August 2021

Sediment removal in the lower Vasse River: Environmental Management Plan for Carter's Freshwater Mussel *Westralunio carteri*

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:
Stephen Beatty, Alan Cottingham, Alan Lymbery
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
s.beatty@murdoch.edu.au

Robyn Paice
Ottelia Ecology
robyn@otteliaecology.com.au

Distribution List:

Organisation	Contact Name	Date
City of Busselton	Mathilde Breton	27/01/2021
City of Busselton	Mathilde Breton	8/02/2021
City of Busselton	Mathilde Breton	10/06/2021
City of Busselton	Mathilde Breton	05/07/2021
City of Busselton	Mathilde Breton	10/08/2021

Document Control:

Document Status	Prepared By	Reviewed by	Date
Draft report V1	Stephen Beatty, Robyn Paice	Alan Lymbery	26/01/2021
Draft report V2	Stephen Beatty, Robyn Paice	Mathilde Breton	02/02/2021
Draft report V3	Stephen Beatty, Robyn Paice	Mathilde Breton Alysia Woodward	09/06/2021
Draft Report V4	Robyn Paice, Stephen Beatty	Mathilde Breton Alysia Woodward	05/07/2021
Final Report	Robyn Paice, Stephen Beatty		10/08/2021

Executive Summary

As part of the implementation of the Lower Vasse River Waterway Management Plan (City of Busselton, 2019), the City of Busselton proposes to remove sediment from an ~750 m reach of the Lower Vasse River in Busselton, Western Australia (WA) (hereafter primary Sediment Removal Site (‘SRS’)). An additional ~1700 m of river upstream may also be targeted for sediment removal in the future.

The Lower Vasse River is known to contain populations of Carter’s Freshwater Mussel (*Westralunio carteri*), which is listed as a threatened species under Commonwealth and WA legislation. As the sediment removal program (hereafter referred to as ‘SRP’) has a high likelihood of negatively impacting the species, a baseline survey was required to assess the distribution and abundance of the species throughout the lower Vasse River to underpin the development of a management plan for the species.

A survey completed in two stages in December 2020 (primary SRS) and May 2021 (upstream to Bypass) found large numbers of *W. carteri,* however the project area experienced an unusual saltwater incursion event just prior to the May survey that has resulted in severe mortality. The May survey and additional targeted survey in the primary SRS in June has confirmed 100% mortality of *W. carteri* downstream of Gwendolyn St. This has removed the potential for impacts from sediment removal in the primary SRS, as no live mussels currently occur. Notwithstanding this, the remaining mussel population upstream of Gwendolyn St requires ongoing protection, as guided by this *W. carteri* management plan (WCMP). In addition, the WCMP provides for monitoring of water quality in primary and future SRSs to ensure conditions are suitable for re-establishment and relocation of *W. carteri*. The WCMP will inform a referral to the Department of Agriculture, Water and the Environment (DAWE).

The current WCMP first considers the known habitat requirements and environmental preferences and tolerances of the species, summarises the potential impacts of the sediment removal on the species, outlines the methods to be used during the relocation program, develops a monitoring program to effectively assess the health of the population during the relocation and reintroduction phases, conducts a risk analysis of the relocation program, develops triggers and respective corrective actions that will be taken, and develops environmental objectives and criteria to measure their achievement. Those environmental objectives and measurement criteria are presented in Table 1.

Table 1. Management objectives and criteria to measure achievement of WCMP

Management Objectives	Management Targets
Minimise the mortality of *W. carteri* at any sediment removal site (SRS) in the Lower Vasse River associated with the SRP	• Collect and remove all live *W. carteri* from the SRS including within a 40 m buffer zone upstream and downstream of the dredging footprint • Relocate all live *W. carteri* from the SRS to Taylor's Lake in Iluka's Capel wetlands. • House the relocated mussels in purpose-built cages for the duration of the sediment removal process
Minimise the mortality of relocated individuals during the SRP	• No more than 2% mortality of relocated *W. carteri* during the SRP
Avoid mortality of the host population of *W. carteri* at the Taylor's Lake relocation site	• No reduction in density of the existing host population of *W. carteri* recorded during the relocation period
Restore the population abundance of *W. carteri* in the sediment removal site following the SRP	• No adverse change in the density and population viability of *W. carteri* in the SRS following SRP

Contents

1 Context, scope and rationale

1.1 Location

For the purposes of defining areas for surveys, sediment removal and relocation, key landmarks are defined in Figure 1. The Project site includes the primary sediment removal site (SRS), being the stretch of the Vasse River from the Butter Factory Boards to the old Boat Ramp on Southern Drive; and future sediment removal areas throughout the lower Vasse River ~1700 m from the boat ramp to the Busselton Bypass. Mussel numbers and relocation requirements are separated into the following sections, with sections 1 and 2 forming the primary SRS:

1. Butter Factory to Causeway
2. Causeway to boat ramp
3. Boat ramp to Strelly St
4. Strelly St to bend 200m upstream
5. Upstream of Strelly St to Fairlawn Rd
6. Fairlawn Rd to Bypass

This *Westralunio carteri* Management Plan (WCMP) includes a proposed temporary relocation site (hereafter referred to as 'the relocation site') within Taylor's Lake, which is part of the Iluka (formerly RGC) Wetlands suite formed at the completion of mineral sand mining (-33.5893, 115.5133, Figure 2). The relocation site is located within State Forest 12 (Coolilup State Forest), within Iluka's mining lease (M 70/63) and is managed by Iluka Resources. Access is restricted and subject to induction through Iluka OHS processes.

1.2 Project description

The City of Busselton proposes to remove sediment from a ~750m section of the Lower Vasse River in Busselton, Western Australia (WA), defined as the primary sediment removal site. Future sediment removal may also be pursued in upstream sections, subject to ongoing planning by the City of Busselton. The sediment removal aims to improve water quality and amenity in the lower Vasse River and is part of the City's Lower Vasse River Waterway Management Plan (City of Busselton, 2019).

The current proposed process for sediment removal is to use a small dredge to pump soft sediment from the bed of the river directly into porous geotextile bags located adjacent to the river. The sediment is dosed with flocculant so that fine sediments are retained in the bag while water is expelled and returned to the river. The bags are then left to allow drying of the sediment prior to removal, reducing the volume of material for disposal.

The Lower Vasse River contains populations of Carter's Freshwater Mussel (*Westralunio carteri*), which is listed as Threatened under Western Australia's *Biodiversity Conservation Act 2016* (BC Act) and the Commonwealth *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act). Dredging has a high likelihood of negatively impacting the species in that section through physically removing individuals during the dredging phase, burying and smothering the mussels, and impacting water quality in particular the potential release of Monosulfidic Black Ooze. Therefore, a baseline survey was required to underpin the preparation of this WCMP in order to mitigate potential impacts to the species during the proposed sediment removal program.

This WCMP has been prepared to guide the temporary translocation program and support environmental approvals under Commonwealth and WA legislation.

1.3 Approvals framework

This WCMP will inform a referral to the Department of Agriculture, Water and the Environment (DAWE) under the EPBC Act pertaining to the proposed sediment removal project (SRP). The proposed SRP will also require a Licence under the WA *Biodiversity Conservation Regulations 2018* and this WCMP will address the approval requirements under those Regulations. The Regulations are administered by the WA Department of Biodiversity, Conservation and Attractions (DBCA).

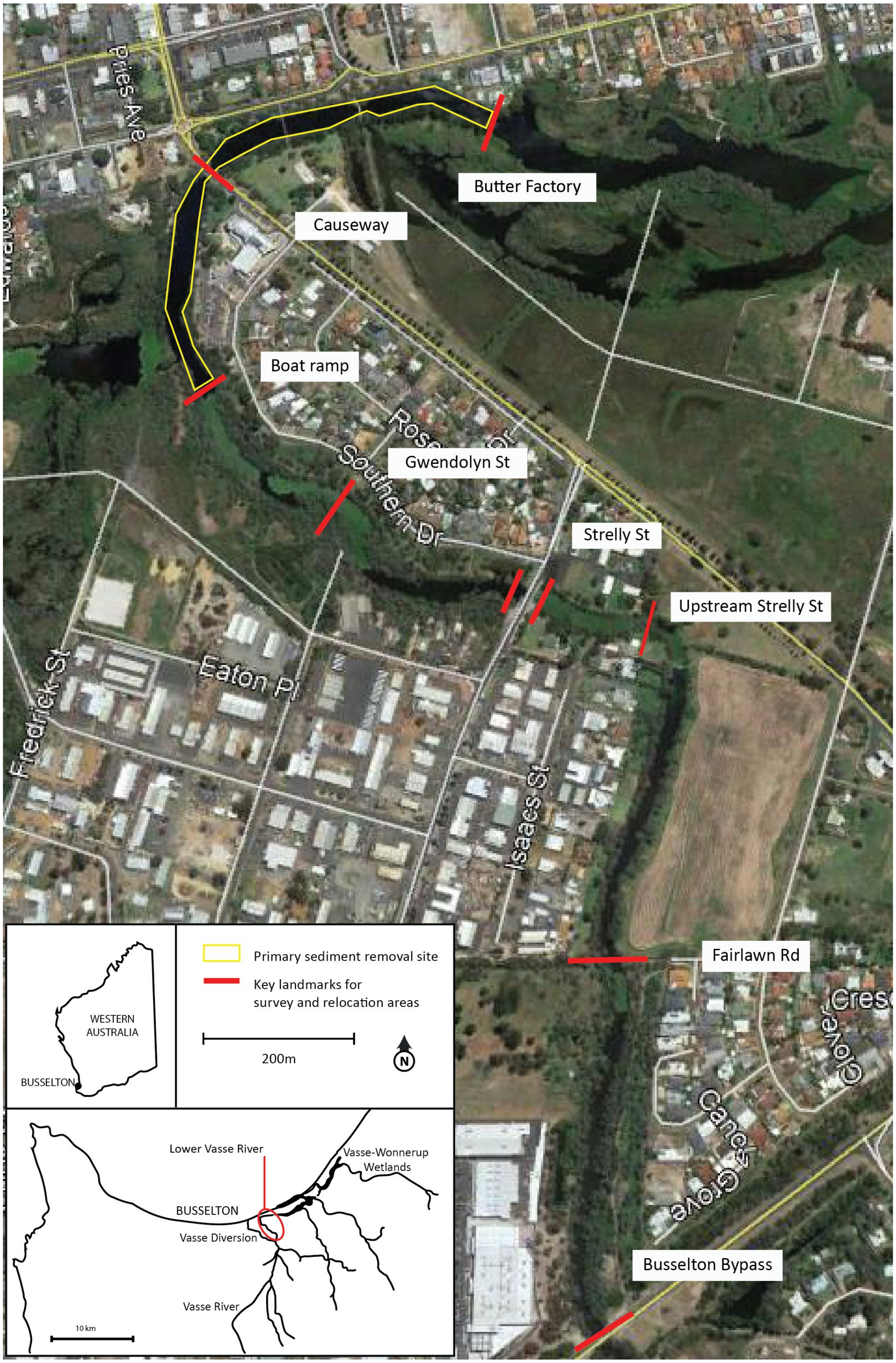

Figure 1: Lower Vasse River project area showing primary sediment removal site (SRS) and location of key landmarks used to describe survey sections and relocation areas.

Figure 2: Location of the proposed relocation site in Taylor's Lake in relation to Lower Vasse River (top left) and other lakes in the Iluka wetland system (top right).

1.4 Existing information on *W. carteri* in the lower Vasse River

The current work complements and builds on a previous survey that was undertaken in January 2019 (Beatty et al. 2019) for a proposed pilot sediment removal trial, which did not proceed. It is also consistent with the ongoing Eastern Link *Westralunio carteri* Environmental Management Plan (ELWCMP) (Beatty and Lymbery, 2019). The ELWCMP was required to manage the species as part of the construction of the Eastern Link bridge across the Lower Vasse River. The implementation of the ELWCMP successfully relocated, housed and reintroduced the *W. carteri* into a sub-section of the Lower Vasse River (LVR) within the 750m primary sediment removal site (Beatty et al., 2019a, 2020). That plan ensured that the abundance of the species within that section was maintained and that negligible mortalities occurred, as demonstrated by six months of post-relocation monitoring (Paice and Beatty, unpubl.).

1.4.1 Previous studies

The first documented records of *Westralunio carteri* in the lower Vasse River were from Lymbery et al. (2008) who found mean density of 2.5 ± 1.2 mussels/m^2 at their Causeway bridge site. As part of the preliminary assessment of the potential impact of the Eastern Link and Causeway Bridge construction on the species, a targeted survey was undertaken within a section of the current proposed sediment removal site (SRS) (Beatty et al., 2017). The latter survey revealed that W. carteri were present at a mean density of ~1.8 and 2.5 mussels/m^2 at the Eastern Link and Causeway Bridge sites, respectively. In June 2020, the mussels that were successfully removed from the LVR and captively housed in Iluka's Capel wetlands as part of the *W. carteri* Eastern Link project management plan (Beatty et al. 2019a, 2020), were reintroduced into the LVR around the newly constructed bridge (Figure 3). As part of a previously proposed sediment removal trial, Beatty et al. (2019) recorded mean mussel densities of 1.35 ± 0.44 mussels/m^2 and 0.85 ± 0.34 mussels.m^{-2} downstream of the Causeway, on the north and south banks respectively (also shown in Figure 3).

Overall, relative to other populations in the south-west, the density of the species in the LVR are lower (e.g. a mean density of 4.4 mussels/m^2 was recorded over 17 survey sites in the South West). The densities in the LVR are also less than those within the Vasse Diversion Drain (VDD). The VDD population was assessed upstream of Chapman Hill Rd within the footprint of the Water Corporation project to reconstruct the VDD levee banks by Lymbery et al. (2019). That survey recorded a density of 7.4 mussels.m^{-2} (±1.76 S.E.). Beatty and Lymbery (2020) also surveyed two reaches upstream of the levee bank reconstruction footprint and recorded mean densities of ~16 (±2.1 SE) and ~22 (±3.6) mussels/m^2 at the two reaches, respectively (Figure 3).

While some information existed on the density and distribution through the middle section of the primary sediment removal site, an additional survey was required to provide a more robust estimate of the distribution and abundance of the species throughout the river in order to guide the current WCMP.

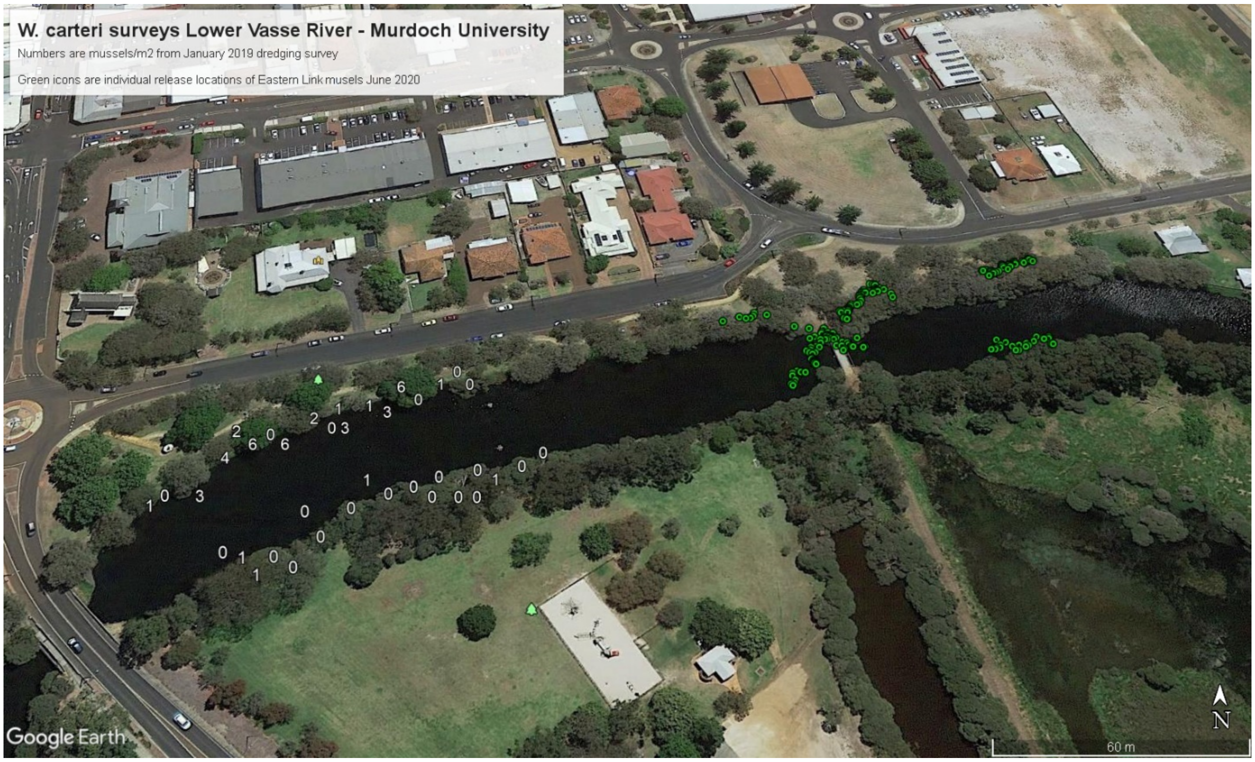

Figure 3: Previous distributional information of W. carteri within middle section of the proposed sediment removal site. N.B. The green dots are the relocated individuals in June 2019 and the numbers are quadrat totals are from the baseline survey prior to the pilot sediment removal trial that did not proceed (Beatty et al. 2019).

1.4.2 Additional surveys to inform this Management Plan

A baseline survey was conducted in two stages in December 2020 and in April-May 2021 and is attached as Appendix 1. The survey in December 2020 focused on the section of river of highest priority for sediment removal (primary SRS), being the ~750m reach between the Butter Factory and the old boat ramp on Southern Drive; and additionally included areas in the vicinity and upstream of Strelly St bridge. Following this, the City extended the area for potential future sediment removal throughout the lower Vasse River ~1700m from the boat ramp to the Busselton Bypass (Figure 1). As a result, a further survey was conducted in

April-May 2021 to inform requirements for *W. carteri* management in this broader area.

Immediately prior to the second stage of the survey, a saltwater incursion event from the Vasse Estuary resulted in increased salinity levels in the river in excess of tolerable limits of *W. carteri* (Klunzinger et al., 2015). This event has severely impacted the population, likely resulting in 100% mortality within the initial survey area. Live mussels are now restricted to small numbers between Gwendolyn St, upstream of the boat ramp, and Fairlawn Rd, and higher abundance retained upstream of Fairlawn Rd. It is critical that remaining live mussels are protected to ensure the long-term conservation of this species in the lower Vasse River.

The initial survey in the primary SRS revealed hotspots of distribution were located upstream of the causeway on the eastern (right) bank, with the western (left) bank also housing relatively high abundances (Appendix 1). The section between the Butter Factory and Causeway housed fewer mussels with the majority on the north (left) bank (Appendix 1). Overall, the estimated number of mussels needing managing within the primary SRS was 3183 ± 309 (Appendix 1,

Table 2). This assumed distribution was limited to within 2 m of each wetted bank margin, with no mussels found in the off-bank grab samples (n=150) that were conducted in the baseline survey. This finding and assumption was also consistent with previous surveys in river habitats including this site by Beatty et al. (2019) and also its known habitat preferences (Klunzinger et al., 2012a).

The initial survey also found mussels around and downstream of the Strelly Street Bridge, but no live mussels in the ~150 m reach upstream of the bridge (Appendix 1, Table 2). It is estimated the number of mussels in the Strelly Street section (i.e. limited to the ~50 m around the bridge) was ~563 ± 278. The large degree of variability in the latter estimate was due to high level of patchiness of density.

Due to the mortality found in the stage 2 of the survey, further targeted survey in June 2021 was undertaken to assess the status of *W. carteri* in the stage 1 area. Intensive searching of the Eastern Link relocation area (left bank) and Strelly St bridge were also completed in April in response to advice regarding salinity levels. This work has confirmed 100% mortality of mussels in the primary SRS and in the vicinity of Strelly St.

The second stage of the survey, following the saltwater incursion event, included three sections: from the boat ramp to Strelly St (~580m); from ~150m upstream of Strelly St to Fairlawn Rd (~475m); and from Fairlawn Rd to the Busselton Bypass (~480m). Total mussel density downstream of Fairlawn Rd was very low and a high percentage of mussels were deceased. Between the boat ramp and Strelly St, only ~103 ± 26 live mussels are estimated to remain, representing 13% of the original abundance, all located upstream of Gwendolyn St. From upstream of Strelly St to Fairlawn Rd, only ~43 ± 16 live mussels occur, representing 4% of the original abundance.

