Survey of Carter's Freshwater Mussel in the Lower Vasse River to inform future sediment removal

June 2021

Prepared for:
City of Busselton

Prepared by:
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute, Murdoch University
& Ottelia Ecology

Harry Butler Institute
MURDOCH UNIVERSITY

Ottelia Ecology
Environmental Services
Aquatic Ecosystem Specialists

Prepared for:
City of Busselton
2 Southern Drive, Busselton WA 6280
Locked Bag 1, Busselton WA 6280

Contact:
Mathilde Breton
Senior Sustainability/Environment Officer
p: (08) 9781 0457
Mathilde.Breton@busselton.wa.gov.au

Prepared by:
Robyn Paice
Ottelia Ecology
robyn@otteliaecology.com.au

Stephen Beatty
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
s.beatty@murdoch.edu.au

Distribution List:

Organisation	Contact Name	Date
City of Busselton	**Mathilde Breton**	**9 June 2021**

Document Control:

Document Status	Prepared By	Reviewed by	Date
Draft report V1	**Robyn Paice**	**Stephen Beatty**	**8 June 2021**

Executive Summary

This report presents the outcomes of a survey of Carter's Freshwater Mussel (*Westralunio carteri*) in the Lower Vasse River, Busselton, to provide information for planning of sediment removal in the river. The City of Busselton proposes to undertake sediment removal from the river and is required to minimise potential impacts of these works on mussels in the river. The Lower Vasse River is degraded with a long history of seasonal algal blooms and the purpose of sediment removal is to provide benefits to water quality, habitat and public amenity.

Carter's Freshwater Mussel is a listed threatened species under the Commonwealth Environmental Protection and Biodiversity Conservation Act (1999), and as such any sediment removal projects must be managed to minimise impacts on this species. The outcomes of the current survey will inform the development of an environmental management plan to ensure appropriate protection and management of mussels during future sediment removal works.

The survey was undertaken in two stages in December 2020 and in April-May 2021. The survey confirmed the presence of mussels throughout the river and determined density and abundance estimates. As found in previous surveys, mussels were confined to bank habitat areas in much of the river and were not found in off-bank sampling downstream of the boat ramp. However, upstream of Fairlawn Rd, mussels are distributed throughout the river in bank and off-bank habitat.

While large numbers of mussels were found with densities varying in different sections of the river, the population has now been substantially impacted by a saltwater incursion event. This event occurred in March-May 2021, before and during the second stage of the survey.

Key outcomes from the Stage 1 survey are outlined below, however it is likely that all mussels in these areas have now died as a result of the saltwater incursion event. Further survey would be required to confirm this.

> <u>Butter Factory to Causeway</u>: The northern bank along Peel Terrace had higher density of mussels than the southern bank, as found previously, and the area around the old rail bridge is also important habitat. Mussel density downstream of the Eastern Link bridge was low, and no mussels were found near the Causeway Bridge or the Butter Factory weir. The total abundance of mussels estimated for this section of the river was estimated to be 1004.
>
> <u>Causeway to old boat ramp</u>: This survey area had more consistent occurrence of mussels throughout and higher density than downstream of the Causeway. Significant hotspot areas were identified along the eastern and western banks directly in front of the City of Busselton administration building. The total abundance of mussels in this section was estimated to be 2112.
>
> <u>Strelly Street Bridge area and upstream</u>: The area under the bridge and just downstream had the highest mussel density found in the study, and these mussels were significantly larger (and older) than those in downstream survey areas. The total abundance of live mussels in the vicinity of Strelly

St Bridge was estimated to be 563. Mussels were absent from the river upstream of the Strelly St bridge, which may be related to a previous infestation of Mexican waterlily throughout this section.

Key outcomes from Stage 2 survey were as follows:

Boat ramp to Strelly St: This area had a very low mean mussel density and large sections of bank with no mussels. The highest mussel densities occurred near Gwendolyn St and upstream to Strelly St. No live mussels were found downstream of Gwendolyn St. The total number of live mussels between Gwendolyn St and Strelly St was estimated at 103, representing 18% survival following the saline incursion, all within bank habitat areas.

Upstream of Strelly St to Fairlawn Rd: Mussel density was higher than downstream of Strelly St and was patchy although no hotspots were identified. Elevated salinity caused severe mortality in this section with only 4% survival. The total number of live mussels was estimated to be 43, all within bank habitat areas.

Fairlawn Road to Busselton Bypass: This section had the highest density of mussels upstream of the boat ramp, although overall density was relatively low and patchy with two significant hot spots identified. Saltwater incursion in this area was limited, resulting in much greater survival rate of 60%. Live mussels were distributed in both bank and off-bank habitats in this shallower section of the river, with a total estimate abundance of 2007 live mussels.

Sediment removal presents risks to mussel health from physical disturbance, potential negative effects on water quality and from smothering by resettling particulates. Prior to the saltwater incursion event, large numbers of mussels present in the river were present requiring a significant management effort to protect them from impacts of sediment removal. The live mussel population is now substantially restricted to the area upstream of Fairlawn Rd, although updated surveying in the Stage 1 area is needed to confirm whether any live mussels remain downstream of the boat ramp. The outcomes of this survey will inform the development of a management plan to facilitate protection of the *W. carteri* population in the lower Vasse River during future sediment removal works.

Contents

Tables and Figures

Context and scope

The City of Busselton proposes to undertake sediment removal from the lower Vasse River, Busselton, as part of its implementation of the Lower Vasse River Waterway Management Plan (CoB, 2019). The aim of sediment removal is to improve water quality, habitat and amenity, as the sediment layer consists of fine organic material with high nutrient content, low oxygen and poor structure. This work is currently proposed for the section of river between the Butter Factory and the old boat ramp, approximately 800m (Figure 1). It is also being considered for a further section upstream from the old boat ramp to the Busselton Bypass.

Carter's Freshwater Mussel (*Westralunio carteri,* CFM) is known to occur in the Lower Vasse River, including in the proposed sediment removal areas. This species is endemic to south-western Australia and is listed as threatened under the *Biodiversity Conservation Act* (2016) and the *Environmental Protection and Biodiversity Conservation Act* (1999). Dredging has been shown to negatively impact mussel populations elsewhere and thus proposed sediment removal has the potential to negatively impact *W. carteri*.

The current project complements previous survey and management work in the lower Vasse River by the authors for the City of Busselton, which overlap the current survey area:

- Survey of the species in order to guide the management of the species during the previously proposed pilot study into sediment removal from the Lower Vasse River by the City of Busselton (Beatty et al. 2019). That study surveyed mussels downstream of the Causeway Rd Bridge to the old footbridge.
- Baseline survey, development of an Environmental Management Plan (Beatty and Lymbery 2019) and temporary relocation of mussels in relation to construction of the Busselton Eastern Link (see recent progress report; Beatty et al., 2020).

As the proposed sediment removal involves a greater area of the Lower Vasse River, additional survey was required to determine the distribution, density and population viability of the species and help to underpin the development of the management plan for the species. The survey requirements initially specified two adjacent areas between the Butter Factory to the old boat ramp and an additional area around and upstream of Strelly Street Bridge; and was subsequently extended to cover the entire river between the Butter Factory and the Busselton Bypass (Figure 1).

A survey of *W. carteri* was required to inform preparation of a management plan for the species to minimise and monitor impacts on the population during sediment removal. This survey was undertaken in two stages:

1. The sections from the Butter Factory to the boat ramp, under and near Strelly Street bridge and upstream to the river bend were surveyed in December 2020.
2. The sections from the boat ramp to Strelly St, and from upstream of Strelly St to the Busselton Bypass were surveyed in April-May 2021.

In March to May 2021, the survey area was affected by abnormally high salinity levels resulting from saltwater incursion from the Vasse Estuary. This increased salinity severely impacted the CFM population prior to the second stage of the survey, and the extent of this impact is addressed in this report.

The results of the current survey for *W. carteri* will be included in the development of a Management Plan and together, these will inform a referral to the Department of Agriculture, Water and the Environment (DAWE) under the Environmental Protection and Biodiversity Conservation (EPBC) Act.

