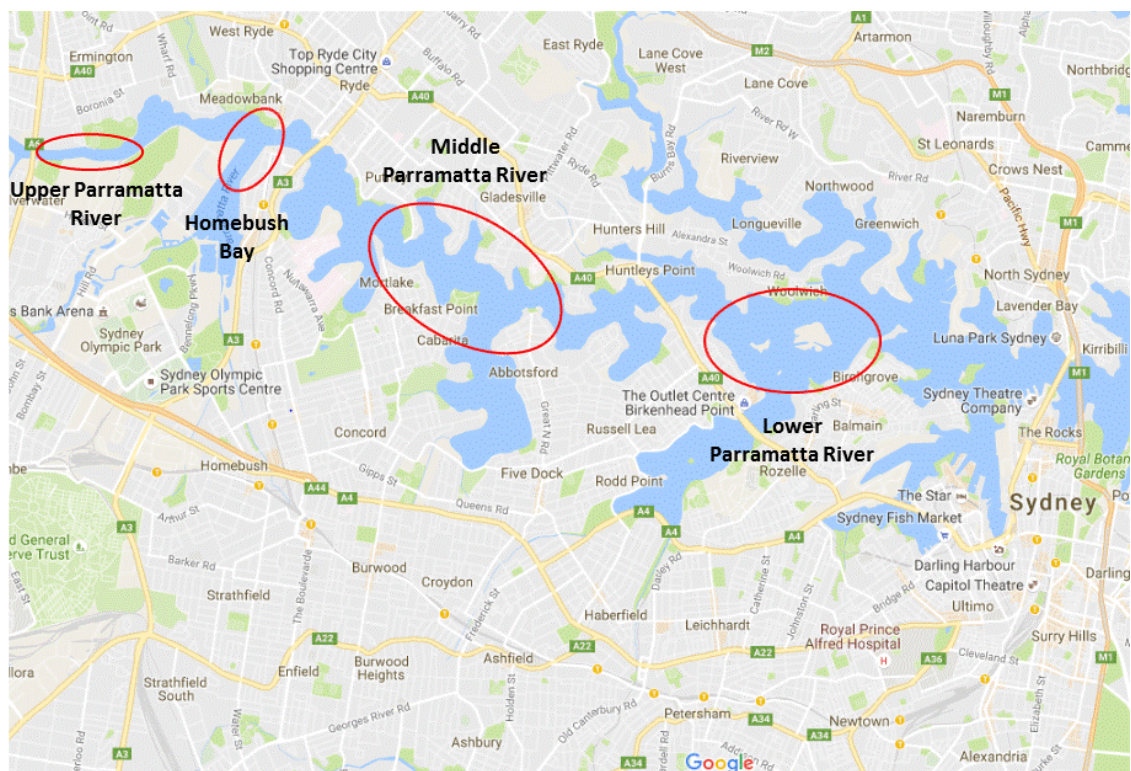


Figure 3: Fish and prawn sampling zones for the Phase 1 and Phase 2 study

Sea Mullet had the highest concentrations of dioxins (on a fresh weight (fw) basis), ranging from 57 – 135 pg TEQ/g fw (Figure 4). This was likely due to the high lipid content of these fish, meaning they have a greater capacity to accumulate these compounds. The lowest concentrations were found in the prawns, with little difference between the two species (Figure 4). It should be noted that the number of School and King Prawn caught in each zone was not sufficient to prepare the targeted number of samples and in UPR insufficient King Prawn were collected to generate any data. The Yellowfin Bream data showed the greatest variability in dioxin concentration between zones, with significantly higher concentrations found in fish caught in HB compared to the other zones (Figure 4). The concentrations of dioxins in all of the species sampled during the Phase 1 study exceeded the threshold limit for the protection of human health (6 pg TEQ/g) (Figure 4).

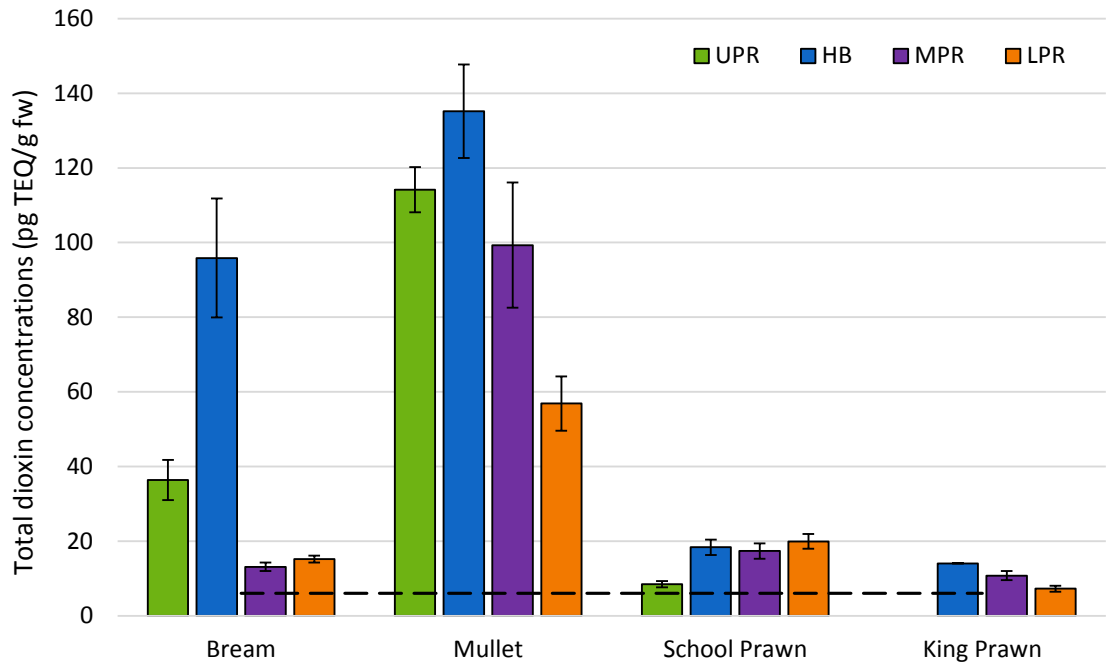


Figure 4: Concentrations of dioxins in fish and prawns sampled during the Phase 1 study in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR). The dashed line represents the threshold limit of 6 pg TEQ/g for human consumption (FSANZ, 2006) and the error bars indicate  $\pm 1$  standard error. No standard error shown for King Prawn in HB as  $n=1$  and insufficient King Prawn were caught from UPR for analysis.

## 3 Methodology for the Phase 2 study

### 3.1 Sample collection

In Phase 2, the same zones and methods were used as outlined for Phase 1 (Figure 3). Sampling times were selected to be consistent with the sampling times used during Phase 1, which ensured that differences due to seasonal variations were minimised. The sampling for Yellowfin Bream took place between 19 and 28 October 2015, the sampling for Sea Mullet took place between 11 and 30 March 2016 and the sampling for both species of prawns took place between 8 and 29 March 2016.

The fish sampling (Yellowfin Bream and Sea Mullet) was undertaken using standard mesh nets set within each of the sampling zones. Each net was deployed for a maximum of three hours before retrieval. To induce fish movement and contact with the net, a splashing method was used, whereby the boat ran up and down the length of the net while staff slapped the surface of the water. After retrieval of the mesh net, the fish were sorted to identify the required species, which were then placed immediately on ice.

The prawns were sampled using an estuary prawn trawler and standard estuary prawn trawl nets. The tow length of the trawl net differed between tows depending on the location, the presence of barriers to trawling (e.g. ferries, submarine cables, moored vessels etc.), channel width, presence of mud and presence of jelly fish. Following each tow, the nets were retrieved and the contents emptied onto sorting trays. The catch was then sorted into species and the School Prawn and King Prawn were set aside until an approximate weight of 2 kg per species was obtained or all individuals were sorted. Samples were then placed into clearly labelled bags and immediately placed on ice.

All samples were delivered to the National Measurement Institute (NMI), Sydney, immediately after disembarking from the boat (or at 9 am if the boat was disembarked prior to this time) for subsequent preparation and analysis.

As a control, samples of all species were also collected from areas in NSW that did not have a history of industrial inputs. These control samples were collected to give an indication of background dioxin concentrations in the same species. The control samples for the Yellowfin Bream were collected from Wallis Lake, which is located approximately 300 km north of Sydney. The control samples for Sea Mullet, School Prawn and King Prawn were collected from the Clarence River, which is located approximately 650 km north of Sydney.

