Effects of anthropogenic pressures on the pollutant loads, body condition and functional traits of seafood species in Southeast Queensland estuaries

A joint project between Healthy Land and Water and USC

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Executive summary

Estuaries provide numerous ecosystem services that are valued by people. Principle among these is the provision of fish habitats for finfish and crustacean species of commercial and recreational significance (Barbier et al. 2011). However, many estuaries globally have become degraded by human activities. Estuaries have had their shorelines hardened by human infrastructure, and estuarine catchments are modified by human land uses, resulting in alterations to runoff regimes, and water quality (Halpern et al. 2008).

These anthropogenic changes to coastal ecosystems result in changes in coastal species health and body condition, food webs, and potentially toxic chemicals accumulating in the flesh of some species. For example, perfluorinated alkylated substances, or PFASs, used often in industrial firefighting foams, can leach into waterways, cause local fish kills, accumulate in fish flesh, and potentially trigger cancer and immune disorders in people (Taylor and Johnson 2016). Structural changes to in-stream habitats modify food webs, alter species' food targets, change population and food web structures (Heery et al. 2017), and potentially proliferate to fish condition, and morphological and dietary changes in some affected species (Schlacher et al. 2007). Whilst most of these consequences can be predicted for fish assemblages in impacted estuaries, the full suite of consequences and their scales have never been studied in unison, in the same systems, to garner a fuller understanding of the consequences of urbanisation for coastal fish and fisheries. Given the importance of coastal ecosystems in the provision of food for people, understanding how the effects of urbanisation on the condition of key seafood species and food safety proliferate throughout the food web is a significant priority for coastal management.

To date, collaborative projects between Healthy Land and Water and University of the Sunshine Coast (USC) have surveyed 22 estuaries for broad patterns in fish assemblages, and then 13 estuaries for habitat-specific patterns in fish assemblages throughout southeast Queensland (SEQ). These surveys, perhaps the most thorough and widest reaching globally, have established:

- the catchment, water quality, and in-stream habitat factors that drive fish diversity and abundance,

- the human factors that impinge most upon the structure (i.e. the number and type) of coastal fish assemblages, and;
- the management interventions that should be prioritised, modified, or increased to optimise the value of southeast Queensland's estuaries for fish.

This project used these extensive surveys as a basis to investigate, for the first time, how water quality, catchment and instream factors combine to influence the safety of seafood caught in southeast Queensland estuaries for human consumption, and how estuarine condition modifies seafood species health more broadly throughout the region. Consequently, this project has three key objectives, separated into three separate chapters of this report.

1. **Objective 1- What are the pollutant loads in southeast Queensland's estuarine fish?**
   Do fish in southeast Queensland estuaries contain dangerous levels of heavy metals, PFASs and associated chemicals, or pesticides?
2. **Objective 2- Do human modifications to estuaries and their catchments change the diet composition and morphology of estuarine fish?**
   What implications do modifications to estuaries, in terms of water quality or habitat changes, have for the condition and functional traits of fish in estuaries?
3. **Objective 3- Whether and how anthropogenic pressures on estuaries can combine to modify the body size and functional traits of giant mud crabs in southeast Queensland, Australia.**
   Given the intensive fishing pressure on particular demographics of giant mud crabs in southeast Queensland, how does fishing pressure and human population size interact with changes in estuarine habitat condition to modify the size and potential value of giant mud crab catches in estuaries?

Human activities in coastal catchments can result in the release of pollutants into waterways and coastal ecosystems, and these pollutants can accumulate in seafood. In **chapter 1**, we quantified the concentrations of a suite of heavy metals, PFASs and pesticides in the flesh of two commercially and recreationally important fisheries species (yellowfin bream *Acanthopagrus australis* and giant mud crab *Scylla serrata*) in 13 estuaries southeast Queensland, Australia; a region with a wide variety of human land uses. No samples contained detectable levels of pesticides, and only two yellowfin bream and four mud crabs contained detectable, but low, levels of PFASs. Only yellowfin bream samples from the Mooloolah River contained mercury levels above food safety standards. These results show that concentrations of potentially harmful pollutants can be low in commercially and recreationally important seafood species, even in regions that are subject to substantial human modifications.

Populations of animals can respond to human impacts by favouring different morphological traits or by exploiting different food resources. In **chapter 2**, we quantified the morphology and diet of four functionally different fish species (n=543 fish) from 13 estuaries with varying degrees of human modification in Queensland, Australia. We found marked differences in the responses of different trophic groups to the environmental conditions of estuaries; principally the extent of seagrass in the estuary, and the amount of shoreline and catchment urbanisation. Here, seagrass and urbanisation correlated with the diet and morphology of zooplanktivores and detritivores; thereby indicating that human modifications may modify these species functional roles. Conversely, environmental variables did not correlate with the diet or morphology of zoobenthivores or piscivores; thereby indicating human modifications may not affect these species functional roles. Our findings demonstrate that anthropogenic impacts to coastal ecosystems extend from the traditionally measured metrics of abundance and diversity.

Human pressures on ecosystems from landscape transformation and harvesting can result in changes to body size and functional traits of affected species. Yet, these effects remain very poorly understood in many settings. In **chapter 3**, we examine whether and how fishing and the attributes of coastal seascapes can operate in concert to change the body size and functional traits of the giant mud crab, *Scylla serrata;* a prized fisheries species. Legal sized (>15cm carapace width) male giant mud crabs were sampled from estuaries in southeast Queensland, Australia that spanned a wide range of the intensity of fishing and coastal landscape transformations human pressures, and a total of 9000 external morphometric measurements made. There was a distinct effect of estuarine landscape context on body size, with the largest individuals captured from systems with bigger inlets and lower extent of intertidal flats. Variation in functional traits was most often associated with variation in fishing pressure and human population size in the catchment. Crabs from areas with less commercial fishing pressure and lower human populations in the catchment had the largest chelipeds. We also found effects of urbanisation (negative correlations), intertidal flats (inconsistent effects) and mangrove extent (positive correlations) on the size of some functional traits. Our results show that human pressures can have sub lethal effects in estuarine seascapes, which alter the body size and functional traits of individuals, and we suggest that these phenotypic responses might have consequences for the fitness and ecological roles of targeted species, and the yields of fisheries catches.

Key conclusions and take-home points

Anthropogenic impacts to estuaries are expanding globally. Quantifying the effects of these impacts on the condition of estuaries, the structure of their ecosystems, and their capacity to deliver key ecosystem services is therefore an important focus for ecologists and managers. In previous studies, we established clear effects of catchment and verge urbanisation, and the loss of marine habitats on the condition of fish assemblages in estuaries in southeast Queensland. In this report, we highlight **two key take-home points**;

- **The effects of human modifications extend beyond only changes to the number and type of animals in estuaries, but also to the condition of size and shape of key seafood species,** and the niches they fill in coastal seascapes.
- There is little value in generalising either land use or perceived impact levels to likely pollutant levels in coastal seafood species because **land use does not correlate with pollutant levels**.

Therefore, the results of this report have important consequences for the value of some coastal fisheries that might be perceived as potentially harmful to people in urbanised systems, and stress the importance of surveying broadly across regions to quantify the effects of anthropogenic impacts on the condition of and pollutant levels in coastal seafood species.

Chapter 1

Diverse land uses and high coastal urbanisation do not always result in harmful environmental pollutants in fisheries species

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Introduction

Ecosystems are increasingly impacted by human activities globally (Halpern et al. 2019). These impacts have implications for the condition and functioning of coastal ecosystems, and the abundance and diversity of animals in coastal seascapes (Mouillot et al. 2013b; Heery et al. 2017). Some impacts, like runoff from catchments that are modified by agricultural, urban or industrial developments can affect how safe seafood captured from coastal ecosystems is for human consumption due to potential exposure to harmful environmental pollutants (Murray et al. 2010; Bosch et al. 2016; Jian et al. 2017). This is of increasing concern to managers because catchment modifications and potential pollutant sources increase and expand in geographic scope globally (Halpern et al. 2019), as does the demand for wild-caught seafood (FAO 2018). Consequently, quantifying the concentrations of potentially harmful environmental pollutants in the flesh of key seafood species, and identifying potential sources and drivers of these pollutants is a key focus for coastal managers (Budtz-Jorgensen et al. 2007; Carbery et al. 2018).

Estuaries are an ideal study system to test for the accumulation of pollutants in seafood because they are subject to substantial runoff from catchments that vary significantly in land use and potential pollutant sources (Crain et al. 2008; Halpern et al. 2008). Pollutants that runoff from degraded and modified catchments concentrate in waters near the mouths of estuaries before being released into the ocean (Wen et al. 2017; Warwick et al. 2018; Barletta et al. 2019). From here, the pollutants often dilute to levels considered less dangerous to people and seafood (Wood et al. 1993). These effects can be worsened by the loss of coastal habitats like marshes, mangroves and seagrasses that act as filter to remove some of these harmful substances from the water column (Knox et al. 2008). Pollutants can bioaccumulate in seafood species through a number of trophic pathways. Therefore, the exposure of seafood species to pollutants is likely contingent on their biology, ecology and behaviour (Suedel et al. 1994; Russell et al. 1999; Liu et al. 2019). For example, species who regularly feed in or on the benthos are potentially at a greater risk of consuming harmful concentrations of sediment-associated pollutants like some heavy metals (Chen and Chen 1999). Accurately quantifying the sources of pollutants of human health concern, and determining whether particular features of catchments (i.e. from relatively natural, to heavily impacted), the marine environment, or species biology and ecology increase the risk of pollutant bioaccumulation is an increasing focus for coastal managers.

There are numerous environmental pollutants that can accumulate in seafood (Hellberg et al. 2012; Thomsen et al. 2018; Ye et al. 2018). Perfluorinated chemicals or perfluoroalkyl substances (PFASs) are emerging contaminants of international concern that are used for numerous domestic, industrial and agricultural purposes (Murray et al. 2010). They have recently been subject to significant media attention globally due to their use in firefighting foams at airports, and their being released into nearby waterways and water tables (Xiao 2017; Food Standards Australia and New Zealand 2018). PFASs are highly resistant to environmental degradation and bioaccumulate in food chains (Lindstrom et al. 2011; Taylor and Johnson 2016). Consequently, there are now several examples globally of PFAS accumulation in the flesh of coastal seafood species (e.g. Taylor and Johnson 2016). There is increasing evidence of potential carcinogenic and immunological effects of PFASs in people, meaning that they are of increasing concern for human health (Grandjean and Clapp 2015; Lau 2015). Heavy metals, especially lead, cadmium, mercury and arsenic, have significant health consequences for people, and occur in high concentrations in many fish species (Jarup 2003; Wang et al. 2005; Verdouw et al. 2010; Yi et al. 2011). Their broad industrial, domestic, agricultural and technological applications mean that they are widely distributed in the biosphere and that their potential sources are diverse (Tchounwou et al. 2012; Bosch et al. 2016). Despite ongoing warnings regarding the health consequences of heavy metal consumption by people, exposure rates remain high in many human populations, especially those that regularly consume seafood (Castro-Gonzalez and Mendez-Armenta 2008; Bosch et al. 2016). Pesticides are a pollutant of significant community concern and are considered a persistent environmental pollutant. Pesticides are used broadly across landscapes due to their importance as control agents for weeds, pests, and diseases, such that there are now no groups of people that remain unexposed to pesticides (Kim et al. 2017). Whilst the most widely reported use of these compounds in coastal catchments is in agricultural areas, they are also used in urban parklands and sporting fields for controlling weeds and pests, and are also present in common household items such as shampoo and building materials (Nicolopoulou-Stamati et al. 2016; Kim et al. 2017). There is evidence that exposure to certain pesticides causes both short term (e.g. skin irritation, dizziness; Fareed et al. 2012; Kim et al. 2017) and long term (e.g. cancer, diabetes; Bassil et al. 2007; Colette Sylvie et al. 2013; Kim et al. 2017) health effects in people. Combined, these three groups of environmental pollutants (PFASs, heavy metals, and pesticides) represent a variety of human health risks and effects (from short-term, non-lethal effects, to long term and potentially lethal effects), sources in coastal areas (from both point and non-point source releases), and potential bioaccumulation pathways, and so are the focal pollutants for this study.

Quantifying the extent and likely drivers of pollutant contamination in seafood is vital in understanding the effects of human impacts on coastal ecosystems, how these impacts might reduce the value of fisheries assets, and whether these sorts of impacts have potential implications for human health. In this study, we quantify the concentrations of a suite of PFASs, pesticides and heavy metals in seafood captured from estuaries in southeast Queensland, Australia. Southeast Queensland estuaries are an ideal region to test for these effects because the region has a broad suite of human impacts, land uses and potential sources of pollutants in the catchment, and these catchments release into estuaries with a diversity of sizes and extent of marine vegetation (Gilby et al. 2017a; Olds et al. 2018b). There has been increasing concern amongst the public in this region about the health risks associated with consuming some coastal seafood species, especially after some recent high-profile spills of pollutants into estuaries in the region. We chose two species from this study region that are important commercial and recreational targets for local fisheries, but also represent two fundamentally different lifecycles of coastal organisms that might result in different exposure to pollutants. Yellowfin bream *Acanthopagrus australis* are one of the most commonly targeted recreational finfish species in Queensland (Webley et al. 2015), and are a generalist consumer in estuarine ecosystems (Froese and Pauly 2019), meaning that they are exposed to multiple pollutant accumulation pathways. Giant mud crabs *Scylla serrata* are a prized recreational and commercial species in Queensland due to their size (often >25 cm carapace width) and ample consumable flesh (Alberts-Hubatsch et al. 2015; Webley et al. 2015). Giant mud crabs are top benthic predators and consume a variety of vertebrate and invertebrate fauna from sediments. They also create large burrows in sediments in coastal ecosystems (Alberts-Hubatsch et al. 2015). Consequently, they are particularly likely to be exposed to sediment-bound pollutants.

Methods

Study region

We collected yellowfin bream and mud crabs from 13 estuaries in southeast Queensland, Australia (Figure 1). These estuaries have previously been sampled for both broad, and habitat-specific patterns in fish assemblages, and so we have a good understanding of the factors influencing the distribution of fish in these estuaries (Gilby et al. 2017a; Gilby et al. 2017d; Gilby et al. 2018c; Olds et al. 2018b). Broadly, the abundance and diversity of fish assemblages are shaped by the extent and connectivity of both natural and anthropogenic habitats in these coastal ecosystems. Some species targeted by fisheries in the region are not adversely affected by significant urbanisation either instream or in the surrounding catchment (Brook et al. 2018b; Olds et al. 2018b), so it is plausible that some fisheries species have long-term exposure to pollutants in some estuaries. Our sampling extent within each estuary therefore followed the extent of these previously completed surveys; from the estuary mouth, to the point in the estuary where long-term winter salinity values (from the previous 10 years of monitoring) averaged 30ppt (EHMP 2019). This range allows for standardisation of the salinity range in which we sample in estuaries that vary widely in hydrology and size, and focused surveys in areas towards the mouths of the estuary where pollutants concentrate before flowing into the open ocean.

We sourced the most recent land use information from the Queensland State Government (Queensland Government 2015b), and clipped the land use layer for the catchment of each estuary sampled (Queensland Government 2015a) in QGIS (QGIS Development Team 2019). We then calculated the cover of primary and secondary land use types within the catchment of each estuary. Overall, 26% of total land use in the region was protected in conservation areas and natural environments, which represents the lowest impact land use category, and likely lowest sources of pollutants. The area of conservation areas and natural environments ranged between 18.6 and 1210 km^2, or 12.7 to 51.0% of land use within catchments (Figure 1, Figure S1, Table S1). Production from relatively natural environments incorporates agricultural production from relatively unmodified ecosystems, including grazing of natural vegetation, and comprised 35% of the region's land use. The area of production from relatively natural environments ranged between 9.9 and 1607.6 km^2, or 6.7 to 46.4% of land use within catchments (Figure 1, Figure S1, Table S1). Intensive uses encompass highly modified landscapes for residential, mining, manufacturing, waste treatment and disposal and other services, and comprised 23% of the total regional land use. The area of intensive uses ranged between 0.7 and 360.0 km^2, or 12.3 to 90.1% of land use within catchments (Figure 1, Figure S1, Table S1). Production from dryland agriculture such as sugar, plantation forestry and other dryland cropping comprised 9% of regional land use. The area of production from dryland agriculture ranged between 38.0 and 844.6 km^2, or 0.49 to 89.1% of land use within catchments (Figure 1, Figure S1, Table S1). Production from irrigated agriculture and plantations (e.g. production from irrigated cropping and pastures; 2%) and waterbodies (e.g. rivers, wetlands and reservoirs; 5%) had the area of lowest land uses throughout the region (Figure 1, Figure S1, Table S1). The estuaries surveyed therefore represent the full range of conditions present in estuaries throughout south-east Queensland (Gilby et al. 2017d), ranging from estuaries that have catchment heavily urbanised by humans, to estuaries with up to 50% of the catchment area protected in conservation areas (Figure 1, 2, Figure S1, Table S1). Consequently, we sampled a range of estuaries from very highly impacted, with many potential sources of pollutants, to estuaries that have much less urbanisation, and so have fewer potential sources of pollutants.

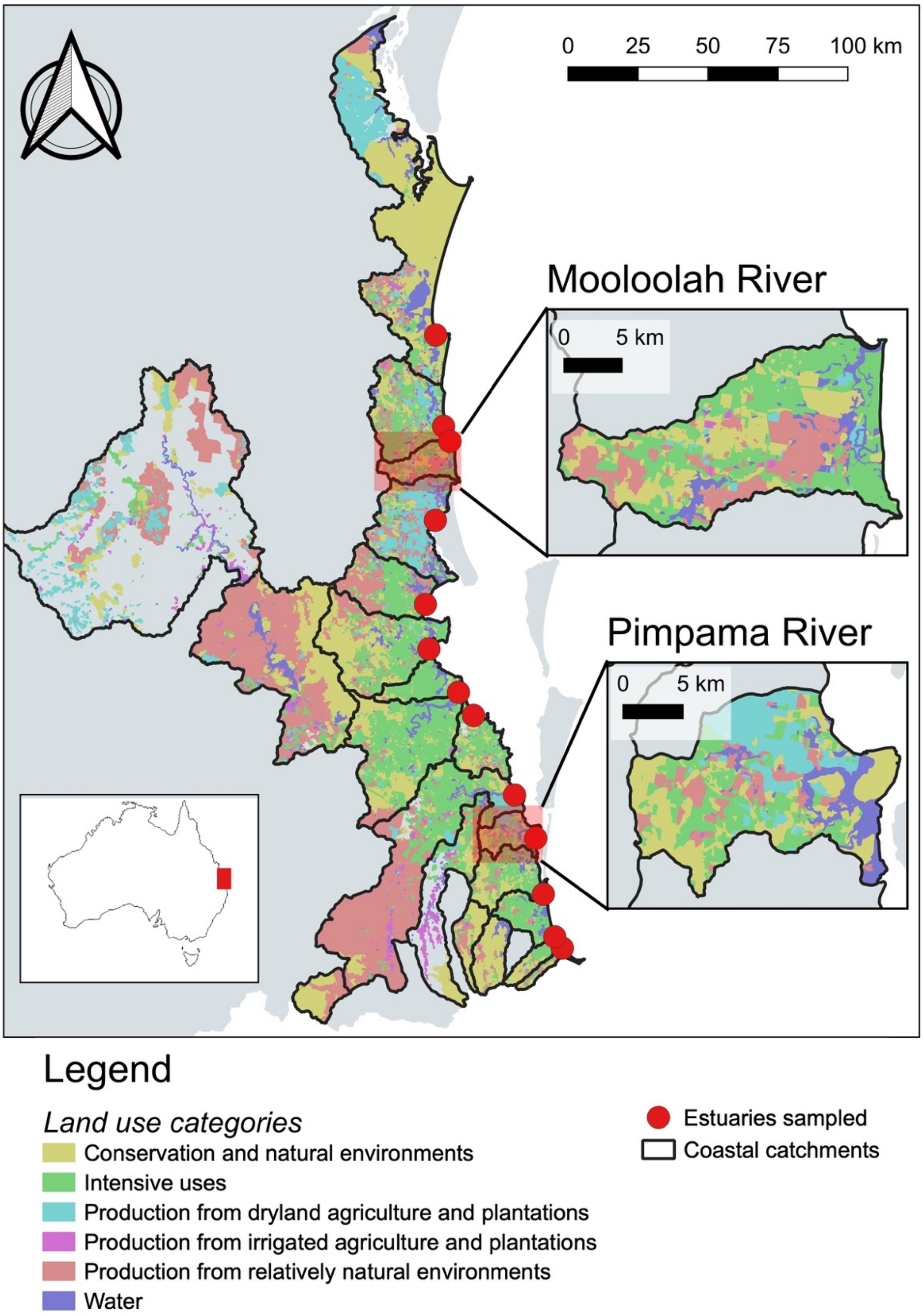

Figure 1 Map of sampled estuaries and their land uses with the surrounding catchment.

Figure 2 Krona Plot of catchment land use types for (a) all 13 catchments in the study and (b) Mooloolah and (c) Pimpama Rivers. Colours match with colours for land use categories in figure 1.