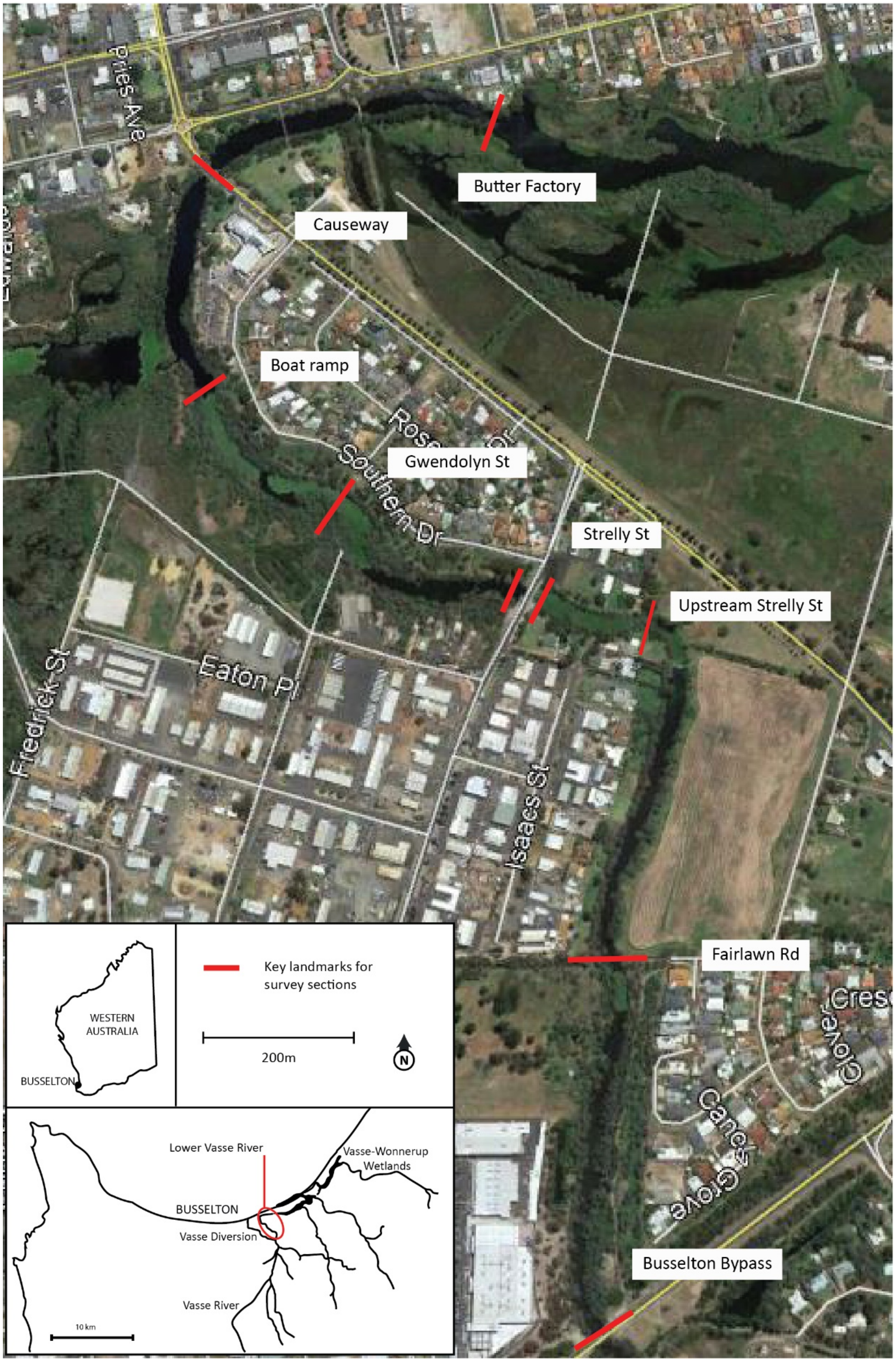


Figure 1. Lower Vasse River mussel survey area showing location of key landmarks used to describe survey sections.

Aim

The aim of the current study was to determine distribution, density and population viability of Carter's Freshwater Mussel in the specified survey areas. The outcomes of this study will extend current knowledge of the mussel population in the river and inform the development of a Management Plan to minimise impacts of future sediment removal on this population. Importantly, the survey will confirm abundance of mussels expected to be managed to mitigate the impact of sediment removal.

Methods

Surveying was undertaken in two stages, covering eight sections of the river (Figure 1):

Stage 1:

- Butter Factory to Causeway (Bussell Hwy)
- Causeway to old boat ramp (on Southern Drive)
- Vicinity of Strelly St
- Upstream of Strelly St to the river bend

Stage 2:

- Boat ramp to Gwendolyn St
- Gwendolyn St to Strelly St
- River bend upstream of Strelly St to Fairlawn Rd
- Fairlawn Rd to Busselton Bypass

Survey methods followed those previously used quantify the distribution and population structure for the species (see Klunzinger et al., 2012a; Klunzinger et al., 2012b, Beatty et al., 2017, Beatty et al., 2019), and included both bank habitat and off-bank survey methods.

Physico-chemical water quality variables were measured at three sites within each of the three survey areas. Measurements were taken at the surface and at 0.5m intervals to the bottom at each bank and in the centre of the river at each site, including temperature, dissolved oxygen, pH, and conductivity.

Bank habitat survey

Bank habitat of wadable depth is known to be the primary habitat for the species (Klunzinger et al., 2012a, Beatty et al., 2019). The benthos was surveyed using 1 m^2 quadrats spaced every 5 m along each bank within the three reaches. Quadrats were placed close to the edge of the bank as this is where greater numbers of mussels were observed. Each quadrat was thoroughly searched for mussels by hand-foraging in sediment and detritus and around rocks and woody debris. Mussel density was determined within each quadrat and the location of each quadrat or grab sample recorded using a GPS. The shell length of each individual collected was measured to the nearest 1 mm, before being returned to the site of capture.

Off-bank habitat survey

Based on previous sampling (Beatty et al., 2019) and known habitat preferences (Klunzinger et al., 2012a), mussels were not expected to be found in off-bank habitats in deeper areas of the river, but considered more likely further upstream where the river is shallower and sediments potentially less anoxic. To provide further confirmation, sampling was undertaken in the survey areas using two methods.

During the first stage of the survey, an Ekman grab (dimensions 25 x 25 cm to a sediment depth of 20 cm) was used to collect sediment samples. Thirteen sites were sampled, including ten between the Butter Factory and the boat ramp, and three upstream of Strelly Street. Intensive sampling was undertaken each site, with ten samples collected from an anchored boat and checked, providing a total of 130 Eckman grab samples in the study area. This method was difficult to implement and did not yield any mussels. Although many samples were taken, this only covered a very small proportion of the river bed.

In the second stage of the survey, off-bank habitat was sampled using 1-metre wide transects from the bank towards the centre of the river every 25m along the banks where water and soft sediment depth allowed safe access. Transect length was measure from the boat in 5-metre intervals and the bed was hand-searched.

Data analysis

To provide estimates of total abundance and assist in future management, mussel density was calculated separately each survey section. Mean density values obtained from quadrats were multiplied by bank habitat length and a conservative width of 2m to estimate total abundances. Mussel density from transect searches was obtained using a width of 1m to provide a transect area and dividing the number of mussels by this area. Mean density was applied to total off-bank habitat areas covered by transect sampling only to avoid over-estimating the number of mussels. This assumes that no mussels occur in the deeper waters, as found by high density Eckman grab sampling. River length surveyed was multiplied by 10m to provide an area, and this area multiplied by mean density to estimate total abundance.

Length-frequency distributions of mussels at each site were plotted to confirm population structure and age distribution estimates following Klunzinger et al. (2014).

Point density and Optimised Hotspot Analysis were conducted on quadrat data within ArcMap to map the densities of mussels in each of the three survey areas. This provided a visual representation of mussel distribution and determined statistically significant hot and cold spots. The optimised analysis generated a Getis-Ord Gi* statistic (z-scores) and the GI_Bin score (the significance of the z-scores).

Analysis of variance (ANOVA) in SPSS with Tukey's post hoc comparisons was performed to determine differences in shell length between survey areas. Normality plots of shell length showed strong correlation of observed values with those expected from a normal distribution for all survey areas, so ANOVA was considered appropriate without transformation of the data.

Results

Mussel density – Stage 1

Stage 1 of the survey was completed from 11th to 17th December 2020, including searching of 378 quadrats in bank habitat along a total bank length of 1.77km (Appendix 1) and 130 Eckman grab samples in off-bank habitat. At the Causeway Road bridge, the river was too deep for use of quadrats and the bridge too low for sediment grabs. The edges of the bridge were manually searched to the greatest extent possible. A total of 394 live mussels were found in quadrats, and 2 additional mussels were found at the Causeway Bridge. No mussels were recorded in the off-bank, deeper habitats.

Summary statistics for the three survey areas are provided in Table 1. As with previous surveys, the distribution of mussels was patchy with large sections of bank with no mussels and others with high-density hot spots (Figure 2). As this survey was done prior to the mortality event, it did not aim to differentiate between dead and live mussels, therefore dead mussels were recorded but not measured during this stage of the survey. A total of 13% of mussels encountered were dead, and these were found mainly between the Causeway and the boat ramp.

Between the Butter Factory and the Causeway, much greater density of mussels occurred on the northern bank (0.99 per m^2) than the southern bank (0.22 per m^2). Very few mussels were found downstream of the Eastern Link bridge, but they occurred consistently around the old rail bridge and upstream, with hot spots near the old fountain pump infrastructure. Along the southern bank, mussels were concentrated near the viewing platform and the old rail bridge (footbridge). The total abundance of live mussels for this section of the river was estimated to be 1004.

In addition to quadrats, the Butter Factory weir structure was searched separately to quadrats with no mussels found. A search of the Causeway Bridge structure on the downstream side found no live mussels in this area (one dead mussel found). On the upstream side, only two mussels were found. This low density at the Causeway is notable because mussel density is higher at other structures in the river (bridges, old fountain infrastructure, viewing platform, old deck opposite City offices).

The Causeway to boat ramp survey area had a greater density of mussels than downstream, particularly on the eastern bank. Overall mean density was 1.73 per m^2, with higher density on the eastern bank (Table 1). A relatively large number of dead mussels were found in this area (53), which is likely related to a mortality event observed in early 2020 in this part of the river, when rapidly dropping water levels left mussels stranded on dry banks (R. Paice pers observation). Mussels were distributed consistently through most of this area with significant hotspots in front of shire offices and on opposite bank near stand of *Typha* and remains of an old deck (Figure 2). The total abundance of live mussels in this section was estimated to be 2112.

In the Strelly Street survey area, mussels were present downstream of and under the bridge but were not found upstream. Downstream of and under Strelly St bridge at the highest density found in the study (5.6 per m^2). The highest densities were found under the bridge, with 31 mussels encountered in one quadrat. The total abundance of live mussels in the vicinity of Strelly St Bridge was estimated to be 563, however the standard error for this estimate is notably large due to the high variation in quadrat density.