### 3.2 Sample preparation and analysis

The fish (Yellowfin Bream and Sea Mullet) were scaled and filleted prior to compositing and homogenising the samples. The skin was left on the fillets as this was consistent with the methodology used for the Phase 1 study. This approach was considered to represent the worst-case scenario due to the high lipid content associated with the skin. For each zone, five composite samples were prepared per species that each consisted of fillets from ten individuals. The fish were randomly allocated to composites and the length and weight of all fish in each composite were recorded.

To prepare the prawns for analysis, their heads were removed and then they were shelled and deveined (i.e. the digestive tract was removed) prior to compositing and homogenising. Five composites were prepared for each zone with the composites containing enough individuals to generate a sufficient amount of tissue for analysis (approximately 200-300 g). The carapace length of each prawn in the composite was measured. This information was recorded as total counts in different carapace size ranges. King Prawn could only be caught in one zone (LPR), therefore, five composites were prepared for that zone but no composites for the other zones.

Identical preparation methods were used for the control composite samples, from Wallis Lake and Clarence River. For these samples however, the aim was to prepare two composite samples per species instead of five composite samples as employed for the Parramatta River samples.

Sample preparation and analysis was conducted based on US EPA 1613B (US EPA, 1994). In brief, the prepared samples were freeze dried and spiked with a range of isotopically labelled surrogate standards. The samples were exhaustively extracted with an organic solvent and then a clean-up was undertaken by partitioning with sulfuric acid followed by distilled water. The samples were further purified using column chromatography on acid- and base-modified silica gels, basic alumina and carbon dispersed on celite.

The samples were analysed using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) after addition of internal standards. This method determines PCDDs, PCDFs and dl-PCBs. Results were provided from NMI for individual congeners as well as total dioxin and furan TEQs (TEQ<sub>DF</sub>) and total dl-PCB TEQs (TEQ<sub>P</sub>) on a fresh weight basis. The overall total dioxin TEQs (PCDDs, PCDFs and dl-PCBs) were then calculated as the sum of the TEQ<sub>DF</sub> and TEQ<sub>P</sub>. In cases where concentrations of individual congeners were below the limit of reporting (LOR), the LOR was used in the TEQ calculation. This approach represents the upper limit of the TEQ and is a conservative estimate. In addition to the dioxin concentrations, NMI provided the lipid content for each of the composite samples and lipid normalised TEQs. Lipid normalised TEQs are calculated by dividing the concentration on a fresh weight basis by the proportion of lipid.

### 3.3 Data analysis and interpretation

The physiology of the fish and prawns were initially considered (lengths, weights, carapace lengths and lipid content) to compare these parameters between the Phase 1 and Phase 2 studies. This was done using an analysis of variance (ANOVA) to test only for a main effect of '*time*' (Phase 1 and Phase 2) for lengths, weights and lipid content. This identified if there were significant differences between the two phases irrespective of zone. Carapace lengths were visually compared using frequency plots.

Total dioxin concentrations (as TEQs) were compared using a two-way ANOVA for each species to determine if there were significant differences between the sampling times (Phase 1 and Phase 2) and zones using the statistical software R (R Core Team, 2016). The ANOVA was done by specifying the main effects of '*time*' and '*zone*' and an interaction term of '*time by zone*' to generate p-values. Differences were considered statistically significant when  $p < 0.05$ . The term significant is only used throughout this report when the difference has been statistically tested.

Prior to using ANOVA, the dioxin concentrations were log-transformed so that the data approximated normality and homogeneity of variance. ANOVA models were validated by examining residual diagnostic plots to ensure that the assumptions of the ANOVA (normality and homogeneity of variance) were met. In all cases, the interaction of time by zone was significant. As a result, the differences were investigated further via *post hoc* multiple comparisons. These were pairwise comparisons of sampling times within each zone and zones within each sampling time. The p-values from these individual tests were corrected for multiple comparisons using the Holm procedure (Neter et al., 1996) to minimise the increased risk of Type I errors. Due to a limited number of King Prawn samples, this statistical approach could not be used for this species. The approach used for King Prawn is outlined in Section 4.4.

Dioxin concentrations in all species were presented on a fresh weight basis as this is considered the most relevant measure for human consumption and consistent with the units used by FSANZ in determining a limit safe for human consumption. The sample lipid content was considered to have a major influence on the accumulation of dioxins in fish and prawns. Therefore, when the lipid content decreased from Phase 1 to Phase 2, lipid normalised data were also compared. This was done to assist in the interpretation of the data, based on the rationale that a decrease in lipid content was likely to be a rate limiting step in accumulation of dioxins. That is, with lower lipid content there is less capacity for the organisms to accumulate dioxins, which may affect the interpretation of the fresh weight TEQ data. In contrast, if the lipid content increased in the Phase 2 study compared to the Phase 1 study, the lipid content was not considered to be limiting, therefore the lipid normalised data were not considered.

The contribution of individual groups of dioxins (PCDDs, PCDFs, PCBs and TCDD) to the total dioxin concentrations were also considered as part of the data interpretation. For all species sampled, the pattern and trend of these results were the same as the total dioxin concentrations. Based on this, the results from these groups of compounds are not presented here.

Fresh weight dioxin concentrations (pg TEQ/g fw) were compared to the FSANZ threshold limit (6 pg TEQ/g). This was done to put the concentrations in fish and prawns into context, however it should be noted that decisions relating to the suitability of fish and prawns for human consumption was outside the scope of this project.

## 4 Comparison of the Phase 1 and Phase 2 results

### 4.1 Yellowfin Bream

Yellowfin Bream generally have discrete home ranges to which they display a relatively high level of attachment. They can however undertake large migrations down the estuary, either in response to adverse physicochemical conditions or to spawn, but often return to the same areas from which they left (Pollock, 1982; Payne et al., 2013). They are carnivorous benthic generalists, feeding on benthic invertebrates, including polychaetes, penaeid and alpheid shrimp, amphipods and bivalves (Pease et al., 1981). They can live up to 14 years but the most commonly encountered ages in commercial fisheries are 3-8 years (Stuart et al., 2015).

In this study, Yellowfin Bream were sampled in sufficient numbers to produce the desired number of composites per sampling zone ( $n = 5$ ) during both phases of the study (Table 1). In addition, sufficient samples were collected from Wallis Lake in Phase 2 to prepare the proposed number of control composites ( $n = 2$ ).

Table 1: The number of composite samples of Yellowfin Bream analysed per zone in each phase

Zone	Phase 1 (October 2005)	Phase 2 (October 2015)
UPR	5	5
HB	5	5
MPR	5	5
LPR	5	5
Control (Wallis Lake)	0	2

#### 4.1.1 Yellowfin Bream physiology

Yellowfin Bream sampled in Phase 2 were slightly longer than those sampled in Phase 1 (Figure 5a). Although this was significant ( $p < 0.0001$ ) the actual difference was only small with the average fish lengths per composite ranging from 23 to 26 cm (average 24 cm) in Phase 1 and 27 to 30 cm (average 28 cm) in Phase 2. The length of the control samples collected from Wallis Lake were consistent with the fish sampled in Phase 2 from Parramatta River. In contrast, the fish weights were not significantly different between the two phases ( $p = 0.097$ ) (Figure 5b). Overall, the average Yellowfin Bream weights per composite sample ranged from 313 to 411 g (average 351 g) in Phase 1 and 306 to 470 g (average 370 g) in Phase 2. The weight of the control samples were slightly lower than the Yellowfin Bream sampled from Parramatta River during both phases.

The lipid content of the Yellowfin Bream sampled in Phase 2 was significantly higher than those in Phase 1 ( $p < 0.0001$ , Figure 5c). In Phase 1, the lipid content ranged from 1.5 to 3.4% (average 2.1%), whereas in Phase 2 it ranged from 2.0 to 5.6% (average 3.7%). Due to this increase, lipid content was not considered to be a limiting factor for accumulation of dioxins in the Yellowfin Bream and therefore only the dioxin concentrations on a fresh weight basis are presented below. The lipid content of the Yellowfin Bream controls were lower than those collected in the Parramatta River (average 1%).