The saltwater incursion had less impact upstream of Fairlawn Rd, with 60% survival in this area due to lower salinity levels. This section of the river is shallower than downstream and provides habitat for *W. carteri* throughout the riverbed. Live mussels were found at low densities of 1.25 ± 0.16 and 0.24 ± 0.06 mussels per m^2 in bank and off-bank habitat respectively. Estimated live mussel abundance in this section was 2007 (1230 ±155 in bank habitat; 777 ± 187 in off-bank habitat).

Table 2: Summary of results from surveys in the Lower Vasse River in Stage 1 (December 2020) and Stage 2 (April-May 2021) from Paice and Beatty (2021) (Appendix 1).

	Live Mussels		**Dead Mussels**	
	Mean Density (per m^2)	Abundance (±SE)	Mean Density (per m^2)	Abundance (±SE)
Stage 1 Survey (Bank habitat only)				
Butter Factory to Causeway (420m):	0.59 ± 0.10	1004 ± 166	0.02 ± 0.01	16 ± 8
Causeway to boat ramp (330m):	1.73 ± 0.19	2112 ± 237	0.40 ± 0.07	244 ± 43
Strelly St bridge vicinity (50m)	5.6 ± 2.78	563 ± 278	0	0
Upstream Strelly St bridge (200m)	1.03 ± 0.12	0	0	0
Stage 2 Survey Bank habitat				
Boat ramp – Gwendolyn St (560m)	0.00	0	0.27 ± 0.07	153 ± 38
Gwendolyn St – Strelly St (670m)	0.15 ± 0.04	103 ± 26	0.69 ± 0.08	459 ± 55
Strelly St – Fairlawn Rd (955m)	0.05 ± 0.02	43 ± 16	1.20 ± 0.12	1146 ± 111
Fairlawn Rd – Busselton Bypass (985m)	1.25 ± 0.16	1230 ±155	0.70 ± 0.09	694 ± 92
Off-bank habitat				
Boat ramp - Strelly St (5800m^2)	0	0	0.05 ± 0.02	99 ± 47
Strelly St - Fairlawn Rd (4750m^2)	0	0	0.11 ± 0.02	477 ± 97
Fairlawn Rd - Busselton Bypass (4800m^2)	0.24 ± 0.06	777 ± 187	0.19 ± 0.05	634 ± 161
Totals in key areas[1]:				
Total primary SRS		0		3451 ± 359[2]
Total boat ramp – Strelly St		103 ± 26		711
Total Vicinity Strelly St		0		563 ± 278[2]
Total Strelly St – Fairlawn Rd		43 ± 16		1623
Total Fairlawn Rd - Bypass		2007		1328

[1]Standard error not provided where number is total of bank and off-bank habitats due to different methods of estimation.
[2]Mussels recorded as live in Stage 1 survey included in dead mussel abundance due to saltwater incursion.

2 Potential impacts and objectives of the Management Plan

2.1 Habitat requirements and environmental preferences of *W. carteri*

The distribution of *Westralunio carteri* has contracted by 49% in less than 50 years mostly due to secondary salinisation 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 also 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}. The intolerance of *W. carteri* to elevated salinity has recently been demonstrated in the Lower Vasse River with the mass mortality of more than 7,000 individuals due to an incursion of saltwater into the river from the Vasse Estuary, when salinity exceeded these LC50 values for an extended period (Figure 4). A detailed account of this event is provided in the Survey Report (Appendix 1).

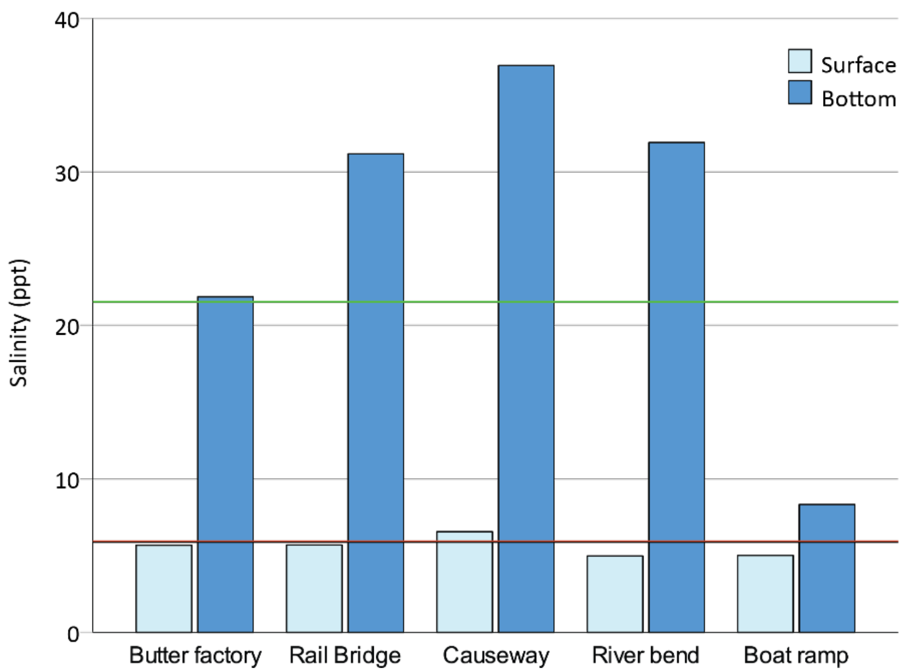

Figure 4: Salinity levels in the primary sediment removal site on 27 April 2021. The red line is the LC_{50} and the green line shows surface salinity at the Rail bridge on 6th April 2021.

As with all freshwater mussels, *W. carteri* are filter feeders and likely play an important role in helping to maintain water quality. For example, a study in the upper Mississippi River estimated that freshwater mussels can filter ~14 billion gallons (53 billion L) per day removing tonnes of contaminants including phytoplankton (Newell 2004). Ongoing research is being undertaken on the filtration capacity of *W. carteri* but initial estimates are that adult mussels can filter more than 2 L of water per day (Lymbery et al., unpubl. data).

Along with salinity, *W. carteri* is likely to be intolerant of prolonged periods of anoxia and elevated temperatures such has been found in other species (Chen et al., 2001). Sedimentation (causing smothering) is also likely to negatively impact *W. carteri* (Klunzinger et al., 2015). Sediments in the lower Vasse River are known to be sulfidic with potential for acidification on exposure to oxygen, including oxygenated water (CoB, 2019). Monosulfidic black ooze (MBO) was also thought to be possibly present. MBO is an organic ooze enriched by iron monosulfides and its disturbance may result in rapid deoxygenation and acidification, producing toxic metal sulfides (Sheldon & Walker, 1989; Bush et al. 2004). Freshwater mussels elsewhere are known to be sensitive to such contaminants (Wang et al., 2013). These sediments also pose a risk to the mussels in-situ, by causing reduced oxygen levels and releasing nutrients into the water column. However, sediment sampling undertaken in March 2018 between the Butter Factory and the old Boat Ramp found the sediment to be sulfidic with very high potential acidity but not monosulfidic.

While the environmental tolerances of *W. carteri* has only properly been assessed for salinity, the ongoing Eastern Link *Westralunio carteri* Environmental Management Plan (ELWCMP) that housed and closely monitored *W. carteri* in Taylor's Lake has recently added valuable information on its field tolerance to temperature and dissolved oxygen, and the efficacy of relocating and housing the species over prolonged periods in cages (Beatty and Lymbery 2019, Beatty et al. 2019a, 2020). By constantly monitoring temperature, oxygen and mussel behavior using valvometers within purpose-built cages (Figure 5, Appendix 2), the project revealed that the species could tolerate temperatures up to at least 32°C without adverse effects on behavior or survival (Figure 6). This information was used to formally amend the management trigger values within the ELWCMP relating to temperature, dissolved oxygen and behaviour. There was also negligible mortality that occurred in the captively held animals in Taylor's Lake. Together, that information underpins the current monitoring program for the relocated mussels from the proposed sediment removal site in the lower Vasse River.

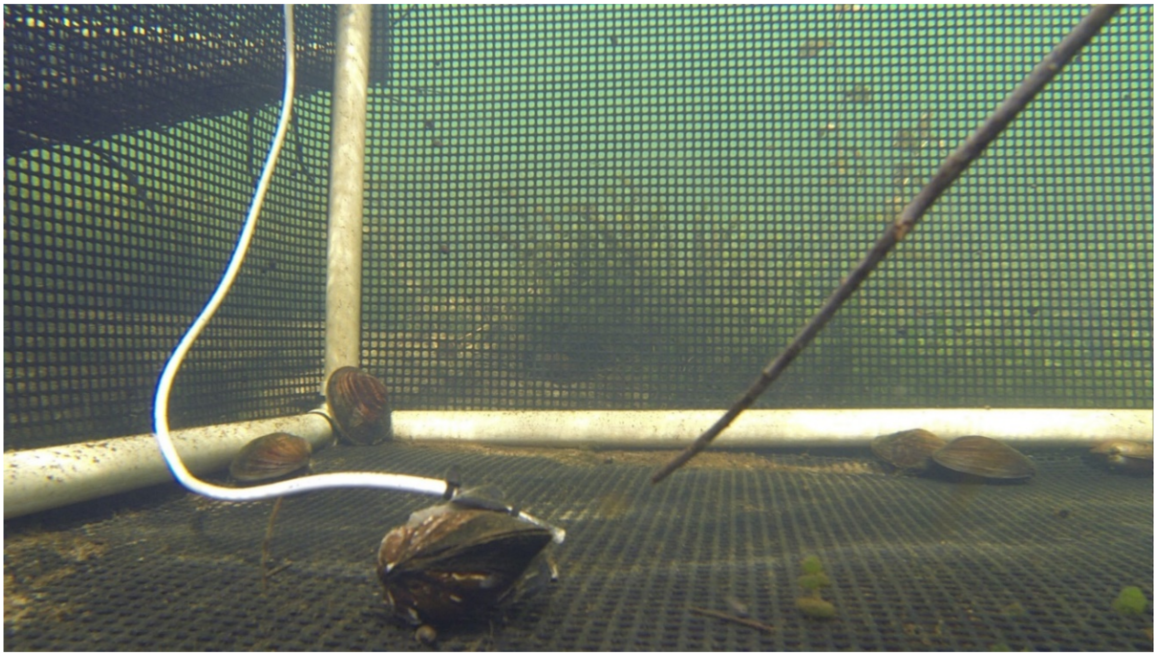

Figure 5: Example of a caged sentinel mussel with a valvometer attached in Taylor's Lake.

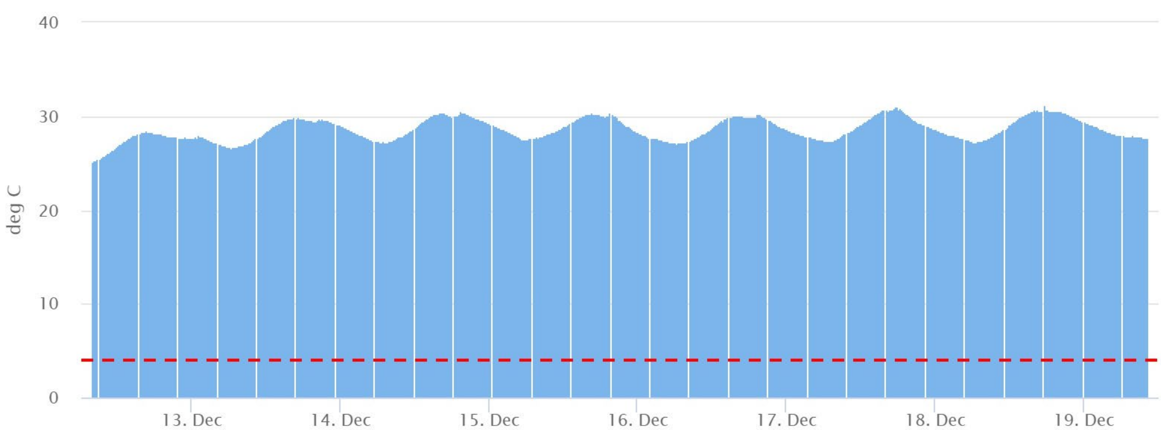

Figure 6: Bottom temperature at the ELWCMP *W. carteri* relocation site in Taylor's Lake, in Iluka's Capel Wetlands during a heatwave in December 2019. N.B. this demonstrated the species could repeatedly tolerate temperatures >30°C and was used to refine management trigger values in that management plan. Red line is not relevant to temperature in this graph (i.e., it is the overlaid dissolved oxygen trigger).

2.2 Potential impacts of the Project on *W. carteri*

The proposed sediment removal program in the lower Vasse River has the potential to impact *W. carteri* in the following ways.

- Direct mortality during dredging due to the physical removal of mussels and disposal of sediment spoils.
- Direct mortality during dredging by the smothering or burying of individuals.
- Indirect mortality of *W. carteri* within and outside of the SRS footprint associated with a decline in water quality, specifically increases in turbidity and disturbance of sulfidic sediments.

The current WCMP assesses the efficacy of the proposed relocation of *W. carteri* as a key management measure to achieve the Objectives (i.e. assesses the residual risk). It also identifies and addresses any potential risks associated with the proposed relocation.

Prior to the saltwater incursion event, large numbers of mussels were present, which would require 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. No live mussels remain in the primary SRS between the Butter Factory and the boat ramp; hence the potential of the SRP to impact mussels in this area is now limited to any long-term effect on potential recolonisation.

Upstream of the primary SRS in the two sections between the boat ramp and Fairlawn Rd, including the Strelly St bridge area, relatively small numbers of live mussels are present. It is considered that sediment removal in this section is feasible in terms of management of *W. carteri* to minimise impacts.

Upstream of Fairlawn Rd to the Busselton Bypass, large numbers of live *W. carteri* remain including 1230 ±155 in bank habitat and 777 ± 187 in off-bank habitat. While this number of mussels can be adequately managed through the temporary relocation approach in this WCMP, sediment removal in this section is not recommended until additional surveys are conducted to determine longer term population decline of the species throughout the reaches following the saltwater incursion for two key reasons:

- Fairlawn Rd to the Busselton Bypass now provides habitat for a substantial proportion of remaining *W. carteri* in the river and it is critical that this area is protected if the population in the lower Vasse River is to persist. Disturbance to this population may interrupt breeding which is essential to the re-establishment of mussels downstream.

- 40% of this population occurs in off-bank habitat, dispersed throughout the river bed at a density of less around one mussel per 4 m^2 over an area of over approximately 3,000m^2. While harvesting from bank habitats is feasible, finding individuals in off-bank habitat at such low density in order to relocate them would be logistically very difficult due to safety issues associated with soft sediments, hidden objects, low visibility and risk of harmful algal blooms.

2.3 Objectives of the *W. carteri* management plan

The primary objective of the current WCMP is to mitigate the potential negative impacts of sediment removal on the abundance and population viability of *W. carteri* in the lower Vasse River in respect of the primary SRS downstream of the boat ramp and potential sediment removal throughout the river upstream to Busselton Bypass. The approach to achieve this objective is to implement a temporary relocation program for sections of the river in alignment with planning of staged sediment removal. In addition, water quality monitoring is required to ensure a return to conditions capable of supporting both the return of relocated individuals and the re-colonisation of the population throughout the river.

In applying this WCMP, the term 'sediment removal site' (SRS) pertains to any section where sediment removal works are proposed in the future. This program will largely replicate the successful ELWCMP that occurred 2019-2020 and which represents a best-practice approach for conducting a relocation program for the species.

Specific objectives of the current WCMP are to:

1) Minimise the mortality of *W. carteri* at the primary SRS and future SRSs (upstream of the boat ramp) during the sediment removal program (SRP).

2) Minimise the mortality of relocated individual *W. carteri* during the SRP.

3) Avoid mortality of the host population of *W. carteri* at the relocation site.

4) Ensure the abundance and population structure of *W. carteri* at the SRS is not significantly different following completion of the SRP.

2.4 Risks associated with the WCMP

The potential risks associated with the proposed relocation are consistent with those that were identified in the ELWCMP, however, the likelihood and consequence of many of these are herein downgraded given the success of that plan:

- Mortality associated with handling stress during collection of mussels and transport to the relocation site.
- Conditions at the proposed relocation site no longer suitable prior to relocation (e.g. poor water quality, evidence of mortality of existing population)
- Predation of caged mussels at the relocation site.
- Removal or other interference with caged mussels by members of the public.
- Water quality decline at the relocation site during the housing period due to external factors (e.g. fire, flooding, or pollution event).
- Mortality from compromised water quality in cages associated with stocking density.
- Mortality from water emersion of cages due to drying of relocation site.
- Risks to the existing wild population at the relocation site of adding captively housed relocated mussels.
- Extended period of impacted water quality at the sediment removal site that necessitates extension of the temporary relocation period causing chronic effects (e.g. reproduction, growth, disease, waste accumulation).

3 Rationale and approach for meeting the objectives

3.1 Species tolerances and relocation site characteristics

The biology and ecology of *Westralunio carteri* has been intensively studied by the Centre for Sustainable Aquatic Ecosystems at Murdoch University (see Klunzinger et al. 2011, 2012a, b, 2013, 2014, 2015; Lymbery et al., 2020). The species is slow-growing and long-lived, with a maximum age of at least 52 years and an age at maturity of 4-6 years. Larval mussels are found on a wide range of fish hosts, while juvenile and adult mussels are relatively sedentary and live on the stream bed, preferring fine-grained sediments such as silt and sand. They have an aggregated distribution, with a maximum recorded density of 512 mussels per m^2.

The Iluka wetlands near Capel were surveyed in April 2019 for habitat quality and *W. carteri* presence and population structure as part of the ELWCMP (S. Beatty, Murdoch University). Several lakes were assessed within the Iluka wetlands chain (see ELWCMP, 2020, Appendix 3), with Taylor's Lake selected based on the following characteristics:

- A very healthy population of *W. carteri* with multiple size cohorts with a density of between 20-55 mussels per square metre,
- Appropriate sediment characteristics (sandy with some silt) that are preferred by the species,
- A depth profile off the bank which ensures that installing and monitoring relocation cages is easily achievable,
- Water quality within the known tolerance of the species (high DO, salinity of 1 ppt) (ELWCP, 2020; Appendix 3).

As stated, in undertaking the relocation program outlined in the ELWCMP 2020, valuable additional data on the tolerances and behaviour of the species has been generated. Salinity is thought to be the key driver of the decline in the species range. 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}. Taylor's Lake maintained very low conductivity and salinity concentrations throughout the relocation period for *W. carteri* (i.e., <~3 $mS.cm^{-1}$ or ~1.5 $g.L^{-1}$, Figure 7).

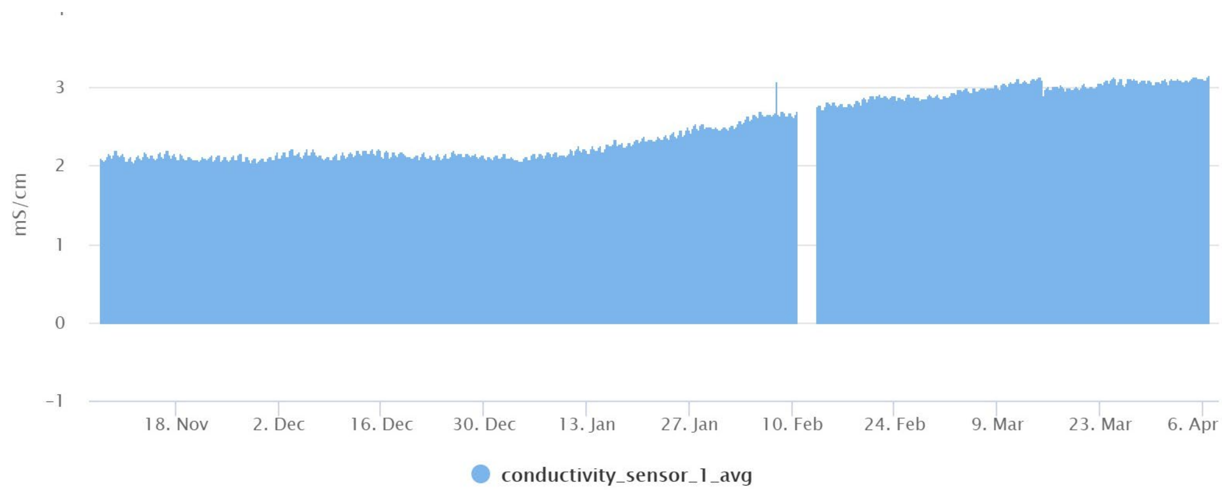

Figure 7: Conductivity in Taylor's Lake during the first three months of captivity.