Specimen collections and pollutant analysis

We collected between three and five legal sized (>25cm total length) yellowfin bream from each estuary (for a total of 57 yellowfin bream) using gill nets, cast nets, and angling between June and September 2018. We collected between 2 and 9 legal sized (>15cm carapace width males only) giant mud crabs from each estuary (for a total of 65 giant mud crabs) between September 2018 and February 2019 using 4 entry crab pots baited with sea mullet *Mugil cephalus*. Upon capture, all specimens were euthanized via blunt trauma to the cranium (according to USC animal ethics protocol ANA18126), placed into food grade Ziploc bags and were frozen at -20°C upon return to the laboratory. We quantified the concentrations of pollutants in the flesh of edible sized portions from each individual; flesh from shelled, legal sized male mud crabs and descaled whole fillets, with the skin on from legal sized yellowfin bream. Overall, we quantified the concentration of 14 heavy metals, including 4 arsenic species, 37 pesticides, and 19 PFASs (Table 1). The concentration of pesticides and arsenic species were quantified using compound samples of all samples of yellowfin bream and mud crabs separately for each estuary. All collections and sample preparation were conducted according to established protocols for the handling and processing of seafood samples for pollutant analysis to avoid any sample contamination (Queensland Department of Environment and Science 2018) (Appendix 1). Samples were analysed according to established protocols at Queensland Health Forensic and Scientific Services.

Results

We identified low occurrence and concentrations of most pollutants in the consumable flesh of yellowfin bream and giant mud crabs in southeast Queensland. Fifty-two (74%) of our pollutants returned no samples with concentrations above the limit of reporting (Table 1). We found concentrations of 12 heavy metals and six PFASs above the limit of reporting in our samples (Table 1). No samples contained concentrations of any pesticides above the limit of reporting (Table 1). Arsenic, copper, mercury, selenium and zinc were identified in all yellowfin bream and giant mud crab samples. Detectable levels of cobalt (63% of yellowfin bream samples), chromium (14% of yellowfin bream samples), nickel (14% of yellowfin bream samples), lead (7% of yellowfin bream samples and 34% giant mud crab samples), antimony (2% of yellowfin bream samples), titanium (21% of yellowfin bream samples) and cadmium (22% of giant mud crab samples) were also identified (Tables 2, 3). Between 80 and 100% of arsenic in samples was arsenobetaine, and we had no samples that recorded concentrations over the limit of reporting for dimethylarsinic acid, monomethylarsonic acid or inorganic arsenic. PFASs were predominantly identified in giant mud crabs. Here, six PFAS compounds were identified in mud crab samples, but never in more than 33% of samples from individual estuaries (Table 3). Only one PFAS compound was detected in yellowfin bream samples, with 50% (n=2) of samples from Coochin Creek containing trace amounts perfluro-octanesulfunic acid (0.002mg/kg) (Table 2).

For system-specific results for each species and pollutant, see Table 2 and 3.

Table 1 List of pollutants sampled and their limit of reporting. Pollutants which had samples of either bream or mud crabs detected above the limit of reporting given are shown in bold.

Pollutant group Limit of reporting (mg/kg)	**Pollutant**
Metals	
0.005	**Arsenic**, Cadmium, **Mercury**, **Lead**
0.01	**Cobalt, Chromium, Manganese, Nickel, Antimony**
0.04	**Titanium**
0.05	**Copper**, **Selenium**, Tin, **Zinc**
Arsenic speciation *	
0.1	**Arsenobetaine**, Dimethylarsinic Acid, Monomethylarsonic Acid, Inorganic Arsenic
Pesticides *	
0.01	Chlordane cis, Chlordane trans, DDD (o,p), DDD (p,p), DDE (o,p), DDT (p,p), Endrin, HCB, HCH-a, HCH-B, Lindane (HCH-y), Methoxychlor, Nonachlor cis, Nonachlor trans
0.02	Aldrin, Dieldrin, Chlordene, Chlordene epoxide, DDE (pp), DDT (o,p), Endosulfan alpha, Endosulfan beta, Endosulfan ether, Endosulfan sulfate, Endrin aldehyde, HCH-gamma, Heptachlor, Heptachlor epoxide, Oxychlordane
0.04	Total Aldrin and Dieldrin, Total Chlordane, Total HCH isomers, Total Heptachlor
0.05	Chlordene (1-hydroxy), Chlordene (1-OH-2,3-epoxy)
0.06	Total DDT, Total Endosulfan,
PFAS	
0.001	**Perflurobutanoic acid**, Perfluropentanoic acid, **Perflurohexanoic acid**, **Perfluroheptanoic acid**, Perfluroctanoic acid, Perflurononanoic acid, **Perflurodecanoic acid**, Perflurobutanesulfunic acid, Perflurohexanesulfunic acid, **Perflurooctanesulfunic acid, Perflurodecanesulfunic acid**
0.002	Perfluroundecanoic acid, Perflurotridecanoic acid
0.004	Total C4-C10 Sulfonic acids
0.005	Perflurododecanoic acid, Perflurotetradecanoic acid, 4:2 Fluorotelomer sulfonic acid, 8:2 Fluorotelomer sulfonic acid
0.02	Total C4-C14 Carboxylic acids

*samples tested on compound samples of all individuals (separated by species) from each estuary only

Table 2 Frequency of occurrence of pollutants detected over the limit of reporting of analysis, and the average values of those samples for those pollutants in yellowfin bream flesh in southeast Queensland, Australia. '-' indicates that no samples recorded values above the limit of reporting. Values in bold are above Australian food safety standards. All units for average values are mg/kg. F= frequency of occurrence, Av=average values.

Estuary	**Arsenic**		**Cobalt**		**Chromium**		**Copper**		**Mercury**		**M'nese**		**Nickel**		**Lead**		**Antimony**		**Selenium**		**Titanium**		**Zinc**		**Perfluro-octanesulfunic acid**	
	F	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**
Brisbane	1	0.91	0.8	0.02	0	-	1	0.23	1	0.11	1	0.15	0.2	0.01	0.4	0.02	0	-	1	0.35	0.2	0.11	1	9.4	0	-
Caboolture	1	1.28	0.25	0.01	0	-	1	0.22	1	0.24	1	0.12	0.25	0.02	0	-	0	-	1	0.53	0.25	0.05	1	9.7	0	-
Coochin	1	1.08	1	0.04	0	-	1	0.24	1	0.24	1	0.18	0	-	0	-	0	-	1	0.37	0	-	1	12.3	0.5	0.002
Currumbin	1	1.47	0.2	0.01	0	-	1	0.27	1	0.35	1	0.06	0	-	0	-	0.2	0.02	1	0.45	0	-	1	12.8	0	-
Logan	1	1.02	1	0.02	0.2	0.01	1	0.35	1	0.19	1	0.09	0.4	0.01	0	-	0	-	1	0.43	0.2	0.04	1	9.5	0	-
Maroochy	1	1.25	0.2	0.02	0	-	1	0.31	1	0.29	1	0.12	0	-	0	-	0	-	1	0.46	0.2	0.05	1	8.9	0	-
Mooloolah	1	2.48	0.6	0.01	0.2	0.29	1	0.24	**1**	**0.51**	1	0.07	0.2	0.01	0	-	0	-	1	0.51	0	-	1	10.0	0	-
Nerang	1	1.15	0.4	0.02	0	0.03	1	0.23	1	0.14	1	0.06	0	0.01	0	-	0	-	1	0.34	0.4	-	1	10.3	0	-
Noosa	1	1.20	0.33	0.02	0.33	-	1	0.22	1	0.26	1	0.24	0.33	-	0	-	0	-	1	0.94	0	0.04	1	11.5	0	-
Pimpama	1	1.54	0.8	0.07	0.2	0.01	1	0.26	1	0.28	1	0.23	0.2	0.01	0	-	0	-	1	0.38	0.6	0.06	1	12.3	0	-
Pine	1	0.93	1	0.03	1	0.02	1	0.32	1	0.18	1	0.14	0.33	0.01	0	-	0	-	1	0.43	0.66	0.06	1	9.6	0	-
Tallebudgera	1	1.68	0.4	0.03	0.2	0.04	1	0.28	1	0.29	1	0.07	0	-	0.2	0.01	0	-	1	0.42	0.2	0.05	1	10.3	0	-
Tingalpa	1	1.43	0.33	0.01	0	-	1	0.19	1	0.12	1	0.20	0	-	0.33	0.01	0	-	1	0.42	0	-	1	8.9	0	-
All estuaries	1	1.34	0.63	0.03	0.14	0.05	1	0.26	1	0.25	1	0.13	0.14	0.01	0.07	0.01	0.02	0.02	1	0.47	0.21	0.06	1	10.4	0.04	0.002

Table 3 Frequency of occurrence of pollutants detected over the limit of reporting of analysis, and the average values of those samples for those pollutants in mud crab flesh in southeast Queensland, Australia. '-' indicates that no samples recorded values above the limit of reporting. All units for average values are mg/kg. F= frequency of occurrence, Av=average values.

Estuary	**Arsenic**		**Cadmium**		**Copper**		**Lead**		**Mercury**		**Selenium**		**Zinc**		**Perfluoro-butanoic acid**		**Perfluoro-decanoic acid**		**Perfluoro-heptanoic acid**		**Perfluoro-hexanesulfonic acid**		**Perfluoro-octanesulfonic acid**		**Perfluoro-octanoic acid**	
	F	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**	**F**	**Av**
Brisbane	1	4.4	0.25	0.02	1	9.0	1	0.01	1	0.04	1	1.11	1	45.25	0	-	0	-	0	-	0	-	0	-	0	-
Caboolture	1	3.0	0.75	0.03	1	7.9	0	-	1	0.03	1	0.95	1	35.00	0.25	0.001	0	-	0	-	0	-	0	-	0	-
Coochin	1	1.3	0.33	0.01	1	5.6	0	-	1	0.04	1	0.81	1	45.67	0	-	0	-	0	-	0	-	0.33	0.001	0	-
Currumbin	1	0.9	0.4	0.02	1	5.0	0.2	0.01	1	0.02	1	0.38	1	44.40	0	-	0	-	0	-	0	-	0	-	0	-
Logan	1	2.5	0.22	0.01	1	7.8	0.22	0.09	1	0.03	1	1.08	1	42.56	0.11	0.0009	0.11	0.001	0.11	0.0009	0.11	0.003	0.11	0.002	0.11	0.007
Maroochy	1	0.9	0.17	0.01	1	10.9	0.33	0.04	1	0.06	1	0.46	1	38.33	0	-	0	-	0	-	0	-	0	-	0	-
Mooloolah	1	1.8	0.66	0.01	1	11.5	0.33	0.01	1	0.03	1	0.64	1	33.00	0	-	0	-	0	-	0	-	0	-	0	-
Nerang	1	0.6	0	-	1	4.7	0	-	1	0.03	1	0.39	1	48.50	0	-	0.5	0.002	0	-	0	-	0	-	0	-
Noosa	1	2.6	0.25	0.01	1	7.1	0.75	0.02	1	0.13	1	1.40	1	50.50	0	-	0	-	0	-	0	-	0	-	0	-
Pimpama	1	2.0	0	-	1	6.5	0.22	0.01	1	0.03	1	0.67	1	36.22	0	-	0	-	0	-	0	-	0	-	0	-
Pine	1	2.6	0	-	1	7.9	0.6	0.01	1	0.03	1	0.63	1	38.40	0	-	0	-	0	-	0	-	0.2	0.001	0	-
Tallebudgera	1	2.0	0	-	1	5.0	0.5	0.01	1	0.03	1	0.77	1	34.25	0	-	0	-	0	-	0	-	0	-	0	-
Tingalpa	1	3.0	0.14	0.02	1	7.2	0.28	0.06	1	0.03	1	0.92	1	39.29	0	-	0	-	0	-	0	-	0	0.001	0	-
Grand Total	1	2.2	0.22	0.02	1	7.5	0.34	0.02	1	0.04	1	0.80	1	40.32	0.03	0.001	0.03	0.002	0.015	0.0009	0.02	0.003	0.06	0.001	0.02	0.007

Discussion

Expansion and intensification of human land use in coastal catchments (Halpern et al. 2019) results in a greater diversity and volume of pollutants being released into waterways (Murray et al. 2010; Bosch et al. 2016; Jian et al. 2017). Because some pollutants can bioaccumulate into the consumable flesh of key seafood species, it is likely that human exposure to pollutants might be high in some heavily modified coastal areas (Wen et al. 2017; Warwick et al. 2018; Barletta et al. 2019). Despite this, we found low occurrences and concentrations of all pollutants surveyed in giant mud crabs and yellowfin bream in southeast Queensland, Australia; a region that we hypothesised would have hotspots for the accumulation of certain pollutants given the extensive and diverse land uses present. Here, only the concentration of mercury in one estuary was above Australian national guidelines for food safety (Australian Government 2017; Food Standards Australia and New Zealand 2018). Given the wide variety of environmental conditions sampled, the original intent of this study was to quantify relationships between land uses and concentrations of key environmental pollutants in southeast Queensland. However, these detailed analyses were not possible given the results found. These results broadly follow the findings of a similar study conducted over a smaller spatial extent in this region (Waltham et al., 2011). We found some differences in the diversity and concentrations of pollutants in the flesh of the two seafood species surveyed. Mud crabs had a lower diversity of heavy metals detected above limits of reporting, but a higher prevalence of PFASs than for yellowfin bream. These levels were, however, consistently lower than trigger levels in local seafood safety guidelines (Australian Government 2017; Food Standards Australia and New Zealand 2018). This supports the notion of different exposure pathways between these two species. Our results highlight the risks of generalising either land use or perceived impact levels to likely pollutant levels in coastal seafood species, and suggest context-specific risk of pollutant accumulation. Therefore, these results have important consequences for the value of some coastal fisheries that might be perceived as potentially harmful to people in urbanised systems, and stress the importance of surveying broadly across regions to quantify potential risks and pollutant levels in coastal seafood species.

We identified concentrations of a variety of heavy metals above the limits of reporting of chemical analyses in our samples. However, these concentrations were essentially all below Australian standards for seafood, some significantly so (Australian Government 2017). The majority of arsenic found in our samples was arsenobetaine, which is considered to be non-toxic (Sloth et al. 2005), and we found no samples with detectable levels of the more dangerous arsenic species. The key exception was that we found concentrations of mercury higher than local food safety standards (set at 0.5 mg/kg) in the Mooloolah River (Australian Government 2017). The Mooloolah River occurs in the northern part of our study region, with 39% of its catchment subject to intensive human use, 21% conserved or in relatively natural condition, and 30% subject to agricultural production on relatively natural ecosystems like grasslands. Consequently, there are no attributes of land use in the Mooloolah River that set it significantly apart from land use in other estuaries. The Mooloolah River does, however, have a large harbor towards the estuary mouth that is a major port for local fisheries; the only estuary in our study with this spatial arrangement around a relatively narrow estuary mouth. However, these attributes are unlikely to be contributing to the patterns found here because mercury accumulation in fish is more influenced by concentrations in sediments and water column, and there's unlikely to be major sources of mercury in the harbor (Calta and Canpolat 2006; Verdouw et al. 2010). The potential causes of the spike in mercury levels could be investigated further in this estuary, especially if further surveys how broader effects across higher trophic level species.

In addition to these low concentrations of heavy metals in our samples, we found low occurrences and low concentrations of both pesticides and PFASs. Concentrations of PFASs were significantly lower than levels that trigger broader investigations in Australian national food safety standard guidelines (Food Standards Australia and New Zealand 2018). No samples had detectable levels of pesticides. This somewhat surprising result has several potential explanations in southeast Queensland. Many of the estuaries we sampled are large estuaries with wide inlets that open either to the ocean or large embayments (Gilby et al. 2017a). Consequently, it could be hypothesised that any pollutant releases are quickly diluted and released into the open ocean via tidal flushing and river flows. These effects might, however, be considered unlikely because we also surveyed relatively small estuaries, with lower flow, narrower estuary inlets, longer water residence times, and a diversity of potential pollutant sources in this study (Gilby et al. 2017a).

We surveyed the concentrations of pollutants in two key coastal fisheries species that have fundamentally different ecological and biological traits. These species were chosen to represent two particular groups of coastal fisheries targets (yellowfin bream- mobile generalist benthic consumer, and giant mud crab- benthic predator). However, there may be attributes of the species chosen that reduce their likelihood of accumulating pollutants. For example, giant mud crabs are top benthic predators, that live in close association with the benthos (Alberts-Hubatsch et al. 2015); we hypothesised that this would increase their exposure to a diversity of pollutants. Whilst this may have been the case for PFASs, where we identified a higher diversity and occurrence of detectable pollutants in giant mud crabs than in yellowfin bream, the giant mud crabs relatively low trophic level may not expose them to significant bioaccumulation risk that other, higher level predators might be exposed to. Therefore, surveying other, higher level consumers may yield different results. For example, surveying large, mobile piscivorous fish that inhabit the mouths of estuaries (like flatheads, trevallies or snappers) might yield different results as the capacity for pollutants to bioaccumulate in these longer-lived, large predatory fishes might be greater (Pourang 1995; Power et al. 2002; El-Moselhy et al. 2014). Therefore, we recommend surveying more broadly across both land use intensity and the trophic spectra to ensure the consistence of our results in the region. Previous studies have, however, found reportable levels of PFASs and other pollutants in the species that we surveyed in this study (e.g. Kamaruzzaman et al. 2012; Taylor and Johnson 2016).

We hypothesised that the diverse land use and extent of urbanisation present in southeast Queensland would result in higher concentrations and diversity of pollutants in seafood. An alternative explanation for the lack of pollutants in our samples might be that the region either is simply not modified enough, or contain enough potential sources of pollutants relative to other regions where these effects have been established (Gu et al. 2015; Jian et al. 2017; Tepe et al. 2017), or that local regulations around pollutant releases are stringent and well enforced enough to limit these effects. Combined, these potential effects may result in the low occurrences and concentration of pollutants detected here. Reported occurrences of large releases of pollutants are rare in southeast Queensland, and are therefore covered extensively by local media. Public concerns regarding potentially harmful concentrations of pollutants often increase around these events, potentially leading to poorer perceptions of the health and condition of coastal fisheries in the long run. In any case, these results highlight the need quantify the concentration and prevalence of pollutants in individual species and regions to establish potential exposure risk of pollutants to people.

In this study, we quantified the concentration of suite of potential environmental pollutants that could be considered harmful to people in two coastal fisheries species within southeast Queensland. Given the diversity of potential environmental pollutants present in coastal ecosystems globally and the diversity of species harvested for seafood (Webley et al. 2015; FAO 2018), the results of this study do not indicate a complete lack of potentially harmful pollutants in all seafood in the region. For example, global studies of flathead mullet *Mugil cephalus* indicate context specific effects of human impacts on pollutant levels in consumable flesh (Waltham et al., 2013). Despite this, the results of this study are positive indication of potentially lower risks of many environmental pollutants that are of strong concern to people within southeast Queensland. They also support the notion that perceived risk and broad land use information might be poor indicators of exposure to pollutants through seafood in some regions. We stress however, the importance of thorough surveys across a wider variety of seafood species and a greater number and diversity of pollutants in order to more thoroughly quantify these affects both within this region and beyond

Supplementary Materials

Table S1a List of sampled estuaries and the area (in km^2) of each primary (in bold) and secondary (in italics) land use categories in each catchment.

Land Use	Currumbin Creek	Tallebudgera Creek	Nerang River	Pimpama River	Logan River	Tingalpa Creek	Brisbane River
Conservation and natural environments	18.62	52.52	209.52	62.46	402.20	71.75	1027.08
Managed resource protection	0.00	1.16	57.03	1.84	5.43	4.33	155.34
Nature conservation	5.99	14.10	103.01	23.41	250.03	39.98	488.46
Other minimal use	12.63	37.26	49.48	37.21	146.74	27.44	383.28
Intensive uses	37.97	44.54	182.90	50.64	583.53	179.35	844.63
Intensive animal production	0.00	0.04	0.22	1.14	17.88	1.65	4.12
Intensive horticulture	0.11	0.00	0.25	0.07	3.89	1.17	1.64
Manufacturing and industrial	0.53	0.15	2.49	0.12	21.64	2.84	55.45
Mining	0.00	0.21	0.74	0.79	8.87	0.90	8.21
Residential	27.64	37.02	124.67	37.96	448.67	136.13	603.61
Services	6.51	6.36	47.58	7.23	68.52	34.74	133.09
Transport and communication	2.70	0.55	4.70	2.49	8.63	0.75	27.84
Utilities	0.11	0.03	1.13	0.44	0.42	0.10	5.34
Waste treatment and disposal	0.37	0.17	1.11	0.39	5.01	1.08	5.32
Production from dryland agriculture and plantations	0.73	1.10	2.37	43.27	95.44	2.17	359.28
Cropping	0.00	0.00	0.34	0.70	0.54	0.00	66.85
Cropping - Sugar	0.00	0.00	0.00	36.05	39.32	0.00	0.00
Grazing modified pastures	0.00	0.00	0.00	0.77	11.60	0.00	29.92
Land in transition	0.10	0.30	1.41	5.75	18.78	1.39	12.92
Perennial horticulture	0.52	0.80	0.06	0.00	0.50	0.53	7.67
Plantation forestry	0.11	0.00	0.57	0.00	24.65	0.03	241.66
Seasonal horticulture	0.00	0.00	0.00	0.00	0.05	0.21	0.25
Production from irrigated agriculture and plantations	0.00	0.00	0.93	0.56	99.32	2.99	76.86
Irrigated cropping	0.00	0.00	0.00	0.00	57.72	0.24	36.23
Irrigated cropping - Sugar	0.00	0.00	0.00	0.00	0.26	0.00	0.00
Irrigated land in transition	0.00	0.00	0.00	0.00	0.18	0.00	0.09
Irrigated modified pastures	0.00	0.00	0.75	0.00	28.22	0.00	6.34
Irrigated perennial horticulture	0.00	0.00	0.16	0.45	3.74	0.84	7.18
Irrigated plantation forestry	0.00	0.00	0.00	0.00	0.00	0.00	2.72
Irrigated seasonal horticulture	0.00	0.00	0.03	0.11	9.20	1.92	24.31
Production from relatively natural environments	9.89	12.11	46.21	24.00	1026.82	16.45	1607.58
Grazing native vegetation	9.89	11.96	42.33	24.00	1024.39	16.45	1119.61
Production forestry	0.00	0.15	3.87	0.00	2.43	0.00	487.97
Water	1.44	2.08	28.41	23.37	40.09	14.05	187.16
Channel/aqueduct	0.08	0.00	0.07	0.00	0.00	0.00	1.00
Estuary/coastal waters	0.21	0.08	0.03	0.00	0.09	0.04	0.01
Lake	0.27	0.00	4.06	0.00	0.01	0.05	0.23
Marsh/wetland	0.00	0.04	0.00	17.70	21.30	6.82	11.14
Reservoir/dam	0.23	0.52	13.65	1.29	9.18	5.89	135.00
River	0.65	1.44	10.60	4.38	9.50	1.25	39.78

Table 1b List of sampled estuaries and the area (in km^2) of each primary (in bold) and secondary (in italics) land use categories in each catchment.