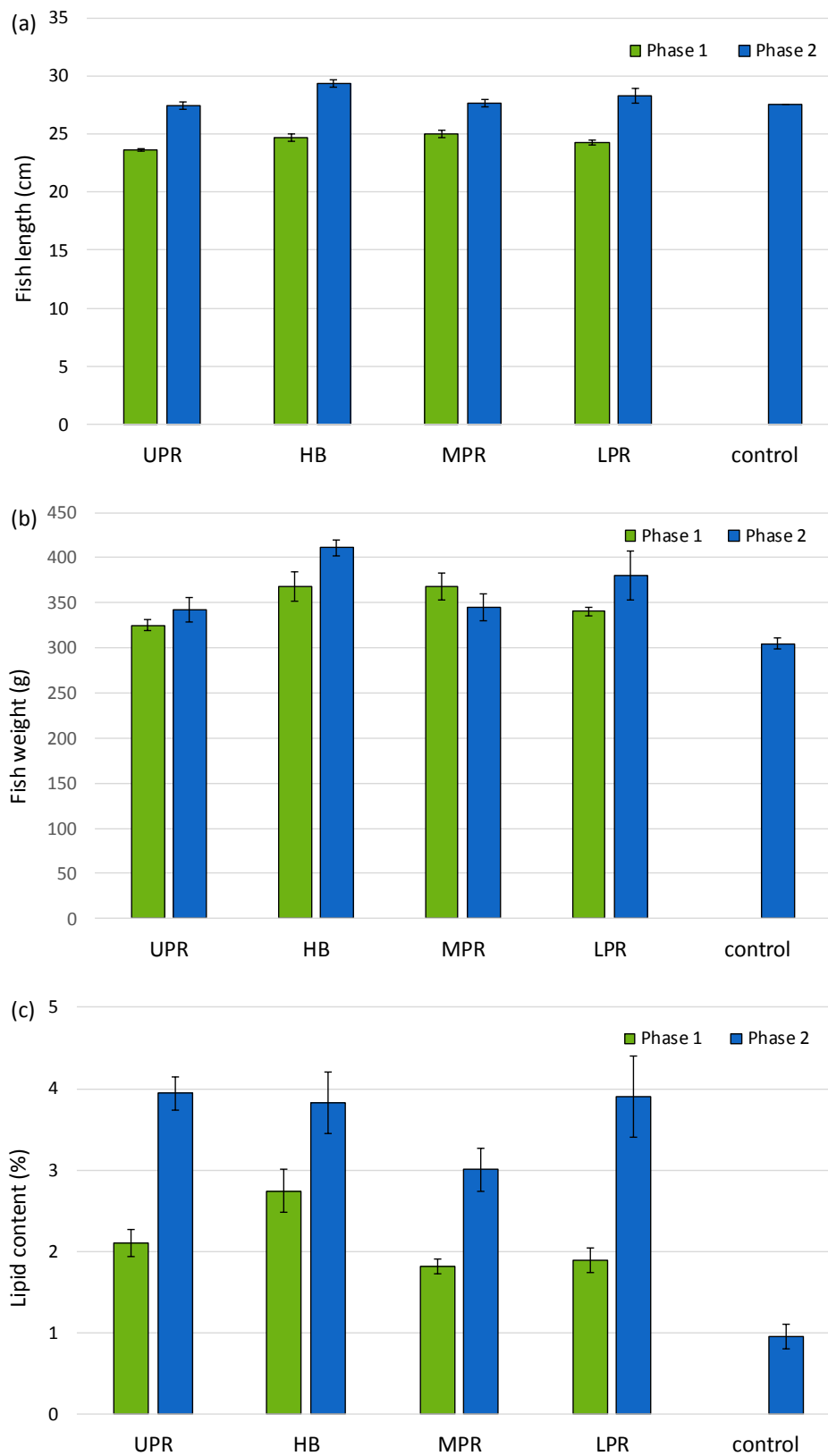


Figure 5: Yellowfin Bream (a) length, (b) weight and (c) lipid content from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Wallis Lake. No controls were collected in Phase 1. Error bars indicate  $\pm 1$  standard error.

## 4.1.2 Dioxin concentrations

Overall dioxin concentrations in Yellowfin Bream sampled from the Parramatta River have not changed substantially from Phase 1 to Phase 2 (main effect of time  $p = 0.54$ ). These concentrations, on a fresh weight basis, are summarised in Figure 6, where the letters indicate significant differences between zones within each phase of the project (i.e. the letters across the two phases do not correspond) and the arrows indicate where there are significant differences between the two phases within a zone. Within each zone, there was no significant difference between the Phase 1 and Phase 2 dioxin concentrations, with the exception of MPR where a small but significant increase was observed from 13 to 23 pg TEQ/g ( $p = 0.028$ ). Within this zone, the concentrations have increased to a level that is not different to the samples collected in UPR ( $p = 0.17$ ). The lack of overall change in dioxin concentration between the Phase 1 and Phase 2 studies may be due to residual dioxin contamination in sediments of the Parramatta River or indicate that the duration of time since the completion of the remediation (remediation finished in 2010) was not sufficient for measureable changes in dioxin concentration in this species.

There were differences in dioxin concentrations in the Yellowfin Bream between zones (Figure 6). In both the Phase 1 and Phase 2 studies, the concentration of dioxins in Yellowfin Bream from HB were significantly higher than in those collected at all of the other zones ( $p \leq 0.008$ ). Overall, the trend across zones was similar between the Phase 1 and Phase 2 studies with UPR having the next highest concentrations followed by MPR and LPR.

In all of the Yellowfin Bream samples collected from Parramatta River, the concentration of dioxins were above those considered acceptable for human consumption, 6 pg TEQ/g (FSANZ, 2006). Dioxin concentrations in the control samples from Wallis Lake were considerably lower than the samples from Parramatta River and were all below those considered acceptable for human consumption at an average concentration of 1.6 pg TEQ/g.



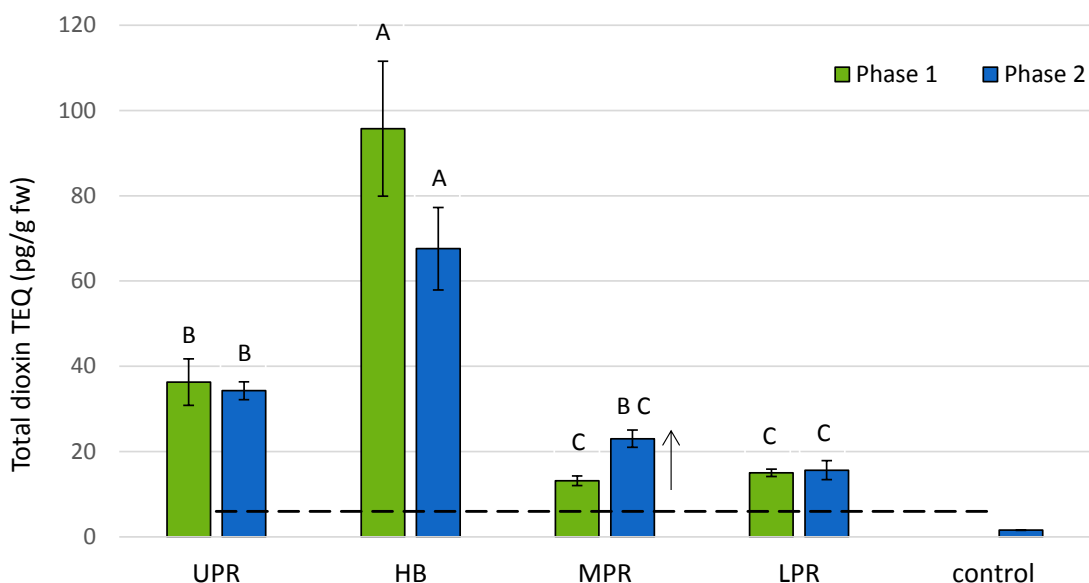


Figure 6: Total dioxin TEQs (fresh weight) in Yellowfin Bream from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Wallis Lake (no controls were collected in Phase 1). Letters indicate significant differences between zones within phases and arrows indicate significant differences between phases within zones. The dashed line represents the limit of 6 pg TEQ/g for human consumption (FSANZ, 2006) and error bars indicate  $\pm 1$  standard error.

