Monitoring and morphometric studies of the European shore crab (*Carcinus maenas*) in Gulf St Vincent 2016/17

Report for the Adelaide & Mount Lofty Ranges NRM Board

Sabine Dittmann, Ryan Baring, Hayley Jessup-Case & Rene Campbell

Flinders University, School of Biological Sciences

GPO Box 2100, Adelaide, SA, 5001

December 2017

Flinders UNIVERSITY

This report may be cited as: Dittmann, S., Baring, R.J., Jessup-Case H., Campbell R. (2017). Monitoring and morphometric studies of the European shore crab (*Carcinus maenas*) in Gulf St Vincent 2016/17. Report for the Adelaide & Mount Lofty Ranges NRM Board. Flinders University, Adelaide.

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Executive Summary

The European shore crab *Carcinus maenas* (hereafter *Carcinus*) is one of the most invasive marine species in the world and has established populations in the Gulf St Vincent. Monitoring the distribution and abundance of *Carcinus* along the Adelaide coastline from the northern mangrove shores to rocky intertidal areas south of the city has confirmed the wide-spread occurrence and increased abundance of this invasive crab.

Monitoring in 2016/17 was carried out using baited traps at two mangrove and two Port River sites, and timed searches on southern rocky shores. This report comprises findings from six surveys between winter 2016 and spring 2017 and the results are presented in conjunction with longer-term data from previous monitoring.

During the current monitoring year, 193 *Carcinus* were caught at the northern mangrove and Port River sites, and a further 104 *Carcinus* were found at southern sites. This brings the total number of this invasive crabs caught in the monitoring since 2012 to over 800 individuals. Catches were higher than in the previous monitoring year and had especially increased at the southern sites.

A seasonal pattern with highest catches of *Carcinus* in winter and spring could be corroborated with findings from the recent monitoring. The highest catches of female and male crabs were made in winter and spring. Female crabs were seldom caught in summer and autumn. *Carcinus* was caught in the mangroves in all seasons, but was rarer in the Port River during the warmer months of the year. At the southern sites, seasonal patterns in *Carcinus* catches were less apparent, with a slight indication for higher numbers in spring.

The size of *Carcinus* caught in the 2016/17 monitoring ranged from 10 to 88 mm carapace width, with a higher frequency of juvenile crabs recorded than in previous monitoring years. Cohorts were apparent from the increase in size over consecutive surveys. Size frequency distributions showed that crabs were smaller at the southern sites, where most of the recruitment appeared to occur, intermediate in size in the Port River, and largest in the mangroves. Sex ratios were male biased in the mangroves and southern sites, but more even or female biased in the Port River. Females with eggs (ovigerous) were previously only recorded from mangrove sites, but in the current monitoring also found in the Port River and, for the first time, at the southern sites.

The frequency of red and green colour morphs, which indicates moulting intervals, showed that green morphs (moulting frequently) were more common in juvenile crabs as well as in male *Carcinus*. Most of the female crabs caught in winter and spring were red, and almost all ovigerous females had not moulted for some time. The large male *Carcinus* caught in the mangroves were mostly red, especially in winter and spring. The colour pattern of the dorsal side of the carapace varied with habitat, with more diverse colour patterns and complexity at the rocky shores compared to predominantly plain dorsal colour of crabs found in mangroves.

Correlations with water quality variables indicated higher catches of *Carcinus* when water temperatures were cooler and dissolved oxygen concentration and saturation higher. Salinity had little influence on the abundance of Carcinus. The native blue swimmer crab *Portunus armatus* was found

as by-catch in baited traps mostly during the warmer summer and autumn months, and different temperature adaptations of these two crab species could reduce competitive overlap.

Allometric growth relationships were found for male and female crabs in each region, with carapace length and height growing at the same rate as carapace width. The weight of the crabs showed a positive allometric relationships with accelerated increase in weight in larger crabs. The frequency of ratios of carapace width to length, and width to height was analysed to explore the presence of *Carcinus aestuarii*, giving inconclusive indications of the presence of this species or hybrids. Most of the crabs found in all regions were *C. maenas* based on the ratios.

Overall, the monitoring has documented that *Carcinus* is widespread along the Adelaide coastline, is increasing in abundance and reproductively active throughout the distribution. The morphometric variation of male and female *Carcinus* across the three regions could further reflect a differential habitat use along the coast in the course of the crab's life cycle, which warrants further investigation.

Recommendations for attempts to reduce the population of this invasive species could target catching ovigerous female *Carcinus* in winter and spring.

1. Introduction

Marine invasive species pose serious threat to coastal ecosystems and native flora and fauna worldwide (Hayes & Sliwa 2003, Elliott *et al.* 2014). Through time, increased shipping traffic has resulted in increased rates of invasion by introduced marine species in coastal ports and harbours via ballast water exchange and hull fouling (Bax *et al.* 2003; Carlton & Cohen 2003; Blakeslee *et al.* 2010; Ojaveer *et al.* 2015). Transportation of introduced marine species can also occur via many other pathways including; aquaculture, fisheries, offshore mining, aquarium industry, recreational boating, diving industry and floating debris (Bax *et al.* 2003). The stressors of climate change and human-induced ecosystem modification can promote favourable conditions for potential marine invasive species (Occhipinti-Ambrogi & Savini 2003; Compton *et al.* 2010; Occhipinti-Ambrogi & Galil 2010; Tepolt & Somero 2014). Usually, eradication or control of alien marine species that have invaded coastal waterways is challenging and risky (Sambrook *et al.* 2014; Atalah *et al.* 2015).

The European shore crab *Carcinus maenas* is listed as one of the top 100 of the IUCN's most invasive species in the world and it has established populations in many countries foreign to its native distribution range of the Atlantic coast of Europe (Lowe *et al.* 2000; Leignel *et al.* 2014; Crafton 2015). In many regions around the world including South Africa, North America and Australia, populations of *C. maenas* have been identified as reproductively active (Carlton & Cohen 2003, McGaw *et al.* 2011, Best *et al.* 2017, and previous reports from this monitoring: Dittmann *et al.* 2016). In regions where *C. maenas* has established populations outside of its native range, results of impacts include voracious feeding on native mussel populations, and effects on fisheries and seagrass decline from burrowing activities in soft sediments (Grosholz & Ruiz 1996, Tan & Beal 2015, Matheson *et al.* 2016). *Carcinus maenas* has also recently been shown to be a major threat to aquaculture oyster leases and oyster restoration efforts in North America (Pickering *et al.* 2017).

Carcinus maenas was introduced in Australia during the 19th century and first recorded in Port Philip Bay, Victoria (Fulton & Grant 1902) and later in Botany Bay, NSW (Ahyong 2005). Australian populations of *C. maenas* are usually found in sheltered, low energy estuaries and bays in soft sediment, seagrass and mangrove habitats (Thresher *et al.* 2003; Dittmann *et al.* 2013, 2015, 2016; Garside & Bishop 2014). In Australia, *C. maenas* was identified as marine invasive species of concern by the Australian National Introduced Marine Pests Coordination Group (Aquenal, 2008) and has been classified as a national priority pest by the Australian Natural Heritage Trust due to various potential risk criteria including; loss of fisheries harvest, out-competing native species, and predation of native species (Hayes *et al.* 2005).

In 1976, *C. maenas* was first recorded in South Australia and has since been identified at various sites throughout Gulf St. Vincent, where there appears to be a self sustaining population that is reproductively active (Zeidler 1978; Rosenzweig 1984; Thresher 1999; Wiltshire *et al.* 2010). Abundances have been variable throughout the years, particularly along the northern metropolitan Adelaide coastline and recent sightings have indicated range extensions further south of Adelaide (Dittmann *et al.* 2016).

Confirmation of invasive species identity is important as it may help to recognise the species' native population and mode of introduction (Yamada & Hauck 2001). Two species of invasive green crab are currently recognised: the Atlantic *Carcinus maenas* and the Mediterranean *C. aestuarii*, and both have established populations in countries outside of their native distribution range (Yamada & Hauck 2001, Leignel *et al.* 2014). There are also growing indications for hybridisation between the two species, which could contribute to the success of their global invasions (Darling *et al.* 2008, Darling 2011, Rius & Darling 2014).

Green crab populations in Gulf St. Vincent, South Australia, have so far been identified as *Carcinus maenas,* yet the more mediterranean climate in South Australia's gulfs provide a habitat more suitable to *C. aestuarii*. Differentiation of *C. maenas* and *C. aestuarii* requires detailed inspection of male pleopods, the shape of the carapace edge between the eyes, and carapace width to length ratio (Yamada & Hauck 2001). Genetic variability and morphological variations due to phenotypic plasticity are also known for both *C. maenas* and *C. aestuarii* (Brian *et al.* 2006, Marino *et al.* 2010, Deli *et al.* 2014). Although genetic techniques can be more accurate in determining this information, these procedures are often expensive and time-consuming. Identification and measurement of morphological features is thus a more feasible approach to detect species differentiation.

Seasonal monitoring was continued at the sampling sites in the mangrove and Port River region north of Adelaide, and on rocky shores south of Adelaide, to detect whether the population size has further increased or spread to other locations. The monitoring also covered the assumed main reproductive period of *C. maenas* in autumn and winter to detect whether ovigerous females are present. Morphometric measurements on crab specimens collected over several monitoring years was carried out to assess morphological variation across habitats and parameters for population studies. Colour patterns were also recorded to detect habitat specific colour variations. The objectives were thus to provide an update on the population size and distribution, a confirmation of the reproductive periodicity and estimate of fecundity, species identification and growth estimates based on morphometric analyses, an analysis of colour variation in relation to habitats.

2. Methods

2.1 Sampling sites and dates

Monitoring of the *Carcinus maenas* (hereafter *Carcinus* unless stated otherwise) population in Gulf St. Vincent during 2016/17 used the same two approaches as in previous years; field trapping and timed search surveys. Field trapping was conducted in the Port River (2 sites: Old Port Reach and Port Adelaide Marina), and mangroves (2 sites: Port Gawler and Middle Beach; Figure 1). Habitats vary throughout the Port River sites with Old Port Reach consisting of a small mangrove cove and mudflats adjacent to the West Lakes water outlet, whereas the Port Adelaide Marina has an expansive bare sediment mudflat close to a boat ramp and marina, and a shipping channel in the deeper subtidal zone. Also, high-rise apartments are close to the Old Port Reach site and the Port Adelaide Marina site is within close vicinity to industrial areas (e.g. AGL Power Station and the Australian Submarine Corporation). The Port Gawler and Middle Beach sites have more similar habitats to each other with expansive mudflats, mangrove forests and seagrass beds. Both mangrove sites are publically accessible and Port Gawler in particular experiences pressures from recreational off-road vehicle use, dumping of illegal rubbish and recreational fishing. A small creek (Salt Creek) is creating a break in the mangroves at Middle Beach, which is part of the Upper Gulf St Vincent Marine Park. Along the rocky shores of the southern metropolitan Adelaide coastline (4 sites; Marino, Hallett Cove, Port Noarlunga South and Aldinga), timed search surveys were conducted to assess if the population of *Carcinus* had continued to increase its range and population size.

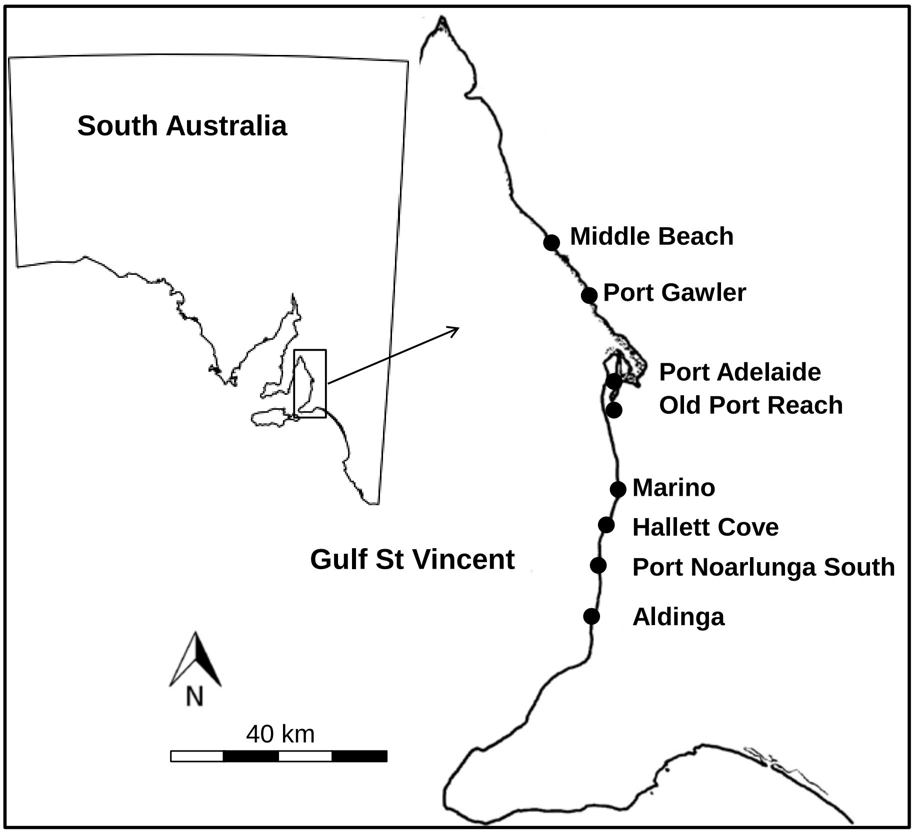

Figure 1: Map of the monitoring sites for *Carcinus* along the greater Adelaide coastline of the Gulf St Vincent. Middle Beach, Port Gawler, Port Adelaide and Old Port Reach constitute the northern sites. Marino, Hallett Cove, Port Noarlunga South and Aldinga the southern sites.

The sampling sites for trapping and timed searches are in a low-energy coastline with a mixed tidal rhythm and a 2-3 m spring tidal range. Sites sampled for field trapping coincided with the new moon period and spring tidal ranges as in previous years. Timed searches at southern field sites were conducted at low tide (<0.6 m level) in order to gain full access to the intertidal rocky shorelines along the southern coastline.

Trapping of *Carcinus* in the field occurred seasonally from July 2016 through to October 2017 (i.e. six sampling events; Table 1). Timed searches at the southern sites occurred seasonally from November 2016 through to October 2017 (i.e. five sampling events from spring to spring; Table 1).

Table 1: Sampling dates for the monitoring of *Carcinus* at the four northern sites, where baited traps were set, and four survey sites for timed searches south of Adelaide, sampled between July 2016 and October 2017. See Figure 1 for the location of the northern and southern sites.

Year	Season	Month	Baited traps	Timed searches
2016	**Winter**	Jul.	7th & 8th	
	Spring	Nov.	3rd & 4th	15th, 16th
2017	**Summer**	Jan.	30th & 31st	
		Feb.		2nd, 3rd
	Autumn	Mar.	27th & 28th	30th, 31st
	Winter	Jun.	26th & 27th	28th, 29th
	Spring	Oct.	25th & 26th	23rd, 24th

2.2 Sampling methods and analysis

Trapping of *Carcinus* occurred with the use of opera house-style traps (67 x 48 cm, opening diameter 7.8 cm, 2 cm mesh size) that had two entry points and were baited with commercially available pilchards. The baited opera house traps were deployed in shallow water at low tide and secured to the sediment with wooden stakes. At the four northern sites, five traps were set in each season and on all occasions the traps were deployed and retrieved 24 hours later.

Carcinus is generally more active at night (Reid & Naylor 1989) and so all traps were set overnight to maximise the possibility of capturing crabs. Upon retrieval of traps, all individuals of *Carcinus* were collected and transported to Flinders University, where they were sexed, their carapace width measured with Vernier callipers across the widest part of the carapace, and ventral colour recorded. Ovigerous females were also recorded when crabs were gravid with eggs. All specimens of *Carcinus* were frozen for future morphometric measurements. Any accidental bycatch was carefully removed from the traps. Fish were identified, counted and immediately released back into the water. Invertebrates (snails, other crabs) were also identified and counted prior to release. Environmental parameters were recorded at each site, with water temperature, salinity, and dissolved oxygen (concentration and saturation) measured using a handheld multiprobe, and salinity also measured with a refractometer. Three replicate readings were taken for each parameter and later averaged.

The total number of *Carcinus* caught per site and survey was divided by the number of traps retrieved to obtain CPUE so that catches could be compared with data from previous years as the number of traps retrieved sometimes differed. The CPUE values can be fractions or smaller than one. The report includes analyses for annual and seasonal patterns which utilise data from previous monitoring (Dittmann *et al.* 2013, 2015, 2016). Seasonal differences in CPUE were tested with a two-way ANOVA and pairwise t-test on untransformed data with Euclidean distance, based on all data since 2012 and using the four sites in northern Adelaide as replicates. A two-way ANOVA was also carried out for season and year differences with data from 2013. Similar tests were carried out for catches from male and female crabs separately, with pairwise means test (Tukey). The tests were run using Origin Pro 2015 or the PRIMER v7 with PERMANOVA add-on software. Test results are presented in tables in an Appendix. Correlation coefficients (Pearson, and in some cases Spearman) were calculated for correlations between crab catches and water quality variables, as well as for some correlations between *Carcinus* and other species found in the baited traps. These correlations and most associated figures were prepared in Origin Pro 2015.

The timed search surveys at each of the four southern sites were carried out across intertidal reef platforms. During the autumn 2017 survey at Port Noarlunga South, two out of the four timed searches were conducted in soft sediments covered by rocks in the adjacent Onkaparinga estuary, as there had been observations of dead *Carcinus* from the mouth of the estuary. As *Carcinus* were found at this location, subsequent sampling in winter 2017 occurred within the Onkaparinga estuary, rather than the rocky intertidal platform. Two timed searches were conducted by each of two researchers per sampling event over 30 minutes each (i.e. four timed searches per site per sampling event) across separate sections of the intertidal reef platform. Researchers conducted the timed searches by slowly walking across the reef platforms at each site, looking underneath small boulders and into overhangs or crevices to locate and catch any *Carcinus* found. All *Carcinus* captured were taken back to Flinders University and frozen (-20° C) before measurements were taken for carapace width and records of sex and colour noted as above. All other crab species were recorded as present in categories of low to high abundances.