There are several factors that likely contribute to an absence of mussels in this reach of river. The more gradual slopes in this shallower section of river would lead to greater drying of banks as water recedes more rapidly on the gradual slopes, leaving mussels more exposed and requiring them to move greater distances and into unsuitable sediments and warm conditions. Contributing to the shallow conditions in this area has been the previous infestation of waterlily, which over time has led to a build-up of sediments. While the lilies have now been controlled, there is now more drying of banks in this section than previously. Further, during the waterlily infestation, oxygen levels in this section were extremely low (Paice, 2018) and would have severely impacted mussels that may have occurred there earlier.

Estimates of mussel abundance from the survey data is important in guiding management during disturbance activities where relation is necessary, as would be the case for dredging. The abundance estimates given in Table 1 were determined using bank length and a bank habitat width of 2m. This is considered conservative, as mussels in the river are found in higher densities closer to the bank, and habitat would be very narrow in parts of the river with steeper banks.

As this stage of the survey was completed prior to the saltwater incursion, it does not reflect the current status of the mussel population. These mussels have been severely impacted by the saltwater incursion event and it is considered likely that 100% mortality has occurred (see further section on this event).

Table 1. Carter's Freshwater Mussel density and abundance in Stage 1 survey areas December 2020.

Survey Area	Total live mussels	Total dead mussels	Mean density ±SE (per m^2)	Maximum density (per m^2)	Estimated mussel abundance ±SE
Butter Factory to Causeway:					
North bank (420m)	78	0	0.99 ±0.18	9	830 ± 262 (133)
South bank (380m)	17	3	0.22 ± 0.07	3	170 ±110 (60)
Total					**1004 ± 166**
Causeway to boat ramp:					
East bank (290m)	140	19	2.0 ± 0.30	10	1160 ± 171
West bank (320m)	92	34	1.44 ± 0.25	13	920 ± 157
Total					**2112 ± 237**
Total – Butter Factory to Boat ramp					**3183 ± 309**
Strelly St bridge and upstream:					
Bridge and downstream (50m)	62	1	5.6 ± 2.78	31	563 ± 278
Upstream north bank (150m)	0	1	0	0	0
Upstream south bank (160m)	0	0	0	0	0
Total					**563 ± 278**

Figure 2. Optimised Hotspot analysis showing significant hotspots in Stage 1 survey areas downstream of the boat ramp.

Mussel density – Stage 2

Stage 2 of the survey was completed from 27th April to 20th May 2021, with 653 quadrats search along a total bank length of 3.17km (Appendix 1). A total of 56 off-bank transects were searched covering $471m^2$. This survey coincided with a mass mortality event of *W. carteri,* therefore live and dead mussels were counted separately, and all were measured. In both bank habitat and off-bank habitat, mussel density increased with distance upstream, and the percentage of live mussels was also higher upstream of Fairlawn Rd to the Busselton Bypass (Table 2). A total of 778 mussels were found in quadrats and 102 in transects and 65% of these were dead.

The lowest density of mussels occurred in the most downstream section between the boat ramp and Gwendolyn St, with mean bank density of 0.27 dead mussels per m^2 and zero live mussels. Most mussel shells were encountered in quadrats on the northern bank halfway along this section (Appendix 1), possibly corresponding to lower density of waterlily. From Gwendolyn St upstream to Strelly St total bank density was higher at 0.84 per m^2 with density of dead mussels much higher than live, accounting for an estimated 82% of total abundance. Off-bank mussel density from the boat ramp to Strelly St was extremely low at 0.05 mussels per m^2 and all mussels encountered here were dead. No significant hot spots were identified in this area, however the greatest densities of live mussels were encountered on the northern bank adjacent to the island in this area. The total number of live mussels between Gwendolyn St and Strelly St was estimated at 103, all within bank habitat areas.

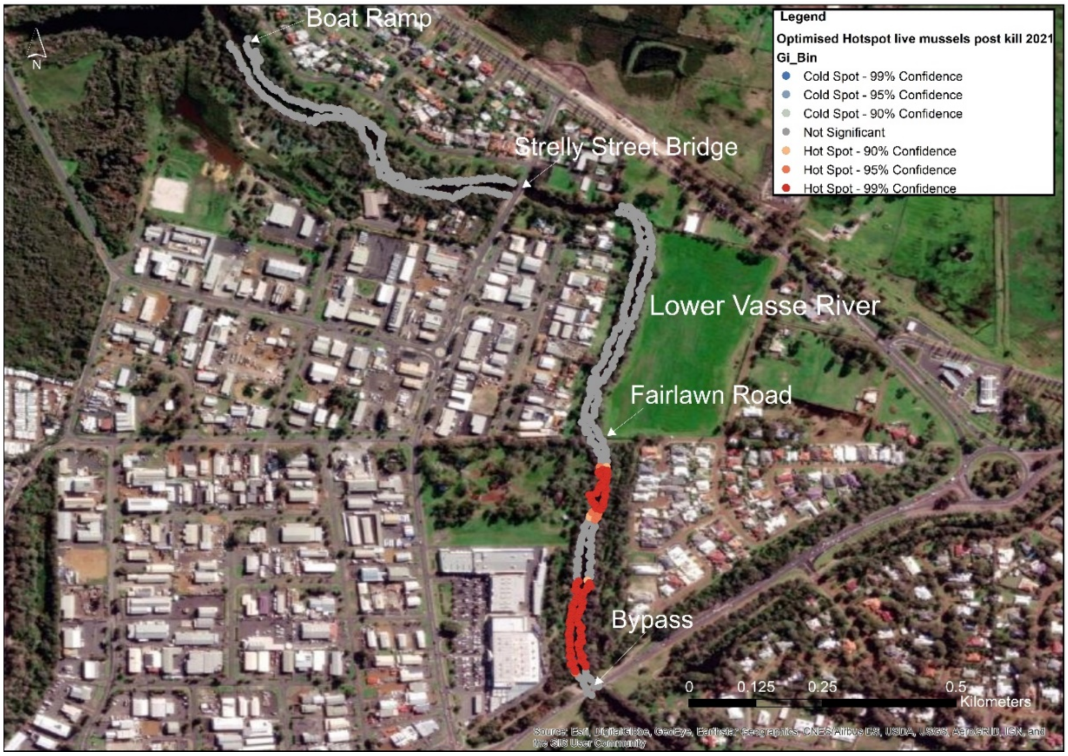
Upstream from Strelly St to Fairlawn total mussel density was higher at 1.25 per m^2 but the proportion of dead mussels was very high (96%) and live mussel density was only 0.05 per m^2 (Table 2). Density was patchy with large areas of bank habitat having no mussels and significant hot spots for dead mussels occurring at two locations on both banks (Figure 3b). No hot spots were identified for live mussels in this section (Figure 3a). Off-bank mussel density was very low at 0.11 mussels per m^2 and 100% of these mussels were dead. The total number of live mussels from Strelly St to Fairlawn Road was estimated to be 43, all within bank habitat areas.

The impact of the mortality event was much reduced in the most upstream section from Fairlawn Rd to the Busselton Bypass. This section had the highest total mussel density both on- and off-bank at 1.25 and 0.43 mussels per m^2 respectively, and also the largest number of live mussels (Table 2). A much higher survival rate was observed with 65% live mussels in bank habitat and 55% live in off-bank habitat. Mussel distribution in this section was patchy, with higher densities observed in a variety of bank habitats and no obvious pattern to off-bank distribution. Live mussel hotspots were observed in two areas on both banks: just upstream of the patch of waterlily approximately 70m upstream of Fairlawn Rd; and a reach of approximately 180m directly downstream of the Bypass (Figure 3a). A hot spot for dead mussels also occurred just upstream of the waterlily patch (Figure 3b). The total number of live mussels present in this section of river is estimated to be 2007, including 1230 in bank habitat areas and 777 in off-bank habitat.

Table 2. Carter’s Freshwater Mussel density and abundance in Stage 2 survey areas April-May 2021.

Bank Habitat	**Dead Mussels**		**Live Mussels**	
	Mean Density (per m²)	**Abundance (±SE)**	**Mean Density (per m²)**	**Abundance (±SE)**
Boat ramp – Gwendolyn St (560m)	0.27 ± 0.07	153 ± 38	0.00	0
Gwendolyn St – Strelly St (670m)	0.69 ± 0.08	459 ± 55	0.15 ± 0.04	103 ± 26
Strelly St – Fairlawn Rd (955m)	1.20 ± 0.12	1146 ± 111	0.05 ± 0.02	43 ± 16
Fairlawn Rd – Busselton Bypass (985m)	0.70 ± 0.09	694 ± 92	1.25 ± 0.16	1230 ±155
Total	**0.77 ± 0.05**	**2452 ± 163**	**0.42 ± 0.05**	**1376 ± 165**
Off-bank Habitat	**Dead Mussels**		**Live Mussels**	
	Mean Density (per m²)	**Abundance (±SE)**	**Mean Density (per m²)**	**Abundance (±SE)**
Boat ramp - Strelly St ($5800m^2$)	0.05 ± 0.02	99 ± 47	0	0
Strelly St - Fairlawn Rd ($4750m^2$)	0.11 ± 0.02	477 ± 97	0	0
Fairlawn Rd - Busselton Bypass ($4800m^2$)	0.19 ± 0.05	634 ± 161	0.24 ± .06	777 ± 187
Total	**0.12 ± 0.02**	**1210 ± 194**	**0.07 ± 0.02**	**777 ± 187**

(a)



(b)