Land Use	Pine River	Caboolture River	Coochin Creek	Mooloolah River	Maroochy River	Noosa River	Grand Total
Conservation and natural environments	358.84	70.76	87.74	57.38	134.16	1210.41	4013.42
Managed resource protection	41.51	0.70	0.92	1.54	3.29	20.89	294.53
Nature conservation	132.31	16.43	45.41	21.63	73.82	808.68	2120.50
Other minimal use	185.01	53.64	41.41	34.20	57.06	380.85	1598.39
Intensive uses	330.18	222.23	96.69	104.41	285.34	124.54	3457.05
Intensive animal production	1.04	2.90	5.53	0.78	2.73	0.54	39.00
Intensive horticulture	0.30	0.90	0.92	0.05	2.88	0.48	13.50
Manufacturing and industrial	3.62	2.22	1.69	1.59	5.36	1.74	104.84
Mining	5.56	3.33	2.40	1.49	2.02	1.41	39.14
Residential	251.17	185.19	69.88	83.63	242.23	104.56	2605.94
Services	54.23	20.23	7.63	12.78	20.24	13.12	511.52
Transport and communication	7.58	5.76	7.78	3.52	8.80	1.34	107.20
Utilities	4.83	0.40	0.00	0.17	0.11	0.00	13.84
Waste treatment and disposal	1.84	1.31	0.87	0.40	0.95	1.34	22.07
Production from dryland agriculture and plantations	15.97	20.08	297.06	5.67	74.23	330.18	1261.75
Cropping	0.05	0.13	0.84	0.18	0.10	5.91	75.63
Cropping - Sugar	0.00	0.00	2.23	0.00	37.32	0.18	116.00
Grazing modified pastures	0.00	0.00	0.00	0.00	0.00	2.52	44.81
Land in transition	9.91	3.09	46.22	3.95	26.03	1.82	137.23
Perennial horticulture	3.79	9.54	44.96	1.29	10.54	0.52	81.37
Plantation forestry	2.22	7.32	202.81	0.25	0.20	319.23	806.09
Seasonal horticulture	0.00	0.00	0.00	0.00	0.04	0.00	0.62
Production from irrigated agriculture and plantations	1.25	10.40	16.43	1.18	13.35	13.52	241.18
Irrigated cropping	0.43	0.58	1.50	0.00	0.82	0.19	97.72
Irrigated cropping - Sugar	0.00	0.00	0.00	0.00	0.00	0.57	0.83
Irrigated land in transition	0.21	0.00	0.00	0.00	0.03	0.00	0.51
Irrigated modified pastures	0.00	1.08	0.23	0.00	1.78	6.78	48.46
Irrigated perennial horticulture	0.22	4.49	10.62	0.96	6.89	5.34	41.69
Irrigated plantation forestry	0.00	0.00	0.00	0.00	0.00	0.00	2.72
Irrigated seasonal horticulture	0.39	4.25	4.09	0.22	3.83	0.65	49.25
Production from relatively natural environments	153.72	163.99	106.13	54.78	115.43	185.54	3610.42
Grazing native vegetation	153.54	155.97	47.99	47.76	114.77	149.76	3006.03
Production forestry	0.18	8.01	58.14	7.01	0.66	35.77	604.39
Water	61.02	24.69	70.57	23.11	35.78	180.57	727.77
Channel/aqueduct	0.00	0.00	0.00	0.00	0.00	0.09	1.24
Estuary/coastal waters	0.67	0.12	2.96	1.90	7.37	75.87	90.13
Lake	0.00	0.56	0.61	0.28	0.41	2.40	11.30
Marsh/wetland	24.37	18.34	56.13	12.75	20.66	99.72	307.13
Reservoir/dam	30.38	1.99	6.80	5.18	6.16	1.04	219.45
River	5.60	3.69	4.07	3.00	1.17	1.46	98.53

Chapter 2

Human modifications change the morphology and functional roles of coastal fish

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Introduction

Increasing and expanding human populations modify ecosystems globally (Vitousek et al. 1997). These landscape transformations modify the condition and connectedness of ecosystems (Kareiva et al. 2007), the structure of food webs (Lotze et al. 2011), and the distribution of biodiversity (Gámez-Virués et al. 2015). Landscape transformations also change the rate and modify the distribution of key ecological functions (Hector and Bagchi 2007; Olds et al. 2018a), and this can result in reductions in the condition of ecosystems (Lotze et al. 2011) and further reductions in ecosystem services (Cardinale et al. 2012; Haddad et al. 2015). However, many species adapt and survive within modified ecosystems, and these species can help maintain key ecological functions that provide resistance to further ecosystem degradation (Clavel et al. 2011; Aronson et al. 2014). Whilst the effects of landscape transformation on biodiversity, food webs, and ecological functions is increasingly well understood, the adaptive capacity of species that persist in modified ecosystems remains poorly understood (Cardinale et al. 2012; Haddad et al. 2015). An organism's functional role is defined by its dietary requirements so structural changes to ecosystems and food webs can modify the suite of available niches, and the broader functioning of ecosystems (Toyama et al. 2018). Therefore, understanding the ways in which different species and functional groups can adapt to human stressors is an important research gap in many ecosystems.

Approximately 40% of the world's population lives within 100 kilometres of coastlines (Chaudhary and Pisolkar 2019). Coastal ecosystems are therefore hotspots for the combined influence of direct (e.g. habitat loss, overharvesting) and indirect (e.g. runoff from modified catchments, sedimentation) anthropogenic impacts that modify the condition and functioning of ecosystems (Halpern et al. 2007). Marine vegetation such as mangrove forests, salt marshes, and seagrass meadows are particularly vulnerable to these threats (Tscharntke et al. 2012; Firth et al. 2016). These ecosystems have a disproportionately large effect on the abundance and distribution of marine fauna, so their loss significantly modifies the broader condition of coastal systems, and the provision of ecosystems services like fisheries (Kornis et al. 2017; Gilby et al. 2018b). For example, between 50 and 65 percent of tidal wetlands have been lost globally due to direct replacement by hardened shorelines (e.g. concrete structures such as seawalls, jetties, and groins) (Kornis et al. 2017). This reduces the availability of quality habitat for fish, and can result in changes in fish species diversity and abundance both locally and throughout coastal seascapes (Connolly 2003; Gilby et al. 2018b). Similarly, the effects of indirect impacts such as eutrophication are increasing (Taira et al. 2018). Effluent discharge and runoff from agricultural lands into coastal systems can cause phytoplankton blooms, which can lead to eutrophication, food web destabilisation, habitat loss and subsequent change in faunal assemblages (Lee et al. 2006). Therefore, increasing anthropogenic modifications to marine habitats alters the composition of fish and invertebrate assemblages within coastal ecosystems (Whitfield and Elliott 2002; Hooper et al. 2005). Many coastal fish species are, however, adaptable to these landscape modifications and can change their diets and therefore their functional role within ecosystems to suit the resources available within ecosystems (Fox and Bellwood 2011; Olds et al. 2018a). The ways in which different species or functional groups respond to these anthropogenic impacts is, however, poorly understood.

Estuaries provide a suite of ecosystem services (e.g. nursery habitat for fish, coastal protection, nutrient sequestration), but are under increasing threat from human activities globally (Elliott et al. 2019). Many estuarine fish species can adapt to ecosystem modification because they are naturally plastic in their diets and morphology (Whitfield and Elliott 2002; Elliott et al. 2007b). Morphological changes in fish are often associated with diet, development, growth rate, nutrition, and linked environmental variables (e.g. habitat structure, water temperature, predation pressure, and currents) (Canty et al. 2018; Floeter et al. 2018). For example, urbanisation and other catchment modifications can change the quality, quantity and composition of food items in estuaries (Schlacher and Wooldridge 1996a; Schlacher and Wooldridge 1996b; Martin et al. 2009). This can modify body condition and organism health (e.g. Whitfield and Elliott 2002; Schlacher et al. 2007), internal (e.g. Mutchler et al. 2014) and external morphology (especially of the head)(e.g. Burress et al. 2016), and the structure and function of feeding appendages (especially of jaws, teeth, and mouth position) (e.g. Eggold and Motta 1992) in some fish populations. Changes in diet amount to changes in ecological niches, and therefore the functional role of species, and so such changes might have broader implications across modified ecosystems (Bruno and O'Connor 2005; Leitão et al. 2018). Changes in fish morphology caused by changes in feeding mechanisms or alterations to body shapes for refuge are often used as a tool to predict the functional roles of fish within ecosystems (Wainwright 1996; Goatley and Bellwood 2009; Bellwood et al. 2014). Because diet and morphological changes can potentially be tied to changes in the condition of estuarine ecosystems and the species that inhabit them (some of which are of commercial and recreational significance), these valuable resources are a priority for conservation and management planning (Halpern et al. 2007; Unsworth and Cullen 2010).

Modifications to ecosystems can lead to changes in the condition, diet and ecological niches of animals, that perform important ecological roles and functions (Cardinale et al. 2012;

Haddad et al. 2015) which may prevent ecosystem collapse or provide ecosystem services (Devictor et al. 2008; Olds et al. 2018a). The effects of landscape transformation have been well established for fish assemblage composition (Clynick et al. 2008), and body condition of animals in coastal ecosystems (Taylor and Miller 2001; Hartman 2003). However, the ways human modifications combine to modify both fish functional roles (i.e. diet) and morphology together remain poorly understood (Meillère et al. 2015). In this study, we test whether human modifications to estuaries and their catchments change the diet composition and morphology of fish. We assess this for four fish species, each representing a key trophic group found in estuaries; zoobenthivore (yellowfin bream *Acanthopagrus australis*), zooplanktivore (southern herring *Herklotsichthys castelnaui*), detritivore (sea mullet *Mugil cephalus*) and piscivore (dusky flathead *Platycephalus fuscus*). We hypothesised that changes in diet and morphology would correlate with changes in environmental conditions of estuaries, especially with respect to the level of urbanisation along the estuary and throughout the catchment, and the amount of marine vegetation (especially mangroves and seagrasses) within the estuary, but that the direction and strength of these effects would differ among functional groups (Figure 1). We also gauged whether changes to morphology are associated with changes to diet and vice versa (Figure 1).

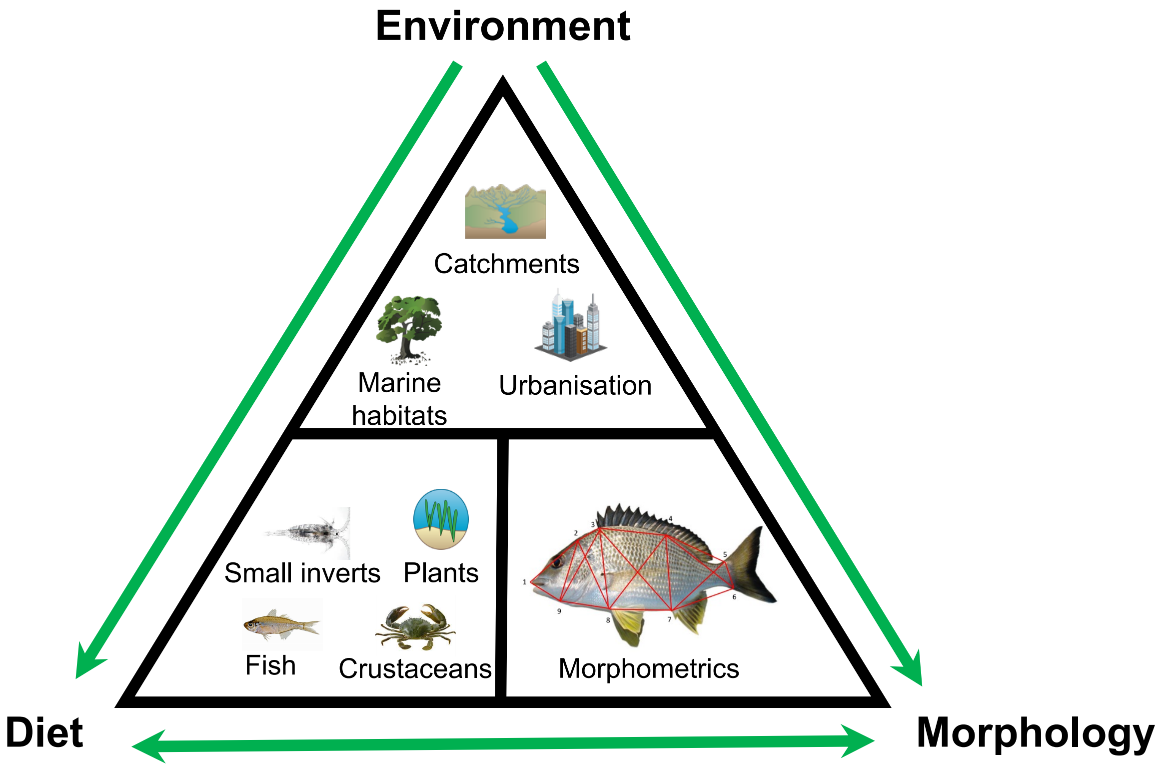


Figure 1 Conceptual diagram illustrating the hypothetical relationship between fish and changes in the environmental conditions of estuaries and their catchments. We specifically tested whether fish morphology and diet composition change in response to anthropogenic landscape modifications (e.g. conversion of wetlands to urban lands), remaining marine vegetation (e.g. seagrass and mangrove areas), and water quality (e.g. turbidity, chl-a; Table 1).

Methods

Study region

Fish were collected from July to September 2018 from 13 estuaries spanning approximately 230 km of coastline in southeast Queensland, Australia (Figure 2). The region is appropriate to test for the effects of urbanisation on estuarine systems due to growing population pressure (~4.7 million people increasing at >2% per year) (ABS 2015) causing an expanding urban footprint at the cost of mangroves and seagrass in the coastal strip and more intensive agriculture in the catchments (e.g. Lee et al. 2006; Elliott et al. 2019). The estuaries sampled encompass a broad suite of environmental conditions, ranging from relatively undisturbed systems (e.g. Coochin Creek), to floodplains where the estuarine banks are entirely urban land (e.g. Nerang River) (Figure 2, Table S1).

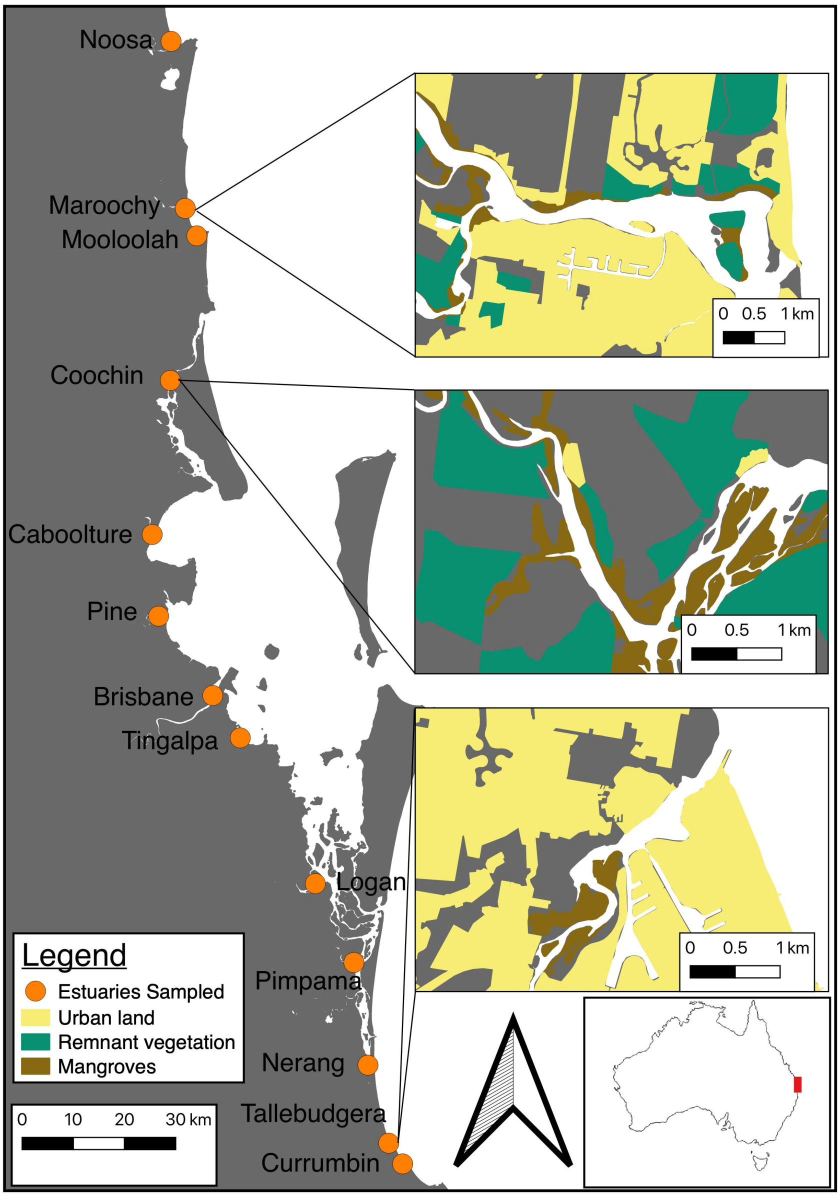


Figure 2 Location of selected estuaries from which fish were sampled for analyses of their body shapes and diet composition in SE Queensland, Australia. Estuaries selected represent a range of habitat complexities within the estuaries. Inset maps give examples of intermediately (top panel), low (middle panel) and highly (bottom panel) urbanised estuaries within this region.

Fish species and sample collections

We selected representative species from four functional groups to examine how human modifications to estuaries may affect estuarine fish species with different biological traits. The species chosen are the most abundant and widespread example of the main functional groups in the region (Gilby et al. 2018b). Yellowfin bream, *Acanthopagrus australis,* is a zoobenthivore that feeds opportunistically on a variety of benthic invertebrates (e.g. molluscs, polychaetes, crustaceans) from a several substrates (e.g. rock, seagrass, mangroves, sand and mud) (Pollock 1982a; Hadwen et al. 2007). Southern herring, *Herklotsichthys castelnaui,* is a small (<15 cm) zooplanktivore common in estuaries of the region during spring and summer (Abrantes and Sheaves 2009; Gray et al. 2011; Waltham and Connolly 2013). Sea mullet, *Mugil cephalus,* is a benthic detritivore abundant throughout the year in coastal waters of eastern Australia (Pastor et al. 1996; Bell et al. 2005). Dusky flathead, *Platycephalus fuscus,* is a piscivore, feeding as an ambush predator on the seafloor (Gray and Barnes 2008). We quantified morphological measurements and diet composition for 543 fish; 118 yellowfin bream (zoobenthivore), 238 southern herring (zooplanktivore) 160 sea mullet (detritivore) and 27 dusky flathead (piscivore) (Table S2).

Fish were captured from the lower reaches of each estuary (to the seaward point in the estuary where winter salinity levels averaged 30 ppt over the previous 10 years)(Gilby et al. 2017c), following the extent of previous surveys conducted in these estuaries (Gilby et al. 2018b), using a combination of gillnets, cast nets and angling. All specimens were immediately euthanised (USC Animal Ethics ANA18126), preserved on ice in the field, transferred to the lab within 3 h and stored at -20°C until processed.

Morphological measurements

Morphological measurements were quantified from photographs taken of each fish and calculated in the *geomorph* package of the R statistical framework (Figure S1) (Adams et al. 2018). Fish images were captured by horizontally mounting (checked using a tubular spirit level) a Nikon W300 digital camera (set on macro photograph mode) on a tripod positioned to fit the entire fish in the frame as closely as possible. Photographs were taken from the left side of each fish only and included a 30 cm ruler as a scalebar for subsequent image analyses. Morphological measurements were based on a standard truss network (Strauss and Bookstein 1982), with additional measurements of the body width, head, jaw and fins of each fish (Table S3, Figures S2). The width metrics (e.g. body width, interorbital width, jaw width, mouth gape) were obtained with Vernier callipers in the laboratory. In total, 35 morphological measurements were made for each individual, yielding a total of 19,005 data points. All morphological measurements were standardised to a proportion of the individual's standard length (SL) for statistical analyses. There were few differences in the average standard lengths of individuals from the different estuaries (Table S2).