2.3 Morphometric analyses, colour patterns and fecundity

Frozen *Carcinus* specimens (800 crabs) that were collected from the monitoring project and accompanying honours student projects in Gulf St Vincent over several years (mainly 2015-2017) were processed for morphometric analysis to explore whether crabs occurring in the southern and northern sites are the same species of *Carcinus*, and estimate further aspects of their population biology. Each frozen crab was thawed and blotted dry, and the sex of the crab, the date, site and intertidal zone and habitat of collection recorded. The wet weight (g) of each crab was measured using electronic scales.

Morphological features based on Clark *et al.* (2001) and Fowler & McLay (2013) were measured for the following dimensions using digital Vernier callipers to the nearest 0.1 mm: 1) carapace width; 2) carapace length; 3) carapace height (also referred to as depth); 4) optical groove width; 5) length of right chela; 6) height of right chela; 7) length of propodus of right chela (excluding pollex); 8) length of left chela; 9) height of left chela; 10) length of propodus of left chela (excluding pollex). The width of the penultimate pleomere and the pleon length (including telson) of each male and female crab were also measured.

Allometric growth was explored using the carapace width (CW) as independent variable and carapace length (CL), carapace height (CH) and the wet weight (WW) as dependent variables. Allometric relationships were analysed separately for male and female *Carcinus* and for each of the main study regions, with most of the measured crabs coming from the mangrove sites. Power functions were fitted to scatter plots, using the allometric equation $y=ax^b$, whereby a is the intercept, x the independent variable (CW), and b the allometric growth coefficient. Plots and curve fittings, as well as accompanying ANOVA tests for the regressions were carried out in ORIGIN PRO 2015 software.

The crab measurements were also applied to assess the identification of *Carcinus*, as certain morphological features are used in the literature as distinguishing characteristics for *C. maenas* or *C. aestuarii* (Table 2). The most useful differentiation that has emerged in the literature is the ratio of carapace width to length (CW/CL). The CW/CL and also the ratio for carapace width to height (CW/CH) were calculated for each *Carcinus* measured, and the frequencies for both ratios were determined for each region.

Table 2: Morphological features used for distinguishing specimens of *Carcinus* collected in Gulf St. Vincent, South Australia as *C. maenas* or *C. aestuarii*. Based on methods by Clark *et al.* (2001), Yamada & Hauck (2001).

Feature	*Carcinus maenas*	*Carcinus aestuarii*	Comments
Carapace width to length ratio (CW/CL) for crabs ≥ 20 mm	Wider carapace 1.29 – 1.36	Narrower carapace 1.22 – 1.27	Good indicator
Shape of three lobes in frontal area (between the eye and sockets)	3 distinct “bumps”, margin scalloped, frontal area does not protrude	Flatter, “bumps” not as distinct, frontal area protrudes beyond eyes	Good indicator
Maximum carapace width	> 90 mm	> 65 mm	Not a good indicator: maximum size is not reached in many locations
Male pleopods (copulatory appendages)	Crescent-shaped, touch at the bend	Parallel and straight, don’t touch	May be a good indicator, may require microscopy for better comparison
Carapace width to height ratio (CW/CH)	Thinner carapace 2.32 – 2.5	Deeper carapace 2.19 – 2.26	May be a good indicator

The twelve morphological features measured were further analysed for morphometric differences in *Carcinus* found in the mangrove region, Port River and southern sites, and for both sexes. Multivariate analyses was carried out on the size measurements using Euclidean Distance for a resemblance matrix and bootstrap procedure to create an MDS plot, as the sample size from the three regions was very different. Bootstrapping is resampling the data set with replacement to estimate a region for group values. Morphological features that accounted for the differences between regions and sex were further analysed with SIMPER. These multivariate analyses were carried out for 680 measured crabs with no missing values (e.g. claw missing), using PRIMER v7.

To investigate the colour or pattern variation of the dorsal carapace of *Carcinus*, a high-resolution photograph was taken of each crab using a Nikon D3100 SLR digital camera and assigned with a corresponding reference number. Contrast has been edited 8-10% to enhance pattern variation, however all crabs were placed under the same lighting conditions and camera settings (NIKON D3100 DSLR camera). Colour and pattern variation categories were assigned for the dorsal surface of each crab (e.g. plain, speckled, splodge etc.) and a key designed to ensure consistency between distinguishing features (Table 3). If a new characteristic was encountered in an individual (i.e. a new colour or pattern not seen in previous specimens), it was added to the key.

Table 3. Distinguishing features and their corresponding name for patterns found on the dorsal surface (carapace) of *Carcinus.* Visual reference can be seen in Figure 28.

Pattern name	Distinguishing features
Plain	Over 75% of the carapace has a uniform, solid colour with little pattern.
Double stripe	Two cream/faint-coloured patches on either side of the carapace near lateral teeth). Often found with other patterns
Cloudy spots	A mixture of large and small dark splodges and spots cover 50-75% of the carapace
Small spots	Small, dark spots cover 50-75% of the carapace
Speckled	Very small spots, often grainy and lighter in colour, cover 50-75% of the carapace
Splodges	Large, uneven cream-coloured patches and "splodges" cover parts of the carapace
Rostrum spot	One patch on the rostrum between the eyes (often cream/faint in colour, but may be dark). Often found with other patterns
Mottled	A mixture of many spots, speckles, splodges and colours covers 50-75% of the carapace.
Tri-stripe	Three cream/faint-coloured patches on either side of the carapace and on the rostrum. Often found with other patterns.

To analyse fecundity, ovigerous females caught in previous and current monitoring were analysed based on methods by Fowler & McLay (2013). The wet weight (g) of each ovigerous female, including their egg mass, was determined using electronic scales. Egg masses were then detached from pleopods and female were weighed again. Three sub-samples were taken from each egg mass (assuming egg condition had not deteriorated after being frozen) and preserved in 70% ethanol. Further analyses (counting number of eggs in each sub-sample) will occur as part of René Campbell's PhD project once further ovigerous females have been found and measured.

3. Results - Seasonal monitoring

3.1 Distribution, abundance and seasonal variation

3.1.1 Northern sites

Throughout the monitoring years since Winter 2012, a total of 642 *Carcinus* have been caught by trapping in the Port River and northern mangrove sites. In the current monitoring period (2016/17), abundances of *Carcinus* were slightly higher than in the previous monitoring year (2015/16; Dittmann *et al.* 2016) with a total of 193 and 151 crabs caught, respectively (six surveys in each period). Catch per unit effort (CPUE) of *Carcinus* across the 2016/17 year from late Winter 2016 to Spring 2017 was 1.6 (193 crabs from 120 traps, Table 4) compared to 1.3 in 2015/16 (151 crabs from 117 traps from Autumn 2015 to Winter 2016).

The total catches of *Carcinus* in 2016/17 were slightly higher at the two mangrove sites (Middle Beach and Port Gawler) compared to the two Port River sites (Port Adelaide and Old Port Reach) (102 versus 91 crabs, respectively, Table 4). The highest single catch was a total of 37 individuals of *Carcinus* in the Spring 2016 survey from Old Port Reach. In the preceding 2015/16 monitoring year, slightly more *Carcinus* had also been captured at the mangrove compared to the Port River sites (Dittmann *et al.* 2016).

Table 4: Summary of total catch, total traps retrieved and CPUE for each site and season for *Carcinus* collected in baited traps in the monitoring period since late winter 2016 (see Table 1). The three cells shaded in grey show (in order left to right) total number of *Carcinus* caught, total number of traps retrieved and overall CPUE (catch per trap) for the monitoring period 2016/17.

Site	Late Winter 2016	Spring 2016	Summer 2016/17	Autumn 2017	Winter 2017	Spring 2017	Site Total	Traps per site	Site CPUE
Middle Beach	9	11	0	1	11	14	46	30	1.5
Port Gawler	18	10	3	3	7	15	56	30	1.9
Port Adelaide	7	5	0	1	21	8	42	29	1.4
Old Port Reach	0	37	0	0	9	3	49	30	1.6
Season Total	34	63	3	5	48	40	193	119	1.6
Traps per season	20	20	20	20	20	20	-	-	-
Season CPUE	1.7	3.2	0.2	0.3	2.4	2	-	-	-

The CPUE of *Carcinus* in the Port River and mangrove region north of Adelaide was higher in Spring 2016, Spring 2017 and Winter 2017 than in Summer and Autumn 2017 (Figure 3). The finding of higher total catches and CPUE around the winter months and spring upheld a seasonal pattern which had emerged over the monitoring years (Figure 4, Table A1). Winter and Spring catches and CPUE in 2016/17 were comparable to values from the same seasons in previous monitoring years. During 2016/17, CPUE and total catches of *Carcinus* were low in Summer and Autumn compared to seasonal CPUE and total catches in respective seasons from 2014 to 2016 (Figure 3). Seasonal CPUE of *Carcinus* over the entire monitoring were always lower in Summer and Autumn compared to Winter and Spring (Figure 4, Table A1). This seasonal pattern was not only irrespective of sites, but also of annual variation, as a two-way ANOVA showed no significant interaction for seasons and years (Table A2).

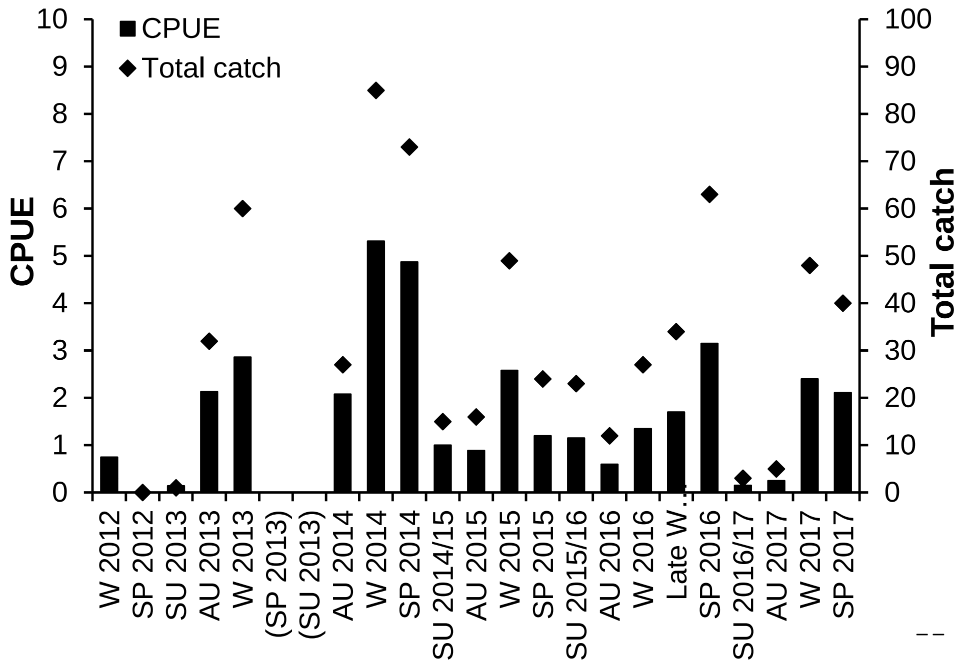

Figure 3: Catch per unit effort (CPUE, in bars) and total catch (diamonds) of *Carcinus* for the monitoring from mid-2012 to Spring 2017 at sites sampled in the Port River region and mangroves north of Adelaide. The sites sampled differed slightly over the 2012/13 and 2014/15 monitoring, as did the lunar phase of sampling, which included neap tides in 2012/13. The Winter 2013 combines the spring and neap tide sampling events carried out that month. No sampling occurred in Spring and Summer 2013.

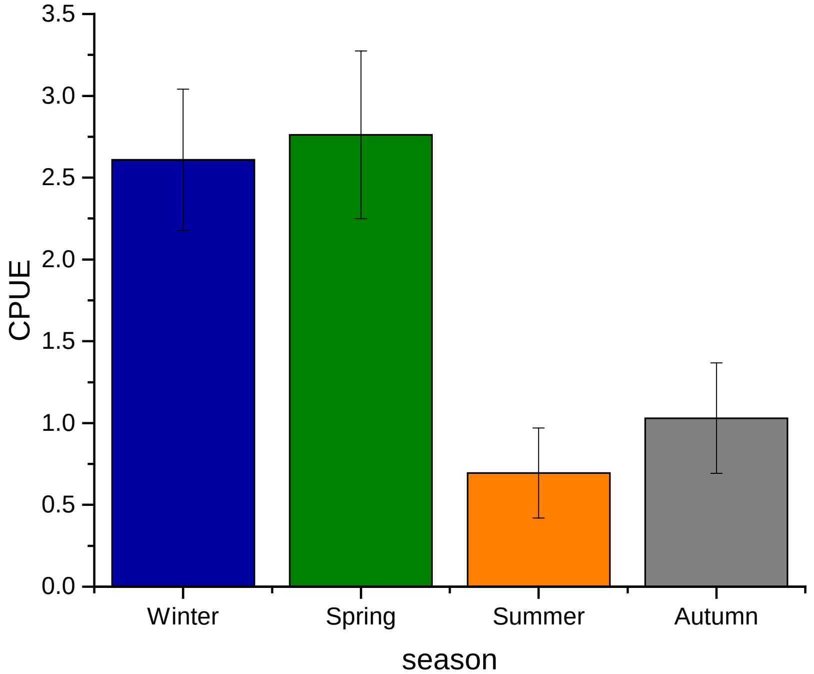

Figure 4: Catch per unit effort (CPUE, average ± SE) of *Carcinus* across the seasons based on monitoring data from Autumn 2013 (from when all sites were sampled more regularly) to Winter 2017 for the mangrove and Port River sites north of Adelaide.

There was, however, some variability in seasonal abundances of *Carcinus* at a finer scale of sites or habitats. Differentiating the four northern sampling sites into Mangrove and Port River showed that the higher CPUE in winter and spring was sometimes driven by catches in the mangroves, and at other times in the Port River (Figure 5). In the two seasons with low abundances (Summer and Autumn), catches came mostly from the mangrove sites (Figure 5). This finding from the current surveys (Figure 5) aligns with previous monitoring years, when *Carcinus* was present in all seasons at the mangrove sites, but rare at the sites in the Port River region in Summer and Autumn (Figure 6).

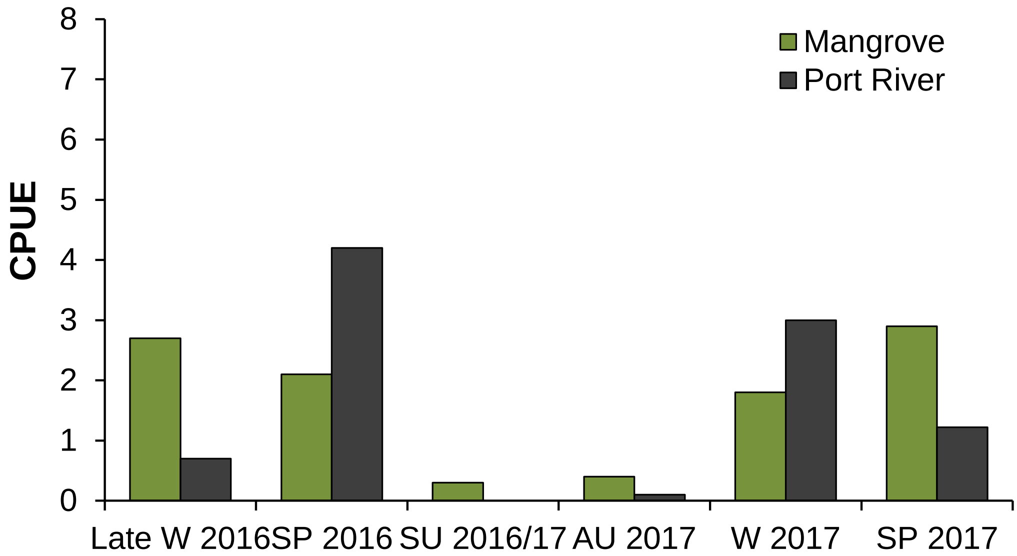

Figure 5: Catch per unit effort (CPUE) of *Carcinus* compared between the Port River region and mangroves north of Adelaide, for the surveys in the monitoring period since late Winter 2016. Based on two sites per region with five traps set at each site and season. Late Winter, Late W; Spring, SP; Summer, SU and Autumn, AU.

At a site level, the general pattern of higher CPUE of *Carcinus* during cooler seasons, particularly Winter and Spring, was consistent through time over the monitoring years from 2012 to 2017 (Figure 6). Pronounced peaks in the CPUE of crabs were found in Winter and Spring at Middle Beach in 2014 and 2015; in Autumn, Winter and Spring at Port Gawler in 2013 and 2014; in Port Adelaide in Winter 2014 and 2017; and Winter 2014 and Spring 2016 at Old Port Reach (Figure 6).

The seasonal difference in catches of *Carcinus* were due to higher catches of male and female crabs in Winter and Spring surveys (Figure 7). CPUE for male crabs were significantly higher in Winter compared to Summer and Autumn, and for female crabs in Spring over Summer (Figure 7, Table A3). CPUE for male crabs was significantly higher at Port Gawler than the other three northern sites (Figure 8, Table A3). CPUE was mostly higher for males than females, but in several Winter surveys (and some autumn and spring surveys), female CPUE exceeded the male catches (Figure 8). Especially at Port Adelaide, female CPUE exceeded male catches of *Carcinus*. Variability in the catches of male and female crabs over time was apparent at each of the four sites in the mangrove and Port River region (Figure 8), but no significant season by site interactions were found statistically (Table A3).