The species survived periods of daily water temperature maxima of at least 32°C in captivity in Taylor's Lake (Figure 6; Appendix 2). This was consistent with the historical water quality data that was sourced from DWER for the population in the lower Vasse River (Table 3). This suggested that the species regularly experiences temperatures >30°C in the lower Vasse River with a maximum recorded spot measurement of 32.7°C.

Given the species can close its valve for extended periods of drying, it was also assumed that intermittent periods of low dissolved oxygen would be tolerated by the species in captivity in Taylor's Lake. The pre-existing data from the lower Vasse River revealed that the 5th percentile for dissolved oxygen measurements (n=1057 measurements) was 0.80 mg/L (Table 3). Moreover, dissolved oxygen concentrations during the period of captivity in Taylor's Lake in 2019-2020 were much greater and did not reach trigger levels set out in the ELWCMP (Figure 8).

Lymbery et al. (2020) recently determined that the species can survive water emersion for up the three months if they are shaded from direct sunlight. The cages used in Taylor's Lake were checked regularly to ensure they remained immersed and avoided any air exposure of the captive mussels.

The species environmental preferences and tolerances, measured against those conditions that were monitored in Taylor's Lake through implementing the ELWCMP, explain why that program successfully mitigated mortality of the species. It also set a best-practice precedent for relocating, housing, monitoring and reintroducing *W. carteri.* The current WCMP largely follows the approach outlined in the ELWCMP (see summary of its activities in Appendix 2).

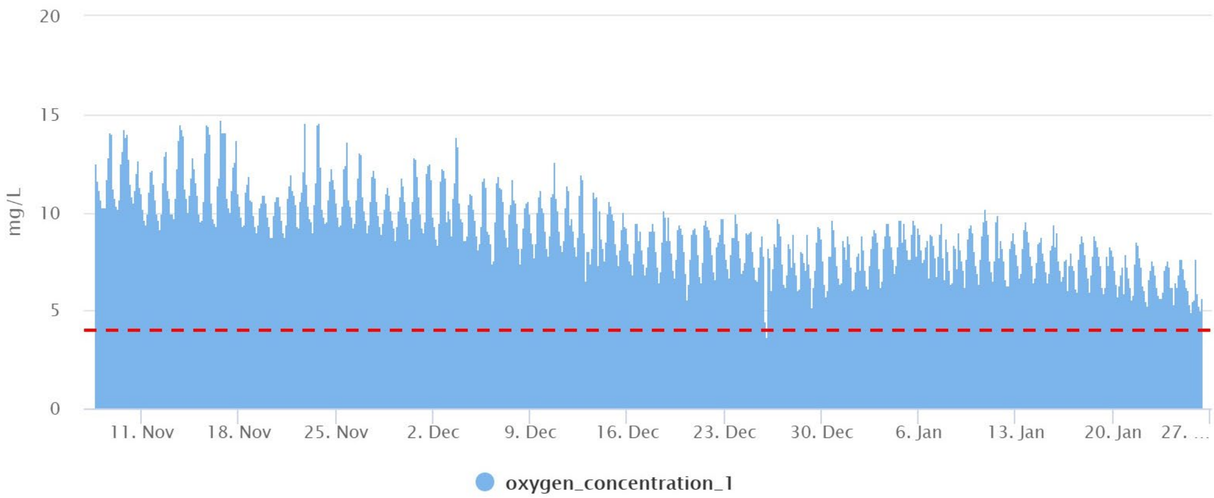

Figure 8: Dissolved oxygen concentration in Taylor's Lake during the first 3 months of captivity. N.B. the proposed trigger value is shown by the red dashed line.

Table 3. Water quality data recorded by Department of Water and Environmental Regulation in the Lower Vasse River (2006-2019, samples = 594-1067) between 2007-2016.

Taylor's Lake				
	Mean (min-max)	S.E.	5th Percentile	95th Percentile
Temperature	19.8 (13.8-25.6)	3.5	14.0	24.7
pH	6.74 (4.97-8.13)	1.19	5.09	7.89
Conductivity (µS/cm)	1990 (1107-3030)	351.8	1181.0	2958.0
Lower Vasse River				
	Mean (min-max)	S.E.	5th Percentile	95th Percentile
Temperature	22.3 (12.1-32.7)	0.11	15.5	27.6
pH	8.16 (6.21-10.43)	0.02	6.82	9.38
Conductivity (µS/cm)	1872 (507-64050)	252.8	579.6	2089.5
Dissolved Oxygen (mg/l)	7.31 (0-25.44)	0.13	0.80	14.06

3.2 Relocation timing

The timing of relocation from the sediment removal site will consider the spawning period of the species. *Westralunio carteri* is a spermcaster, with males spawning in July/August. Larvae (glochidia) are retained by the female until October/November, when they are released (Klunzinger 2008, PhD Thesis, Murdoch University). Translocation will be timed to avoid the spawning/brooding period.

Depending on approvals and logistical considerations (including OHS considerations around algal blooms that generally occur between December-June), the sediment removal program (SRP) is proposed to commence in late 2021 – early 2022. The translocation is proposed to occur just prior to the SRP commencing and will avoid the winter/spring spawning/brooding period. The potential translocation as part of future sediment removal activities upstream of the Boat Ramp will be similarly timed.

Due to the mortality event in 2021, no live mussels occurred in the primary SRP and very low numbers occurred upstream to Fairlawn Rd. If the SRP is not undertaken within before June 30 2023, further survey will be required to determine the extent of re-establishment of the mussel population in the river, and the numbers of mussels requiring relocation will be amended accordingly.

3.3 Mussel cages

The current project will use the purpose-built mussel cages within Taylor's Lake that were used as part of the ELWCMP (Figure 9). Up to 60 cages will be used to house the relocated mussels and they will be monitored during the relocation period. All caged mussels will also be able to be readily collected for the reintroduction to the sediment removal site once conditions are suitable post-construction.

Figure 9: Mussel cages in Taylor's Lake that will be used to house relocated individuals.

3.4 Stocking densities

The number of *W. carteri* requiring relocation prior to the sediment removal in the primary SRS between the Buttery Factory boards and old boat ramp was estimated to be ~3183 ± 309 SE in the Stage 1 survey (Table 2). However, the recent saltwater incursion event has resulted in 100% mortality of these mussels. The total numbers of live mussels estimated from the remaining potential sediment removal areas are 103 between the boat ramp and Strelly St (confined to upstream of Gwendolyn St); 43 between Strelly St and Fairlawn Rd (confined to upstream of 200m from Strelly St); and 2007 between Fairlawn Rd and Busselton Bypass (Table 4).

The current WCMP enables flexibility in the numbers that can be housed at Taylor's Lake as required. There is ample space available around the banks of Taylor's Lake to accommodate the estimated number of *W. carteri* potentially requiring relocation from future SRS areas.

Stocking densities within cages will be within those recorded for the species at other sites in the South West region. The species has a highly aggregated population structure, with densities across 31 sites throughout the range varying from 2-512 mussels.m^{-2} (Ma 2018 PhD Thesis, Murdoch University). The cage density allowed for in the ELWCMP was 50 mussels.m^{-2} was greater than the mean density across all south-western Australia sites of 29.5 mussels.m^{-2}, but only 1/10th of the maximum recorded density. Moreover, the species has recently been shown to occur at densities of up to ~140 mussels.m^{-2} in the Vasse Diversion Drain as part of surveys underpinning a proposed *W. carteri* management plan for the levee bank works (Beatty and Lymbery, 2020). Therefore, we propose to have a maximum stocking density of 70 mussels per 1 m^2 cage.

The area of suitable habitat at the temporary relocation site is approximately 300 m^2 allowing for placement of up to 50 cages with >6.0m^2 of habitat, enabling ample spacing to avoid potential impacts on light attenuation and water flow, and mitigate the potential for density dependent effects (see below). This maximum number of cages would provide housing for up to 3500 mussels at any given time.

Table 4. 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%

3.5 Inclusion of buffer zones around the sediment removal site

The current proposed process for sediment removal is to use a small dredge to pump soft sediment from the bed of the river directly into porous geotextile bags located adjacent to the river. The sediment is dosed with flocculant so that fine sediments are retained in the bag while water is expelled and returned to the river. The bags are then left to allow drying of the sediment prior to removal, reducing the volume of material for disposal. This minimises impacts on turbidity and sulfidic sediments, as the sediments are mostly retained in the bags and are not exposed to air. However, some suspended solids may pass through the bag, and the action of the dredge is also likely to create disturbance of the sediments, increasing turbidity of the surrounding waters and potentially reducing oxygen levels. Return waters may have elevated nutrient levels and heavy metals released from sediments.

Impacts of return waters will be reduced by undertaking works when river flow is minimal and by installation of PVC curtains either side of sediment removal sites to retain any sediment arising during the dredging process. A 40 m buffer zone is proposed upstream and downstream of the SRS, giving a total maximum of 820 m of streamline for *W. carteri* removal from the lower Vasse River. The 40 m buffer zone is provided as a conservative measure to prevent impacts to *W. carteri* in the event of any unexpected sediment disturbance during installation and removal of the PVC curtains.

3.6 Monitoring of water quality at the sediment removal site

Water quality monitoring at each sediment removal site will be undertaken to assess the impacts of sediment removal on water quality during works and to determine suitability of habitat for the return of mussels to the site. This is to be undertaken regardless of the presence or absence of live mussels in a given section to assess impacts on water quality on potential re-colonisation by *W. carteri* throughout the river. PVC curtains will not be removed until water quality is within acceptable ranges as determined by baseline water quality monitoring.

Prior to dredging, in order to provide baseline water quality, monitoring of dissolved oxygen, pH, temperature, nutrients (total nitrogen, total phosphorus, nitrate plus nitrite, ammonium, dissolved organic nitrogen, filterable reactive phosphorus) and dissolved heavy metals will be undertaken at three locations in the SRS. Dissolved oxygen, pH and temperature will be measured in situ along a depth profile; while depth-integrated samples will be collected for analysis for nutrients and heavy metal analysis.

During the dredging works, the monitoring program will include the following components:

- Bi-weekly monitoring (vertical profiles) of dissolved oxygen, pH and temperature at three locations in the SRS.
- Daily monitoring of dissolved oxygen, pH and temperature of return waters.
- Daily monitoring of nutrients and dissolved heavy metals of return waters and at three locations in the SRS open waters.
- Bi-weekly monitoring of dissolved heavy metals of return waters and at three locations in the SRS open waters.

Post-dredging, monitoring will be reduced to weekly monitoring of all parameters at three locations in the SRS for at least one fortnight, or until results are within 10% of baseline condition.

3.7 Host population monitoring

The risks to the host population of *W. carteri* in Taylor's Lake from temporarily housing the mussels from the lower Vasse River include competition for benthic habitat space, reduction in water quality, predation and disease transmission. These are again ranked as low as per the ELWCMP given the size of Taylor's Lake, the high abundance and healthy status of the host population that was closely monitored as part of conducting the ELWCMP.

There was no predation on caged mussels during the ELWCMP housing period. We again do not expect an increase in predator numbers at the translocation site and therefore we do not expect any increase in predation rate on the host population. Mussels are not the major prey of any predator species and we have no evidence of any difference in predation rate (from broken shells left by birds or middens left by Rakali) in relation to mussel density.

3.8 Post-sediment removal habitat and water quality assessment

Given that the benthic habitat will be substantially altered by sediment removal, a comprehensive assessment of the habitat and water quality in the SRS will determine its suitability for re-introduction of the relocated mussels prior to this occurring. Monitoring of the water quality at the SRS as described above will contribute to this assessment.

The sediment removal will result in increased depth of the channel and may result in a change in bank angles; but will not alter the riparian vegetation that offers shading and inundated roots that are favoured by the species. An assessment of the remaining benthic substrate post-sediment removal will be required to ensure that the substrate is not too hard for burrowing.

Given the predicted long-term improvement in overall water quality that will result from the sediment removal program along with the overall Lower Vasse River Waterway Management Plan, it is anticipated that there will be a long-term improvement in the viability of the mussel population in the lower Vasse River and that mortalities of reintroduced mussels will be minimal.

3.9 Monitoring health of reintroduced mussels

It is important to conduct a thorough monitoring program of the reintroduced mussels to determine their health and viability post reintroduction (see criteria in Table 5, Table 6). All relocated mussels will be individually tagged (shellfish tags) to enable the monitoring of the health of individual mussels.

Monitoring of the population at the SRS following reintroduction will occur at one, two and six month intervals in order to detect mortalities. Our previous studies of mussel tolerance to water quality parameters such as salinity and silting have found mortality to be rapid (< 2 weeks). Detecting non-lethal effects, such as reduced fertility, which might affect population size over the longer term, would require monitoring over a longer time period. Juvenile mussels first appear in the sediment in January, so annual surveys in summer/autumn would provide an indication of recruitment success.

3.10 Contingency for permanent relocation

Reintroducing mussels to the SRS post sediment removal is recommended as they will have a long-term benefit from a living streams perspective in terms of assisting with water quality and creating habitat. In addition, the populations of mussels within the lower Vasse River have already been severely impacted by recent saltwater incursion, and further permanent removal may have long-term consequences for viability of the species in the river. Moreover, it is possible that the mussels in the lower Vasse River may have locally adapted to those conditions (including cyanobacterial blooms, extreme temperatures, periods of depleted oxygen).

However, in the unexpected event that the water quality or habitat at the SRS is found to be unsuitable post-dredging, or that the water quality or mussel health management triggers are met during the period of captivity in Taylor's Lake, a survey will occur of the lower Vasse River between the Busselton Bypass and the Diversion Drain to identify alternative relocation habitats and the new site/s approved by DAWE/DBCA prior to release. It is expected that an acceptable site for permanent relocation (if required) will be present along the lower Vasse River upstream of the Busselton Bypass. This is due to the approximately 4 km of river stretch that includes substantial areas of similar riverine morphology, geology, catchment and fringing vegetation as downstream; and the recorded presence of mussels at in this reach (Lymbery et al 2008, DBCA fauna database 2017).

We emphasise, however, that releasing the mussels upstream is a less preferred option compared the proposed relocation / reintroduction program. Although mussels have been found in upstream habitats, density is very low (Lymbery et al. 2008) and a permanent increase in density may pose risks for population viability. Should a site not be able to be identified in the lower Vasse River, the final option will be to relocate the mussels upstream to the known suitable habitats in the Vasse Diversion Drain with the assumption that over the long-term, the species will recolonize the SRS via transfer of their larvae on fishes. This is the least preferred option, as recolonisation is likely to be a very slow process particularly given the lack of suitable native fish hosts present in the lower Vasse River compared with introduced species such as Goldfish, which are not suitable hosts (see Beatty et al. 2014).

3.11 Risk assessment

The risk posed to translocated individuals and the host community is assessed as low to medium based on the following factors:

(1) The success of the ELWCP that the current WCMP will largely replicate. The ELWCMP resulted in no mortality of captively held *W. carteri* over an 8 month period of captivity.

(2) The proposed stocking densities within the cages are over ~2.5 times less than have been recorded for the species in other systems.

(3) The cages will also be spaced well apart to further decrease the potential for density dependent effects.

(4) The water quality and habitat at the relocation site are suitable for the species based on the ELWCMP telemetered data from 2019-2020 and historical data.

(5) The 10 mm mesh width on the cages will ensure free water flow and the flushing of all organic wastes.

(6) No morbidity or mortality associated with infectious disease has ever been recorded in the Vasse River or in other sites where *Westralunio carteri* has been surveyed; between 2009 and 2011, 582 mussels were dissected for analysis of reproductive biology and health status, with no pathogens or parasites observed.

(7) The major predators of freshwater mussels generally are water birds and some mammals, such as water-rats. The cages that will be used will be constructed of hi-strength plastic mesh and fully sealed to prevent access by predators. Therefore, while increasing the time in captivity will increase the probability of predators being present, it will not affect the low risk of predation by animals.

4 Environmental management plan provisions

The following outlines the approach of the proposed relocation program to ensure it successfully mitigates the potential negative impact on *W. carteri*. It identifies management actions that will be implemented as part of the WCMP; the residual risk following the undertaking of those actions; and the timeframe for conducting each action (Table 5). Also outlined is the pre-relocation, relocation period and post-release monitoring program to assess the performance of WCMP in meeting its management targets, including triggers for instigation of corrective actions and the reporting framework (Table 6). A conceptual diagram detailing key elements of the management actions and monitoring program is also provided below (Figure 10).

Relocation is not currently required for the primary SRS due to the recent mortality event resulting in no live mussels in that area. However, the approach is provided to allow for future sediment removal throughout the river as planning progresses. Current survey outcomes must be used to guide the implementation of the WCMP (i.e. completed within two years of planned sediment removal).

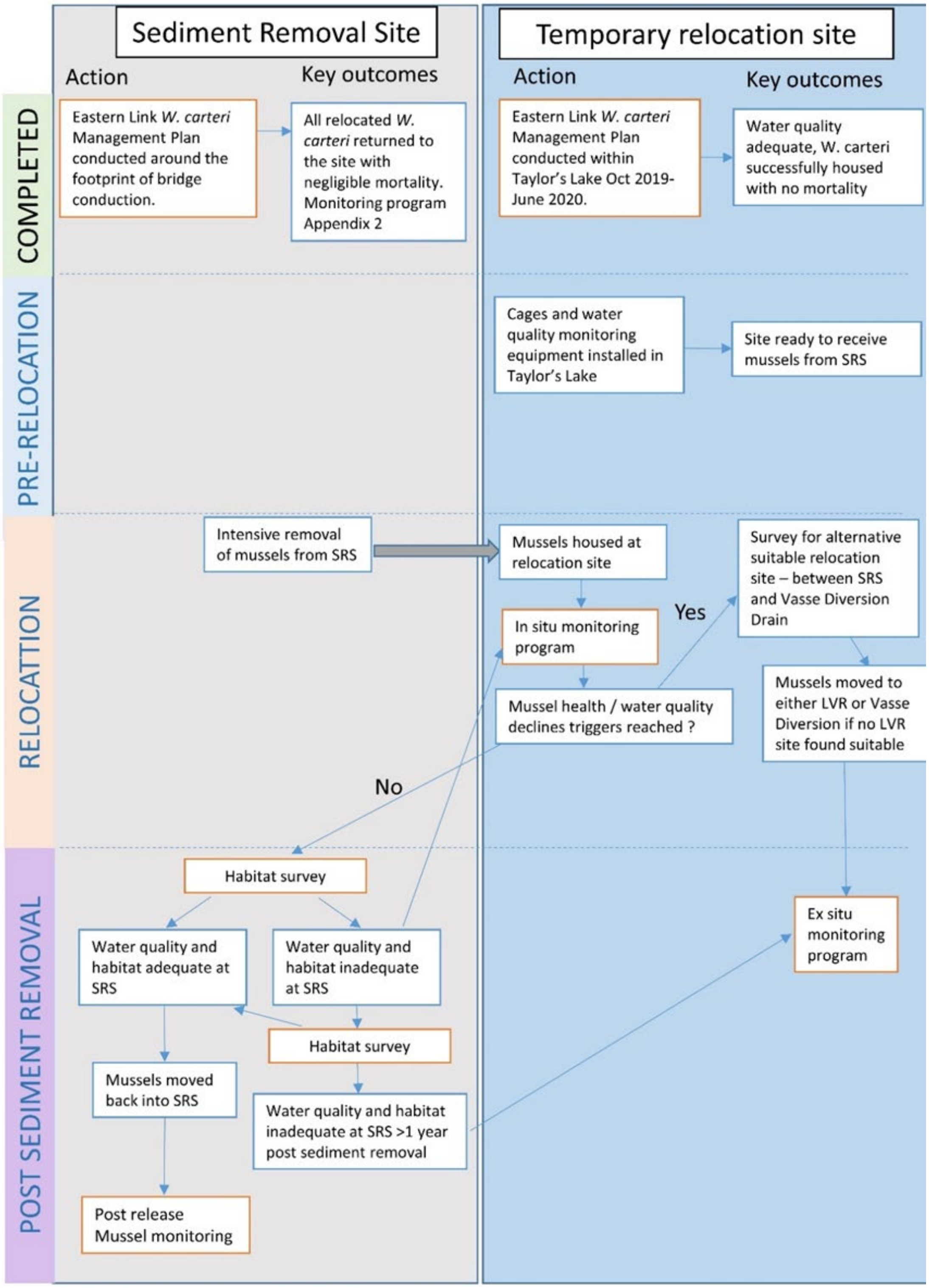

Figure 10: Conceptual flow chart of key elements of the management actions and monitoring program of the WCMP. See Tables 5 and 6 for details. N.B. Eastern Link *W. carteri* Management Plan has been noted as completed in the flow chart above as it is a successful precedent that underpins the current WCMP. The flow chart also applies to the future sediment removal sites, upstream of the Boat Ramp.