Diet measurements

Visual quantification of gut content was conducted following procedures in Linke et al. (2001) and Gilby et al. (2011). Gut content was identified to the lowest taxonomic level possible under a dissecting microscope, and recorded as the percentage of total gut volume (Linke et al. 2001; Gilby et al. 2011). A total of 425 different dietary items were identified from 543 gut dissections across our dataset. Where fish were captured using angling, any bait material identified in the stomach was excluded from the analysis.

Environmental variables

Twelve environmental variables were chosen based on their importance in predicting fish abundance and diversity in previous studies of estuaries in the region (Gilby et al. 2018b; Olds et al. 2018a) (Table 1). These variables can be broadly grouped into three categories. Firstly, site-specific variables are those which quantified cover (in m^2) of marine habitats and urbanisation within a 500 m buffer of each fish capture site. This buffer distance was selected based on the scales of movement of fish in estuaries over single tidal cycles within this region (e.g. Gilby et al. 2017b). Secondly, estuary-scale variables are quantified as the extent of urbanisation within catchments, or the extent of marine ecosystems (intertidal flats, seagrass and mangroves) in each study estuary. We normalised each of these metrics for the size of the catchment because larger catchments and estuaries will have bigger areas of each habitat type. We accounted for the potential effects of catchment size by also including catchment size as a variable in the analysis. Thirdly, water quality data are collected monthly by a regional water quality monitoring program across the study region (EHMP 2018). Because water quality monitoring sites did not precisely match the sites where we captured fish, water quality values were interpolated to capture sites using inverse distance weighting (IDW) interpolations in QGIS. We chose to analyse the average of interpolated values from the 6 monthly monitoring events prior to fish capture because six months values correlated significantly with both three and one month values (EHMP 2018), and because this longer time period enabled quantification of the longer-term effects of any declining water quality at each site.

Table 1 Environmental variables included in statistical models, and their definitions. 'Estuary-scale measures' refer to factors measured at the scale of the watershed, or the entire sampled stretch of the estuary, whereas 'site-scale measures' are attributes of individual fish capture sites measured at smaller spatial scales (within 500 m of the site where individual fish were captured).

Factor	***Definition***
Site-scale	
Intertidal flats area	Area (in m^2) of intertidal sandbanks, intertidal flats, and flood-and ebb-tide delta within a 500m buffer of each fish capture site (Queensland Government 2018)
Mangrove area	Area (in m^2) of mangrove coverage within a 500m buffer of each fish capture site
Urban area	Area (in m^2) of urbanised land cover within a 500m buffer of each fish capture site (Queensland Government 2018)
Seagrass area	Area (in m^2) of seagrass within a 500m buffer of each fish capture site (Queensland Government 2018)
Estuary-scale	
Intertidal flats-catchment	Area (in percentage) of intertidal sandbanks, intertidal flats, and flood and ebb-tide delta for the whole estuary, corrected for the total area of the estuary (from the mouth to the upper limit of mangroves) (Heap et al. 2001) (Queensland Government 2018)
Mangroves-catchment	Area (in m^2/m) of mangrove coverage within the whole estuary corrected for total length of the estuary that supports mangroves (to account for the size of the estuary) (Queensland Government 2018)
Urban-catchment	Percentage of the catchment classified as urbanised, including residential, utilities, and manufacturing and industrial areas (Queensland Government 2018)
Seagrass-catchment	Area (in percentage) of seagrass coverage within the whole estuary, corrected for the total area of the estuary (from the mouth to the upper limit of mangroves) (Queensland Government 2018)
Catchment Size	Total area (in m^2) of the estuary (Queensland Government 2018)
Water Quality	
Turbidity	Average water column turbidity levels (in nephelometric turbidity units) interpolated (inverse distance weighting) to each fish capture site over a 6 month sampling period from June-December 2018 prior to fish capture (EHMP 2018)
Salinity	Average salinity (in PPT) interpolated (inverse distance weighting) to each fish capture site over a 6 month sampling period from June-December 2018 prior to fish capture (EHMP 2018)
Chlorophyll A	Average water column chlorophyll a concentration (in mg/L) interpolated (inverse distance weighting) to each fish capture site over a 6 month sampling period from June-December 2018 prior to fish capture (EHMP 2018)

Statistical Analysis

Conceptually, our analytical approach was to test each species separately for correlations between the suite of environmental metrics indexing anthropogenic landscape transformations of estuaries with two sets of multivariate data; one for morphology and one for diet composition. We then identified the main variables driving these differences using multivariate BEST analyses and visualised these results using non-metric multi-dimensional scaling ordinations (nMDS).

We tested for correlations between environmental variables, and the diet and morphology (separately) of each species using the BEST procedure in PRIMER E (Clarke and Gorley 2006). BEST was based on a normalised Euclidean distance dissimilarity matrix for environmental variables and morphometrics, and fourth-root transformed Bray Curtis dissimilarity measures for diet data. Where a significant correlation was found between the suite of environmental metrics and the dependent variables, we identified the combination of four or fewer environmental variables that best explained variation in diet or morphology. We visualised the directionality of these relationships by plotting vectors of variables from the best-fit model and dependent variables with correlation values greater that 0.4 on nMDS ordinations.

We tested for correlations between diet metrics and morphometrics of each species using the RELATE procedure in PRIMER (as opposed to the BEST procedure) because we could not infer directionality or causality of one suite of variables on the other (i.e. we could not be certain that diet was modifying morphology, or vice versa). We visualised the directionality of these relationships by plotting vectors from both datasets with correlation values greater than 0.4 on nMDS ordinations.

Results

Correlations between environment, morphology and diet variables

The environmental conditions of estuaries correlated significantly with both the morphology and diet of the southern herring and sea mullet (Figure 3). There was no significant effect of environmental variables on the diet or morphology for yellowfin bream, and no correlation between yellowfin bream diet and morphology. A significant correlation between the morphology and diet of dusky flathead was found, but environmental variables were not correlated with either diet or morphology (Figure 3).

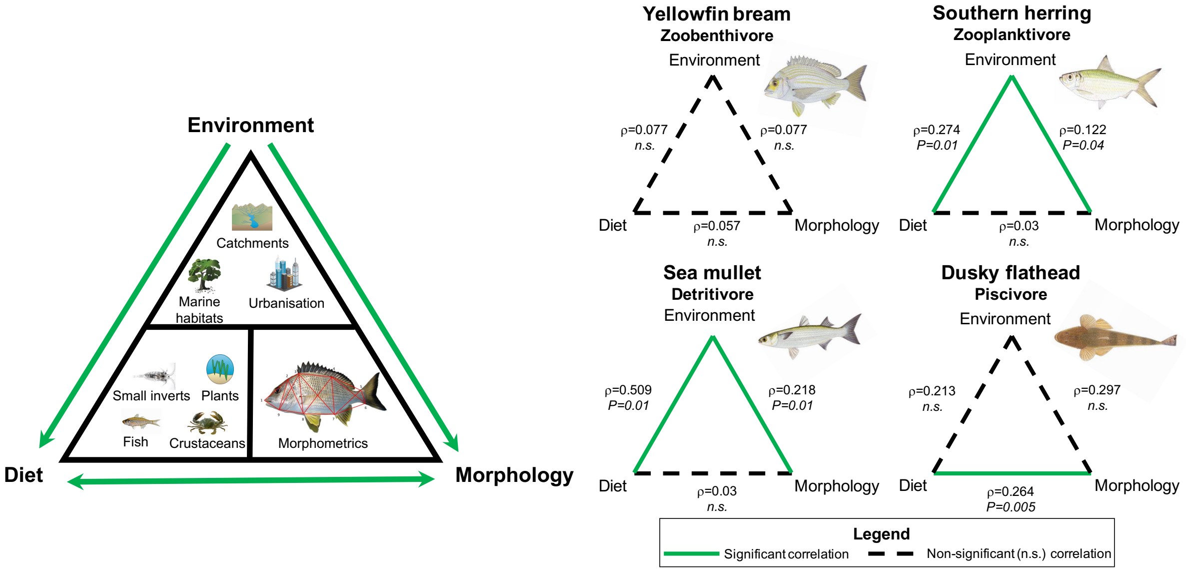


Figure 3 Relationships between the three main multivariate matrices measured. The sides of the triangle represent the analysis (BEST procedure) testing for multivariate correlations between the set of environmental variables (Table 1) and the diet composition and morphology of the four fish species representing the main trophic groups. The base of the triangle represents a similar test (RELATE procedure) between diet and morphology (ρ=Spearman's Rho, n.s.= P > 0.05). Here, the diet and morphology of southern herring and sea mullet correlates significantly with the environmental conditions in estuaries. Also, the diet and morphology of dusky flathead correlates with each other, but not with environmental conditions. We found no correlations at all for yellowfin bream. T**ext regarding relationship between each variable can be found in the results.**

Table 2 Best fit models from significant BEST analyses for correlations between environmental metrics and morphometrics, and environmental metrics and diet composition. ρ=Spearman's rho.

Species	**BEST model**	ρ	**P**
a. Environmental metrics and morphology			
Southern herring (zooplanktivore)	Seagrass area + Urban area + Mangroves-catchment + Catchment size	0.122	0.04
Sea mullet (benthic detritivore)	Seagrass area + Urban area + Chlorophyll-A + Catchment size	0.218	0.01
b. Environmental metrics and diet			
Southern herring (zooplanktivore)	Seagrass Area + Catchment Size	0.274	0.01
Sea mullet (benthic detritivore)	Seagrass Area + Urban Area + Mangroves-catchment + Seagrass-Catchment	0.509	0.01

Variables modifying diet and morphology

Southern herring- zooplanktivore

Environmental metrics that best explained variation in the morphology of southern herring were the area of seagrass at the site of fish collection, the extent of urban structure at the site of fish collection, the percentage of mangroves within the catchment and catchment size (Figure 4a, Table 2a). There were two prominent morphological changes evident. Firstly, fish that were captured at sites with greater seagrass area nearby, in estuaries with smaller areas of mangroves, had a longer pre-orbital length (morphometric PO), and larger pectoral fin height (morphometric PH) (Figure 4a). Secondly, fish in heavily urbanised estuaries draining a small catchment had broader shoulders (morphometric HP) (Figure 4a).

Environmental metrics that best explained variation in the diet of southern herring were the extent of seagrass near the collection site and catchment size (Figure 4b, Table 2b). Diet composition correlated with the environment in two ways. Firstly, individuals from sites with more seagrass in estuaries with larger catchments ate more harpacticoid (benthic) copepods and gammarid amphipods (Figure 4b). Secondly, individuals from sites with intermediate areas of seagrass and intermediately sized catchments ate more crab larvae (Figure 4b).

In summary, we found consistencies in the effects of the size of the catchment in which fish were captured and the extent of seagrass nearby to the capture site for both the morphology and diet of southern herring. Here, fish captured in estuaries with larger catchments and greater extents of seagrass nearby tended to contain fish with longer pre-orbital length and larger pectoral fin height that consume a greater proportion of gammarid amphipods and harpacticoid copepods in their diets.

Sea mullet- benthic detritivore

Environmental metrics that best explained variation in the morphology of sea mullet were the extent of seagrass and urbanisation nearby to the capture site, and average chlorophyll-a concentrations at the site of fish collection, and total catchment size (Figure 4c, Table 2a). Individuals captured at sites with higher chlorophyll-a concentration, in larger catchments, at sites with lower extent of urban structure and seagrass nearby, tended to have larger thoraxes (morphometrics VBL, RDA, RDPF, DBL, FDA), wider peduncles (morphometric PW), a longer distance between the peduncle and anal fin (morphometric PA), and a shorter jaw (morphometric JL). Therefore, the thorax and tail region of fish tended to be larger, and the jaw length shorter in fish from larger, more productive (and potentially nutrient-rich) estuaries, with less seagrass.

Environmental metrics that best explained variation in the diet of sea mullet were the area of seagrass and the degree of urbanisation near the capture site, and the extent of mangroves and seagrass in the catchment (Figure 4c, Table 2b). Fish captured at sites with more seagrass and urban structure nearby, and in estuaries draining catchments with more seagrass and fewer mangroves consumed more gammarid amphipods (Figure 4d).

In summary, we found consistencies in the associations between the extent of seagrass and urban structure nearby the capture site on both the morphology and diet of sea mullet: fish captured at sites with greater extents of seagrass and urban structure nearby tended to contain fish with smaller thoraxes and longer jaw lengths, that also tended to consume a greater proportion of gammarid amphipods in their diets.

Dusky flathead- piscivore

We found a significant correlation between the diet and morphology of dusky flathead. These correlations extend in two ways. Firstly, fish with longer thoraxes (morphometric VBL) that were flatter (dorsal fin to anal fin length; RDA) ate more fish and fewer mud crabs (likely juvenile *Scylla serrata*) and penaeid prawns (Figure 4e). Conversely, fish with shorter thoraxes (morphometric VBL), and less flat (dorsal fin to anal fin length (RDA) with longer heads (HL) ate more mud crabs and penaeid prawns but fewer fish (Figure 3e).

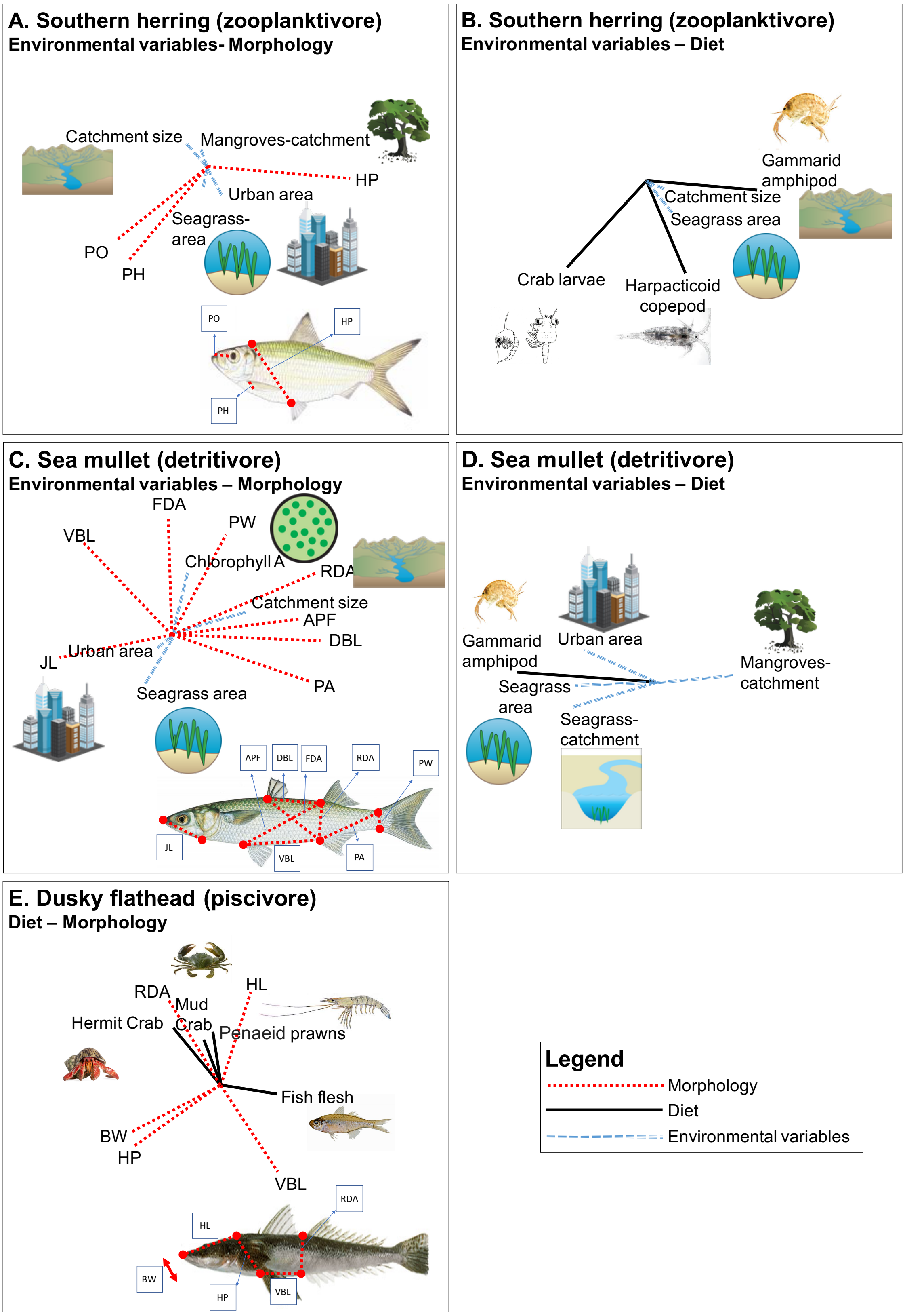


Figure 4 Non-metric multidimensional scaling (nMDS) ordinations vectors illustrating significant relationships from Figure 3 for; A) environmental variables and morphology for southern herring, B) environmental variables and diet for southern herring, C) environmental variables and morphology for sea mullet, D) environmental variables and diet for sea mullet, and E) diet and morphology for dusky flathead. Ordinations of centroid values for each estuary are provided in Figure S3. Note- there is only one panel for dusky flathead as only one pair of variables correlated significantly.

Discussion

The effects of human modifications to ecosystems on the abundance and diversity of animals have been thoroughly quantified in a number of settings (Foley et al. 2005). Many species can, however, adapt well to anthropogenic changes (Hoffmann and Sgro 2011; Bell 2013). The ways in which these species change their functional roles and morphology might have significant consequences for ecosystem functioning in impacted ecosystems (Villéger et al. 2010; Mouillot et al. 2013a; Gagic et al. 2015). In this study, we found consistent effects of seagrass extent and urbanisation on the diet and morphology of southern herring (zooplanktivore) and sea mullet (detritivore) in southeast Queensland estuaries. Diet is strongly tied to environmental conditions, as the availability and quality of some resources (e.g. plankton and detritus) depends on environmental conditions and the presence of key ecosystems (e.g. seagrasses) in estuaries (Telesh 2004; López-Vila et al. 2019). Seagrass has been shown in many systems to be significant in structuring coastal food webs (Connolly and Waltham 2015), especially when it is in close proximity to urbanised shorelines (Gilby et al. 2018b), and our results support these findings. In this sense, the effects of seagrass and urbanisation appear to extend beyond modifications to the structure of fish assemblages (Heck Jr et al. 2008; Gilby et al. 2018b), to effects on the morphology, diet and functional role of some species in modified estuaries (Unsworth et al. 2007; Villéger et al. 2010). Conversely, yellowfin bream (zoobenthivore) and dusky flathead (piscivores) were not significantly affected by the condition of estuaries in this study, and so their morphology and functional role may be resistant to broader landscape transformations.

In this study, changes to the morphology and diet of southern herring (zooplanktivores) and sea mullet (detritivores) correlated most with the extent of seagrass and shoreline urbanisation nearby to the capture sites, and the broader context of the estuary (i.e. catchment size). Both seagrass extent and urbanisation are key predictors of the structure of fish assemblages (Brook et al. 2018a; Gilby et al. 2018b) and the distribution of ecological functions across seascapes in this region (Henderson et al. 2019), and are key indicators of human impacts on coastal systems globally (Waycott et al. 2009; Bishop et al. 2017; Heery et al. 2017). Seagrass meadows are a threatened but pivotal habitat for a range of animals in coastal ecosystems because they provide nurseries, sequester carbon and nutrients, and provide a food source (including through detrital pathways) (Heck Jr et al. 2008; Waycott et al. 2009; Connolly and Waltham 2015). Seagrass harbours grazing benthic amphipods that feed directly on the seagrass and free living benthic harpacticoid copepods are abundant in productive seagrass meadows (Touchette and Burkholder 2000; Jankowska et al. 2019). Gammarid amphipods and harpacticoid copepods were more abundant in the diets of sea mullet (detritivore) when captured from areas with more seagrass. Gammarid amphipods, on average made up 0.1% of the gut volume in individuals from estuaries with no or little seagrass, compared with 16% in individuals captured from areas with more seagrass. Harpacticoid copepods, on average made up 0.1% of the gut volume individuals from estuaries with no or little seagrass, compared with 1.5% in individuals captured from areas with more seagrass. This may have simply been an incidental effect of greater seagrass availability, rather than a concerted switching of diet by these generalist detritivores. Nevertheless, such changes constitute a genuine change in the broader diets of these detritivores. Greater seagrass extent resulted in southern herring (zooplanktivore) with longer pre-orbital lengths (PO); this modification may allow fish to better capture invertebrates associated with seagrass (Walters and Bell 1994; Meyer et al. 2019). Increased pectoral fin heights (PH) increases manoeuvrability which can, arguably, be an advantage in seagrass meadows via increased manoeuvrability (Webb 2002; Drucker et al. 2005; Liao 2007). Seagrass increases quality and availability of detritus in warm temperate estuaries, which potentially attracts detritivore species (Franco et al. 2008). Therefore, this study has established that changes in seagrass extent can be implicated in potential functional changes within trophic groups and has consequences for our understanding of the role that seagrass plays in shaping functional roles at a species-specific level.