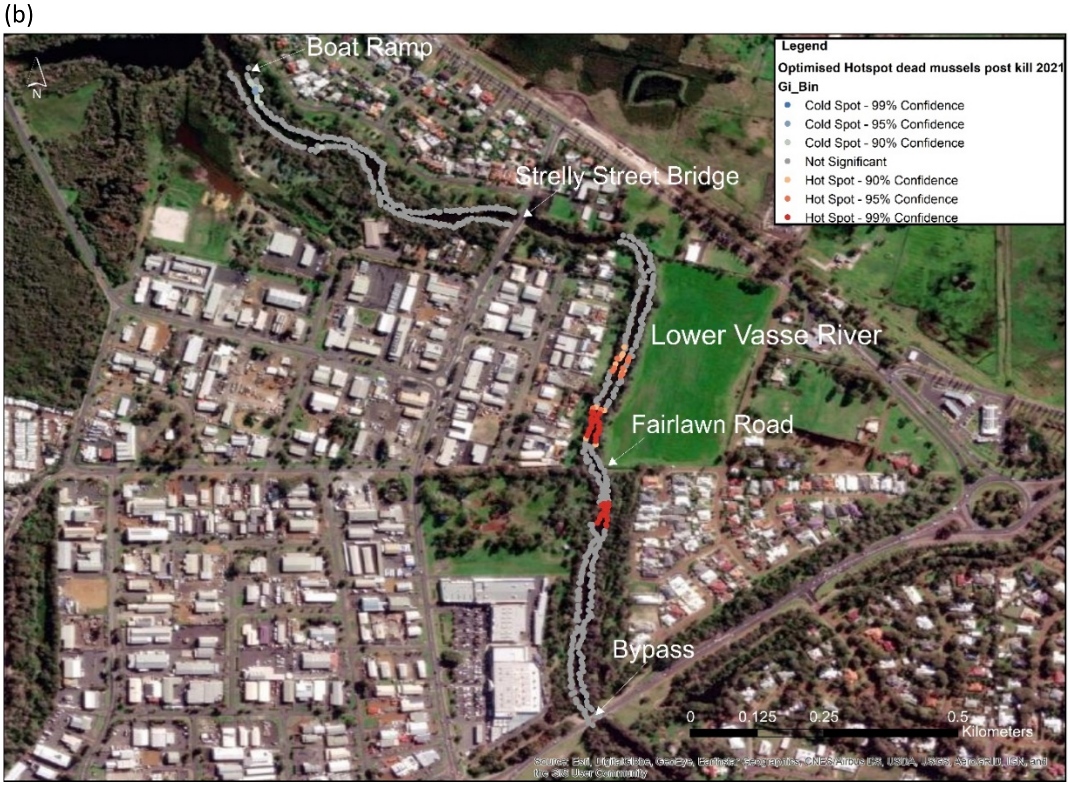


Figure 3. Optimised Hotspot analysis showing significant hotspots of live (a) and dead (b) *W. carteri* in Stage 2 survey areas from the boat ramp to the Busselton Bypass.

Population structure of *W. carteri*

The two most downstream survey areas had comparable length-frequency distributions, with the strongest size cohorts in both areas being 65-75 mm (Figure 4a) and similar mean mussel length (68-69 mm). This is similar to the length-frequency distribution previously found in this part of the river and these individuals were likely to be approximately 15-20 years of age (Klunzinger et al. 2014; Beatty et al., 2017, Beatty et al., 2019).

Upstream of the boat ramp, mussels were generally larger, with most ranging from 70-90mm (Figure 3e, Figure 5). Analysis of variance found the difference in shell length between survey areas was significant overall ($p < 0.001$), with post hoc tests revealing that mussels in all areas upstream of the boat ramp were significantly larger than downstream ($p < 0.05$), while the two downstream survey areas did not differ significantly from one another ($p = 0.99$).

There was a lack of small mussels in the vicinity of Strelly St (Figure 4c), with all mussels greater than 65mm. Between the boat ramp and Strelly St, mean size of live and dead mussels were the same (78mm, Figure 5a and b). Between Strelly St and Fairlawn Rd, the small number of remaining live mussels had a much narrower size range (73 - 85mm) than dead mussels (57-96mm), though means were similar (77-80) (Figure 4 c and d). This section had the most very large mussels, with 16 dead individuals greater than 90mm.

The section from Fairlawn Road to the Bypass had the largest number of small mussels, and all those in this section less than 55mm were live (Figure 5e). This resulted in mean size being smaller than for dead mussels (live 78 mm, dead 82 mm $p<0.001$). The effect was small and the overall length frequency distributions were similar (Figure 5e, f), however it is notable that all smaller mussels survived in this section.

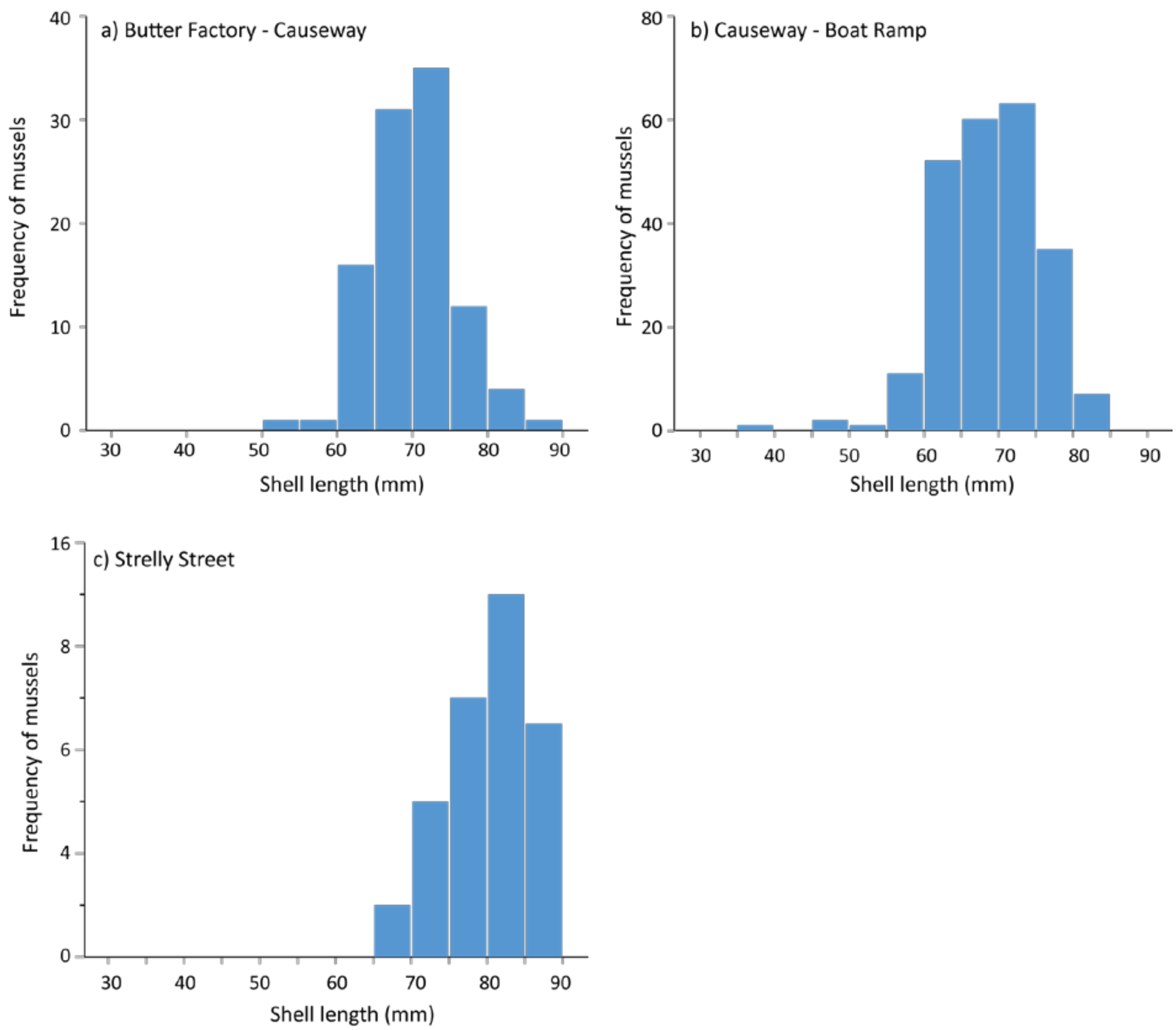


Figure 4. Length-frequency distribution of *W. carteri* from bank habitats in Stage 1 survey areas.