## 4.2 Sea Mullet

Sea Mullet are a schooling fish that inhabit estuaries up to and beyond the tidal limit. They tend to remain within the estuary until they mature, but are not known to display a high level of site-attachment. Mature Sea Mullet (> 3 years old) run to sea to spawn, and this run often commences in March (Pease et al., 1981). Following spawning, Sea Mullet return to estuaries, but often to an estuary further north of the one from which they emigrated. They are omnivorous and detritivorous, feeding mainly on detritus, diatoms, and small amounts of animal material (Pease et al., 1981). They can live up to 12 years but the most commonly encountered ages in commercial fisheries are 3-8 years (Stewart et al., 2015).

In this study, Sea Mullet were sampled in sufficient numbers to produce 5-6 composite samples per zone during both phases of the study (Table 2). In addition, sufficient numbers were also collected from Clarence River to prepare the proposed number of control composite samples (n = 2).

Table 2: The number of composite samples of Sea Mullet analysed per zone and in each phase

Zone	Phase 1 (March 2006)	Phase 2 (March 2016)
UPR	5	5
HB	6	5
MPR	6	5
LPR	5	5
Control (Clarence River)	0	2

#### 4.2.1 Sea Mullet physiology

Sea Mullet sampled in Phase 2 were significantly longer and weighed more than those sampled in Phase 1 ( $p < 0.0001$ ) (Figure 7). This difference was more pronounced for the weights, which had an average weight per composite ranging from 423 to 701 g (average 551 g) in Phase 1 compared to 830 to 1260 g (average 1020 g) in Phase 2. The average fish lengths per composite ranged from 30 to 36 cm (average 32 cm) and 39 to 45 cm (average 42 cm) in the Phase 1 and Phase 2 study, respectively. The control Sea Mullet collected from the Clarence River in Phase 2 were a similar length to those collected from Parramatta River but weighed less.

Similar to Yellowfin Bream, the lipid content of Sea Mullet sampled in Phase 2 was significantly higher than in Phase 1 ( $p < 0.0001$ ) (Figure 7). The change in the lipid content for this species was considerable. It ranged from 4.5 to 17% (average 7.6%) in Phase 1 and increased to a range from 12 to 20% (average 15%) in Phase 2. Due to this increase, lipid content was not considered to be a limiting factor for dioxin accumulation and therefore the results are only presented below on a fresh weight basis. The lipid content of the control Sea Mullet samples collected from Clarence River was lower than that in the Sea Mullet from Parramatta River.

It is unknown why such large differences were observed in the lipid content of both the Yellowfin Bream and Sea Mullet between the two phases. This could be due to many factors that can influence the lipid content of an organism, including diet, water quality and climatic conditions.

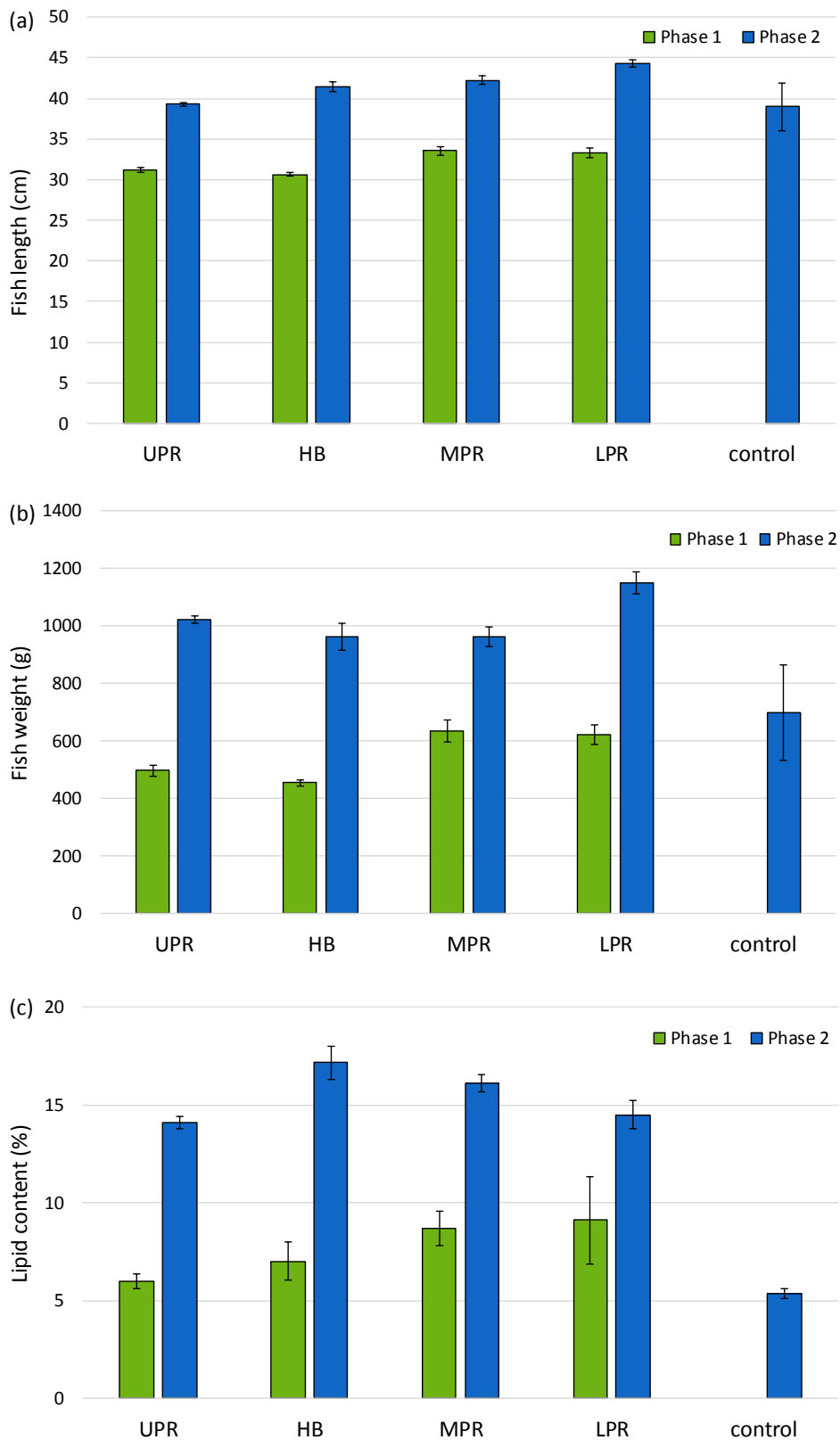


Figure 7: Sea Mullet (a) length, (b) weight and (c) lipid content from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River. No controls were collected in Phase 1. Error bars indicate  $\pm 1$  standard error.

## 4.2.2 Dioxin concentrations

Similar to the Yellowfin Bream, overall there was no considerable change in the dioxin concentration (on a fresh weight basis) in Sea Mullet from Phase 1 to Phase 2 (Figure 8; main effect of time  $p = 0.94$ ). The concentration at MPR, showed a significant increase ( $p = 0.034$ ), from an average concentration of 99 to 140 pg TEQ/pg, which was consistent with the increase observed for Yellowfin Bream. This increase at MPR resulted in dioxin concentrations that were not significantly different from HB ( $p = 0.65$ ). The cause of the increase at this zone is unknown but it could be due to residual contaminated sediment from HB moving downstream. Sediment sampling would be required to confirm if this is the case. In contrast, at LPR there was a significant decrease in the average dioxin concentration ( $p = 0.015$ ) from 57 to 35 pg TEQ/g and at UPR and HB there was no change ( $p \geq 0.14$ ). The limited difference observed in dioxin concentrations between Phase 1 and Phase 2 may again be due to residual dioxin contamination in the Parramatta River or indicate that that a longer duration post-remediation is required to observe any measurable decreases.

The Sea Mullet showed varying concentrations across the different zones but the magnitude of the differences were not as large as those observed for the Yellowfin Bream. This may be due to the limited level of site-attachment that these species show, therefore the dioxin concentrations reflect exposure from throughout the Parramatta River rather than only the zone they were caught in. In Phase 1, Sea Mullet from HB had dioxin concentrations that were similar to UPR and significantly higher than those from MPR and LPR. Whereas, in Phase 2 the concentrations in fish from HB and MPR were significantly higher than the other zones. Overall, this shows that the dioxin concentrations in Sea Mullet from HB were either the same or higher than those from the other zones.