Table 5: Risk based management actions for the sediment removal project. N.B. these apply all potential future sediment removal projects.

Management Objective	Management Actions	Residual risk of impact	Timeframe
Minimise the mortality of *W. carteri* at the sediment removal site (SRS)	• Undertake an intensive removal program of *W. carteri* within and adjacent to the SRS, guided by outcomes of recent surveys	Low. • The collection site will include a buffer zone 40 m upstream and downstream of the SRS (within silt curtains) • Mussels will be intensively removed by hand up to 2m from each bank over two passes • A depletion curve will be plotted on the second pass and mussel collection halted only when no further mussels are found after 30 minutes of intensive searching	• Prior to any disturbance of the riverbanks or bed associated with the sediment removal program and to avoid spawning period
Minimise the mortality of relocated individuals during SRS	• Rapid relocation in insulated, aerated containers to the relocation site at Taylor's Lake • Maintain mussels in purpose-built cages in perennial habitat at the relocation site • Submerge cages to reduce visibility to the general public • Each cage will be coded and location recorded (using GPS), each will be secured to star pickets • Undertake regular inspection of sub-sample of population • Undertake real-time constant monitoring of mussel activity in cages using valvometers	Low. • Relocation site has been shown to be suitable in housing captive mussels through the Eastern Link *W. carteri* Management Plan (ELWCMP) • Relocation site has adequate space (300 m^2) to house the estimate number of relocated mussels (i.e., 3183 (±608) of up to 70 mussels / cage • Relocation site is managed by Iluka Resources on a mining lease and is closed (locked gate) to public access • Relocation site has adequate space for estimated number of relocated mussels to house the	• Transport time from Project sites to Relocation site is 30 minutes • Daily inspection of random sub-sample of caged mussels for the first three days of relocation • Weekly inspection for the first one month of relocation • Monthly inspection for the remainder of relocation period • Continuous real-time (telemetered) monitoring of mussel activity throughout relocation period

Management Objective	Management Actions	Residual risk of impact	Timeframe
		number of cages at the proposed stocking density • Regular monitoring will trigger further management actions if required (see Table 6)	
Avoid mortality of the host population of *W. carteri* at the relocation site	• House the relocated mussels in cages to avoid direct competition for benthic habitat and minimise the risk of spread of disease by direct contact between relocated and host individuals • Regularly monitor health of host populations and water quality to ensure that conditions at relocation site can support expanded mussel population without impacting the host population	Low. • ELWCMP found no impact on the host population in Taylor's Lake of temporarily housing mussels • Density of existing host population is substantially less than found at other sites • There has been no recorded morbidity or mortality of parasites associated with infectious disease for the species • Regular monitoring will trigger further management actions if required (see Table 6)	• Monthly assessment of the host population at the relocation site will occur following the techniques in Beatty et al. (2017) • Continuous monitoring of sub-sample of mussels will occur using valvometers that will provide early warning of an adverse conditions affecting both the host and relocated mussels
Restore the population abundance of *W. carteri* post-sediment removal in the SRS to equal the pre-removal abundances	• Assessment of the water quality and habitat suitability of the lower Vasse River SRS following sediment removal will occur prior to re-locating the mussels • Relocation of mussels to original stream section into habitats known to be suitable for the species. The relocation density will be within that of the population was originally recorded to avoid any potential density dependent impacts on the population and to ensure the ecosystem services (particularly	Low. • ELWCMP demonstrated success in reintroducing mussels to the lower Vasse River • Mussels will only be relocated to the SRS once sediment removal program on the lower Vasse River is completed • As a 100% survival rate of relocated *W. carteri* is anticipated • Assessment of the water quality and habitat (including depth, substrate composition, temperature, dissolved oxygen, pH, turbidity) at the sediment removal	• Monitoring of the water quality at the SRS will occur during the sediment removal period to help inform future dredging projects in the lower Vasse River • Assessment of the water quality and habitat suitability of the SRS to house mussels will occur within one month following the completion of sediment removal

Management Objective	Management Actions	Residual risk of impact	Timeframe
	water filtration) provided by the species are maintained at the SRS • Monitoring program of the relocated mussels will occur after being placed back into the SRS to confirm ongoing viability	site will be made prior to the decision to re-stock the mussels • Mussels will be relocated to the SRS and re-stocked at densities within the original range that existed prior to construction • In the event of the water quality and/or habitat at the SRS site remaining unsuitable for a prolonged period of time (i.e. >1 year post construction phases), additional management actions will be taken (see Table 6)	• Weekly monitoring of water quality will continue until a return to baseline conditions • Reintroduction of mussels will occur following the confirmation of meeting appropriate water quality and habitat criteria (see Table 6) at SRS • Monitoring of the relocated mussel population will occur one, two and six months after being relocated at the SRS and will include density and population structure being assessed

Table 6: Management targets to measure success of management actions. N.B. these apply to both the primary SRP between the Buttery Factory and the boat ramp and also the potential future sediment removal projects upstream to the Busselton Bypass.

Management Objectives	Management targets	Monitoring	Trigger value	Corrective Action
Minimise the mortality of *W. carteri* at the sediment removal site (SRS) on the Lower Vasse River	• Remove all *W. carteri* from the SRS including within a 40 m buffer zone upstream and downstream of the SRS • Relocate all captured *W. carteri* to confirmed suitable habitat at Taylor's Lake	• Mussels will be intensively removed by hand up to 2m from each bank over two passes. • A depletion curve will be plotted on the second pass and mussel collection halted only when no further mussels are found after 30 minutes of intensive searching	• Minimum time of 30 minutes on second pass of intensive searching with no further *W. carteri* detected	• Continue mussel collection until they are undetectable at the SRS
Minimise the mortality of relocated individuals during the SRP	• No significant amount of mortality of relocated *W. carteri* during the SRP	• Daily inspection of caged mussels for the first three days of relocation • Subsample of 100 mussels randomly checked for viability (valve closure on touching; checking of closed mussels) • Weekly assessments of caged mussels will then occur for the first one month and then monthly inspections for the remainder of the relocation phase • Continuous real-time (telemetered) monitoring of mussel activity throughout	• Water emersion (i.e. drying) of any cages during inspection • Death of >1% of mussels in cages, indicated by gaping valves, during inspection • Valve closure, as indicated by valvometer activity, >50% of *W. carteri* for a period of >12 hours • Water temperature >32°C and/or dissolved oxygen <2 ppm for a continuous period of >12 hours: and valve closure, as indicated	• Site visit (within 24 hours of trigger detection) to assess possible cause of stress • If cause cannot be rectified by onground actions in Taylor's Lake (e.g. relocation of cages to more suitable habitat in the lake), all relocated *W. carteri* transferred to appropriate site either on the lower Vasse River downstream of the Vasse Diversion Drain, or within the Vasse Diversion Drain upstream of Chapman Hill Rd.

Management Objectives	Management targets	Monitoring	Trigger value	Corrective Action
		relocation period (as per the ELWCMP) • Continuous real-time monitoring of water quality (temperature, dissolved oxygen)	by valvometer activity, >50% of *W. carteri* for a period of >12 hours	• *W. carteri* transported to new site in insulated, aerated containers • *W. carteri* released at total (host plus reintroduced mussel) densities not exceeding maximum existing mussel density at the new site
Avoid mortality of the host population of *W. carteri* in Taylor's Lake	• No significant amount of mortality or reduction in density of the existing host population recorded during the relocated period	• Regularly (monthly) assessment of the health of host populations and water quality to ensure that conditions at relocation site supports expanded mussel population without impacting the host population • Daily inspection of sub-sample of caged mussels for the first three days of relocation • Weekly inspection for the first two months of relocation. Fortnightly inspection for the remainder of relocation period • Continuous real-time monitoring of water quality (temperature, dissolved oxygen)	• A decline in host population density of 20% (0.5 SD) (compared with pre-relocation densities) • Water temperature >32°C and/or dissolved oxygen <2 ppm for a continuous period of >12 hours: and valve closure of sentinel mussels (as a proxy of stress of the host population), as indicated by valvometer activity, >50% of *W. carteri* for a period of >12 hours	• Site visit (within 24 hours of trigger detection) to assess possible cause of stress on host population • If cause cannot be rectified by onground actions in Taylor's Lake (e.g. relocation of cages to more suitable habitat in the lake), all relocated *W. carteri* transferred to appropriate site either on the lower Vasse River downstream of the Vasse Diversion Drain, or within the Vasse Diversion Drain upstream of Chapman Hill Rd – as described above

Management Objectives	Management targets	Monitoring	Trigger value	Corrective Action
Restore the population abundance of *W. carteri* post-sediment removal to equal the pre-removal abundances	• No change in the density and population viability of *W. carteri* at the SRS following the completion of sediment removal	• Prior to restocking at the SRS, water quality and habitat will be measured (including temperature, dissolved oxygen, pH, turbidity burrowable substrate) • Population resampled one, two and six months after restoration, and density and population structure assessed	• Inadequate water quality (i.e. key triggers of water temperature >32°C and/or dissolved oxygen <2 ppm for a continuous period of >12 hours); pH, turbidity and TN/TP checked to ensure within tolerance of mussels and similar to baseline levels • Inadequate habitat suitability (for >50% of relocation stream-length): i.e, burrowable benthic substrate less than a depth of 10 cm and/or average grain size >500 µm, mean depth within 2m of the water's edge at sediment removal site <0.3m) • Reduction in population density of 10% (0.5 SD) of pre-translocation densities • Abnormal mussel mortality i.e. 5% dead mussels, as	• Mussels continue to be maintained in Taylor's Lake sites until water quality and habitat in SRS is adequate • Should population decline once relocated back to SRS, all living mussels collected and relocated upstream (see previous section) • In the event of the water quality or habitat at the SRS being unsuitable for a prolonged period of time (i.e. >1 year post sediment removal), a survey will occur of the lower Vasse River between the Project sites and Diversion Drain to identify alternative relocation habitats and the new site/s approved by DAWE/DBCA prior to release (as per previous corrective actions) • The mussels will be relocated into the approved site/s, limiting the overall density to within the range that exists at the new relocation site/s

Management Objectives	Management targets	Monitoring	Trigger value	Corrective Action
			indicated by empty shells	

5 Roles and responsibilities

Implementation of the WCMP will be managed by senior members of the Centre for Sustainable Aquatic Ecosystems (CSAE, Murdoch University) and Ottelia Ecology who have successfully managed all previous relocation projects for *W. carteri* including the Eastern Link *W. carteri* Environmental Management Plan. They will be responsible for:

- Supervising and coordinating the removal of *W. carteri* from the sediment removal site (with the help of City of Busselton Staff, and public volunteers to increase efficiency if required).
- Rapid relocation of *W. carteri* to the relocation site.
- Installation of the purpose-built holding cages.
- Installation of the valvometers to monitor the behavior of the relocated mussels.
- Ongoing monitoring of the telemetered data and ongoing assessment of trigger levels.
- Water quality monitoring of the SRS.
- Assessment of habitat suitability for return of relocated mussels.
- Determination of alternative contingency site(s) if required.

City of Busselton will be responsible for:

- Coordinating all aspects of the sediment removal program from the lower Vasse River.
- Notifying adjacent landholders of relocation site and activities.
- Notifying CSAE and Ottelia of successful planned proposals for sediment removal works in the lower Vasse River.
- Scheduling the *W. carteri* relocation prior to sediment removal commencing.
- Notifying CSAE and Ottelia of the cessation of the sediment removal program.

6 Audit and review

The progress and efficacy of the WCMP will be reviewed under the following schedule:

- Real-time monitoring of the rate of removal of *W. carteri* from the SRS. This will generate a catch curve that will trigger the cessation of the fishout.
- Within 2 weeks following the initial relocation of *W. carteri* a report will be produced that provides an assessment of the success of the fishout and relocation status of *W. carteri* including numbers, size distribution, water quality, and valvometer data assessment.
- Progress report on the status of the relocated *W. carteri* will be produced at the half-way point of SRP, or 6 months following initial relocation of *W. carteri* (whichever is sooner) outlining the status of the relocated mussels (health, behavior, water quality).
- Within 4 weeks following completion of the sediment removal in the lower Vasse River, a report will be produced on the status of the water quality and habitat conditions of the site including a recommendation as to its suitability for relocating mussels back to their capture sites.
- Following the restocking of the mussels back into the SRS in the lower Vasse River, a report will be produced as to the status of the mussels at the restocked site (6 weeks after restocking).
- A final report will be produced eight months following the restocking that provides an assessment of all aspects of the WCMP, including the status of the restocked mussels in the Lower Vasse River.
- All reports will be provided to DBCA under the Regulation 40 License and the DAWE under the EPBC Act approval.
- Should a catastrophic event occur at any time which impacts upon the mussel project, causing >10% mussel mortality in either the translocated or host population, the Department of Biodiversity, Conservation and Attractions will be notified within 48 hours with a statement of facts and proposed mitigation actions for approval.

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Appendix 1: Survey of Carter's Freshwater Mussel in the Lower Vasse River to inform future sediment removal

R. Paice and S. Beatty

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**	**1 February 2021**
City of Busselton	**Mathilde Breton**	**8 February 2021**
City of Busselton	**Mathilde Breton**	**9 June 2021**
City of Busselton	**Mathilde Breton**	**10 June 2021**

Document Control:

Document Status	Prepared By	Reviewed by	Date
Draft report V2	**Robyn Paice**	**Stephen Beatty**	**14 January 2021**
Draft report V2	**Robyn Paice**	**Stephen Beatty**	**4 February 2021**
Draft report V3	**Robyn Paice**	**Stephen Beatty**	**8 June 2021**
Final report	**Robyn Paice**		**10 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.

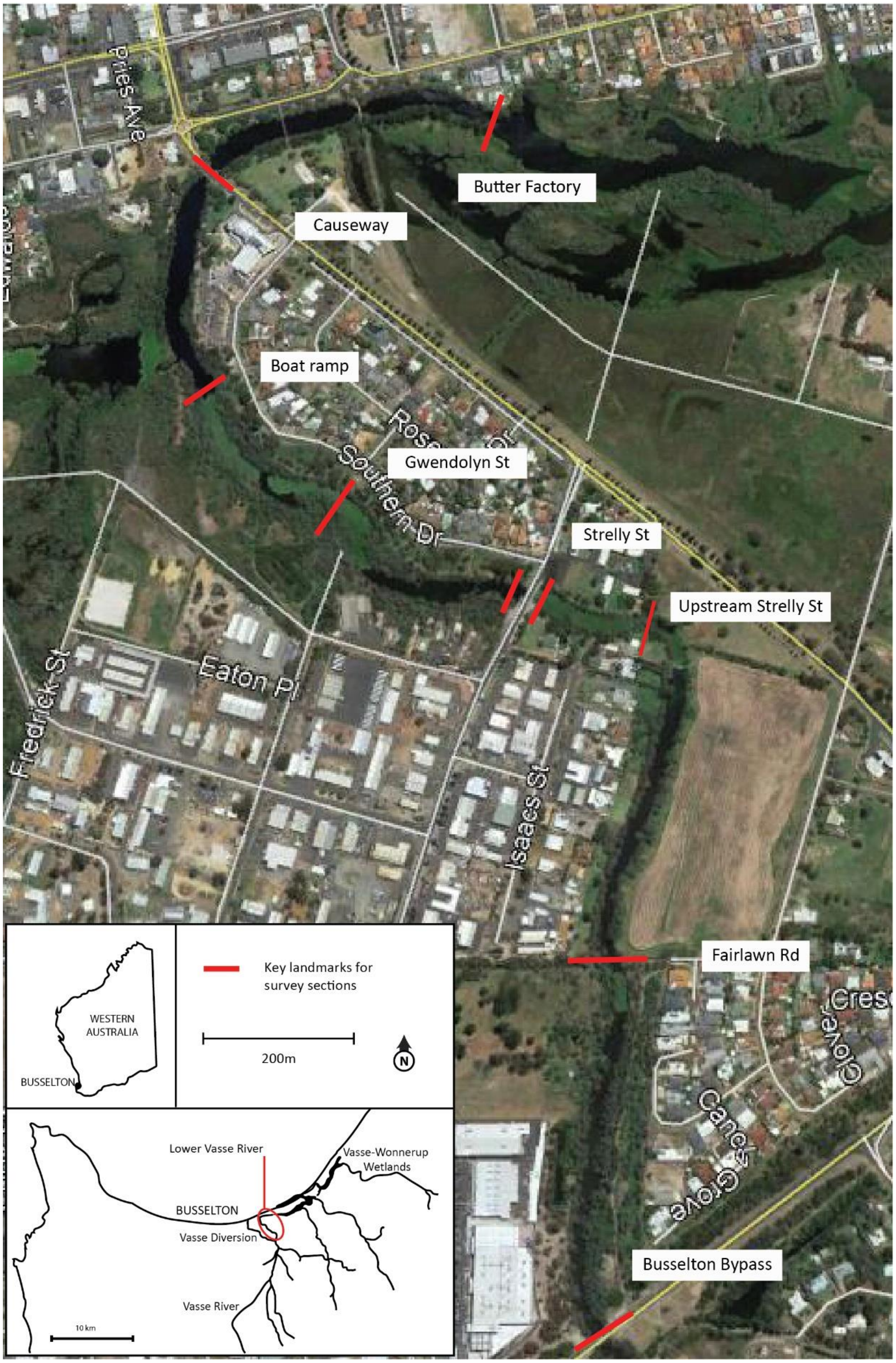


Figure 1. Lower Vasse River mussel survey area showing location of key landmarks used to describe survey sections.

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 required to be managed during future sediment removal works.

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 survey area in Stage 1. 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. Stage 2 coincided with water quality transect measurements by the Department of Water and Environmental Regulation, and this data was provided.

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 each section. 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 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, off-bank sampling was undertaken in the survey areas using two methods.

During the first stage of the survey, an Ekman grab was used to collect sediment samples to check for mussel presence (dimensions 25 x 25 cm to a sediment depth of 20 cm). 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 small proportion of the total riverbed area.

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.

	Live Mussels		Dead Mussels	
Survey Area	Mean Density (per m^2)	Abundance (±SE)	Mean Density (per m^2)	Abundance (±SE)
Butter Factory to Causeway (750m)				
North bank (420m)	0.99 ±0.18	830 ± 262 (133)	0	0
South bank (380m)	0.22 ± 0.07	170 ±110 (60)	0.04 ± 0.02	16± 8
Total (420m)		1004 ± 166	0.02 ± 0.01	16 ± 8
Causeway to boat ramp:				
East bank (290m)	2.0 ± 0.30	1160 ± 171	0.27 ± 0.10	78 ± 0.29
West bank (320m)	1.44 ± 0.25	920 ± 157	0.53 ± 0.10	170 ± 32
Total (330m)		2112 ± 237	0.40 ± 0.07	244 ± 43
Total		**3183 ± 309**	**0.20 ± 0.04**	**268 ± 50**
Strelly St bridge and upstream (200m)				
Bridge and downstream (50m)	5.6 ± 2.78	563 ± 278	0	0
Upstream to bend (150m)	0	0	0	0
Upstream south bank (160m)	0	0	0	0
Total (200m)		**563 ± 278**	**0**	**0**

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.