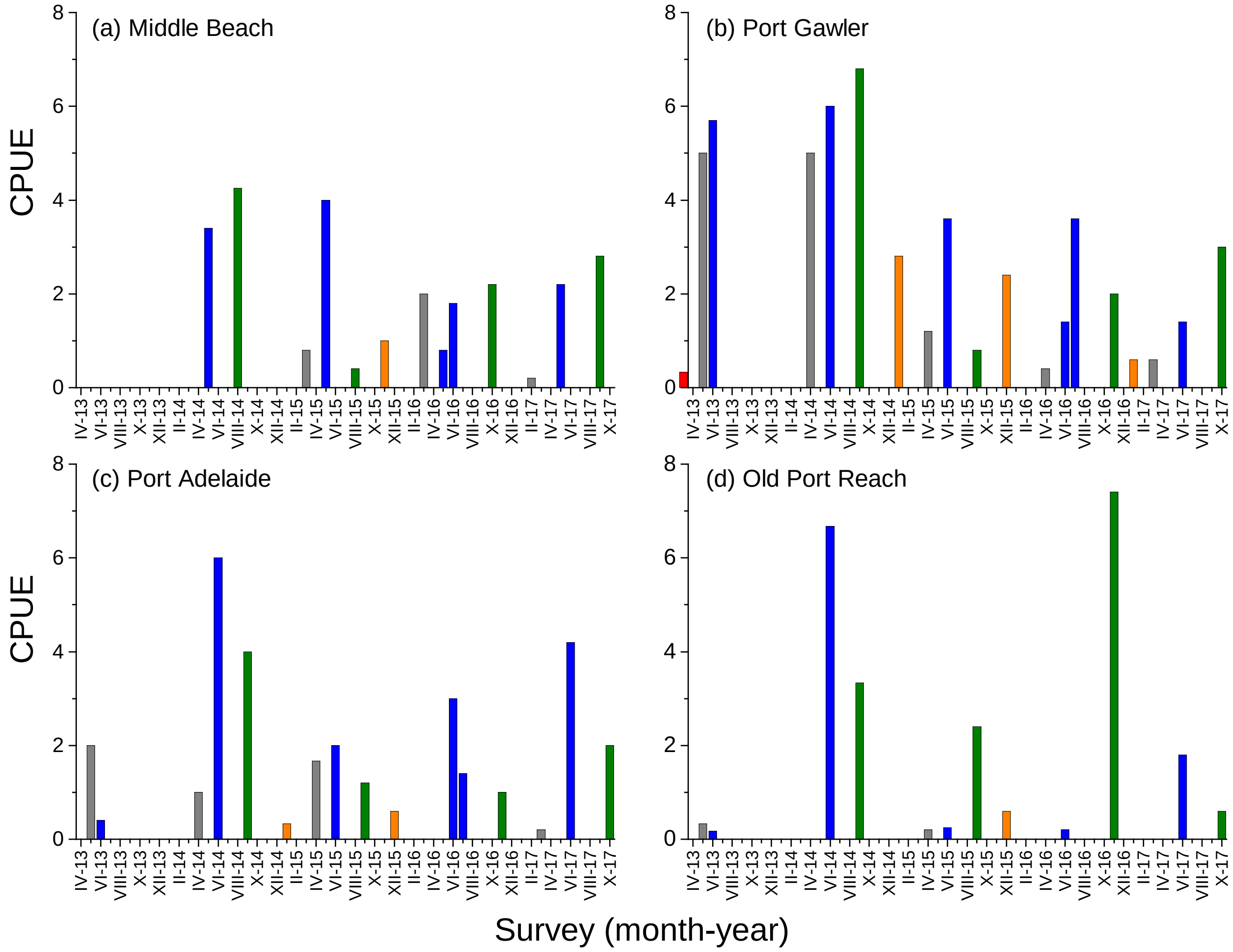

Figure 6: Catch per unit effort (CPUE) of *Carcinus* at the sites in the mangrove region ((a) Middle Beach and (b) Port Gawler), and the Port River region ((c) Port Adelaide and (d) Old Port Reach) over time (month in Roman numbers and year with last two digits) since Autumn 2013, when all sites were sampled more regularly. Seasons are indicated by the same colours of the bar as in Figure 4, with winter = blue, spring = green, summer = orange, autumn = grey.

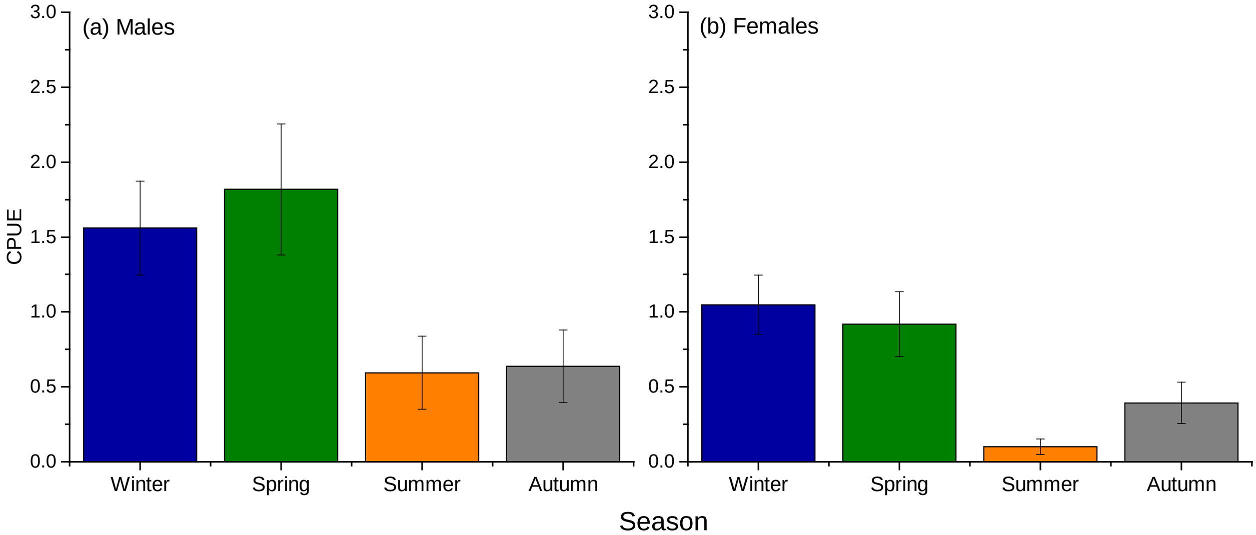

Figure 7: Catch per unit effort (CPUE, average ± SE) of a) male and b) female *Carcinus* across the seasons based on monitoring data from Autumn 2013 (from when on all sites were sampled more regularly) to Winter 2017 for the mangrove and Port River sites north of Adelaide. Seasons are indicated by the same colours of the bar as in Figures 4 and 6, with winter = blue, spring = green, summer = orange, autumn = grey.

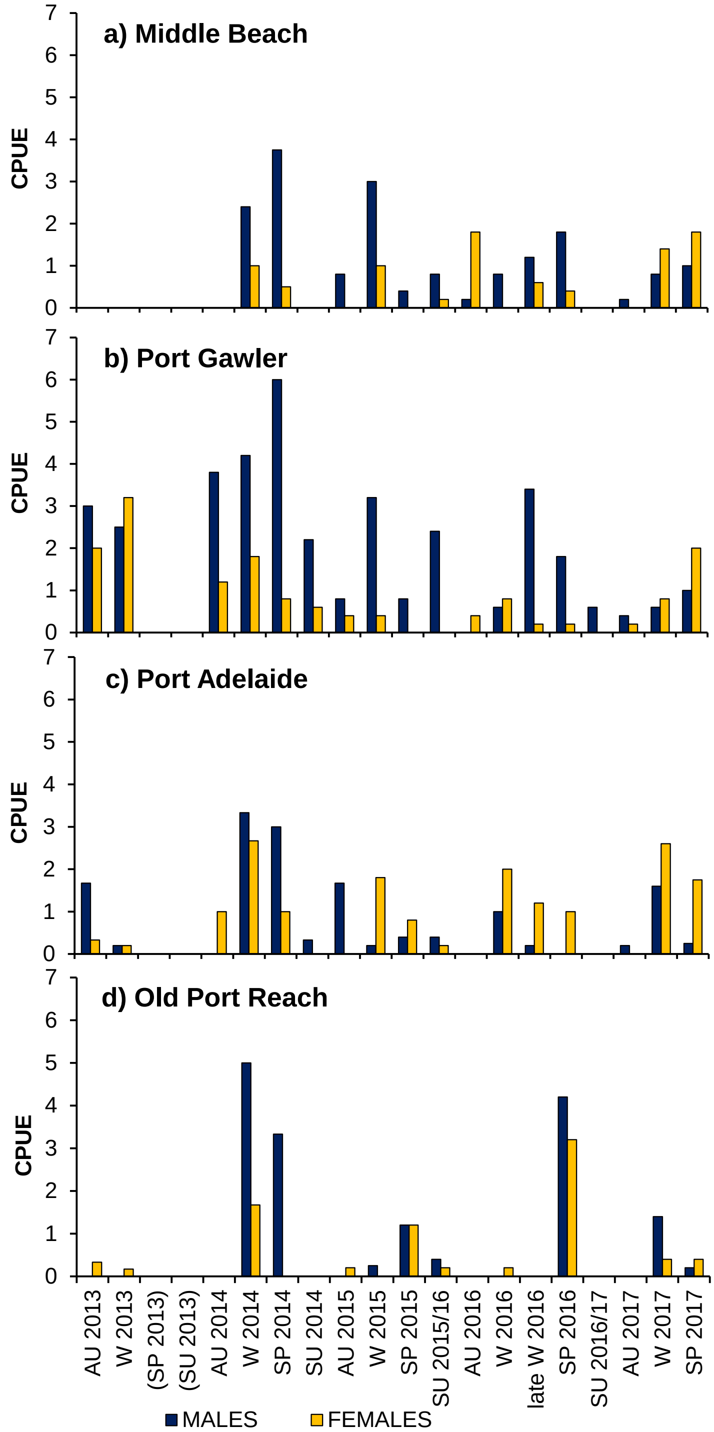

Figure 8: Catch per unit effort (CPUE) of male (blue) and female (yellow) *Carcinus* at each of the four sites surveyed since Autumn 2013. No sampling occurred in Spring and Summer 2013, and Middle Beach was not sampled in Winter 2013.

3.1.2 Southern sites

The timed search surveys in the 2016/17 monitoring at four coastal sites along the southern Adelaide/Fleurieu Peninsula region yielded 104 individuals of *Carcinus*, most of which were found at Aldinga (Figure 9). The monitoring in 2016/17 further confirmed the presence at Hallet Cove, and at the mouth of the Onkaparinga River at Port Noarlunga South (Figure 9). The surveys gave lowest numbers for *Carcinus* at Hallett Cove, followed by the Onkaparinga estuary, and highest numbers from Aldinga. No *Carcinus* were detected during any of the timed searches at Marino.

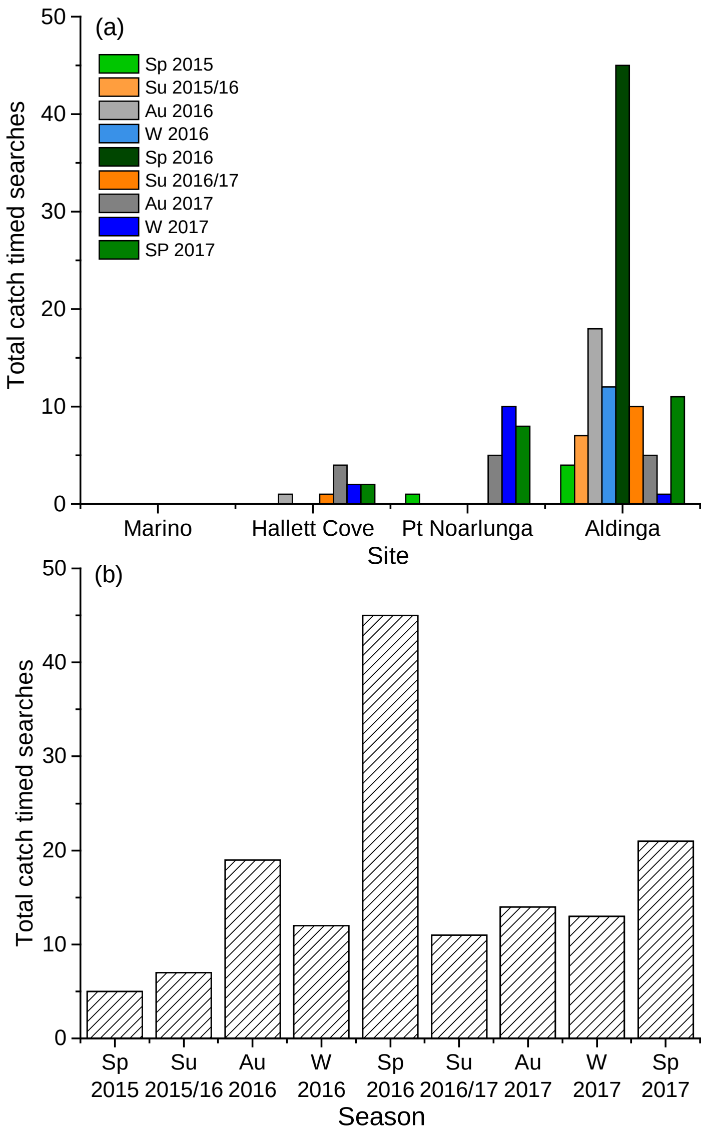

Figure 9: Total catches of *Carcinus* from intertidal rocky shores at southern sites along the metropolitan Adelaide/Fleurieu Peninsula coastlines in 2016/17, based on times searches. (a) catches from each site and survey; (b) total catch (sum) from all southern sites by season (Sp = Spring, Su = Summer, Au = Autumn, W = Winter).

At Hallett Cove, the spatial expanse of the rocky shoreline is very large and complex, and timed searches may not capture the entire crab population present at the site. At Port Noarlunga South, the crab records were high since the autumn 2017 survey, from when on surveys took place further in the estuary close to the footbridge, where rocks are overlying sand, compared to previous surveys carried out at the mouth of the estuary. At Aldinga, the crabs were found mainly in a limited section of the intertidal reef that has flat rocks on top of sand. Of the 84 *Carcinus* obtained at Aldinga since Winter 2016, about half of the individuals (45 crabs) were recorded in one survey in Spring 2016 (Figure 9a). The crab numbers recorded in the four replicate searches per site and survey ranged from 2 – 21 individuals, indicating a high spatial patchiness. In the following three surveys, less *Carcinus* were found, and sightings were lower than in the Autumn and Winter season of the previous year. However, more *Carcinus* were found again in Spring 2017 at the Aldinga reef (Figure 9a).

Seasonal patterns were not as apparent for the southern sites compared to the northern sites. Apart from the peak abundance from spring 2016, about 16 *Carcinus* were found on average across the sites per survey (Figure 9b). Since timed searches commenced in Spring 2015, 147 crabs were found in times searches along the southern metropolitan coast, where *Carcinus* is now established.

3.2 Size-frequency distributions

Size ranges of *Carcinus* recorded in the 2016/17 monitoring from northern and southern sites ranged from 9.9 – 88.3 mm carapace width (CW), which corresponded with the size range found in all surveys since 2012 (Figure 10). Female *Carcinus* were smaller (maximum CW 75.3 mm) than males (maximum CW 88.3 mm; Figure 11). While the size-frequency distributions for all surveys were skewed to larger sizes, they were more even and slightly bimodal for 2016/17 (Figures 10 and 11). Both female and male *Carcinus* contributed to the first mode at about 32 mm CW, the second mode at 64 mm CW was mostly made up of females, and at 80 mm CW by males (Figures 10 and11).

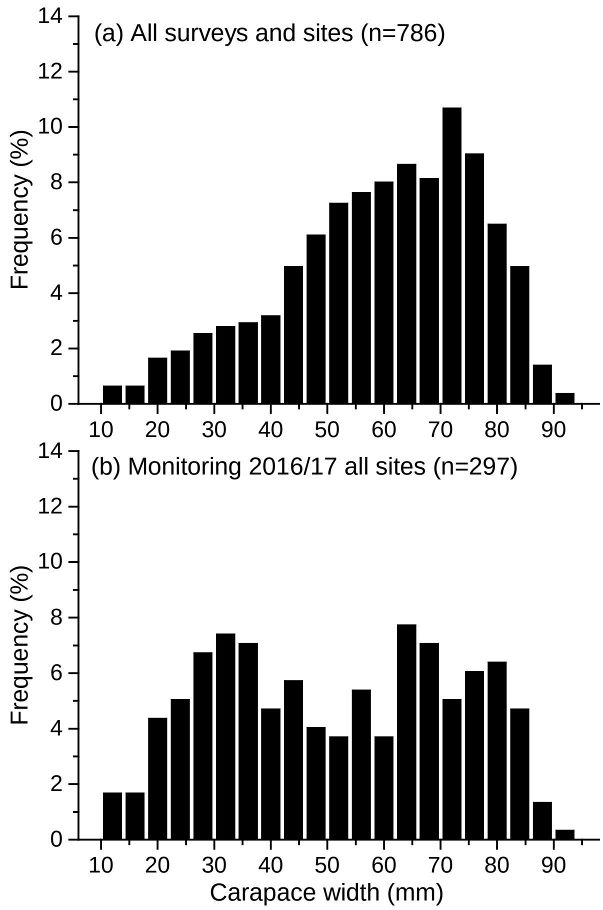

Figure 10: Size-frequency distribution (in percent frequency) of *Carcinus* carapace width from crabs caught across all sites (a) in all surveys since 2012, and (b) from late Winter 2016 to Spring 2017. Based on catches from baited traps and timed searches along the Adelaide coast from Aldinga in the south to Middle Beach in the north.