Total dioxin concentrations in the Sea Mullet in Phase 2 ranged up to 194 pg TEQ/g, which is approximately 30-times higher than the limit of 6 pg TEQ/g recommended by FSANZ (2006) for human consumption. Similar to the Yellowfin Bream, all of the Sea Mullet samples collected in Phase 2 had concentrations exceeding the 6 pg TEQ/g threshold. In contrast, dioxin concentrations in the control Sea Mullet collected from the Clarence River were considerably lower than this threshold at an average concentration of 0.5 pg TEQ/g.

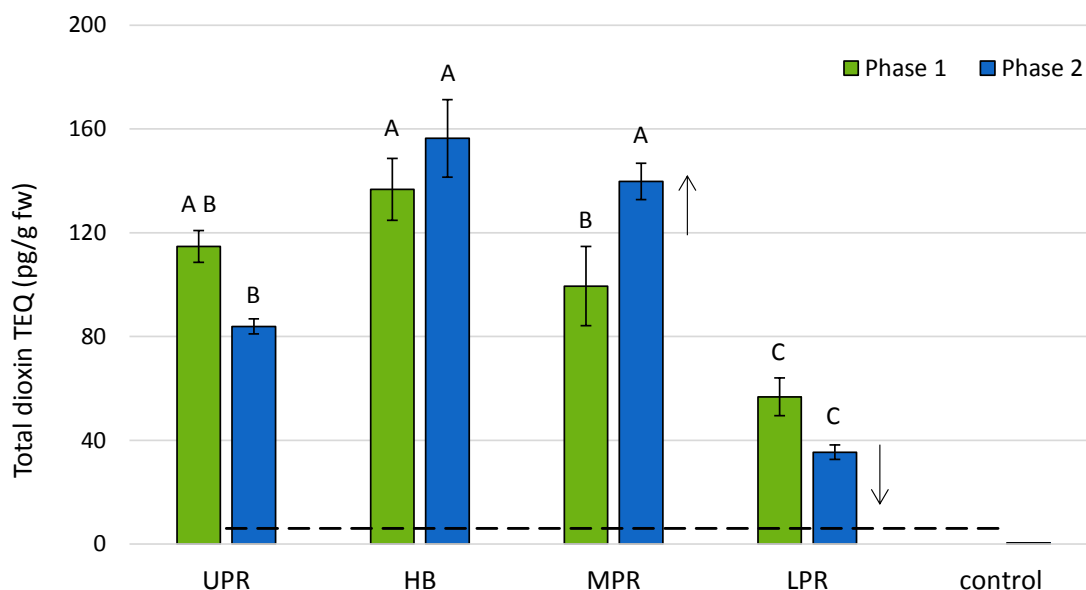


Figure 8: Total dioxin TEQs (fresh weight) in Sea Mullet from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River (no controls were collected in Phase 1). Letters indicate significant differences between zones within phases and arrows indicate significant differences between phases within zones. The dashed line represents the limit of 6 pg TEQ/g for human consumption (FSANZ, 2006) and error bars indicate  $\pm 1$  standard error.

### 4.3 School Prawn

School Prawn can tolerate a wide range of salinities and will usually distribute along the length of an estuary (Ruello, 1973). School Prawn that settle in the upper estuary may move increasingly closer to the mouth of the estuary as they grow (Ruello, 1971), but where the estuary lacks a strong salinity gradient they are just as likely to remain in the same location from settlement to emigration. Prawns generally emigrate to the sea once they reach an appropriate size, usually during the 3<sup>rd</sup> quarter of the moon on the ebb tide (Racek, 1959). However, heavy rainfall and the influx of fresh water into the estuary can also stimulate this migration (Glaister, 1978).

They are opportunistic omnivores feeding on material that they pick up from the sediments, including detritus and small amounts of animal material (crustaceans, annelid worms, minute bivalves and foraminifera) (Ruello, 1973). The School Prawn are largely an annual species but they are thought to live up to 2.5 years (Flood, et al., 2014).

School Prawn could not be caught in sufficient numbers to produce the desired five composites from all zones in Phase 1, whereas, sufficient samples were collected from all four zones in Phase 2 (Table 3). Sufficient School Prawn were collected from Clarence River to produce the required number of control composite samples (n=2).

Table 3: The number of composite samples of School Prawn analysed per zone and in each phase

Zone	Phase 1 (March 2006)	Phase 2 (March 2016)
UPR	3	5
HB	2	5
MPR	2	5
LPR	3	5
Control (Clarence River)	0	2

#### 4.3.1 School Prawn physiology

Carapace length of all School Prawn used in composites were recorded prior to shelling and de-veining in both phases. Overall, there was a slight difference in the size distribution of prawns between the two phases, but differences were minor with the majority of prawns in both phases falling within a size range 16.1 - 22 mm (Figure 9a).

Lipid content of School Prawn sampled in Phase 2 was significantly lower ( $p < 0.0001$ ) than that in Phase 1. Lipid levels were about 3-times lower in Phase 2 compared to Phase 1 with an average of 0.43 and 1.25%, respectively (Figure 9b). Consequently, the concentrations of dioxins on both a fresh weight and lipid weight basis are discussed below. There are many factors that could have influenced this change in the lipid content in the prawns. In general, the lipid reserves in prawns are used to deal with starvation, onset of gonad development, and moulting. Prawns do not store a great deal of lipid and therefore a large decrease in lipid content will limit their capacity to accumulate dioxins. The lipid content of the control School Prawn from Clarence River were more consistent with the Phase 2 Parramatta River samples at an average of 0.2%.

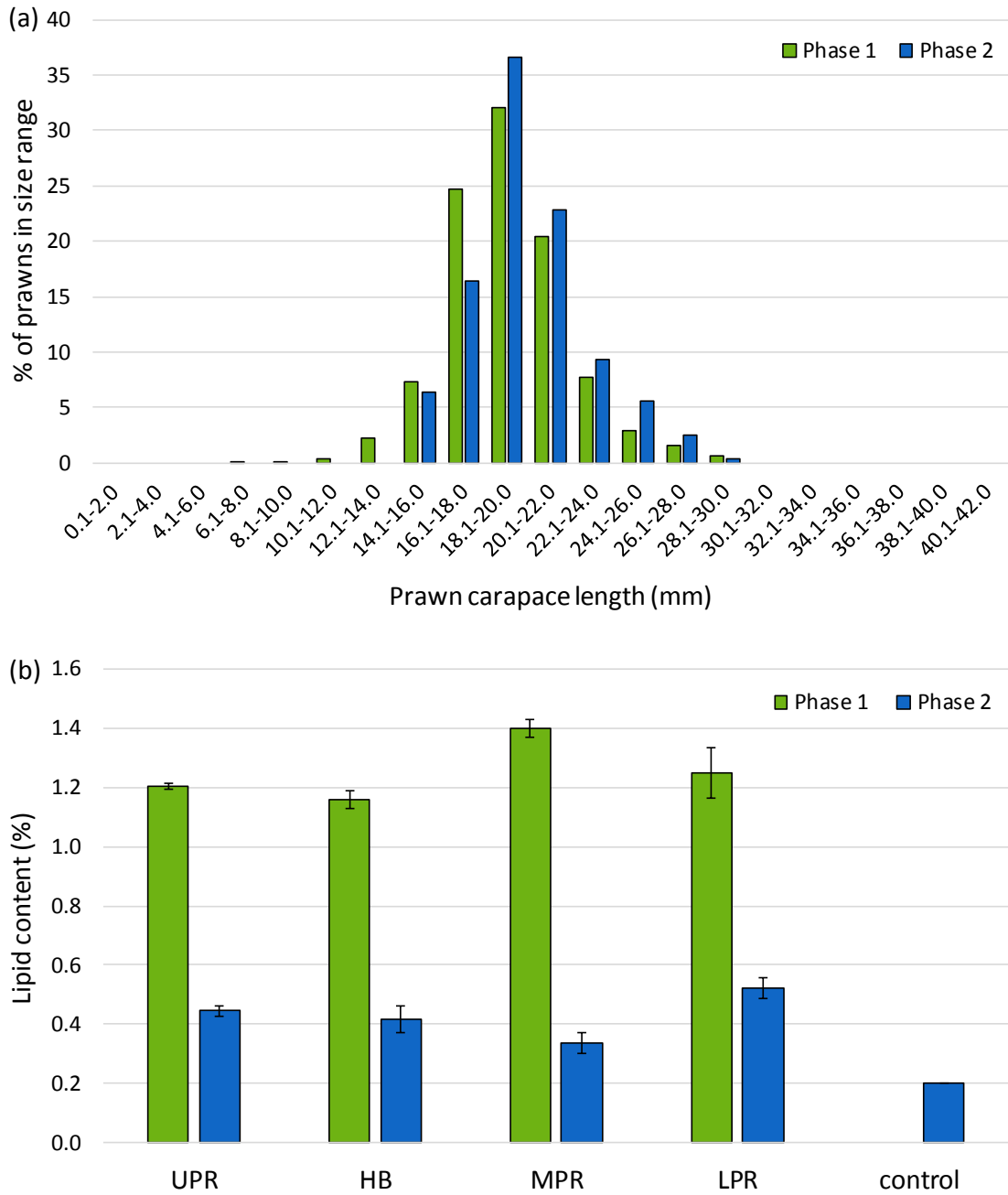


Figure 9: School Prawn (a) carapace length and (b) lipid content from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River. No controls were collected in Phase 1. Error bars indicate  $\pm 1$  standard error.