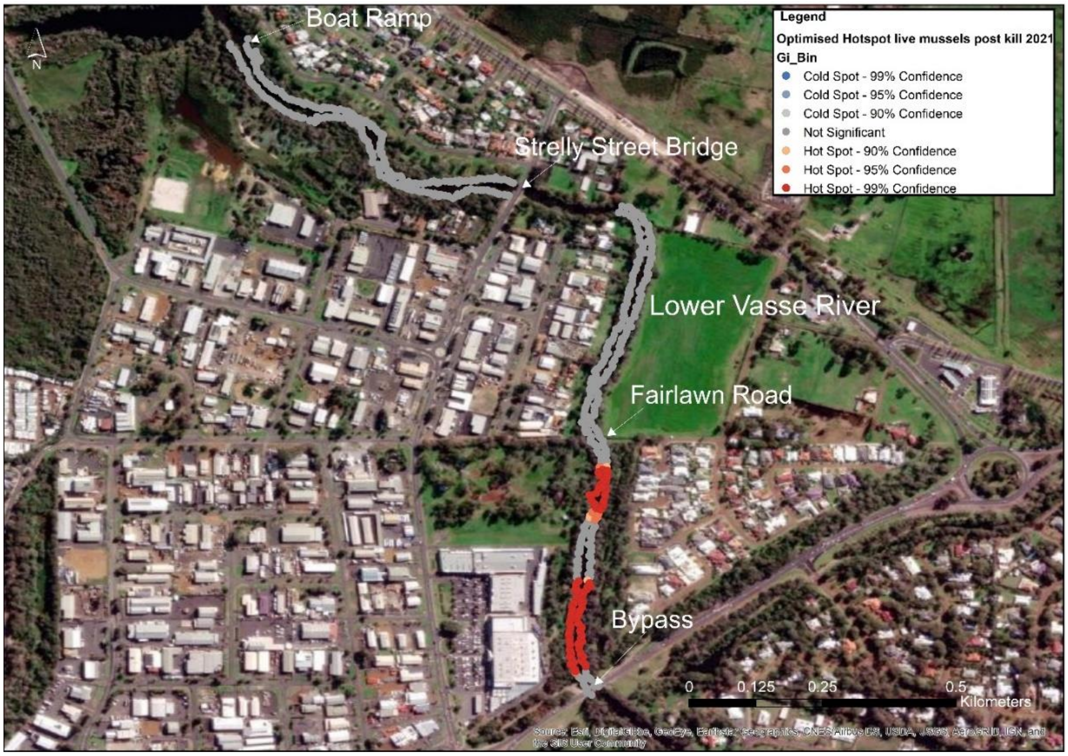
Upstream from Strelly St to Fairlawn total mussel density was higher at 1.25 per m^2 but the proportion of dead mussels was very high (96%) and live mussel density was only 0.05 per m^2 (Table 2). Density was patchy with large areas of bank habitat having no mussels and significant hot spots for dead 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	**Live Mussels**		**Dead Mussels**	
	Mean Density (per m^2)	**Abundance (±SE)**	**Mean Density (per m^2)**	**Abundance (±SE)**
Boat ramp – Gwendolyn St (560m)	0.00	0	0.27 ± 0.07	153 ± 38
Gwendolyn St – Strelly St (670m)	0.15 ± 0.04	103 ± 26	0.69 ± 0.08	459 ± 55
Strelly St – Fairlawn Rd (955m)	0.05 ± 0.02	43 ± 16	1.20 ± 0.12	1146 ± 111
Fairlawn Rd – Busselton Bypass (985m)	1.25 ± 0.16	1230 ±155	0.70 ± 0.09	694 ± 92
Total	**0.42 ± 0.05**	**1376 ± 165**	**0.77 ± 0.05**	**2452 ± 163**
Off-bank Habitat	**Live Mussels**		**Dead Mussels**	
	Mean Density (per m^2)	**Abundance (±SE)**	**Mean Density (per m^2)**	**Abundance (±SE)**
Boat ramp - Strelly St (5800m^2)	0	0	0.05 ± 0.02	99 ± 47
Strelly St - Fairlawn Rd (4750m^2)	0	0	0.11 ± 0.02	477 ± 97
Fairlawn Rd - Busselton Bypass (4800m^2)	0.24 ± 0.06	777 ± 187	0.19 ± 0.05	634 ± 161
Total	**0.07 ± 0.02**	**777 ± 187**	**0.12 ± 0.02**	**1210 ± 194**

(a)



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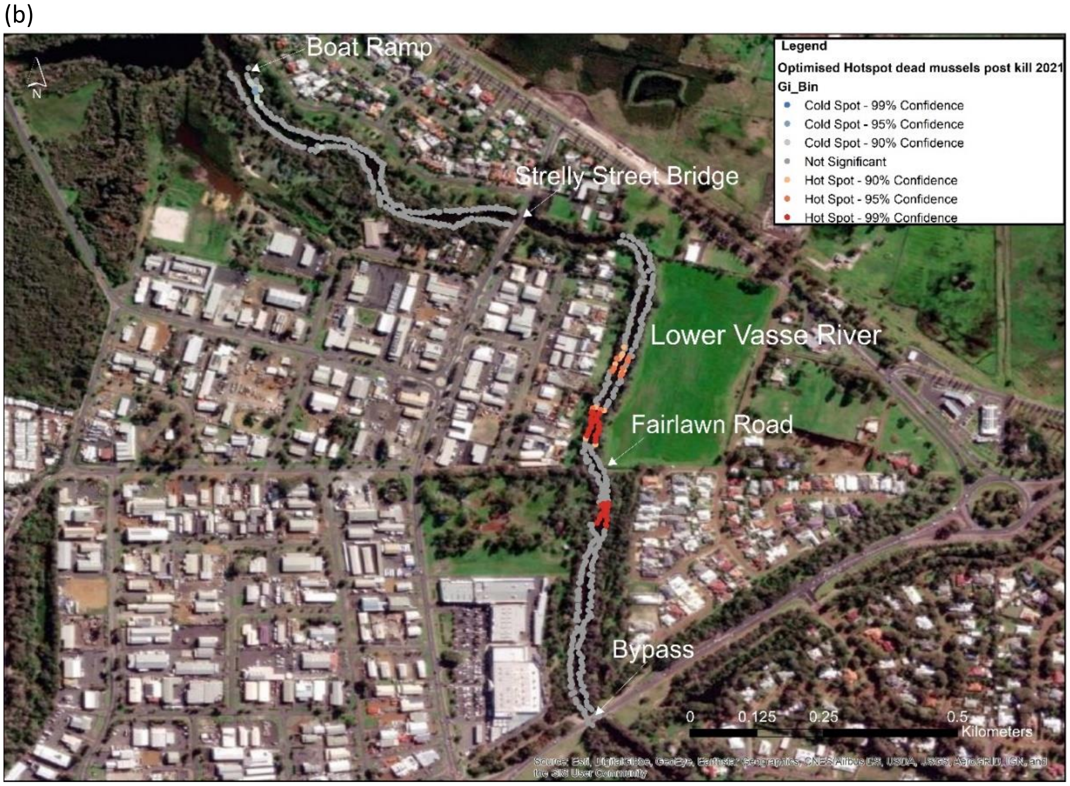

Figure 3. Optimised Hotspot analysis showing significant hotspots of live (a) and dead (b) *W. carteri* in Stage 2 survey areas from the boat ramp to the Busselton Bypass.

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 mussels 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 size 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.

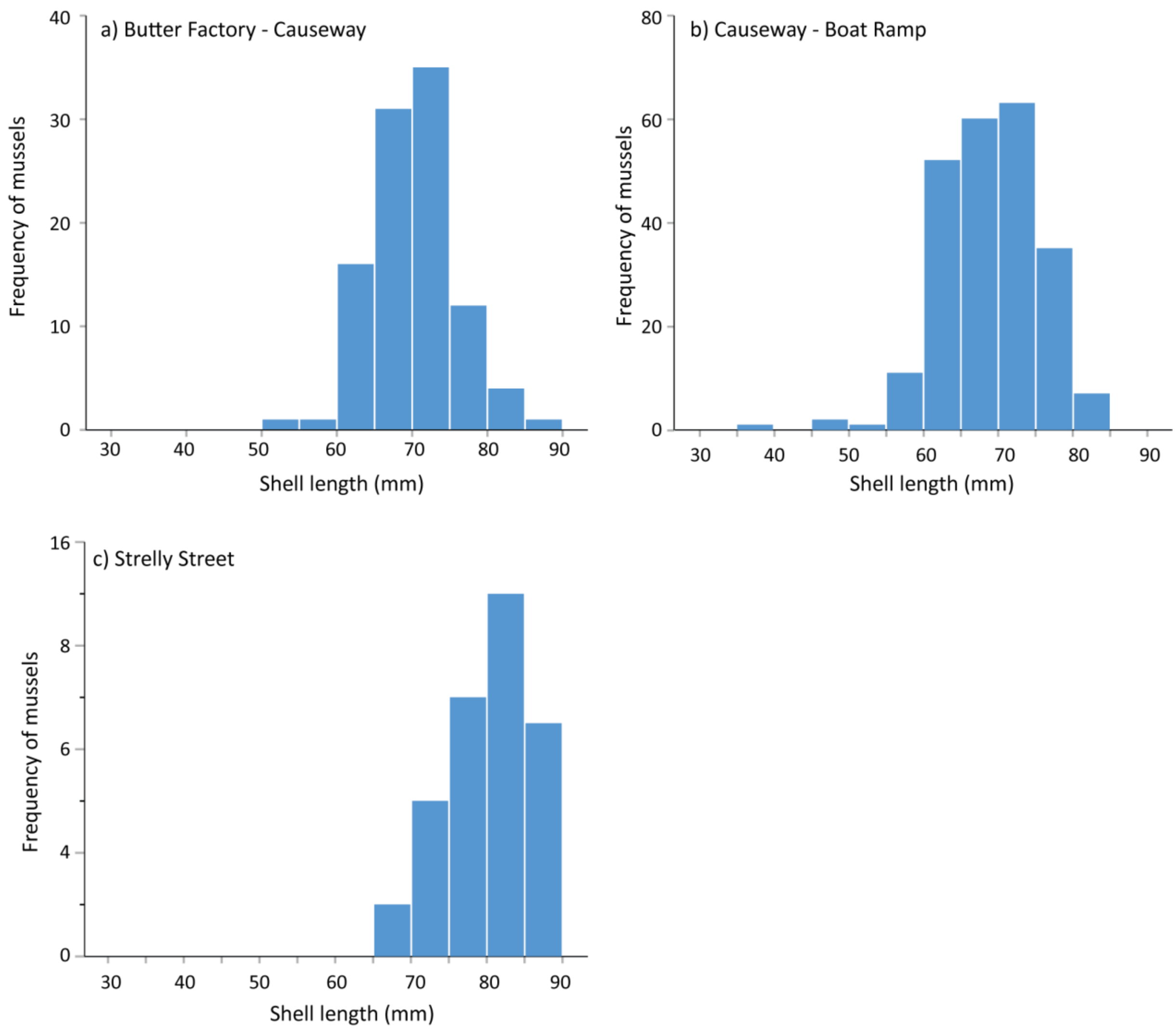

Figure 4. Length-frequency distribution of *W. carteri* from bank habitats in Stage 1 survey areas.

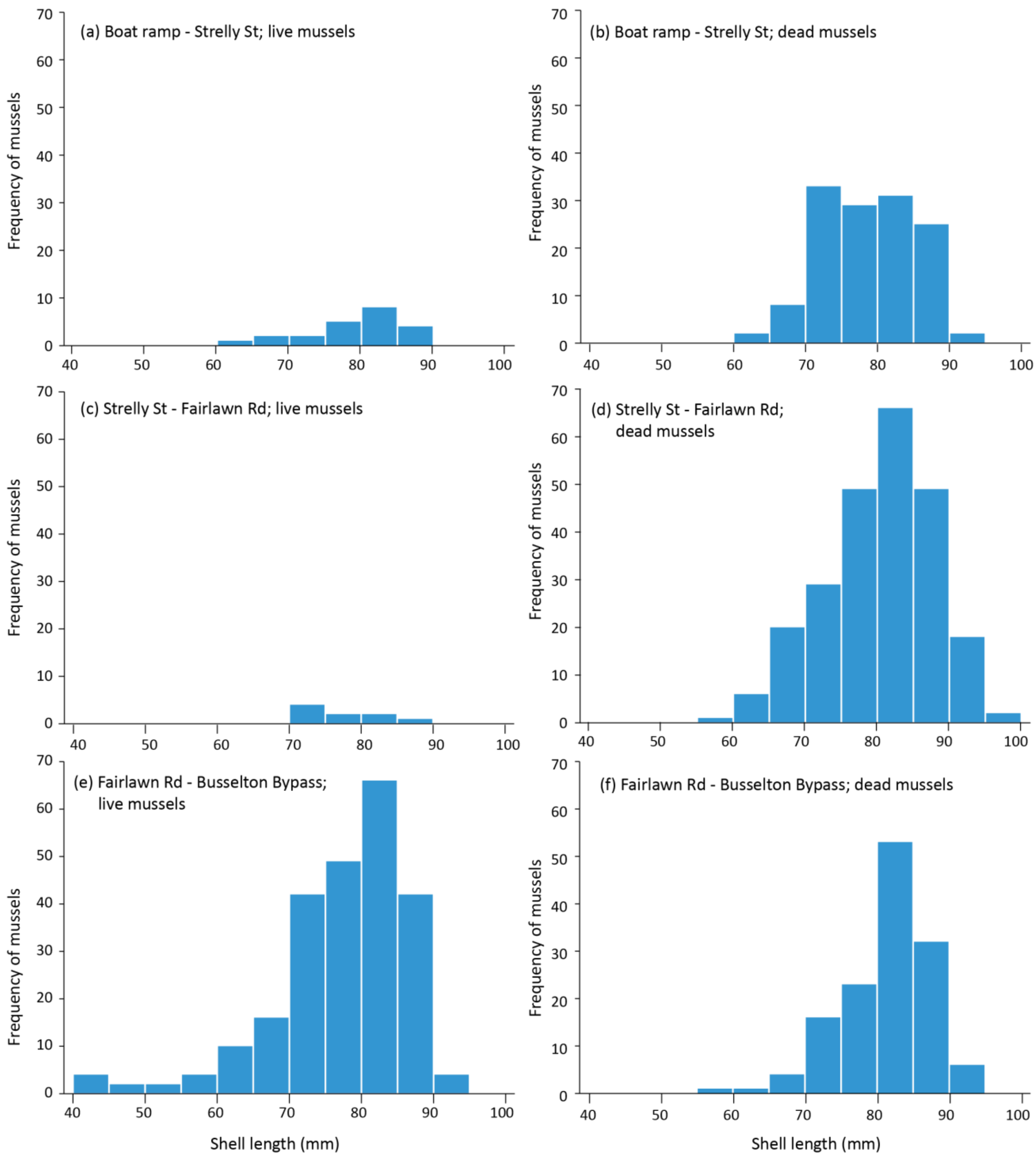

Figure 5. Length-frequency distribution of *W. carteri* from bank habitats in Stage 2 survey areas.

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 measurements from throughout the survey area during the time of the Stage 2 survey shows more detail. Very high salinity occurred 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 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}.

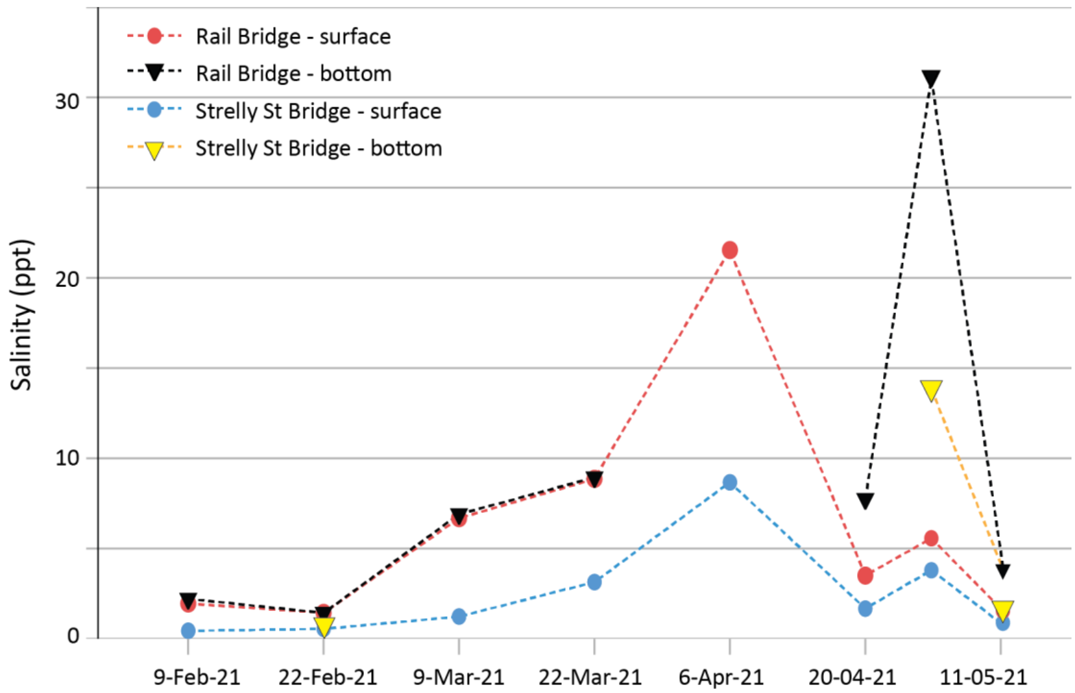

Figure 6. Salinity levels in the lower Vasse River from February to May 2021 (data from Department of Water and Environmental Regulation, 2021).

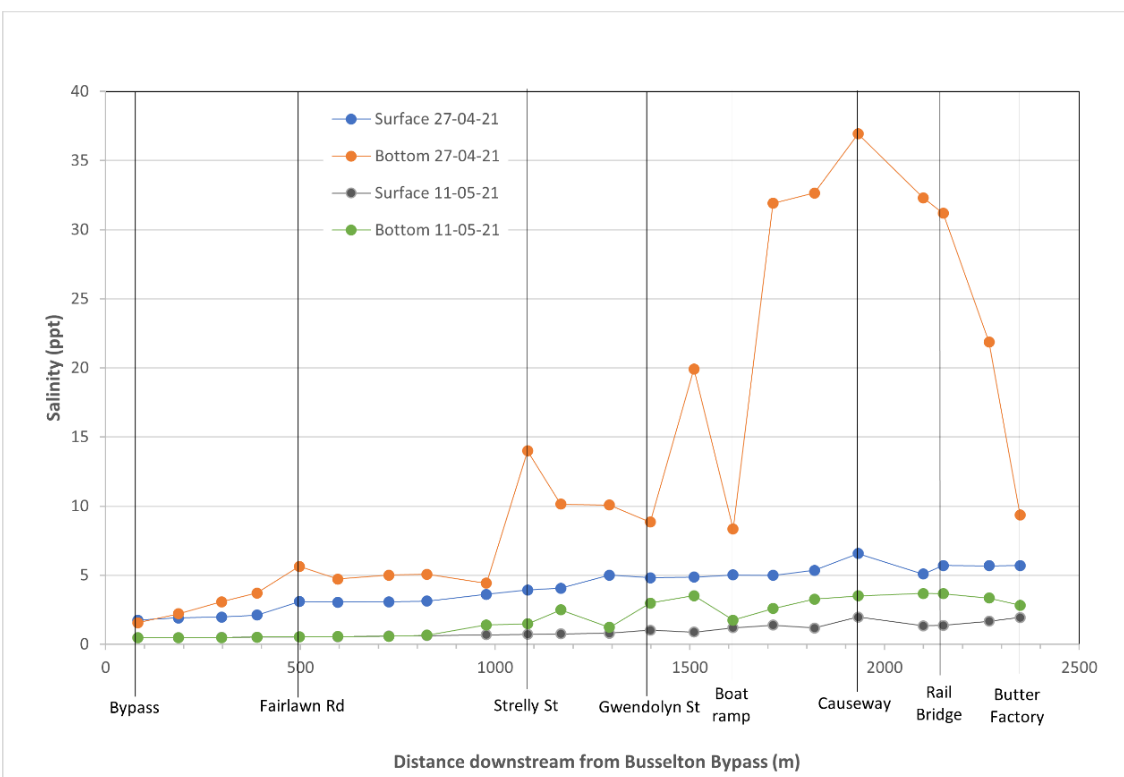

Figure 7. Salinity transect data throughout the lower Vasse river during the Stage 2 survey in April and May 2021 (data from Department of Water and Environmental Regulation, 2021).

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 23.1˚C in December 2020 and 22.6 ˚C in April 2021. During both surveys, the highest temperatures occurred in the less-shaded 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.