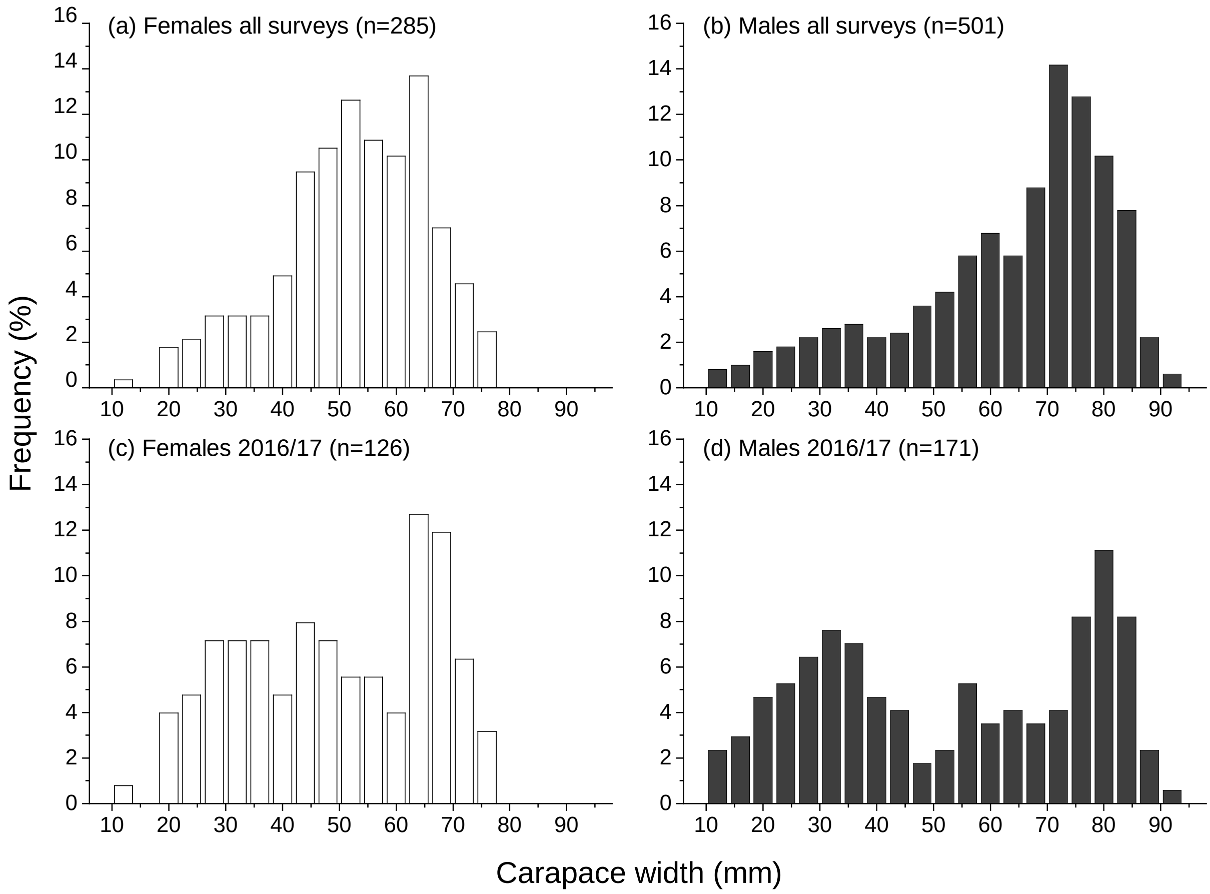

Figure 11: Size-frequency distribution (carapace width CW, in percent frequency) of female (a, c) and male (b, d) *Carcinus* caught in traps across all sites and in all surveys since 2012 (a, b), and from late winter 2016 to Spring 2017 (c, d). Based on catches from baited traps and timed searches along the Adelaide coast from Aldinga in the south to Middle Beach in the north.

Size-frequency distributions of *Carcinus* varied between the study regions, with larger crabs found at the two mangrove sites (Figure 12a, b), medium sized crabs at the Port River sites (Figure 12c, d), and mostly smaller crabs on the rocky shores at the southern sites (Figure 12e, f). These size distributions are based on catches from all seasonal surveys in the 2016/17 monitoring.

Plotting size-frequency distributions (SFD) for consecutive surveys for each region illustrates several cohorts of *Carcinus*. At the mangrove sites, cohorts are apparent for example from Summer 2014/15 to Spring 2015, and from Spring 2015 to Spring 2016 (Figure 13). At the Port River sites, cohorts are not very distinct, but a shift to larger sizes occurs from winter into spring surveys (Figure 14). The SFD for spring surveys also appeared bimodal, e.g. for Spring 2016. The most distinct cohorts are apparent for the southern sites, especially between Spring 2016 and Spring 2017 (Figure 15), indicating that many of the crabs found in the recent spring survey at the southern sites are still going back to a recruitment event a year before.

The analysis of size-frequency distributions across the three regions over time indicate that *Carcinus* are utilising the habitat along the Adelaide coastline differently during their life cycle and may be connected through larval or adult migration. Recruitment occurs at the southern sites, where more juvenile crabs were found. The Port River region appears to be a transition area, and larger mature crabs occur predominantly in the mangrove habitats along the northern Adelaide shores.

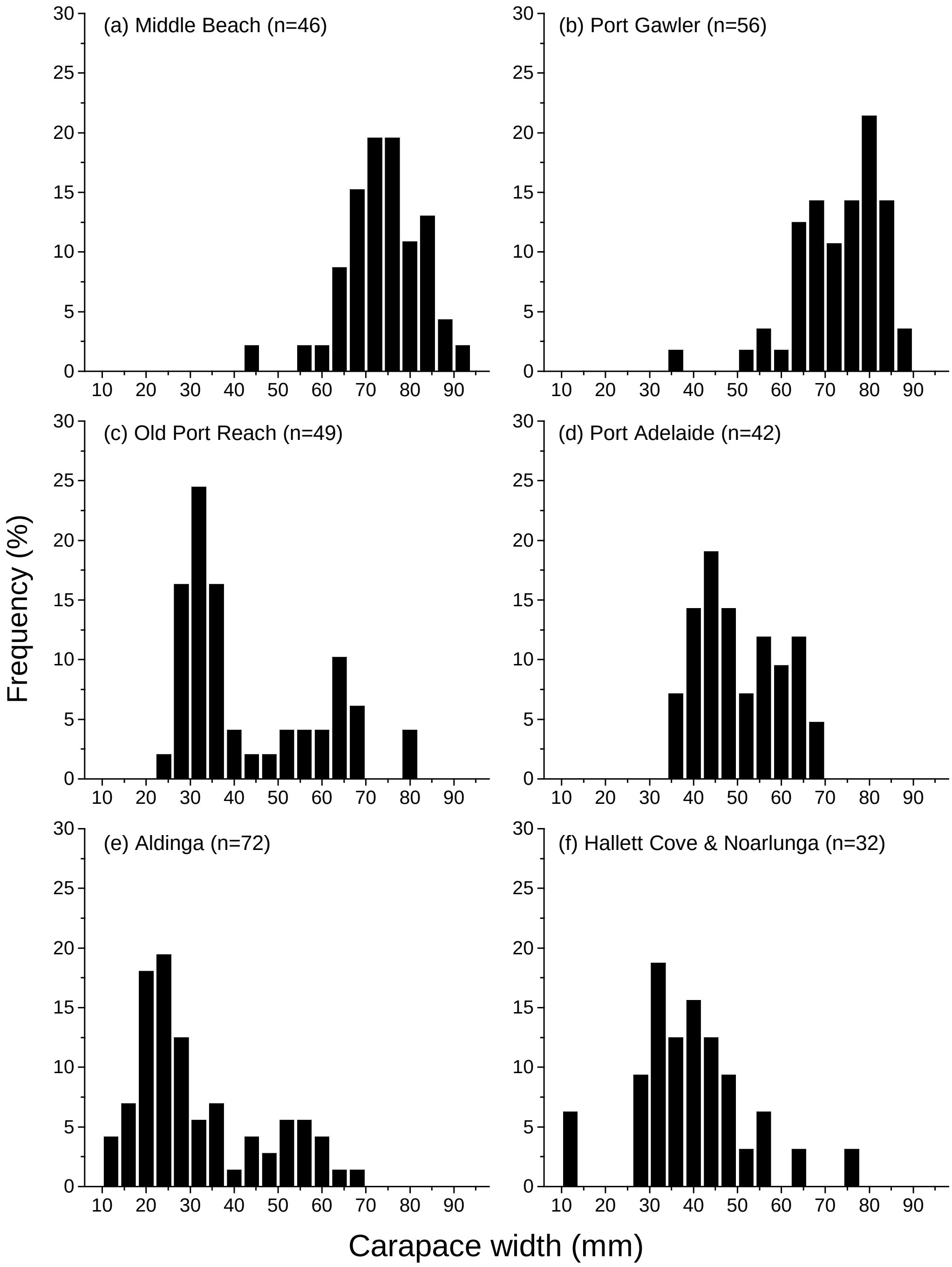

Figure 12: Size-frequency distribution (in percent frequency) of *Carcinus* caught in traps or during timed searches at each of the sampling sites at the seasonal sampling events from late winter 2016 to Spring 2017. Hallett Cove and Noarlunga were combined due to low numbers of crabs at each of the nearby sites.

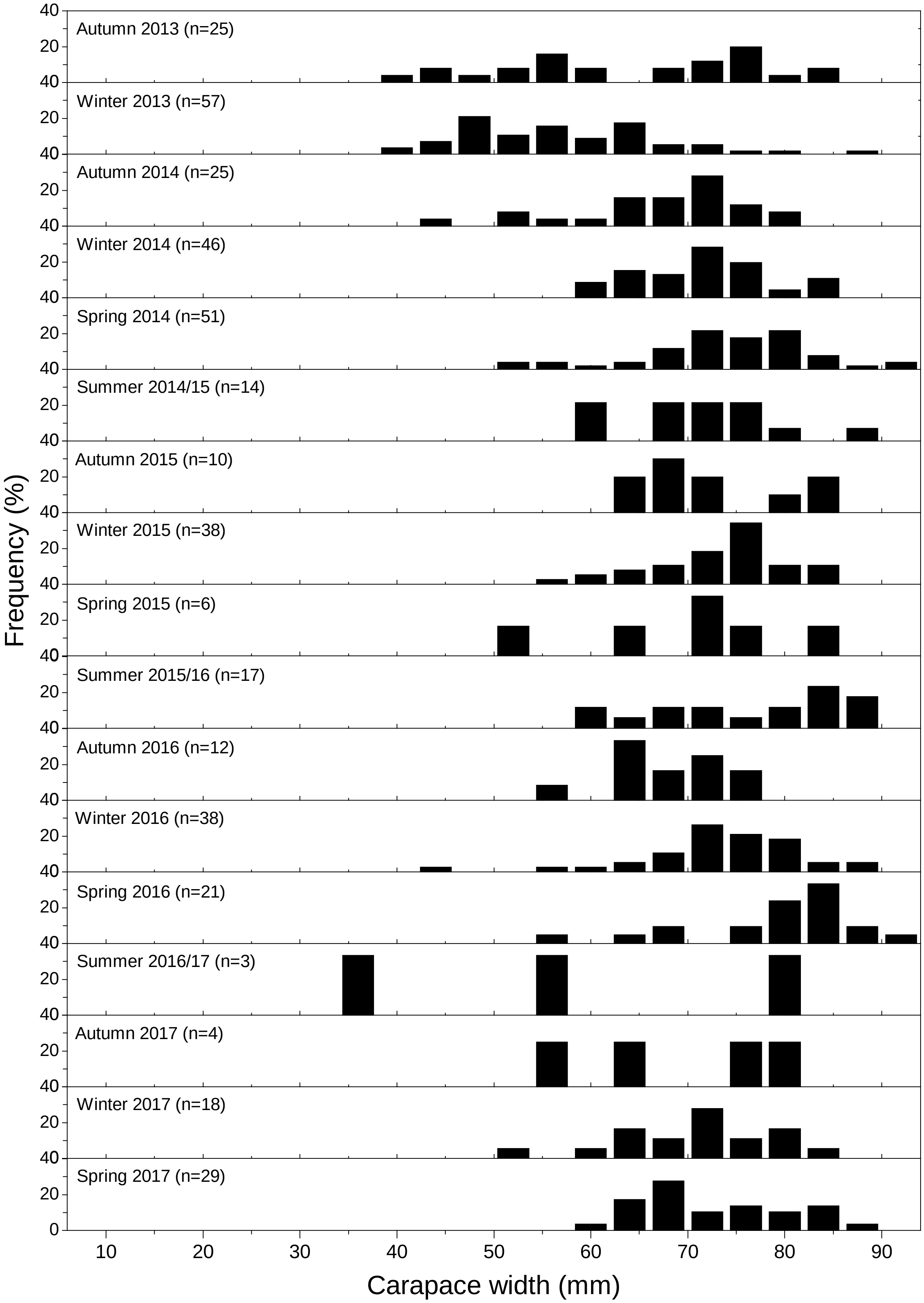

Figure 13: Size-frequency distribution (in percent frequency) of *Carcinus* carapace width from mangroves at the northern sites based on seasonal timed searches. Note that no monitoring occurred in spring and summer 2013 and the seasonal sequence is consecutive only from Autumn 2014. The Winter and late Winter surveys in 2016 were combined for this plot.

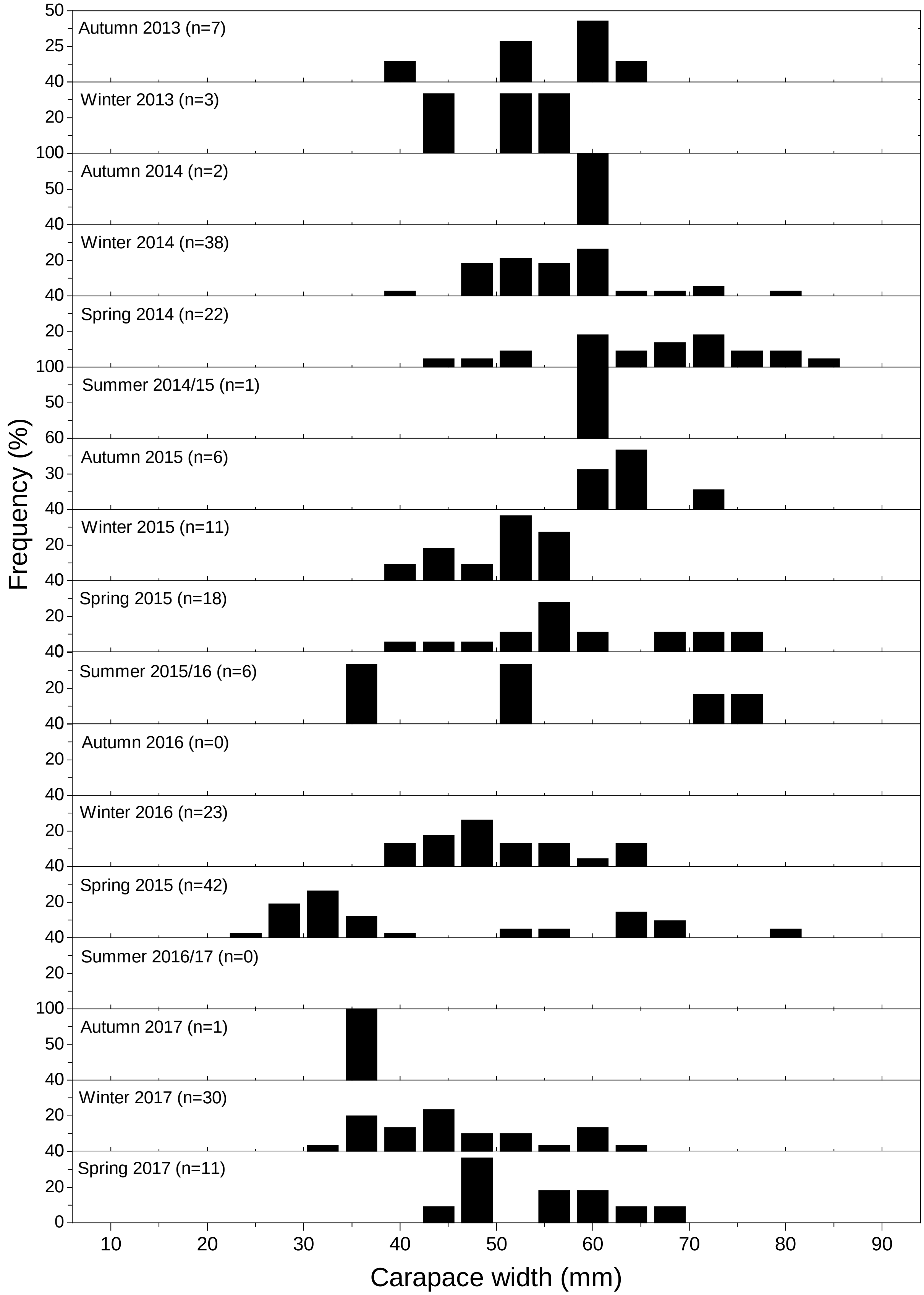

Figure 14: Size-frequency distribution (in percent frequency) of *Carcinus* carapace width from Port River sites based on seasonal timed searches. Note that no monitoring occurred in spring and summer 2013 and the seasonal sequence is consecutive only from Autumn 2014. The Winter and late Winter surveys in 2016 were combined for this plot.

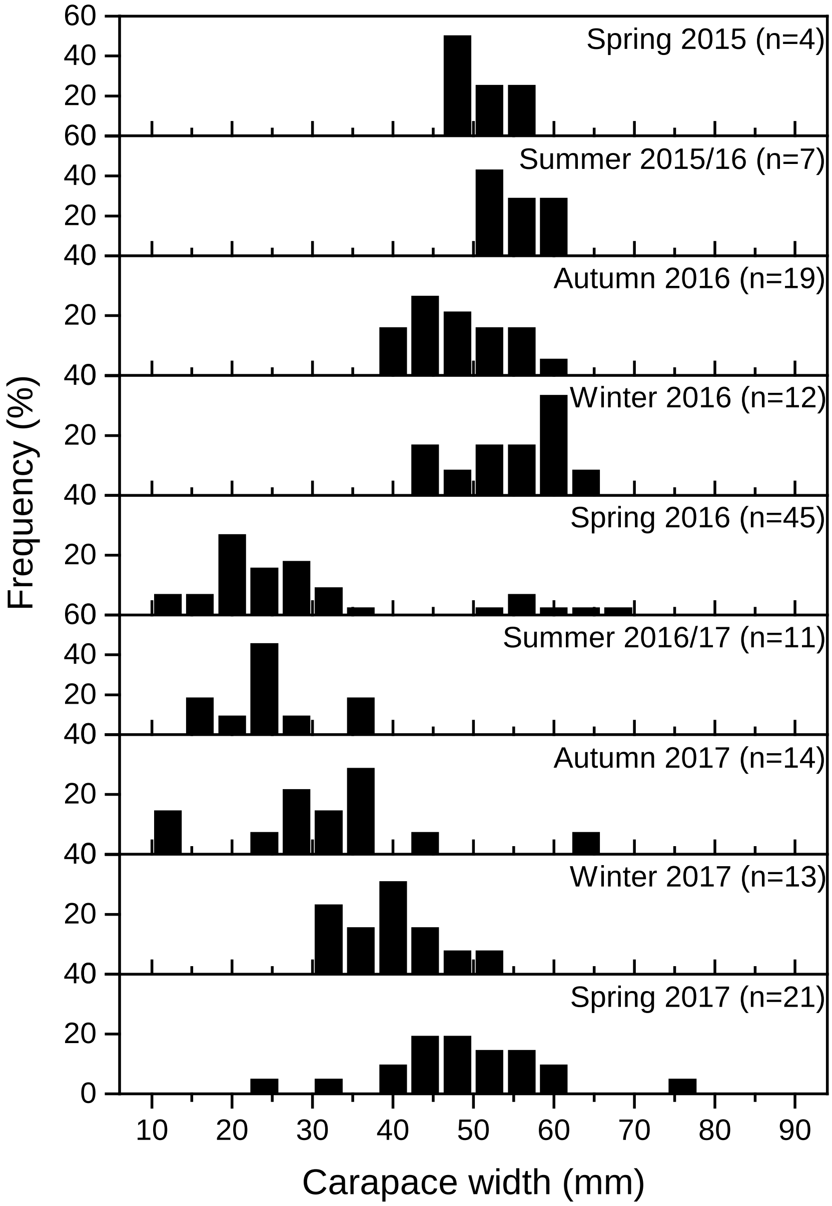

Figure 15: Size-frequency distribution (in percent frequency) of *Carcinus* carapace width from southern rocky shore sites based on consecutive seasonal timed searches since late 2015.