Anthropogenic nutrient enrichment alters the community structure and biomass of plankton; a vital primary producer in estuaries (Paerl et al. 2010). Environmental conditions are linked to zooplankton abundance in estuaries, and this is positively correlated with growth rates of planktivorous fish (Anderson and Sabado 1995). Larger catchments often have greater nitrogen and phosphate loads which influence plankton and the condition of seagrass ecosystems (Touchette and Burkholder 2000; Rabalais 2002), and these patterns were supported by our results. There can also be greater hydrodynamic flow in larger estuaries with less mangroves, and this could change swimming adaptations required by fish (e.g. Katopodis et al. 2019). In contrast, smaller catchments with lower seagrass extent appeared to increase crab larvae consumption in zooplanktivores. The spatial distribution of crab larvae is modified by temperature (Baylon 2010), salinity (Dos Santos et al. 2019), dissolved oxygen (Roman et al. 2019), depth and water currents (Eggleston et al. 1998). Crabs are also found in higher abundance in mangrove dominated systems (Walton et al. 2006). Given these broad effects of human modification on zooplankton assemblages, consistencies in the effect of catchment size and seagrass on the diet and morphology of zooplanktivore may therefore extend to other zooplanktivores present in estuaries within this region.

Higher primary production by phytoplankton improves food availability for detritivores, increases fish health and often results in higher body condition and larger thorax sizes (Holmlund and Hammer 1999; Gido 2002; Vanni et al. 2005). Jaw length and morphology is tightly tied to fish feeding traits (Wimberger 1991). In this sense, changes in jaw structure in sea mullet in this study may have developed to take better advantage of different detrital sources (e.g. microphytobenthos vs seagrass detritus) (Wainwright and Richard 1995). Modification to detrital pathways can have significant consequences for the structure and functioning of coastal ecosystems (Pitt et al. 2018). We found detritus in the stomachs of all sea mullet but did not quantify its original source. Therefore, the effects on diet that we identified (i.e. differences in fauna consumption) were likely incidental. Implications for detritivores, especially the sea mullet which is a commercially important fishery globally, should be explored in more detail.

Some species that remain in urbanised ecosystems are resistant to ecosystem-wide changes because either their functional niches are not modified by impacts, or they consume a wide enough diet that they simply consume whatever is available (Elmqvist et al. 2003; Devictor et al. 2008; Olds et al. 2018a). In this sense, we found no significant effects of environmental conditions on the diet or morphology of either yellowfin bream (zoobenthivore) or dusky flathead (piscivore). The lack of pattern in yellowfin bream may be due to the extensive movements they make across estuaries ecosystems and the diverse range of prey they consume (Pollock 1982a; Hadwen et al. 2007). Yellowfin bream can occur in high abundance in many marine ecosystems (Taylor et al. 2013; Gannon et al. 2015), and can be hyperabundant around urban structures, especially when other catchment-wide impacts (like habitat loss and sedimentation) are not too high (Brook et al. 2018a; Olds et al. 2018a). Urbanisation impacts may be lessened for such generalist species as they are able to adapt with broad and variable diets across different ecosystem configurations (Clavel et al. 2011; Olds et al. 2018a). Conversely, dusky flathead are ambush predators in marine environments (Douglas and Lanzing 1981), and feed predominantly on fish and small crustaceans; prey that remain in abundance even in some urbanised estuaries (Clynick and Chapman 2002; Sheaves 2005). Morphological changes in piscivores often result from changes in predation method, which reflects the prey availability within an ecosystem and success of prey capture in the predation-prey interaction (Eklöv and Diehl 1994). Therefore, the degree of modifications in the estuaries we sampled may not have been sufficient to modify the functional niche of piscivores. We did find, however, that dusky flathead that consumed a greater proportion of fish had larger thorax size and narrower heads. This may be an adaptation to the maximum size of prey targeted (Scharf et al. 2000) and the potentially more nutritious fish-dominated diet (Barnes et al. 2011).

Consequently, more intense urbanisation, and significant reductions in small fish abundance in particular, may lead to morphological changes in dusky flathead in the future. Combined, however, this lack of effect of anthropogenic impacts on the diet or morphology of yellowfin bream and dusky flathead could indicate that these species, and species with a similar ecological niche, may be more resistant to ecosystem modifications than both zooplanktivores and detritivores.

Quantifying the temporal scale over which changes to fish diet and morphology occur may be instructive in optimising management outcomes as the scales over which they change may differ. For example, diet may change over a period of hours or days due to environmental fluxes, whereas morphology may change over years or decades depending on whether changes are genotypic or phenotypic. For genetic change to occur within the species tested, conditions must be consistent over generational scales, and the populations must be semi or totally closed (Vieira et al. 2016; Kelley and Evans 2018); both unlikely in the systems studied (Pollock 1982b; EHMP 2019). Consequently, the changes we found in species morphology are likely to be localised anatomical responses within the anatomical plasticity spectrum of individuals. Further analysis (perhaps of genetic stocks and flows) of these patterns is, however, required to make substantive conclusions regarding these effects.

Changes to the diet and morphology of fish can alter their functional roles (Hjelm et al. 2003), have knock on effects for the functioning and condition of coastal ecosystems (Villéger et al. 2010), and the provision of key ecosystem services (i.e. good condition harvestable fish), and has important consequences for the management of human-modified coastal ecosystems. Our results highlight the importance of human modifications in driving adaptive change in fish. Each trophic group plays a pivotal role in shaping the structure of ecosystems through their diet and ecological functions, so changes to the functional roles of species in urbanised ecosystems has the potential to alter the functioning of entire ecosystems (Olds et al. 2018a; Price et al. 2019). We found consistent, and strong, effects of seagrass and urbanisation on the diet and morphology of our representative zooplanktivore and detritivore species. Seagrass has been identified as a globally threatened habitat that provides shelter, nurseries and resources for many marine organisms (Beck et al. 2001; Jackson et al. 2001), shaping assemblage structure, functional niches and ecological food webs across seascapes (Heck Jr et al. 2008; Waycott et al. 2009). Properly managing coastal ecosystems for the persistence of such structurally complex coastal ecosystems is vital (Elliott et al. 2007a; Barbier et al. 2011) because the effects of these habitats can potentially expand to changes in diet, morphology and functional roles in some species of fish. The ways in which human modifications to ecosystems extend through both assemblages and within individual species should therefore be more closely considered when quantifying the functional changes to ecosystems following human impacts.

Supplementary Materials

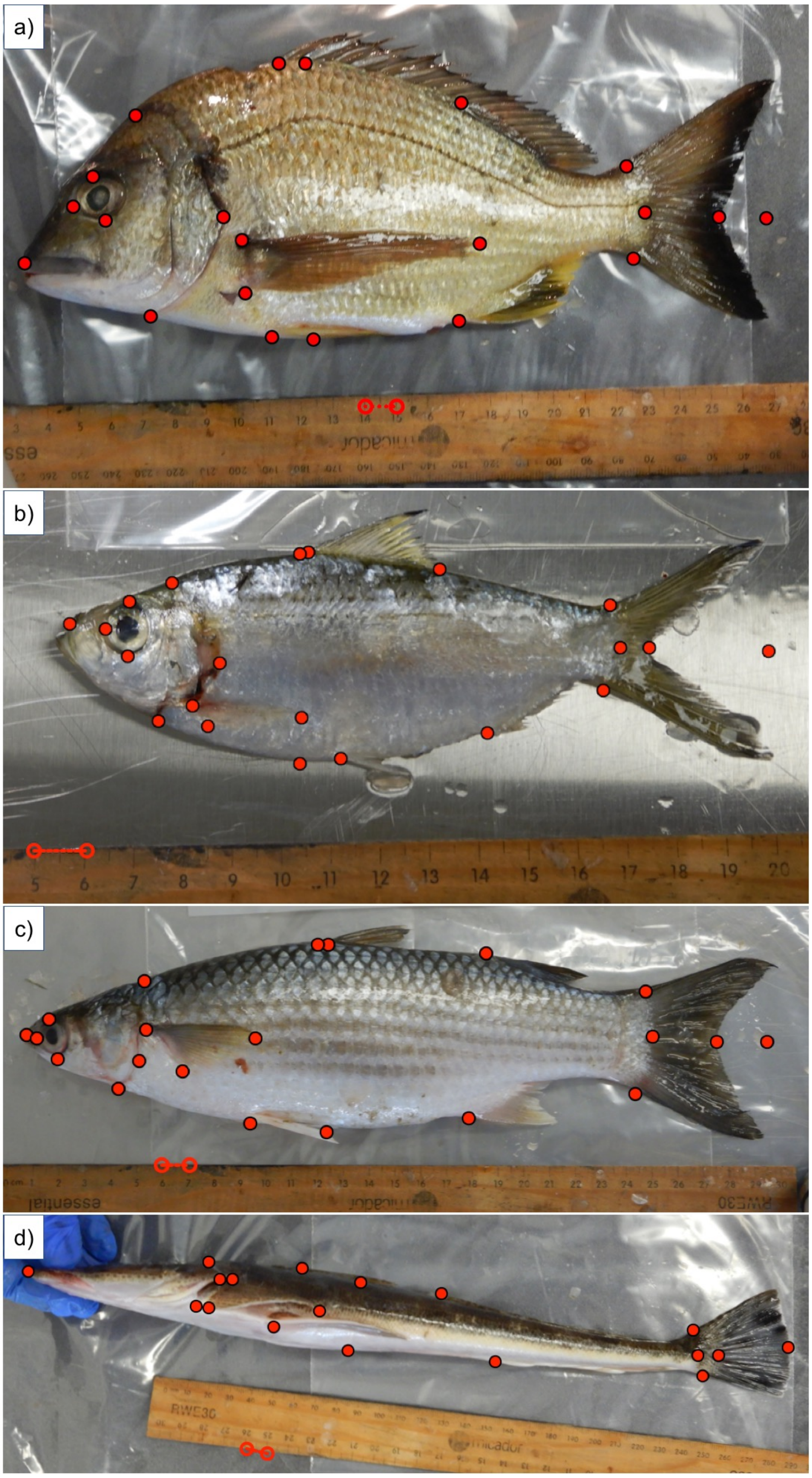
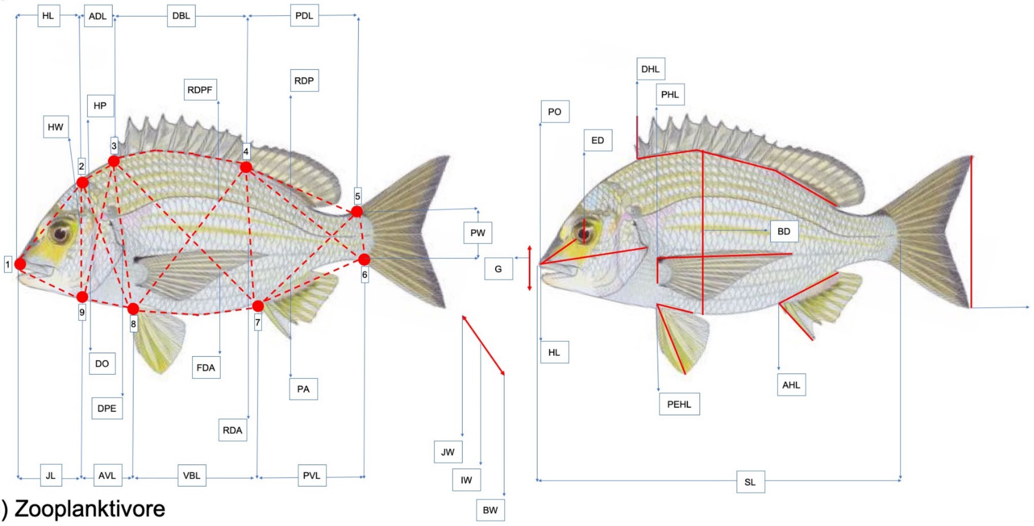
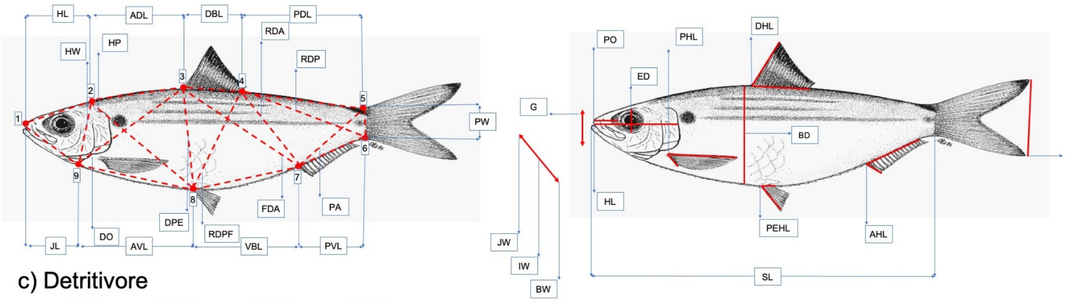


Figure S1 Example images of landmark morphology measurements made using the package “geomorph” in R for each of the species tested; A) yellowfin bream *Acanthopagrus australis* (zoobenthivore), B) southern herring *Herklotsichthys castelnaui* (zooplanktivore), C) sea mullet *Mugil cephalus* (benthic detritivore) and D) dusky flathead *Platycephalus fuscus* (piscivore).

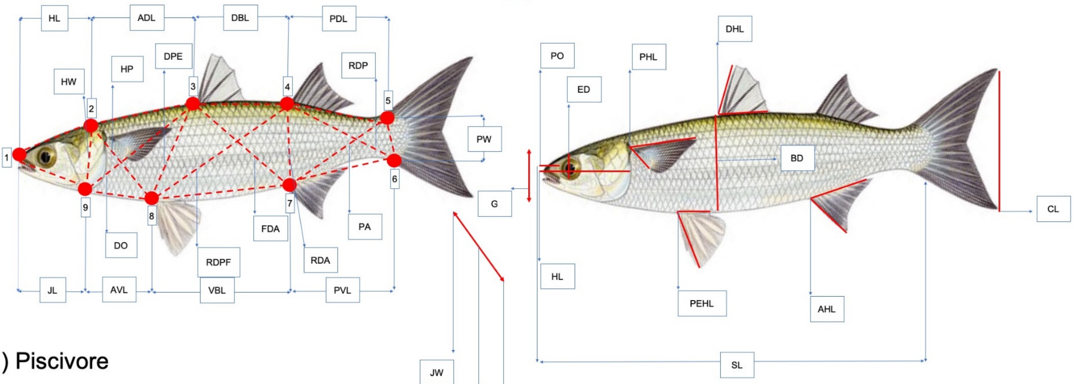
a) Generalist Zoobenthivore



b) Zooplanktivore



c) Detritivore



d) Piscivore

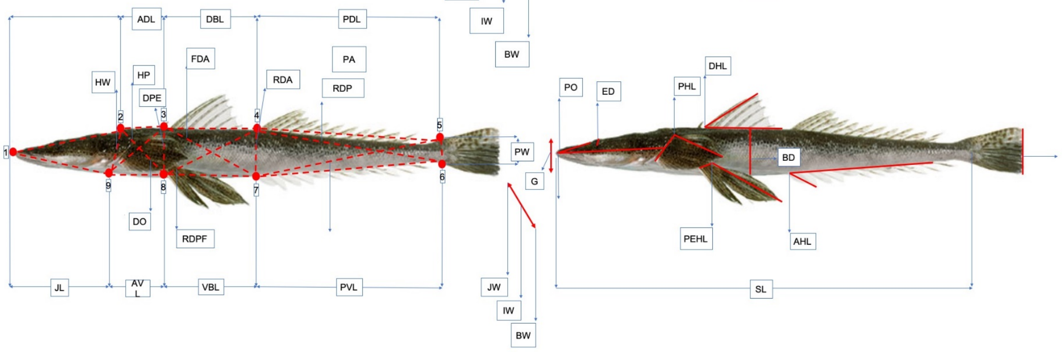


Figure S2 Morphological measurements for each of the four species analysed; a) yellowfin bream *Acanthopagrus australis* (zoobenthivore), b) southern herring *Herklotsichthys castelnaui* (zooplanktivore), c) sea mullet *Mugil cephalus* (benthic detritivore) and d) dusky flathead *Platycephalus fuscus* (piscivore).

Figure S3 Non-metric multidimensional scaling ordinations of estuary centroids for species with significant correlations between environmental variables, morphology or diet (See Figures 3, 4). Estuaries nearer to each other are more similar.

Table S1 List of estuaries sampled and their environmental conditions.

Estuary	Site-scale								Water Quality						Catchment-scale				
	Intertidal flats area (m^2)		Mangroves area (m^2)		Urban area (m^2)		Seagrass Area (m^2)		Turbidity (NTU)		Salinity (ppt)		Chl A (ug/L)		Urban-Catchment (%)	Seagrass-catchment (%)	Mangroves-catchment (m^2/m)	Intertidal flats-catchment (%)	Catchment size (m^2)
	Av.	SD	Av.	SD	Av.	SD	Av.	SD	Av.	SD	Av.	SD	Av.	SD					
Brisbane	163072.0	74335.4	89213.0	30951.5	228126.8	127081.2	0.0	0.0	6.2	0.3	29.7	0.9	1.9	0.2	11	0	112	6.6	6847608456
Caboolture	68444.9	78347.1	403802.3	62029.5	39676.6	85080.7	0.0	0.0	8.5	2.2	18.1	4.5	2.0	0.2	38	0	327	18.0	353183634
Coochin	103198.5	114542.6	217745.0	159810.9	11172.9	32923.3	835.2	1045.1	10.7	0.5	22.4	1.9	2.6	0.0	15	0	230	0	333607314
Currumbin	6145.0	7641.7	82808.7	63699.9	629715.5	149506.9	737.4	559.9	4.2	0.5	27.9	1.9	1.9	0.5	42	0.9	61	15.9	145844787
Logan	66173.1	69593.4	134377.9	125864.8	29741.1	121277.4	0.0	0.0	12.4	1.0	25.5	2.7	2.8	0.6	15	0	343	1.4	3007398831
Maroochy	63929.2	84061.8	103844.6	59560.1	307707.9	171418.3	233.4	790.9	11.3	2.8	15.1	3.1	3.7	0.7	41	0.2	240	17.0	609126641
Mooloolah	0	0	30339.5	40362.3	378157.7	63214.1	0.0	0.0	17.0	4.5	19.6	3.5	2.3	0.6	40	0	60	0	189088949
Nerang	9430.1	10662.4	0	0	550009.2	130791.5	868.8	970.4	4.3	0.2	25.3	0.3	2.4	0.1	39	0.5	0	4.1	479319933
Noosa	54299.9	40696.3	185538.7	44387.9	285988.0	120259.3	980.8	2628.1	9.4	0.8	14.1	3.4	1.4	0.1	8	0.1	125	0.6	783054729
Pimpama	55074.7	67818.3	234210.5	218006.0	140628.4	146301.4	0.0	0.0	7.2	0.6	28.7	3.4	2.1	0.9	28	0.8	469	44.6	136081856
Pine	32554.8	75983.9	186248.7	8967.8	213107.0	178290.6	0.0	0.0	7.0	0.3	19.3	3.4	4.0	0.4	31	0	455	5.9	690469265
Tallebudgera	24129.5	31128.6	96229.4	60435.2	419845.8	164243.7	340.6	661.7	1.7	0.4	31.1	1.8	1.1	0.2	42	0.3	51	8.3	145844787
Tingalpa	0	0	198232.6	33216.7	259223.8	70061.8	0.0	0.0	6.7	1.0	18.3	4.7	4.4	0.8	64	0	218	14.0	199003829

Table S2 Sample size and body size (SL standard length) of fish analysed.

Species	Estuary	Number of Individuals Measured (n)	Standard Length (mm) mean	s
Yellowfin bream	All sites	118	179	47
	Brisbane	15	150	29
	Caboolture	10	143	66
	Coochin	11	146	45
	Currumbin	9	201	22
	Logan*	6	230	60
	Maroochy	19	176	39
	Mooloolah	8	193	53
	Nerang	5	209	36
	Noosa	8	191	40
	Pimpama	5	206	28
	Pine	6	202	17
	Tallebudgera	5	207	13
	Tingalpa	11	167	43
Flathead	All sites	27	346	99
	Brisbane	2	353	155
	Caboolture	4	293	165
	Coochin	3	325	87
	Nerang	4	357	47
	Noosa	3	337	52
	Pimpama	5	402	92
	Pine	6	340	107
Southern herring	All sites	238	88	12
	Brisbane*	20	106	13
	Caboolture	20	85	7
	Coochin	20	83	8
	Currumbin	19	89	7
	Logan	20	82	12
	Maroochy	20	82	9
	Mooloolah*	19	105	8
	Nerang	19	77	3
	Noosa	20	92	7
	Pimpama#	18	76	8
	Pine	20	88	7
	Tallebudgera	20	87	8
	Tingalpa	3	75	5
Sea mullet	All sites	160	219	52
	Brisbane	15	224	24
	Caboolture	9	226	18
	Coochin#	9	103	10
	Currumbin	11	225	25
	Logan	20	236	32
	Maroochy	10	226	39
	Mooloolah	9	196	122
	Nerang	17	243	22
	Noosa	9	243	21
	Pimpama	10	243	28
	Pine	20	225	26
	Tallebudgera	5	227	34
	Tingalpa#	16	191	59

*indicates values significantly higher than the average, whilst # indicates values significantly lower than the average

Table S3 Morphometric measurements for all four functional groups, their acronyms and definitions.