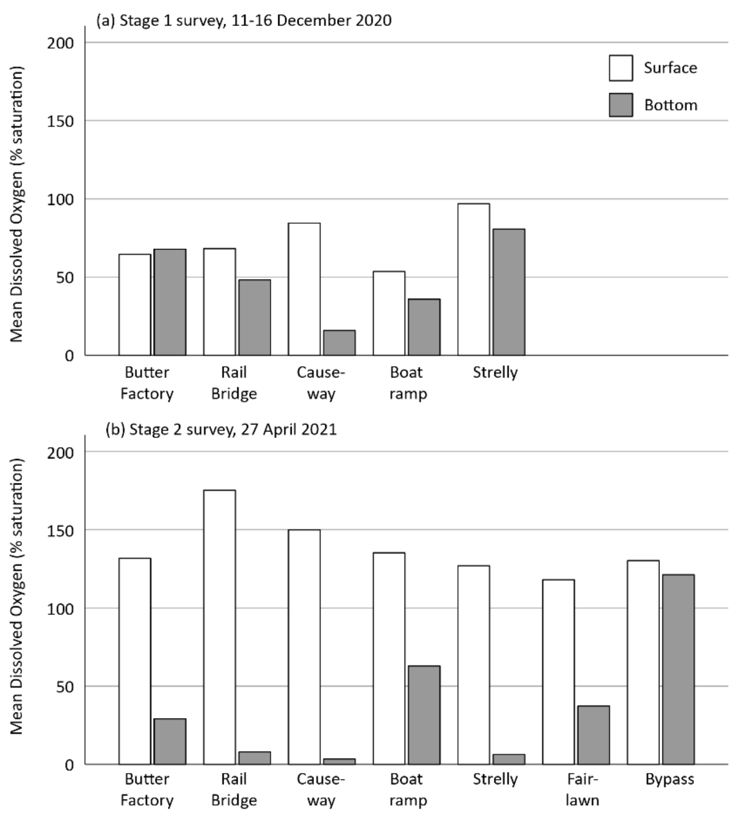

Figure 8. Dissolved oxygen concentrations in the river during the two survey periods.

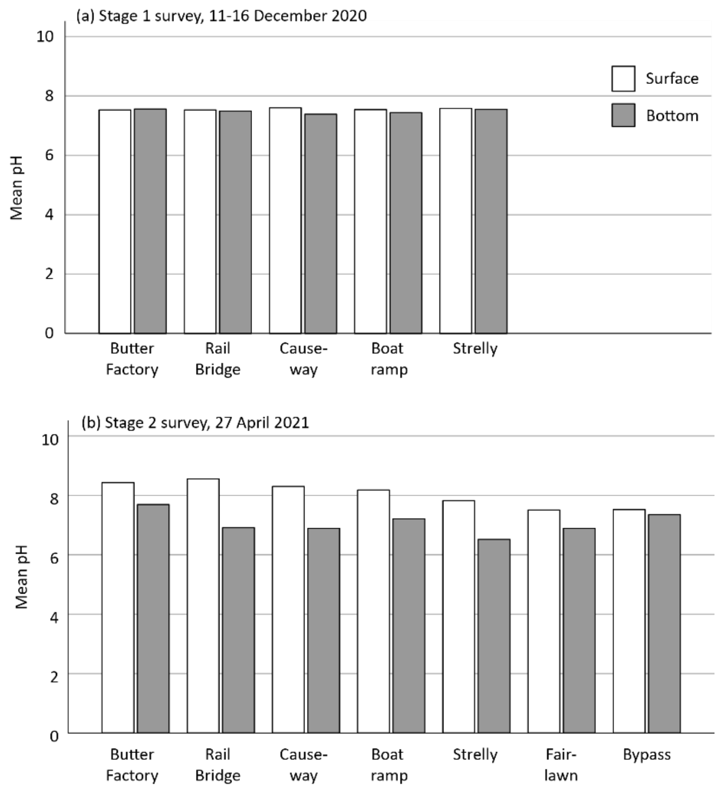

Figure 9. Levels of pH in the river during the two survey periods.

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 reduced water quality. 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 habitat 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 with an estimated abundance of 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 riverbed, 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 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.

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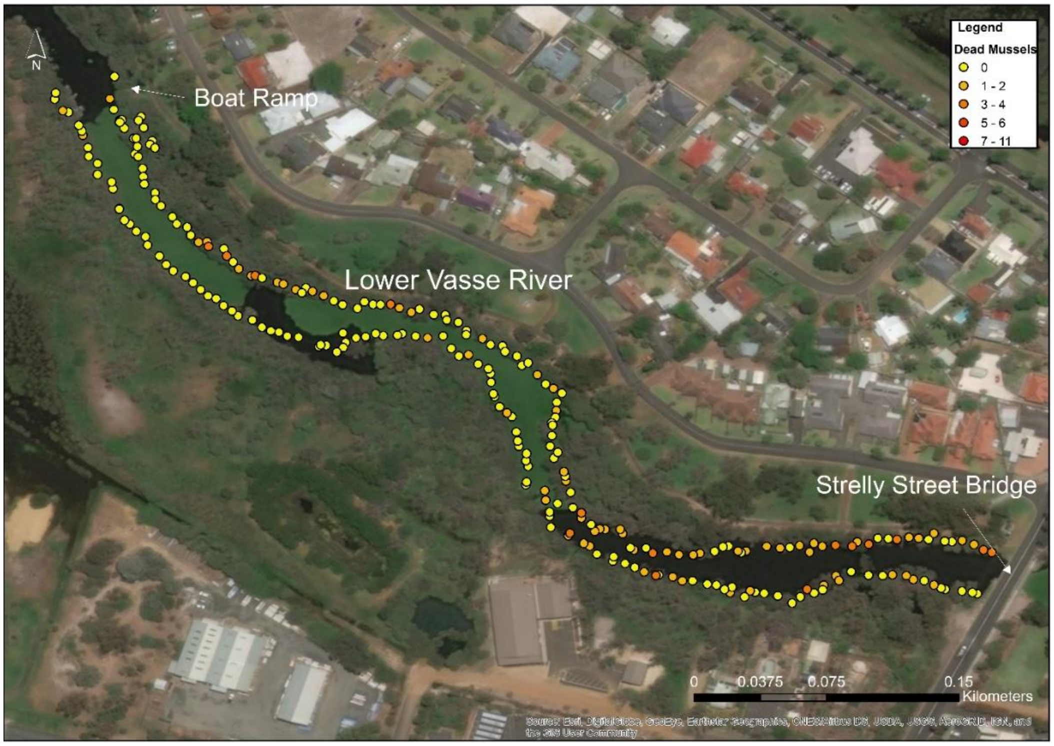
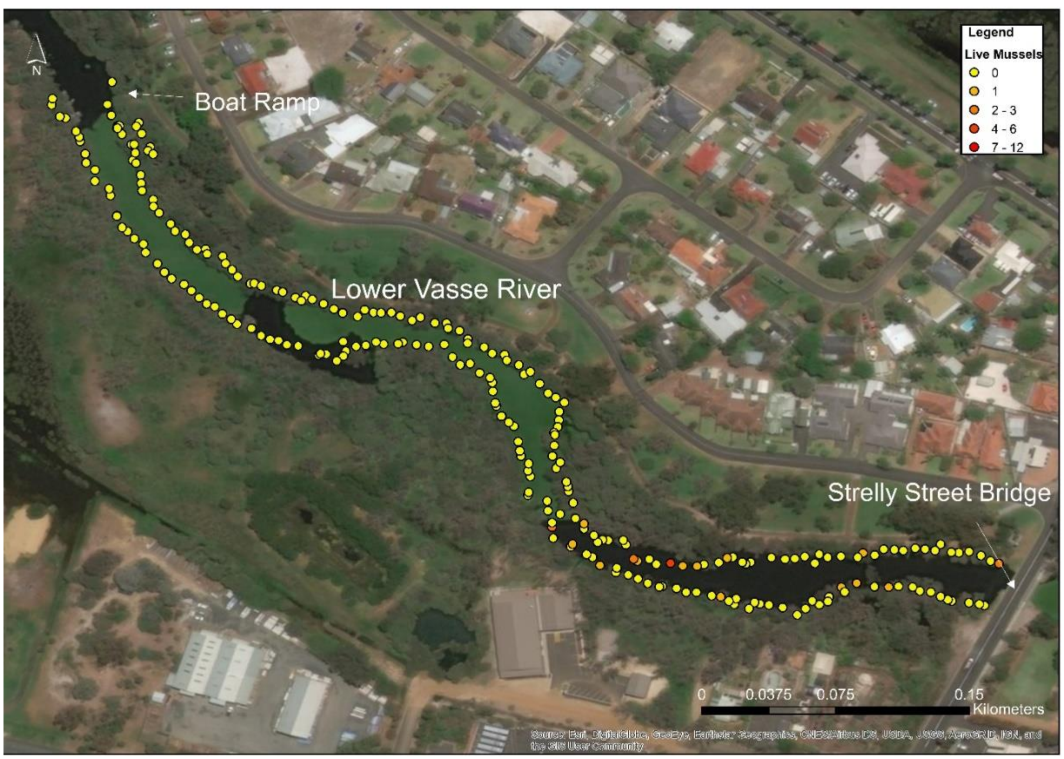
Appendix 1. Location of quadrats showing mussel density in each quadrat.

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.



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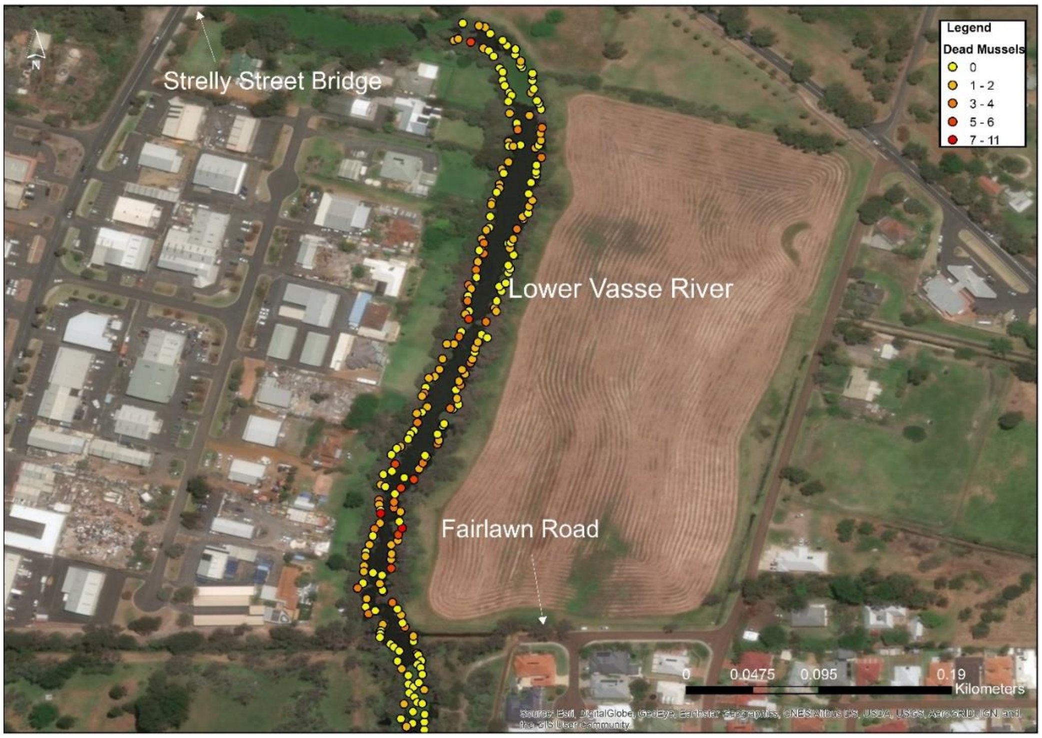
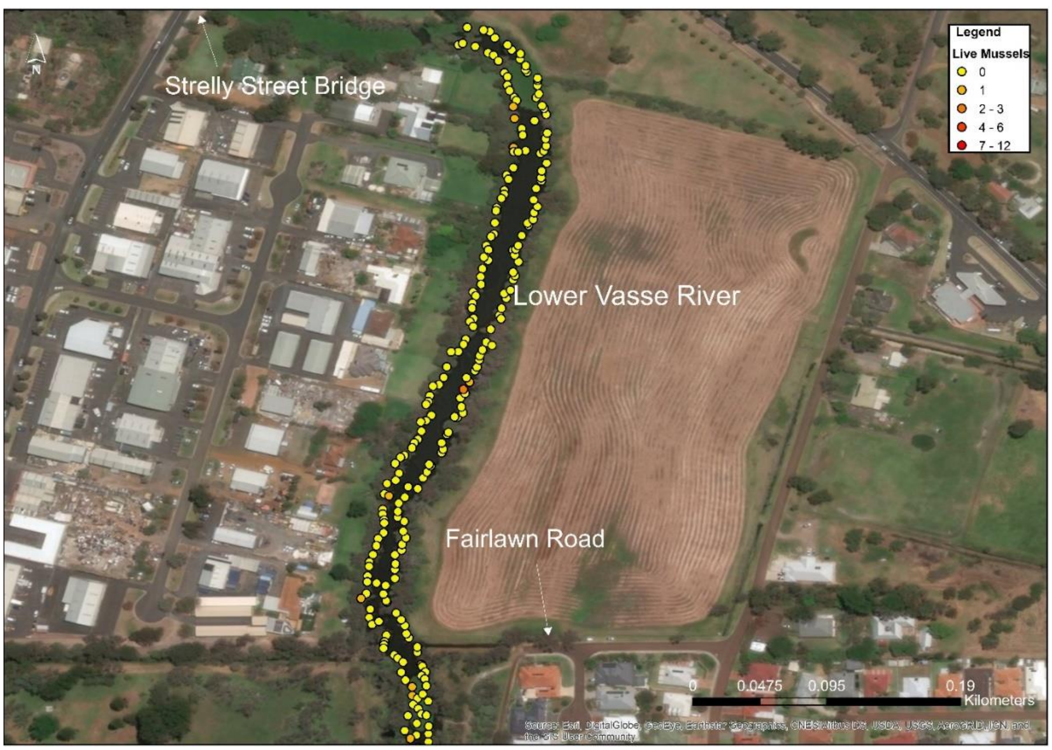
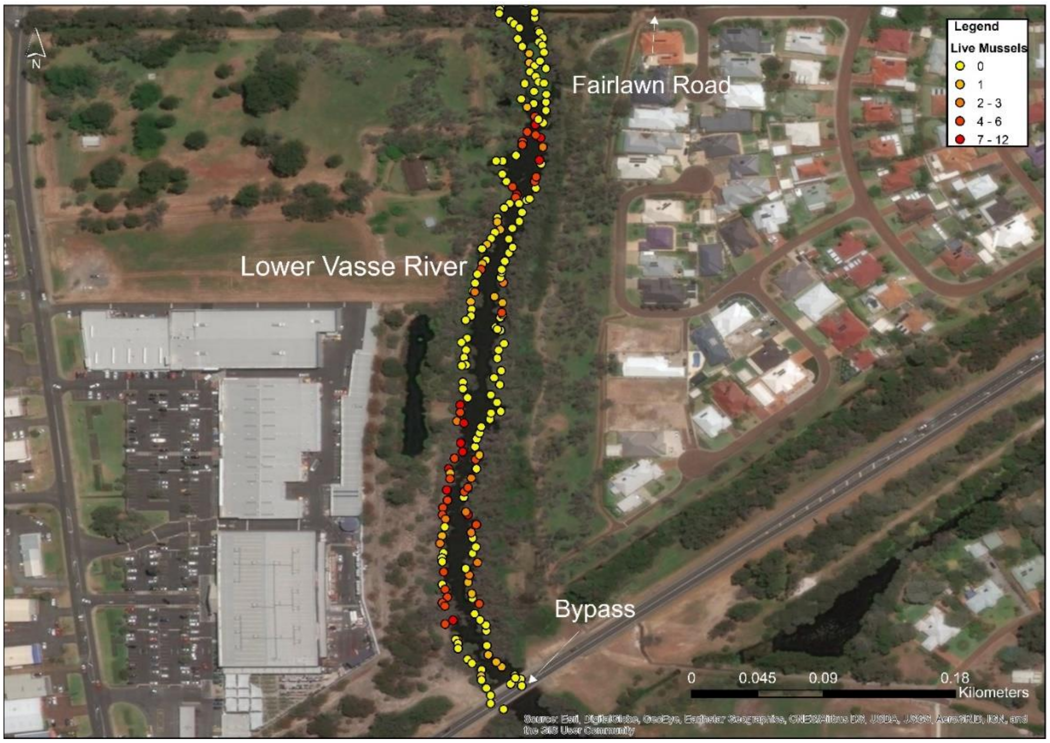


Location of the 1-m² quadrats surveyed for *W. carteri* in the survey area from upstream of Strelly St to Fairlawn Rd and density of dead mussels found in April-May 2021.



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-m^2 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.



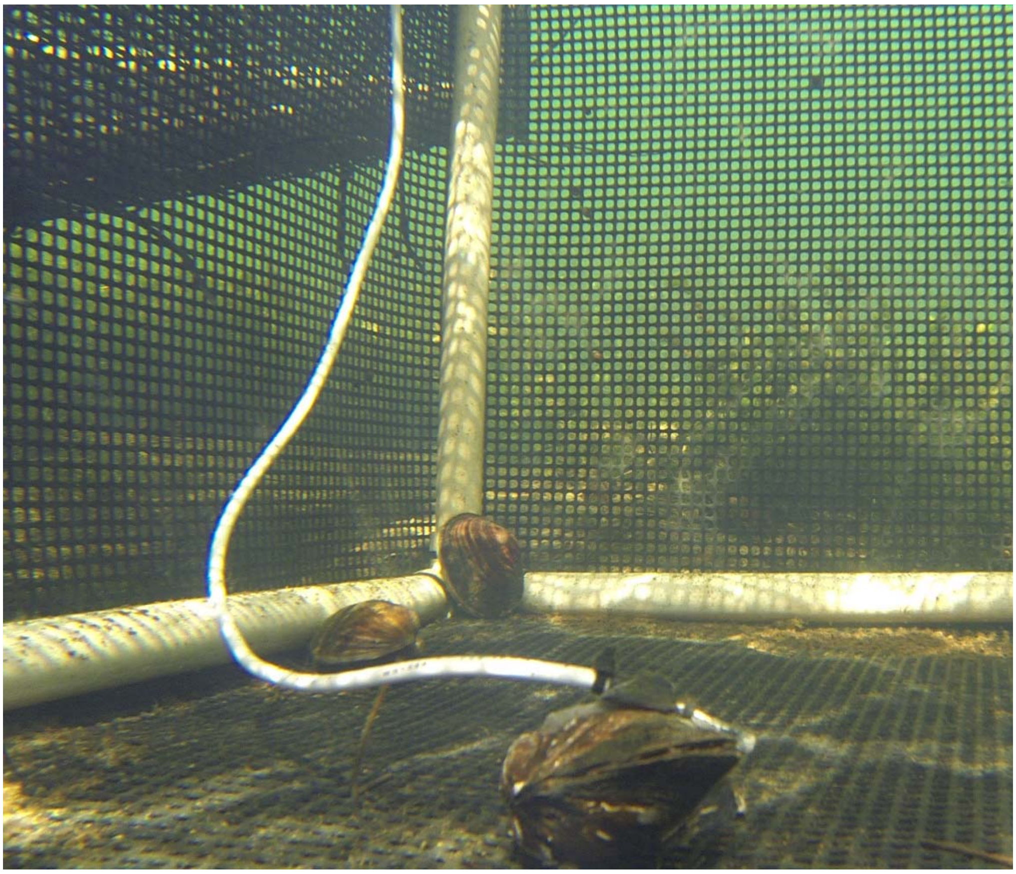
Location of the 1-m^2 quadrats surveyed for *W. carteri* in the survey area from Fairlawn Rd to Busselton Bypass and density of live mussels found in April-May 2021.

Appendix 2: Status of relocated Westralunio carteri: Eastern Link project Progress report August 2020

August 2020

Status of relocated *Westralunio carteri*: Eastern Link project

Progress report



Prepared for:
City of Busselton

Prepared by:
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
Murdoch University

Ottelia Ecology

Prepared for:

Daniell Abrahamse

Manager Engineering and Technical Services

City of Busselton

Prepared by:

S. Beatty, R. Paice*, A. Cottingham, K. Hastings, A. Lymbery
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
Murdoch University
https://harrybutlerinstitute.org/
*Ottelia Ecology
www.otteliaecology.com.au

Distribution List:

Organisation	Contact Name	Date
City of Busselton	Daniell Abrahamse	20/08/2020
Accendo Australia	Kirsten Muir-Thompson	20/08/2020
Kim Williams	DBCA	20/08/2020

Document Control:

Document Status	Prepared By	Reviewed by	Date
Draft progress report Version 1	S. Beatty, R. Paice, A. Cottingham, K. Hastings, A. Lymbery	David Morgan	15/08/2020

The authors acknowledge the Noongar people who are the Traditional Custodians of the land on which this research took place.

Frontispiece: Mussel with valvometer fitted in Taylor's Lake in November 2019.