The size frequency distributions for male and female *Carcinus* in the monitoring period from late Winter 2016 to Spring 2017 also indicate the progression of cohorts (Figure 16). Juvenile crabs were mainly found in Spring and Summer, and a higher frequency of larger crabs for males and females in the Winter and Spring sampling events. The greatest range in sizes for male crabs was during the spring 2016 survey. In comparison, females occurred with a wide range of sizes in Spring 2016, Winter 2017 and Spring 2017 (Figure 16). Few large crabs of either sex were caught in summer (Figure 16).

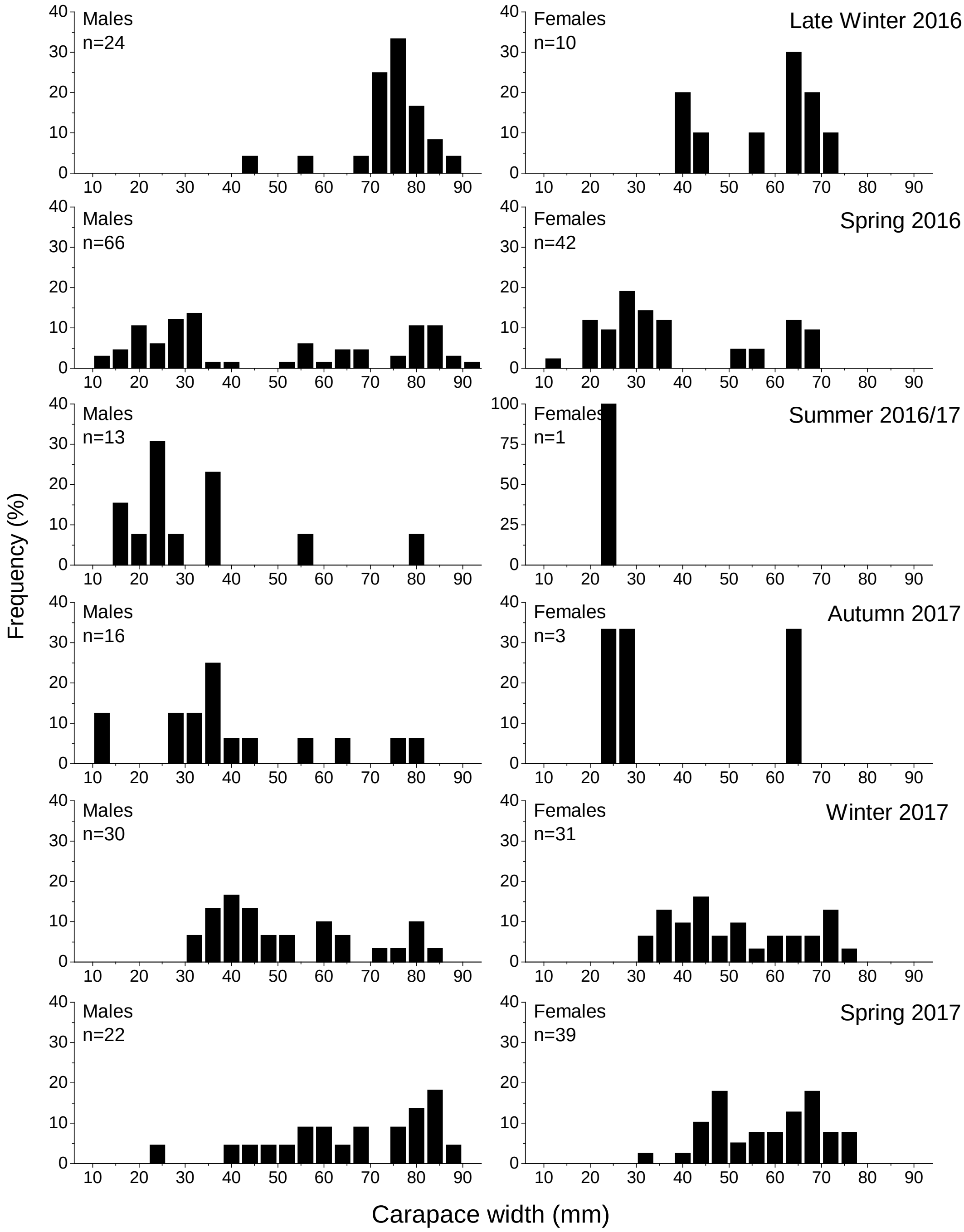

Figure 16: Size-frequency distribution (in percent frequency) of male (left column) and female (right column) *Carcinus* caught in traps (pooled across all sites from the northern and southern regions) during each sampling occasion of the current monitoring period in 2016/17. Note different values on y-axes.

3.3 Sex composition and presence of ovigerous females

The total catch of 297 *Carcinus* across all surveys and seasons in 2016/17 from the northern and southern sites consisted of 171 males and 126 females. Male crabs outnumbered female crabs in the mangrove sites and at the southern sites, but more female than male crabs were caught in the Port River region during the 2016/17 monitoring. The sex ratio changed between seasonal surveys in each study area (Table 5). In late Winter 2016 (Port River only), Winter 2017 and Spring 2017, female crabs outnumbered male crabs.

Table 5: Total numbers of male and female *Carcinus* caught in each region over the monitoring period since late winter 2016, and sex ratios for the catches in each season. The southern sites were not surveyed in late Winter 2016.

	Mangrove			Port River			Southern sites		
	Males	Females	Sex ratio	Males	Females	Sex ratio	Males	Females	Sex ratio
Late winter 2016	23	4	1 : 0.17	1	6	0.17 : 1			
Spring 2016	18	3	1 : 0.17	21	21	1 : 1	27	18	1 : 0.67
Summer 2016/17	3	0	1 : 0	0	0		10	1	1 : 0.10
Autumn 2017	3	1	1 : 0.33	1	0	1 : 0	12	2	1 : 0.17
Winter 2017	7	11	0.64 : 1	15	15	1 : 1	8	5	1 : 0.63
Spring 2017	10	19	0.53 : 1	2	9	0.22 : 1	10	11	0.91 : 1

Catches of female and male *Carcinus* were low in Summer and Autumn at the mangrove and Port River sites in the current monitoring, and female crabs were also less abundant in these months at the southern sites (Figure 17). Over several years, the relative sex composition reveals no clear pattern across the surveys and regions, but female crabs accounted for a higher percentage of crab catches mostly in surveys from late autumn, winter or spring months (Figure 18).

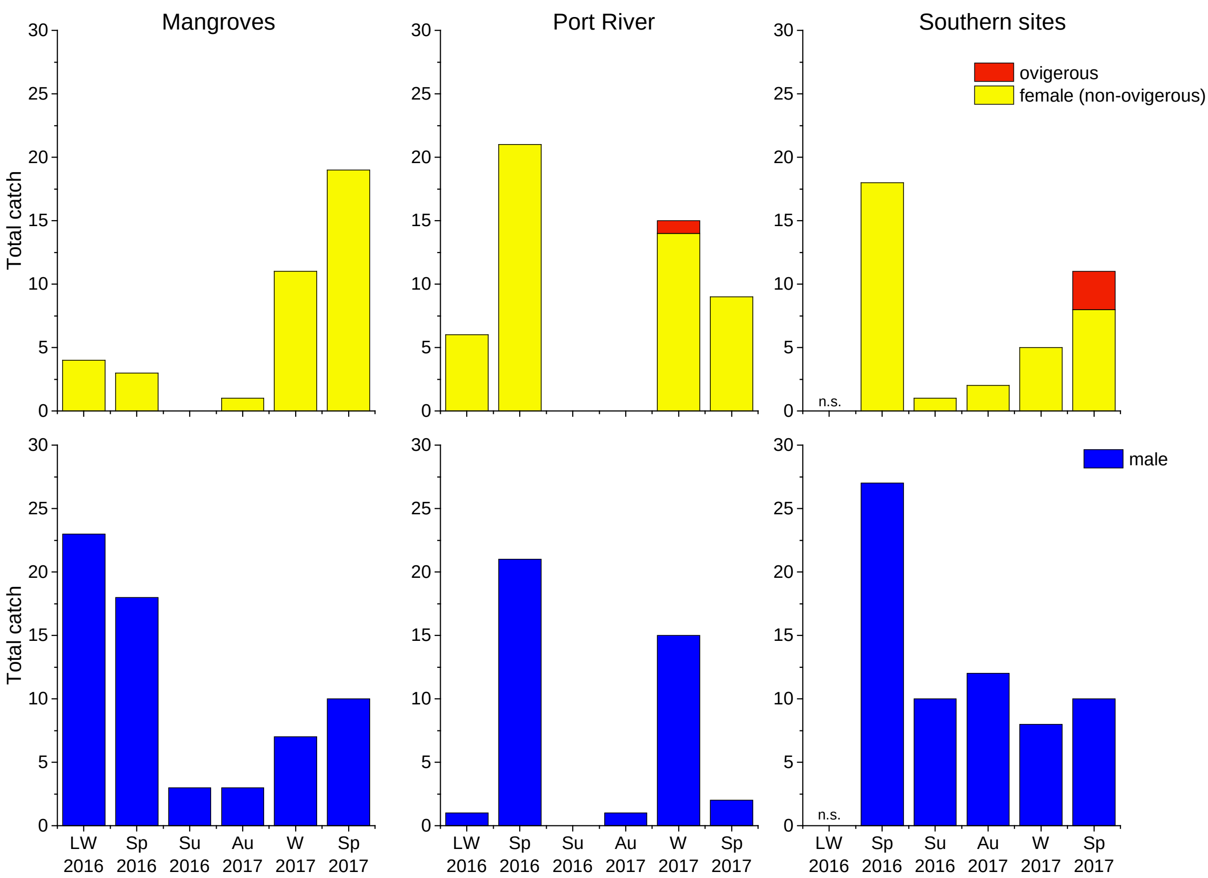

Figure 17: Total catches of female and male crabs for each region and survey month of the 2016/17 monitoring, based on baited traps in the mangroves and Port River, and timed searches at the southern rocky shore sites. For female crabs, numbers of egg-bearing (ovigerous) crabs are indicated in red. The southern sites were not sampled (n.s.) in late winter 2016.

Ovigerous females were encountered in the Port River in Winter 2017, and for the first time also at the southern sites (at Port Noarlunga South and Hallett Cove) in Spring 2017, but were overall rare (Figure 17). Ovigerous females have been mostly recorded in winter and spring, but constitute only a small percentage of the *Carcinus* catches (Figure 18). A total of 26 ovigerous females have been found in the monitoring since Autumn 2013 so far, 22 of them in winter surveys, three in spring and one in late autumn. Ovigerous females occurred mostly in the mangroves, with a total of 18 egg-bearing female *Carcinus* found at Port Gawler (15 crabs) and Middle Beach (3 crabs). Five ovigerous females were found at sites in the Port River and three at the southern rocky shores. The smallest female *Carcinus* bearing eggs was 33 mm carapace width (CW), and the largest 69 mm CW, and most ovigerous females were about 50 mm CW.

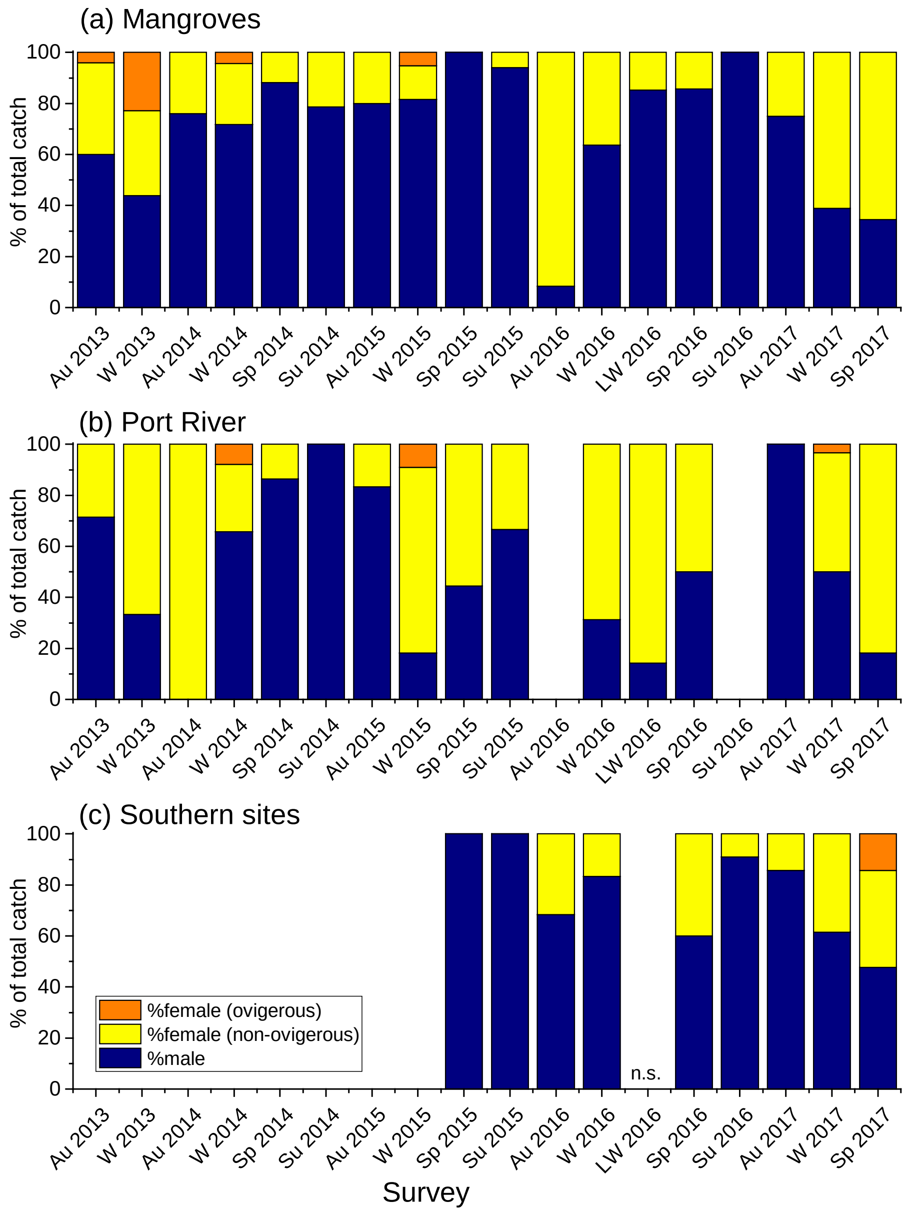

Figure 18: Relative composition of male and female (ovigerous and non-ovigerous) *Carcinus* of the total catch for each survey and region. Crabs were caught in baited traps at the mangrove and Port River sites, and with timed searches since Spring 2015 at the southern sites. The southern sites were not sampled (n.s.) in Late Winter 2015. No crabs were found at the Port River in the surveys in Autumn 2016 and Summer 2016.

3.4 Colour morphs

Over all surveys of the 2016/17 monitoring, green male *Carcinus* (133) were more common than red male crabs (38), and this prominence of green colour morphs for male crabs was most pronounced at the Port River and southern sites (Figure 19). Red colour morphs of male crabs were mostly found in winter and spring surveys, and more common at the mangrove sites (Figure 19). For female *Carcinus*, the occurrence of red and green colour morphs was overall more balanced (67 red and 59 green crabs). However, regional differences were apparent, with red female crabs dominating in the catches from the mangrove sites, green female crabs from the southern sites, and a mix of red and green females being recorded from the Port River (Figure 19).

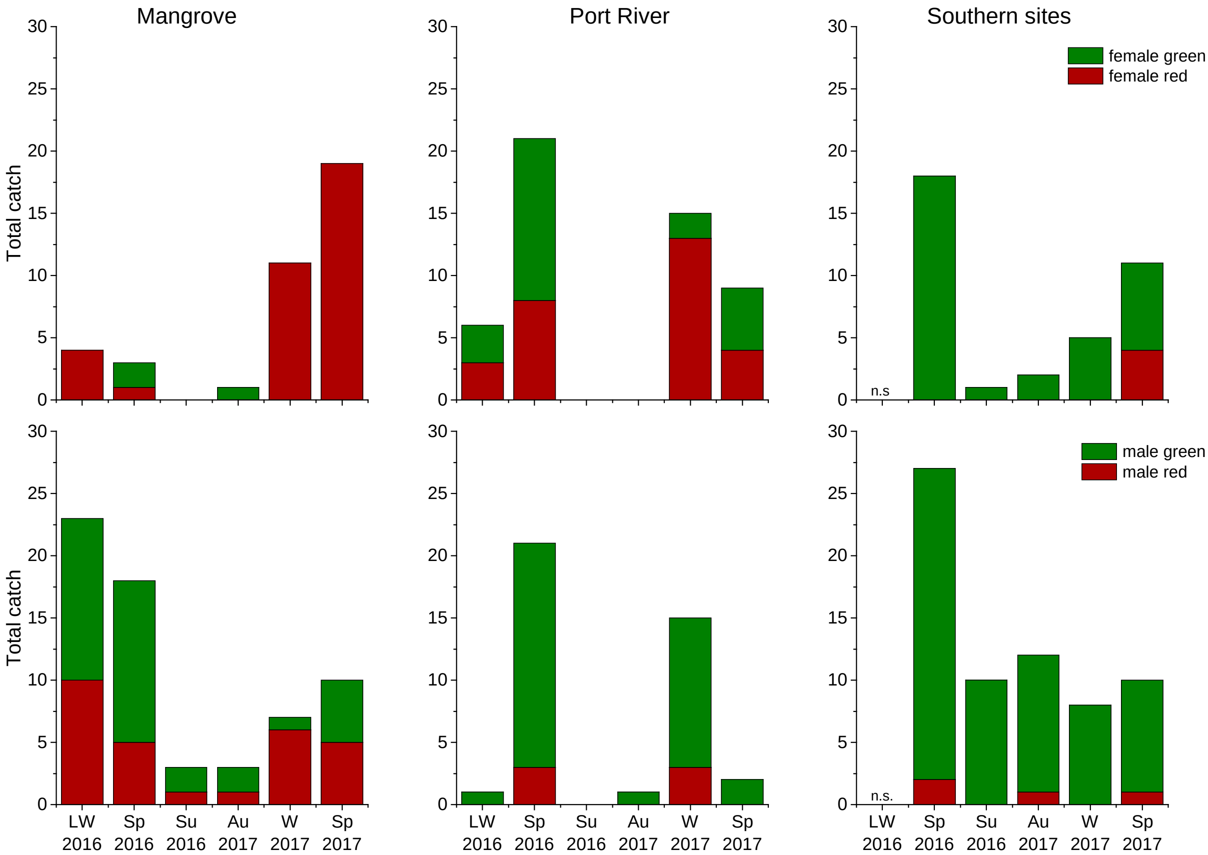

Figure 19: Total catches of red and green specimens of female and male *Carcinus* for each region and survey month of the 2016/17 monitoring, based on baited traps in the mangroves and Port River, and timed searches at the southern rocky shore sites. The southern sites were not sampled (n.s.) in late winter 2016.