Body region	Landmark	Acronym	Description
Head			
	Head length (1-2)	Hl	Length from the most anterior point of the head to the base of the head.
	Head width (2-9)	Hw	Distance between the base of the head to the opening of the operculum
	Jaw length (9-1)	Jl	Length from the opening of the operculum to the most anterior point of the head.
	Pre-orbital length	Po	Straight-line measurement taken from the tip of the snout to the anterior of the eye.
	Eye diameter	Ed	The greatest distance between the margins of the eyes orbit.
	Head length	Hl	The straight-line measurement of the head taken from the front of the upper lip to the membranous posterior end of the operculum.
	Jaw width	Jw	Measurement of the width of the jaw opening
	Interorbital width	Iw	Measurements of the area on top of the head between the eyes at the widest point of the edges of eye orbits.
	Gape	G	Straight-line measurement taken from the tip of the snout to the mouth angle; opening of the mouth, measures transversely.
Thorax			
	Anterior dorsal length (2-3)	Adl	Length from the base of the head to the front of the dorsal fin.
	Head to pelvic fin (2-8)	Hp	Distance from the base of the head to the front of the pelvic fin.
	Front of dorsal fin to pelvic fin (3-8)	Dpe	Distance from the front of the dorsal fin to the front of the pelvic fin.
	Front of dorsal to operculum (3-9)	Do	The distance from the front of the dorsal fin to the opening of the operculum.
	Anterior ventral length (8-9)	Avl	Length from the front of the pelvic fin to the opening of the operculum.
	Dorsal base length (3-4)	Dbl	Length between the front of the dorsal fin and the back of the dorsal fin.
	Ventral base length (7-8)	Vbl	Length between the front of the anal fin and the front of the pelvic fin.
	Rear of dorsal fin to pelvic fin (4-8)	Rdpf	Distance from the rear of the dorsal fin to the front of the pelvic fin.
	Front of dorsal fin to anal fin 1 (3-7)	Fda	Distance from the front of the dorsal fin to the front of the anal fin.
	Rear of dorsal fin to anal fin 4-7	Rda	Distance from the rear of the dorsal fin to the front of the anal fin.
	Body depth	Bd	The vertical distance from the dorsal margin of the body to the ventral margin of the body measured at the bae of the pectoral fin where it attaches to the body; fins or fin bases are not included in the measurement.
	Body width	Bw	Measurement of the greatest width of the body.
Tail			
	Posterior dorsal length (4-5)	Pdl	Length between the rear of the anal fin and the base of the tail.
	Peduncle width (5-6)	Pw	Width of the tail.
	Posterior ventral length (6-7)	Pvl	Length between the base of the tail and the front of the anal fin.
	Peduncle to anal fin (5-7)	Pa	Distance from the top of the peduncle to the front of the anal fin.
	Rear of dorsal fin to peduncle (4-6)	Rdp	Distance from the rear of the dorsal fin to the bottom of the peduncle.
Fins			
	Pectoral fin height/length	Phl	The vertical length of the pectoral fin from the base to the top of the pectoral fin and the horizontal length of the pectoral fin from base to tip.
	Dorsal fin height/length	Dhl	The vertical distance from the base of the longest dorsal fin and the horizontal length from the longest dorsal fin to the end of the dorsal fin.
	Pelvic fin height/length	Pehl	The vertical length of the pelvic fin from the base of the top of the pelvic fin and the horizontal length of the pelvic fin from base to tip.
	Anal fin height/length	Ahl	The vertical length of the anal fin from the base to the top of the anal fin and the horizontal length of the anal fin from base to tip
	Caudal length	Cl	Total length of the caudal fin.

Chapter 3

Urbanisation and fishing alter the body size and functional traits of a key fisheries species

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Introduction

Human impacts on ecosystems are widespread and growing in frequency and intensity (Søndergaard and Jeppesen 2007; Bishop et al. 2017; Halpern et al. 2019). These impacts modify both habitats and the animals that inhabit them in a many ways and at multiple spatial scales (Worm et al. 2006; Hooper et al. 2012). For example, human impacts to ecosystems can reduce the rate and modify the distribution of key ecological functions (Hector and Bagchi 2007; Olds et al. 2018a) and change the assemblage composition of plants and animals (McCauley et al. 2015; Gilby et al. 2018c), potentially altering the provision of ecosystem services (Cardinale et al. 2012; Haddad et al. 2015). Some anthropogenic impacts have particularly strong consequences for specific components of ecosystems; this can result in disproportionate changes in population structure (Pillans et al. 2005), body condition and organismal health of these species (Acevedo-Whitehouse and Duffus 2009). These impacts can propagate to changes in the morphology and functional traits of individuals, as species adapt either genetically or phenotypically to environmental variation (Villéger et al. 2010; Mouillot et al. 2013a; Gagic et al. 2015). Determining how anthropogenic pressures impact on the body size, morphology and functional traits of species that have economic, social or functional significance is, therefore, a priority for both conservation and fisheries management.

The joint effects of fishing, habitat loss and degradation, and runoff from modified catchments widely affects the health of marine ecosystems and species (Halpern et al. 2008; Barbier et al. 2011; Teichert et al. 2016). For example, fishing significantly modifies coastal faunal assemblages, and has either direct (through destructive fishing methods) (Lambert et al. 2014; Clark et al. 2015) or indirect and cascading effects (Mumby et al. 2006; Gilby and Stevens 2014) on the structure and condition of marine habitats and faunal assemblages. Whilst there are well-established challenges in quantifying the catch and effort of commercial and, particularly, recreational fishing sectors (McCluskey and Lewison 2008; Lancaster et al. 2017), the consequences of fishing are perhaps some of the most widely studied and best understood anthropogenic impacts on coastal systems (Pauly et al. 2005; McCauley et al. 2010). However, the effects of fishing do not occur independently of broader landscape transformations and other anthropogenic impacts that reduce the condition of ecosystems. For example, fishing impacts may operate in concert with impacts from habitat loss and water quality (Halpern et al. 2008). The consequences of joint effects from fishing and landscape transformation have been documented in coastal seascapes (e.g. Micheli et al. 2013; Gilby et al. 2016), but the potential implications of these impacts for variation in body size, morphology and functional traits are rarely measured.

Estuaries are highly valuable sites for human settlement, resource use, and recreation (Barbier et al. 2011), resulting in substantial coastal development and coastal fishing pressure and associated change in the condition of estuarine ecosystems (Cloern et al. 2016). Further, the effects of runoff from degraded catchments are concentrated in the lower reaches of estuaries before being discharged into the ocean (Hopkinson and Vallino 1995). Consequently, the joint effects of a suite of anthropogenic impacts that threaten coastal ecosystems can be prominently manifested in estuaries. However, estuaries are also pivotal ecosystems for many commercially, recreationally and economically important species that use them either periodically (i.e. for spawning or as nurseries) or more permanently (i.e. estuarine resident species) (Elliott et al. 2007c; Whitfield 2017). It is for this reason that estuaries are widely regarded as a priority for research that seeks to both understand the ecosystem-wide effects of human activities, and design effective management and restoration interventions to mitigate these threats (Barbier et al. 2011; Gilby et al. 2016; Elliott et al. 2017; Gilby et al. 2018a).

Giant mud crabs (*Scylla serrata)* are an ecologically and economically important portunid in muddy, mangrove-dominated estuaries of the Indo-West-Pacific (Alberts-Hubatsch et al. 2015). The species is highly-prized by consumers because of its large body size (to >23 cm carapace width) and chelipeds containing ample consumable flesh, and is therefore targeted heavily by both commercial and recreational fishers (Fratini et al. 2010; Dumas et al. 2012; Alberts-Hubatsch et al. 2015). In some regions, giant mud crab catch is both size- and sex-biased. For example, in Queensland, Australia, only male crabs with carapace widths greater than 15 cm may be kept by fishers. This catch bias can result in significant changes in size and sex ratios in wild populations (Pillans et al. 2005). Removing the competitive-dominant, territorial, and aggressive larger males from a population through fishing might reduce competition between conspecifics for space and mates. In systems where the abundance of male giant mud crabs is higher, they may need to spend a greater proportion of their time competing with other males, and/or a greater amount of energy growing larger body size and chelipeds to outcompete conspecifics (Beattie et al. 2012). Similarly, sub-dominant males in systems with large males (i.e. due to less fishing) may have larger claws to defend themselves against larger conspecifics. Simultaneously, however, the abundance, size and functional traits of giant mud crabs might also be shaped by the condition of the surrounding ecosystem, especially the availability of high quality foods and habitats (especially their preferred muddy mangrove habitats) (Fratini et al. 2010; Alberts-Hubatsch et al. 2015; Unhalu et al. 2015). Male giant mud crabs are an ideal species to test the effects of anthropogenic pressures on body size and shape in coastal species for three reasons: 1) they are specifically targeted in some fisheries; 2) they are highly competitive, a trait that may make them more susceptible to habitat loss because of behavioural displacement; and 3) they have small home ranges as adults (usually <1 km) (Hyland et al. 1984; Bonine et al. 2008), making it plausible that any changes in size and/or functional traits reflect the conditions of an estuary. Whilst the effects of anthropogenic pressures (including fishing, habitat loss and catchment runoff) on giant mud crab population structure and abundance are relatively well understood (e.g. Pillans et al. 2005; Bonine et al. 2008; Webley et al. 2009; Fratini et al. 2010; Meynecke et al. 2012), any impacts from fishing and landscape transformation that may jointly alter crab functional traits remain untested.

Here we assess whether and how anthropogenic pressures on estuaries can combine to modify the body size and functional traits of giant mud crabs in southeast Queensland, Australia. Southeast Queensland is an ideal study region for this test as the region has multiple estuaries that span a broad gradient of environmental conditions, catchment land use change (particularly urbanisation), and fishing effort (Gibbes et al. 2014; Gilby et al. 2018c). There is also a significant commercial and recreational demand for giant mud crabs (Webley et al. 2015), a continual growth in human population size in the region (Australian Bureau of Statistics 2017), and changes to the condition, area, and distribution of key marine ecosystems (especially seagrasses and mangroves) (Manson et al. 2003; Saunders et al. 2013). We hypothesised that male giant mud crabs captured from areas where fishing pressure was lower would be larger and have larger chelipeds. This is because higher densities of male giant mud crabs might cause greater competition amongst males, thereby driving an increase in the size of chelipeds to increase individuals competitiveness. Similarly, we hypothesised that there would be additive effects of the extent of marine ecosystems on body size and functional traits of male giant mud crabs by providing access to food and territory (i.e. crabs from estuaries with extensive mangroves might be larger), and by mediating the competitiveness of male giant mud crabs with each other (by providing define territories and refuges).

Methods

Study region and giant mud crab collections

We collected giant mud crabs from 13 estuaries in southeast Queensland, Australia (Figure 1). Estuaries were chosen to represent a gradient in coastal modifications, ranging from relatively 'natural' systems with abundant mangroves to highly urbanised ones (Gilby et al. 2018c)(Table S1). In each estuary, crabs were collected from the lower reaches, operationally defined to extend from the mouth to long-term (>10 year) salinity values averaging >28ppt (EHMP 2019). We sampled the lower reaches for three key reasons; 1) to keep the sampling extent consistent between estuaries that differed in size, 2) because these reaches are the central locations for crab fishing pressure in the region, and 3) because the effects of the loss of marine habitats and degraded catchments is centralised in the lower reaches of estuaries. We collected two to nine males of legal size (>15cm carapace width) from each estuary between September 2018 and February 2019. We collected this demographic of mud crab as fishers are permitted to keep only male giant mud crabs greater than 15 cm carapace width in Queensland. Crabs were sampled with traps (four per estuary) baited with sea mullet; trapped crabs were euthanised in an ice slurry within a few minutes after retrieval and deep-frozen (-20°C) within 2 hours upon return to the laboratory.

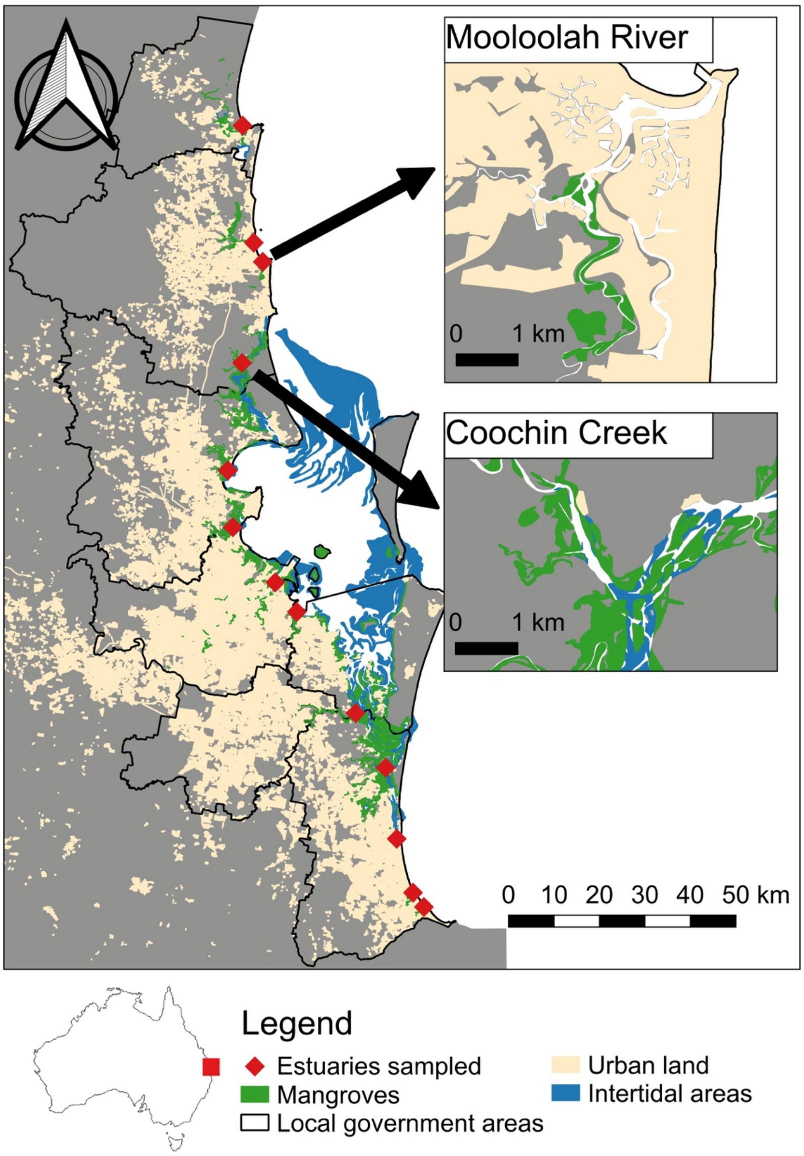

Figure 1 Map of study region and estuaries sampled in southeast Queensland, Australia. Inserts illustrate estuaries with intensive human modifications of the floodplain and catchment (Mooloolah River, top) and more natural settings with substantial areas of mangrove forest remaining (Coochin Creek, bottom).

Functional trait measurements

We weighed each giant mud crab to the nearest 1/10 of a gram using laboratory scales. We quantified functional traits from standardised still images analysed with the geomorph package in R (Adams et al. 2018). Images of crabs were captured in 'planar bird eye perspective' by mounting a digital camera (Nikon W300 in macro mode; 24-120mm equivalent lens) on a tripod. We took images of the ventral and dorsal surface (including all walking legs) and the inside of the left and right chelipeds. From the four images taken for each individual, we measured 145 morphometrics in four categories (Figure S1): 1.) the dorsal carapace ('Top'; henceforth labelled '*T*'), 2.) the ventral carapace ('Underside'; labelled '*U*'); 3.) the chelipeds (labelled '*C*'); and 4.) the walking legs (labelled 'L'). Morphometric values for walking legs and chelipeds were averaged between right and left body sides, thus yielding a total of 90 morphometrics per individual to be included in the numerical analyses. To account for variation in overall body size, we standardised all morphometrics by carapace width (i.e. x' = x / carapace width).

Statistical analyses

We gauged the association between body size and functional traits with environmental variables that fall, broadly, into three groups (Table 1): 1) at the estuarine scale we modelled the influence of estuarine inlet size and the area and extent of mangroves and intertidal flats; 2) to account for potential smaller-scale environmental effects (for individuals with small home ranges) we modelled the influence of variation in mangrove, tidal flats and urban land within 1 km of capture sites, and; 3) to index broad pressures from fishing and development, we used data on commercial crab catches and human population size in three subregions of our study region (Table 1, Table S1, Figure S2). These were based on the available commercial fishing data grids provided by the Queensland State Government (Queensland Department of Agriculture and Fisheries 2019), that divided our sampling reach into a northern, central and southern region, and population census by the Australian Bureau of Statistics (Figure 1, Table 1). The variables intertidal flats area correlated strongly (>0.7 Pearson's R) with intertidal flats catchment, estuary mouth width, urban area, and mangroves area, and total mangroves and urban catchment percentage correlated strongly (>0.7 Pearson's R) with urban area and mangroves corrected, so these variables were not included in subsequent analyses. The remaining variables did not covary.

We used generalised additive models (GAMs) in the mgcv package of R (Wood 2017) to test for correlations between environmental variables and giant mud crab carapace width, weight, and standardised weight (weight divided by carapace width). To minimise overfitting, we ran GAMs with all possible combinations of three environmental variables or fewer

(Barton 2018), and restricted GAM fits to three polynomial lines or fewer (k=3). Best fit GAMs were identified using AIC values. We calculated relative variable importance by summing the weighted AIC values for each model containing the variable of interest, with values nearer to 1 indicating greater importance of the variable in explaining overall patterns.

We used a distance-based linear model (distLM) in PrimerE to identify the suite of variables that best correlated with all functional traits (Anderson et al. 2008). The distLM model was calculated on normalised functional traits, using a stepwise approach and Akaike information criterion (AIC). The best fit distLM model was visualised using distance-based redundancy analysis (dbRDA). We identified the functional traits most driving these relationships (i.e. 'important' functional traits) by overlaying Pearson's vectors over the ordination space with correlations greater than 0.5. We used GAMs to further examine patterns between variables from the best-fit distLM model, and important functional traits. GAMs followed the same procedures as outlined above.

Results

Body Size

Body size was consistently associated with variation in width of the estuarine inlet and the extent of intertidal flats (Figure 2). Whilst some patterns were non-linear, typically the heaviest individuals and those with the largest carapace widths were captured in estuaries with wider inlets and smaller intertidal flats upstream of the mouth (Figure 2). Estuary mouth width and the total extent of intertidal flats were, however, not significant for corrected weight (Figure 2C). Urban area was included in the best fit model for carapace width, but neither urban area or mangrove area individually were significant predictors of giant mud crab body size.

Functional traits

The gross morphology of giant mud crabs correlated best with commercial fishing pressure, human population size, urban area, and extent of mangroves and intertidal flats in the estuary (Figure 2). Twelve morphometrics correlated (R>0.5) with the ordination space (Figure 2B, C). We found distinct clusters of giant mud crabs from areas of high, intermediate and low commercial fishing pressure in ordination space (Figure 2A). dbRDA vectors suggest that crabs from regions with lower commercial catches and fewer people in the broader catchment tend to have larger morphometrics, especially of the carapace and walking legs. Crabs from estuaries where populations are high, but commercial catches are of intermediate intensity are morphometrically distinct, tending to have larger chelipeds.

Finally, crabs from estuaries with the highest commercial catches and highest human populations in the catchment tended to have smaller and narrower chelipeds.

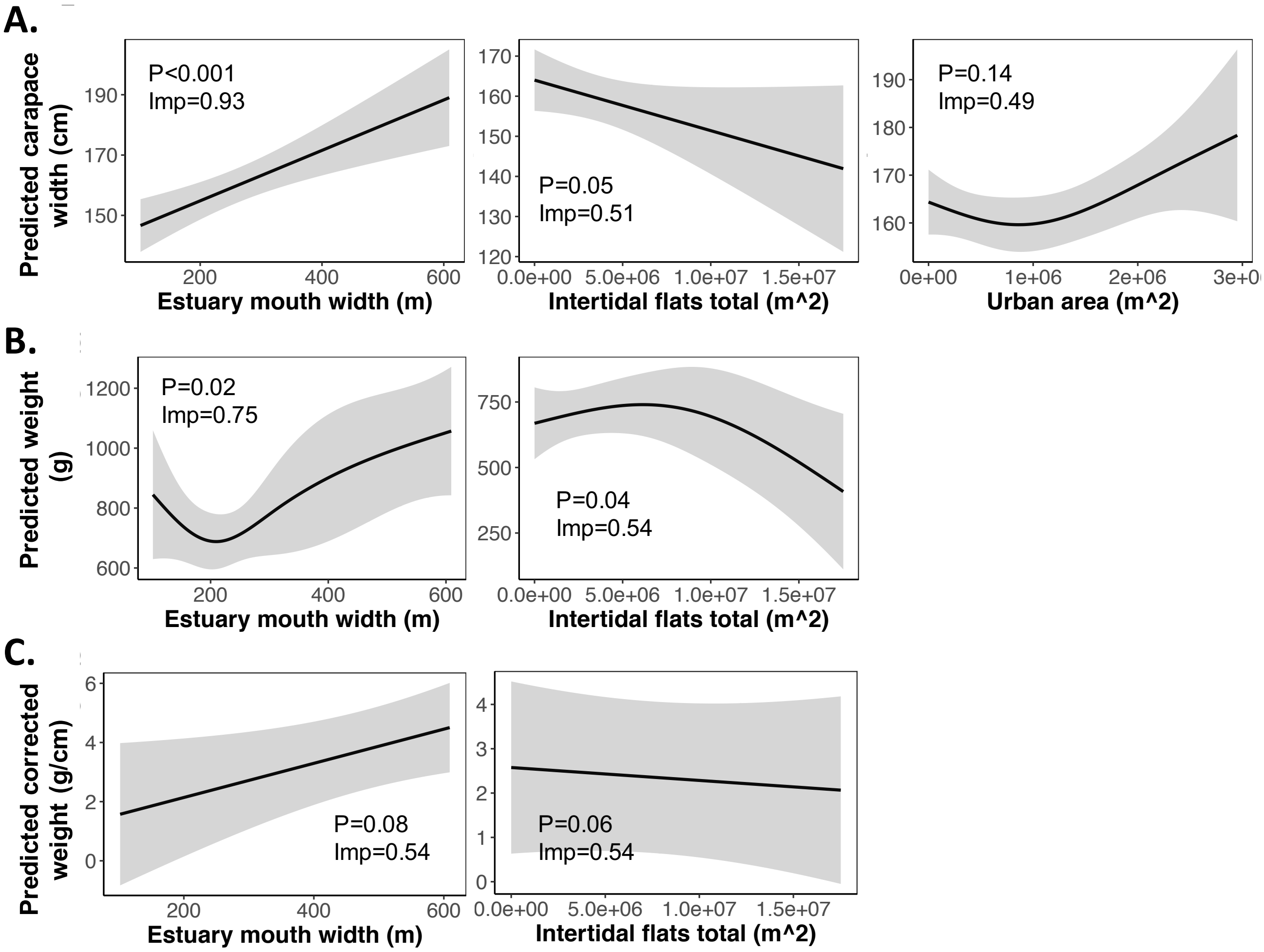

Figure 2 Generalised additive model output plots showing variables included in the best fit models for A) carapace width, B) body weight, and C) standardised body weight (weight / carapace width). P values are those from the best fit model, and importance values (Imp.) scale from 0 being not important to 1 being very important in explaining overall patterns. All error measurements are 95% confidence intervals. We calculated relative variable importance by summing the weighted AIC values for each model containing the variable of interest, with values nearer to 1 indicating greater importance of the variable in explaining overall patterns.