### 4.3.2 Dioxin concentrations

Dioxin concentrations in School Prawn (on a fresh weight basis) were significantly lower in Phase 2 compared to Phase 1 at all of the zones ( $p < 0.0001$ ), by about 3-fold (Figure 10; main effect of time  $p < 0.0001$ ). This trend differs from that observed for the two fish species where overall no change was observed. As lipid concentrations in School Prawn also decreased by 3-fold between phases, potentially limiting dioxin accumulation, lipid normalised dioxin concentrations were also considered (Figure 11). When lipid normalised, there were no significant differences in dioxin concentrations between Phase 1 and Phase 2 in the different zones, with the exception of LPR. In LPR there was a significant decrease in the lipid normalised dioxin concentration ( $p = 0.007$ ).

These results suggest that although there was a large decrease in dioxin concentrations in School Prawn on a fresh weight basis between phases, this may be driven by the decrease in lipid content of the prawns, rather than a change in their level of exposure over time. As the lipid content is lower, the prawns do not have as much capacity to accumulate dioxins and this may explain the overall lower fresh weight dioxin concentrations. If the lipid content of the prawns in Parramatta River should increase in the future as a result of different seasons or a change in diet, the fresh weight concentrations of dioxins may also increase as they would have more capacity to accumulate these compounds.

Despite the large decrease in fresh weight dioxin concentrations from Phase 1 to Phase 2, there were also significant differences between zones within each sampling period ( $p = 0.022$ ). In Phase 1, fresh weight dioxin concentrations in School Prawn from HB, MPR and LPR were not significantly different ( $p \geq 0.31$ ). The concentrations from these zones ranged from 15 to 23 pg TEQ/g, whereas the concentrations at UPR were significantly lower, ranging from 7 to 10 pg TEQ/g. In Phase 2, although there were some significant differences observed between the zones, the magnitude of these differences were lower than in Phase 1.

In Phase 1, all of the School Prawn dioxin concentrations were higher than the 6 pg TEQ/g threshold limit (FSANZ, 2006). In Phase 2, the average concentration of dioxins were marginally below the 6 pg TEQ/g for all zones except HB, where the average was 7.2 pg TEQ/g. It should be emphasized that although dioxin concentrations were below the 6 pg TEQ/g limit, it is not clear if an increase in lipid content would result in an increase in the dioxin concentration. The control samples collected from the Clarence River had concentrations that were considerably lower than the 6 pg TEQ/g limit, with an average concentration of 0.2 pg TEQ/g.



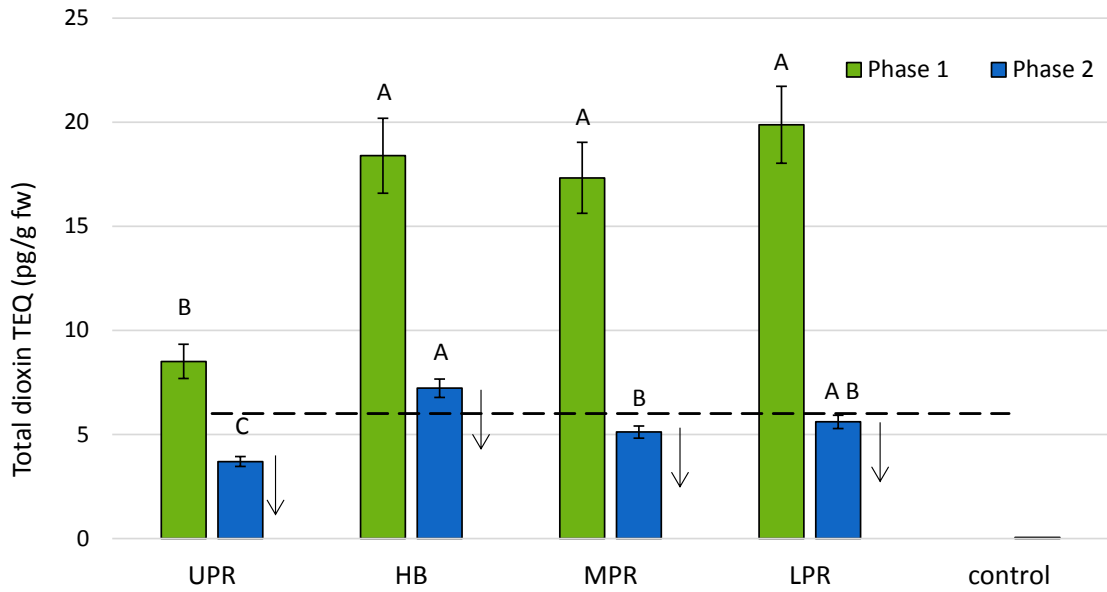


Figure 10: Total dioxin TEQs (fresh weight) in School Prawn from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River (no controls were collected in Phase 1). Letters indicate significant differences between zones within phases and arrows indicate significant differences between phases within zones. The dashed line represents the limit of 6 pg TEQ/g for human consumption (FSANZ, 2006) and error bars indicate  $\pm 1$  standard error.

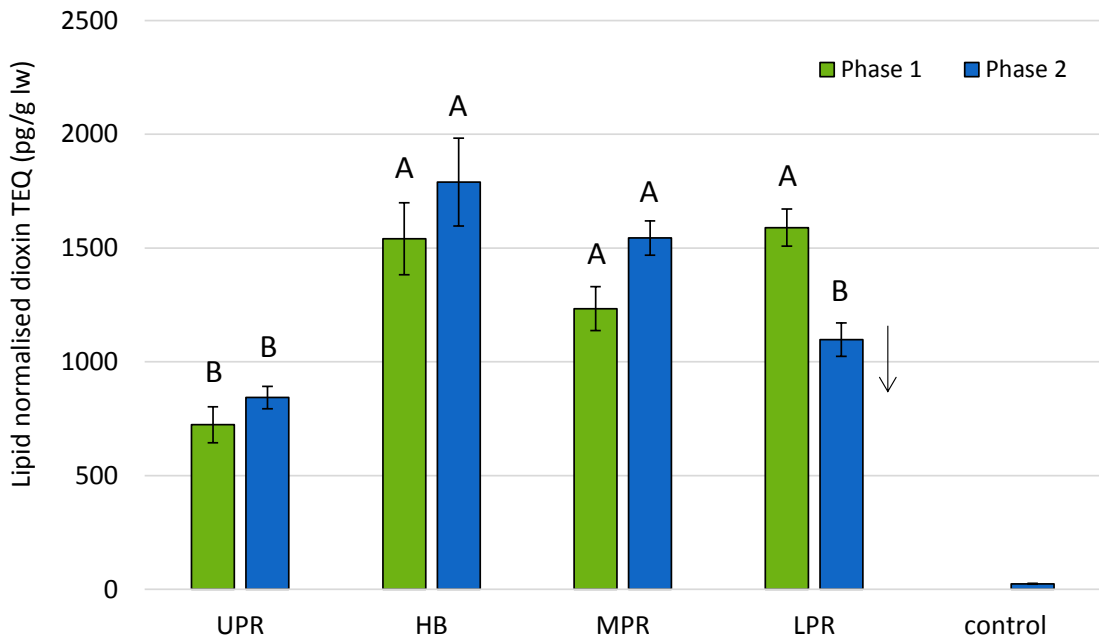


Figure 11: Lipid normalised total dioxin TEQs in School Prawn from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River (no controls were collected in Phase 1). Letters indicate significant differences between zones within phases and arrows indicate significant differences between phases within zones. The error bars indicate  $\pm 1$  standard error.