The pattern for the current monitoring period was similar to the relative contribution of colour morphs to the total catches of female and male *Carcinus* seen in surveys since Autumn 2013 (Figure 20). Some of the previous surveys recorded a higher percentage of red male and female crabs at the Port River sites. Most of the crabs at the mangrove sites were red, indicating they had not moulted for some time. Female crabs were mostly of the red colour morph (Figure 20) and 88% of the ovigerous females found since 2013 were red. Over most of the surveys, red crabs were more prevalent during the cooler months between autumn, winter and spring.

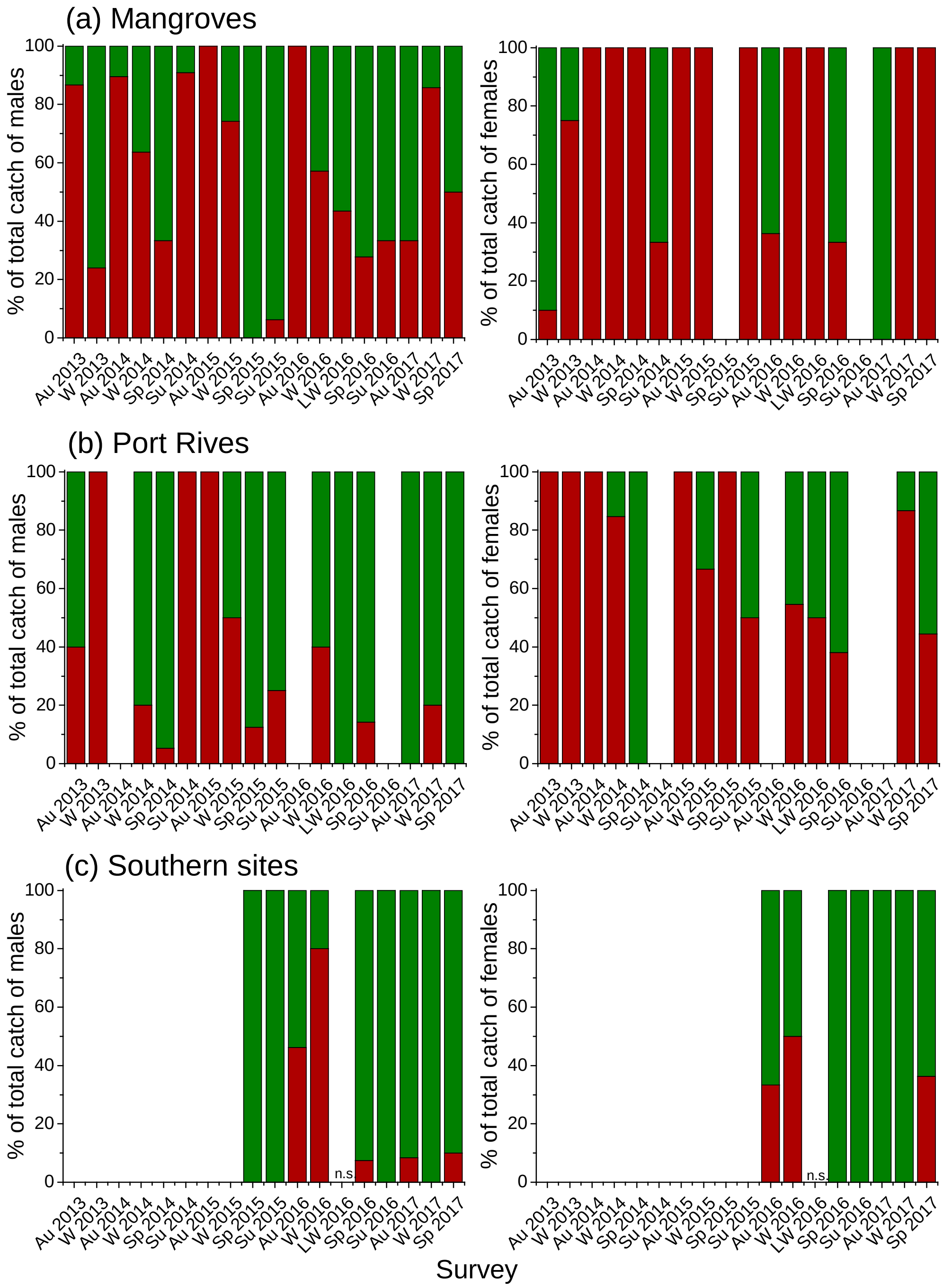

Figure 20: Relative composition of red and green colour morphs for male (left column) and female (right column) *Carcinus* in percent of the total catch for each survey and region. Crabs were caught in baited traps at the mangrove and Port River sites, and with timed searches since Spring 2015 at the southern sites. The southern sites were not sampled (n.s.) in Late Winter 2015. No crabs were found at the Port River in the surveys in Autumn 2016 and Summer 2016.

The size-frequency distributions of green and red colour morphs for male and female *Carcinus* showed a similar pattern based on data from the 2016/17 monitoring and all surveys since Autumn 2013 (Figure 21). Most of the smaller crabs of both sexes were of a green colour morph, and the smallest red crabs about 30 mm carapace width (CW). The green colour morph occurs in crabs of all sizes, but the frequency of red colour morphs was more prevalent in larger male and female crabs.

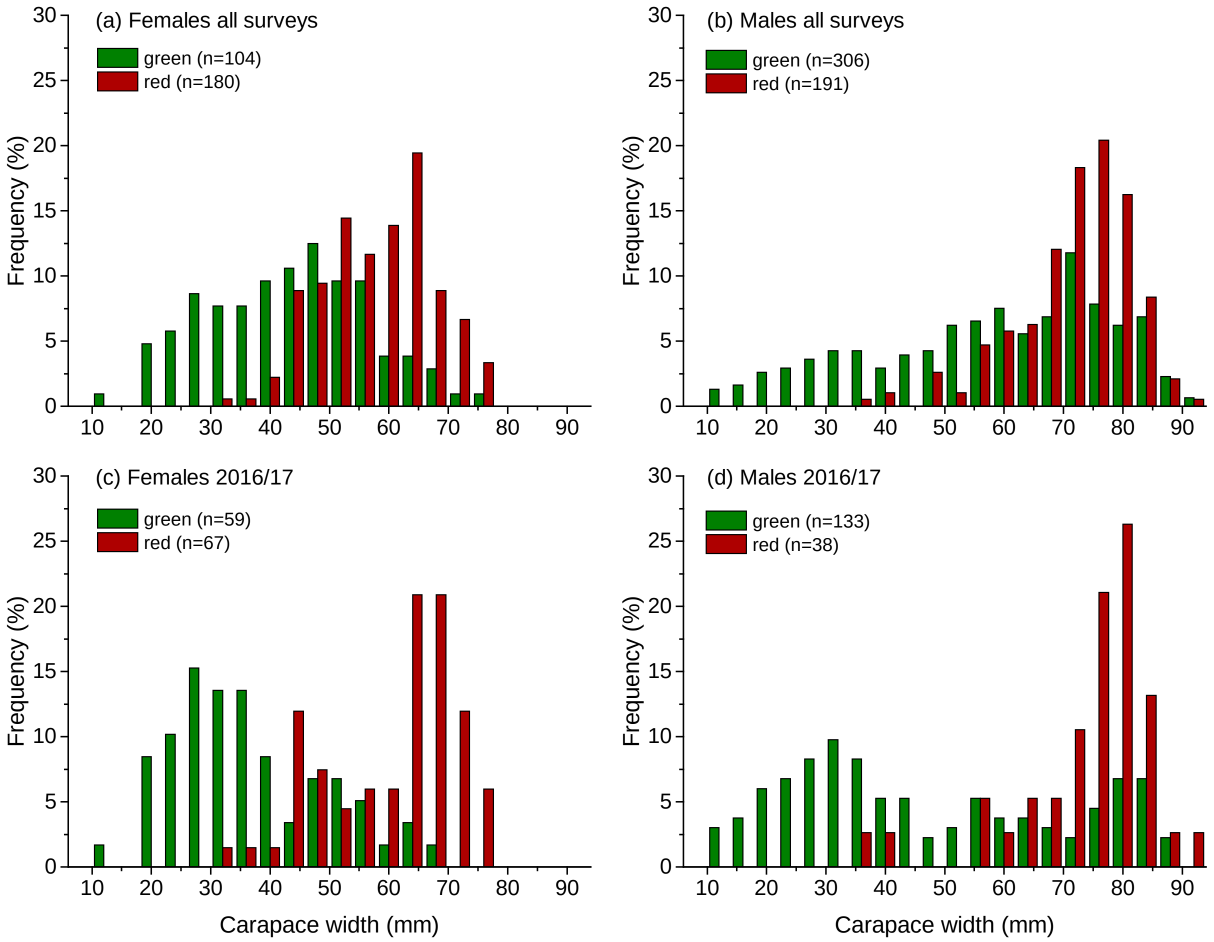

Figure 21: Size-frequency distribution (in percent frequency) of red and green colour morphs of female (a, c) and male (b, d) *Carcinus* caught in traps from several sites in the mangrove and Port River region sampled (a, b) over the entire monitoring since Autumn 2013, and with timed searches since Spring 2015 at the southern sites, and (c, d) during the 2016/17 monitoring at all sites.

3.5 Water quality and catch of *Carcinus*

For the northern sites, water quality was measured during most surveys, and tests between these variables and catches of *Carcinus* showed correlations especially for water temperatures and dissolved oxygen (Table A4). A negative correlation was found between *Carcinus* catches and water temperature, mostly due to lower catches of female crabs in warmer waters (Figure 22). Ovigerous females were only found when water temperatures were cool (10 - 17 °C). Salinity had little influence on the catches, apart from a negative correlation at the Port River sites (Table A4). Catches of *Carcinus* were lower when dissolved oxygen (DO) concentrations and saturation levels were low, which was most pronounced at the Port River sites (Figure 22). Apart from the water temperature effect on catches of female *Carcinus*, water quality had less effect on the crabs at the mangrove sites compared to the Port River sites (Figure 22, Table A4). Looking at the combined effect of temperature, salinity and dissolved oxygen (as either concentration or saturation), no significant effects were found with the CPUE of *Carcinus* (for total catch, and separately for male and female CPUE) (Table A5 for results of Multivariate General Linear Model).

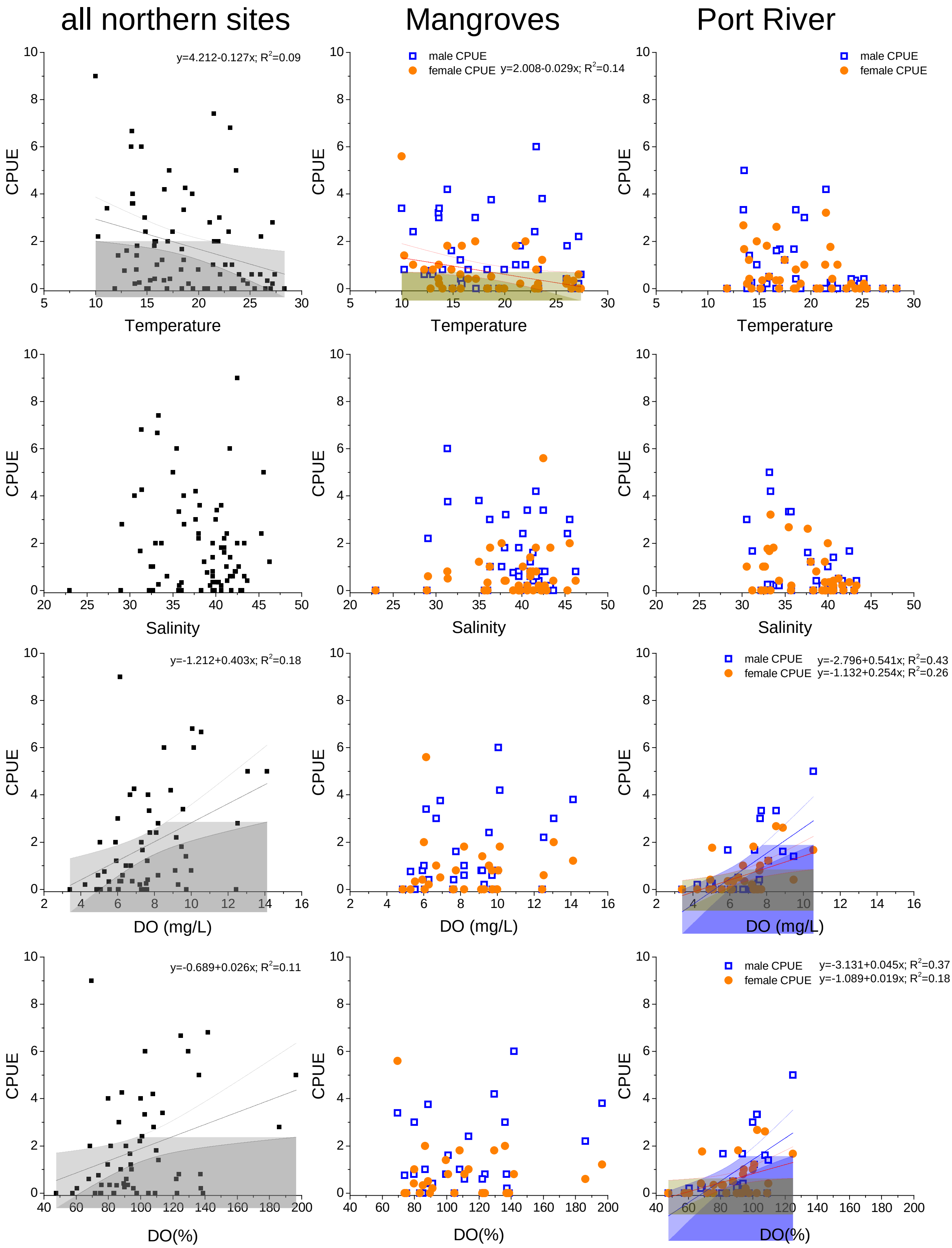

Figure 22: Scatterplots of catch per unit effort (CPUE) and water quality parameters for all *Carcinus* at all northern sites based on monitoring since 2012, and for the mangrove and Port River sites over all monitoring surveys. CPUE for male (blue) and female (orange) *Carcinus* are indicated separately in the scatterplots for mangroves and Port River. Where significant correlations were found, equations are included, as well as linear fits (straight lines) and 95% confidence interval bands.

3.6 Occurrence of other species in traps

Quantitative analyses can be carried out with the number of native blue swimmer crabs (*Portunus armatus*) found inside the baited traps, and significantly less *P. armatus* occurred in traps with high (> 6-10) numbers of *Carcinus* (Figure 23; Spearman correlation coefficients and *P* values for Mangrove sites: 0.306, $P < 0.001$, and for all northern sites 0.172, $P = 0.0014$). This pattern is also affected by the different seasonal occurrence of the two species of Portunidae and does not necessarily indicate competition. The bycatch of *P. armatus* increased with temperature and

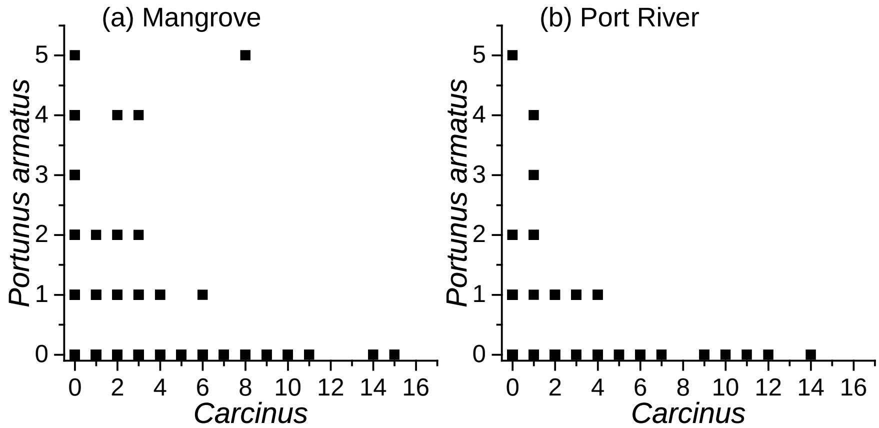

Figure 23: Scatterplot of catch (total numbers per trap) of the blue swimmer crab *Portunus armatus* against the catch of *Carcinus* per trap for (a) the mangrove sites, and b) the Port River, based on the entire monitoring since 2012.