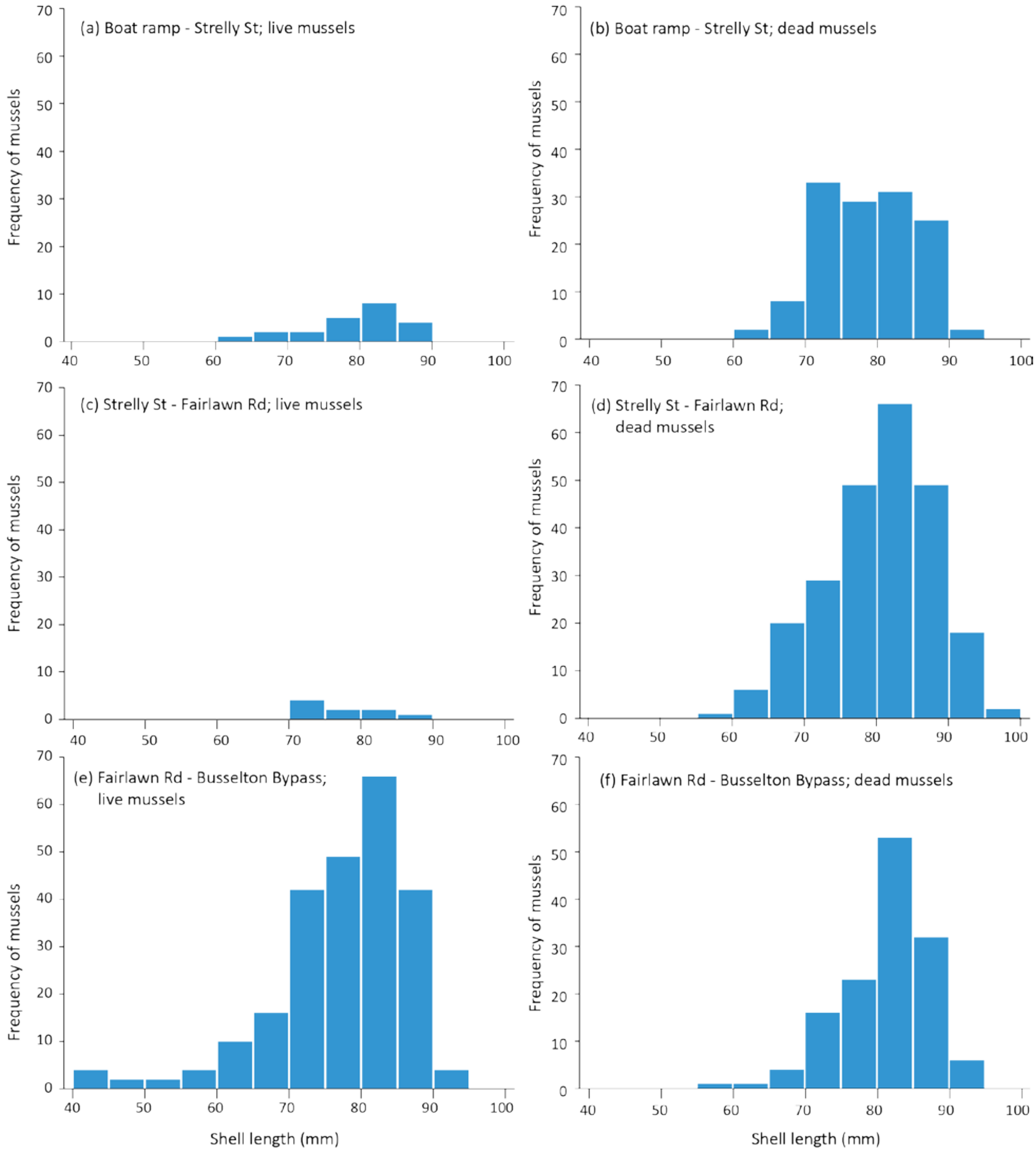


Figure 5. Length-frequency distribution of *W. carteri* from bank habitats in Stage 2 survey areas.

Impact of saltwater incursion

The lower Vasse River is a freshwater ecosystem, with salinity levels typically ranging from around 0.25ppt in winter to 1.8 ppt in early autumn (Department of Water and Environmental Regulation (DWER), 2019). Its hydrology has been markedly altered, including the establishment of surge barrier at the exit channel of the Vasse Estuary, which prevents tidal inflows. This barrier is actively managed, allowing tidal flushing during summer and autumn to improve water quality in the Vasse Estuary exit channel. This has not previously impacted the Vasse River as it has been hydrologically disconnected from the estuary at this that of year. In March 2021 salinity levels in the lower Vasse River increased to well-above normal levels due to saltwater incursion from the Vasse Estuary, owing to greater than expected connectivity between the two water bodies.

Increased salinity was first apparent at the old rail bridge site in early March, with salinity reaching 6.9ppt, and subsequently increased through April and May 2021 (Figure 6). Available data shows a peak on 6 April 2021 of 21.5 ppt at the rail bridge and 8.9ppt at Strelly St, with higher peaks on 27 April of 31.2 ppt and 14.0 ppt in bottom waters at these sites, respectively. This data suggests a tidal interaction and due to Gaps in measurements it is not clear what maximum salinity was reached and when. However, it is evident that salinity levels in the river reached excessively high levels during this period.

Transect data from throughout the survey area during the time of the Stage 2 survey shows more detail very high salinity in bottom waters between Strelly St and the Butter Factory, with decreasing salinity moving upstream and generally lower salinity in surface waters (Figure 7). In late April, salinity between the Butter Factory and Fairlawn Rd salinity was 3.1-5.7 ppt in surface waters and 4.4-36.9 ppt in bottom waters. Upstream of Fairlawn salinity was 0.8-2.0 ppt in surface waters and 1.6-3.7 ppt in bottom waters. By 11 May salinity levels had decreased substantially throughout the river to less than 2.0 ppt in all surface waters; less than 0.5 ppt in bottom waters upstream of Strelly St; and less than 4 ppt in bottom waters downstream of Strelly St.

The levels of salinity in the river during this time exceeded the tolerance levels of *W. carteri,* particularly downstream of Fairlawn Rd. Mussels are intolerant of elevated salinity, with increased salinity in freshwater ecosystems considered a key factor in the decline of the species (Klunzinger et al. 2015). Its distribution has contracted by 49% in less than 50 years mostly due to secondary salinization and is now confined to freshwater lentic and lotic systems with salinities <3 ppt (Klunzinger et al. 2015). It is assumed that the species has been lost from the Sabina River and the Buayanup River in the Geographe Bay catchment (Lymbery et al. 2008; Klunzinger et al. 2015). The tolerance of the species to salinity has been determined by Ma (2018 PhD Thesis, Murdoch University) with the LC_{50} values of two populations ranging between 5.87-5.96 gL^{-1}.

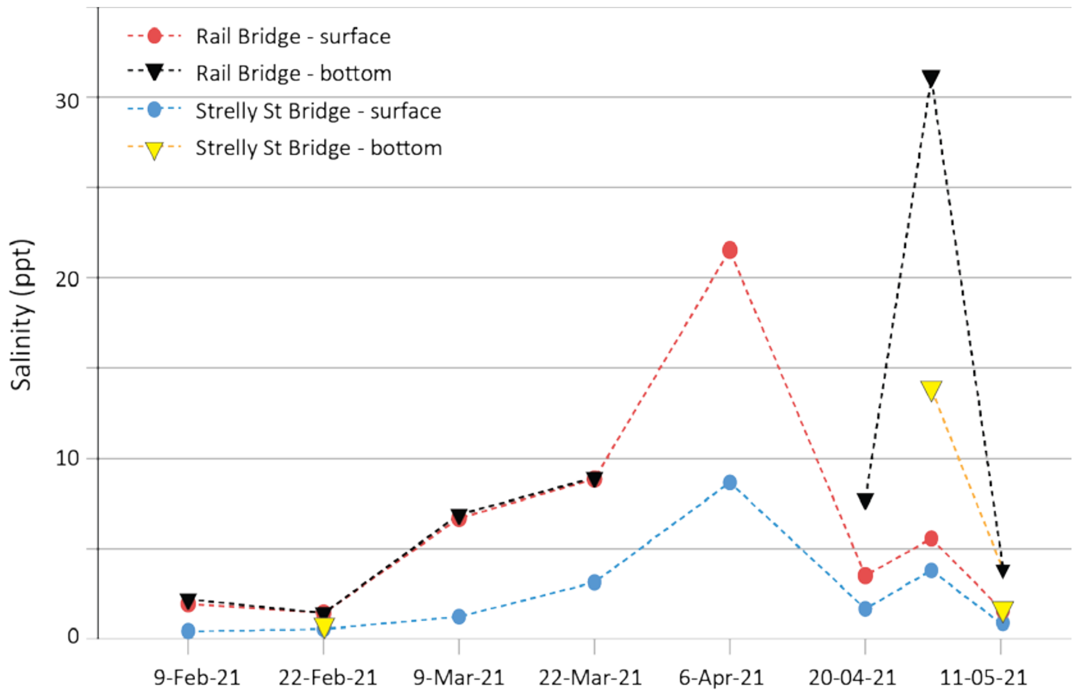


Figure 6. Salinity levels in the lower Vasse River from February to May 2021 (data from Department of Water and Environmental Regulation, 2021).

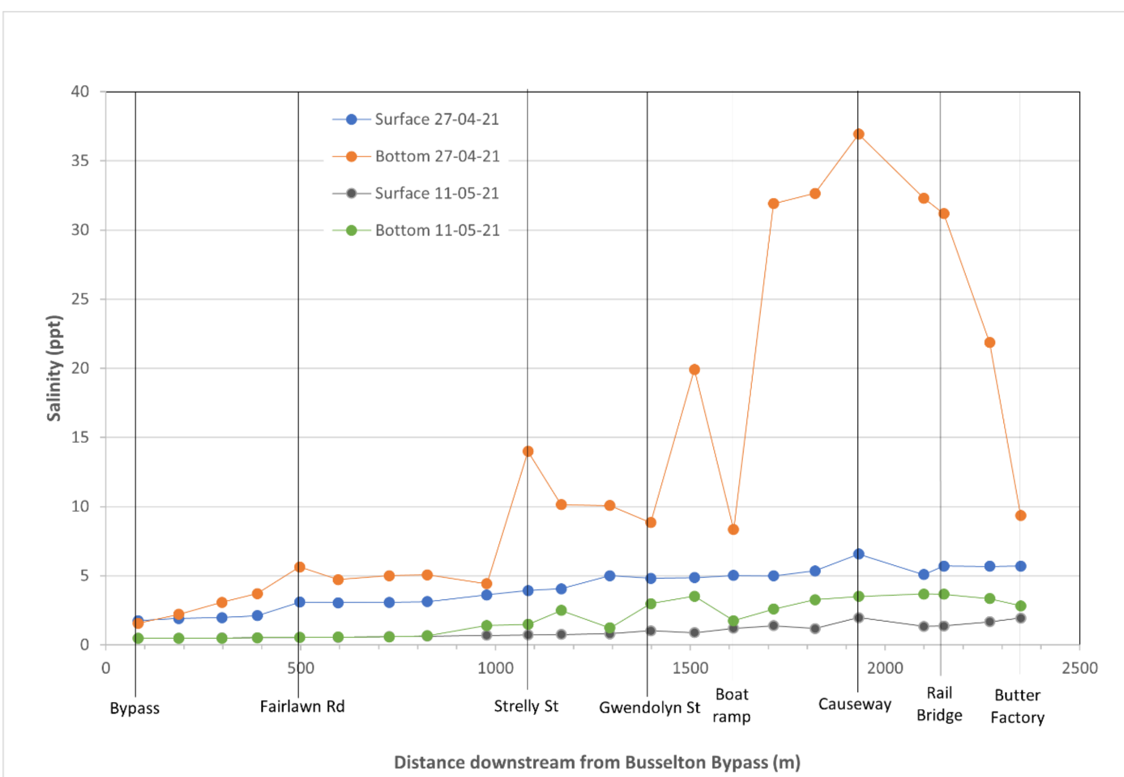


Figure 7. Salinity transect data throughout the lower Vasse river during the Stage 2 survey in April and May 2021 (data from Department of Water and Environmental Regulation, 2021).