Each of the environmental variables from the best fit distLM correlated significantly with at least one of the important morphometrics (Figure 3). However, the shape of associations was variable. Main cross carapace morphometrics (morphometrics T13 and T16; i.e. the 13th and 16th morphometrics of the top of the carapace) (Figure 2B) were largest in areas with moderate human populations (Figure 3, 4A, S3). Morphometric T13 was also higher in areas with greater intertidal flat extent (Figure S3). No environmental predictor correlated significantly with the underside of the body (Figure 3). The length of the end segment of the second walking leg was shorter in areas with greater area of urban development nearby (Figure 3, 4A).

Whilst there were inconsistent effects of environmental variables on morphometrics of the carapace and walking legs, cheliped morphometrics were consistently lowest in areas with high commercial fishing pressure and were always highest in areas of low human populations (Figure 4B, S4). Here, commercial fishing pressure correlated significantly with all of the important cheliped morphometrics, and human population correlated significantly with five of seven (Figure 3). There were also consistent correlations between urban area on cheliped morphometrics. Urban area was associated with some cheliped morphometrics: individuals captured from sites with low urban land use nearby had the largest C6 and C15 morphometrics (Figure 4B, S4). Morphometric C30 was largest in areas with moderate commercial fishing pressure, and greater corrected mangrove extent (Figure 4B, S4).

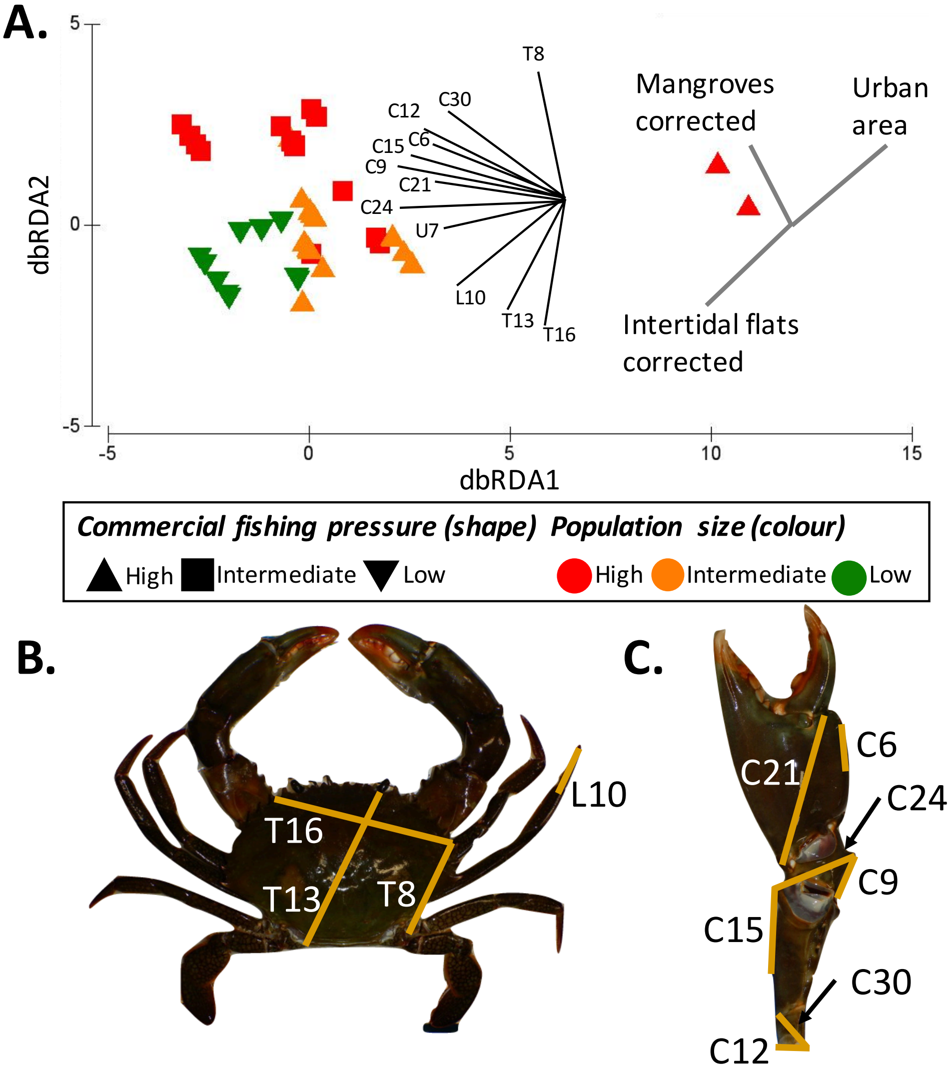

Figure 3 Distance-based redundancy analysis (dbRDA) of relationships between environmental variables and the morphometrics of giant mud crabs from the best-fit distance-based linear model (distLM) (A.), and visualisations of the morphometrics that correlated best with the ordination for legs and carapace measurements (B.) and chelipeds (C.).

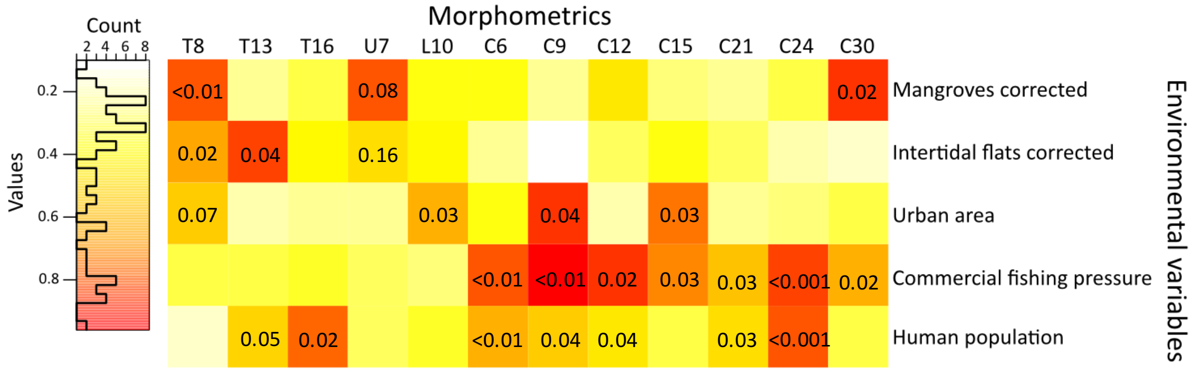

Figure 4 Heat map of importance values for environmental variable in the generalised additive models (GAMs) models for each morphometric that correlated strongly with results from multivariate analyses (see Figure 3). Importance values range from 0 (being the least important variables; white), through to 1 (being the most important variables; dark red). Values within boxes indicate P values of variables included in the best fit model. Legend on the left provides a histogram the distribution of values across the range. T=top of carapace; U=underside of carapace; L=legs; C=chelipeds. 'Corrected' values account for the fact that larger estuaries will naturally have a greater extent of marine habitats, and so values are corrected for the length of the estuary (for mangroves) or the area of the estuary (for intertidal flats). We calculated relative variable importance by summing the weighted AIC values for each model containing the variable of interest, with values nearer to 1 indicating greater importance of the variable in explaining overall patterns.

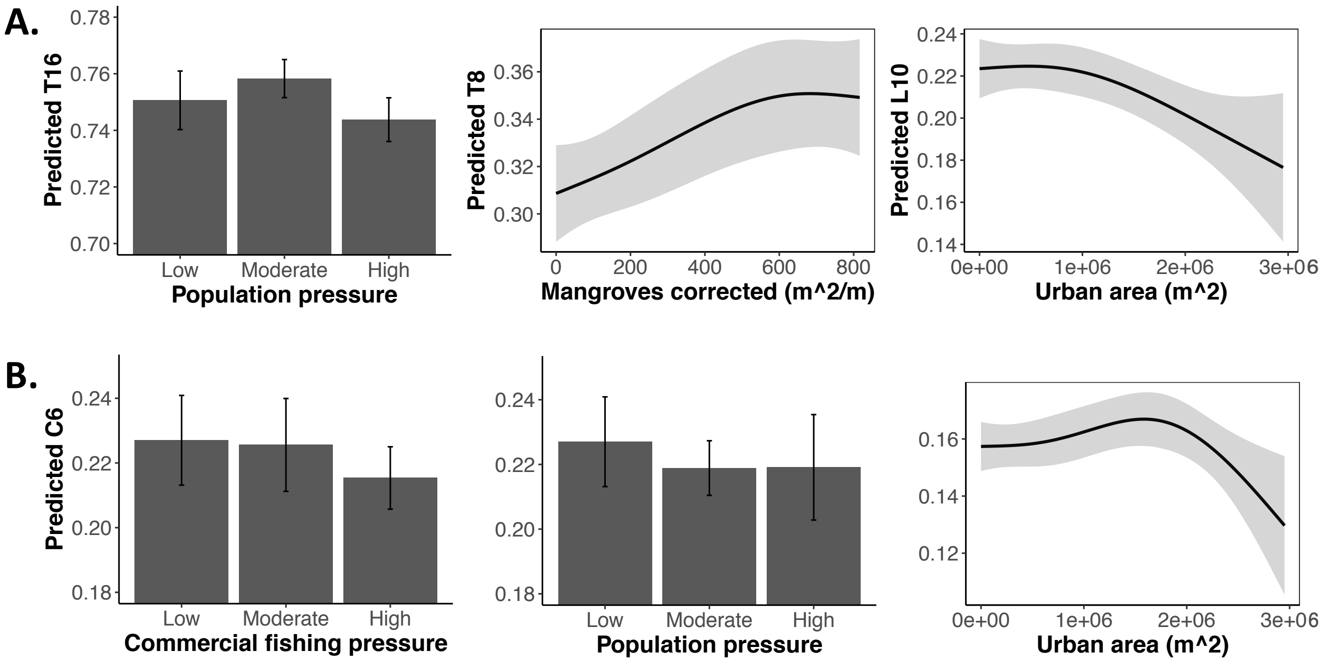

Figure 5 Example generalised additive model output plots showing usual trends for morphometrics from the carapace and legs (A.), and the chelipeds (for morphometric C6 only) (B.). All error measurements are 95% confidence intervals. See Figures S3 and S4 for the remaining plots from the best fit models for all other morphometrics.

Discussion

The cumulative influence of human impacts can reduce the body size and change the functional traits of some species within modified ecosystems (Acevedo-Whitehouse and Duffus 2009; Villéger et al. 2010; Mouillot et al. 2013a; Gagic et al. 2015). Some behavioural or ecological traits of species may make them more vulnerable to particular human impacts. For example, sex- and size-biased fishing practices and landscape transformations may combine to affect the condition and functional traits of highly-valued, and often highly competitive and territorial species (Beattie et al. 2012; Alberts-Hubatsch et al. 2015; Unhalu et al. 2015). This can affect the health of wild populations, and the value of catches. In this study, we found significant correlations between the extent of marine ecosystems, urbanisation and fishing pressure on the body size and/or functional traits of giant mud crabs in southeast Queensland. Giant mud crab carapace size and weight was greatest in larger estuaries with lower extents of intertidal flats, thereby indicating that both natural attributes of estuaries, and the ways in which humans modify estuaries can affect the body size of giant mud crabs. Correlations between cheliped size and fishing pressure and increases in urban footprint around coastal ecosystems is of concern to consumers because the flesh from the chelipeds is some of the most prized for consumption. Further reductions in the size of individual crabs (as measured by weight or carapace width), especially if linked with reductions in the relative size of chelipeds may reduce the value of catches in the future. Given the social and economic importance of giant mud crabs throughout their range (Alberts-Hubatsch et al. 2015), understanding how these sub-lethal effects of anthropogenic impacts proliferate both through their population size and structure, as well as their body size and functional traits, is vital in optimising management responses.

We found that legal sized male giant mud crabs were larger and heavier in estuaries with wider estuary mouths and lower extent of intertidal flats in southeast Queensland. Estuary mouth width is a good proxy for the size and volume of estuaries in this region (Gilby et al. 2017d). Larger estuaries with larger estuary mouths have greater oceanic water flow in the lower reaches and therefore lower residence time of low saline water during runoff events, potentially resulting in greater temporal periods and broader extent of high salinity water to allow giant mud crabs to grow larger, especially during early developmental phases (Nurdiani and Zeng 2007; Alberts-Hubatsch et al. 2015). This greater water flow may also reduce the residence time of poor water quality runoff on in these larger estuaries. Large male giant mud crabs prefer to inhabit subtidal areas of narrow, mangrove-lined estuaries, and are often captured at the mouths of small inlets that drain tidally within these estuaries. It is likely that these sorts of seascape compositions are less common in estuaries with extensive intertidal flats. Giant mud crabs also make routine movements for foraging, and the extent of these movements likely depends on the composition of the seascape in which the giant mud crab lives (Alberts-Hubatsch et al. 2015). For example, giant mud crabs that occur in open embayments with extensive intertidal flats exhibit significantly larger home ranges that the crabs studied in narrow estuaries in this study (1 km in estuaries versus 3.7 km in open embayments) (Hyland et al. 1984). This increased energy expenditure and movement of individuals across extensive intertidal flats would likely have an effect on the amount of energy used for growth and weight gain. Adult giant mud crabs are top benthic predators consuming a variety of macroinvertebrates and carrion (Prasad and Neelakantan 1988; Alberts-Hubatsch et al. 2015). Extensive areas of low-complexity intertidal flats may not provide the same abundance and diversity of these food items as more channelized estuaries with more direct access between mangroves and adjacent deeper waters. Large estuaries with less extensive intertidal flats may also have higher flow rates from river outputs, meaning that giant mud crabs in these estuaries may receive greater feeding opportunities from catchments, in turn resulting in a lower requirement for moving to find food and meet their dietary needs. Animals that move less might also be less likely to encounter a competitor. Combined, these results indicate the importance of accounting for the broader landscape context of estuaries when considering the influence of human impacts on the body size of coastal species.

We found consistent effects of commercial fishing pressure and human population size in the surrounding catchment on the functional traits of giant mud crab, especially their chelipeds. These effects were consistent between both multivariate and univariate analyses of our functional trait data. Here, greater human populations and commercial fishing pressure correlated with smaller cheliped morphometrics. There are several potential mechanisms underlying this correlation. Male giant mud crabs are highly territorial and compete for territory and mates with other males within their range (Beattie et al. 2012). Males with larger chelipeds have an edge in these competitive interactions (Yoshino et al. 2011). In systems where many male giant mud crabs are removed due to fishing, the necessity to have large claws to outcompete conspecifics is significantly reduced. Conversely, there might be a genetic underpinning of this change if there is biased removal of male crabs with disproportionately large chelipeds. This genetic change might, however, be considered unlikely because of 1) homogenisation of the larvae over large spatial scales due to offshore spawning by females (Alberts-Hubatsch et al. 2015), and 2) the unlikeliness of fishers biasedly remove individuals with larger chelipeds as fishers would keep legal sized crabs even if they had proportionately smaller chelipeds. Therefore, the changes observed in this study are likely to be within the phenotypic spectra of the males following settlement, and related to competition and competitiveness amongst the males within this sex-biased fishery.

Whilst the metrics of fishing pressure included in this study are coarse, categorical metrics, the included variables reflect the best possible data available in the region to quantify these effects. For commercial fishing, data is not available at a finer spatial scale due to privacy issues surrounding the identification of individual fishers. Our human population size variable is used in this analysis as a proxy for both broader impacts within the catchment, and likely local recreational fishing pressure. Collecting accurate recreational fishing pressure information is a consistent challenge for coastal ecologists (McCluskey and Lewison 2008; Steffe et al. 2008; van Poorten et al. 2015), and the finest information available publically in this region covers most of the estuaries sampled in a single data point (Webley et al. 2015). Including this metric that uses population size as a proxy for likely effort is likely fair given the abundance of people across the entire region that undertake recreational fishing activities (Webley et al. 2015). Nevertheless, this is the first attempt, to our knowledge, of attempting to correlate these two key pressures on crab fisheries (Dumas et al. 2012; Alberts-Hubatsch et al. 2015) with key metrics of the functional traits and body size of the catch.

We found very few consistent effects of our environmental variables on the functional traits of the walking legs or the carapace. Whilst we did find a correlation between urban area and the extremities of the second walking legs, it’s difficult to establish causality between this variable and changes in the morphometric without further evidence of changes in other similarly positioned segments. In this sense, there’s no obvious mechanism underlying change in the size of this particular leg segment, and so the patterns found here are likely a coincidental correlation. In this study, increases in our metrics of the carapace don’t necessarily mean larger body size as they are scaled with carapace width. Consequently, our results indicate changes in the proportions of the carapace relative to carapace width, and therefore changes in the depth and breadth of certain areas of the carapace with different environmental conditions. Consequently, these inconsistencies potentially infer that variability in these morphometrics is due to specific adaptations to movement or burrowing in estuaries with different conditions. We did, however, find a correlation between human population size and morphometrics T13 and T16; two major, cross carapace morphometrics. These patterns are likely related to the above-described patterns between fishing pressure and the morphometrics of the chelipeds, and related to reduced competitiveness of males with conspecifics in areas of higher fishing pressure. Irrespective of these potential effects, the lack of consistent patterns across our carapace morphometrics, and the relatively low number of variables that did correlate with environmental variables may mean that the variables we used are not those causing variation in these the carapace or legs, or that there is genuinely little predictable variation in these metrics.

Giant mud crabs are a commercially, recreationally and socially important species throughout their range. Impacts to their populations will therefore have diverse ramifications for people. With increasing demand for giant mud crab flesh, and increasing fishing pressure and coastal development across their range (all concomitant with increasing human population), quantifying the effects of human impacts, the spatial scale and influence of those impacts, and optimising management around these impacts to ensure maximum abundance and condition of their populations will become increasingly important (Ewel 2008; Dumas et al. 2012; Alberts-Hubatsch et al. 2015). In this study, we identified a combination of natural and human-associated variables that modify the body size and functional traits of giant mud crabs in southeast Queensland. Intensive fishing pressure and reduced marine habitat extent are well understood pressures on the population size and structure of giant mud crabs (Pillans et al. 2005; Fratini et al. 2010; Unhalu et al. 2015). The results of this study show that these stressors can now be more strongly implicated in modifications to giant mud crab body size and functional traits. Giant mud crabs are significant ecological engineers in the systems they inhabit due to their large body zise and extensive burrowing actions. Changes in the functional traits of this species may eventually affect this capacity. Our results support the assertions of other studies regarding the importance of properly managing fishing effort of these larger, site-attached portunids in coastal ecosystems (Dumas et al. 2012), but add to this existing understanding by highlighting the potential influence of such stressors on the value of catches, and not just the volume of catches. Ecological restoration and marine reserves that are implemented with the goal of conserving and enhancing giant mud crab stocks must more closely consider the landscape context of the actions, and account for the potential effects of competitive interactions and variations in functional traits on the outcomes of those actions.