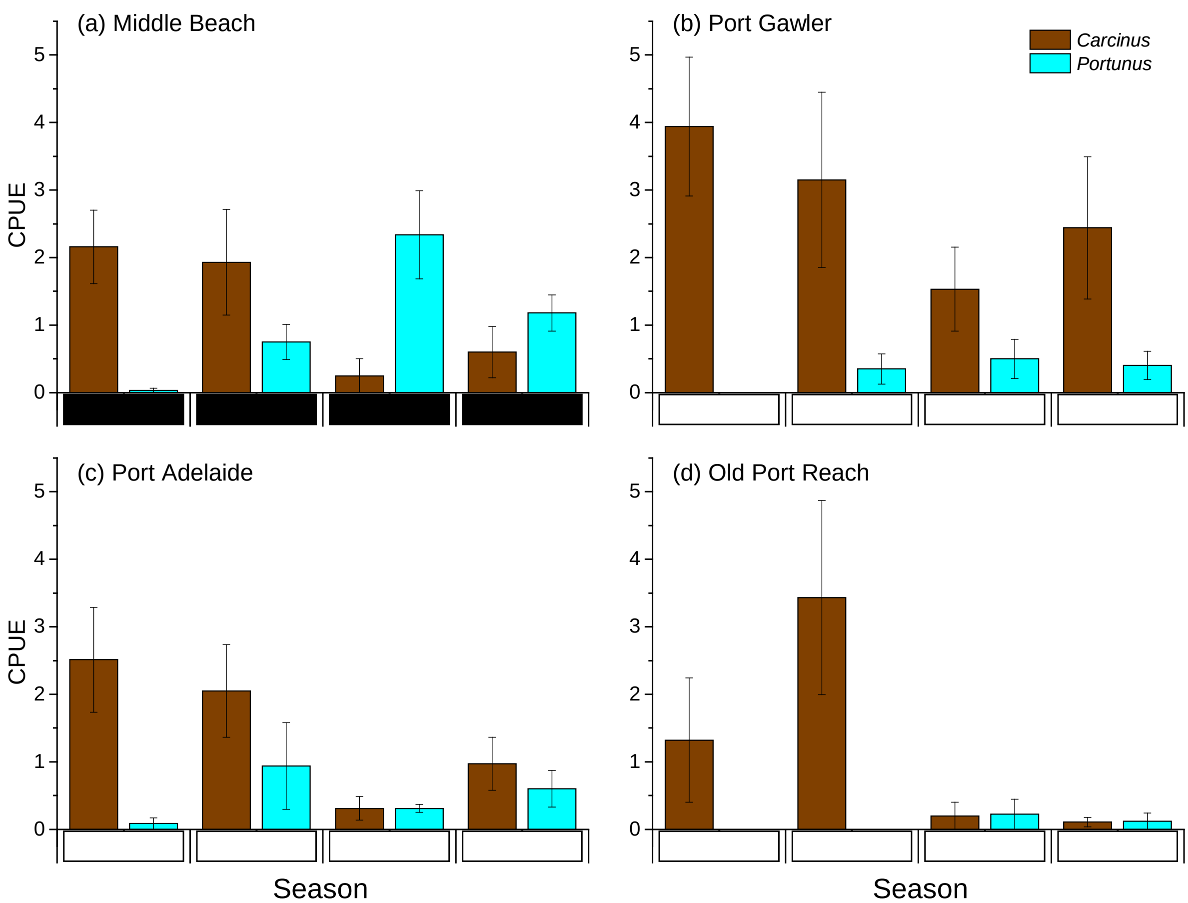

Figure 24: CPUE for the introduced *Carcinus* and the native *Portunus armatus* for each of the northern monitoring sites and averaged (± SE) across seasons for surveys since 2012.

decreased with higher salinities (Figure A1). The native *P. armatus* was thus more common in the warmer seasons between spring and autumn and rarely found in the traps during winter surveys, when catches of *Carcinus* were highest (Figure 24). This was most pronounced at the mangrove sites, in particular at Middle Beach (Figure 24).

The baited traps at the northern monitoring sites often contain specimens of other native scavengers. Pebble crabs (*Bellidilia laevis*) and scavenging snails (*Nassarius* sp.) are small sized and can move freely in and out of the baited traps through the coarse mesh. Correlations between the catch of *Carcinus* per trap and these native scavenging species can thus not be made.

3.7 Morphometric analyses, colour patterns and fecundity

3.7.1 Allometric relationships

Allometric growth coefficients (*b*) were similar between male and female *Carcinus* and the three study regions (Table 6). Carapace length (CL) and height (CH) of male and female *Carcinus* showed isometric growth with carapace width (CW) in each region (Figure 25, Table 6). There was a slight indication of negative allometric growth for CL and CW for male *Carcinus* at the southern sites. Weight had a positive allometric relation with CW, with an accelerated increase in weight over size commencing from about 50 mm CW (Figure 25, Table 6). This positive allometric growth for weight occurred in males and females in each region. All allometric relationships were significant (ANOVA, $P < 0.01$).

Table 6: Power functions for allometric relationships between the carapace width (independent variable) of *Carcinus* and the carapace length (CL), carapace height (CH) and wet weight (WW) (all as dependent y-variables) for male and female crabs in each region (see Figure 25). R^2 is the adjusted coefficient of determination indicating goodness of fit. *b* is the allometric growth coefficient and allometry is indicated as = for isometry, + for positive allometry, and – for negative allometry.

Region	y-Variable	Sex	Power function	R^2	*b*	Allometry
Mangrove	CL	Male	$CL = 0.7828\ CW^{0.9879}$	0.9794	0.9879	=
		Female	$CL = 0.79233\ CW^{0.9913}$	0.9725	0.9913	=
	CH	Male	$CH = 0.3899\ CW^{1.0184}$	0.9564	1.0184	=
		Female	$CH = 0.3556\ CW^{1.0526}$	0.9030	1.0526	=
	WW	Male	$WW = 0.00018\ CW^{3.0484}$	0.9406	3.0484	+
		Female	$WW = 0.00041\ CW^{2.8322}$	0.9472	2.8322	+
Port River	CL	Male	$CL = 0.7842\ CW^{0.9894}$	0.9942	0.9894	=
		Female	$CL = 0.7921\ CW^{0.9883}$	0.9892	0.9883	=
	CH	Male	$CH = 0.3986\ CW^{1.0104}$	0.9894	1.0104	=
		Female	$CH = 0.3721\ CW^{1.0346}$	0.9807	1.0346	=
	WW	Male	$WW = 0.00004\ CW^{3.4090}$	0.9801	3.4090	+
		Female	$WW = 0.00041\ CW^{2.8225}$	0.9800	2.8225	+
Southern sites	CL	Male	$CL = 0.8323\ CW^{0.9763}$	0.9954	0.9763	-
		Female	$CL = 0.8049\ CW^{0.9873}$	0.9953	0.9873	=
	CH	Male	$CH = 0.4153\ CW^{0.9990}$	0.9897	0.9990	=
		Female	$CH = 0.3249\ CW^{1.0657}$	0.9720	1.0657	=
	WW	Male	$WW = 0.00054\ CW^{2.7664}$	0.9783	2.7664	+
		Female	$WW = 0.00005\ CW^{3.3534}$	0.9283	3.3534	+

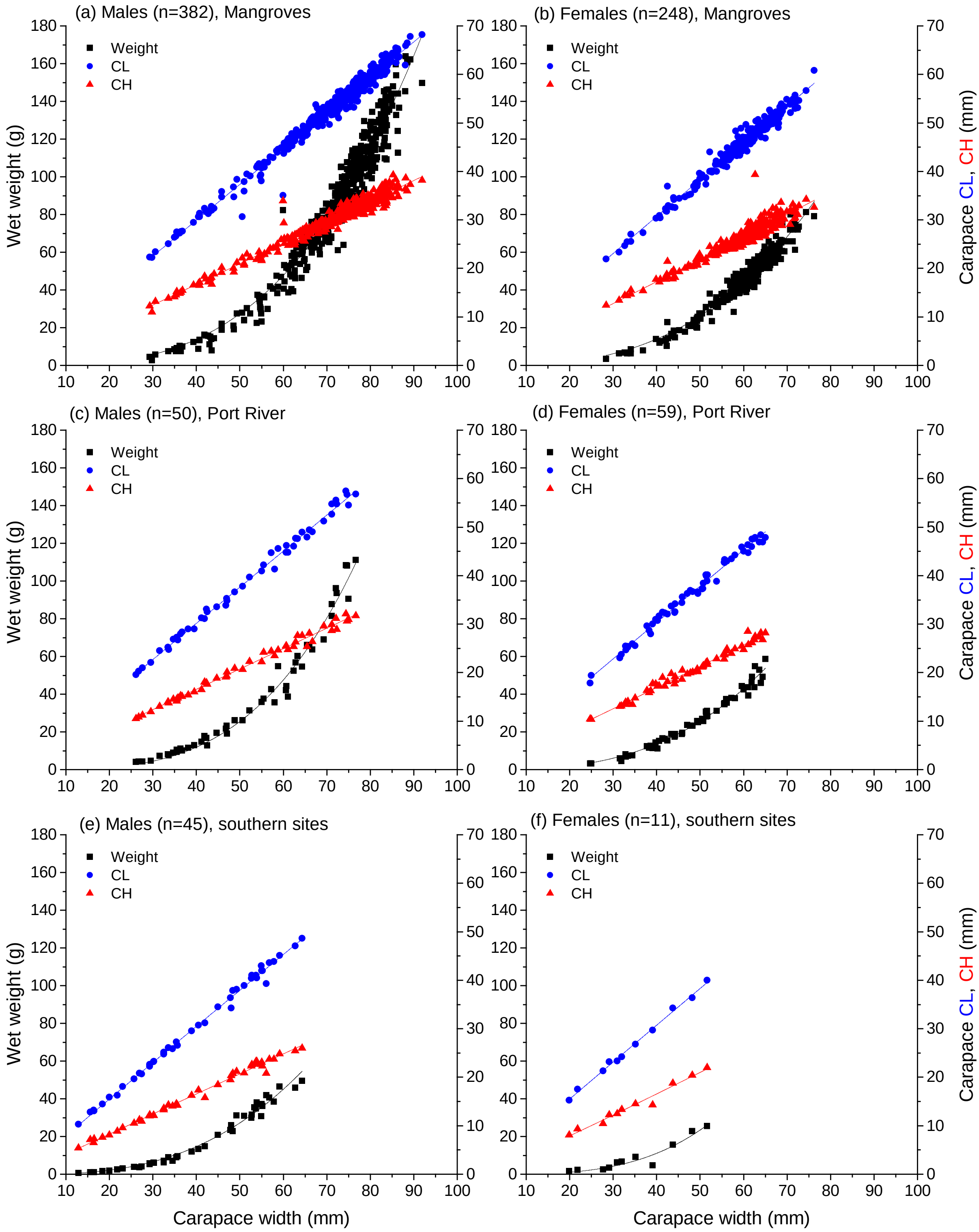

Figure 25: Allometric relationships between carapace width and carapace length (CL, blue symbols), carapace height (CH, red symbols) as well as the wet weight (black symbols) of *Carcinus* from each of the main regions and separated for males and females. The lines are showing allometric fit and corresponding details of the power functions are given in Table 6. Based on nearly 800 crabs collected over recent years.

3.7.2 Species identification

Ratios used in the literature to differentiate between *C. maenas* and *C. aestuarii* were calculated based on the morphometric measurements taken for nearly 800 crabs, and indicate that specimens found in Gulf St Vincent are predominantly *C. maenas* (Figure 26). Out of the measured crabs, 93% had a carapace width to length ratio (CW/CL) > 1.29, typical for *C. maenas*, and only about 3 % had a CW/CL ratio < 1.27 which is a good indicator for *C. aestuarii*, and 4% were undefined. Compared between the three regions, a CW/CL ratio < 1.27 occurred more at the southern sites (Figure 26). Using the less strong indicator of carapace width to height ratio, 79% were *C. maenas,* about 12 % *C. aestuarii*, and 9 % undefined. The CW/CH ratio < 2.26 was more common in the mangroves (Figure 26). Based on these ratios, specimens of *C. aestuarii*, or hybrids between the two species could be present, yet current findings are inconclusive.

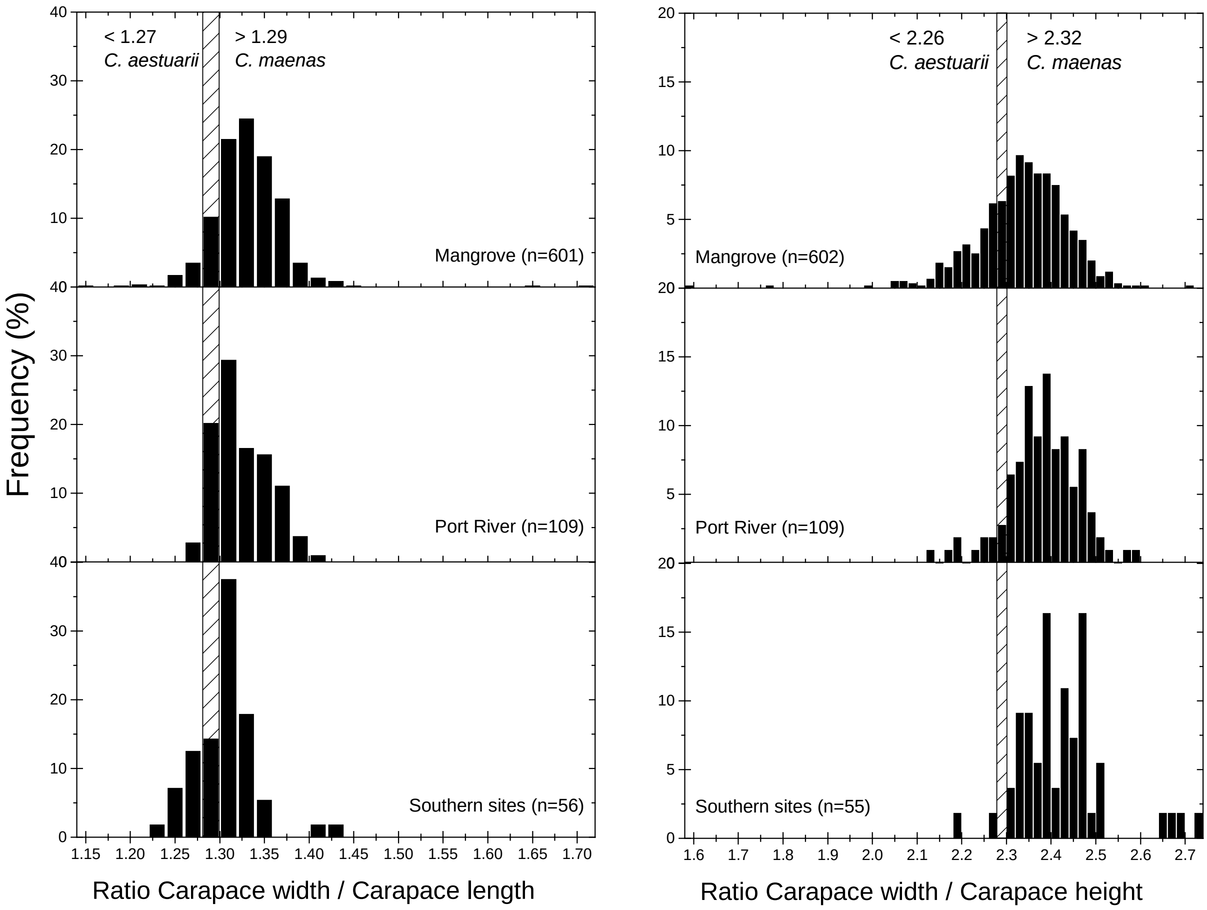

Figure 26: Frequency of two ratios based on morphometric measurements (carapace width, length and height) in 767 *Carcinus* collected from the three main regions. Values for the ratios more typical for either of the two species are indicated. The shaded section indicates ratios that are undefined and could be indicative of either species, or hybrids.

3.7.3 Morphometric variation

Using twelve morphometric variables measured from several hundred *Carcinus* showed morphological differences for male and female crabs, as well as for the three main regions (Figure 27). The morphometric features of male *Carcinus* found in the mangroves were most distinct from the other regions. Differences between male and female *Carcinus* were mainly due to carapace width and the lengths of right and left chelae (SIMPER analyses). The difference between the regions (southern sites, Port River and mangroves) was mainly due to carapace with and also lengths. This corresponds with the changes in the size-frequencies across the regions seen in chapter 3.2.

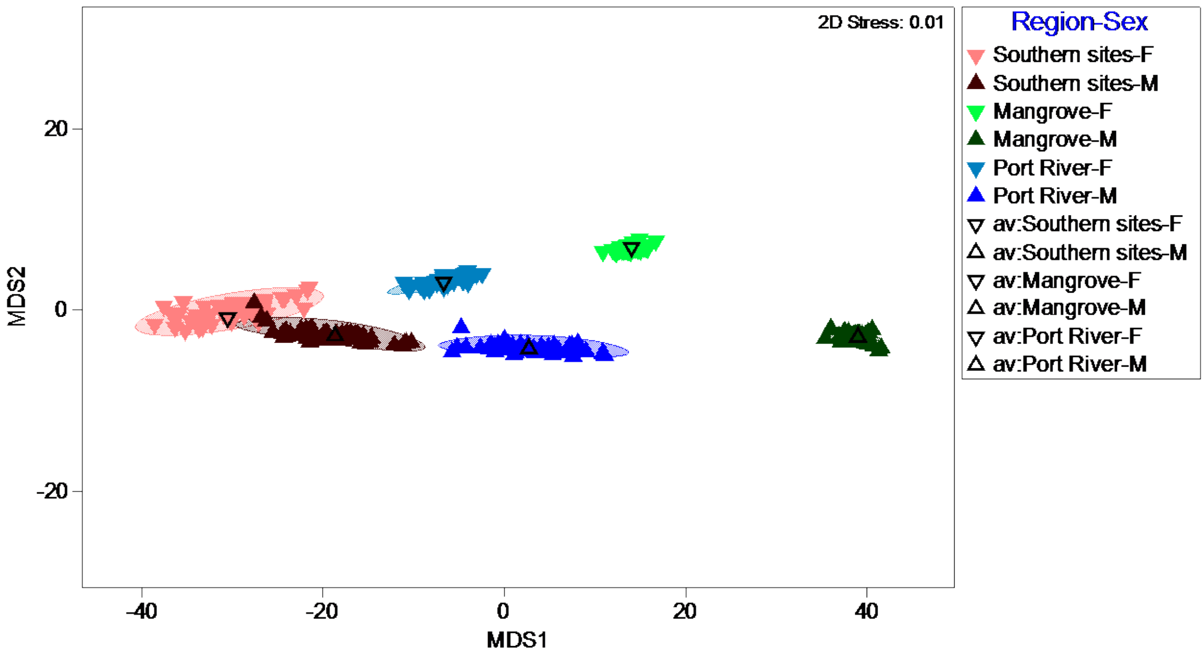

Figure 27: Bootstrap mMDS (multidimensional scaling) ordination plot of morphological features determined from female (F) and male (M) *Carcinus* sampled across three regions. The bootstrap averages for the regions are indicated with open black symbols.