Introduction and Scope

The *Carter's Freshwater Mussel Westralunio carteri Environmental Management Plan (WCMP) – January 2019* (hereafter referred to as the WCMP) (Beatty et al. 2019a) detailed the requirements for a temporary translocation program for the EPBC listed Carter's Freshwater Mussel *W. carteri*. The WCMP was instigated in October 2019 prior to the construction phase of the *Busselton Eastern Link and Causeway Bridge Duplication Project*.

The methods and results of the removal program and status of the relocated mussels in Taylor's Lake over the first month of relocation are summarised in the progress report in November 2019 (Beatty et al., 2019b). That demonstrated that 171 live *W. carteri* were removed from the bridge construction site in the lower Vasse River (LVR) to Taylor's Lake in October 2019. Capture rates of *W. carteri* from the LVR suggested that the vast majority of the species were removed from the site. It also revealed that all relocated mussels were surviving in the cages in Taylor's Lake after one month of relocation.

In line with the requirements outlined in the WCMP, an assessment was conducted of the water quality and habitat conditions within the LVR prior to the relocated *W. carteri* being moved back into the system in April 2019. A report was then provided to DBCA and the City of Busselton (R. Paice, Appendix 1)).

The current progress report summarises the status of the mussels for the entire ~8 month housing period in Taylor's Lake. It also summarises the results of the pre-relocation water quality and habitat assessment (detailed in Appendix 1) and outlines the relocation process of *W. carteri* back into the LVR including detailing the results of the first post-relocation status assessment. This report thus fulfils key components of the *Management Objectives and Targets* and *Audit and Review* in the WCMP.

Methods

Status of relocated *W. carteri* during the housing period

Regular checks on the mussels in the 10 cages in Taylor's Lake were undertaken during the relocation period at approximately the following frequency:

- daily for the first three days
- weekly for two months
- fortnightly for three months
- monthly for two months

On each occasion, mussels were first checked for responsiveness: open mussels in each cage were counted and were touched to see if they would close, and the number responsive mussels recorded. All open mussels checked in this way were responsive. However, most mussels were closed during monitoring, likely in response to disturbance of the cages during the process.

On 21st January 2020 the mussel with the valvometer attached in cage 10 was found to be dead when it was removed from the water, indicating that observations of closed mussels can be unreliable. Following this it was decided to physically check mussels by removing from the water, rather than relying on responsiveness of a small number.

Status of host population of *W. carteri* in Taylor's Lake during the housing period

Two surveys of the wild mussel population in Taylor's Lake were undertaken on 6/12/2019 and 29/1/2020 in the shallow water zone between the between the cages housing relocated mussels and the shore (Figure 1). For each survey, ten replicate 1x1m quadrats were randomly placed along the shallow water zone. Within each quadrat wild mussels were collected by hand via searching along the surface of the sand and up to 10cm below the surface for buried mussels. All mussels collected from each quadrat were counted and measured before being placed carefully back into quadrat area.

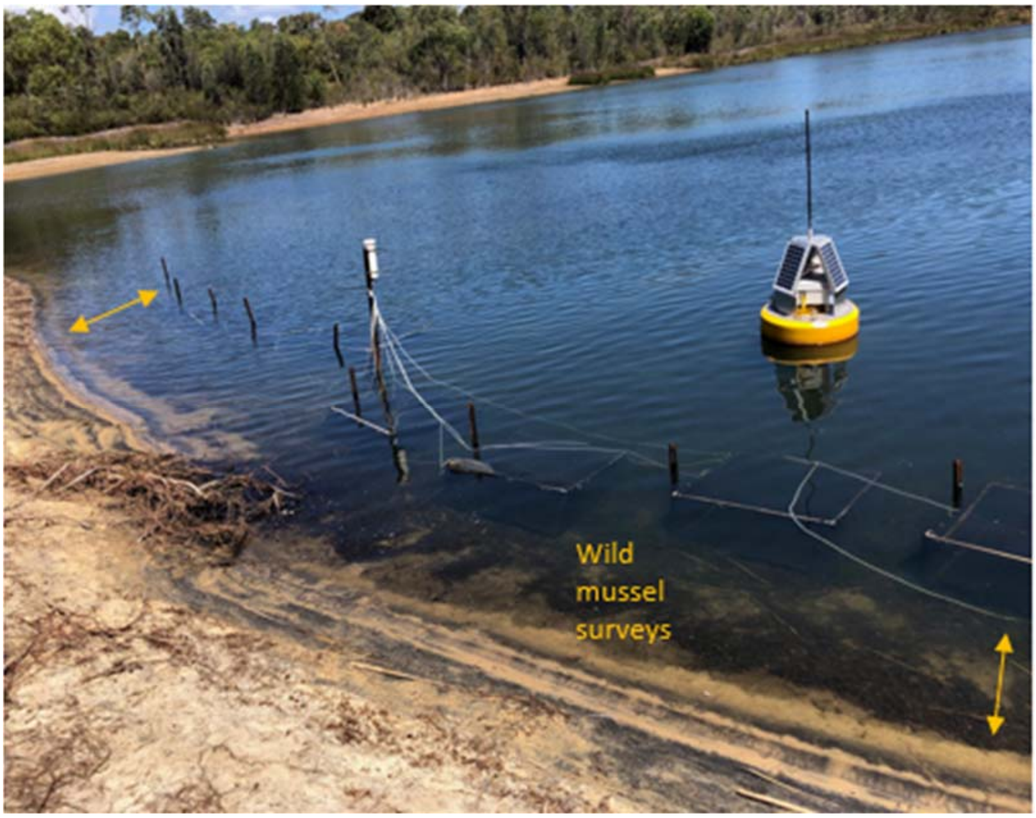


Figure 1: Approximate location of random quadrats used to survey wild mussels at Taylor's Lake.

Habitat and water quality assessment of Lower Vasse River prior to re-introduction

Water quality and habitat assessments were conducted within and upstream of the LVR construction site on April 29th 2020. Visual assessments were made of the benthic habitat throughout the construction footprint on both sides of the LVR including bank angle, water depth and substrate types (Appendix 1). Water quality profiles were taken on three occasions at the construction footprint (five hours apart) to quantify changes in water quality over time. To compare water quality within the footprint to control sites, assessments were also made at five additional sites upstream of the construction footprint to a distance of 600m. Linear models with Tukey's post-hoc pairwise tests were employed to determine whether there were significant differences in temperature, pH, dissolved oxygen, turbidity and salinity among the sites.

Reintroduction of *W. carteri* to the lower Vasse River

Following the above habitat and water quality assessment of the lower Vasse River confirming the site to be suitable for reintroduction of *W. carteri* (see Results), relocation of the mussels to the lower Vasse River was undertaken on June 10th 2020. Mussels were removed from cages, measured to the nearest 1-mm shell length (SL) and marked with numbered shellfish tags (*Hallmark Print*) prior to transport (Figure 2). Water from the Vasse River was mixed 50:50 with Taylor's Lake water to provide some acclimatisation in the insulated transport containers prior to release.

Using knowledge of suitable habitat gained from the fishout, mussels were returned at appropriate sites within their original capture zone. Each individual mussel's tag number and release location was recorded using a GPS.

Figure 2: Shellfish tags being fitted to mussels at Taylor's Lake in June 2020 prior to reintroduction in the lower Vasse River.

Post-relocation monitoring

Follow-up monitoring of relocated mussels was undertaken one month after release in line with the WCMP guidelines (i.e. 10th July 2020). Water levels in the river had increased somewhat so that sampling for mussels was difficult. The water was very cold (see Results), limiting the personnel time spent in the water, and conditions were too turbid for effective snorkelling. Searching for mussels occurred within arm's reach along each bank for 45 minutes. All mussels captured were measured, tag number recorded and location of capture recorded using a GPS.

Results

Status of *W. carteri* during the relocation period

The original dissolved oxygen and temperatures triggers for corrective actions to occur of relocated mussels in Taylor’s Lake were too conservative based on early data of water temperature in Taylor’s Lake. Therefore, the triggers were revised (13/5/20) and approved by DBCA so that corrective actions were required if: *Water temperature >32°C and/or dissolved oxygen <2 ppm for a continuous period of >12 hours: and valve closure, as indicated by valvometer activity, >50% of W. carteri for a period of >12 hours.* These revised triggers were not reached throughout the eight-month holding period.

Four mortalities occurred during the translocation period: two in cage 10 on 21st January and 6th February 2020; and two when all mussels were removed for relocation to the Vasse River (Table 1). Valvometer activity of sentinel mussels revealed that the all undertook prolonged periods of valve openings. On average, the valves were open for >70% of the time and closures were typically short, i.e. < 15 minutes (Figure 3).

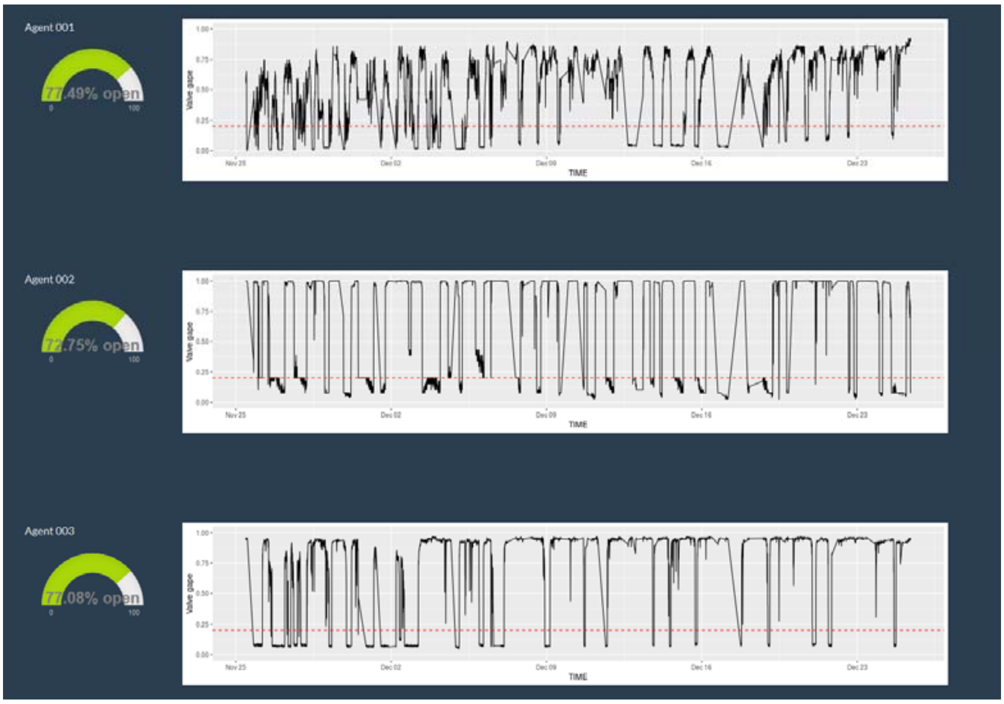


Figure 3: Live-streamed data for three of ten of the *W. carteri* fitted with valvometers in Taylor’s Lake. The gauge plots show average time spent open and line plots the behaviour with time. Valves fully open are represented by values of ~ 1 (top) and valve closures values of <0.2 (denoted by the dashed red line).

Water levels in Taylor’s lake declined by around 0.6m during the translocation period. Cages were moved to deeper water on 6th February to prevent exposure of caged mussels to increasingly shallow conditions.

Table 1: Results from monitoring of caged mussels during relocation period.

Sample date	Mussel counts in each cage											Notes
	1	2	3	4	5	6	7	8	9	10	Total	
09/10/2019	19	10	10	18	19	19	19	19	19	19	171	
10/10/2019	19	10	10	18	19	19	19	19	19	19	171	
11/10/2019	19	10	10	18	19	19	19	19	19	19	171	
15/10/2019	21	11	11	15	16	18	20	19	20	20	171	Valvometer repairs and redistribution of mussels
18/10/2019	21	11	11	15	16	18	20	19	20	20	171	
21/10/2019	21	11	11	15	16	18	20	19	20	19	170	Cage 10 mortality
08/11/2019	21	11	11	15	16	18	20	19	20	19	170	
18/11/2019	21	11	11	15	16	18	20	19	20	19	170	
22/11/2019	21	15	15	15	16	18	20	19	20	19	178	Additional 8 mussels added
28/11/2019	21	15	15	15	16	18	20	19	20	19	178	
06/12/2019	21	15	15	15	16	18	20	19	20	19	178	Wild mussel survey
19/12/2019	21	15	15	15	16	18	20	19	20	19	178	
03/01/2020	21	15	15	15	16	18	20	19	20	19	178	
16/01/2020	21	15	15	15	16	18	20	19	20	19	178	
29/01/2020	21	15	15	15	16	18	20	19	20	19	178	Wild mussel survey
06/02/2020	21	15	15	15	16	18	20	19	20	18	177	Cage 10 mortality Cages moved to deeper water
14/02/2020	21	15	15	15	16	17	21	19	20	18	177	
20/02/2020	21	15	15	15	16	17	21	19	20	18	177	
05/03/2020	21	15	15	15	16	17	21	19	20	18	177	
19/03/2020	21	15	15	15	16	17	21	19	20	18	177	
17/04/2020	21	15	15	15	16	17	21	19	20	18	177	
15/05/2020	21	15	15	15	16	17	21	19	20	18	177	
10/06/2020	21	15	15	15	16	17	21	19	20	18	175	Return to river 2 mortalities in cages

Status of host population of *W. carteri* in Taylor's Lake during the housing period

Wild mussel abundance was high in both the December and January surveys, with a total of 268 and 421 individuals collected respectively (Table 2). The mean per quadrat density of wild mussels was much higher in January (42.1) compared to December (26.6), which likely reflected a concentration of the population as water levels dropped over the season. Abundance of wild mussels per quadrat was highly varied in both seasons, ranging from 13-42 mussels/m^2 in December and 25- 67 mussels/m^2 in January; reflecting the known patchiness of its distribution. Importantly, densities of host mussels in Taylor's Lake post-relocation of Vasse River mussels to cages were significantly higher than was recorded pre-relocation in September 2019 and thus no adverse effect of the relocation of the Vasse mussels was recorded on the host population.

The average shell length of wild mussels was 48cm in December and 44.8cm in January. The sizes of mussels collected reflected a range of different age classes. In December mussel shell length ranged from 13 to 72 cm and in January from 21 to 81 cm (Table 2, Figure 4).

Table 2: Summary of abundance and size of wild mussels recorded at Taylors Lake directly adjacent to caged relocated mussels.

	Dec 6 2019	**Jan 29 2020**
Abundance		
Total number of mussels	266	421
Mean density per m^2	26.6	42.1
Max	42	67
Min	13	25
Shell Length (mm)		
Mean	48.1	44.8
Max	72.0	81.0
Min	25.0	21.0

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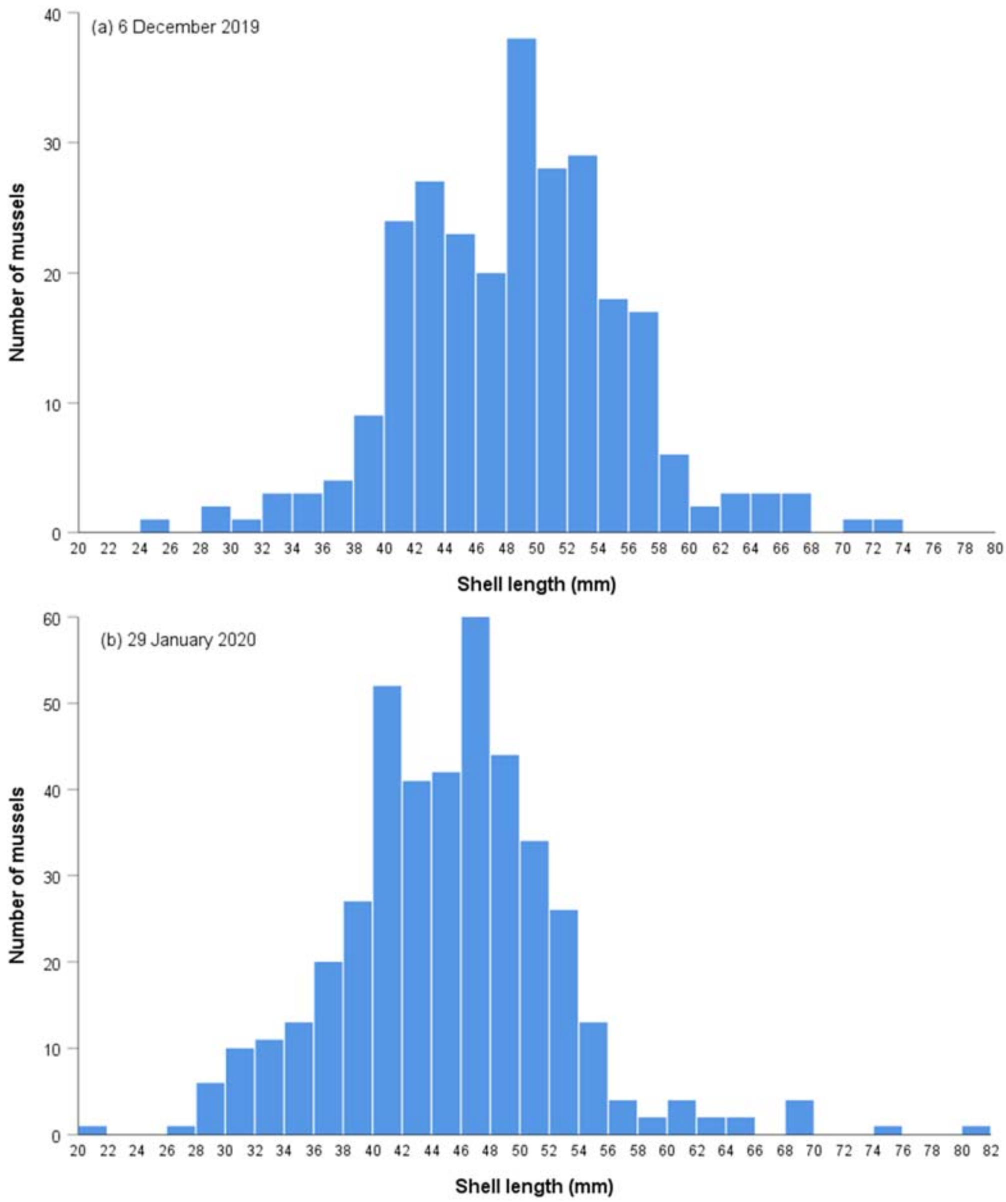

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Figure 4: length-frequency distribution of *W. carteri* in Taylors lake in December 2019 (a) and January 2020 (b).

Habitat and water quality assessment of Lower Vasse River prior to re-introduction

Habitat assessment throughout the bridge construction footprint in April 2020 revealed that there was ~40 m of hard, rocky substrate around the bridge that was deemed unsuitable for *W. carteri* (Figure 5, Appendix 1). Therefore, it was proposed reintroduce the mussels to the outer sections upstream and downstream of the bridge footings. As the WCMP stated that mussels would be reintroduced back into the site at similar densities as they were originally found, a variation to the WCMP was prepared and approved by DBCA, that also included adding individual shellfish tags to the relocated mussels so that the fate of individuals could be determined in the post-introduction monitoring program. The increased density of restocking at the upstream and downstream sections of the site was deemed to have negligible risk as the species were in relatively low abundances originally and the species is known to reach much higher densities in other reaches of the Vasse River.

Water quality at the construction footprint site (Table 3) was well within the known field tolerances of the species and well within the trigger values for the relocation site outlined in the WCMP. There were significant overall differences in temperature ($p = 0.037$), dissolved oxygen ($p < 0.01$), and pH ($p < 0.01$) among the sites. However, the construction site was not consistently poorer than the other control sites. For example, dissolved oxygen was lowest at the control site that was ~600 m upstream of the construction site and turbidity at the construction site was the second lowest of all sites assessed (Table 3).

Based on the above pre-relocation assessment in April 2020, it was confirmed that the mussels could be re-introduced to the construction site.

Table 3: Water quality at the Eastern Link bridge construction footprint and control sites upstream in April 2020.

Site	Temp (C)	Dissolved oxygen (%)	Conductivity (mS/cm)	pH	Turbidity (NTU)
Bridge construction footprint	18.17 (0.20)	73.97 (0.47)	1.26 (0.04)	7.99 (0.07)	10.33 (0.62)
OLB + 100m	18.30 (0.45)	110.83 (1.33)	1.51 (0.01)	8.30 (0.09)	16.33 (0.79)
OLB + 200m	18.10 (0.30)	90.53 (1.32)	1.50 (0.03)	8.13 (0.11)	32.25 (2.09)
OLB + 400m	17.60 (0.36)	74.93 (1.57)	1.46 (0.05)	7.85 (0.09)	11.00 (0.50)
OLB + 600m	16.63 (0.23)	35.50 (1.60)	1.37 (0.07)	7.46 (0.20)	9.33 (0.38)
Old Rail Bridge (OLB)	17.67 (0.05)	98.10 (1.61)	1.50 (0.03)	8.05 (0.09)	35.67 (2.92)

mussels were alive and the population continued to be represented by a wide size range (as per previous baseline survey by the authors in April 2019 during the assessment of the suitability of the site for relocation).