## 4.4 King Prawn

King Prawn are generally only able to tolerate a narrow salinity range and occur closer to the mouth of the estuary (Taylor et al., 2016). Similar to the School Prawn, King Prawn emigrate to sea once they reach an appropriate size (Racek, 1957). They are omnivorous and detritivorous, feeding mainly on detritus and small amounts of animal material (Suthers, 1984). They are largely annual species but are thought to live up to 3 years (Flood et al., 2014).

King Prawn could not be caught in sufficient numbers to produce the desired five composites from all zones during both phases (Table 4). In Phase 2, LPR was the only zone where sufficient King Prawn were sampled to prepare any composites. Due to this, the dataset was incomplete for this species and data interpretation is limited. There were sufficient King Prawn sampled from Clarence River to produce the two control composite samples.

*Table 4: The number of composite samples of King Prawn analysed per zone and in each phase*

<b>Zone</b>	<b>Phase 1 (March 2006)</b>	<b>Phase 2 (March 2016)</b>
UPR	0	0
HB	2	0
MPR	3	0
LPR	3	5
Control (Clarence River)	0	2

### 4.4.1 King Prawn physiology

The distribution of King Prawn carapace length in Phase 1 and Phase 2 were similar, with the majority of individuals having carapace lengths ranging from 14.1 to 24 mm (Figure 12a). As King Prawn were only collected from LPR in Phase 2, this was the only zone where a comparison between the two phases could be made (Figure 12b). Overall King Prawn showed a similar trend to the School Prawn with a decrease in lipid content during Phase 2 compared to Phase 1. This decrease was about 2-fold, from 1.3% during Phase 1 to 0.62% during Phase 2. Due to the decrease in lipid content, dioxin concentrations on both a fresh weight and lipid weight basis are discussed below.

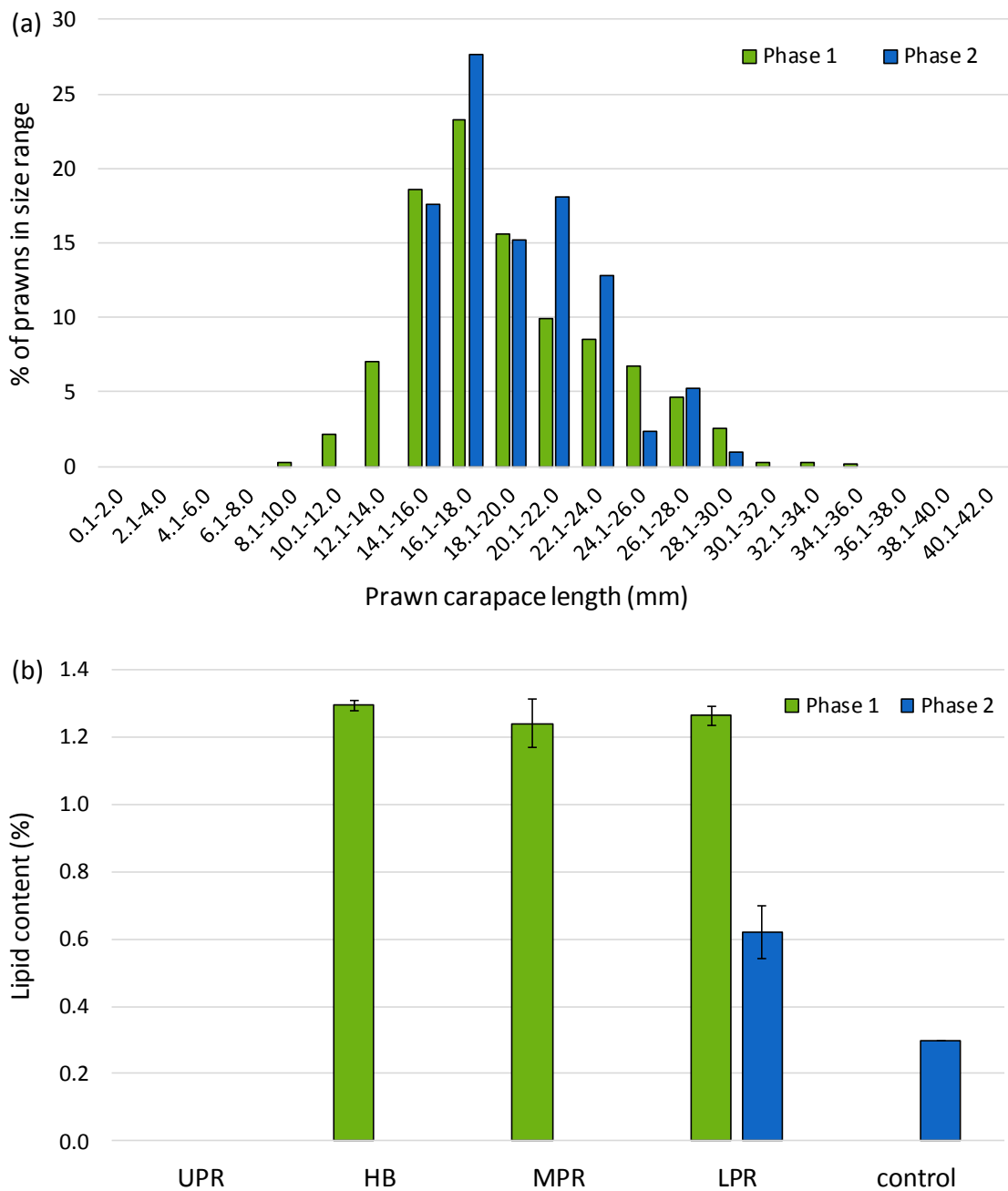


Figure 12: King Prawn (a) carapace length and (b) lipid content from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River. No controls were collected in Phase 1. No bars indicate insufficient prawns were collected within that zone. Error bars indicate  $\pm 1$  standard error.

#### 4.4.2 Dioxin concentrations

Due to the limited number of King Prawn caught during both phases, the statistical analysis used for the other species could not be applied. For this species, the dioxin concentrations in the different zones in Phase 1 were tested using a one-way ANOVA with a *post hoc* test (Tukey's) and differences in the concentrations at LPR between the two phases were tested using a t-test.

A comparison between the Phase 1 and Phase 2 results could only be made for LPR. In this zone, the dioxin concentrations (on a fresh weight basis) significantly decreased from an average of 7.3 pg TEQ/g in Phase 1 to 4.8 pg TEQ/g in Phase 2 (Figure 13). Due to King Prawn only being caught in one zone at both sampling times, limited conclusions can be made, but they suggest a similar result to those observed for the School Prawn with a decreasing trend over time on a fresh weight basis. However, when the data were normalised to the lipid content (Figure 14), the decrease observed with the fresh weight data was no longer evident and instead, the lipid normalised concentration at LPR were not significantly different (Figure 14,  $p = 0.078$ ). Similar to the School Prawn, this result suggests that the decrease in dioxin concentration on a fresh weight basis may be driven by the decrease in lipid content rather than a change in the exposure of the organisms.

Differences between zones could only be investigated for the Phase 1 results. This showed that there was a decreasing trend in dioxin concentration in the King Prawn moving from HB down the Parramatta River (Figure 13).

In Phase 1, dioxin concentrations in all of the samples were above the 6 pg TEQ/g threshold limit concentration (FSANZ, 2006). At LPR in Phase 2, the average concentration was marginally lower than this limit (4.8 pg TEQ/g). Similar to School Prawn, it is unknown if this concentration could increase with an increase in lipid content. The concentrations of dioxin in the control samples collected from the Clarence River were considerably lower than the limit concentration at 0.14 pg TEQ/g.