Results of the Stage 2 survey indicate that this saltwater incursion has caused a mass mortality of *W. carteri* in the lower Vasse River, with 7408 mussels, representing 77% of this population, estimated to have died (Table 3). This impact has been most profound downstream of Fairlawn Rd, with an estimated mussel mortality of 94%, coinciding with the reach of river that experienced the most extreme salinity levels. Mortality was 100% downstream of Gwendolyn Street, while there has been 13% survival in bank habitat only between Gwendolyn and Fairlawn Road. No live mussels were found in off-bank habitat downstream of Fairlawn Rd. During the survey period, many mussels were observed floating in the river. Subsequent settling throughout the river would have occurred, therefore it is not clear whether mussels were previously distributed in off-bank habitats in these areas.

Upstream from Fairlawn Rd to the Busselton Bypass, mortality was much lower at 40% overall and live mussels were present in both bank and off-bank habitat, with 64% and 55% survival respectively. This section of river is shallower, and mussels are likely to occur throughout the riverbed. Dead mussels in this area may be a combination of those that have died in situ and those that have floated, drifted, and settled. Interestingly, this section had the greatest abundance of smaller mussels, and all mussels less than 55mm in this section were live. Of note is the presence of a concrete structure in the river at Fairlawn Rd, which may be an old river crossing site or weir. This creates a shallow area that may have limited the extent of saltwater incursion and thus contributed to the greater survival of mussels upstream of this point.

It is inferred that there has been 100% mortality of mussels in the Stage 1 survey area due to the higher salinity levels; the total mortality observed downstream of Gwendolyn St; and additional checks on mussel status in the vicinity of Strelly St bridge in the Eastern Link relocation area finding no live mussels. However, resurveying of the Stage 1 area would be required to confirm the extent of remaining live mussels here. Therefore, it is recommended that the section downstream of the boat ramp is re-surveyed, with a focus on sections of bank previously found to have the greatest mussel density.

Estimates of mortality rates assume that all mussels died due to the saltwater incursion and were not already dead prior to this. While partial shells were excluded from the survey to minimise the inclusion of previously dead mussels, there remains some uncertainty in the densities and abundances attributed to elevated salinity. Notwithstanding this uncertainty, it is the abundance of remaining live mussels in the river that is of most importance to future management.

Table 3. Summary estimates of mortality of *Westralunio carteri* resulting from the saltwater incursion event as determined by this survey.

Survey area	Dead mussel abundance	Live mussel abundance	Total mussel abundance	% Mortality	% Live
Strelly St – boat ramp	711	103	814	87%	13%
Fairlawn - Strelly	1623	43	1666	97%	3%
Bypass - Fairlawn	1328	2007	3334	40%	60%
Strelly Bridge	563	0	563	100%	0%
Boat ramp - Butter Factory	3183	0	3183	100%	0%
Total all areas	**7408**	**2153**	**9561**	**77%**	**23%**

Other water quality variables

Temperatures were moderate with little stratification present throughout the survey area and average values of in 23.1˚C in December 2020 and 22.6 ˚C April 2021. During both surveys, the highest temperatures occurred in the open waters between the Causeway and the Butter Factory and upstream of Strelly St. Dissolved oxygen in surface waters was at acceptable levels in December, however was low in bottom waters between the rail bridge and the boat ramp (Figure 8a). The lowest levels were in bottom waters downstream of the Causeway (15.9%), where the river is at its deepest. The survey was undertaken prior to the onset of the seasonal algal bloom, during which time oxygen levels and pH are markedly higher. Considerable oxygen stratification was evident throughout most of the river in April 2021, with surface waters above 100% saturation and bottom waters very low at most sites (Figure 8b). Low dissolved oxygen concentrations have been observed in the river previously and are a concern for mussel health. These low oxygen levels may be related to salinity stratification apparent in the river during this time, as the denser more saline waters do not mix with the more oxygenated surface waters. Conversely, higher algal productivity in surface waters would contribute to increased oxygenation and pH. During the Stage 1 survey, pH was consistent throughout the survey area and throughout the water column with an average value of 7.52 (Figure 9). In April 2021, pH was higher in surface waters (mean 7.8) than in bottom waters (mean 7.2), particularly downstream, where surface water pH exceeded 8.

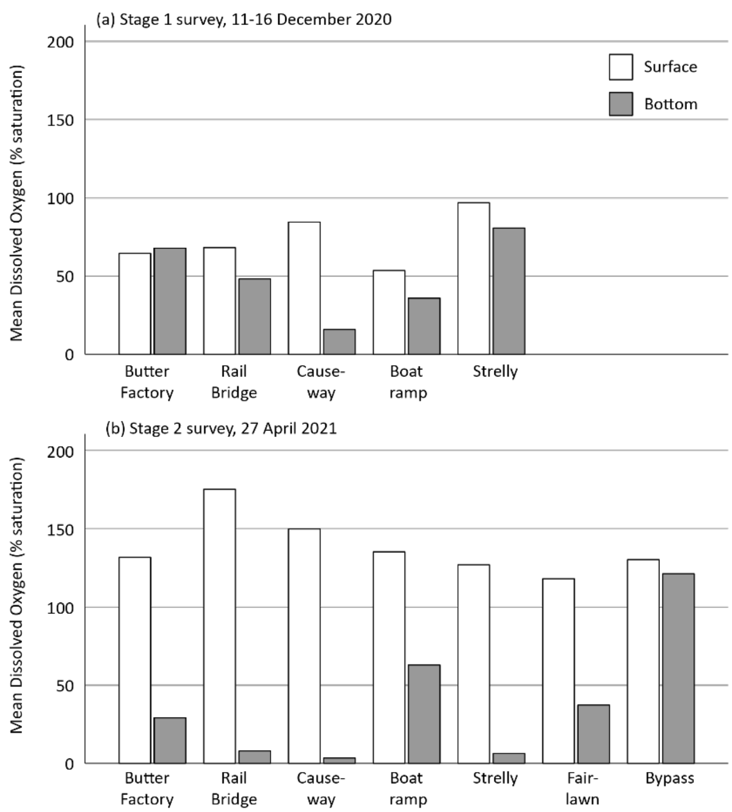


Figure 8. Dissolved oxygen concentrations in the river during the two survey periods.

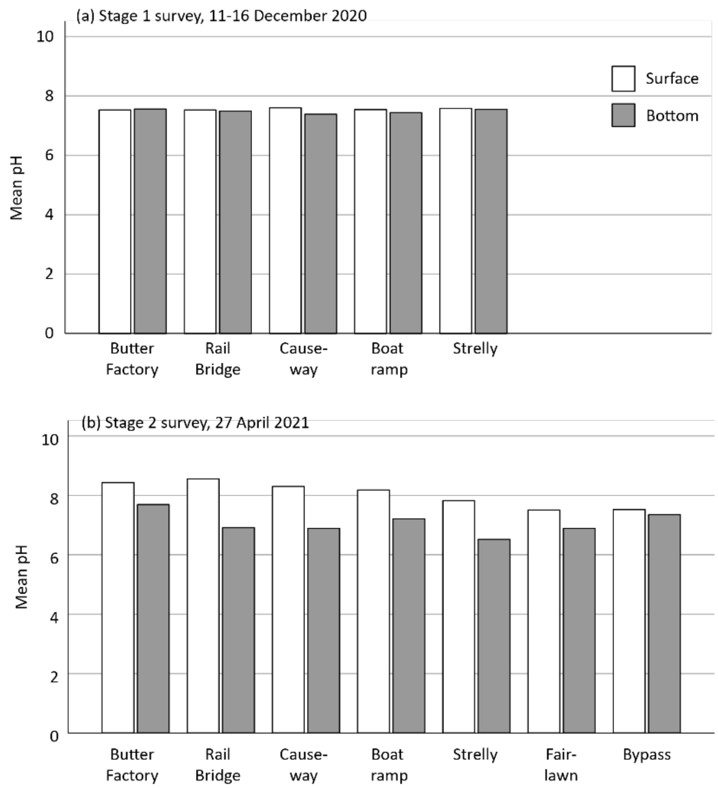


Figure 9. Levels of pH in the river during the two survey periods.

Implications and Recommendations

The City of Busselton is planning sediment removal from the Lower Vasse River, and the results of this survey will inform a management plan for management of *W. carteri* to minimise impacts from sediment disturbance and water quality impacts. The survey confirmed the presence of mussels throughout the river and determined density and abundance estimates, however the population has now been substantially impacted by a saltwater incursion event. This event has resulted in a high likelihood that no live mussels are present downstream of Gwendolyn St, although their presence downstream of the boat ramp would need to be confirmed by further survey. The Stage 1 survey also found no mussels upstream of Strelly St to the river bend.