A total of 172 *W. carteri* were removed from the lower Vasse River within and adjacent to the Eastern Link construction site on the 8/10/19 (Figure 4). A substantial decline in the number of mussels removed during each consecutive pass on each bank (Table 1) suggesting that the process would have resulted in all or the vast majority of individuals being relocated.

Table 1: Number of *W. carteri* removed from the Eastern Link project site.

Bank	Pass	Number caught
right	1	47
right	2	11
right	3	3
left	1	85
left	2	20
left	3	6
TOTAL		**172**

Figure 4: Distribution of the of the *W. carteri* removed from the Eastern Link project site. Approximate location of the new bridge is also indicated (shaded region).

Western side of bridge

Eastern side of bridge

Figure 5: Habitat at the construction site during the pre-relocation assessment in late April 2020. N.B. the rocky substrate around the bridge that was deemed unsuitable for relocation of *W. carteri.*

Monitoring of reintroduced *W. carteri* in the lower Vasse River

Water quality during the post release monitoring continued to be within the known tolerance of *W. carteri* (Table 4). The first monitoring event one month after reintroduction of *W. carteri* into the LVR encountered 36 live mussels during the sub-sampling of the site: 29 of these were tagged and 7 had no tags. It was unclear as to whether the untagged mussels were relocated mussels that had shed their tags, or were mussels that had moved into the original relocation site in the ~9 month holding period. One dead tagged mussel was recorded. Most mussels were found on the northern bank (24), including the dead mussel and one mussel with no tag. On the southern bank, 13 mussels were found, of which only 7 had tags.

Table 4: Water quality in lower Vasse River during post-release monitoring 10th July 2020 (Old rail bridge).

Variable	Surface (0.2m)	Bottom (1.14m)
Temperature (°C)	11.7	11.5
Dissolved oxygen (%)	59.3	56.8
Dissolved oxygen (mg/L)	6.4	6.17
Conductivity (mS/cm)	0.961	1.032
Salinity (ppt)	0.48	0.51
pH	7.30	7.20
Turbidity	11.09	10.6

Ongoing activities

Monitoring of the reintroduced mussels is scheduled to occur at 2 and 6 months. Movement patterns of tagged relocated mussels will be determined over this period using the recapture data. A further 10 of the original sentinel mussels continue to be housed in cages in Taylor's Lake to obtain a full 12 months of activity. This was undertaken with approval from DBCA in order to provide a baseline of the species activity that may be useful for future monitoring projects. A final report will be prepared in February 2021 (i.e. 8 months after reintroduction of the mussels into the LVR, and 2 months after the final monitoring event) that will collate all activities of the project against those outlined in the WCMP.

References

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.

Appendix 1

Eastern Link habitat assessment for mussel relocation

29 April 2020

General: The footprint of the bridge extends along approximately 40m of river. Where the bridge intercepts the river bank, habitat is dominated by large rocks (>300mm diameter) and bank slope is steep, quickly dropping to 1m beyond exposed rocks. Sediment beyond the rocks is hard, presumed to be a road-base material, but samples could not be obtained. River bank substrate beyond footprint is clayey sand extending to soft organic sediments at varying distance from bank, depending on bank slope.

The rocky areas adjacent to the bridge probably do not provide suitable mussel habitat due to steep banks, large rocks, hard substrate and lack of shade. Mussels relocated among rocks would be very difficult to recapture for monitoring. However, beyond the bridge footprint the banks have not been impacted and retain the original habitat values, so would provide suitable relocation sites.

NW corner: Footprint extends 5.5m from bridge, rock extends 3.5m from bridge. Large submerged rocks visible 2m from bank, river depth beyond this 1m, with hard substrate. This leaves 2m of earth river bank with clayey sand substrate of original river, with bank slope to 3m from water's edge ranging from 0-0.5m to 0.45-1.25 near rocks. Vegetation is cleared with kikuyu present, but site receives shade from northern trees.

NE corner: Footprint extends 7m from bridge, rock extends 5m from bridge. Submerged rock extends 1m from bank and depth beyond this is ~1m and substrate hard. This leaves 2m of sandy riverbank downstream of rocks within footprint, but bank is steep: 0.45m at edge of bank and 1.1m 1m from edge of bank. Some fringing vegetation and shade.

SW corner: Footprint extends 10.7m from bridge, with rock along entire length. Depth 0.7m off edge of rocks. Submerged rocks and hard substrate beyond. Bank has been scalped back within footprint about 2m from natural edge. Original river bank upstream of footprint with riparian vegetation retained including overhanging shrubs.

SE corner: Width of footprint 7.5m from bridge, with rock along entire length. Very steep bank with depth off rocks >1m. No shade. Original river bank

Appendix 3: Relocation program for Westralunio carteri: Eastern Link project Nov 2019

November 2019

Relocation program for *Westralunio carteri*: Eastern Link project

Progress report

Prepared for:
City of Busselton

Prepared by:
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
Murdoch University

Prepared for:

Daniell Abrahamse
Manager Engineering and Technical Services
City of Busselton

Prepared by:

S. Beatty, A. Cottingham, R. Paice, A. Lymbery
Centre for Sustainable Aquatic Ecosystems
Harry Butler Institute
Murdoch University, South St
Murdoch, Western Australia 6150

Distribution List:

Organisation	Contact Name	Date
City of Busselton	Daniell Abrahamse	21/11/2019
Accendo Australia	Kirsten Muir-Thompson	21/11/2019
Andrew Webb	DBCA	21/11/2019

Document Control:

Document Status	Prepared By	Reviewed by	Date
Draft progress report Version 1	S. Beatty A. Cottingham, R. Paice, A. Lymbery	David Morgan	15/11/2019

The authors acknowledge the Noongar people who are the Traditional Custodians of the land on which this research took place.

Frontispiece: Mussel relocation housing cages in Taylor's Lake October 2019.

Introduction and Scope

As part of the Busselton Eastern Link Projects ('the Project'), the *Busselton Eastern Link and Causeway Bridge Duplication Projects Carter's Freshwater Mussel Westralunio carteri Environmental Management Plan (WCMP) – January 2019* (hereafter referred to as the WCMP) was prepared by Beatty et al. (2019). This outlined the requirements for a temporary translocation program for the EPBC listed Carter's Freshwater Mussel *W. carteri* to support environmental approvals under Commonwealth and WA legislation.

The current progress report summarises the management activities associated with the first month of the *W. carteri* relocation project and assesses how it is fulfilling the Management Objectives and Targets of the WCMP as it relates to the Eastern Link (Tables 2 and 3 in the WCMP)).

Methods

Mussel fishout from Eastern Link

A pre-relocation baseline survey of *W. carteri* was conducted at the Eastern Link construction site on 18/9/19 to ensure accurate baseline data prior to relocation. The survey followed the methods outlined in Beatty et al. (2017) as stipulated in the WCMP (monitoring actions in Table 4 of the WCMP).

Subsequently, the fishout of *W. carteri* from the Eastern Link construction site occurred on the 8/10/19 were collected from each bank along a 70 m stretch of the Project site; which included a buffer zone 20 m upstream and downstream of the sediment containment area (within the planned silt curtains).

Each bank was searched intensively in a grid formation by four people with three passes occurring in an upstream direction. The location of each mussel individual was logged using a GPS, removed from the river, and promptly placed in insulated, aerated containers.

Relocation of *W. carteri from the Eastern Link construction site*

A pre-relocation baseline survey of *W. carteri* was also conducted at the Taylor's Lake relocation site on 18/9/19. This also a fulfilled a monitoring action in Table 4 of the WCMP. Due to the fewer than expected *W. carteri* detected during the baseline survey within the Eastern Link construction site (see Results below, i.e.) compared to what was expected as outlined the WCMP (that was based on adjacent sections of the lower Vasse River, see Beatty et al., 2019a), 10 x 1m^2 cages were constructed and installed in Taylor's Lake. Based on the densities detected in the baseline survey, this number of cages would ensure that more than twice the predicted number of mussels that would need to be relocated would be catered for (i.e., based on a maximum stocking density of 50 mussels/m^2 as stipulated in the WCMP). Each cage was secured to steel posts spaced across the relocation site (at locations with a minimum depth of 0.5 m). Each cage was coded and location recorded (using GPS).

Mussels from the Eastern Link construction site were transported in batches to the Taylors Lake holding site in aerated insulated containers where they were then promptly measured and placed into the cages.

One sentinel mussel was randomly selected from each cage and each fitted with a valvometer, and an automated, solar powered telemetered monitoring system was installed on the bank (Figure 1). Initial issues with the adhesive used to affix the valvometers meant that the initial sentinel individuals were swapped for a different group of randomly selected individuals. The original group were cleaned of their fixative with no adverse reaction to the process subsequently observed. This is the first time valvometers have been fixed to the species and the trigger value proposed in the WCMP (i.e. valuve close of >50% of the population for >12 hrs) was assessed by remotely monitoring the telemetered valve activity of the sentinel mussels.

A subsample of mussels in each cage were also physically monitored from site visits that occurred on the following days (8/10/19, 9/10/19, 10/10/19, 11/10/19 (valvometer check), 21/10/19, 24/10/19, 28/10/19, 1/11/19, 8/11/19). On each occasion, a subsample were assessed for activity by gently touching each shell and recording valve closure response. As mussels were found to be highly sensitive to movement around the cages (i.e., elicit a closure prior to be able to visually observe the event), from the 24th of October those closed mussels were also checked for status by gentle handling each mussel to ensure valve was actively shut.

The population status of the resident population of *W. carteri* present at Taylor's Lake was also assessed by sampling 10 randomly placed quadrats on the benthos adjacent to the relocation cages. All mussels were assessed (as alive or deceased), measured and released promptly at the site of capture.

Figure 1: The relocation cages, valvometer and water quality monitoring loggers at Taylor's Lake in November 2019.

Figure 2: A Western Pygmy Perch (likely host fish for larval mussels) in Taylor's Lake.

Figure 3: Sentinel mussels fixed with valvometers at Taylor's Lake in October 2019.

Results

Mussel fishout

Prior to the relocation of the lower Vasse River mussels from the Eastern Link project site, the survey of the resident population within Taylor's Lake adjacent to the relocation cages revealed all

Status of relocated mussels

The lower Vasse River mussels were successfully relocated into the 10 cages in Taylor's Lake (transport time <30 minutes). Of the 172 mussels moved, one was already deceased (was miss-identified as a closed shell alive on relocation) and was thus excluded. All six monitoring events after initial relocation indicated that all mussels assessed were alive on all monitoring occasions (Table 2, Figure 5).

Valvometer activity in the second group of sentinel mussels revealed that the all undertake prolonged periods of valve openings (>~87%, Figure 6); a very interesting finding and that which contrasts with marine species, which undertake much longer periods of value closure. Dissolved oxygen and temperature for the initial month of relocation revealed that readings were within the proposed management trigger values in the WCMP.

Table 2: Status of mussels in each cage in Taylor's Lake over the first month of monitoring. N.B. percentages of open individuals that responded to stimuli by closing their valves are shown for each cage, and the value in parentheses indicate the percentage of actively closed mussels in each cage (i.e. actively closed).

	Percentage of relocated mussels alive through valve response (or active valve closure)					
Cage number	**9-Oct**	**10-Oct**	**24-Oct**	**28-Oct**	**1-Nov**	**8-Nov**
1	100	100	100 (100)	100 (100)	100 (100)	100 (100)
2	100	100	100 (100)	100 (100)	100 (100)	(100)
3	100	100	100 (100)	(100)	100 (100)	(100)
4	100	100	100 (100)	100 (100)	100 (100)	100 (100)
5	100	100	100 (100)	100 (100)	100 (100)	100 (100)
6	100	100	100 (100)	100 (100)	100 (100)	100 (100)
7	100	100	100 (100)	100 (100)	100 (100)	100 (100)
8	100	100	100 (100)	100 (100)	100 (100)	100 (100)
9	100	100	100	100 (100)	100 (100)	100 (100)
10	100	100	100 (100)	(100)	100 (100)	100 (100)

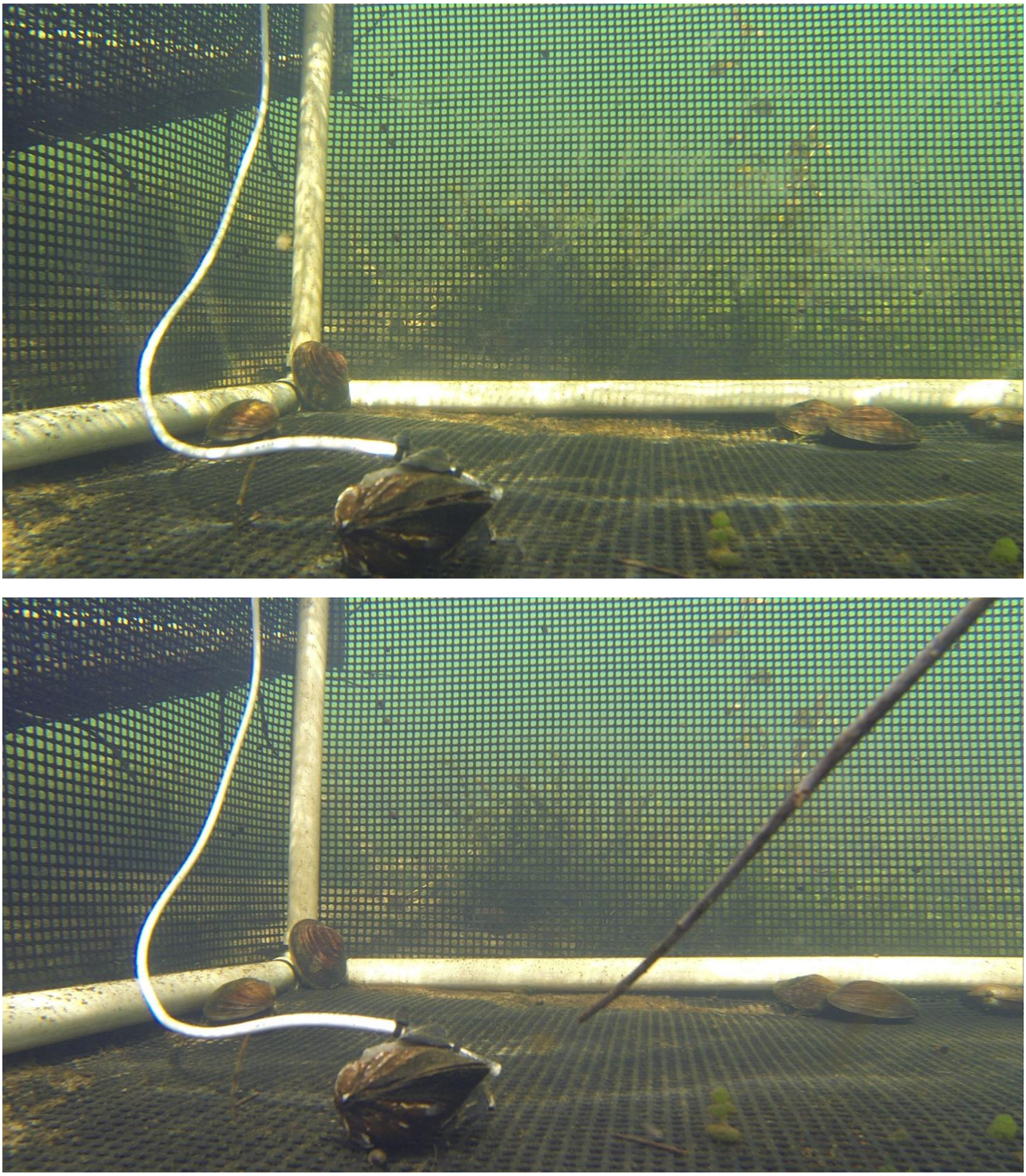

Figure 5: Example of a sentinel mussel being open (top image), and then closed upon stimuli (bottom image).

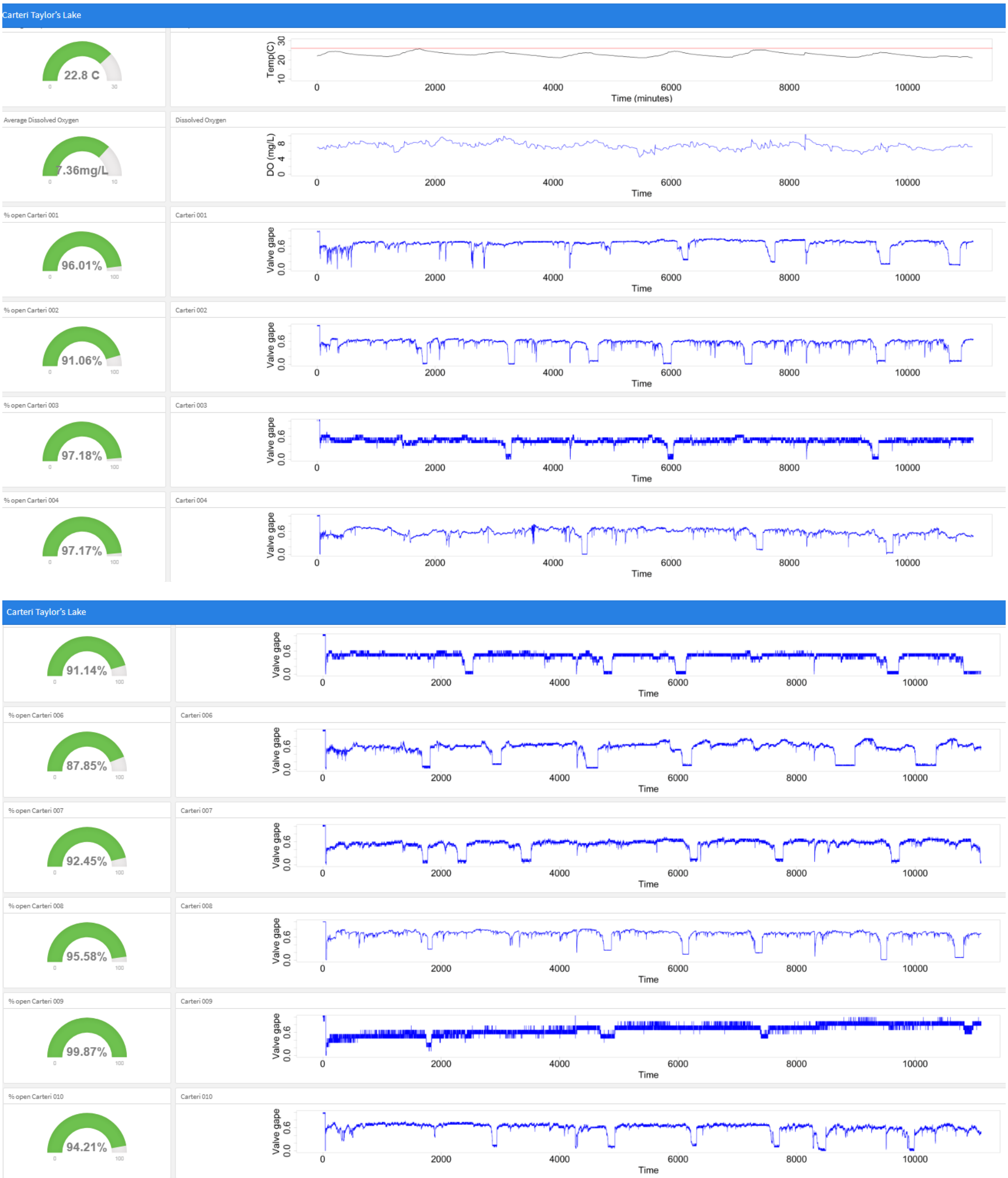

Figure 6: Extracted temperature, dissolved oxygen (top to figures) and the 10 valvometer activity readings in Taylor's Lake. Note the green values to the left show the percentage period of valve opening for each mussel over the sample period.

Ongoing activities

The mussels will continue to be physically monitored weekly up until the 8th of December and then fortnightly for the remainder of the relocation period (in line with the WCMP). Sentinel mussels and water quality triggers will also continue to be monitored remotely against trigger values in the WCMP.

References

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.