Supplementary materials

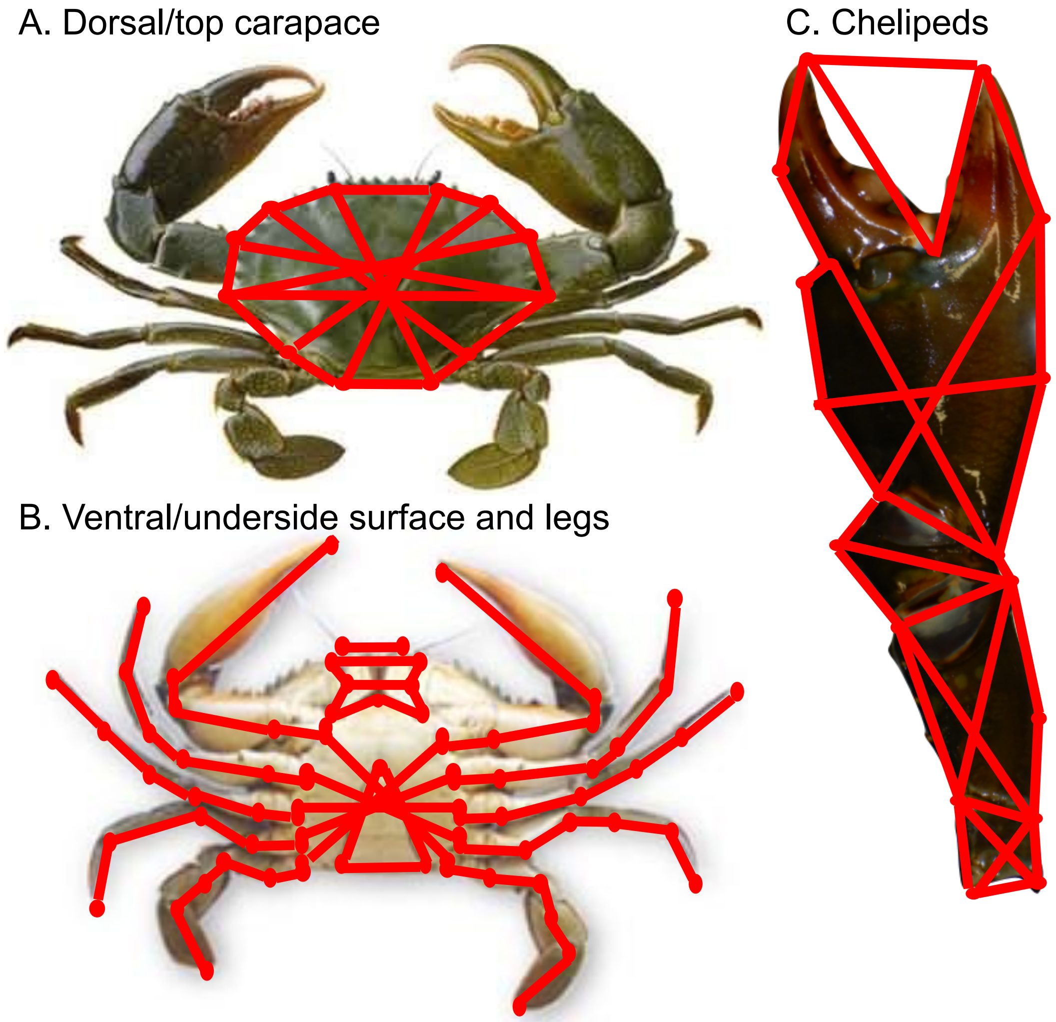

Figure S1 Giant mud crab morphometrics used in this study.

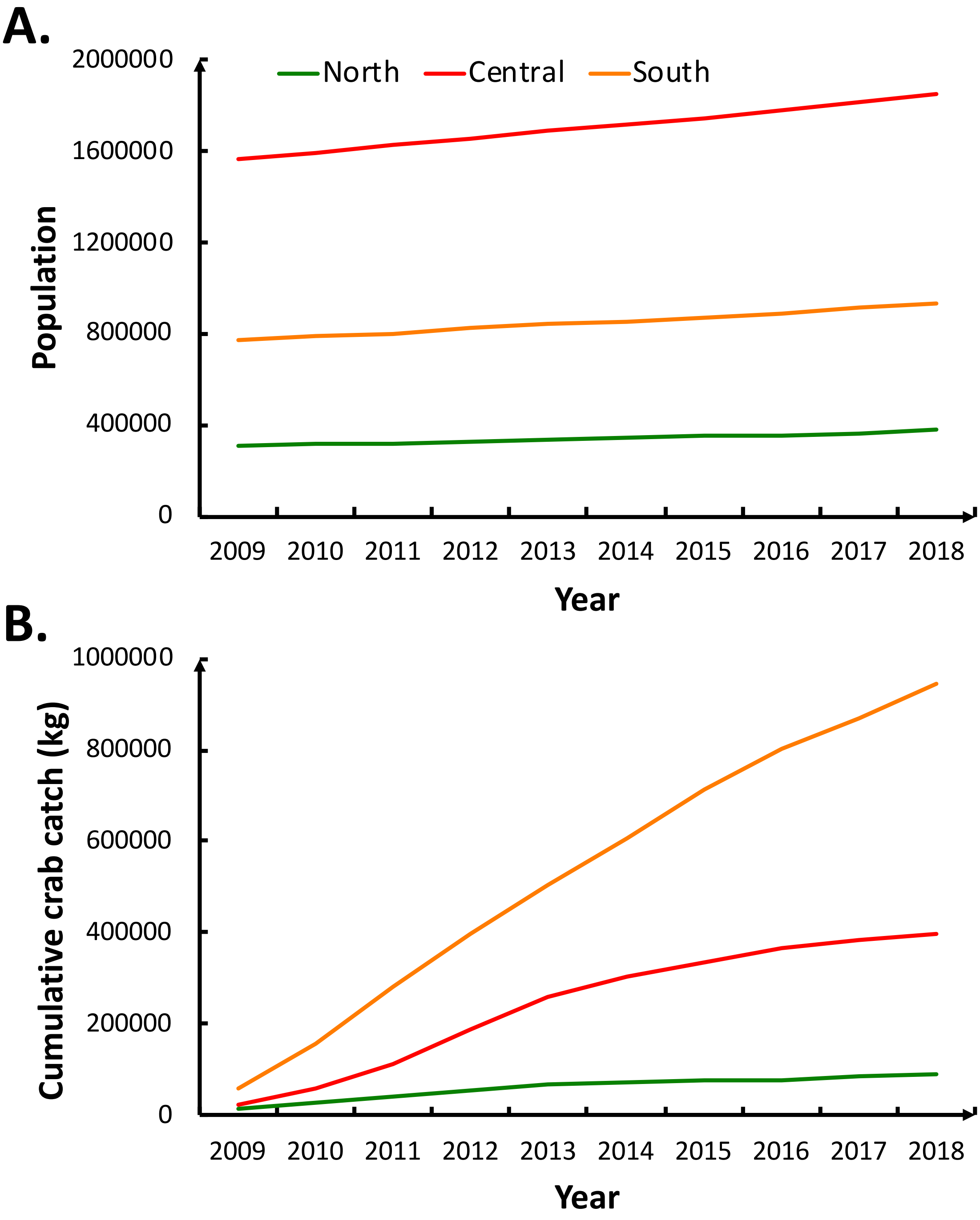

Figure S2 Population size (A.) and cumulative commercial giant mud crab catch (B.) for each of the three study regions in southeast Queensland. Commercial fishing data sources from the Fisheries Queensland QFish Data Base, and population data sourced from the Australian Bureau of Statistics.

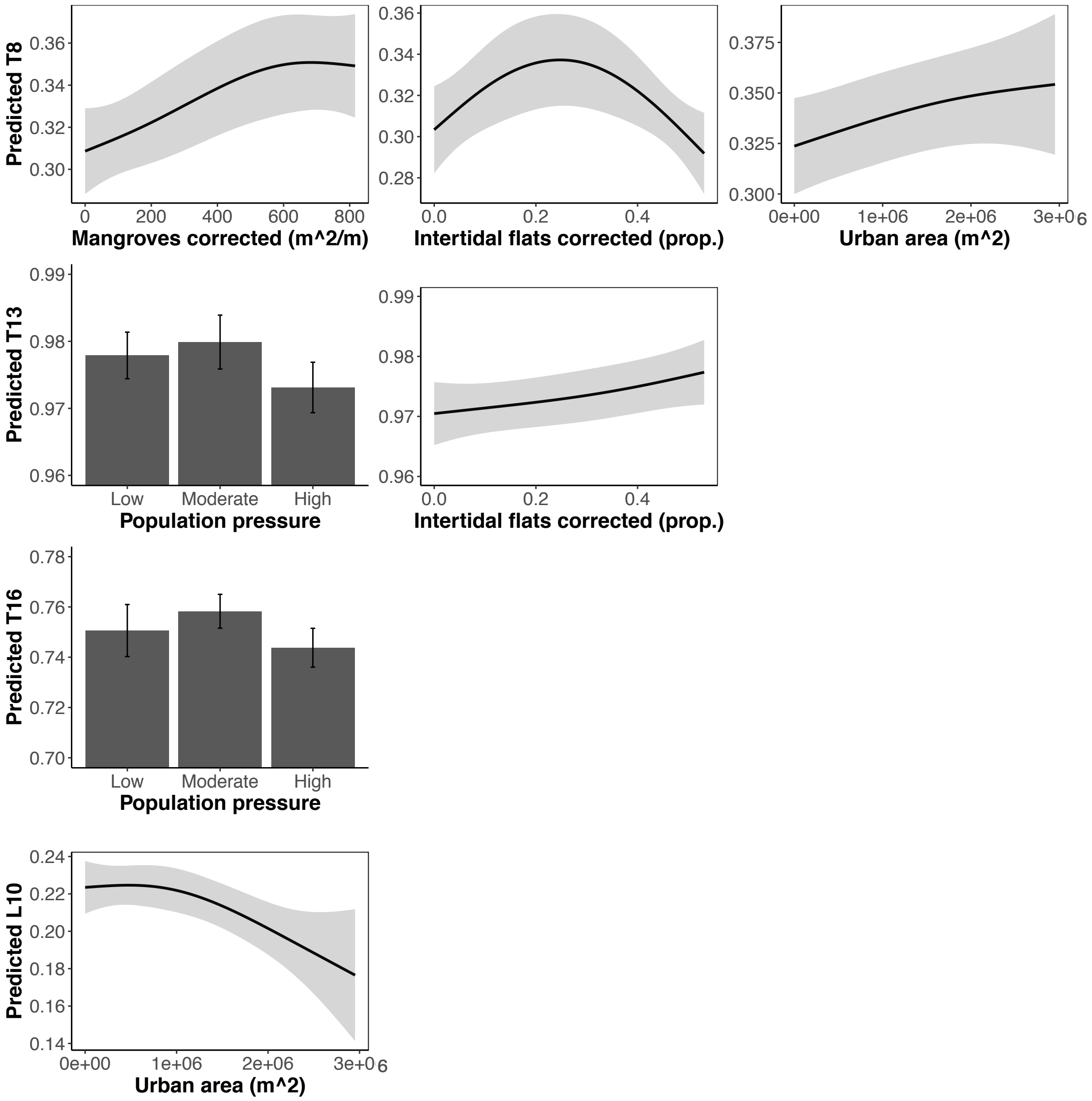

Figure S3 Generalised additive model (GAM) outputs of the variables in the best fit models for the top of the carapace (T) and walking leg (L) morphometrics that correlated strongly during multivariate analyses. All error measurements are 95% confidence intervals.

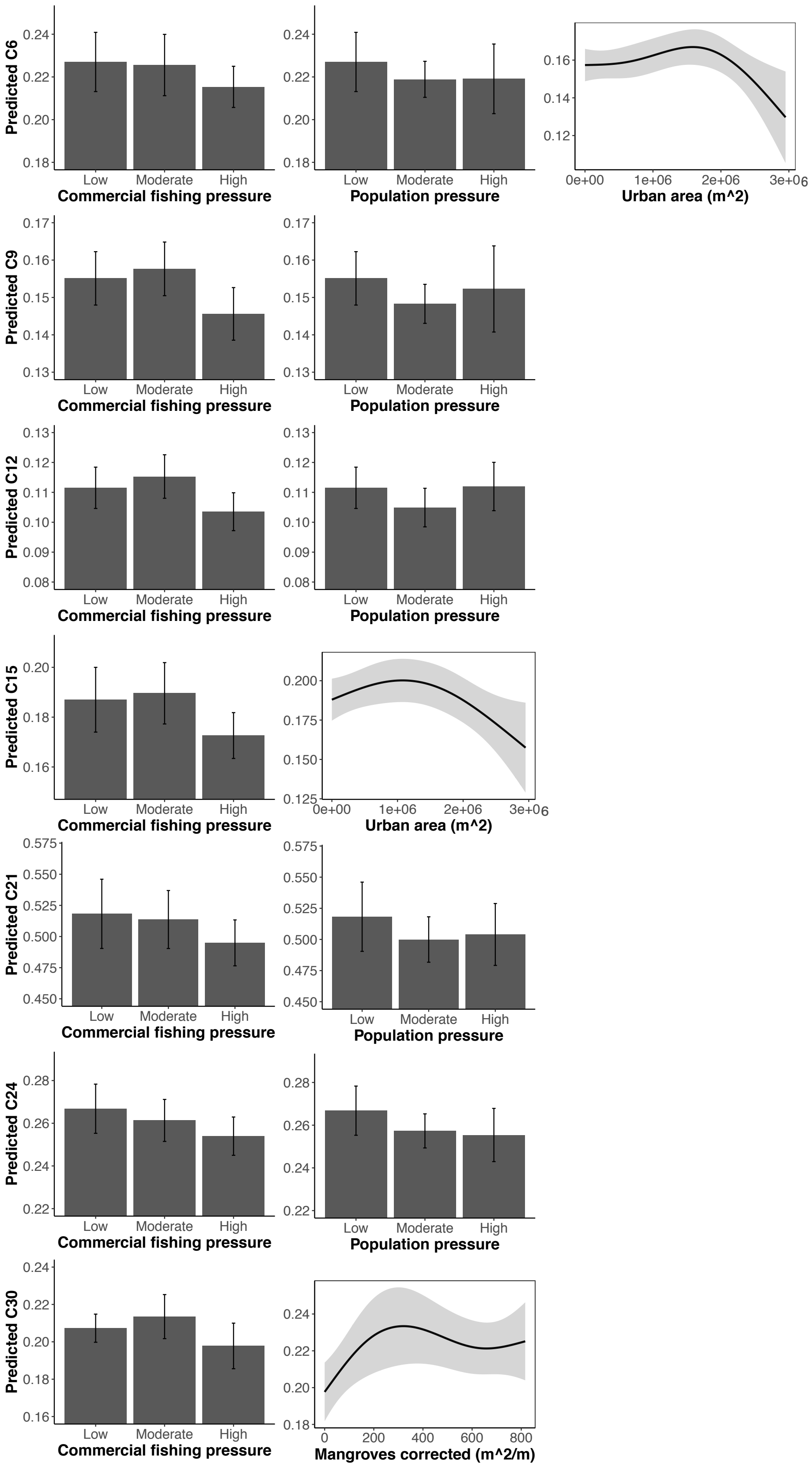

Figure S4 Generalised additive model (GAM) outputs of the variables in the best fit models for cheliped morphometrics that correlated strongly during multivariate analyses. All error measurements are 95% confidence intervals.

Table S1 List of sampled estuaries, number of crabs caught in each estuary, and average values of environmental variables included in statistical analyses.

Estuary	Number of giant mud crabs captured	Estuary mouth width (m)	Total mangroves (m^2)	Mangroves corrected (m^2/m)	Intertidal flats total (m^2)	Intertidal flats corrected (proportion)	Mangrove area (m^2)	Urban area (m^2)	Intertidal flats area (m^2)	Commercial fishing pressure	Population pressure
Brisbane	4	608	7820020	10	17519000	0.297	909323	313321	1040286	Moderate	High
Caboolture	4	312	5230670	669	9157040	0.531	1051334	917002	462912	Moderate	High
Coochin	3	161	1613210	545	1596840	0.000	634133	57840	92013	Low	Low
Currumbin	5	142	257848	45	373731	0.117	15687	1949572	0	High	Moderate
Logan	9	276	10927800	816	1247500	0.068	357829	615579	0	High	Moderate
Maroochy	6	191	4337910	144	1286230	0.459	643560	1080076	53426	Low	Low
Mooloolah	3	102	600604	42	0	0.000	150054	1660380	0	Low	Low
Nerang	2	214	0	0	193188	0.083	0	2489383	0	High	High
Noosa	4	210	3866210	199	5147730	0.048	422475	620628	202101	Low	Low
Pimpama	9	152	4265600	623	1381690	0.504	973522	0	459768	High	Moderate
Pine	5	609	6871430	631	6730240	0.087	1050241	168025	759099	Moderate	High
Tallebudgera	4	171	361848	66	370540	0.141	357609	2380172	11457	Moderate	High
Tingalpa	7	215	2026710	352	2821460	0.415	683172	1615013	9210	High	Moderate

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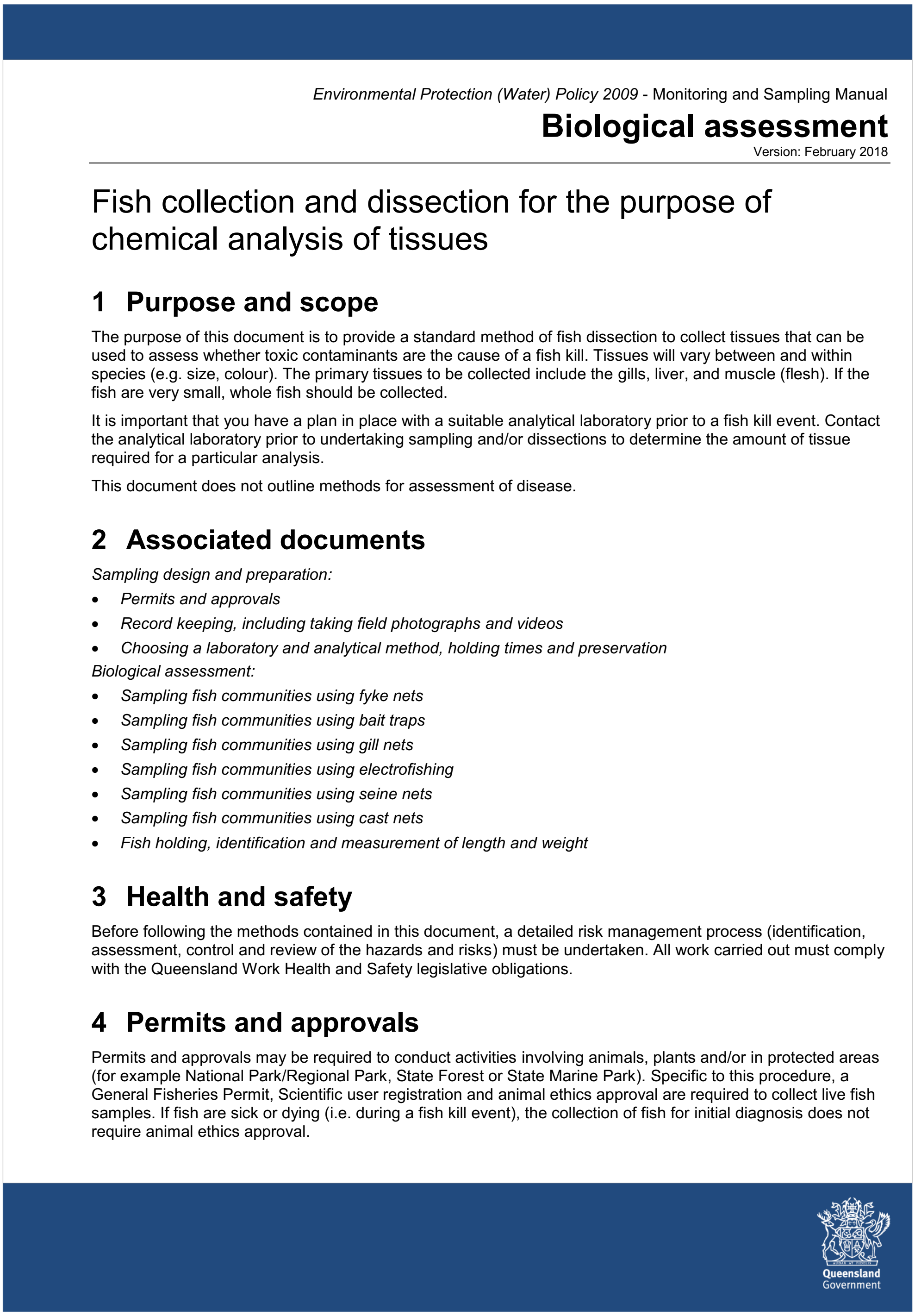
Appendices



Environmental Protection (Water) Policy 2009 - Monitoring and Sampling Manual

Biological assessment

Version: February 2018

Fish collection and dissection for the purpose of chemical analysis of tissues

1 Purpose and scope

The purpose of this document is to provide a standard method of fish dissection to collect tissues that can be used to assess whether toxic contaminants are the cause of a fish kill. Tissues will vary between and within species (e.g. size, colour). The primary tissues to be collected include the gills, liver, and muscle (flesh). If the fish are very small, whole fish should be collected.

It is important that you have a plan in place with a suitable analytical laboratory prior to a fish kill event. Contact the analytical laboratory prior to undertaking sampling and/or dissections to determine the amount of tissue required for a particular analysis.

This document does not outline methods for assessment of disease.

2 Associated documents

Sampling design and preparation:

- *Permits and approvals*
- *Record keeping, including taking field photographs and videos*
- *Choosing a laboratory and analytical method, holding times and preservation*

Biological assessment:

- *Sampling fish communities using fyke nets*
- *Sampling fish communities using bait traps*
- *Sampling fish communities using gill nets*
- *Sampling fish communities using electrofishing*
- *Sampling fish communities using seine nets*
- *Sampling fish communities using cast nets*
- *Fish holding, identification and measurement of length and weight*

3 Health and safety

Before following the methods contained in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the Queensland Work Health and Safety legislative obligations.

4 Permits and approvals

Permits and approvals may be required to conduct activities involving animals, plants and/or in protected areas (for example National Park/Regional Park, State Forest or State Marine Park). Specific to this procedure, a General Fisheries Permit, Scientific user registration and animal ethics approval are required to collect live fish samples. If fish are sick or dying (i.e. during a fish kill event), the collection of fish for initial diagnosis does not require animal ethics approval.

Queensland Government

Appendix 1 Fish collection and dissection protocols (Available from USC on request)