To ensure no impacts on mussels, temporary relocation during the process according to an approved management plan is recommended. This approach was used successfully during construction of the Eastern Link bridge (Beatty et al., 2020). Low densities of live mussels remain in bank between Strelly St and Gwendolyn St and between the bend upstream of Strelly St and Fairlawn Rd, with an estimated total abundance of 146 mussels in this area. Upstream of Fairlawn Rd to the Busselton Bypass, higher live mussel densities occur and estimated abundance is 2007 mussels. This number of mussels, although higher than encountered during the Eastern Link program (around 180 mussels: Beatty et al., 2020) is considered manageable for a similar approach. Previous management of has focused on bank habitats, due to this being the primary habitat, and the 1230 mussels could be found similarly. However, the shallower waters in this area support an estimated 777 mussels in off-bank habitat. These mussels are dispersed throughout the river bed, and finding them over an area of over 3000m^2 in order to relocate them would be logistically very difficult due to safety issues associated with soft sediments, hidden objects and poor water quality. Downstream of Strelly St, live mussels are unlikely to occur in off-bank habitats.

Potential impacts of sediment removal on mussels include physical disturbance, reduced water quality and smothering by resettling of suspended particles. The sediments in the Lower Vasse River have high nutrient concentrations and are sulfidic (CoB, 2019), and disturbance may increase nutrient concentrations and turbidity and pose a risk of acidification when oxygenated in water or air. The suspension of sediments with high organic content may deplete oxygen in the water column. Although previous sediment investigations have not found monosulfidic black ooze (MBO) (CoB, 2019), there is uncertainty as to the risk of deoxygenation and acidification associated with disturbance.

Despite the risks involved in sediment removal, it is acknowledged that this approach has merit in terms of outcomes for ecological health of the Lower Vasse River. In addition to contributing to nutrient enrichment problems, the sediments provide a hostile benthic habitat for aquatic fauna and flora. However, while the sediment structure may be improved, there may be no new mussel habitat created due to increased depth. Increased depth in the river may also result in additional stratification, which can result in lower oxygen levels in bottom waters.

Although updated surveying of live mussel abundance downstream of the boat ramp is needed, the likelihood that no live mussels remain in this downstream area creates an opportunity to remove sediments with minimal impact on the population. A similar situation exists upstream of Strelly St bridge to the bend in the river, where the survey found no mussels. The absence of mussels and the shallow conditions present here may present an opportunity for sediment removal to contribute to restoration of mussel habitat by improving the benthos and increasing the period of bank inundation.

References

Beatty S., Paice R., Cottingham A., Hastings K., Lymbery A. (2020). Status of relocated *Westralunio carteri*: Eastern Link project Progress report. Report to City of Busselton. Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, Ottelia Ecology.

Beatty, S., Lymbery, A. (2019). Busselton Eastern Link Project: Carter's Freshwater Mussel *Westralunio carteri* Environmental Management Plan. Report to Strategen. Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University.

Beatty, S., Ma, L., Lymbery, A. (2017). *Baseline assessment of Carter's Freshwater Mussel, Westralunio carteri, at proposed bridge construction sites on the lower Vasse River.* Report to Strategen Environmental. Freshwater Fish Group and Fish Health Unit, Centre for Fish and Fisheries Research, School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia.

Beatty, S., Ma, L., Watsham, J., Lymbery, A., (2019). Proposed Lower Vasse River Sediment Removal Trial: Assessment of potential impacts on Carter's Freshwater Mussel. Report to City of Busselton. Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University.

City of Busselton (2019) Lower Vasse River Waterway Management Plan. City of Busselton, Western Australia.

Department of Water and Environmental Regulation (DWER) (2019). Routine monitoring data 2006-2019 extracted from Water Information Reporting online tool. https://www.water.wa.gov.au/maps-and-data/monitoring/water-information-reporting.

DWER (2021). Routine monitoring data 2021 extracted from Water Information Reporting online tool. https://www.water.wa.gov.au/maps-and-data/monitoring/water-information-reporting.

Klunzinger, M. K., Beatty, S. J., Morgan, D. L., & Lymbery, A. J. (2012a). Distribution of *Westralunio carteri* Iredale, 1934 (Bivalvia: Unionoida: Hyriidae) on the south coast of south-western Australia, including new records of the species. *Journal of the Royal Society of Western Australia*, 95, 77-81.

Klunzinger, M. W., Beatty, S. J., Morgan, D. L., Lymbery, A. J., & Haag, W. R. (2014). Age and growth in the Australian freshwater mussel, *Westralunio carteri*, with an evaluation of the fluorochrome calcein for validating the assumption of annulus formation. *Freshwater Science*, 33(4), 1127-1135.

Klunzinger, M. W., Beatty, S. J., Morgan, D. L., Pinder, A. M., & Lymbery, A. J. (2015). Range decline and conservation status of *Westralunio carteri* Iredale, 1934 (Bivalvia : Hyriidae) from south-western Australia. *Australian Journal of Zoology*, 63, 127-135.

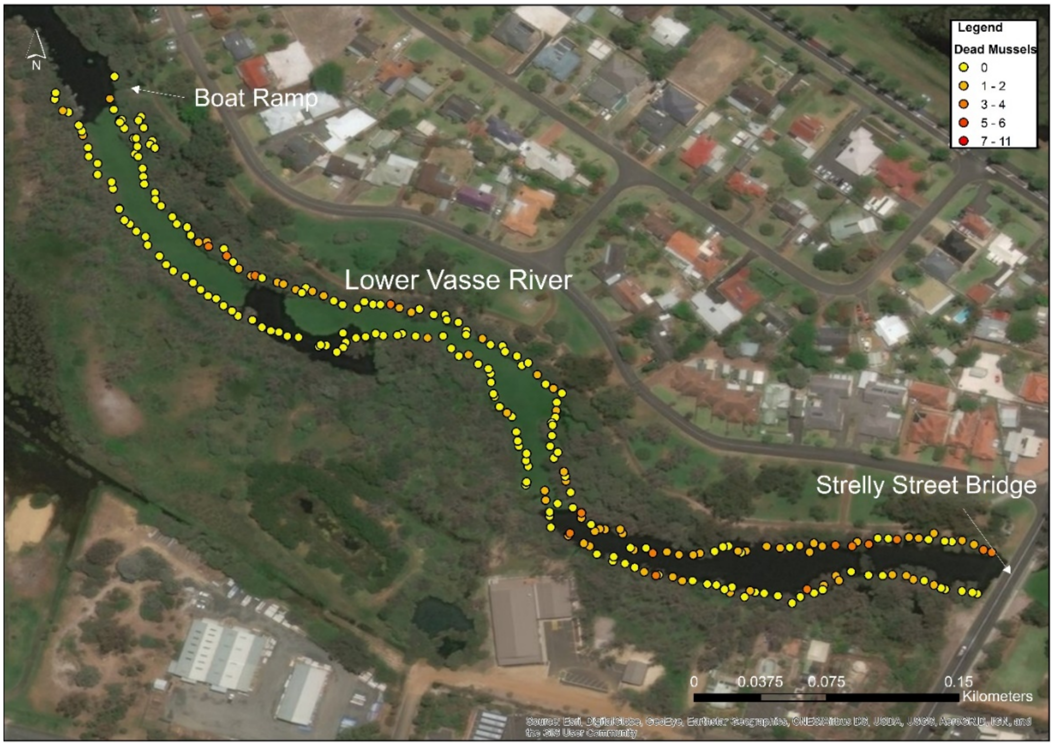
Klunzinger, M. W., Morgan, D. L., Beatty, S. J., Thomson, G. J., & Lymbery, A. J. (2012b). Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for an endemic freshwater mussel of south-western Australia. *Australian Journal of Zoology* 60, 26-36.

Lymbery, A., Lymbery, R., Morgan, D., & Beatty, S. (2008). *Freshwater Mussels (Westralunio carteri) in the catchments of Geographe Bay, south-western Australia.* Prepared for the Water Corporation, Western Australia. Fish Health Unit. Murdoch, Western Australia.

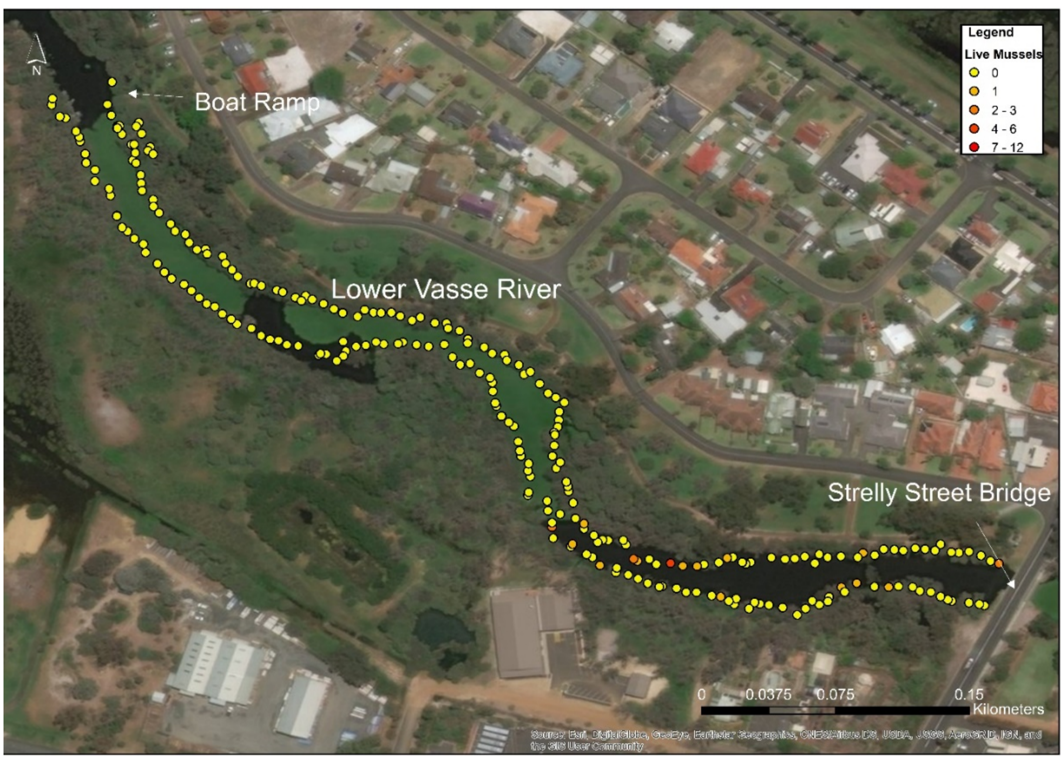
Paice, R. (2018) Influence of Mexican waterlily (*Nymphaea mexicana*) on ecology and water quality in the lower Vasse River, Busselton. Report for the Department of Water and Environmental Regulation. Ottelia Ecology.