3.7.4 Colour patterns

The dorsal carapace colour of *Carcinus* varies with colour depth and pattern complexity (Figure 28). The most prominent pattern was a 'plain' dorsal carapace, found in 68% of the crabs analysed (Table 7). The following most common colour patterns were 'cloudy spots' and 'small spots' with 18% and 6% respectively. Regional differentiation emerged, as 76% of the *Carcinus* from the mangroves had a plain coloured carapace, compared to 24% from the more rocky habitats at the southern sites (Table 7). In the Port River, *Carcinus* colour patterns were more varied than in the mangroves, and the largest diversity of colour patterns appeared for *Carcinus* collected at the southern sites (Table 7).

Table 7: Comparative table for the occurrence of dorsal patterns (see Table 3 for pattern detail) in percentage of *Carcinus* caught in the three regions (n = number of individuals measured).

Pattern	Mangroves (n=602)	Port River (n=109)	Southern sites (n=58)	total (n=766)
Plain	76	43	24	68
Cloudy spots	17	24	21	18
Small spots	3	14	26	6
Mottled	1	13	5	3
Tri-stripe	1	4	9	2
Speckled	1	1	9	1
Splodges	1	0	0	1
Rostrum spot	0	2	5	1
Double stripe	0	0	2	0

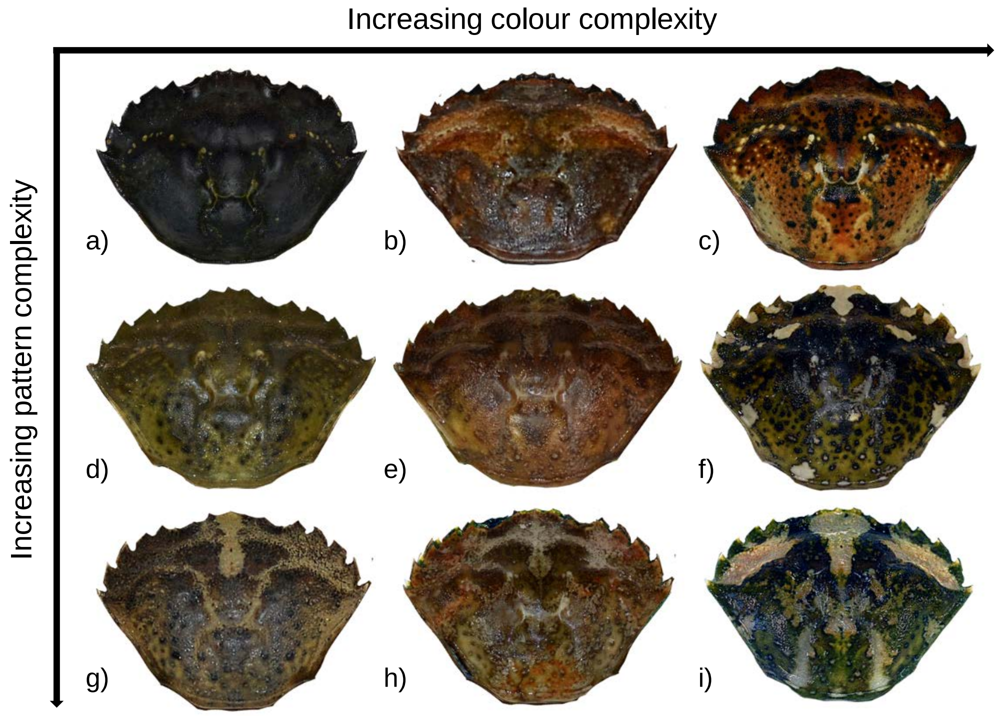

Figure 28. Example of colour and pattern variation in *Carcinus* collected from sites along Adelaide's metropolitan coastline, South Australia. Crabs depicted are a mixture of sexes, sizes and northern or southern sites. Pattern characteristics are distinguished in Table 3 and representative examples shown here for a) Plain, b) Double stripe, c) Cloudy spots, d) Small spots, e) Speckled, f) Splodges, g) Rostrum spot, h) Mottled, i) Tri-stripe.

3.7.5 Fecundity

So far, three gravid females were measured (Table 8) and three sub-samples of their egg mass preserved. The weight of the egg mass varied between 2 and 7 g, independent of the overall weight of the crab. Further analyses on fecundity will occur once additional ovigerous females have been prepared and measured.

Table 8: Details and measurements (CW = carapace width) of gravid female *Carcinus* to be used for fecundity analyses. The crabs were collected at Old Port Reach (OPR) and Port Gawler (PG).

Date collected	Site	CW (mm)	Weight (g) – with eggs	Weight (g) – without eggs	Weight (g) – eggs
27/06/2017	OPR	35.06	11.80	9.41	2.39
8/07/2016	PG	58.5	48.11	40.14	7.97
21/06/2016	PG	61.61	52.44	50.61	1.83

4. Discussion

Findings of the 2016/17 monitoring provide further information on the temporal and spatial population structure, abundance and distribution of *Carcinus* along the Adelaide coast from mangroves along the northern samphire coast to rocky shores of the northern Fleurieu peninsula. The report highlights the persistence of this invasive crab population along the coast, a continued range extension, increase in numbers, and a differential habitat use throughout the life cycle of *Carcinus*. Monitoring of *Carcinus* began in 2012 and since then there have been oscillations in the numbers of the crabs, with higher catches in the current monitoring than in the previous monitoring period. In particular, catches during timed searches along several sites of the southern Adelaide coastline were higher than previously found.

Understanding of the seasonal pattern of abundance is consolidating with catches from the monitoring, reflecting higher abundances in the cooler months of the year, and in particular a larger number of female crabs in nearshore habitats during winter and spring, which appears to overlap with their main reproductive period. Although *Carcinus* can tolerate higher temperatures, avoidance of temperatures > 28°C through migration has been reported (Hopkin *et al*. 2006), and this upper temperature limit corresponds well with the findings from this monitoring.

Carcinus was reproductively active (presence of ovigerous females) in the northern and also the southern sites. The number of ovigerous females caught was, however, low, similar to findings from monitoring of *Carcinus* on the coast of New South Wales (Garside *et al.* 2015a). Multiple reproductive cycles have been recorded from *Carcinus* populations elsewhere (Yamada *et al.* 2005; Leignel *et al.* 2014), but consecutive size-frequency plots give little indication to this occurring in Gulf St Vincent.

The size-frequency distributions were similar to previous monitoring (Dittmann *et al*. 2016), but showed a higher frequency of juvenile crabs as well as crabs just over one year old (Crothers 1968; Best *et al.* 2017). *Carcinus* is reaching maturity at about 30-40 mm carapace width (Best *et al*. 2017). Immature and juvenile *Carcinus* were mainly collected from southern sites and the Port River, suggesting specific habitat preferences for juvenile and adult crabs. Juvenile C. maenas prefer more complex structures for shelter such as mussel beds, rocks, seagrass and algae (Moksnes 2002, Cosham *et al.* 2016) and may also burrow deeper into soft sediment habitats as observed in North American populations (Aman & Grimes 2016). The more varied dorsal colour pattern of juvenile crabs found at the southern sites might be an adaptation to reduce detection by predators (Nokelainen *et al*. 2017). Different colour and pattern variations in relation to the complexity of the surrounding habitat have been described for *Carcinus* (Todd *et al*. 2012, Stevens *et al*. 2014) and can be phenotypic plasticity that facilitates their establishment in a non-native area.

The findings indicated that *Carcinus* may be recruiting on to the southern rocky shores, migrate north through the Port River area to the mangroves, where only larger crabs occurred. Such differential habitat use throughout their life cycle would require connectivity through larval dispersal (south from northern sites) as well as movement of adult crabs northward along the metropolitan coast into the mangroves. Life cycle movements of *Carcinus* with planktonic and benthic dispersal between different estuarine habitats are known to occur in the native range (Baeta et al. 2005). The method of catching

Carcinus differs between the southern (timed searches) and northern sites (baited traps), but no juvenile crabs were detected at any time during observations while deploying and retrieving the traps at the northern sites. The pattern is thus unlikely affected by the different approaches. However, further dedicated studies on movements, connectivity and habitat use of *Carcinus* will be needed to fully understand their life cycle and associated risks of further dispersal.

Morphometric assessments were inconclusive about the presence of two species of *Carcinus* or their hybrids in Gulf St. Vincent. Based on ratios of carapace width to length and height, *C. maenas* and *C. aestuarii* have been differentiated in other regions of the world (Clark *et al.* 2001; Yamada & Hauck 2001). Based on the measurements of *Carcinus* caught from Gulf St Vincent so far, the vast majority of them are *C. maenas*, yet the presence of *C. aestuarii* or hybrids cannot be fully ruled out based on these morphometric relationships. Hybrids of these two species of *Carcinus* have been confirmed from other regions, such as Japan, where these crabs are not native (Darling 2011). Molecular analyses of crab specimens from Gulf St Vincent are commencing as part of the PhD project by René Campbell, who will also carry out further analysis of the colour patterns and fecundity.

To reduce the population size of *Carcinus*, attempts could be made for targeted efforts to remove ovigerous females over the winter and spring period. Removal of adult crabs through catches has had the opposite effect of increasing population sizes by reducing intraspecific competition (Duncombe and Therriault 2017). By catching ovigerous females, the larval supply for the next generation would be reduced. However, this requires considerable effort given the low number of ovigerous females found. Yet the monitoring results allow to advice on the timing and locations for such removal.

Competitive interactions with native species are little understood, and require more targeted investigations. Interactions could be competition for prey and habitat, but also predatory relationships. Species of *Portunus* have been found to prey on *Carcinus* and reduce their numbers on other coastlines (Garfield *et al*. 2015b). Based on by-catch of *Portunus armatus* in the baited traps, it appears that the two species of Portunidae may only temporarily overlap in nearshore habitat, with the introduced *Carcinus* being more abundant in cooler months of the year and abundances of *P. armatus* increasing in the warmer seasons. However, inverse patterns occurred between the abundances of *Carcinus* and *P. armatus* between Middle Beach and Port Gawler for summer and autumn seasons. Port Gawler is a popular site for recreational fishing of *P. armatus*, whereas Middle Beach has been protected as a marine park for several years. These interactions deserve further investigation to explore whether protection can lead to a higher abundance of native *P. armatus*, which could reduce the abundance of the introduced *Carcinus* through biocontrol (Atalah *et al*. 2015).

5. Acknowledgements

We would like to thank Edward Bonner for volunteering during several of the field surveys over the previous year. Orlando Lam Gordillo carried out some of the statistical tests. Funding was provided by Adelaide and Mount Lofty Ranges Natural Resources Management Board (AMLR NRM), and we thank Tony Flaherty from the AMLR NRM Board for his interest and discussions on the European shore crab.

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Appendix

Table A1: Test outcome from two-way Permutational ANOVA for differences in the CPUE between seasons (fixed factor) and sites (random factor), and pairwise tests for differences between seasons. Based on data from Autumn 2013 to Spring 2017 when all sites were more regularly sampled. Over these monitoring years, five autumn and winter surveys were carried out, four spring and three summer surveys. Significant differences are indicated in bold.

Source	df	MS	Pseudo-*F*	$P_{(perm)}$
Main test				
Season	3	18.311	11.840	**0.002**
Site	3	7.880	2.526	0.066
Season x Site	9	1.545	0.495	0.872
Residual	55	3.119		
Pairwise t-tests for seasons				
winter, spring				0.854
winter, summer				**0.029**
winter, autumn				**0.029**
spring, summer				**0.040**
spring, autumn				0.083
summer, autumn				0.139

Table A2: Test outcome from two-way Permutational ANOVA for differences in the CPUE between seasons (fixed) and years (fixed), based on data from Autumn 2013 onward when all sites were more regularly sampled. Significant differences are indicated in bold.

Source	df	MS	Pseudo-*F*	$P_{(perm)}$
Main test				
Season	3	15.211	6.278	**0.001**
Year	4	11.718	4.836	**0.003**
Season x Year	8	3.552	1.466	0.196
Residual	55	2.423		

Table A3: Test outcome from two-way ANOVA for differences in the CPUE for male and female crabs between seasons (fixed) and sites (random), based on data from Autumn 2013 onward when all sites were more regularly sampled. Pairwise tests were carried out for differences between seasons, and for male crabs also between sites. Significant differences are indicated in bold.

Source	df	MS	*F*	*P*	MS	*F*	*P*
		Male crabs			Female crabs		
Main test							
Season	3	6.620	9.426	**0.004**	3.173	5.259	**0.028**
Site	3	6.007	3.280	**0.026**	0.523	0.948	0.426
Season x Site	9	0.701	0.383	0.941	0.603	1.093	0.389
Residual	55	1.831			0.552		
Pairwise t- tests for season							
winter, spring				0.532			0.720
winter, summer				**0.026**			0.064
winter, autumn				**0.038**			0.108
spring, summer				0.063			**0.031**
spring, autumn				0.101			0.152
summer, autumn				0.828			0.106
Pairwise t- tests for season							
PG, PA				**0.017**			
PG, OPR				**0.042**			
PG, MB				**0.036**			
PA, OPR				0.776			
PA, MB				0.508			
OPR, MB				0.823			

Table A4: Pearson Correlations Coefficients (Corr.) for *C. maenas* caught over all monitoring years at the northern sites, and separately for the two mangrove and Port River sites each. Correlation coefficients are given for all crabs, and separately for male and female crabs. Significant *P* values are highlighted in bold.

	All Northern sites		Mangroves		Port River	
	Corr.	*P*	Corr.	*P*	Corr.	*P*
All crabs CPUE						
Temperature (°C)	-0.301	**0.007**	-0.294	0.065	-0.311	0.058
Salinity (ppt)	-0.099	0.384	-0.004	0.98	-0.331	**0.042**
DO (mg/L)	0.419	**0.001**	0.233	0.233	0.691	**<0.001**
DO (%)	0.334	**0.012**	0.149	0.449	0.62	**<0.001**
Male crabs CPUE						
Temperature (°C)	-0.206	0.071	-0.161	0.322	-0.269	0.103
Salinity (ppt)	-0.138	0.228	-0.114	0.484	-0.306	0.061
DO (mg/L)	0.481	**<0.001**	0.338	0.078	0.655	**<0.001**
DO (%)	0.439	**<0.001**	0.311	0.107	0.609	**<0.001**
Female crabs CPUE						
Temperature (°C)	-0.338	**0.003**	-0.368	**0.019**	-0.291	0.076
Salinity (ppt)	-0.009	0.935	0.155	0.34	-0.279	0.089
DO (mg/L)	0.192	0.157	0.014	0.944	0.513	**0.005**
DO (%)	0.068	0.619	-0.122	0.536	0.426	**0.024**

Table A5: Test outcomes from multivariate General Linear Model (GLM) for tests using either a) dissolved oxygen (DO) saturation of b) concentration.

a) GLM with DO (%).

GLM Source	P
(Intercept)	0.554
Temperature	0.306
Salinity	0.386
DO (%)	0.507
Temperature-Salinity	0.176
Temperature-DO (%)	0.336
Salinity-DO (%)	0.425
Temperature-Salinity-DO (%)	0.245

	AIC
CPUE	193.62
Male CPUE	154.18
Female CPUE	124.89

Multivariate test

Source	Residual df	df	Dev	P
(Intercept)	55			
Temperature	54	1	19.361	**0.005**
Salinity	53	1	13.256	**0.014**
DO (%)	52	1	29.202	**0.001**
Temperature-Salinity	51	1	1.218	0.740
Temperature-DO (%)	50	1	0.599	0.874
Salinity-DO (%)	49	1	2.368	0.505
Temperature-Salinity-DO (%)	48	1	5.469	0.224

Univariate tests

	CPUE		Male CPUE		Female CPUE	
Source	Dev	P	Dev	P	Dev	P
(Intercept)						
Temperature	7.781	**0.006**	2.54	0.095	9.039	**0.006**
Salinity	5.461	**0.032**	7.106	**0.019**	0.698	0.420
DO (%)	12.808	**0.001**	15.729	**0.001**	0.665	0.401
Temperature-Salinity	0.641	0.799	0.092	0.799	0.484	0.799
Temperature-DO (%)	0.18	0.870	0.007	0.945	0.412	0.870
Salinity-DO (%)	0.075	0.840	0.249	0.840	2.044	0.415
Temperature-Salinity-DO (%)	1.048	0.555	0.479	0.559	3.941	0.191

b) GLM with DO (mg/L)

GLM Source	P
(Intercept)	0.360
Temperature	0.205
Salinity	0.236
DO (mg/L)	0.287
Temperature-Salinity	0.104
Temperature-DO (mg/L)	0.207
Salinity-DO (%)	0.231
Temperature-Salinity-DO (mg/L)	0.147

	AIC
CPUE	192.40
Male CPUE	154.31
Female CPUE	123.69

Multivariate test

Source	Residual df	df	Dev	P
(Intercept)	55			
Temperature	54	1	19.361	**0.007**
Salinity	53	1	13.256	**0.018**
DO (mg/L)	52	1	28.571	**0.001**
Temperature-Salinity	51	1	1.852	0.597
Temperature-DO (mg/L)	50	1	3.471	0.360
Salinity-DO (mg/L)	49	1	2.638	0.470
Temperature-Salinity-DO (mg/L)	48	1	7.822	0.111

Univariate tests

	CPUE		Male CPUE		Female CPUE	
Source	Dev	P	Dev	P	Dev	P
(Intercept)						
Temperature	7.781	**0.009**	2.54	0.088	9.039	**0.009**
Salinity	5.461	**0.050**	7.106	**0.024**	0.689	0.445
DO (mg/L)	12.114	**0.002**	15.411	**0.001**	1.046	0.288
Temperature-Salinity	1.012	0.660	0.302	0.740	0.538	0.740
Temperature-DO (mg/L)	1.873	0.427	0.983	0.580	0.615	0.580
Salinity-DO (mg/L)	0.161	0.769	0.425	0.769	2.052	0.424
Temperature-Salinity-DO (mg/L)	1.594	0.411	1.007	0.411	5.221	0.108

Figure A1: Scatterplots of catch per unit effort (CPUE) and water quality parameters for *Portunus armatus* found as by-catch in the baited traps, from all northern sites based on monitoring since 2012. Where significant correlations were found, linear fits (straight lines) and 95% confidence interval bands are included.