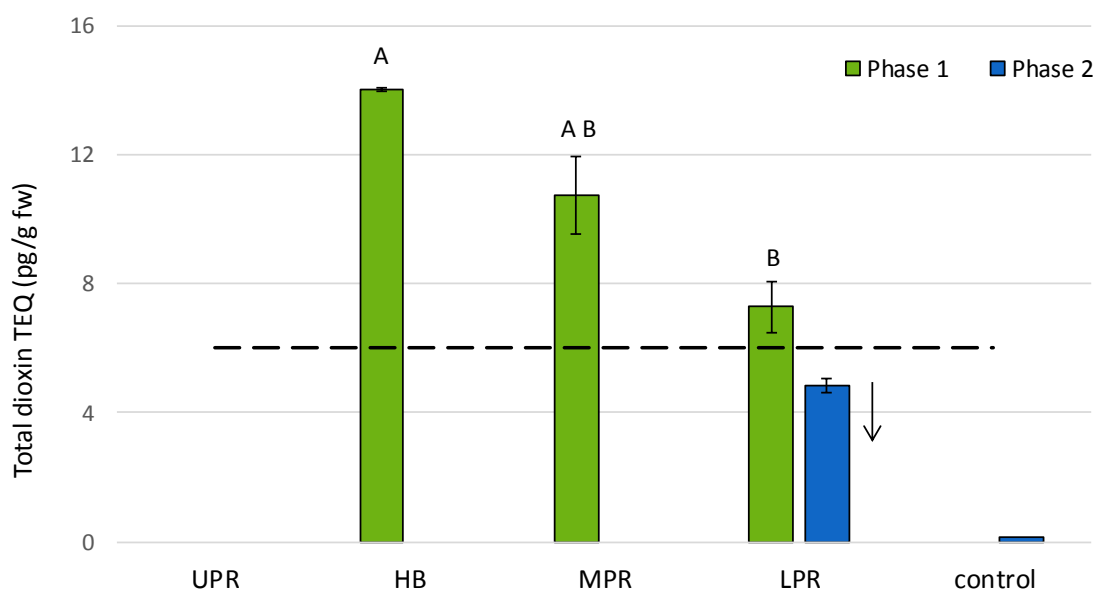


Figure 13: Total dioxin TEQs (fresh weight) in King Prawn from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River (no controls were collected in Phase 1). No bars indicate insufficient prawns were collected. Letters indicate significant differences between zones within phases and arrows indicate significant difference between phases within zones. The dashed line represents the limit of 6 pg TEQ/g for human consumption (FSANZ, 2006) and error bars indicate  $\pm 1$  standard error.

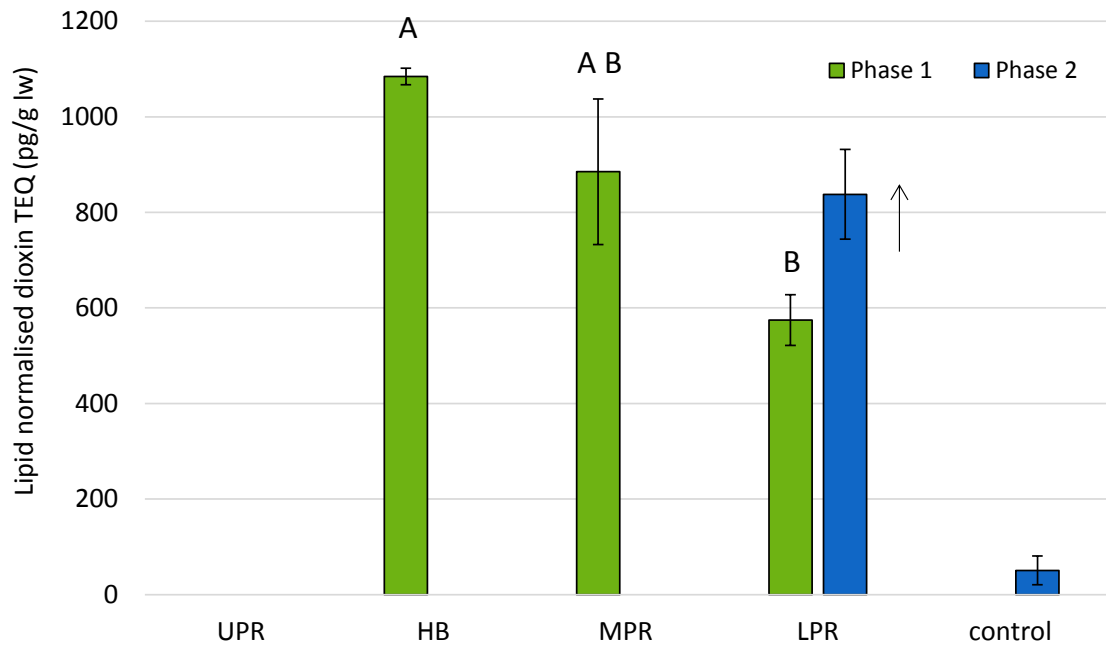


Figure 14: Lipid normalised total dioxin TEQs in King Prawn from Phase 1 and Phase 2 in Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR) and the controls from Clarence River (no controls were collected in Phase 1). No bars indicate insufficient prawns were collected. Letters indicate significant differences between zones within phases and arrows indicate significant differences between phases within zones. The error bars indicate  $\pm 1$  standard error.

## 5 Conclusions

This report presents the results from a follow-up study carried out in 2015/16 (referred to as the Phase 2 study) investigating the concentrations of dioxins in fish (Yellowfin Bream and Sea Mullet) and prawns (School and King Prawn) in Homebush Bay and the Parramatta River. The results were compared to a previous study conducted in 2005/06 (referred to as the Phase 1 study), which took place prior to the remediation of Homebush Bay in 2006-2010.

Four zones were targeted during both phases of the study, Upper Parramatta River (UPR), Homebush Bay (HB), Middle Parramatta River (MPR) and Lower Parramatta River (LPR). Overall, for both of the fish species, the concentrations of dioxins did not change considerably when the post-remediation results (Phase 2) were compared to the pre-remediation results (Phase 1). This may be due to the presence of residual contamination in Parramatta River or indicate that insufficient time had passed between the two phases of the study to result in measurable changes for these species. The concentrations of dioxins (on a fresh weight basis) in both of the prawn species showed a large decrease. On a lipid normalised basis, the decrease in concentration was not evident, suggesting that the decrease on a fresh weight basis may be driven by the drop in lipid content that was observed during the Phase 2 study. This decreased lipid content may limit the ability for the prawns to accumulate dioxins. If the lipid content of the prawns should increase as a result of seasonal differences or a change in diet, the overall fresh weight concentrations may increase as their capacity to accumulate dioxins will increase.

For all of the species, the concentration of dioxins measured in the samples collected from Homebush Bay were equal to or greater than the concentrations at all the other zones. The dioxin concentrations in all of the species (fish and prawns) were compared to the Food Standards Australia New Zealand limit for dioxins in fish and seafood for human consumption (6 pg TEQ/g (pg of dioxin toxicity equivalence per g fresh weight)). The concentration of dioxins in all of the fish samples were considerably higher than this threshold limit. In contrast all of the prawn samples were marginally lower than this limit, with the exception of the HB School Prawn samples. It should be emphasised however, that decisions relating to the suitability of fish and prawns for human consumption was outside the scope of this project and it is recommended that the current recreational fishing advisories remain. It is also noted that Sydney Harbour and Parramatta River has been closed as a commercial fishery and that commercial fishing in the area is not permitted.

## 6 Abbreviations

Term	Definition
%	Percent
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
ANOVA	Analysis of variance
Cm	Centimetre
DDT	Dichlorodiphenyltrichloroethane
dl-PCB	Dioxin like polychlorinated biphenyl
EMP	Environmental Management Plan
FSANZ	Food Standards Australia New Zealand
fw	Fresh weight
g	Gram
HB	Homebush Bay
HRGC	High resolution gas chromatography
HRMS	High resolution mass spectrometry
km	Kilometre
LOR	Limit of reporting
LPR	Lower Parramatta River
lw	Lipid weight
mm	Millimetre
MPR	Middle Parramatta River
n	Number of replicates
NMI	National Measurement Institute
p	P-value
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
pg	Picogram
RMS	Roads and Maritime Services
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEF	Toxicity equivalence factor
TEQ	Toxicity equivalence
TEQ <sub>DF</sub>	Dioxin and furan toxicity equivalence
TEQ <sub>P</sub>	Dioxin-like polychlorinated biphenyl toxicity equivalence
UPR	Upper Parramatta River

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