Appendix 1. Location of quadrats showing mussel density in each quadrat.

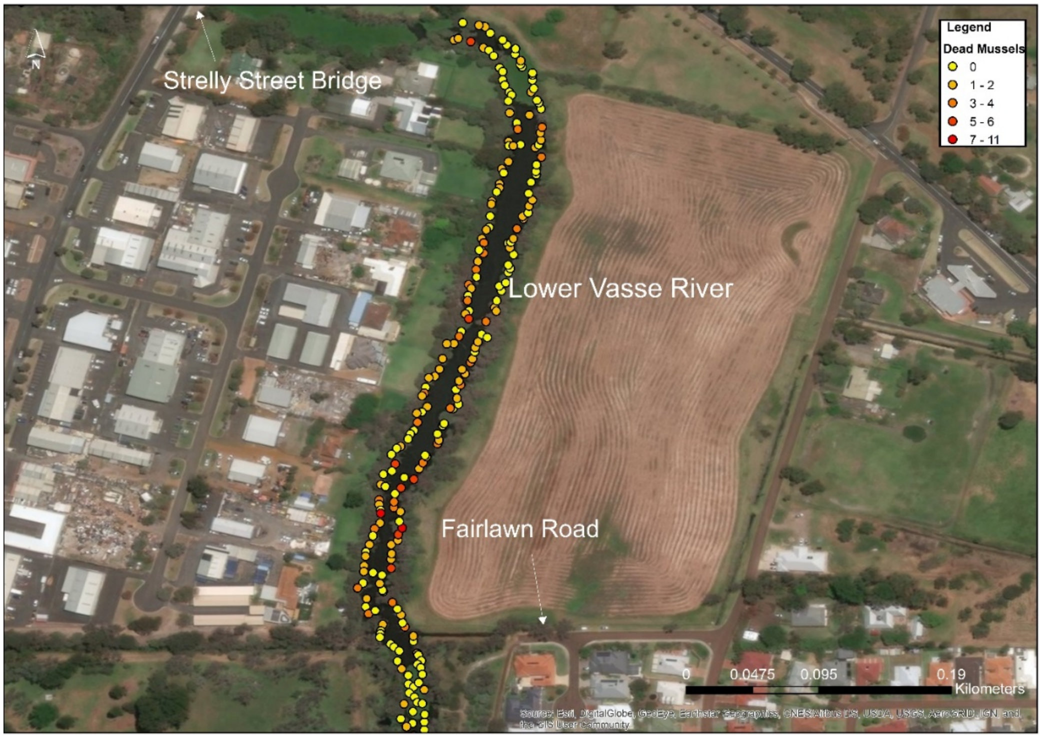
Location of the 1-m^2 quadrats surveyed for *W. carteri* in the survey area downstream of the boat ramp and live mussel density found in December 2020. These mussels are likely to have since died due to saltwater incursion.



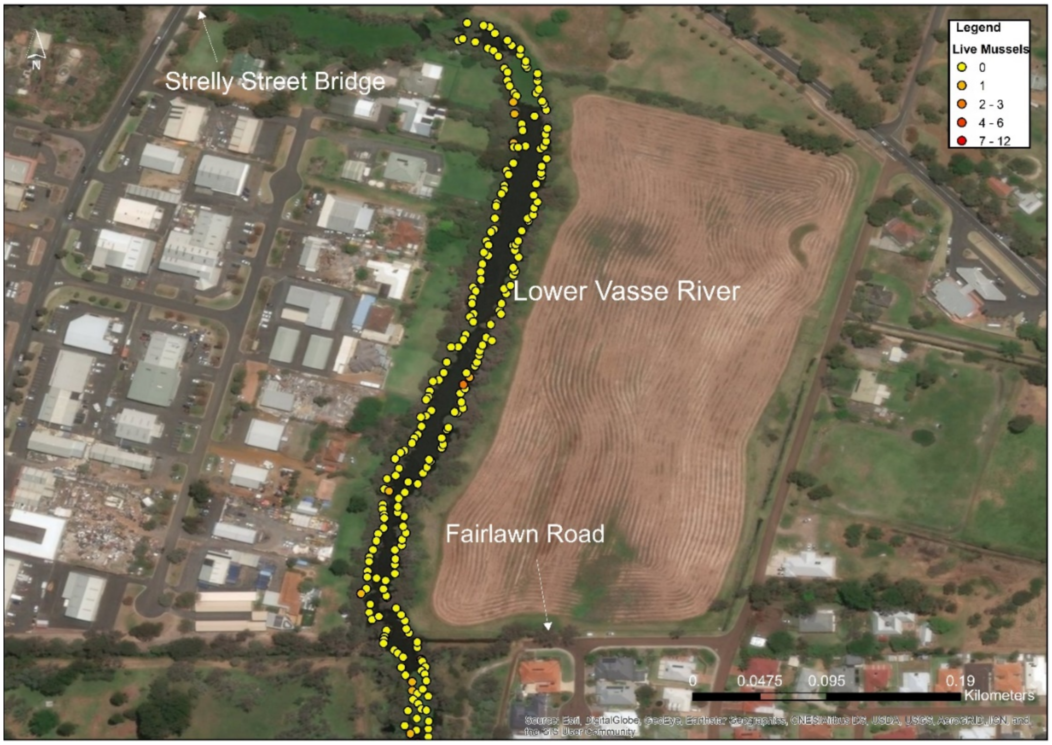
Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from the boat ramp to Strelly St and density of dead mussels found in April-May 2021.



Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from the boat ramp to Strelly St and density of live mussels found in April-May 2021.

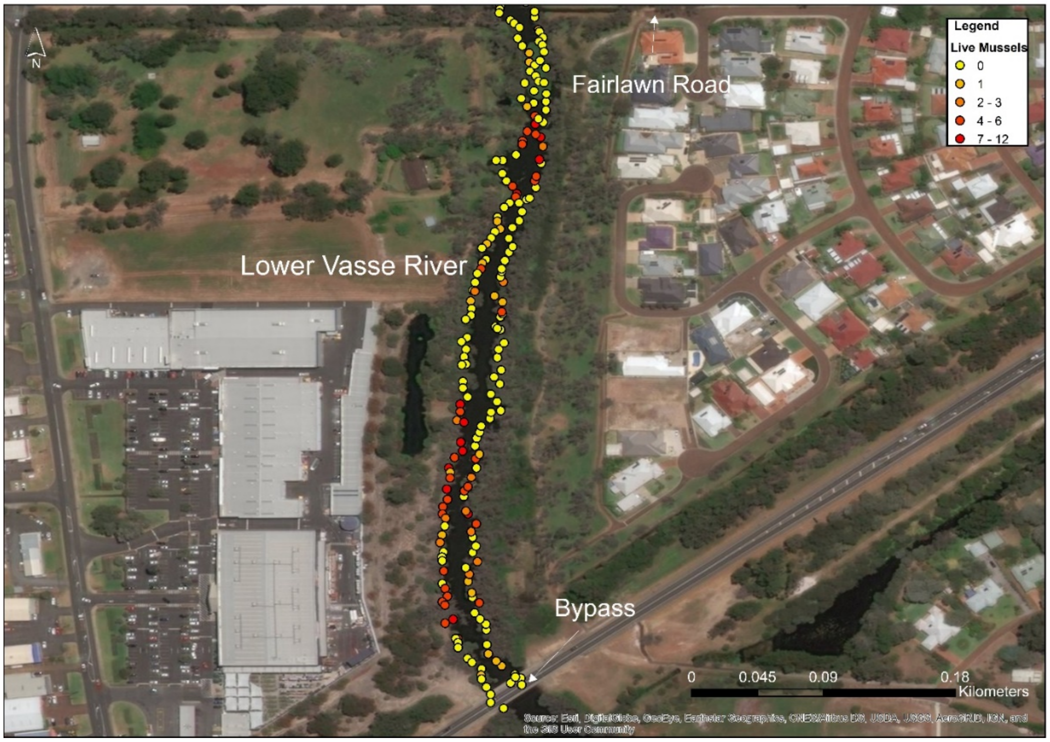


Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from upstream of Strelly St to Fairlawn Rd and density of dead mussels found in April-May 2021.



Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from upstream of Strelly St to Fairlawn Rd and density of live mussels found in April-May 2021.

Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from Fairlawn Rd to Busselton Bypass and density of dead mussels found in April-May 2021.



Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from Fairlawn Rd to Busselton Bypass and density of live mussels found in April-May 2021.